EDQM and European Pharmacopoeia: State-of-the-art Science for Tomorrow's Medicines

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Poster Session

Hidden in the Woods:

Quality Markers of Conifer-Derived Essential Oils

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1 Introduction

The needles of conifers, which are distributed all over the world, are source of precious conifer-derived essential oils (CEOs), which might show an interesting potential of antibacterial activity. EOs are mixtures of natural substances mainly categorized as monoterpenes, sesquiterpenes and their oxygenated derivatives. Genuine EOs in this study are obtained from different *Pinus sylvestris* (*P. sylvestris*, PS) worldwide. Genuine EOs can be characterized by their qualitative fingerprint obtained by gas chromatography coupled to a flame ionization detector (GC-FID). Furthermore the chemical fingerprint can be analyzed to find quality markers to define a genuine EOs and the absolute amount of four selected compounds is determined by a GC-FID method [1].

2 Materials and Methods

Genuine EOs were obtained by steam distillation (Ph. Eur.) of needles of PS collected in Poland (P1) and Russia (R1-R6). Additionally, *P. sylvestris* EOs were purchased from several Swiss suppliers (PS 1-4). The chemical composition of the EOs and the quantification was analyzed by GC-FID (Thermo Fisher Scientific Focus GC) equipped with a DB-WAX capillary column (30 m x 0.25 mm x 0.25 µm). Peaks were identified by comparing with reference substances or by GC coupled to a mass-spectrometer (Thermo Fisher Scientific Trace Ultra GC – Thermo DSQ II MS) due to their fragmentation pattern.

3 Results and Discussion

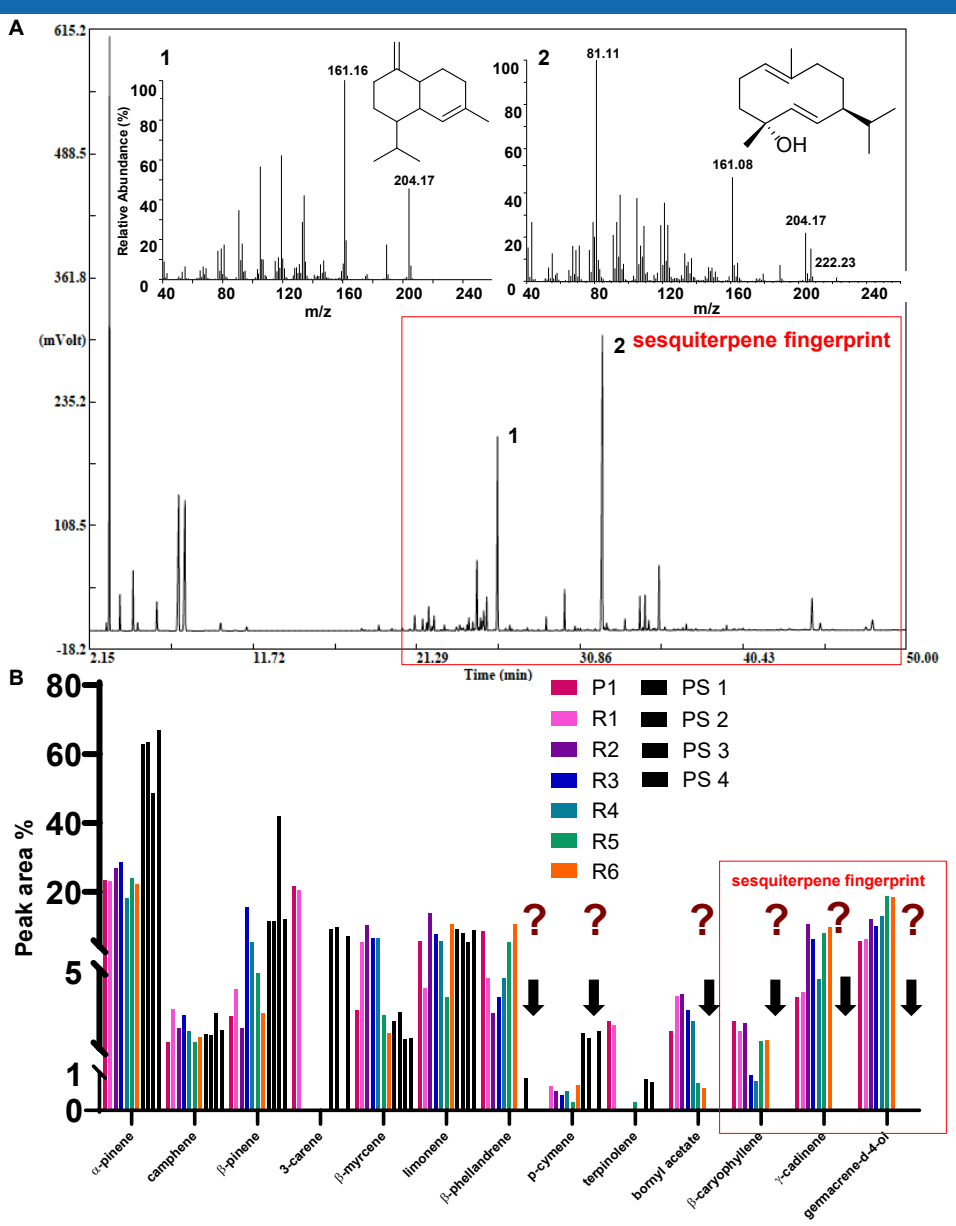

Figure 1: A) The chromatographic profile shows the terpenoid fingerprint of R6 with a large sesquiterpene area (red box). The two largest peaks of the sesquiterpene area were identified as γ-cadinene $C_{15}H_{24}$ (1) and germacrene-d-4-ol $C_{15}H_{26}O$ (2). γ-cadinene and germacrene-d-4-ol are used as quality markers for the genuine EOs. **B)** The class of monoterpene hydrocarbons was predominant in all the EOs whereas PS 1-4 consisted only of monoterpene hydrocarbons and did not contain any compounds of the sesquiterpene area. Additionally, the amount of β-phellandrene and p-cymene varies among the genuine EOs and PS 1-4.

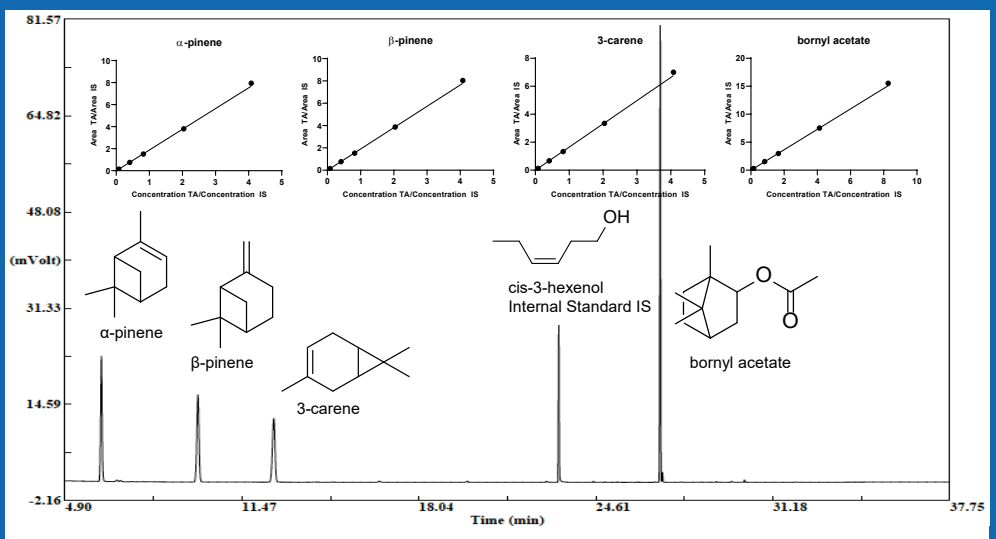

Figure 2: A simple, reliable, sensitive, accurate and precise method for the absolute quantification of α-pinene, β-pinene, 3-carene and bornyl acetate was developed. The method shows high accuracy (92 – 107%) and low imprecision (< 6.5%) for all target analytes (TA) at QC low, medium and high level.

Table 2: A) Strong linearities ($1/x^2$ weighted) were obtained for all four TA. The relative response factor RRF for each compound to the IS is calculated. Concentration range, regression equation, R^2, RRF and LoD are presented in Table 2A. **B)** The developed method was successfully applied to measure the content of the four compounds in the EOs. Prior to analysis the genuine EOs were diluted 1:50 and PS 1-4 1:500, respectively. The absolute concentration was calculated with the respective RRF.

A

	α-pinene	β-pinene	3-carene	bornyl acetate
Concentration [mM]	0.25 – 12.5	0.25 – 12.5	0.25 – 12.5	0.25 – 25.3
Calibration curve (1/x2)	y = 1.88 x - 0.01	y = 1.91 x - 0.01	y = 1.66 x - 0.01	y = 1.83 x - 0.01
R^2	0.9994	0.9995	0.9994	0.999
RRF	1.84	1.82	1.59	1.68
LoD [mM]	0.003	0.005	0.007	0.002

B

	1:50							1:500			
Concentration [mM]	**P1**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**PS 1**	**PS 2**	**PS 3**	**PS 4**
α-pinene	2.38	9.89	1.86	3.42	2.20	3.27	1.62	6.00	6.83	4.96	5.77
β-pinene	0.28	2.00	-	1.86	0.63	0.65	0.20	1.08	1.24	4.44	1.06
3-carene	2.77	3.66	-	-	-	-	-	1.01	1.22	-	0.76
bornyl acetate	< 0.25	1.49	0.28	0.36	0.29	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	0.25

4 Conclusion

For a proper evaluation of the quality of CEOs
- Analysis of the chromatographic fingerprint
- AND absolute quantification of selected compounds

Genuine EOs of *P. sylvestris*
- Large sesquiterpene fingerprint
- γ-cadinene and germacrene-d-4-ol
- Absolute quantification of monoterpenes: α-pinene, β-pinene, 3-carene and bornyl acetate

Analysis of the chromatographic fingerprint and absolute quantification of several compounds is needed for standardizing antibacterial and pharmacological activity tests of the CEOs.

5 References

1. Başer, K.H.C. and G. Buchbauer, *Handbook of essential oils : science, technology, and applications*. Second edition. ed. 2016, Boca Raton: CRC Press, Taylor & Francis Group. xv, 1109 pages.

6 Acknowledgement

The authors would like to thank Danielle Lüthi (Institute of Pharmaceutical Analytics, ETH Zurich) and the team of Systema Natura GmbH for their analytical support and sample provision.

OMCL Network contribution to regulatory action with respect to the "sartan crisis"

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Introduction

Since the start of the "sartan crisis" in summer 2018, Official Medicines Control Laboratories (OMCLs) of the General European OMCL Network (GEON), coordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM), have been involved in testing activities to determine nitrosamines in drug substances (DS) and drug products (DP) of the sartan group. The laboratories have developed a number of test methods to identify and quantify N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA) traces in sartans on the basis of different analytical principles.

In addition, the laboratories have supported European authorities in the evaluation of methods used by concerned companies in the testing of their sartan DS and contributed to a referral under article 31 of Directive 2001/83/EC on "Angiotensin-II-receptor antagonists (sartans) containing a tetrazole group", coordinated by the European Medicines Agency (EMA). The OMCLs also supported GMP inspectors by testing samples drawn during inspections in connection with sartan recalls.

Experts from the Network have been involved in different discussion groups established in Europe on this topic, and since mid-July 2018 have run their own testing group, which meets via teleconference on a regular basis for scientific exchange and coordination of sampling and testing activities.

OMCL methods for determination of nitrosamines in sartans

The following Network members have contributed to the development of specific in-house methods:

- the Irish OMCL in the Public Analyst's Laboratory in Galway (PALG)
- the French OMCL at the ANSM site in Montpellier
- the German OMCL at the "Chemisches und Veterinär-Untersuchungsamt (CVUA) Karlsruhe"
- the OMCL at Swissmedic
- the German OMCL at the "Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)" in Bavaria.

These methods are publicly available and can be accessed on the EDQM website (https://www.edqm.eu/en/ad-hoc-projects-omcl-network):

LGL methods

- LC-MS/MS (AB Sciex Qtrap) method for the quantitative determination of NMBA in losartan DS.
- GC-MS screening method for the determination of NDMA and NDEA in sartan DS (valsartan, irbesartan, losartan, candesartan, olmesartan).
- LC-MS/MS method suitable for the determination of NDMA and NDEA in irbesartan, valsartan and losartan DS and DP.

Swissmedic method

- Limit test method based on GC-MS (Liquid-direct-injection) which allows simultaneous determination of NDMA and NDEA in sartans.

CVUA Karlsruhe methods

- UHPLC-APCI-MS/MS method which allows simultaneous determination of NDMA and NDEA in sartans; the method is now validated for valsartan, irbesartan, losartan and candesartan containing film-coated tablets with or without hydrochlorothiazide.
- UHPLC-APCI-MS/MS method applicable to the detection and quantitative determination of NDMA in valsartan DP.

PALG method

- Headspace GC-MS (single quad) method applicable to the determination of NDMA in DS and corresponding powdered tablets of the sartan group.

ANSM method

- HPLC-UV method applicable to the determination of NDMA and NDEA in sartan DS (valsartan, losartan, irbesartan, candesartan and olmesartan.
- HPLC-UV method applicable to the determination of NDMA in DS and corresponding powdered tablets of valsartan.

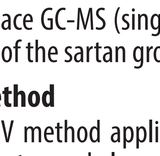
The published methods from the OMCL Network are compared below.

Analytical methods used

	DE_BW CVUA	IE_PAL PALG	CH_Swiss medic	DE_BY LGL	DE_BY LGL	DE_BY LGL	FR_ANSM
Analytical technique	LC-MS/MS	GC-MS (HS)	GC-MS (liquid DI) limit test	GC-MS (DI)	LC-MS/MS	LC-MS/MS	HPLC-UV
Analytes(s)	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NMBA	NDMA, NDEA
Sample (DS and/or DP)	DP	DS and DP	DS and DP	DS	DS and DP	Losartan DS	DS and DP

HS: Head Space; DI: Direct Injection; DP: Drug Product; DS: Drug Substance

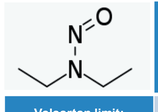
LOQs compared to interim limits proposed by the Article 31 referral for NDMA



	DE_BW CVUA LC-MS/MS (DP)	CH_Swissmedic GC-MS (liquid DI) limit test (DS and DP)	DE_BY LGL GC-MS (DI) (DS)	DE_BY LGL LC-MS/MS (DS and DP)	FR_ANSM HPLC-UV (DS)	Health Canada GC-MS/MS (DI)
Valsartan limit: 0.300 ppm / day	0.10 ppm	0.03 ppm	0.10 ppm	0.236 ppm	0.04 ppm	0.005 ppm (DS and DP)
Irbesartan limit: 0.320 ppm / day	0.10 ppm	0.03 ppm	0.10 ppm	0.079 ppm	0.04 ppm	0.005 ppm (DS and DP)
Losartan limit: 0.640 ppm / day	0.10 ppm	0.03 ppm	0.10 ppm	0.492 ppm	0.05 ppm	0.005 ppm (DS and DP)
Candesartan limit: 3.000 ppm / day	0.10 ppm	0.03 ppm	0.10 ppm	-	0.25 ppm	0.005 ppm (DS)
Olmesartan limit: 2.400 ppm / day	0.10 ppm	0.03 ppm	0.10 ppm	-	0.25 ppm	0.005 ppm (DS)

HS: Head Space; DI: Direct Injection; DP: Drug Product; DS: Drug Substance

LOQs compared to interim limits proposed by the Article 31 referral for NDEA

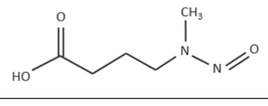


	DE_BW CVUA LC-MS/MS (DP)	CH_Swissmedic GC-MS (liquid DI) limit test (DS and DP)	DE_BY LGL GC-MS (DI) (DS)	DE_BY LGL LC-MS/MS (DS and DP)	FR_ANSM HPLC-UV (DS)	Health Canada GC-MS/MS (DI)
Valsartan limit: 0.082 ppm / day	0.04 ppm	0.03 ppm	0.08 ppm	0.061 ppm	0.08 ppm	0.007 ppm (DS and DP)
Irbesartan limit: 0.088 ppm / day	0.04 ppm	0.03 ppm	0.08 ppm	0.0195 ppm	0.09 ppm	0.007 ppm (DS and DP)
Losartan limit: 0.177 ppm / day	0.04 ppm	0.03 ppm	0.08 ppm	0.149 ppm	0.10 ppm	0.007 ppm (DS and DP)
Candesartan limit: 0.820 ppm / day	0.04 ppm	0.03 ppm	0.08 ppm	-	0.40 ppm	0.007 ppm (DS)
Olmesartan limit: 0.663 ppm / day	0.04 ppm	0.03 ppm	0.08 ppm	-	0.50 ppm	0.007 ppm (DS)

HS: Head Space; DI: Direct Injection; DP: Drug Product; DS: Drug Substance

In green: suitable sensitivity
In black: borderline sensitivity
In red: insufficient sensitivity

LOQ for NMBA in Losartan DS



	DE_BY LGL LC-MS/MS (DS)
Losartan limit: 0.64 ppm / day	0.0286 ppm

Analytical challenges of methods

Interim limits

Acceptable daily intake		"Limit"* for Valsartan	"Limit"* for Losartan
NDMA	96 ng /day	0.3 ppm	0.64 ppm
NDEA	26.5 ng /day	0.08 ppm	0.18 ppm

* Currently considered acceptable based on maximum daily dose

For illustration, typical LOQs achieved for Valsartan tablets

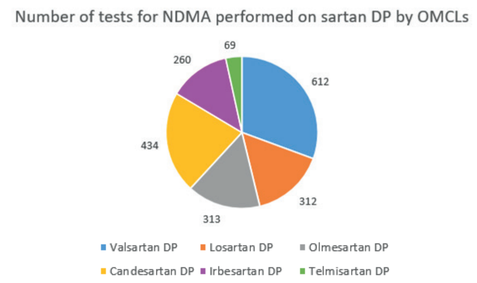
	LC/MS/MS	GC/MS/MS
NDMA	0.1 ppm	0.1 ppm
NDEA	0.04 ppm	0.06 ppm

Limit to be achieved after the transition period, which will last for 2 years: < 0,03 ppm

Number of tested samples and findings

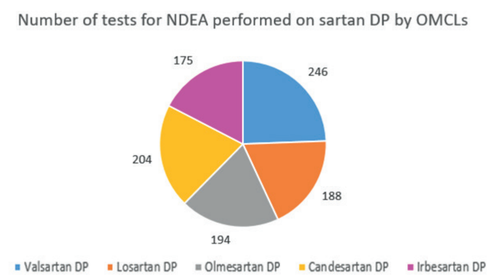
Samples tested by OMCLs for NDMA (cut-off date: 15/04/2019)

	DP	DS
Valsartan	612	141
Losartan	312	16
Olmesartan	313	13
Candesartan	434	10
Irbesartan	260	20
Telmisartan	69	49
Total	2000	249



Samples tested by OMCLs for NDEA (cut-off date: 15/04/2019)

	DP	DS
Valsartan	246	200
Losartan	188	149
Olmesartan	194	43
Candesartan	204	85
Irbesartan	175	160
Total	1007	637



NDMA OOS Findings by OMCLs by 15/04/2019

	DP	DS
Valsartan	253	70

NDEA OOS Findings by OMCLs by 15/04/2019

	DP	DS
Valsartan	36	53
Losartan	2	1
Irbesartan	29	28

NMBA OOS Findings by OMCLs by 15/04/2019

	DP	DS
Losartan	0	13

Conclusion

During the sartan crisis, the importance of OMCL lab testing once again became evident. The testing activities of the OMCLs supported decisions on batch recalls and CEP (Certificate of suitability to the monographs of the European Pharmacopoeia) suspensions. The published methods developed by the Network members were recommended by regulators as a starting point for MAHs to fulfil obligations for the systematic testing of sartans (DS) for nitrosamines. Moreover, they will be further utilised for the development of future general methods of the European Pharmacopoeia.

This incident has also demonstrated the need for close collaboration between assessors, inspectors and OMCLs.

Decisions on future market surveillance programmes as follow-up activities to the sartan crisis, including the extension of testing to other potential nitrosamines and/or other molecule groups, will require further close communication and exchange of expertise among the stakeholders. Depending on the direction taken, further development work by OMCLs to amend existing or establish new test methods might be necessary.

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Study on Analytical Methods of Multiple Nitrosamines in Sartan Pharmaceuticals

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1. Introduction

In June 2018, TFDA received the alarm from abroad about the incident for the contamination of valsartan active pharmaceutical ingredients (APIs). It was proved that a probable human carcinogen *N*-Nitrosodimethylamine (NDMA) was detected in valsartan API produced by Zhejiang Huahai.

NDMA is a member of the class of *N*-nitrosamines, which is also classified in IARC Group 2A (The agent is probably carcinogenic to humans). Generally, nitrosamines are found in both natural conditions and in industrial manufacturing applications such as pesticides, rubbers, dyes, and pharmaceuticals. In industry, it is conjectured that they are formed as by-products in manufacturing processes using materials such as amines, nitrates, and nitrites under a range of pH conditions from different mechanisms, such as nitrosation by nitrite. A recent issue for unexpected detection of NDMA, NDEA and NMBA in sartan medicines has been reported, which a threat has raised for safety of mediation in many countries. To inspect the situation of contamination for sartan medicines in Taiwan, it is necessary to develop an analytical method in order to monitor the nitrosamine impurities. However, many studies have focused on the determination of nitrosamines in tobacco, food, water and cosmetics. Instead, an analytical method of nitrosamines detection in pharmaceuticals, such as sartans, still remains vacancy and is urgent to be developed. In this study, it was aimed to establish a feasible and sensitive LC-MS/MS and GC-MS/MS method for determination of 17 nitrosamines (Figure 1) in sartans.

2. Materials and Methods

2.1 Chemicals and reagents

N-Nitrosodibutylamine (NDBA), *N*-Nitrosodiethylamine (NDEA), *N*-Nitrosodimethylamine (NDMA), *N*-Nitrosodipropylamine (NDPA), *N*-Nitrosodimethylamine-d_6 (NDMA-d_6) and *N*-Nitrosodipropylamine-d_{14} (NDPA-d_{14}) were purchased from AccuStandard (CT, USA). *N*-Nitrosoethylisopropylamine (NEIPA) was purchased from BOC Sciences (NY, USA). *N*-Nitrosodiisopropylamine (NDiPA) was purchased from Chem Service (PA, USA). *N*-Nitrosodiethanolamine (NDELA), *N*-Nitrosomorpholine (NMOR) and *N*-Nitrosopyrrolidine (NPYR) were purchased from Sigma-Aldrich (MO, USA). *N*-Nitrosodiphenylamine (NDPhA) and *N*-Nitrosopiperidine (NPIP) were purchased from Supelco (PA, USA). *N*-Nitrosodicyclohexylamine (NDCHA), *N*-Nitrosodiisobutylamine (NDiBA), *N*-Nitrosodiisononylamine (NDiNA), *N*-Nitrosodiisopropanolamine (NDiPLA), *N*-Nitrosomethylethylamine (NMEA), *N*-Nitroso-*N*-methyl-4-aminobutyric acid (NMBA), *N*-Nitrosodiethylamine-d_4 (NDEA-d_4), *N*-Nitrosodiphenylamine-d_{10} (NDPhA-d_{10}), *N*-Nitroso-*N*-methyl-4-aminobutyric acid-d_3 (NMBA-d_3) were purchased from TRC (ON, Canada).

Methanol and formic acid of LC-MS grade were purchased from Sigma-Aldrich (MO, USA). Acetonitrile of LC-MS grade was purchased from J.T Baker (NJ, USA). Water of LC-MS grade was purchased from Scharlau (Barcelona, Spain).

2.2 Equipment

2.2.1 LC-MS/MS

- Waters Acquity UPLC system
- AB SCIEX QTRAP 6500

➢ **LC column**

- Waters XSelect HSS T3 column (15 cm × 3 mm i.d., 3.5 μm)

➢ **LC-MS/MS conditions**

- Sample injection: 10 μL
- Column temperature: 40℃
- Flow rate: 0.6 mL/min
- Mobile phase: Gradient analysis
- A: 0.1% formic acid in water
- B: 0.1% formic acid in acetonitrile/methanol (2:8)
- Detection time: 0.0 - 8.5 min
- Ion source: APCI+
- Nebulizer current: 5 μA
- Curtain gas: 25 psi
- Ion source gas: 50 psi
- Temperature: 400℃
- Detection mode: sMRM (Table 1)

2.2.2 GC-MS/MS

- Agilent Technologies System (GC 7890B, MS 7000C Triple Quad)

➢ **GC column**

- Agilent DB-WAX Ultra Inert column (30 m × 0.25 mm i.d., 0.25 μm)

➢ **GC-MS/MS conditions**

- Sample injection: 2 μL
- Injection mode: pulsed splitless mode
- Injection temperature: 250℃
- Interface temperature: 250℃
- Carrier gas: He
- Flow rate: 1.0 mL/min
- Oven temperature: 80℃ (3 min) →250℃ at 20℃/min (3 min)
- Electronic impact (EI): 70 eV
- EI source temperature: 230℃
- Q1 and Q2 temperature: 150℃
- Detection mode: MRM (Table 1)

2.3 Analytical procedure

2.3.1 Sample preparation for GC-MS/MS analysis

Sample 100 mg
↓Add 500 μL of IS (20 ng/mL)
↓Add methanol to 5 mL
↓Sonicate (30 min)
↓Filter (0.22 μm PVDF)

2.3.2 Sample preparation for LC-MS/MS analysis

Sample 250 mg
↓Add 250 μL of IS (400 ng/mL)
↓Add methanol 250 μL
↓Votex and Sonicate (5 min)
↓Add water 4.5 mL
↓Votex and Sonicate (5 min)
↓Centrifuge 3000 × g (5 min)
↓Filter (0.22 μm PVDF)

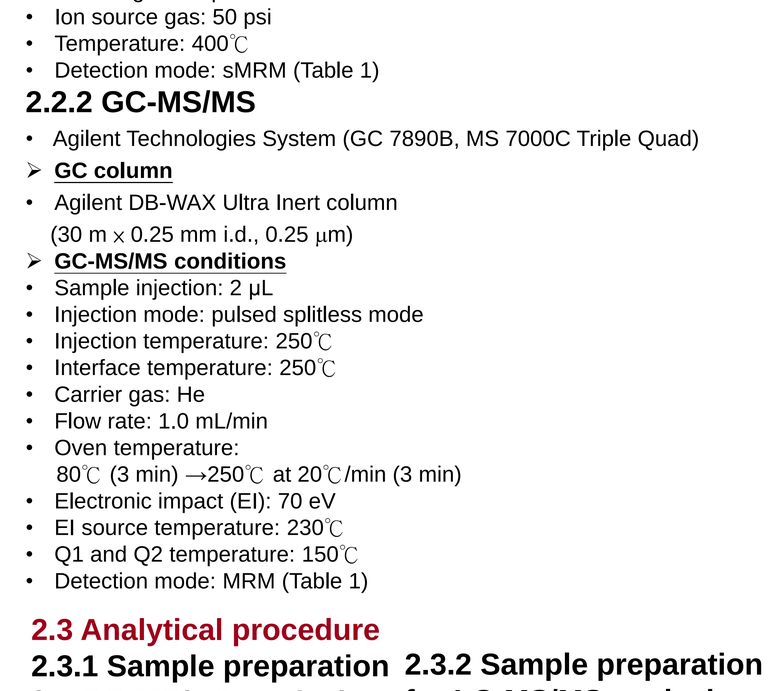


Figure 1. Chemical structures of the 17 nitrosamines and 6 internal standards.

Table 1. MRM transitions of compounds included in the methods.

No.	Compound	M.W.	GC-MS/MS		LC/MS-MS	
			Quantifier	Qualifier	Quantifier	Qualifier
1	NDMA	74	74>42	74>44	75>58	75>43
2	NDEA	102	102>85	102>56	103>75	103>47
3	NMBA	146	-	-	147>117	147>87
4	NDiPA	130	130>88	116>44	131>89	131>43
5	NEIPA	116	116>99	88>71	117>75	117>47
6	NDELA	134	-	-	135>74	135>104
7	NDiPLA	162	-	-	163>88	163>70
8	NDPA	130	130>113	130>43	131>89	131>43
9	NMEA	88	88>71	88>42	89>61	89>29
10	NMOR	116	116>86	116>56	117>87	117>86
11	NPIP	114	114>84	114>97	115>69	115>41
12	NPYR	100	100>55	100>70	101>55	101>41
13	NDBA	158	116>99	158>99	159>57	159>103
14	NDiBA	158	115>84	103>57	159>57	159>103
15	NDiNA	298	169>99	218>225	299>57	299>71
16	NDCHA	210	210>128	210>111	211>129	211>83
17	NDPhA	198	169>168	169>167	-	-
18	NDMA-d_6	80	80>46	-	81>46	-
19	NDEA-d_4	106	106>88	-	107>77	-
20	NMBA-d_3	149		-	150>120	-
21	NDPA-d_{14}	144	110>78	-	145>50	-
22	NDPhA-d_{10}	208	179>177	-	-	-
23	NDELA-d_8	142	-	-	143 > 111	-

3. Results and Discussion

3.1 LC-MS/MS

For the LC-MS/MS system, 16 nitrosamines standard can be analysed excluding NDPhA, which is decomposes during ionization. However, 4 nitrosamines (NDBA, NDiBA, NDiNA, NDCHA) coelute with the sartan ingredient and their signals are suppressed. The remaining 12 nitrosamines could be detected simultaneously in spiked sartan samples (candesartan, irbesartan, losartan, olmesartan and valsartan). In this method, 12 nitrosamines and 5 internal standards elute within 8.5 minutes (Figure 2), and the total run time is 15 minutes to flush out the high concentration of sartan matrix. The linearity from 2.5 to 50 ng/mL (IS 20 ng/mL) was evaluated by the R value, and R was above 0.9977 for all compounds. The recovery of intra-day and inter-day was 87.5-113.9 % for spiked samples in 5 different sartan APIs, while the range of precision was 2.9 - 12.1 % (Table 2). However, only the recovery of NDiPA, NDPA and NEIPA in irbesartan API was not within the range of 100.0 ± 20.0 %. The limit of quantitation (LOQ) for 12 nitrosamines were 0.05 μg/g.

3.2 GC-MS/MS

For the GC-MS/MS system, 16 nitrosamines standard can be analysed excluding NMBA. However, NDELA and NDiPLA signals are too weak and unable to achieve good sensitivity, so only 14 nitrosamines are suitable for GC-MS/MS analysis. In this method, 14 nitrosamines and 4 internal standards could be detected simultaneously within 14.5 minutes (Figure 3). The linearity from 1 to 50 ng/mL (IS 20 ng/mL) was evaluated by the R value, and R was above 0.9995 for all compounds. The recovery of intra-day and inter-day was 97.0-104.8 % for spiked samples in 5 sartan APIs, while the range of precision was 0.5 - 12.2 % (Table 3). Only the recovery of NPYR in low concentration of olmesartan and valsartan API exceeds 120%. The limit of quantitation (LOQ) for 14 nitrosamines were 0.05 μg/g.

The two methods complement each other and the compounds that have less acceptable recoveries in either method can be compensated and measured by the other methods.

3.3 Analysis of sartan samples

The methods are being applied to analyze 181 drug substances and 419 drug products, including valsartan, losartan, irbesartan, candesartan, olmesartan, telmisartan, azilsartan and pioglitazone pharmaceuticals (Figure 4). The test results showed that NDMA was detected in valsartan; NDEA was detected in irbesartan, losartan and valsartan. NMBA was detected in losartan and valsartan. NMOR, NPIP and NDPhA were also detected in valsartan. When these impurities were found in the APIs, the corresponding drug products were also found to contain these impurities.

4. Conclusions

Two fast and sensitive methods were developed using LC-MS/MS and GC-MS/MS techniques to detect 17 nitrosamines at low concentration in sartan drugs. The optimized methods are completed within 15 minutes with good reproducibility and recovery. More work will be done to further improve the sensitivity and recovery of the two methods in the future.

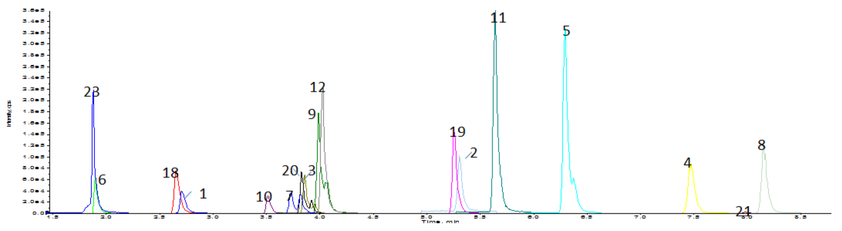


Figure 2. The MRM chromatograms of 12 nitrosamines and 5 internal standards analyzed by LC/MS/MS. (The peak number corresponding to peak name is shown in Table 2)

Table 2. LC/MS/MS – Accuracy and precision expressed as the recovery (%) and relative standard deviation (RSD in %) of the mean of analysis performed on at least three different days.

Compounds	R	Spiked concentration	Intraday (n=3, , 5 matrices)		Interday (n=9, , 5 matrices)	
		(ng/mL)	Accuracy	Precision	Accuracy	Precision
NDMA	0.9988	2.5	101.6	6.4	101.5	7.7
		25	106.0	4.7	104.9	3.8
		50	102.6	3.1	103.3	4.2
NDEA	0.9994	2.5	107.5	5.0	110.3	5.3
		25	106.6	4.5	106.3	5.0
		50	104.2	6.5	104.1	6.2
NMBA	0.9977	2.5	107.9	6.2	108.1	6.0
		25	100.4	7.8	99.1	7.8
		50	99.5	7.7	98.3	6.5
NDiPA*	0.9994	2.5	95.9	8.8	97.1	8.8
		25	104.3	5.9	106.3	7.0
		50	105.7	7.1	106.4	7.6
NEIPA*	0.9992	2.5	98.7	8.5	100.2	10.1
		25	97.7	11.1	100.3	12.1
		50	96.1	7.0	97.0	7.7
NDELA	0.9989	2.5	99.5	6.8	100.5	7.1
		25	105.4	3.8	106.1	4.0
		50	104.7	5.1	106.4	5.2
NDiPLA	0.9995	2.5	106.1	2.9	108.4	5.7
		25	98.8	6.6	102.8	7.7
		50	100.7	5.0	100.9	6.6
NDPA*	0.9996	2.5	89.2	6.4	87.5	6.8
		25	99.2	8.7	100.7	8.6
		50	100.9	5.0	100.8	7.1
NMEA	0.9994	2.5	95.7	6.4	94.4	6.7
		25	96.1	3.8	93.5	5.4
		50	97.5	9.0	93.1	8.5
NMOR	0.9993	2.5	107.9	5.9	110.1	6.0
		25	104.4	6.1	103.7	6.7
		50	108.9	5.8	103.7	7.6
NPIP	0.9983	2.5	111.0	5.8	113.9	4.5
		25	102.0	8.0	104.2	9.4
		50	98.7	7.2	101.6	7.9
NPYR	0.9992	2.5	95.6	5.1	92.1	6.5
		25	95.2	5.8	92.3	7.2
		50	96.7	4.9	91.9	6.4

* Only data from samples spiked into 4 different matrices are shown.

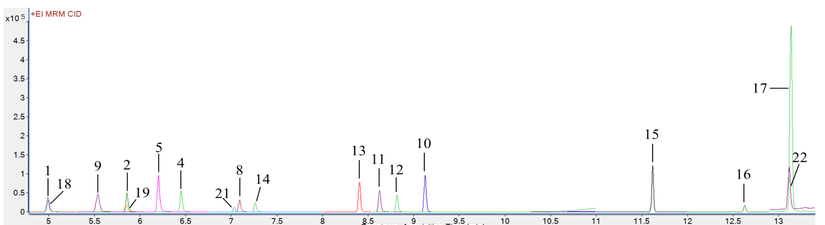
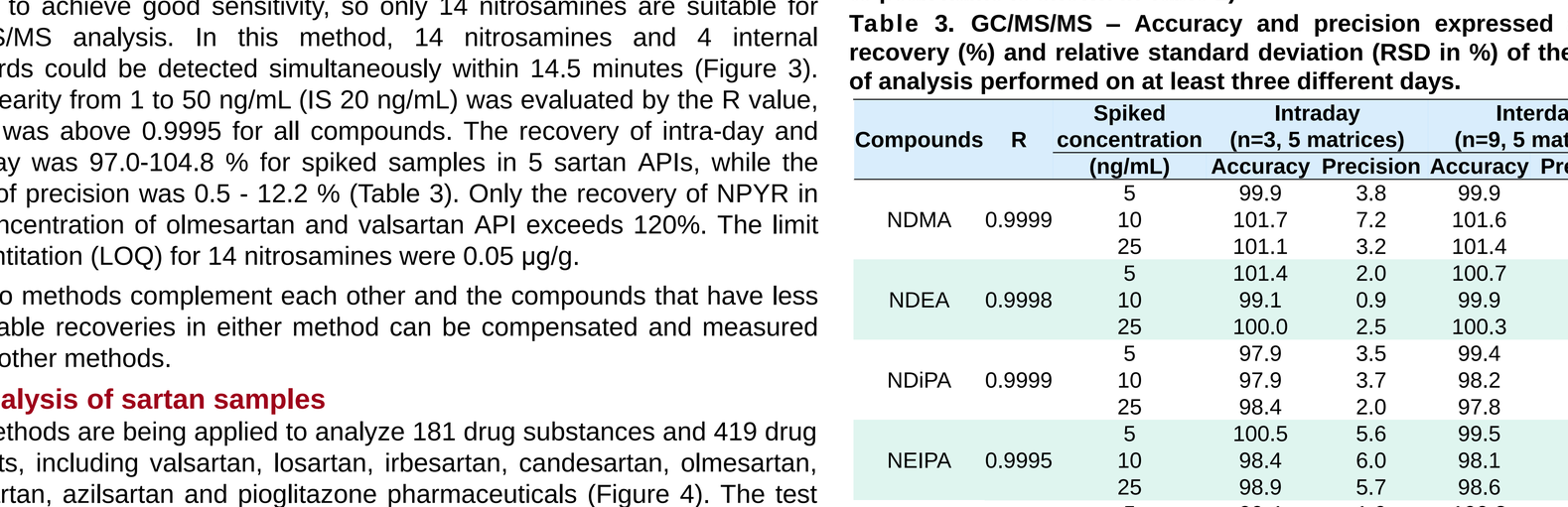


Figure 3. The MRM chromatograms of 14 nitrosamines and 4 internal standards analyzed by GC/MS/MS. (The peak number corresponding to peak name is shown in Table 2)

Table 3. GC/MS/MS – Accuracy and precision expressed as the recovery (%) and relative standard deviation (RSD in %) of the mean of analysis performed on at least three different days.

Compounds	R	Spiked concentration	Intraday (n=3, 5 matrices)		Interday (n=9, 5 matrices)	
		(ng/mL)	Accuracy	Precision	Accuracy	Precision
NDMA	0.9999	5	99.9	3.8	99.9	4.8
		10	101.7	7.2	101.6	4.9
		25	101.1	3.2	101.4	3.0
NDEA	0.9998	5	101.4	2.0	100.7	2.0
		10	99.1	0.9	99.9	1.5
		25	100.0	2.5	100.3	2.0
NDiPA	0.9999	5	97.9	3.5	99.4	3.8
		10	97.9	3.7	98.2	4.7
		25	98.4	2.0	97.8	3.3
NEIPA	0.9995	5	100.5	5.6	99.5	4.7
		10	98.4	6.0	98.1	5.9
		25	98.9	5.7	98.6	5.8
NDPA	0.9997	5	99.4	1.0	100.8	2.0
		10	100.3	0.8	101.5	1.9
		25	102.2	0.9	101.7	1.8
NMEA	0.9999	5	103.3	2.2	102.0	2.3
		10	99.8	3.0	99.9	2.7
		25	100.5	2.7	101.2	3.0
NMOR	0.9998	5	97.0	4.7	99.3	5.4
		10	97.5	3.2	98.6	4.8
		25	98.2	3.9	99.0	4.6
NPIP	0.9998	5	99.4	3.5	100.9	3.6
		10	99.1	3.9	100.1	3.6
		25	100.5	3.2	100.6	2.9
NPYR*	0.9998	5	103.6	3.0	102.1	3.7
		10	98.3	3.9	99.2	3.8
		25	100.1	5.4	99.7	4.3
NDBA	0.9998	5	102.3	4.0	104.1	4.4
		10	102.4	3.6	103.7	3.1
		25	103.0	4.4	103.0	4.0
NDiBA	0.9999	5	102.6	2.6	102.2	2.9
		10	101.7	3.4	102.4	3.1
		25	102.6	2.5	102.4	2.2
NDiNA	0.9998	5	100.6	6.6	101.1	10.8
		10	98.9	7.3	99.1	11.6
		25	99.7	9.7	100.2	12.2
NDCHA	0.9998	5	99.9	6.0	100.5	7.0
		10	98.5	4.1	98.8	6.7
		25	99.0	5.5	98.9	7.4
NDPhA	0.9998	5	103.5	5.6	104.8	5.9
		10	101.2	3.3	102.2	3.4
		25	100.8	0.5	101.8	2.2

* Only data from samples spiked into 3 different matrices are shown.



	Valsartan	Losartan	Irbesartan	Olmesartan	Candesartan	Telmisartan	Azilsartan	Pioglitazone
Total cases	192	266	63	26	10	19	19	5
Detected	56	36	5	0	0	0	0	0
NDMA detected	53	0	0	0	0	0	0	0
NDEA detected	11	4	5	0	0	0	0	0
NMBA detected	1	32	0	0	0	0	0	0
NMOR detected	2	0	0	0	0	0	0	0
NPIP detected	2	0	0	0	0	0	0	0
NDPhA detected	1*	-*	-*	-*	-*	-*	-*	-*

Figure 4. The testing results of *N*-nitrosamines determination in sartan active pharmaceutical ingredient and finished drug products in Taiwan.
*To be determined.

Elemental Analysis for Monograph and ICH-Q3D Applications, the Approach of a Contract Laboratory

Alan Cross, Technical Specialist – Metals Laboratory
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Introduction

This poster provides an overview of how a contract laboratory deals with elemental analysis requests from customers, particularly in support of monograph testing requirements or for ICH-Q3D compliance. This will cover how monograph testing e.g. for Nickel in Stearic acid differs from the ICH-Q3D requirements, in terms of flexibility of analysis, required limits of detection and the use of tools such as an elemental screen to evaluate a sample for the 24 elements of interest in the ICH-Q3D regulations. Approaches to method validation will also be incorporated including the application of the ICH-Q2 document to spectroscopic techniques. Sample preparation and analytical methodology will also be covered, in particular looking at why ICP-MS, ICP-OES or AAS is selected for a particular analysis, and explain the rationale behind the preparation of these samples.

Analytical Selection

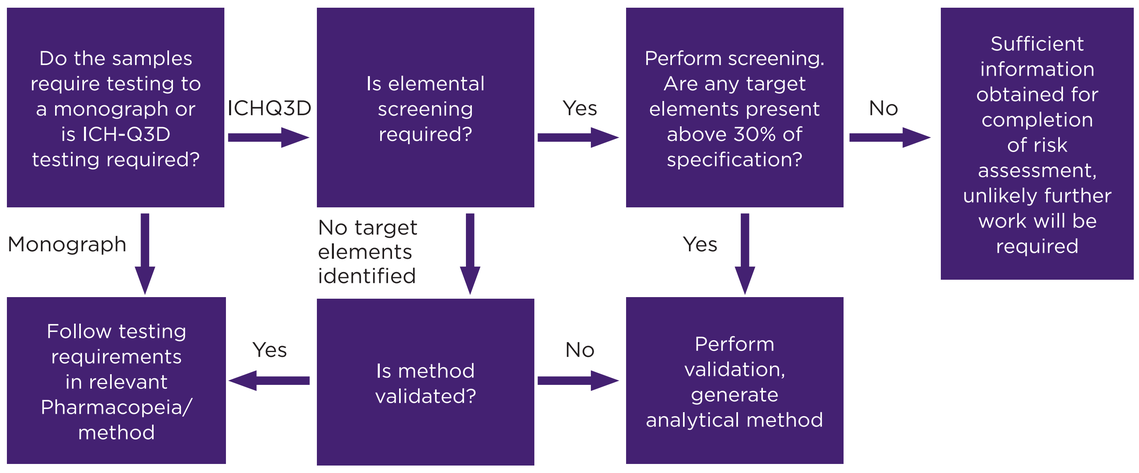
Generally the first step taken when a sample is received is deciding on how best to deal with the sample. Several factors must be considered prior to analysis, the procedure by which the decision is made generally follows the process outlined in the flow chart.

Monograph Testing

For monograph testing the decision is fairly simple, as the material, elements and pharmacopeia will be defined so testing can proceed as per the relevant monograph.

Typically preparation of samples for elemental testing according to monographs will either be a simple dissolution in aqueous/acid solutions or some form of digestion, either using microwave digestion or ashing of the sample to destroy organic material and release the analytes of interest so they can be made into a solution for analysis. Other preparation techniques may also be utilised, for example sugars are commonly dissolved in acetic acid and extracted into a solvent phase using a chelating agent to allow the concentration of the analytes (commonly nickel and lead) to be detectable by the analytical instrumentation.

Monograph testing for elemental impurities is typically carried out using Atomic Absorption Spectrometry (AAS). Typically the prepared solutions are aspirated into a flame and the absorbance compared to that of standards of a known concentration. In some cases, other variants of this method are used to obtain the required detection limits, for example hydride generation for arsenic or graphite furnace for lead and cadmium.



Element	Class	Daily Permissible Exposure (µg/day)		
		Oral	Parenteral	Inhalation
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2a	50	5	3
V	2a	100	10	1
Ni	2a	200	20	5
Tl	2b	8	8	8
Au	2b	100	100	1
Pd	2b	100	10	1
Ir	2b	100	10	1
Os	2b	100	10	1
Rh	2b	100	10	1
Ru	2b	100	10	1
Se	2b	150	80	130
Ag	2b	150	10	7
Pt	2b	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

ICH-Q3D Testing

- Control of elemental impurities covered by this document is a relatively recent requirement. The process is based on performing a risk assessment to evaluate the likelihood of elemental contamination. 24 elements are specified in the list with levels and testing requirements based on toxicity, likelihood of presence (either intentionally added i.e. as a catalyst or as a contaminant e.g. lead) and route of drug administration.
- Screening for the 24 elements can be used to assess the elemental contamination in the sample. If this is shown to be significantly low in numerous lots, e.g. <30% of specification in 3 lots, then no further controls may be required.
- If the screen does show a risk of elemental impurities, or if the customer has decided that they require a more rigorous approach, then a validation will be required. This can take the form of a simple limit test to show that the sample is below a specification, but more commonly, a full quantification validation is performed as this can provide more useful information for trending. The validation procedure typically follows the parameters outlined in the ICHQ2 document, but may also take other forms, such as the approach set out in USP Chapter <233>.
- Once validated, a method will typically be drawn up establishing the correct testing parameters. This allows for routine analysis to take place. In most cases this analysis will be carried out by ICP-MS as this technique is capable of multi-element analysis to the required ppb levels.

Other Types of Analysis

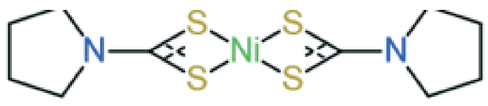
- Assay testing: Sometimes elemental analysis is carried out to ensure the correct level of an element is present, for example calcium present as the counter ion in an API or an essential element such as iron in a vitamin product. Usually this analysis will take place using ICP-OES or AAS as low detection limits are not required.
- Problem solving: Occasionally issues will arise during the manufacture of drug products, such as decolourisation in the final dose, degradation/loss of API or corrosion/ rouging on process equipment. Elemental analysis can be utilised as one of a range of tools to identify the root cause of these issues. Sometimes the testing will be focused due to knowledge of the process, e.g. looking for copper as the source of green tint to a product. A powerful tool is an elemental screen. This is a screen to give an approximate elemental composition of a product looking at 70+ elements. Often a comparison is carried out between “good” and “bad” lots to identify differences, which can then be used to focus investigations into the causes of problems.
- Extractable and Leachable testing: These studies can provide useful information about the migration of metallic contaminants from packaging into the drug product as set out in USP <1664>. Both organic and inorganic solutions can be analysed for elevated levels of metallic components following treatment versus blanks, though there are many different approaches to this procedure, the ICH-Q3D element list, plus other common elements not included such as iron and aluminium, are useful for assessing risks

Sample Preparation

Often overlooked when considering metal analysis, the preparation of samples is a key step in obtaining good analytical results. In the majority of cases the objective is to obtain a clear digest. This way it is possible to ensure that the analytes of interest are in solution and are readily available for analysis. Samples are rarely received in a condition that allows for direct analysis so some form of preparation will be required. In the simplest case, a dissolution into a dilute acid can be performed, but often a stronger digest is required. This generally involves heating the sample in the presence of a strong acid, either as a hotplate digestion, or under pressure using a microwave digester. In the majority of cases, nitric acid is used for the digestion, sometimes with the addition of small amounts of peroxide for organic materials, but in some cases, depending on the matrix or analytes required other acids must be used.

Microwave digestion is frequently used for preparation of samples as it allows high temperature, high pressure digestion to occur, removing much of the matrix and allowing the analytes of interest to be brought into solution. It is useful to have a variety of options for digestion of samples to allow flexibility in through-put and digestion strength. Typically there are 2 types of microwave digesters:

- Rotor based. Individual PTFE vessels are used, sample is weighed into a sealable vessel and acids are added and placed onto rotor in a microwave. Samples are heated as they would be in a conventional microwave. Digested solutions are then made to volume for analysis.
- Single reaction chamber digestion. Samples are weighed into disposable glass test tubes, or re-usable quartz or fluoropolymer tubes and loosely capped. These are then placed into a chamber which is pressurised with nitrogen. This allows for efficient digestion of different sample types in the same run. The choice of tube is dependent on the analysis required, disposable tubes may have background concentrations of some elements such as sodium or boron, fluoropolymer tubes must be used when HF is used in the digestion mixture.



In pharmacopeia methods simple dilution is commonly employed. Extraction into a solvent with use of a chelating agent (such as APDC, shown above) is used for sugary matrices. Microwave digestion is also used particularly for waxy matrices such as stearic acid or magnesium stearate.

Ashing is sometimes utilised, though less common. This technique is more frequently used for preparation of samples for nutritional analysis or assay of alkali/earth elements.

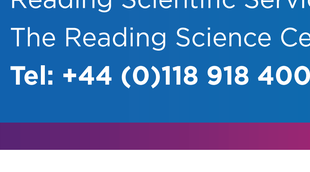
Bromine and iodine analysis can also be carried out by ICP-MS, but reactions with acids generate volatile gases so an alkaline digest is usually employed for these analytes.

Hotblock digestion is an efficient way to digest simpler matrices. Samples are weighed into volumetric tubes, acids are added and placed into a heated graphite block. This technique allows the digestion to be monitored to ensure complete digest.

Once cooled the samples can then be simply made to volume in the same tubes, minimising loss of analyte through transfer or contamination from unclean reaction vessels. However, the samples may have highe levels of residual carbon which may be problematic for arsenic or selenium determinations by ICP-MS.

Analysis

Once a suitable digest has been obtained, analysis can be carried out on the samples. The choice of which technique is used is dependent on the analytes required, the sample matrix (especially the level of total dissolved solid in the digest), the detection level (DL) needed and whether the testing is to be carried out to a specific technique. At RSSL, we have a full range of analytical techniques available, allowing for flexibility to work with most sample types:



Atomic Absorption Spectrometry (AAS)

- Well established, simple technique
- Typically ppm levels of detection
- Deals well with heavy matrix samples
- Well used in many pharmacopeias
- Toxic elements have high DL’s in flame mode
- Requires analyst supervision during analysis
- Not typically used for ICH-Q3D testing as each analyte needs separate analysis and DL’s are challenging for most elements
- Graphite furnace and vapour generation may be used to overcome this

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

- Well established, simple technique
- Utilises argon as a plasma gas – running cost implications
- Typically sub-ppb levels of detection
- Matrix can have a big effect <0.2% TDS recommended
- Interference correction often used due to interferences
- Not typically used in monograph testing
- Is the preferred instrument for ICH-Q3D testing as fast simultaneous analysis of multiple elements possible
- Can also be used for screening for risk assessment purposes

Hydride/Vapour AAS and Graphite (GFAAS)

- Uses the same principle as flame AAS
- Mercury vapour of volatile metal hydrides utilised
- Improved DL’s over AAS for certain elements, usually ppb levels
- Used for arsenic and mercury in monograph testing
- Graphite Furnace AAS (GFAAS) uses a small graphite tube which can be rapidly heated to temperatures in excess 2800ºC causing atomisation of the analyte of interest
- Very low detection limits possible, matrix effects are minimal
- Used frequently in monograph testing for low level determinations of elements such as Cadmium, Nickel and Palladium

Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES)

- Not commonly used for monograph testing though a couple of monographs do exist
- Utilises argon as a plasma gas – running cost implications
- Typically ppb levels of detection
- Better tolerance to heavy matrices compared to ICP-MS
- Less often used for ICH-Q3D testing, due to level of detection, but may be utilised as less prone to matrix issues
- Fast simultaneous analysis of multiple elements possible
- Sampling valves improve analysis time, reducing cost of analysis

Conclusion

The key objective for a contract lab is to deliver precise results to its customers, working to their requirements and often also to tight time-scales. A contract lab can also be useful for assisting with trouble-shooting and root cause analysis testing. For the contract lab, this requires significant investment, not only in instrumentation, but also training, qualification, maintenance and development. In addition, communication between the contract lab and the client is critical to ensure successful outcomes.

The recent implementation of the ICH-Q3D regulation has meant a greater focus on elemental control, with pharmaceutical manufacturers and their suppliers needing to demonstrate the safety of their products as well as still conforming to the existing raw material specifications in the relevant monographs. The contract lab can be a useful resource for assisting in the control of elemental impurities in drug products, from carrying out screening for the risk assessment procedure to developing methodologies, performing validation and carrying out routine analysis for this purpose, as well as carrying out the more routine monograph testing. The experience of dealing with a wide range of sample types can be a useful resource and is often invaluable in the progression of development projects.

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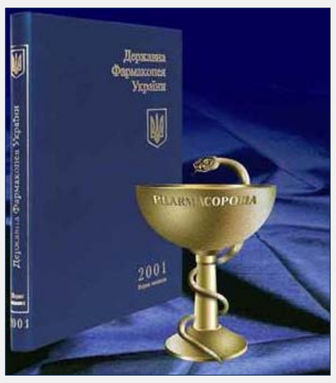

Experience in assessment of pharmacopoeial procedure performance quality within proficiency testing scheme

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INTRODUCTION

The proficiency testing scheme (PTS) is an essential tool to assess performance of measurement laboratories. According to the ISO 17043:2010 and other relevant documents, the usual practice of PTSs is to evaluate only the participants' final results, disregarding the way they were obtained.

Among other fields where PTS is used, the pharmaceutical sector is the most formalized one. It is common for quality control laboratories to test medicinal products according to clearly established and validated compendial procedures. This means using the same methods incorporated in Pharmacopoeia. In fact, results obtained with a failure to comply with pharmacopoeial requirements are not legitimate.

Therefore, it is essential to evaluate the "quality" of the performance along with result itself. In this case "quality" is compliance of the analysis procedure to the pharmacopoeial requirements.

Such approach allows to:

- Provide feedback to participants in order to -
  - understand the sources of negative testing results;
  - provide information on other existing issues;
- Provide feedback to pharmacopoeia -
  - Assess the quality of pharmacopoeial procedures;
  - Indicate needs and suggest ways to improve the texts.

GOALS AND OBJECTIVES

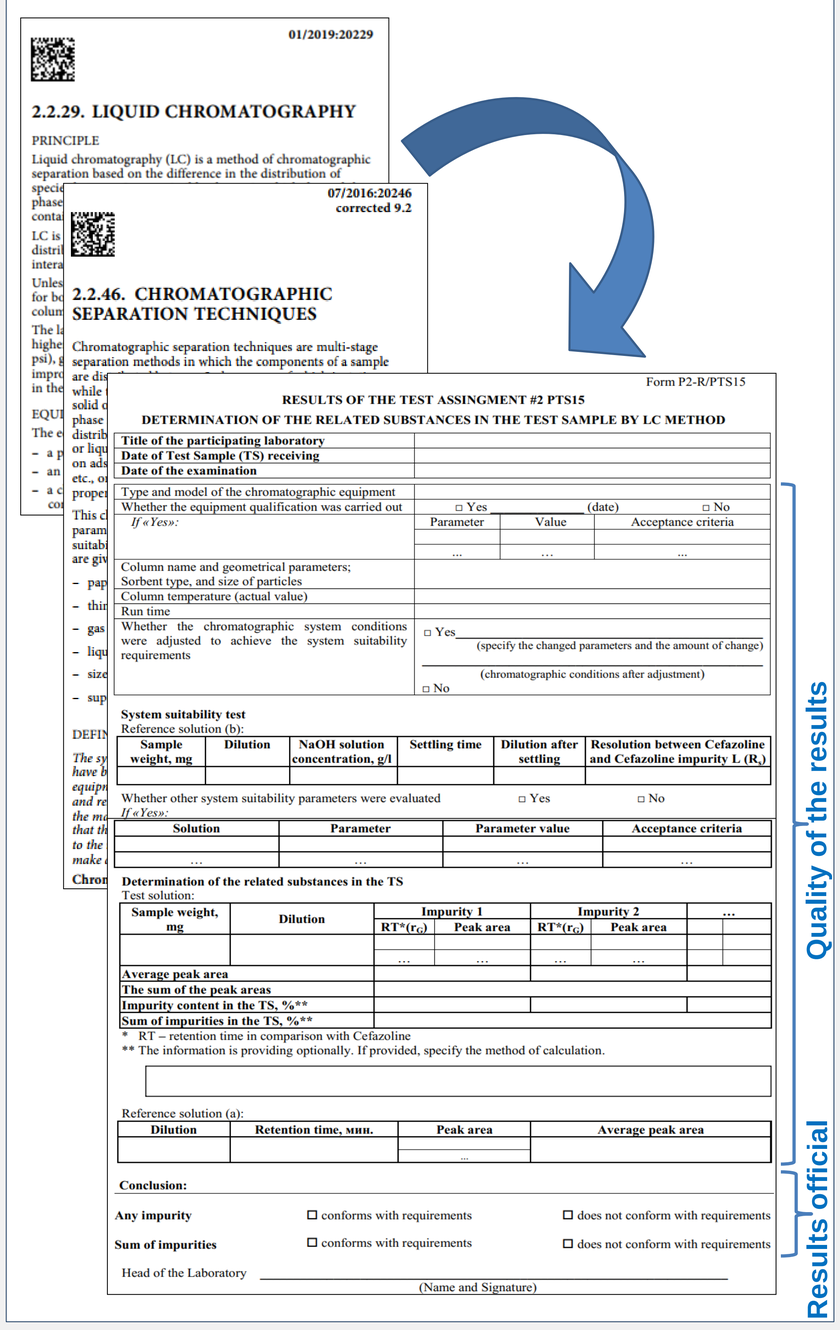
- ✓ To assess the quality of the PTS participants' results
- ✓ To evaluate the compliance of laboratory performance with pharmacopoeial requirements
- ✓ To elaborate the evaluation tool
- ✓ To provide feedback to participants and Pharmacopoeia

METHODOLOGY

In order to assess the quality of Participants' Results, a Form is elaborated for each testing assignment. Form questions reflect common criteria of correct testing methods gathered from monographs and general chapters. Participants must provide information on procedures used to complete the testing assignment.

For example, PTS Round #15 testing assignment form for related substances test of TS Cefazoline for Injection was elaborated considering the requirements of the BP/SPhU «CEFAZOLINE INJECTION» monograph and Ph.Eur. GCs 2.2.29 and 2.2.46.

Questions were related to such methodological aspects as Chromatographic System permissible adjustments, System Suitability and its correct parameter calculations along with common laboratory practice.



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2.2.29. LIQUID CHROMATOGRAPHY

PRINCIPLE

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of

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corrected 9.2

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample

Form P2-R/PTS15

RESULTS OF THE TEST ASSINGMENT #2 PTS15

DETERMINATION OF THE RELATED SUBSTANCES IN THE TEST SAMPLE BY LC METHOD

Title of the participating laboratory	
Date of Test Sample (TS) receiving	
Date of the examination	

Type and model of the chromatographic equipment			
Whether the equipment qualification was carried out	□ Yes _____ (date)		□ No
If «Yes»:	Parameter	Value	Acceptance criteria
	…	…	…
Column name and geometrical parameters; Sorbent type, and size of particles			
Column temperature (actual value)			
Run time			
Whether the chromatographic system conditions were adjusted to achieve the system suitability requirements	□ Yes _____ (specify the changed parameters and the amount of change) _____ (chromatographic conditions after adjustment) □ No		

System suitability test
Reference solution (b):

Sample weight, mg	Dilution	NaOH solution concentration, g/l	Settling time	Dilution after settling	Resolution between Cefazoline and Cefazoline impurity L (R_s)

Whether other system suitability parameters were evaluated □ Yes □ No
If «Yes»:

Solution	Parameter	Parameter value	Acceptance criteria
…	…	…	…

Determination of the related substances in the TS
Test solution:

Sample weight, mg	Dilution	Impurity 1		Impurity 2		…	
		RT*(r_G)	Peak area	RT*(r_G)	Peak area		
		…	…	…	…		
Average peak area							
The sum of the peak areas							
Impurity content in the TS, %**							
Sum of impurities in the TS, %**							

* RT – retention time in comparison with Cefazoline
** The information is providing optionally. If provided, specify the method of calculation.

Reference solution (a):

Dilution	Retention time, мин.	Peak area	Average peak area
		…	

Conclusion:

Any impurity □ conforms with requirements □ does not conform with requirements

Sum of impurities □ conforms with requirements □ does not conform with requirements

Head of the Laboratory _____
(Name and Signature)

Quality of the results

Results official

RESULTS AND DISCUSSION

Since 2001 15 rounds of PTS were conducted. They included 20 methods incorporated to the Ph. Eur. Laboratories prevalently from Ukraine, as well as neighboring countries which recognized the Ph. Eur. Belorussia, Georgia, Moldova, Russia have taken part in the PTS. We elaborated Forms for participants to fill out. The data, provided by participants, demonstrated the quality of participants results in respect to pharmacopoeial requirements. Participants mainly demonstrate satisfactory/positive test results, however, the participants' performance of procedures deviates from pharmacopoeial requirements. Such violations of compendial procedures can question legitimacy of results. Also, these issues lead to reputational damage for controlling laboratories. We have listed some results of quality evaluation of pharmacopoeial procedures carried out by PTS participants.

HPLC

Although, the PTS participants demonstrates satisfactory results among the rounds (Table 1.), there are lots of deviations from pharmacopoeia text in their performance (see the diagrams below).

Table 1. Statistical assessment of the HPLC results by PTS rounds

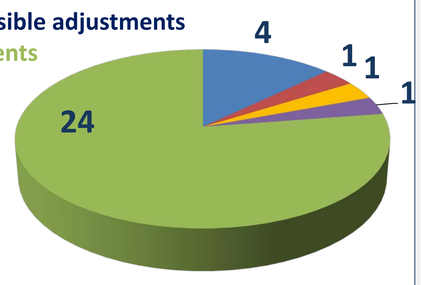
# of round	Year	Procedure	Number of participants	Criteria* (max μ)	Negative results (μ)	Conclu-sion**
2	2002	Assay test of Caffeine in the TS (0,4%solution)	10	3.3	2	OK
3	2003	Assay test of Lincomycin in the TS	20	--	0	OK
8	2010	Related substances test of TS Lincomycin h/cl	33	5.4	1	OK
13	2016	Assay and Related substances test of TS Piracetam	33	5.4	0	OK
					4	OK
15	2018	Related substances test of TS Cefazoline for Injection	31	5.2	2	OK

* - maximum allowed number of negative results depending on the number of participants by binomial distribution for 95% probability
** - criterion on satisfactory result of method performance in the round (**OK**): μ ≤ max μ (*SPhU 2.2, 5.3.N.1 Statistical analyze of Chemical testing results)*

Round #15

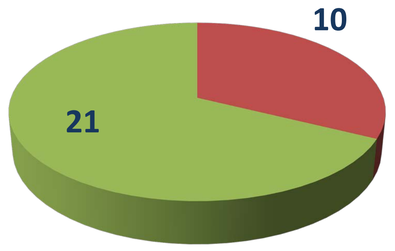
❖ **Chromatographic System permissible adjustments**

+ in compliance with the requirements
- particle size was adjusted
- the temperature was deviated more than 5 °C
- injection volume was increased
- no information was provided



❖ **System Suitability**

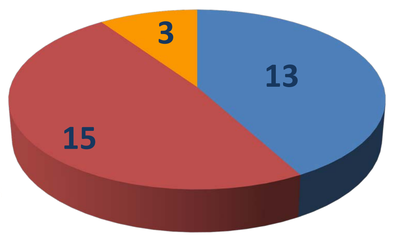
+ other required system suitability parameters were determined
- only peak resolution specified in the monograph was determined



According to the GC 2.2.46 **LOQ and Symmetry factor** should be considered

➢ **Limit of Quantification**

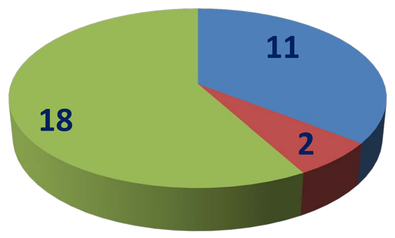
- no calculation of the LOQ
- signal/noise was calculated instead of the LOQ
- went wrong in the calculations



In fact, 42% of the participants performed the analysis under conditions where the LOQ was exceeded the required value!

➢ **Symmetry factor**

+ in compliance with the requirements
- no calculation of the Symmetry factor
- calculations was performed for the wrong solution



100% of participants have broken the pharmacopoeial requirements

❖ **Repeatability of impurities content**

GC 2.2.46 does not require the repeatability control for the tests for related substances. The calculation of RSD, % for impurities content performed by PTS's organizers by raw data revealed significant differences

- ➢ For impurity J in **400** times (RSD from 0.05 % to 15.2 %);
- ➢ For impurity E in **100** times (RSD from 0.1 % to 9.33 %).

It would be useful to include monitoring of the repeatability for the related substances test to the Pharmacopoeia.

Dissolution test

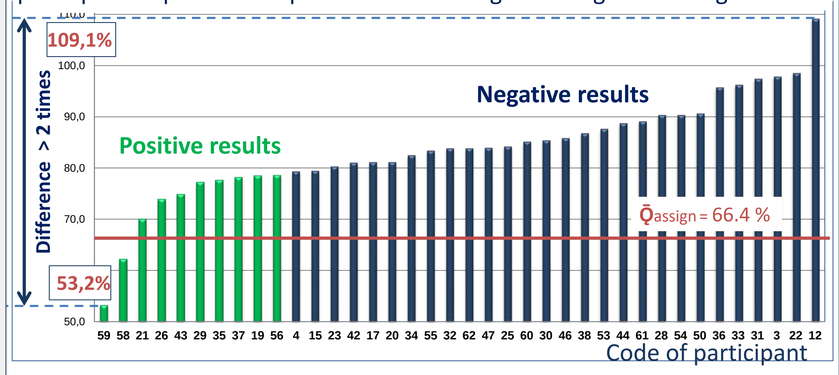
Table 2. Statistical assessment of Dissolution test results by PTS rounds

# of round	Year	Procedure	Number of participants	Criteria* (max μ)	Negative results (μ)	Conclu-sion**
4	2004	Dissolution of TS Paracetamol tablets	19	4.2	1	OK
10	2013	Dissolution of TS Furocemide tablets	38	5.8	28	SOS!
12	2015	Dissolution of TS Nitrosorbide tablets	23	4.4	3	OK

* - maximum allowed number of negative results depending on the number of participants by binomial distribution for 95% probability
** - criterion on satisfactory result of method performance in the round: μ ≤maxμ (*SPhU 2.2, 5.3.N.1 Statistical analyze of Chemical testing results)*

Round #10

During the certification the Test Sample was assigned as not meeting the specification of the «Furosemide tablets» monograph (Q < 80 %). **28** PTS participants reported false positive results and got the negative testing results.

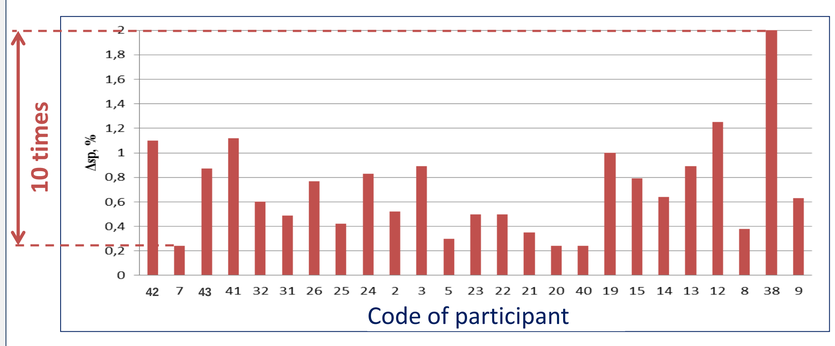


The majority of the results are shifted upwards consequent on increasing amount of dissolved API with any alignments violation (such as shaft and vessel verticality; centering; basket and paddle wobble). Low level of dissolution media de-aeration and delay in samples withdrawal are also lead to the results increasing. With such difference among the participants ' results

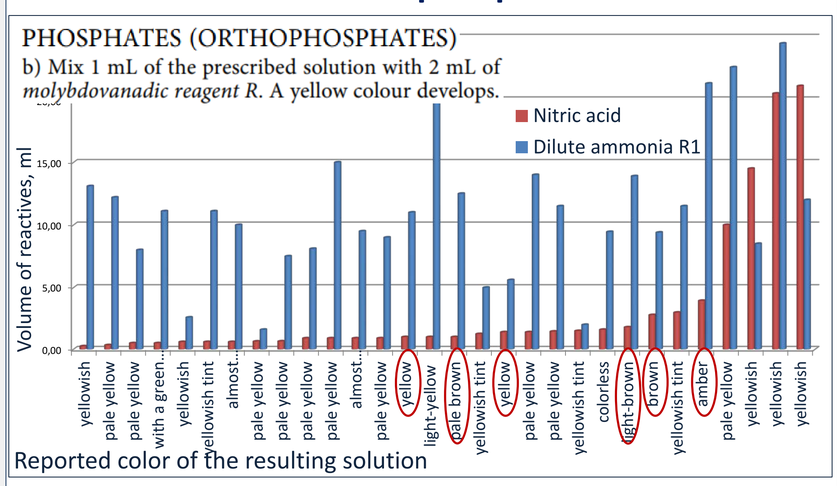
Is the Dissolution test reliable enough and meets discriminatory requirements?

Round #12

Pharmacopoeia waived the strict procedure for solution preparation. Thus, uncertainty of the Isosorbide dinitrate Standard solution preparation differentiates more than 10 times between participants. This affects the total uncertainty of the procedure.



Identification of Phosphate-ion in API of Dexamethasone sodium phosphate



The Pharmacopoeial sample preparation procedure demonstrates the lack of standardization. It is difficult to distinct the target yellow color of solution with molybdovanadic reagent in brown/yellow solution obtained in the sample preparation. The information was addressed to the Ph. Eur.

Feedback to the SPhU (State Pharmacopoeia of Ukraine)

According to the evaluation results, some improvements were made in the SPhU text: the estimated uncertainty value for the spectrophotometry procedures for the purpose of validation was included to the national GC *5.3.N.2[N] Validation of analytical procedures and tests,* Identification by TLC method of SPhU monograph «Ciprofloxacin tablets» was improved in the part of results interpretation. Statistical approaches to the PTS results assessing are incorporated to the national GC *SPhU 2.2, 5.3.N.1 Statistical analyze of Chemical testing results.*

CONCLUSION

The tool for evaluation the compliance of laboratory performance with pharmacopoeial requirements was elaborated. Consequent on the evaluation the need for pharmacopoeial procedures further standardization coupled with personnel training was demonstrated.

IS UNIFORMITY PRECISE ENOUGH? THE CASE OF INHALER PRODUCTS

ELEZOVIĆ A. [1]

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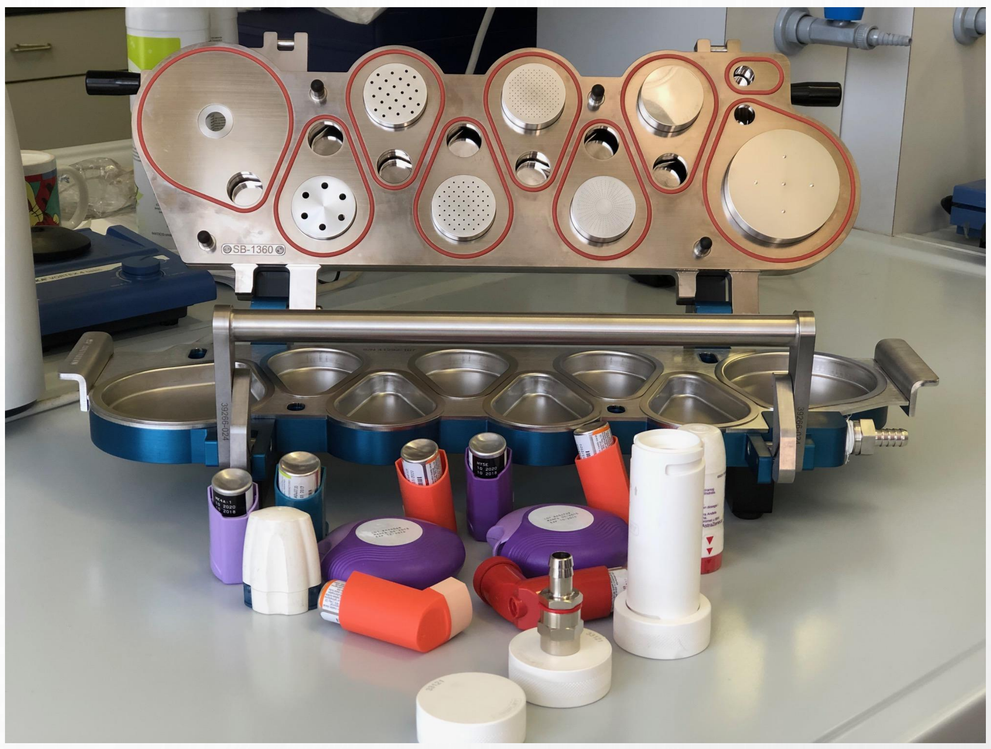
INTRODUCTION

Variability of doses in solid oral dosage forms is regulated by European Pharmacopoeia monograph 2.9.40. Uniformity of dosage units. Monograph defines variation of doses with the accepted value (AV) which is based on standard deviation (SD) of measurement results. Generally, quality requirement of AV < 15, implies that SD < 6,25. On the other hand, Uniformity of delivered dose, which regulates variability of doses of inhalation products, has set its quality requirements at limits of 25% and 35 % around declared value. In such way, theoretically, differences between the lowest and the highest measurements can be more than 40% and the product will still satisfy quality requirements. Because of that, question can be raised about the need of evaluation of precision of delivered dose from inhalation devices. More so, influence of assessment of value based on delivered dose, instead of declared dose needs to be evaluated.



MATERIAL AND METHODS

In this work, we obtained and analysed the results of Uniformity of delivered dose of 11 inhaler products produced by 7 different manufacturers and containing 6 different active ingredients. The results where additionally processed according to the monograph 2.9.40. The results were calculated according to Case 2 from Table 2.9.40.-2. for the cases where difference between declared dose and delivered dose was reported.

Table 1. Results

Inhaler	Declared dose	Declared delivered dose	T value[1]	Variation of results[2]	Average delivered dose[2]	RSD[2]	AV	AV (case a)[3]
1	250 µg	210,7 µg	118,65	105,72 - 115,07 %	109,85%	2,61%	6,9	6,9
2	120 µg	103,2 µg	116,28	99,91 - 116,29 %	107,69%	4,97%	12,86	12,86
3	50 µg	-	-	66,63 - 75,35 %	72,37%	4,00%	**30,08**	6,95
4	160 µg	-	-	97,23 - 104,32 %	99,98%	2,71%	6,51	6,51
5	80 µg	-	-	90,88 – 103,26 %	95,76%	3,80%	11,47	8,73
6	125 µg	-	-	84,31 - 104,35 %	96,16%	6,32%	**16,92**	14,59
7	100 µg	-	-	81,85 - 93,15 %	87,61%	4,39%	**20,12**	9,23
8	12 µg	10,1 µg	-	89,47 - 107,51 %	94,19%	4,89%	**15,57**	11,26
9	125 µg	-		69,74 - 94,27 %	83,69%	8,42%	**32,24**	**17,43**
10	50 µg	-	-	92,60 - 101,81%	98,59%	3,18%	7,52	7,52
11	100 µg	-		97,95 - 104,57%	100,63%	2,42%	6,00	6,00

1. - target value for calculation of AV; calculated as % of Declared dose in relation to Delivered dose
2. - measurement results
3. – first case of calculation of Av (Average delivered dose > 98,5 % and < T)

RESULTS AND DISCUSSION

All tested samples passed the test for Uniformity of Delivered Dose. As for the Uniformity of dosage units, out of 11 analysed samples, 5 had AV > 15. Relative standard deviations were in the range of 2,42 to 8,42 % and could not be correlated to the calculated AV. All samples that had AV > 15, except one, had the same value for declared and delivered dose. As a consequence, the results were lower than declared dose and, due to the addition of difference between average value to declared value to standard deviation for calculation of AV, that difference significantly increased the value of AV.

Although 45% of tested samples did not satisfy the requirements of monograph 2.9.40., the results indicate that the majority of them could pass if differentiation between the delivered and declared dose would be made. Due to the influence that deviation from target value has on AV, properly defined value of delivered dose has pivotal role on determination of dose precision in preparation for inhalation in accordance to Ph.Eur. monograph 2.9.40. It might be considered to broaden the scope of monograph Uniformity of Dosage Units.

REFERENCE

1. Council of Europe. European Pharmacopoeia. 9th ed. Strasbourg: Council of Europe; 2017.

HRVATSKA FARMAKOPEJA
Izdanje 4.8

HALMED

Five years of on-line Croatian pharmacopoeia (HRF): an example of implementation of the Ph. Eur. in a member state

P. Jaksic, G. Benkovic, D. Mikulcic, Agency for medicinal products and medical devices (HALMED), Croatia

Introduction

First edition of the Croatian pharmacopoeia (HRF) in independent country was published in 2007. In November 2013 Agency for Medicinal Products and Medical Devices (HALMED) continued to publish HRF in electronical form only. Since then, three new editions have been published (HRF 2.0, 3.0 and 4.0) as well as 16 supplements to the third and fourth edition.
HRF is regularly aligned with all editions and supplements of the European Pharmacopoeia (Ph. Eur.).

Goals

- ✓ **Help to implement** Ph. Eur. requirements as official standards in Croatia (state and professional obligation);
- ✓ **Develop** and maintain Croatian professional pharmaceutical terminology in pharmacy;
- ✓ **Translate** texts according to the user needs (open query address);
- ✓ **Actively participate** in the European Pharmacopoeia creation;
- ✓ **Publish** at least one new text in each edition/supplement;
- ✓ Continuous **improvement** of application;
- ✓ Regular **user training**;
- ✓ **Promote** Croatian Pharmacopoeia.

Croatian Pharmacopoeia activities

- National Delegation in the European Pharmacopoeia Commission
- National Pharmaocopoeia Authority (NPA)
- Croatian Pharmacopoeia Committee
- Ph. Eur. Working Parties
- HALMED Standard Terms Working Party

Croatian Pharmacopoeia Committee

11 representatives

HALMED professional body (session 4 times a year) that supports cooperation with Ph. Eur. and implementation of Ph. Eur. in all pharmaceutical areas.

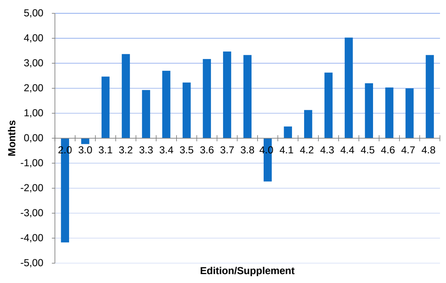
HRF since 2013

- 3 new editions and 16 supplements to the third and fourth edition with new translations of:
  - ➢77 monographs
  - ➢19 general monographs
  - ➢61 general texts
  - ➢16 other texts (for information only)
- Revision of Croatian titles of monographs for herbals („Botanic species, herbal part, herbal drug preparation„ + synonym)
- Numerous improvements of the HRF application
- Pharmacopoeial dictionary

HRF vs. Ph. Eur.

	Ph. Eur.	HRF	%
Content	1	1	100,00
1-general notices	6	6	100,00
2-Methods of analysis	294	108	36,73
3-Containers	27	13	48,15
4-Reagents	2773	658	23,73
5-General texts	47	17	36,17
6- General monographs	21	19	90,48
7-Dosage forms	30	30	100,00
8-Vaccines	144	2	1,39
9-Immunosera	9	2	22,22
10-Radiopharmaceutical preparations	80	1	1,25
11-Sutures	11	2	18,18
12-Herbal drugs	310	15	4,84
13-Homeopathic preparations	43	1	2,33
14-Substances	1780	107	6,01
All together	5576	982	17,61

Publication date vs. implementation date



Texts for information only

- **Comments** from Pharmeuropa
- **Resolution** CM/Res(2016)1
- **Unlicensed Medicines** – translation of BP chapters
- **English-Croatian dictionary** of pharmacopoeial terminology

Continuous promotion

Through work in Ph. Eur. Commission, NPA
Participation at Ph. Eur. Training Course

Various publications

IX · 2014 - 1./2. BILTEN HRVATSKE LJEKARNIČKE KOMORE

troškovi odnose na plaću (vrijednost rada) ljekarničkog tima, koji je zakonski propisan, te na pokrivanje režijskih troškova ljekarne. Izračuni pokazuju da bi vrijednost usluge izdavanja lijeka na recept, koja bi omogućila podmirenje dijela troškova ljekarne vezanih za opseg posla ugovoren s HZZO-om, trebala biti gotovo dvostruko veća nego što je sada.

Svrha je izradbe novoga modela ugovaranja u ljekarničkoj djelatnosti omogućiti da ljekarne budu od HZZO-a plaćene za onaj dio posla koji upravo i imaju ugovoren s HZZO-om. Na taj bi način ljekarne mogle uspješno poslovati i podmirivati svoje troškove, neovisno o veleprodajnim rabatima, bonusima od proizvođača lijekova te neovisno o promjenama cijena lijekova. ■

Nova izdanja Hrvatske farmakopeje

Dana 11. studenoga 2013. u *Agenciji za lijekove i medicinske proizvode* (HALMED) svečano je predstavljeno novo izdanje Hrvatske farmakopeje (HRF). Predstavljanju su prisustvovali predstavnici Hrvatske ljekarničke komore, Hrvatskog farmaceutskog društva, Hrvatskog veterinarskog instituta i brojni kolege. Uslijedila su prigodna predavanja s kratkim prikazom povijesti hrvatske farmakopeje, procesa nastanka monografije, suradnje s Europskom farmakopejom (*European Pharmacopoeia*, Ph. Eur.) te sadržaja i načina pretraživanja *on-line* izdanja HRF.

Ubrzo nakon toga objavljeno je, početkom siječnja 2014. sljedeće izdanje, Hrvatska farmakopeja 3.0, potom 17. siječnja 2014. Dodatak 3.1, a 21. ožujka 2014. Dodatak 3.2.

Zakonska podloga

Svrha je farmakopeje unaprijediti javno zdravstvo putem osiguranja priznatih za-

Cooperation with professional associations and academia

HALMED web-page

Oral presentations
Courses for students, trainees, specialists

Conclusion

Continuous improvement and intensive activities related to HRF have increased awareness of pharmacopoeial quality standards in the country, thus establishing HRF as an useful tool for implementation and promotion of the European Pharmacopoeia requirements.

Validation of a new UHPLC method for related proteins in insulin and insulin analogues as an alternative to the European Pharmacopoeia RP-HPLC method

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2. European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe - 7, allée Kastner, CS 30026, F-67081 Strasbourg (FRANCE)

Introduction

Six monographs for insulins and insulin analogues are currently described in the European Pharmacopoeia (Ph. Eur.) with corresponding Chemical Reference Substance (CRS) distributed by the European Directorate for the Quality of Medicines & HealthCare (EDQM) [1].

Insulin lispro, insulin aspart, insulin glargine and human insulin are produced by method-based on recombinant DNA (rDNA) technology, while bovine and porcine insulins are obtained by extraction from animal pancreas. Hence, the presence of impurities and insulin related proteins highly depends on the production process of the drug substance.

Their content is limited in the respective monographs and controlled by a liquid chromatography (LC) test for related proteins using the normalisation procedure. With the exception of insulin glargine, the LC method described in the monographs of the other insulins, makes use of similar chromatographic conditions, e.g. the insulins and most of the impurities are separated isocratically (followed by gradient rinsing steps) on a C18 column using a mobile phase composed of acetonitrile and an acidic sodium sulfate buffer. The LC methods come with several disadvantages including long run time and low resolution. Therefore, we aimed to develop a state of the art, rapid and standardised method for quantification of related proteins for insulin and insulin analogues.

To investigate if UHPLC could be a valid alternative to the LC methods currently described in Ph. Eur. for insulin related proteins, first the transfer of LC methods to UHPLC was checked for insulins (human, bovine, porcine) and insulin analogues (aspart and lispro). As a second step, the main goal of this study was to develop a single method that provides higher resolution and at least equal or preferably improved sensitivity. Making the method faster was not the objective. Thus, a low flow rate was preferred if it led to higher resolution and/or sensitivity.

Method

During the method development, it was shown that the most suitable column for insulin related substances separation was the Acquity CSH C18 column which comes with the particle modification called "Charged Surface Hybrid". However, to be suitable as compendial method, the future method should not be limited to one specific column with a special particle treatment. It was therefore decided to develop the UHPLC method based on common C18 stationary phases using a buffer salt and acetonitrile.

The final chromatographic conditions selected for the single proposed method are as follows:

Column:

- Size: l = 0.15m, Ø = 2.1mm
- Stationary phase: octadecylsilyl silica gel for chromatography R (1.7 µm) with a pore size of 30 nm
- Temperature: 45 °C

Mobile phase:

Solution S: Dissolve 33g of ammonium sulfate R in water for chromatography R and dilute to 1000.0 mL with the same solvent. Adjust the pH of this solution to 2.5 with phosphoric acid R

- mobile phase A: Mix 30 volumes of solution S, 15 volumes of water R and 5 volumes of acetonitrile R1
- mobile phase B: Mix 60 volumes of solution S and 40 volumes of acetonitrile R1

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 5	45	55
5 – 20	45 → 40	55 → 60
20 – 30	40 → 0	60 → 100
30 – 31	0 → 45	100 → 55
31 – 45	45	55

Flow rate: 0.15 mL/min
Detection: spectrophotometer at 214 nm
Injection: 1.5 µL
Quantification: area normalisation

Results

A much higher resolution was achieved for all insulins tested with the developed method compared to the current Ph. Eur. method.

The increased sensitivity and selectivity led to the detection and quantification of a higher amount of unknown impurities using the UHPLC method in all cases except for insulin aspart. For porcine and bovine insulins, a peak that was coeluting with the main peak in the current compendial method was separated using the new UHPLC method. This peak was identified by UHPLC-MS experiments as being B30-des-Alanine-insulin, which was also confirmed by microTOF direct infusion and specific digestion with bovine carboxypeptidase A.

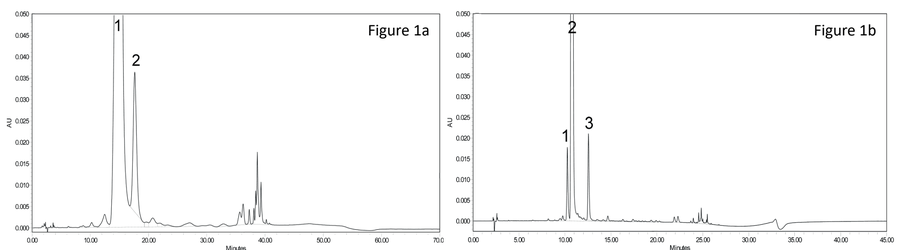

Figure 1a – Chromatograms obtained from a test solution of bovine insulin using the current Ph. Eur. LC method. Peak label: 1: bovine insulin, 2: A21-Asp bovine Insulin

Figure 1b – Chromatograms obtained from a test solution of bovine insulin using the new UHPLC-UV method.

Peak label: 1: B30-des-Ala bovine insulin, 2: bovine insulin, 3: A21-Asp bovine insulin

	Human Insulin		Porcine Insulin		Bovine Insulin		Insulin Lispro		Insulin Aspart	
	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
Resolution	3.3	0.25%	3.3	0.25%	3.3	0.25%	8.6	0.09%	8.4	0.24%
Purity	99.19%	0.01%	97.25%	0.02%	93.76%	0.02%	99.42%	0.02%	98.81%	0.04%
B30-des-Ala	n/a	n/a	0.34%	0.87%	1.77%	0.09%	n/a	n/a	n/a	n/a
A21-Asp	0.16%	5.12%	0.90%	1.20%	2.21%	2.42%	0.17%	3.26%	0.46%	4.74%
Others	0.65%	n/a	1.51%	n/a	2.26%	n/a	0.75%	n/a	0.73%	n/a
Main peak retention time	14.3 min	0.95%	15.2 min	1.00%	12.7 min	1.1%	13.5 min	1.07%	13.0 min	1.39%

Table 1a – Results obtained with the new UHPLC method (n=12)

Validation

The current Ph. Eur. general chapter 2.2.46. acknowledges that since the stationary phases are described in a generic way in monographs and there is such a variety available commercially, with differences in chromatographic behaviour, some adjustments of the chromatographic conditions may be necessary to achieve the prescribed system suitability requirements. However those adjustments are valid as long as the method is not fundamentally modified.

For a geometrical transfer from LC to UHPLC the tolerances in the adjustment of the chromatographic conditions to meet system suitability criteria described in 2.2.46. are not pertinent. The downsized method is considered as an alternative method to conventional LC and, as a consequence, specific validation is required.

Validation of the new UHPLC method was carried out according to ICH Q2 guidelines

Specificity

To demonstrate specificity, impurities were produced by applying the following conditions:

- Acidic (0.1 M HCl), alkaline (0.1 M NaOH) and oxidative degradation (0.3 % H2O2)
- freeze/thaw cycles
- forced degradation at elevated temperature (50 °C/27% relative humidity, 1 week)

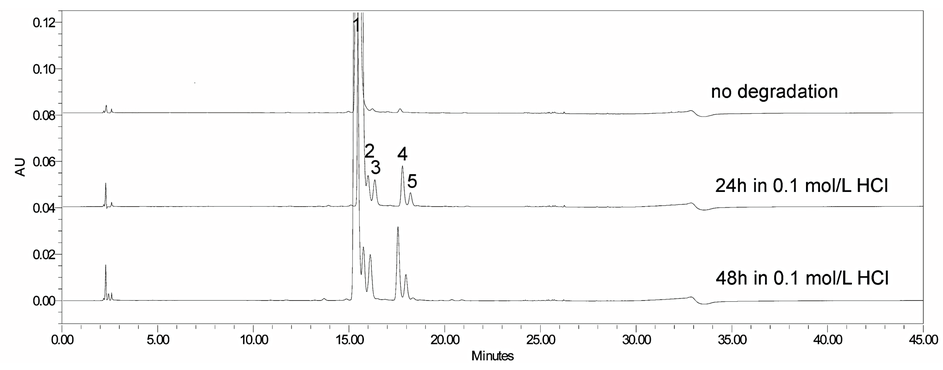

Figure 2a – Chromatogram obtained by UHPLC from a test solution of human insulin produced from a stressed stock solution Peak label: 1: human insulin, 2-3-5: degradation products, 4: A21-Asp human insulin

Robustness

In order to assess the robustness of the method, different changes applied to the chromatographic conditions were tested:

	Robustness criteria		
Changes applied	S/N of 0.05 % impurity ≥ 10	Symmetry of the principal peak 0.8 – 1.5	Resolution: - human/porcine insulin peaks ≥ 2.5 - insulin/A21Asp peaks ≥ 5.0
Flow rate (±0.02 mL/min)	✓	✓	✓
Detection wavelength (±2 nm)	✓	✓	✓
Column temperature (±5 °C)	✓	✓	✓
Gradient delay time (±2 min)	✓	✓	✓
Gradient slope and mobile phase composition (±1 % mobile phase B)	✓	✓	✓
Buffer salt concentration (±0.5 g/L)	✓	✓	✓
pH (±0.2 units)	✓	✓	✓
Column dimension (1.7, 1.8 and 1.9 µm; Ø: 2.0 and 2.1 mm; 12, 17.5 and 30 nm pore size)	✓	✓	✓

Other validation parameters

For each insulin, solutions of 0.8 mg/mL were spiked with a 0.4 mg/mL solution of bovine or human insulin to obtain impurity levels of 0.05/0.25/0.5/1.0/2.0 and 3.0 % respectively to check linearity and accuracy. These spiked solutions were prepared in triplicate.

Parameter	UHPLC method	Acceptance criteria
Linearity	$R^2 > 0.9995$	$R^2 > 0.9990$
Limit of quantification	< 0.02%	< 0.05%
Accuracy (Recovery)	Within the range for all insulins	90.0% - 110.0%
Precision (% rsd of the average peak area, n=6)	≤ 5% for peaks ≥ 0.1% ≤ 15% for peaks < 0.1%	≤ 10% for peaks ≥ 0.1% ≤ 20% for peaks < 0.1%
Intermediate precision (% rsd of the average peak area, n=12)	Within the acceptance criteria for all insulins. See Table 1a	≤ 10% for peaks ≥ 0.1% ≤ 20% for peaks < 0.1%

Conclusion

A single UHPLC-UV method for human, bovine, porcine insulins as well as insulin lispro and aspart was developed and validated according to ICH Q2 guidelines. The new method is superior to the current European Pharmacopoeia LC methods because of its improved selectivity and shorter run time. The method is based on gradient elution and employs a commonly available stationary phase (conventional C18 column) which makes it an appropriate method for pharmacopoeial use. It may therefore represent a valid alternative to the LC methods currently described in the European Pharmacopoeia for insulins. More data and discussion can be found in the authors' publication [2] .

Acknowledgement

The authors are grateful to all staff of the EDQM Laboratory, in particular to Stéphanie Moneret, Remmelt Vanderwerf and Manuela Fernandes for excellent technical assistance and to Andrea Lodi for the scientific advice provided.

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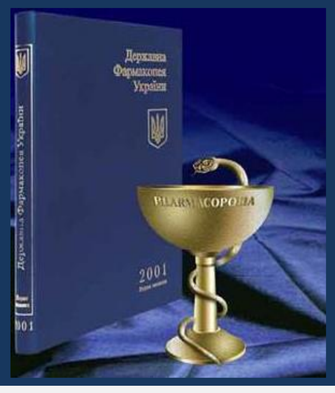
References

[1] European Pharmacopoeia 9th Edition Vol. 9.6. 2018, Strasbourg, France. Monographs for insulin aspart (2084), insulin glargine (2571), insulin lispro (2085), human insulin (0838), bovine insulin (1637), porcine insulin (1638)
[2] Wahl O, Jorajuria S, Development and validation of a new UHPLC method for related proteins in insulin and insulin analogues as an alternative to the European Pharmacopoeia RP-HPLC method, J Pharm Biomed Anal. 2019 Mar 20;166:71-82



Use of second-order derivative spectra for the identification of Sunset Yellow in medicines

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INTRODUCTION

Synthetic food colourings that provide a pleasing orange colour either individually or in combination with each other (red and yellow) are widely used in pharmaceutical preparations to enhance their appearance and taste.

To identify synthetic food colourings, visible absorption spectrophotometry, thin layer chromatography, and high performance liquid chromatography are used. The methods vary greatly in the cost and speed of analysis. Therefore, in the development of identification methods, it is essential to choose the optimal analytical method reasonably, considering the properties of the object of analysis.

AIM

To develop and validate an analytical procedure for identification of the synthetic food colouring Sunset Yellow FCF (E110) in the pharmaceutical preparation 'Vitamin C 500, chewable tablets', where the colouring is distributed in the tablet mass.

METHODS AND MATERIALS

Object of study:

- tablets 'Vitamin C 500, chewable tablets' (Pharmaceutical company 'Zdorovye', Ukraine). The pharmaceutical preparation is scored tablets of the flat-cylindrical shape of light yellow colour with specks to orange or orange-pink colour with specks.

Analytical instruments:

- spectrophotometers Shimadzu UV-2600; Perkin Elmer Lambda 25; and Agilent Cary 4000
- an analytical balance Mettler Toledo XP 205DR;
- volumetric apparatus, Class A

The analytical procedure:

- Visible absorption spectrophotometry;
- Solvent: *Water R* (Ph. Eur.)

Table 1. Characteristics of the colourings used to obtain the orange colour (manufacturer: Sigma-Aldrich)

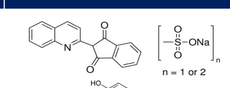
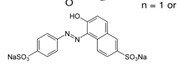
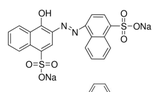
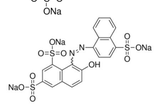
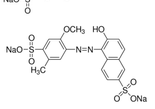
Name	Cas #	Colour	Formula	Purity, %
Quinoline Yellow (E 104)	8004-92-0	Yellow		> 98
Sunset Yellow (E 110)	2783-94-0	Orange		> 98
Azorubine (E 122)	3567-69-9	Red		> 98
Ponco 4R (E 124)	2611-82-7	Red		> 98
Allura Red (E 129)	25956-17-6	Red		> 98

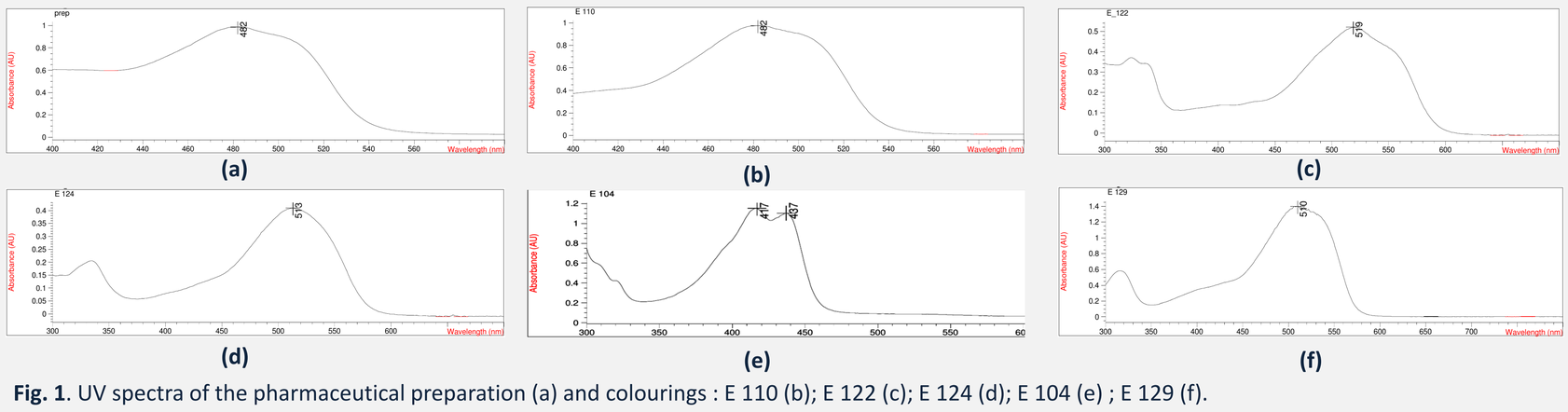
Table 2. A comparative analysis of existing research methods

The analysis method	Advantages	Drawbacks
Visible absorption spectrophotometry	The fastest method; does not require high standardization; potentially can be used without a reference standard of the analyte	The most demanding method for sample preparation; insufficient selectivity in case of spectral overlap
Thin layer chromatography	The lowest requirements for sample preparation; sufficiently high selectivity	Requires disposable, quite expensive TLC plates; time-consuming; requires a reference standard of the analyte and standardization of the procedure*
High performance liquid chromatography	The highest selectivity and sensitivity	Requires expensive and complex equipment; quite demanding requirements for sample preparation; time-consuming; requires a reference standard of the analyte and standardization of the procedure*

* the selection of stationary and mobile phases and demonstration of robustness of the procedure for different batches or suppliers of the sorbent is required

Visible absorption spectrophotometry is **preferable** for **routine analyses**.
The adequate sample preparation and selectivity have to be ensured.

RESULTS AND DISCUSSION

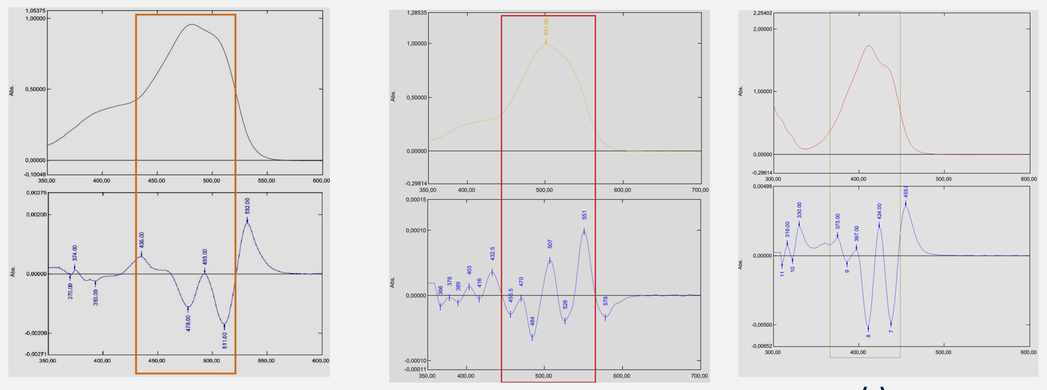


(a) (b) (c) (d) (e) (f)

Fig. 1. UV spectra of the pharmaceutical preparation (a) and colourings : E 110 (b); E 122 (c); E 124 (d); E 104 (e) ; E 129 (f).

- The placebo components do not affect the identification of colourings. Suitable sample preparation is OBSERVED.
- The spectra are rather flat, with close maxima. The specificity of the identification is NOT OBSERVED.
- The spectra have a complex structure (shoulders and inflections).
- **Derivative spectra allows increasing the reliability of the identification**.

To identify a specific colouring, it is proposed to use an 'information window' within which the presence of characteristic maxima and minima is checked.



(a) (b) (c)

Fig. 2. UV spectra of the colourings and their second order derivatives : E 110 (a); E 129 (b); E 104 (c).
Note: The frame shows the 'information window' for the colouring being identified.

Table 3. Maxima of the absorption spectra and their derivatives

Name	Colour	Absorption maximum, initial spectrum, nm	Absorption maxima, second derivative, nm	Absorption minima, second derivative, nm
E 104	Yellow	411, 437	375, 397, 424	386, 411, 438
E 110	Orange	482	435, 493	478, 511
E 129	Red	511	470, 507, 551	456.5, 484, 526
E 104 + E 129	Orange	411, 437, 511	374, 392, 422,456, 510,516	386, 410, 439, 507, 513, 541

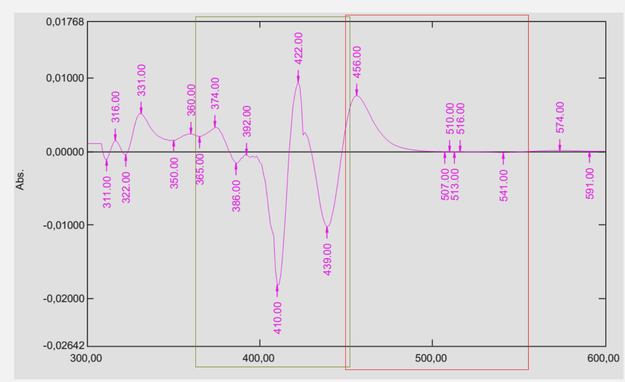


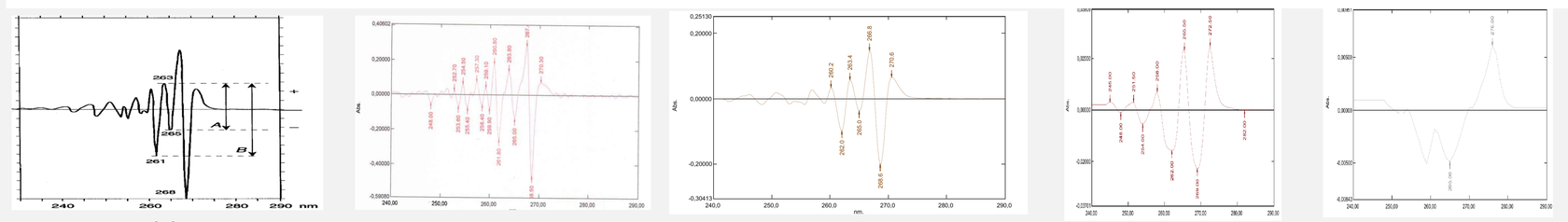
Fig. 3. The second order derivatives of the spectra of the mixture of colourings E 104 and E 129.

The specificity of the identification is OBSERVED when **derivative spectra** are used.

The suggested approach makes it possible **not to use a reference standard** to identify colourings, which is a significant advantage.

Spectrophotometer Qualification

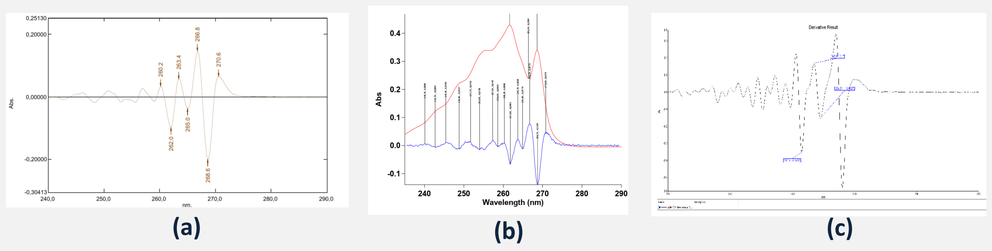
The settings of the spectrophotometer used to identify colourings by default (spectral slit-width of 1 nm, the sampling interval of 1 nm, scan speed 'fast') result in a spectrum that does not meet the requirements of the general chapter 2.2.25 of the Ph. Eur. for the resolution power.



(a) (b) (c) (d) (e)

Fig. 4. The UV derivative spectrum from Ph. Eur. *2.2.25* (a) and experimental UV derivative spectra for the sampling interval of 0.1 nm (b), 0.2 nm (c), 0.5 nm (d), and 1.0 nm (e). Spectrophotometer: Shimadzu UV-2600.

(b) and **(c)** correspond to the Ph. Eur. requirements, whereas **(d)** and **(e)** do not.
The conformity to the Ph. Eur. *(2.2.25)* is observed only for the sampling interval no wider than 0.2 nm.
The experiment was replicated on other analytical instruments (Fig. 5).



(a) (b) (c)

Fig. 5. Experimental UV derivative spectra obtained on spectrophotometers Shimadzu UV-2600 (a); Agilent Cary 4000 (b); Perkin Elmer Lambda 25 (c).
Sampling interval: no more than 0.2 nm.

The setting of the **data sampling interval of 0.1-0.2 nm** on the spectrophotometers Perkin Elmer Lambda 25, Agilent Cary 4000, and Shimadzu UV-2600 allows **meeting** the **requirements** of the **Ph. Eur. *(2.2.25)*** for the **resolution power**.
The **slit-width of 0.1 to 1.0 nm** and **any scan speed** ('fast', 'medium', or 'slow') **do not affect the result**.

CONCLUSIONS

- A spectrophotometric procedure for the identification of the E110 in tablets of vitamin C was developed and validated. The specificity and robustness were evaluated in the intra-laboratory experiment.
- The second order derivative spectrum of E110 increases the specificity of the procedure, which allows us not to use a reference standard.
- We propose to supplement the National Part *2.2.25* of the State Pharmacopoeia of Ukraine with recommendations on setting the data sampling interval.

International Conference on the 'EDQM & European Pharmacopoeia: State-of-the-Art Science for Tomorrow's Medicines'
19-20 June 2019, Strasbourg, France

Pharmacopoeial Reference Standards for Elemental Impurities

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2 European Commission, Joint Research Centre (JRC), Geel, Belgium
3 Physikalische-Technische Bundesanstalt (PTB), Braunschweig, Germany
4 Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany

Abstract

Elemental impurities (EI) in medicinal products for human use are limited according to ICH guideline Q3D, which is in force since December 2017 in the European Union. As a consequence the relevant texts of the European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopeia (USP) have been modified to reflect and complement ICH Q3D, providing details on the analytical methods to be used. In those chapters (Ph. Eur. 2.4.20., USP <233>), it is stated that for the quantification of elemental impurities, certified reference materials (CRM) from a national metrology institute (NMI) or reference materials that are traceable to the CRM of an NMI should be used.

The Ph. Eur. has so far implemented elemental impurity standards of this type for the four most important elemental impurities i.e. those corresponding to ICH Q3D Class 1: lead, cadmium, mercury and arsenic.

Details on the development of those four reference standards are provided, which was undertaken in partnership with an accredited CRM producer (JRC, European Commission), a national metrology institute (PTB, Germany), and a designated institute and accredited CRM producer (BAM, Germany). The reference standards were established and characterised according to rigorous metrological principles and are supplied with extended supporting information as required for the intended use.

After successful completion of the project, the four reference standards have been added to the Ph.Eur. catalogue and are in distribution. It is expected that another three elemental impurity standards will be implemented and made available to users within the next three years.

ICH Q3D Guideline for Elemental Impurities

ICH Q3D [1] is a harmonized guideline to ensure globally consistent control of elemental impurities in pharmaceutical products. The document describes a risk-based approach to ensure control for elements likely to be present in drug products and ingredients for human use by 3 routes of administration and divides them in three classes. In addition, safety-based limits for 24 elemental impurities are defined. The guideline was adopted by the participating regulatory authorities and published in December 2014.

Human toxicants that have limited or no use in the manufacture of pharmaceuticals.

Route-dependent human toxicants divided in sub-classes 2A and 2B based on their relative like-lihood of occurrence in the drug product.

Relatively low oral toxicity but could warrant considerationin the risk assessment for inhalation and parenteral routes.

Table V.1 (5.1): Elements To Be Considered in the Risk Assessment

Element	Class	If intentionally added (all routes)	If not intentionally added		
			Oral	Parenteral	Inhalation
Cd	1	yes	yes	yes	yes
Pb	1	yes	yes	yes	yes
As	1	yes	yes	yes	yes
Hg	1	yes	yes	yes	yes
Co	2A	yes	yes	yes	yes
V	2A	yes	yes	yes	yes
Ni	2A	yes	yes	yes	yes
Tl	2B	yes	no	no	no
Au	2B	yes	no	no	no
Pd	2B	yes	no	no	no
Ir	2B	yes	no	no	no
Os	2B	yes	no	no	no
Rh	2B	yes	no	no	no
Ru	2B	yes	no	no	no
Se	2B	yes	no	no	no
Ag	2B	yes	no	no	no
Pt	2B	yes	no	no	no
Li	3	yes	no	yes	yes
Sb	3	yes	no	yes	yes
Ba	3	yes	no	no	yes
Mo	3	yes	no	no	yes
Cu	3	yes	no	yes	yes
Sn	3	yes	no	no	yes
Cr	3	yes	no	no	yes

Implementation of ICH Q3D in the EU and Ph. Eur.

ICH guideline Q3D on elemental impurities was finally adopted by the EMA Committee for Human Medicinal Products in December 2014 [2]. The date of coming into effect for new marketing authorisation applications was June 2016 and forauthorised medicinal products December 2017.

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Implementation of ICH Q3D in the European Pharmacopoeia transformed the guideline into a legally binding document. The application date was January 2018 (Ph. Eur. 9.3.) and the following Ph. Eur. chapters and general monographs have been revised:

- 5.20. Elemental Impurities
- 2.4.20. Determination of elemental impurities
- 2619 Pharmaceutical Preparations
- 2034 Substances for Pharmaceutical use

Implementation of ICH Q3D by FDA, USP and JP [3,4]

ICH Q3D will be effective for existing products
– New NDA/ANDA effective June 01, 2016
– Existing products effective January 01, 2018

FDA

USP <232> Elemental impurities limits (August 2017)
USP <233> Elemental impurities procedures (January 2018)
(<231> Heavy Metals deleted January 2018)

JP draft for public comments, March to May 2018
New general test 2.66 Elemental impurities - Procedures
Publication of JP17 Supplement II in May 2019

JP Japanese Pharmacopoeia

(The harmonization of the elemental impurities procedure (G-07 Metal Impurities) is ongoing between EP, USP and JP within the PDG (Pharmacopoeial Discussion Group)).

Conclusion and Achievements

A highly successful collaboration has been established between leading European institutes to develop, characterise, produce and establish four elemental impurity reference standards that are traceable to the SI via the CRM of an NMI. These are the first pharmacopoeial reference standards suitable for the quantification of elemental impurities according to Ph. Eur. 2.4.20. or USP <233>. As of January 2018 these four standards are available in the EDQM CRS catalogue. Detailed information on the assigned value and the associated expanded uncertainty is included in the leaflets accompanying the reference standards.

Acknowledgement

The authors are very thankful for the great support and excellent work of the processing group of JRC-Geel, René Meinhardt, Maren Koenig, Dorit Becker, Antje Cossmer, Nicole Langhammer, Andreas Schulz, Volker Görlitz, Carola Pape, Ursula Schulz, Reinhard Jährling and Janine Noordmann.

References

[1] ICH Guideline Q3D (www.ich.org)
[2] EMA/CHMP/ICH/353369/2013
[3] Elemental Impurities in Drug Products Guidance for Industry FDA CDER; https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM509432.pdf
[4] JP draft for public comments, New general tests 2.66 Elemental Impurities – Procedures; https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/pub-comments/jp/0008.html

Requirements Elemental Impurities Reference Materials

Certified reference materials (CRM) from a national metrology institute (NMI), or reference materials that are traceable to the CRM of an NMI should be used.

Establishment of Pharmacopoeial Reference Standards

Reference materials or standards with a known content of the target element are required for the quantification of elemental impurities. The European Pharmacopoeia envisioned the establishment of suitable reference standards. However, there was a lack of specific know-how and technical equipment at EDQM. As a consequence a feasibility study was initiated to define the project scope and to identify competent and equipped partners:

European Commission
Directorate General Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Reference Materials Unit
Retieseweg 111, 2440 Geel, Belgium

BAM Bundesanstalt für Materialforschung und -prüfung

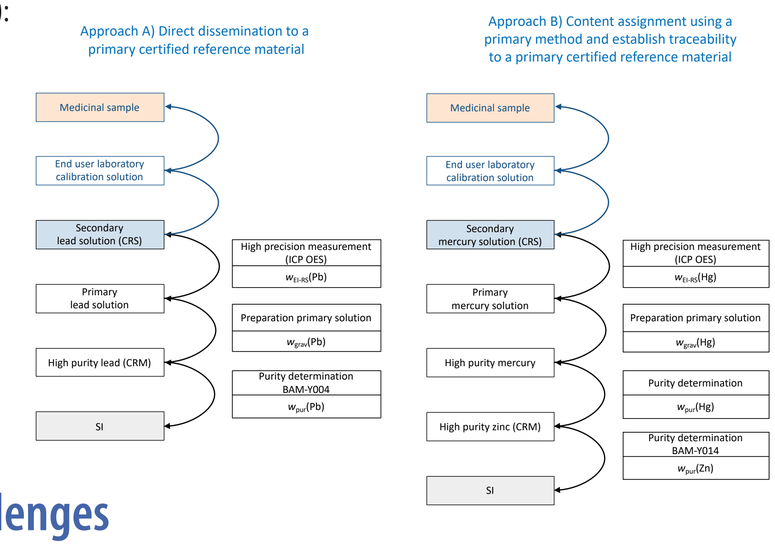
PTB Messen ▪ Forschen ▪ Wissen

First Project Phase for ICH Q3D Class 1 Elements

To mitigate the overall project risk at first the project focussed on the elements classified by ICH Q3D as class 1: lead, cadmium, mercury and arsenic.

Traceability to the SI

A key necessity was the traceability of the element content to the SI (International System of Units) to allow metrologically reliable and reproducible determinations. This included new and specific approaches to be developed by the partners in charge (BAM and PTB):

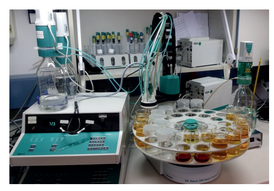


Project Challenges

At the beginning it was necessary to bridge the mind-set gap between metrologists and pharmaceutical analysts. Furthermore, concepts had to be tested and developed to achieve traceability to the SI without primary reference material of the same element. Technical solutions had to be implemented to ensure homogeneity of the concentration in the ampoules throughout the filling process. The possible leaching or absorption of element by contact materials had to be excluded by pre-testing. And finally, the synchronisation of the elemental impurity reference standards with the implementation of ICH Q3D in January 2018 demanded a tight timescale.

Outlook

On the basis of the established excellent cooperation another three elemental impurity standards will be implemented and made available to users within the next three years. Furthermore, long term stability studies are ongoing.



Element	Reference Standard	EDQM Catalogue code	w in mg/g	U(w) in mg/g	U_{rel}(w) in %	k	SI Traceability	High precision measurement	Purity determination
Pb	**Lead solution CRS 1**	Y00001996	0.9996	0.0050	0.50	2	Lead CRM BAM-Y004	ICP OES	(purity of BAM-Y004 well known)
Cd	**Cadmium solution CRS 1**	Y00001997	1.0012	0.0050	0.50	2	Zinc CRM BAM- Y014	EDTA Titration	EDTA Titration
As	**Arsenic solution CRS 1**	Y00002004	1.001	0.015	1.5	2	Copper CRM BAM- Y001	ICP OES	KI/$Na_2S_2O_3$ Titration
Hg	**Mercury solution CRS 1**	Y00002003	0.999	0.015	1.5	2	Zinc CRM BAM-Y014	ICP OES	EDTA Titration

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The European Pharmacopoeia – Facilitating PAT applications and CM

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INTRODUCTION

Process Analytical Technology (PAT) applications are essential to enable Quality by Design (QbD) and Continuous Manufacturing (CM).
The European Pharmacopoeia (Ph. Eur.) Commission and its working parties have made great efforts to facilitate the implementation of PAT applications in quality control.
The General Notices, General Methods and General Chapters of the Ph. Eur., which provide legally binding quality standards in 38 European countries, have been revised or elaborated to provide state-of-the-art methods and to support PAT applications.

Flexibility is provided by the **General Notices**, which apply to all texts of the Ph. Eur.

- **Alternative methods (first introduced in 1988)**

Compliance with Ph. Eur. reference methods is required, but alternative methods may be used, if the same pass/fail result is achieved. Approval from the competent authority is needed in any case.

- **Waiving of tests**

Tests may be omitted based on validation data or other suitable justification.

- **Parametric release (1997), Enhanced approaches (2014)**

The General Notices allow an enhanced approach to quality control using PAT and/or real-time release testing strategies as an alternative to end-product testing..

FLEXIBILITY

- **Uniformity for large sample sizes (2012)**

Chapter 2.9.47 allows sample sizes that are markedly larger than 30 units to be tested for Uniformity of Dosage Units (UDU). It is considered that compliance with this chapter demonstrates compliance with 2.9.40, the UDU test that uses small sample sizes.

- **Near-Infrared Spectroscopy (2013)**

Chapter 2.2.40 introduces PAT concepts and is complemented by the relevant EMA guideline.

- **Raman Spectroscopy (2016)**

Chapter 2.2.48 covers the potential use of this technique within a PAT environment, including hand-held instruments. It further describes updated requirements adapted to different instrument types.

- **Alternative microbiological methods (2017)**

Chapter 5.1.6 takes account of technological developments and provides information on the use of PAT.

- **X-Ray Fluorescence Spectrometry (2017)**
- **IR Absorption Spectrophotometry (2018)**
- **UV/Vis Spectrophotometry (2019)**

These chapters (2.2.37, 2.2.24 and 2.2.25) have been updated to take account of PAT applications and to introduce recent instrument usages. For example IR spectroscopy focusses on ATR and Fourier-transform transmission instruments.

MODERN METHODS

SUPPORTIVE GENERAL TEXTS

FUNCTIONALITY-RELATED CHARACTERISTICS

- **5.21 Chemometric Methods Applied to Analytical Data (2015, rev. 2019)**

Chemometrics has proven to be well suited for PAT and CM. The investigation of large data sets and processing of intricate signals requires alternative analytical tools to those used in a one-variable-at-a-time approach.

- **5.24 Chemical Imaging, CI (2017, rev. 2019)**

CI can be used in process development, improvement or understanding as well as root cause analysis.

- **5.25 Process-Analytical Technology (2019)**

A dedicated chapter on PAT will be published in 2019. It introduces into the Ph. Eur. the concept of interfacing analytical techniques with a manufacturing process. This chapter defines the different interfacing modes and compares conventional testing with PAT. It also refers to the other Ph. Eur. texts that support PAT applications.

Functionality-related characteristics (FRCs) have regularly been included in non-mandatory sections of excipient monographs since 2005.
These sections highlight characteristics that may be critical material attributes for specific manufacturing processes like CM.
Whether they are critical or not needs to be identified during development.
The introduction of FRCs contributes to the desired regulatory flexibility.

CONCLUSION

Ph. Eur. quality standards and requirements apply regardless of the control strategy. They not only ensure quality by end-product testing, but also allow and support QbD/PAT applications – essential for Continuous Manufacturing (CM) – to foster innovative approaches in the development and production of medicinal products and their ingredients. The new general chapter 5.25 summarises the implementation of PAT in the Ph. Eur.

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Establishment of the first Ph. Eur. Biological Reference Preparation for Hepatitis E virus RNA for NAT testing

Council of Europe, European Directorate for the Quality of Medicines & HealthCare, Biological Standardisation Programme

The Biological Standardisation Program (BSP) exists since 1994 and is co-funded by the Council of Europe (EDQM) and the EU Commission. The BSP focuses on the standardisation of the methods and tools for the quality control of biologicals by establishing reference standards and validating new methods with particular focus on reducing, refining and replacing the use of animals (3Rs initiative). The scope of the studies includes biotech products, vaccines and sera for human use, vaccines and sera for veterinary use, blood products and contaminants and allergens. These activities are supervised by the BSP Steering Committee which is composed of the chairs of Ph. Eur. Groups of Experts 6 (Biological and biotechnological substances), 6B (Human plasma and plasma products), 15 (Human vaccines and sera), 15V (Veterinary vaccines and sera) as well as co-opted experts and delegates from the EU Commission, EMA, BWP, IWP and WHO and the EDQM Director. Here we present the results of a study to establish the first Ph. Eur. Biological Reference Preparation (BRP) for Hepatitis E virus RNA for NAT testing. The publication of the study report is openly accessible [1].

Introduction

Hepatitis E virus (HEV) is a single stranded RNA virus belonging to the Hepeviridae family. HEV is a major cause of acute hepatitis where genotypes 1 & 2 HEV are transmitted by the faecal-oral route and is associated with contamination of drinking water. In industrialised areas with safer water sanitation, autochthonous cases of HEV occur and infection with HEV appears more prevalent than originally believed. These cases are mainly caused by genotype 3 HEV originating from undercooked animal meat.

HEV in plasma is not inactivated by solvent/detergent (S/D) treatment. Because of the widespread detection of HEV in donor plasma and the evidence of transmission of HEV by S/D plasma, the European Pharmacopoeia (Ph. Eur.) monograph 1646: Human plasma (pooled and treated for virus inactivation) was revised to include nucleic acid amplification technique (NAT) testing for HEV RNA and was implemented in January 2015 [2] .

The Ph. Eur. monograph states that each test run should contain a positive control with a concentration of 2.5 $\log_{10}$ IU of HEV RNA per millilitre (mL).

The IU for HEV RNA is defined by the World Health Organization (WHO) First International Standard (IS) for HEV RNA (6329/10), which has been prepared using a genotype 3a HEV strain.

Since the WHO IS availability is limited, a new Ph. Eur. Biological Reference Preparation (BRP) had to be established further to the decision to include nucleic acid testing (NAT) for the detection of HEV RNA in the Ph. Eur. texts.

To this purpose, an international collaborative study (coded BSP127) was launched in the framework of the Biological Standardisation Programme (BSP) of the European Directorate for the Quality of Medicines & HealthCare (EDQM, Council of Europe) and the Commission of the European Union (EU).

Participants

24 laboratories from14 countries worldwide (Europe, Asia, Americas) enrolled in the study; 23 laboratories reported data.

Materials

The candidate BRP was prepared from a human plasma positive for genotype 3f HEV kindly donated by Dr Cornelia Adhoch (Robert-Koch Institut, Berlin, Germany).

The HEV positive plasma was diluted in pooled citrated human plasma tested negative for HBV, HCV, HIV-1/2, HEV RNA, anti-HEV IgM and anti-HEV IgG and freeze-dried in screw-cap glass vials (0.5 mL/vial).

The fill volume CV was 1.0% (n=30). The residual moisture was determined by Karl Fischer analysis on vials filled with 0.5 mL of plasma diluent and distributed throughout the freeze-drier. The mean residual moisture of the diluent plasma was 0.6% (n=10).

The homogeneity of the filling/freeze-drying was assessed using HEV RT-PCR. The crossing threshold or cycle threshold (C_T) values were determined for 25 vials of the candidate BRP; the mean C_T value was 28.9, with a CV of 0.79%, indicating that the filling was of acceptable homogeneity.

The candidate BRP was stored at -20°C.

Accelerated thermal degradation stability study showed that the candidate BRP is stable at low temperature; a drop of 0.3 log10 IU/mL in titre (relative to the WHO IS) was observed after storage at +20°C for 6 months. It was difficult to compare stability at higher temperatures with the WHO IS because of problems encountered in reconstituting vials of the IS after prolonged incubation.

Temp. (°C)	Time (months)	Titre ($\log_{10}$ IU/mL)
-20	3	4.55
	6	4.52
+4	3	4.53
	6	4.43
+20	3	4.43
	6	4.19
+37	3	3.91
	6	3.50
+45	3	3.73
	6	2.74

Methods and statistical analyses

All participants were asked to perform 3 assay runs using their routine method for HEV RNA. For each run, a new set of samples had to be used. All dilutions had to be performed using HEV negative plasma.

- For quantitative tests, participants were requested to use the WHO 1st IS for HEV RNA (6329/10) to create a standard curve and samples reported directly in IU/mL.
- For laboratories performing qualitative assays, participants were requested to assay the candidate BRP alongside the 1st IS for HEV RNA (6329/10) by a series of one $\log_{10}$ dilution steps to obtain an initial estimate of an end-point. They were requested to perform 3 subsequent assays, with half-$\log_{10}$ dilutions around the end-point estimated in the first assay.

Standard electronic data reporting sheets and a method reporting form were provided to all participants.

Quantitative assay results were to be reported in IU/mL. Where real-time PCR was used C_T values for the respective dilutions were to be reported.

Qualitative assay results were reported as positive or negative, i.e. HEV RNA detected or negative; C_T, S/Co or RLU values were also requested to be reported if real-time PCR or transcription-mediated assays were used.

- For quantitative assays, the mean potency for the candidate BRP relative to the WHO 1st IS for HEV RNA (6329/10) was estimated using a mixed linear model with random factors *laboratory, assay run and test dilution*.
- For qualitative data analysis, results from all assays were pooled to give the number of positives out of the total number tested at each dilution. If it is assumed that a single 'detectable unit' will give a positive result, and that the probability of a positive result follows a Poisson distribution, the EC63 (the dilution at which 63% of the samples are expected to be positive) was chosen as the end-point. For each dilution series, this end-point was estimated by means of a probit analysis. Within the same evaluation, relative potencies were also estimated.
- For assays reporting C_T values, these were evaluated for both qualitative and quantitative methods (relative to WHO IS) using a parallel line model for each laboratory and assay run, as well as combined for all evaluable (i.e. valid) assay runs.
- Parallel line and sigmoid evaluation model as well as the combination of assays were performed according to methods as described in the Ph. Eur. general chapter 5.3. "Statistical analysis of results of biological assays and tests". The statistical analysis was performed with SAS®/STAT software and CombiStats software [3].

Results

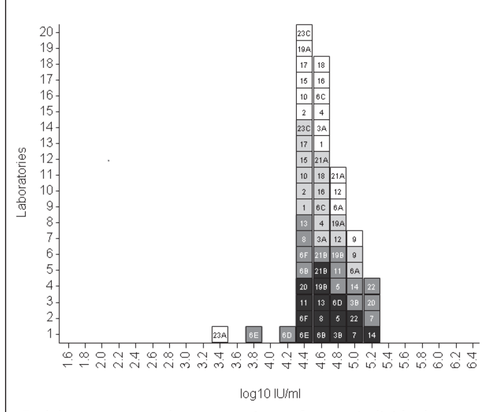
As expected, qualitative data were more variable than quantitative data.
In general, there was a good agreement between qualitative and quantitative assay data

Overall mean estimates (in $\log_{10}$ IU/mL) from quantitative and qualitative assays

CI: confidence interval; SD: standard deviation

	Estimate ($\log_{10}$IU/mL)	95% CI		Median	SD	Min	Max	Range
Quantitative (standard curve)	4.61	4.44	4.78	4.53	0.33	3.47	5.03	1.55
Quantitative (C_T)	4.62	4.51	4.73	4.57	0.21	4.38	5.07	0.69
Qualitative (end-point)	4.68	4.45	4.91	4.71	0.41	3.80	5.27	1.47
Qualitative (C_T and S/Co)	4.70	4.55	4.85	4.65	0.34	4.23	5.70	1.46
Overall	4.61	4.51	4.72	4.61	0.31	3.47	5.27	1.79

Histogram of mean estimates per assay and laboratory from quantitative and qualitative assays (expressed in $\log_{10}$ IU/mL)



Each box represents the mean estimate from an individual laboratory. The number in the box is the laboratory code. Values from Lab 23 are based on 1 assay run; the samples were inconsistently detected.

Conclusion

Based on these results, in February 2016, the Ph. Eur. Commission adopted the candidate material as the Hepatitis E virus RNA for NAT testing BRP batch 1, with an assigned unitage of 2.1×10^4 IU/vial (4.32 $\log_{10}$ IU/vial) [1, 4].

Acknowledgements

Dr Sally Baylis (Paul-Ehrlich-Institut, Langen, Germany) was the Project Leader of this study. Dr K-M O Hanschmann (Paul-Ehrlich-Institut, Langen, Germany) performed the statistical analyses.

The starting material for the BRP was generously donated by Dr Cornelia Adhoch (Robert Koch Institut, Berlin, Germany). Dr Johannes Blümel (Paul-Ehrlich-Institut, Langen, Germany) is thanked for his contribution to this study.

The contribution of Roswitha Kleiber, Dr Micha Nübling (Paul-Ehrlich-Institut, Langen, Germany) and all participating laboratories is gratefully acknowledged.

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[4] Ph. Eur. Hepatitis E virus RNA for NAT testing BRP batch 1, Cat. Code: Y0001873, *available from www.edqm.eu*)

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Case Reports of Defective Influenza Vaccine on Taiwan's Batch Release Procedure in 2018

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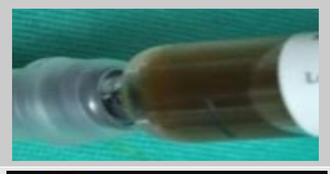
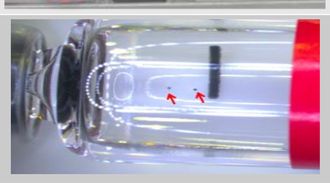
Introduction

According to Taiwan's Pharmaceutical Affairs Act 74, an official batch release for human biologics has been performed. In 2018, sixty two lots of influenza vaccines with a total of 6,799,637 doses were tested, but not all lots were approved for release, since many cases of defective influenza vaccines were reported. The information about defective influenza vaccines is summarized in the Table1.

Table 1. Information about defective influenza vaccines

Case	Abnormality	Reporting Date	Picture
1	Discoloration (Dark-brown color)	26/10/2018	
2	Foreign matter in the vaccine	29/10/2018	
3	Abnormal black particles in the vaccine	07/11/2018	
4	Abnormal white fibers in the vaccine	09/11/2018	
5	Black particles seemed to be in the vaccine	18/12/2018 29/29/2018 01/01/2019 02/01/2019	

Results

Case 1 One discolored syringe of trivalent influenza vaccine (0.5 mL dosage) was reported at one vaccination site on October 26, 2018. Subsequently, the discolored one was sent back to manufacturer for investigation. It was inferred that the discoloration might be attributed to the usage of the defective plunger stopper from the manufacturer's investigation report.

Elements	Element contents in the vaccine (ppm)		
	Discolored vaccine*	Retained vaccines of the same lot	Retained vaccines of the different lot
Pb		Not detected	Not detected
Zn		Not detected	0.008
Sn		Not detected	0.002
Fe	0.074	0.023	0.017
Cr		Not detected	Not detected
S	4900	5.04	4.83

*Result was provided from the manufacturer's investigation report.

Figure 1. Determination of the element contents in the vaccine.
The element contents of plunger stoppers were determined by an inductively coupled plasma mass spectrometry (ICP-MS). From the manufacturer's investigation report, the sulfur (S) content in the discolored one is extremely high (about 4900 ppm). The content of extractable metals in the retained vaccines, either the same lot or different lot with the discolored one, is conformed with the ISO 7886-1 requirement (total amount of plumbum (Pb), stannum (Sn), znic (Zn) and ferrumr (Fe) should not exceed 5 ppm; the amount of cadmium (Cr) should not exceed 0.1 ppm). The sulfur (S) content in the retained vaccines is much lower than in the discolored one.

Case 2 The foreign matter in one syringe of trivalent influenza vaccine (0.25mL dosage) was reported at one vaccination site on October 29, 2018. Subsequently, this foreign matter was analyzed by the Fourier-transform infrared spectroscopy (FT-IR).

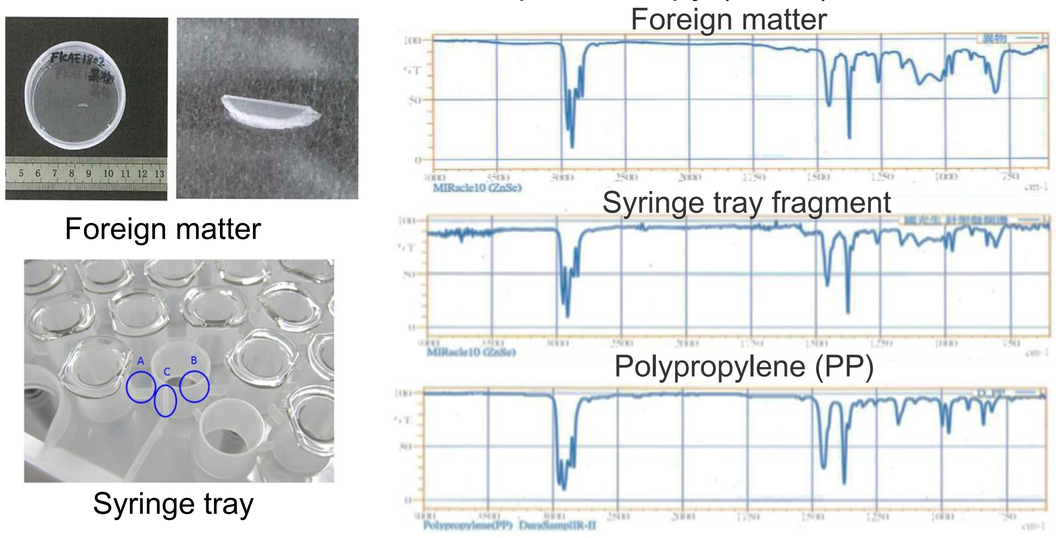


Figure 2. Analysis of the foreign matter in the vaccine
The material composition of foreign matter is polypropylene (PP), which is the same as the syringe tray fragment.

Case 3 Four syringes of trivalent influenza vaccine (0.5 mL dosage) with abnormal black particles in the vaccine were found on November 7, 2018 during our batch release procedure. Subsequently, these particles were analyzed by the Fourier-transform infrared spectroscopy (FT-IR) microscope.

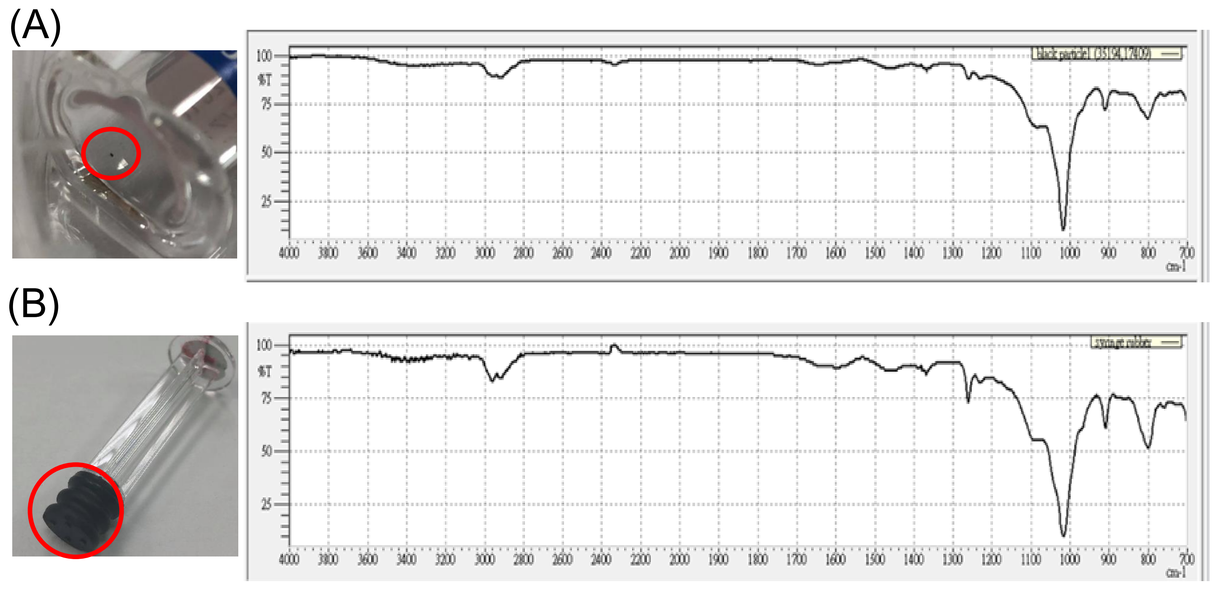


Figure 3. Analysis of abnormal black particles in the vaccine.
The spectrum of the abnormal black particles (A) is similar to the spectrum of the stopper on the plunger rod (B).

Case 4 Nine syringes of tetravalent influenza vaccine (0.5 mL dosage) with abnormal white fibers in the vaccine were found on November 9, 2018 during our batch release procedure. Subsequently, these fibers were analyzed by the Fourier-transform infrared spectroscopy (FT-IR) microscope.

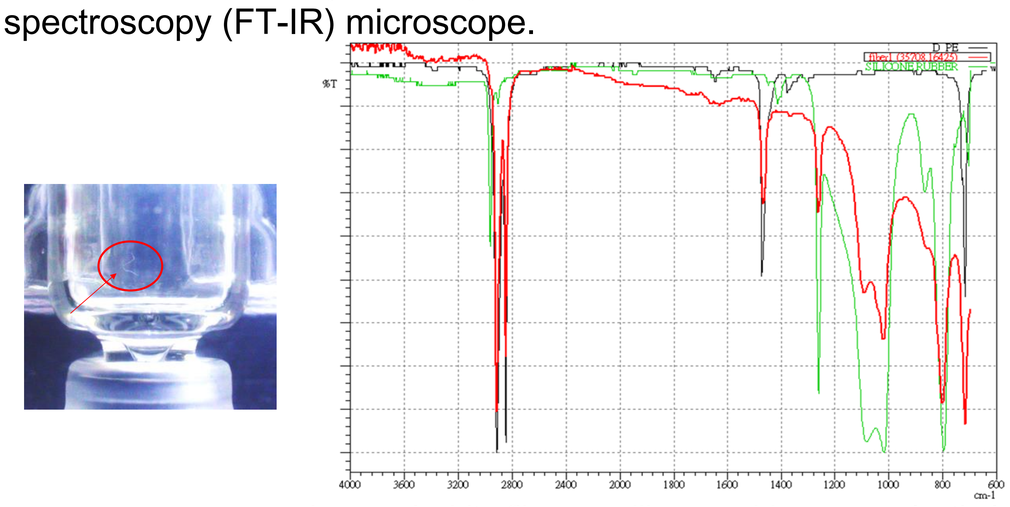


Figure 4. Analysis of abnormal white fibers in the vaccine.
The spectrum of the abnormal white fibers was complex, and explained by the presence of silicon rubber and polyethylene (PE).

Case 5 Medial staff from the vaccination sites reported that there were sixteen syringes of trivalent influenza vaccines (0.25 mL dosage), in which black particles seemed to be.

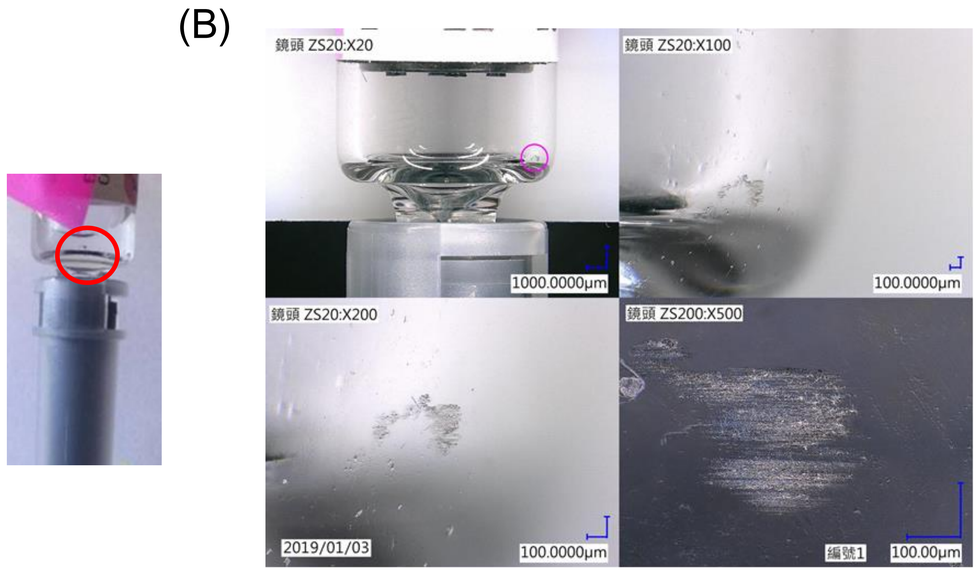


Figure 5. Observation of reported vaccine by digital microscope.
(A) Medical staff reported that a black particle seems to be in the vaccine.
(B) Through high-solution image, it is the scratch marks, not the black particle, on the outer layer of the syringe.

Conclusion

Based on the testing results and manufacturer's investigation reports, the root cause of each case was inferred, and summarized in the Table 2.

Table 2. Root cause analysis of each case

Case	Root cause analysis
1	The discoloration is caused by the chemical reaction between the vaccine and carbonized particles from the defective plunger stopper.
2	The material composition of foreign matter is the same as the material of syringe tray.
3	The abnormal black particles might be a piece of plunger stopper of the implicated syringe.
4	The white fiber agglomerate was complex, and explained by the presence of polyethylene (PE) and silicon rubber.
5	It is the scratch marks on the outer layer of the syringe.

In response to the above-mentioned cases and prevent the recurrence of releasing defective influenza vaccines into markets, Based on the risk-based approach, we increase the sampling ratio and strengthen visual checking on our batch release procedure.

Combining qNMR with LC-UV for better accuracy

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Introduction

qNMR: solution-state 1HqNMR with a certified reference material (CRM) as an internal standard is a primary ratio method with universal molar response. An uncertainty level as low as 0.1 % can be obtained when certain criteria are respected (e.g. pulse delay time, baseline separation and correction, integration region, signal-to-noise ratio, etc. [1]). The peak area of the analyte selected for quantification may include the contribution of related structure impurities (RSIs) which is a drawback in qNMR. Signals due to water, residual solvents and inorganics (not detectable) are in general separated from the analyte signal, resulting in a *% m/m* "as is" content which requires a correction for included RSIs ,if any.

LC-UV: one of the advantages of LC-UV is its high selectivity; many Ph. Eur. monographs describe methods with full separation between the analyte and its RSIs. One of the options to quantify such impurities is by using a dilution of a test solution (e.g. 0.1 % V/V or 2.0 % *V/V*) as an external standard. The result of % peak area RSIs is used as a chromatographic purity (CP) in calculating the mass balance (MB) result in *% m/m* "as is" content of the analyte in the sample:
MB = [100 − % *m/m water* − *% m/m* residual solvents − *% m/m* inorganics] × (100 − *area* % RSIs)/100 (1)

It is proposed to use LC-UV results on RSIs to correct qNMR results in order to improve accuracy.

Experimental conditions

NMR:
Instrument: Ascend 400 MHz Avance III HD Nanobay equipped with a 5 mm BBI probe (Bruker)
Software: Topspin 3.4 (Bruker)
Internal standards: TraceCERT® CRMs for quantitative NMR (Merck)

LC-UV:
Instrument: Alliance e2695 Separation Module with a 2487 Dual λ Absorbance Detector set at 210 nm (Waters)
Software: Empower 3 (Waters)
Stationary phase: Inertsil ODS-2 column of 250 x 4.6 mm, particle size 5 µm
Mobile phase, flow rate, injection volume and temperature settings: see Ph. Eur. monograph No 0582 [2]

The concept

NMR analyses of a folic acid (FA) sample had revealed the unexpected presence of about 0.2 % m/m of a small impurity which could be characterised as pterin-6-aldehyde [3]. Figure 1a shows the chemical shifts of the two singlets caused by this impurity in the aromatic region of the NMR spectrum of FA which was zoomed in on the baseline.

What if the singlet of proton P7 was overlapped by the FA signal as simulated in figure 1c? Then a qNMR result for FA No 7 would be overestimated and, subsequently, could be corrected with the aid of the amount of pterin-6-aldehyde as determined by LC-UV. Bearing in mind that qNMR displays universal molar response, one should subtract 0.2 × 441/191 = 0.5 % m/m (% *m/m* content impurity × $M_{FA}/M_{impurity}$) to obtain an accurate result. Small molecules give rise to higher integrals than large molecules at similar concentrations.

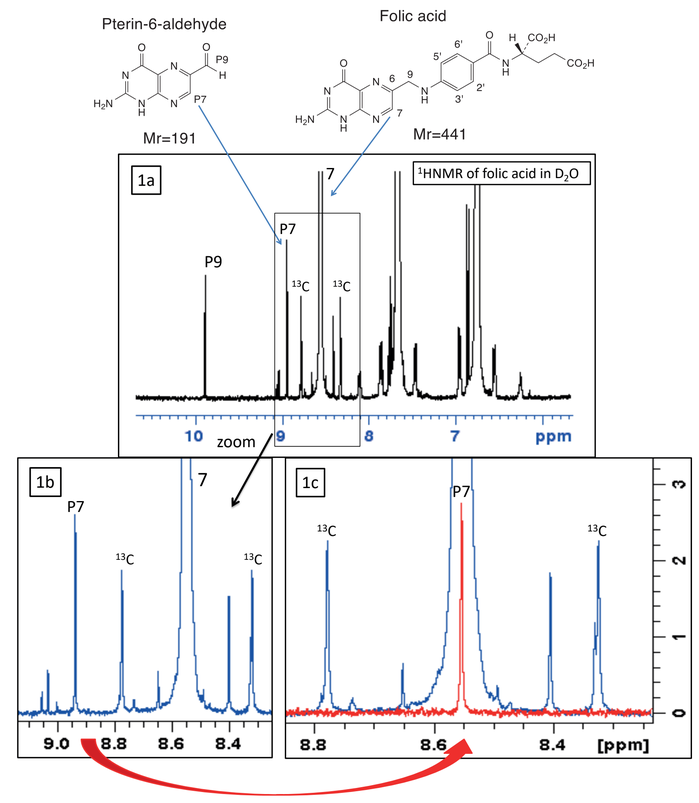

Figure 1: NMR spectra of the simulated overlap of impurity proton P7 by proton No 7 of folic acid.

Conclusion

qNMR results of analytes that are biased can be corrected by subtracting the contents of overlapped RSIs as determined by LC-UV. The information given in Ph. Eur. monographs on such analytes proved to be very helpful.
LC-UV and qNMR are complementary techniques; the combination brings about perfection.

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Calculations in qNMR

Content of X in % *m/m* = $I_X/I_{IS} \times N_{IS}/N_X \times M_X/M_{IS} \times a_{IS}/a_X \times P_{IS}$, (2)
where X stands for the analyte, I is the integral value, N is the number of nuclei, M is the molar mass, a is the amount weighed and *P* is the purity of the internal standard (*IS*).
Correction for RSIs found by LC-UV and overlapped in qNMR:
corrected content of X = qNMR content of X − (*% m/m RSI* × M_X/M_{RSI}) (3)

An example: clindamycin HCl

The Ph. Eur. monograph on Clindamycin HCl [2] describes an LC-UV method for the evaluation of its RSIs by the 2.0 % V/V dilution of a test solution. Three structures of potential RSIs are given in this monograph: impurities A, B and C as depicted in figure 2. The anomeric proton of clindamycin (see ↙ in figure 2) was selected for quantification by NMR. The anomers of the impurities were expected to be (partly) overlapped by the main signal. Therefore the qNMR result had to be corrected by subtracting the amounts of impurities A, B and C as determined by LC-UV, which resulted in a slightly negative bias of 0.3 % with respect to the mass balance. Figure 2 shows the satisfactory separation of clindamycin and its RSIs by LC-UV and, in contrast, their overlap in the integration region of the NMR spectrum.

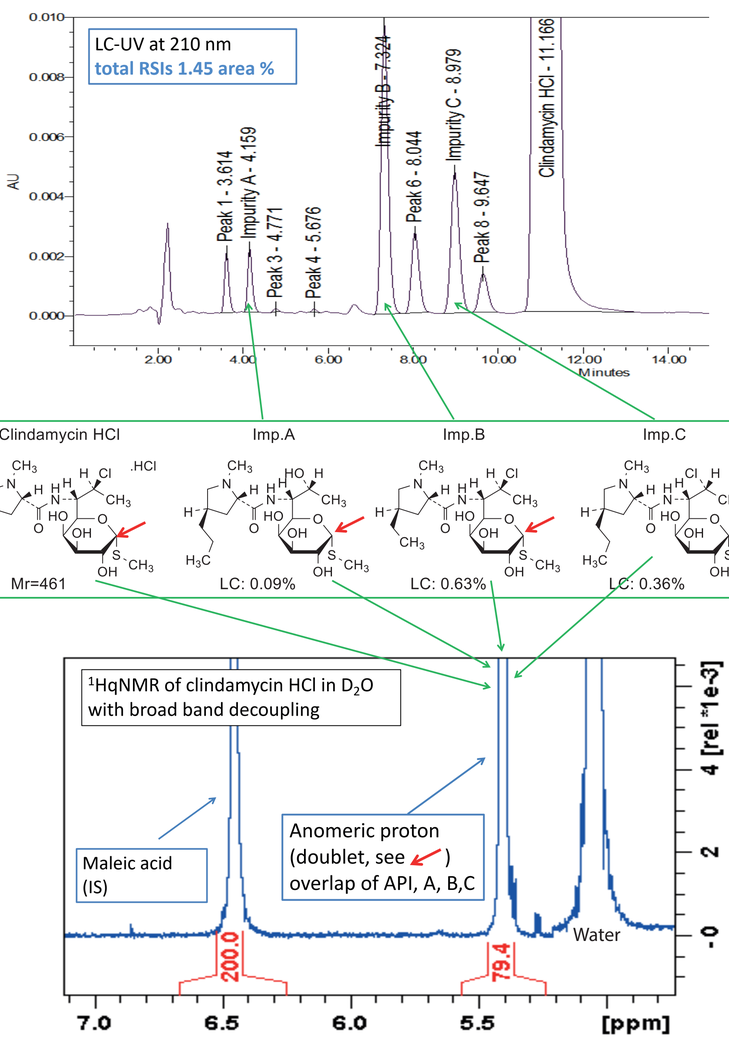

Figure 2: LC-UV chromatogram (top), structures of clindamycin and RSIs (middle) and qNMR spectrum (bottom).

Content of clindamycin HCl in % *m/m*:
MB (1) found: [100 − % *m/m* water − % m/m residual solvents − *% m/m* inorganics] × (100 − *area* % RSIs)/100 = [100 – 3.89 – 0 – 0.03] x (100 – 1.45)/100 = 94.7 %
qNMR (2) found: 95.4 %; correction for overlapped RSIs, A + B + C (3): 95.4 – 1.0 = 94.4 % (ratio $M_X/M_{RSI} \approx 1$)

Acknowledgement

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NIBSC Confidence in Biological Medicines

The development and establishment of an erythropoietin CRS with defined dimer content for SEC system suitability qualification

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INTRODUCTION

Erythropoietin (EPO) is a biotherapeutic medicine widely used to treat anaemia resulting, for example, from chemotherapy. Requirements of the European Pharmacopoeia (2) stipulate that the level of EPO-dimer must be quantified in EPO Active Pharmaceutical Ingredients (with a limit of 2%) using SEC HPLC. However, quantification is hampered by the lack of reference preparations containing stable measurable levels of EPO-dimer (2)which would allow verification of the suitability of the analytical system used. We describe here the development and establishment of a lyophilised, chemically cross-linked EPO preparation as a system suitability reference material for the size-exclusion chromatographic separation of EPO (Project BSP 137).

MATERIALS AND METHODS

Trial Preparation: Glutaraldehyde is widely used to cross-link proteins (3). A highly dimerised EPO was made following optimisation of the glutaraldehyde to protein ratio, incubation period and temperature and this was then diluted into monomeric EPO to give approximately 2% dimer. EPO (total content 100µg) formulated in 3% trehalose, 0.3% arginine, 0.01% Tween20, 0.45% NaCl, 20mM NaP buffer pH 7.4 was dispensed and successfully freeze dried, without changing the dimer content (4).

Candidate Chemical Reference Substance (cCRS) Preparation: Following the success of the small scale study, EDQM supplied sufficient amounts of EPO (a mixture of α and β forms) to fill and freeze dry a cCRS batch at NIBSC with a target of approximately 2% cross-linked dimer. This yielded a total of 5,318 vials at 100µg EPO per vial in the same formulation, with a dry mass of 0.412g/vial, a CV of fill of 0.59%, a residual moisture content of 1.59% w/w (by coulometric Karl Fischer titration) with an inert headspace of nitrogen gas (oxygen content 1.22%).

Collaborative Study: The preparation was evaluated in six laboratories in 5 countries using the Ph Eur method for SEC HPLC described (1). Dimer content and resolution of the dimer from the monomer peak was determined (Table 1, Fig 1). Stability post reconstitution was studied and thermal stressing was undertaken at NIBSC with storage at elevated temperature to assess the impact on the lyophilised material.

RESULTS

Lab	Oligomer		Dimer				Monomer	
	Rel Ret	%	Rel Ret	%	Resolution	P/V ratio	Retention (min)	%
1	0.83	0.16	0.89	3.33	1.72	6.73	18.1	96.5
2	0.81	0.1	0.87	3.38	1.87	10.8	31.8	96.5
3	0.78	0.03	0.89	3.83	1.50	4.93	14.3	96.1
4	0.84	0.17	0.90	3.60	1.73	5.71	18.0	96.2
5	0.79	0.02	0.90	3.00	1.39	3.99	27.7	97.0
6	0.82	0.17	0.88	3.41	1.88	18.63	36.3	96.4
Mean	0.81	0.11	0.89	3.42	1.68	8.46	n.a.	96.5

Table 1: SEC HPLC evaluation of cCRS dimerised EPO lyophilised preparation—each laboratory result is a mean of 4 determinations.

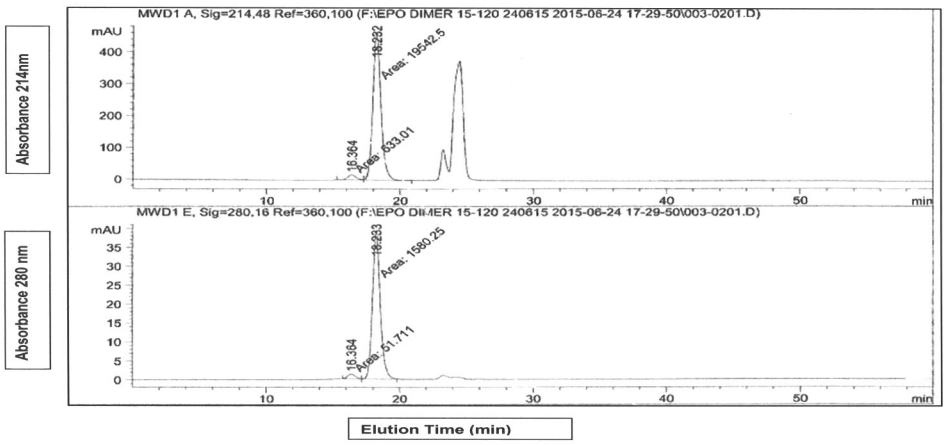

Fig 1: SEC HPLC of dimerised EPO preparation showing absorbance at 214 and 280nm (peaks after monomer are excipient-related)

All six laboratories were able to resolve the EPO dimer from the monomer with a relative retention of 0.87 to 0.90 (dimer/monomer) and a resolution of 1.4 to 1.9. With laboratory 5 excluded, the overall mean dimer content was 3.51% (with CV = 6.0%).
Five laboratories undertook stability testing on the reconstituted cCRS material. A satisfactory stability under refrigerated conditions (+2-8°C) could be established for up to 3 days after reconstitution of the cCRS.
The accelerated and real time stability studies undertaken indicate that though some aggregate forms over time the material remains suitable for purpose.

CONCLUSIONS

Based on the study results, the cCRS was adopted as Ph. Eur. EPO for SEC system suitability CRS batch 1 by the Ph. Eur. Commission at its 159th session in November 2017. The revisions proposed were captured in the Ph. Eur. 9.6 (in force on 1 January 2019).

The CRS is available from the EDQM under catalogue number Y0002009. An example chromatogram is provided with the CRS.

Given its good stability, the CRS may be shipped at ambient temperature to users and should be stored at -20°C upon receipt and until use. The CRS will be monitored at regular intervals

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Particular thanks go to Roche Diagnostic GmbH, Germany, and Janssen-Cilag, Switzerland, for the supply of the starting materials.

Intermediate Finished Product Monographs in a European Scope

Author: Johannes Mauhart, MSc

Abstract

In the scope of European regulatory guidance, API mixes prepared due to workability purposes or reasons other than safety and stability do not fall under the scope of API guidance and the associated regulatory framework. Therefore, API mixes have to be treated as intermediate finished products with all consequent implications, excluding them from the ASMF and CEP procedures.
This can be critical if the intermediate finished product manufacturer belongs to a different company group than the finished product manufacturer and obtaining the full documentation for intermediate finished products is impeded by intellectual property arguments.
In order to facilitate and harmonise the approach to intermediate finished products, the concept of intermediate finished product monographs is proposed.
A monograph would circumvent any implications to the quality of the finished product due to intellectual property discussions, as compliance to the monograph could be established by CEP-like procedures. Furthermore, from a supply point of view, it is easier to establish comparability of intermediate finished products if they comply with a monograph of the European Pharmacopeia. This enables the setting of international standards through centralised assessments and leads to shorter overall review times regarding intermediate finished product documentation.
As a model monograph, paracetamol (97%) granulated with povidone (3%) using purified water is discussed and different concepts for possible implementation are presented.

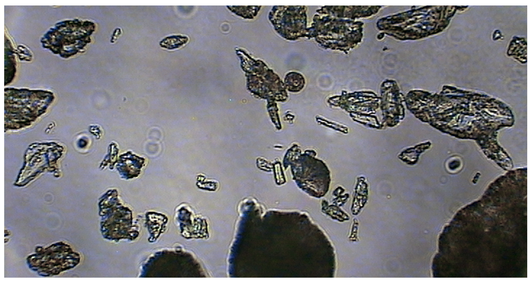

Figure 1: Photograph of paracetamol granules under the microscope with 40x magnification

Figure 2: Photograph of paracetamol granules

Introduction

Harmonisation and streamlining of regulatory procedures and requirements through adequate guidance is a great success. The tripartite of the European Medicines Agency (EMA), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the European Directorate for the Quality of Medicines & HealthCare (EDQM) have come a long way to set the scene for the regulatory landscape as it is known today.
The 'Quality Working Party questions and answers on API mix'[1] and the corresponding statement from the Certification of Substances Department of the EDQM[2] define API mix as a mixture of an API with one or more excipients. The consensus is that API mixes prepared due to workability purposes or reasons other than safety and stability do not fall under the scope of API guidance and the associated regulatory framework. Therefore, API mixes have to be treated as intermediate finished products (IFPs) with all consequent implications, excluding them from the ASMF and CEP procedures.
As long as the companies involved in the manufacture of the finished product belong to the same group, the transfer of documentation is without disruption. In our globalised world with its highly granular and differentiated industries however, this is rarely the case.
Manufacturers of IFPs frequently provide just a skeleton of documentation to the finished product manufacturer, especially if the finished product manufacturer is a small or middle-sized company, because the negotiating power of these companies is limited. GMP audits can verify if there is sufficient documentation on the IFP, but these data are regularly not available for regulatory submissions. Additional documentation is often only provided after explicit request from the authorities.
In order to facilitate and harmonise the approach to IFPs, the concept of IFP monographs is proposed. A monograph of the European Pharmacopeia would circumvent any implications to the quality of the finished product due to intellectual property discussions, and compliance to the monograph could be established by CEP-like procedures. This also applies to a supply point of view.
Monographs for IFPs enable the setting of international standards through centralised assessments leading to shorter overall review times regarding intermediate finished product documentation.

Model Monograph

Paracetamol (97%) granulated with povidone (3%) using purified water is widely used in the manufacture of paracetamol tablets, and the production of paracetamol granulate requires specialised knowledge and equipment. Therefore, the paracetamol granules are commonly manufactured by contractors, leading to possible quality implications as discussed in the introduction.
A monograph for paracetamol (97%) granulated with povidone (3%) using purified water would have to state specifications to be fulfilled and corresponding validated analytical methods for establishing compliance to the monograph. Ideally, these methods would derive from monographs of their components.

CEP Procedure for IFPs

- the scope of the CEP procedure should be extended to include IFPs
- centralised assessment facilitates setting international standards
- ability to observe and address trends in industry practice
- reduction of overall review times

Impact on GMP and CTD

Table 1: Overview of possible changes to the regulatory environment

Production Step		CTD Section		Quality Regime
Current	Proposed	Current	Proposed	
Starting material for API		3.2.S		non-GMP
API intermediate				GMP API
API / API-Mix (safety or stability reasons)	API			
IFP / API-Mix (workability)	IFP / API-Mix (all)	3.2.P	'3.2.I'	GMP Finished product
Finished Product		3.2.P		

Like APIs, IFPs are commonly traded goods on the market. The concept of IFPs allows harmonisation to include API mixes prepared for stability and safety reasons in the scope of IFPs instead of keeping them as special cases in the API regime.
The well-established CTD document structure of the market authorisation dossier can easily be extended to include IFPs by providing a separate section for the IFP and mentally replacing the term '3.2.P - finished product' with '3.2.I - intermediate finished product'.
Introducing a separate section for IFPs would first enable a better distinction between the description of manufacturing and control of IFPs and second a clear separation to the finished product part of the CTD structure.

Impact on the Variation Guideline

The classification of variations[3] is also affected by the concept of IFPs. For example, changes to the specifications of an IFP are better presented using variations of type 'B.II.d.1) - Change in the specification parameters and/or limits of the >intermediate< finished product' than by 'B.II.b.5) - Change to in-process tests or limits applied during the manufacture of the finished product'.

Retest periods for IFPs

According to the 'Note for Guidance on Start of Shelf-Life of the Finished Dosage Form'[4] the date of batch release should not exceed 30 days from the production date, which is defined as follows: 'The date of production of a batch is defined as the date that the first step is performed involving combining the active ingredient with other ingredients…'. This definition clearly applies to IFPs and leads to the discussion, whether a shelf-life or a retest period should be defined for an IFP. Low turnover of products, minimum order quantities of IFPs and complex supply chains often delay the manufacture of the finished product, leading to a reduced effective shelf life of the finished product. Together with inefficient production and supply chain issues, continuous supply of finished product to the market is challenging, especially for critical medicinal products with a small market volume.
If a retest-date can be assigned to IFPs, which is stated on a CEP, above discussed challenges are addressed. However, any impact of a re-tested IFP on the quality of the finished product still has to be assessed by the finished product manufacturer and supported with data if necessary.

Concepts of Implementation

Figure 3: Illustration of the presented concepts of implementation

IFP monographs can be implemented using different concepts:

- as extensions of active substance and excipient monographs - 'delta approach'
  - focuses on the intricacies of the IFP
  - references the monographs of its components
  - states amendments or non-applicability of the referenced specifications and methods
  - states additional limits and testing required

- as self-sufficient monographs - 'holistic approach'
  - lists all the specific information required to establish compliance with the monograph

While the delta approach persuades with its expected brevity and traceable scientific reasoning, amendments of the referenced monographs become more difficult with increasing numbers of IFP monographs, as they have to be checked for consistency and applicability of the amendments.

List of Abbreviations

ASMF	Active Substance Master File
API	Active Pharmaceutical Ingredient
CEP	Certification of Suitability
CTD	Common Technical Document
EDQM	European Directorate for the Quality of Medicines & HealthCare
EMA	European Medicines Agency
GMP	Good Manufacturing Practice
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFP	Intermediate Finished Product
TSE	Transmissible Spongiform Encephalopathy

1 Quality Working Party questions and answers on API mix, EMA/CHMP/CVMP/QWP/152772/2016

2 Certification of suitability to the Monographs of the European Pharmacopoeia – API-Mix (or mixtures) and CEPs, PA/PH/CEP (16) 70

3 Guidelines of 16.05.2013 on the details of the various categories of variations, on the operation of the procedures laid down in Chapter II, IIa, III and IV of Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products and on the documentation to be submitted pursuant to those procedures

4 Note for Guidance on Start of Shelf-Life of the Finished Dosage Form, CPMP/QWP/072/96

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Gebro Pharma

Importance of orthogonal methods for the characterisation of reference standards, illustrated by the example of α-tocopherol CRS

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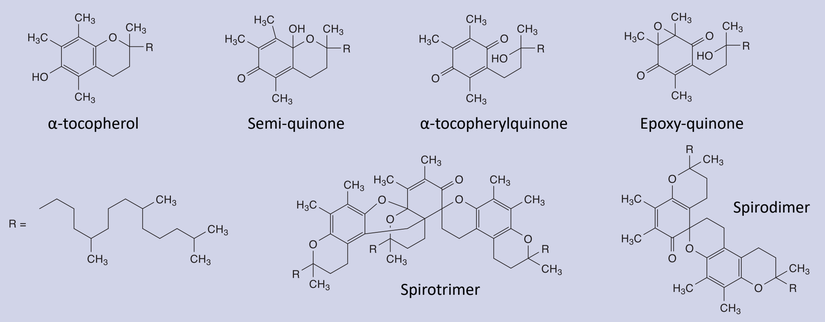
Introduction

The **assigned content** of a Ph. Eur. chemical reference substance (CRS) established for assay purposes is usually calculated from the results of the determination of impurities (organic, inorganic, water and solvents) by applying the principle of mass balance. When possible, the assigned content is verified by comparing with the result obtained by independent methods, in order to ensure the absence of a bias.

α-Tocopherol CRS is used *inter alia* as external standard in the gas chromatography (GC) assay of the Ph. Eur. monographs for *RRR*-α-tocopherol and all-*rac*-α-tocopherol. The establishment of α-tocopherol CRS is particularly challenging since the substance is a liquid and highly sensitive to oxidative degradation. It is of paramount importance that oxidation be avoided during handling and establishment of the CRS candidate material, and that methods appropriate for detection and quantification of potential impurities arising from oxidation be applied.

The **oxidation** mechanism of α-tocopherol is intricate and has not yet been fully elucidated. A major pathway includes the homolytic cleavage of the phenolic hydrogen bond that results in formation of a free radical, that is subsequently oxidised to semi-quinones and finally to α-tocopherylquinone. The latter is known to further oxidise into epoxy-quinones that in turn can dimerise and trimerise [1]. The chemical structure of α-tocopherol and some of its oxidation products is given in Figure 1.

Figure 1: Chemical structure of α-tocopherol and some of its oxidation products.



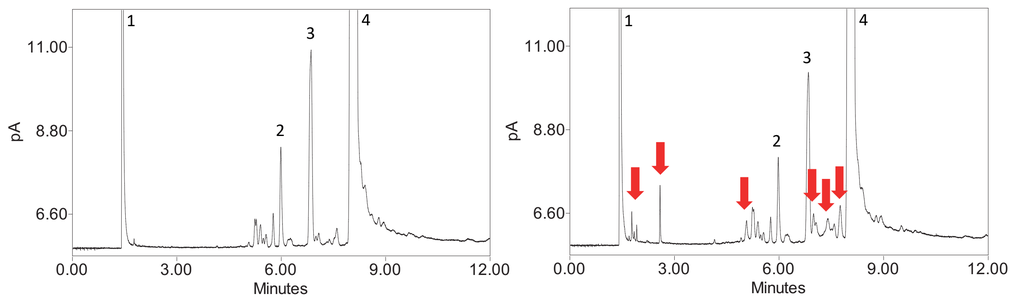
Mass balance / gas chromatography

The major impurities expected to be present in α-tocopherol are of organic nature, i.e. related substances, because usually the contribution of inorganic matter, water and solvents to the mass balance is negligible. In this study, **related substances** were quantified using the corresponding GC test described in the Ph. Eur. monographs for *RRR*-α-tocopherol and all-*rac*-α-tocopherol. Interestingly, the aforementioned oxidation products are not mentioned as specified impurities or other detectable impurities in the monographs.

The chromatograms obtained upon injection of test solutions of α-tocopherol provided by a manufacturer and intentionally oxidised* α-tocopherol are shown in Figure 2. Upon oxidation, the total amount of impurities, estimated by area normalisation, was found to increase by about 1 % and some additional impurity peaks were detected. However, employing squalane as internal standard the area of the peak due to α-tocopherol was found to decrease by about 5 %. The **disparity** between increase of impurities and decrease of α-tocopherol appears to point at oxidation products either having a lower response (unlikely) or not being detected by the GC method. α-Tocopherylquinone** was found to co-elute with α-tocopherol. GC/MS experiments could not elucidate the nature of the additional peaks nor pinpoint the location of other, known oxidation products in the chromatogram.

* Oxidation was performed in-house by exposure to air and heating. ** An authentic sample could be obtained.

Figure 2: GC analysis of α-tocopherol before (left) and after (right) oxidation. 1 = blank (sample solvent), 2 = related substance (impurity A), 3 = related substance (impurity B), 4 = α-tocopherol. Red arrows indicate additional peaks upon oxidation.



Liquid chromatography coupled to UV and MS

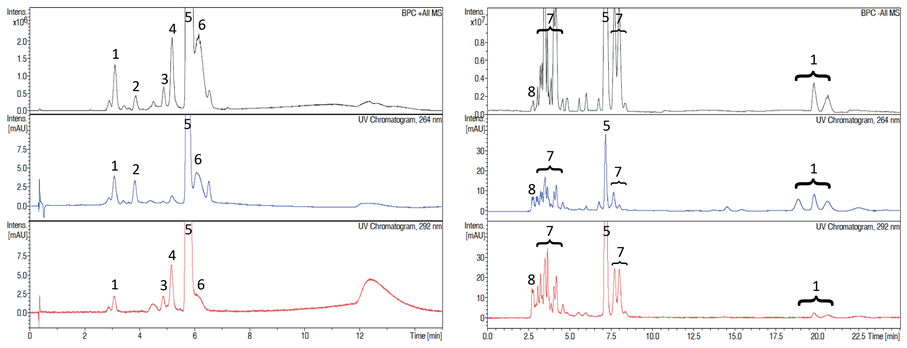
In order to further investigate the oxidation, two LC methods were applied:

- a **reversed-phase gradient LC method** used by a manufacturer to assay α-tocopherol in a finished product, employing an Acquity UPLC BEH C18 column and a mobile phase consisting of water and methanol.
- a **normal-phase isocratic LC method** reported to separate dimers and trimers of α-tocopherol [2], employing a Zorbax Rx Sil column and a mobile phase consisting of hexane and dioxane.

The UV absorbance was monitored using a diode-array detector. Furthermore, the LC instrument was coupled to a time-of-flight mass spectrometer (TOF MS). Typical chromatograms of oxidised α-tocopherol are given in Figure 3. Apart from α-tocopherylquinone, the peaks were only tentatively assigned based on their UV and mass spectrum.

The reversed-phase method could detect and separate several, monomeric oxidation products; dimers and trimers were however not readily detected. The normal-phase method on the other hand was able to detect and separate several dimers and trimers, as well as some monomeric oxidation products. The difference in UV response and absence of authentic samples of most oxidation products render quantification cumbersome.

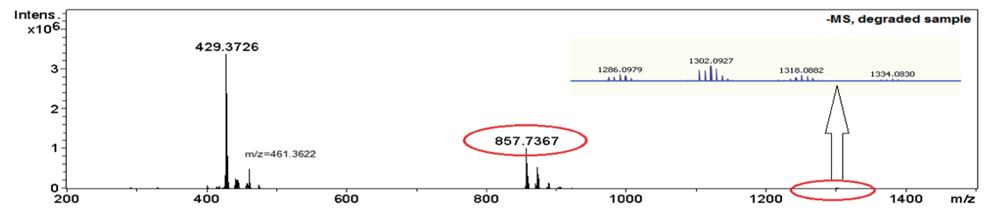
Figure 3: LC analysis of α-tocopherol after oxidation. Left: reversed-phase method; right: normal-phase method. 1 = epoxy-quinones, 2 = α-tocopherylquinone, 3 + 4 = related substances (impurities A and B), 5 = α-tocopherol, 6 = semi quinones, 7 = dimers, 8 = trimers. Peak assignment is tentative except for the underlined substances.



Direct infusion mass spectrometry

Direct infusion TOF-MS experiments were carried out using ESI and APCI in both positive and negative mode, focussing on the detectability of **dimers and trimers**. The best results were obtained using APCI in negative mode. Upon infusion of a solution of oxidised α-tocopherol, the m/z of dimers and trimers were detected, whereas these m/z were absent prior to oxidation. A typical mass spectrum of oxidised α-tocopherol is given in Figure 4.

Figure 4: Mass spectrum of α-tocopherol after oxidation.
The signals due to dimers and trimers are highlighted (red circles).



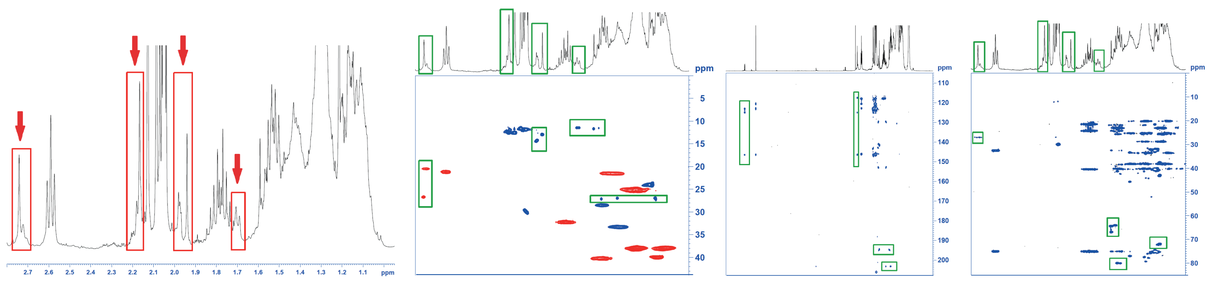
Nuclear magnetic resonance spectrometry

Nuclear magnetic resonance spectrometry (NMR) experiments were performed on a 400 MHz instrument using deuterated acetone as solvent.

Qualitative NMR

NMR spectra obtained for oxidised α-tocopherol are provided in Figure 5. Upon oxidation, additional characteristic signals were observed in the 1D proton spectrum, most prominently in the chemical shift region between 1.5 and 3.0 ppm, and in the 2D proton-carbon spectra. The nature of the signals remains however to be elucidated; it could be excluded that they are due to α-tocopherylquinone.

Figure 5: Excerpts of NMR spectra of α-tocopherol after oxidation.
From left to right: 1D proton spectrum; 2D proton carbon spectrum 1 (HSQC); 2D proton carbon spectrum 2 (HMBC); 2D proton carbon spectrum 3 (HMBC). Additional signals that appear upon oxidation are highlighted (red arrows and green boxes).



Quantitative NMR

Quantitative NMR (qNMR) was performed using an internal standard (1,2,4,5-tetrachloro-3-nitrobenzene). Only the proton signal at about 2.6 ppm, corresponding to the -CH2- group of the dihydrobenzopyrane moiety closest to the unsaturated ring, was found to be sufficiently separated to allow for quantification. However, due to slight overlap with other, minor signals the qNMR results were only considered to be an estimation. Since oxidation takes place close to the chosen protons, the signal is expected to be selective for α-tocopherol vis-à-vis of its oxidation products. Comparing the content estimated by qNMR to the one estimated by applying mass balance taking account of the impurities detected by GC, the former content was found to be lower (by about 5 to 6 %) for intentionally oxidised α-tocopherol, whereas it was within about 0.5 % prior to oxidation.

Conclusion

Based on the results obtained, it appears that the content of organic impurities in α-tocopherol CRS candidate materials may be underestimated applying the GC test for related substances of the corresponding Ph. Eur. monograph, if impurities arising from oxidative degradation are present. Several independent methods including LC coupled to UV and MS, direct infusion MS and NMR were each found to provide complementary, qualitative information regarding the presence of oxidation products. In addition, quantitative NMR seems to be a valuable tool for selective determination of the content of α-tocopherol. Correlation of the information obtained from each method and quantification of oxidation products is complicated by the complex mechanism of α-tocopherol oxidation, the plethora of potential oxidation products present, for most of which authentic samples are not available, and differences in UV response. Upon characterisation of α-tocopherol CRS candidate materials and monitoring of the fitness for purpose of the CRS, application of multiple, orthogonal methods in addition to the aforementioned GC test for related substances seems to be key in order to detect a potential bias on the assigned content related to potential oxidation by-products.

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HPLC Resolution of a Potentially Serious Global Health Crisis

Authors: Clydewyn M. Anthony, PhD., Richard Nguyen. USP (USA)

Abstract

A few years ago there was a potentially serious health issue in Pakistan and Paraguay which required the immediate intervention of both The United States Pharmacopeial Convention (USP) and The Food and Drug Administration (FDA).

This crisis resulted in the deaths of adults and children who had ingested Dextromethorphan Cough Syrup. It was later determined and confirmed that toxic levels of the controlled substance, levomethorphan, an enantiomer of dextromethorphan, was found in the drug formulation and was responsible for the resulting deaths. USP was thus charged with developing a quantitative procedure for monitoring levomethorphan and simultaneously incorporating this method as a revision to the documentary standard within its compendium.

Methodology

Sample Preparation:

- **Buffer:** 1.54 g of ammonium acetate in 1 L of water, adjusted with phosphoric acid to a pH of 4.1
- **Diluent:** Methanol and water (90:10)
- **Standard solution**: 10 µg/mL of USP Dextromethorphan RS in Diluent
- **Sample solution:** 10.0 mg/mL of Dextromethorphan in Diluent

Chromatographic system (HPLC)

- **Detector: UV** 225 nm
- **Column:** 4.6-mm × 25-cm; 5-µm packing L88
- **Mobile phase:** Methanol and Buffer (90:10)
- **Flow rate:** 1 mL/min
- **Injection volume:** 4 µL

Analysis

- **Samples**: Standard solution and Sample solution
- Calculate the percentage of levomethorphan ($C_{18}H_{25}NO$) in the portion of Dextromethorphan taken:

  Result = $(r_U/r_S) \times (C_S/C_U) \times 100$

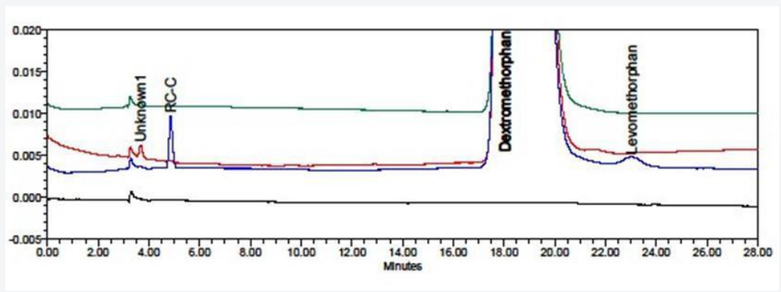
  - r_U = peak response of levomethorphan from the Sample solution
  - r_S = peak response of dextromethorphan from the Standard solution
  - C_S = concentration of USP Dextromethorphan RS in the Standard solution (mg/mL)
  - C_U = concentration of Dextromethorphan in the Sample solution (mg/mL)
- Acceptance criteria: NMT 0.10%

Conclusions

The existing USP Dextromethorphan monograph, at that time, did not include a quantitative procedure for the determination of its enantiomer, levomethorphan. Hence a chiral HPLC method was developed to bring the monograph "Up-to-Date" and simultaneously address obvious safety concerns associated with the enantiomer. The proposed HPLC method separated levomethorphan and dextromethorphanone (another impurity, dextromethorphan Related Compound C) from dextromethorphan; and allowed quantitation to satisfy acceptance criteria requirements for these impurities (0.10%). Hence, manufactured lots which tested higher than the specified limit of levomethorphan can be rejected thus preventing potential safety issues.

Results

Figures: (Left) Overlay chromatograms of injections of samples and standard solution (Right) Linearity plots of Related Compound C and Levomethorphan



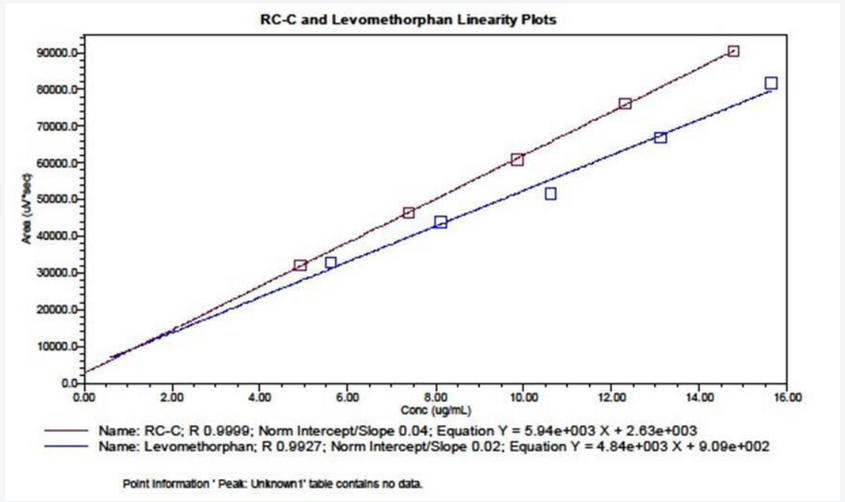
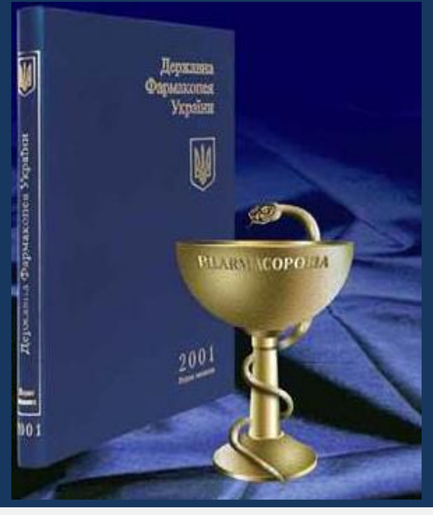


Table: Validation Characteristics and Results

Parameter	Sample	Acceptance Criteria	Results
Specificity	*Diluent, Standard, and Sample Solutions*	No interference at the retention times of Levo and RC-C	Met criteria
Linearity	Five, *Linearity solutions* covering 50 - 150% levels	Correlation coefficient ≥ 0.99 Normalized ± 5%	>0.99 (both peaks) 2% for Levo 4% for RC-C
Accuracy and Precision	*Accuracy/Precision solutions* at 50%, 100%, and 150% levels prepared in triplicate	The average recoveries at each level should be within 90% - 110% %RSD of the 9 results ≤5%	Levo: 96%, 96.3%, 96.3% %RSD (N=9) - 5.6% RC-C: 105.7%, 103.6%, 102.5% %RSD (N=9) = 1.6%
Intermediate Precision	Six Iat 100% level by a different analyst, using a different instrument and different columnon different days	%RSD of 6 results ≤5% %RSD of 15 results ≤10%	%RSD (N=6) 2.3% for RC-C 1% for Levo %RSD (N-15) 2.2% for RC-C 5.2% for Levo
Quantitation Limit	QL solution (5 µg/mL of each Levo and RC-C)	%RSD of peak area of each impurities ≤10%	2.8% for Levo 0.8% for RC-C
S/N ration	Standard solution	report	37 for Levo 227 for RC-C
Standard Stability	*Standard solution* injected periodically for 24 hours	The change in the peak area response from initial time point to final time point should be within ± 5%	8% peak area decreased after day 1 for Levo No change for RC-C



An approach to the method transfer for assay of desloratadine in film-coated tablets

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INTRODUCTION

Transfer of analytical procedures (TAP) is crucial for ensuring the quality of medicines.

The WHO guidelines on transfer of technology in pharmaceutical manufacturing (WHO TRS 961, Annex 7) provide general principles and requirements for the TAP, which should be adjusted on a case-by-case basis.

Analytical procedures should be developed, validated and transferred considering the risk of incorrect decisions on compliance when performing routine analyses in the receiving unit (RU).

The acceptable level of the risk can be assessed using the concept of uncertainty described in the General Text *5.3.N.2[N] Validation of Analytical Procedures and Tests* of the State Pharmacopoeia of Ukraine (SPhU).

AIM

To develop a metrologically sound approach to TAP for assay of desloratadine in film-coated tablets

- based on the risk assessment of incorrect decisions on compliance in routine analyses,
- considering information about risks observed during validation of analytical procedures.

METHODS AND MATERIALS

- film-coated tablets of desloratadine, 5 mg per tablet (a pilot scale batch of Alerdez, Borshchahivskiy CPP, Ukraine; the weight of one tablet is about 108 mg)
- model solutions of desloratadine
- an SPhU reference standard of desloratadine (assigned value of 99.7% for spectrophotometric assay)
- a spectrophotometer Lambda 25, 1-cm cuvette *(Perkin Elmer)*
- an analytical balance *Mettler Toledo XP 205DR*
- volumetric apparatus ISO Class A

Analytical procedure:

- UV-Vis spectrophotometry
- Diluent 0.1 M HCl
- Grind 20 tablets to a visibly homogenous mass. Prepare the test solution with the concentration of desloratadine of 0.020 mg/mL
- $A^{282nm} \approx 0.64$ absorption units

RESULTS AND DISCUSSION

The procedure for assay of desloratadine in film-coated tablets was validated according to the SPhU approach.

The expanded uncertainty of measurement results for 9 model solutions (Δ_{Fact} = 0.55%) did not exceed the maximum allowable value for medicines with content limits of ± 5% (Δ_{Target} = 5%×0.32 = 1.6%), i.e. Δ_{Fact} satisfied the criterion for insignificance in relation to content limits.

The between-tablets technological variation was evaluated by the results of the test *Uniformity of Dosage Units* (UDU) at the stage L2: *RSD* (30) = 3.0 %. As the variability introduced by sample preparation and measurements is insignificant for *RSD* (30), the analytical procedure for UDU was optimized so that the results take account of the technological variation only. Consequently, the obtained value for *RSD* (30) can be considered as an estimate of the technological variation.

The predicted uncertainty introduced by sample preparation was evaluated by the recommendations of the *Technical Guide for the Elaboration of Monographs* (EDQM) and General Text *5.3.N.2[N]* of the SPhU, and regarded as normal laboratory practice.

The following risks were detected:

1. When validating the procedure using model solutions, the requirements for validation characteristics were met, yet the uncertainty associated with the sample preparation (test portions/dilutions) exceeded that adopted by normal laboratory practice;

2. In the first test portions taken from the tablet mass ground to powder, the concentration of the tablet shell was observed, whereas in the last test portions – the concentration of the tablet core.

Acceptable homogeneity of the tablet mass obtained by grinding the tablets to powder was achieved for the test portions equivalent to 4 tablets. When smaller test portions were used, an unacceptably high variation in the assay results was detected.

Actions suggested to control the risks:

1. To control the quality of sample preparation in routine analyses, the specific absorption index for the reference standard of desloratadine was validated for the RU spectrophotometer.

The average value was 3.226E + 06 with a relative one-tailed confidence interval of ± 0.72% for a 95% reliability level.

If the specific absorption index for the reference standard of desloratadine deviates from the established value more than by the confidence interval, it is necessary to carry out the analysis as for anomalous or suspect results.

2. To control the quality of sample preparation of tablet mass, the following procedure was suggested:

Grind 20 tablets of desloratadine (about 2.2 g) to visible uniformity (for approx. 5 min). Take 4 test portions (about 410 mg) from the obtained tablet mass in succession. Conduct an analysis of the first and the fourth test portions under the procedure.

The results of assay obtained for the first and the fourth test portions, in per cents of the nominal content, should not differ by more than $\Delta_{Target} \times \sqrt{2}$ = 1.6% ×√2 =2.3%.

The Design Proposed for the Transfer Protocol:

From the tablet mass obtained by grinding 20 tablets of desloratadine (about 2.2 g) to visible uniformity (for approx. 5 min), 4 test portions (about 410 mg) are taken in succession and subjected to the analysis following the procedure.

Requirements for the precision:

The one-tailed 95 % confidence interval for a single measurement should not exceed Δ_{Target} = 1.6%.

Requirements for the accuracy:

The technological component of the variation for the analysis of 20 tablets ground to powder in two laboratories (Δ_{Tech}) and the total variation resulting from Δ_{Tech} and Δ_{Target} (Δ_{Σ}) were evaluated:
Δ_{Tech} = 1.14%; Δ_{Σ} = 2.0%.

During validation, the general mean for a given batch of desloratadine tablets was established as X_{true} = 98.9%.

The criterion for the maximum permissible difference between the single measurement result and X_{true} was formulated as follows: the greatest difference from 98.9% should not exceed Δ_{Σ} = ±2.0%.

The procedure was tested in the sending and receiving units (SU and RU, respectively). The results are shown in Tab. 1.

Table 1. The results of TAP for assay of desloratadine in film-coated tablets conducted in the sending and receiving units.

No	Assay results X_i, % from the nominal value	
	RU	SU
1	98.3	99.2
2	99.5	99.2
3	99.7	99.8
4	99.5	99.3
Mean	99.3	99.4
RSD	0.64	0.28
$t_{one\text{-}tailed}$ = 2.35		
C.I.	1.51 < 1.6	0.66 < 1.6
Max Difference	0.9 %	< 2.0 %

CONCLUSIONS

An experimental design for TAP for assay of desloratadine in film-coated tablets with content limits of ±5% was developed considering the risks observed in validation of the procedure.

The developed analytical procedure relies on the uncertainty concept and metrologically justified criteria, which allows us to control the acceptable level of the risk of incorrect decisions on compliance with specifications.

International Conference on the 'EDQM & European Pharmacopoeia: State-of-the-Art Science for Tomorrow's Medicines'

19-20 June 2019, Strasbourg, France

Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

Testing of Plasma-derived Medicinal Products at the Paul-Ehrlich-Institut

Gerrit J.K. Praefcke, Andreas Hunfeld, Susanne Breitner-Ruddock

Paul-Ehrlich-Institut, Division for Haematology and Transfusion Medicine, Langen, Germany

Abstract

In order to improve and assure the safety, efficacy and quality of medicinal products derived from human blood or plasma, the Official Control Authority Batch Release (OCABR) of these products has been introduced in 1994 both, in the German medicines law as well as in the European legislation where it is now laid down in the Council Directive 2001/83/EC, amended by Directive 2004/27/EC. Since the beginnings, the Paul-Ehrlich-Institut in Langen, the German Federal Institute for Vaccines and Biomedicines, has been responsible for the OCABR of blood products in Germany. The release procedure comprises the review of the documentation of the production and testing performed by the manufacturers to ensure compliance with the marketing authorisation. Critical parameters of the products and of the starting materials and excipients are tested experimentally by the OMCL according to the product specific guidelines of the European Directorate for the Quality of Medicines & HealthCare (EDQM). Furthermore, for national German release, also the packaging including the labelling, the product information and the compliance with the Falsified Medicines Directive 2011/62/EC are reviewed. The Paul-Ehrlich-Institut is accredited as a testing laboratory according to ISO 17025 by the national German accreditation body (DAkkS). As a member of the Official Medicines Control Laboratory (OMCL) Network of the EDQM, the Paul-Ehrlich-Institut is regularly undergoing mutual audits by other OMCLs and takes part in the proficiency testing scheme of the EDQM. Testing of biological medicines which are not subject to OCABR is performed in the scope of centrally authorised product (CAP)-testing and during pre-authorisation testing (PAT). Other activities include the participation in collaborative studies for the development of international standards and reference preparations, product testing performed for other national authorities, the participation in GMP inspections and the development of novel test systems for the analysis for biomedicines. Currently, Section 3/1 – 'Product Testing of Immunological Biomedicines' of the Division of Immunology, and Section 7/3 – 'Batch Release Blood Products, Logstics' of the Division of Haematology / Transfusion Medicine release over 1,800 product batches of immunoglobulins, human albumins, coagulation factor concentrates, plasma protease inhibitors and SD plasma and over 2,000 plasma pools per year. The time required for official batch release has been continuously decreased by laboratory automation, electronic data management, introduction of the parallel testing procedure and by the electronic submission of batch release applications. Currently, an electronic system for the dispatch of the release certificates is under development which will enable even shorter handling times and thereby accelerated availability of the medicinal products for the patients.

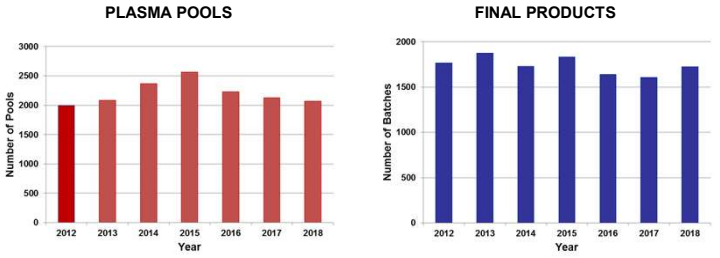
Plasma-derived Medicinal Products Released by PEI

Section 3/1 – 'Product Testing of Immunological Biomedicines' and Section 7/3 – 'Batch Release Blood Products, Logstics perform EU-official control authority batch release for a broad spectrum of medicinal products derived from human blood and plasma.

- **COAGULATION FACTORS**
  - Fibrinogen (Factor I)
  - Fibrin sealant
  - Factor VII
  - Factor VIII
  - Factor IX
  - Factor X
  - Factor XIII
  - Prothrombin Complex Concentrate
  - Protein C
- **INHIBITORS**
  - Antithrombin
  - Alpha1-Proteinase Inhibitor
  - C1-Esterase Inhibitor
- **HUMAN ALBUMINS**
  - Human Albumin 25 %
  - Human Albumin 20 %
  - Human Albumin 5 %
- **SD-PLASMA**
- **IMMUNOGLOBULINS**
  - Human Normal Immunoglobulin
  - Human anti-D Immunoglobulin
  - Human Cytomegalovirus Immunoglobulin
  - Human Hepatitis A Immunoglobulin
  - Human Hepatitis B Immunoglobulin
  - Human Rabies Imunoglobulin
  - Human Tetanus Immunoglobulin
  - Human Varicella Immunoglobulin

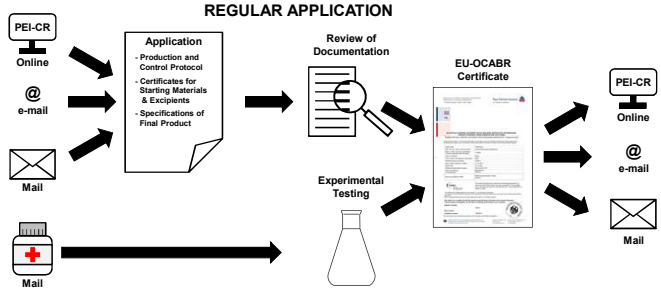
Batch Release Statistics of PEI

With of over 2,000 plasmapools and 1,800 batches of medicinal products derived from human plasma which are on average experimentally tested and released each year, the PEI is a prominent member of the General European OMCL-Network (GEON).

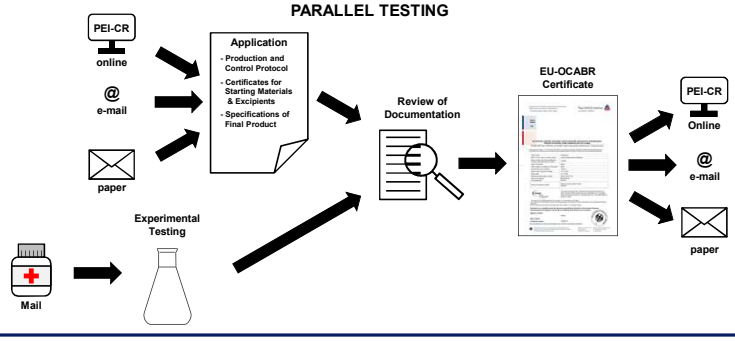


Batch Release Process of Plasma-derived Products

Applications for regular batch release can be submitted by mail, e-mail or via the PEI-CR portal. After successful experimental testing and review of the documentation by the OMCL, the certificate is dispatched by mail or e-mail while the MAH receives an online notification.

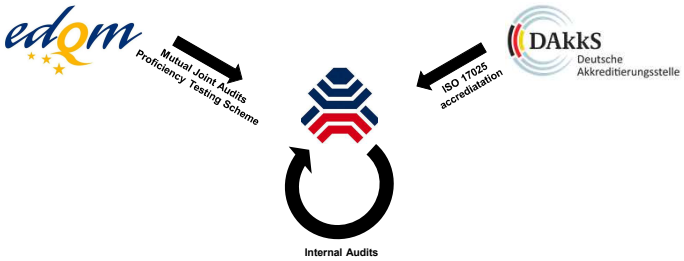


The parallel testing procedure accelerates the batch release process. Release testing of final products is performed by the manufacturer and the OMCL at the same time. After submission of the full documentation, batches can be released within a few days.



Quality Management System of PEI

The Paul-Ehrlich-Institut has regularly passed accreditations by the German Accreditation body DAkkS to guarantee a 100% implementation of the standard ISO 17025. Further audits organised by the EDQM and internal audits augment the quality management system.



Expertise and Activities beyond OCABR

The laboratories of the Paul-Ehrlich-Institut involved in batch release of plasma-derived products contribute their expertise also in other activities at the national, European and international level to improve the safety, efficacy and quality of medicinal products.

- **Regulatory Research**
  - Assay Development
  - Analysis of Quality Defects
- **Product Testing**
  - Pre-Authorisation Testing
  - Testing of Centrally Authorised Products
  - Testing of Products for other OMCLs
- **Collaborative Studies**
  - WHO International Standards
  - EDQM Biological Reference Preparations
- **Inspections**
  - Attendance of National GMP Inspections
- **Training**
  - Organisation of Workshops
  - Training of Assessors, Junior Scientists and International Guests
- **Commitees and Workgroups**
  - Ph. Eur. Group of Experts 6b
  - Human OCABR Advisory Group
  - Biological Standardisation Commitee

Collaboration Between World Health Organization and United States Pharmacopeial Convention, Inc., to help Medicines Quality Control Laboratories ensure Quality of Medicines

Author: Hari Ramanathan (hr@usp.org), with contributions from Rutendo Kuwana, World Health Organization

Abstract

The U.S. Pharmacopeial Convention (USP), based in Maryland, USA is a global health organization working to advance UN Sustainable Development Goal 3 – ensure healthy lives and promote well-being for all – USP develops standards that works to ensure the quality, safety, and benefit of medicines and foods around the world. USP's standards are used worldwide. World Health Organization (WHO), headquartered in Geneva, Switzerland, is a specialized United Nations (UN) agency with the primary role to direct and coordinate international health within the UN system. WHO and USP have a longstanding history of collaboration and have been working to improve global access and use of safe and effective quality medicines by setting public standards and best practices for medicines and health technologies.

USP's Global Public Health Division and WHO's Regulatory Systems Strengthening (RSS) team have been partnering to strengthen regional and in-country pharmaceutical regulatory capacity by supporting and training national labs and regulatory agencies.

USP established the Networks of Official Medicines Control Laboratories (NOMCoL) in different regions of the world which provides an appropriate forum to strengthen the performance and technical capacities of the OMCLs (Official Medicines Control Laboratory). Through these networks, OMCLs share relevant information and promote collaboration by learning from within. USP's support of these networks helps build the local/regional capacity of the OMCLs, which play a critical role in effectively ensuring the quality of medicines available in their communities.

The WHO RSS team provides technical assistance and conducts periodic scientific conferences to provide a pragmatic platform for OMCLs that are in the WHO PQ scheme to network, share ideas, best practices and global trends.

USP and WHO Regulatory Systems Strengthening (RSS) teams have collaborated in co-providing technical assistance, training and guideline development in the areas of auditing of QCLs, implementation of ISO 17025:2017, risk-based market surveillance, harmonization, reliance and recognition, work sharing and networking.

These activities highlight the need to strengthen global collaboration with like-minded partners to ensure maximum utilization of resources and a robust approach towards ensuring the quality of essential medicines in Low- and Middle-Income countries.

Introduction

USP's mission:

- To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.

USP Global Public Health initiatives:

- NOMCoL (Network of Official Medicines Control Laboratories)
- Technology Review Program
- Global Health Standards Program
- Complementary Standards Program

WHO promotes access to assured quality assured medicines:

- Underpinned by numerous World Health Assembly resolutions on access to quality assured medicines and functioning regulatory systems
- Sets international norms and standards, regulatory system strengthening, efforts to combat substandard and falsified products, and the prequalification program

USP and WHO are technical partners

- Improve laboratory capacity to help ensure access to affordable medical products that are safe, of good quality and effective
- Guide OMCLs interested and enrolled in WHO PQ to strengthen their QMS
- Help with compliance in procedures, techniques

NOMCoL

African Medicines Quality Forum, est. 2009

- 47 member countries

NOMCoL-MENA (Middle East/ North Africa), est. 2010

- 8 labs in 8 countries

NOMCoL- Asia Pacific, est. 2013

- 11 labs in 10 countries

Partnerships

Partnership avoids duplication and use, reliance, recognition and work sharing. Partnership in providing technical assistance

- WHO and USP apply a multi-stakeholder approach leveraging on capabilities, resources, and mutual interests.
- Partners to practice and/or follow similar norms to improves resilience, efficiency and agility of assistance provision.

Partnerships

World Health Organization

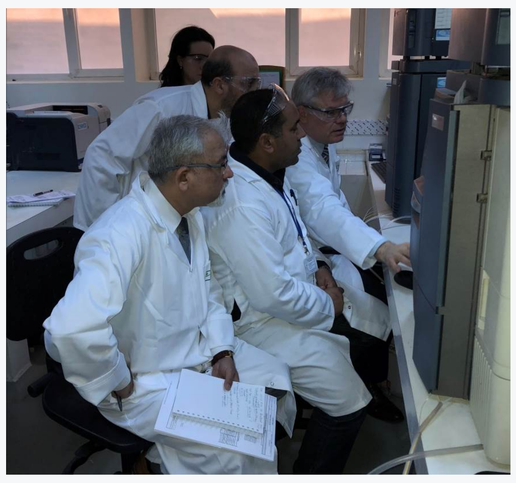
National Medicines Quality Control Laboratories Peer Auditors training workshop
12-15 June 2017
Harare, Zimbabwe

USP led discussion on Root Cause Analysis at Zimbabwe workshop

NOMCoL Asia Pacific | Network of Official Medicines Control Laboratories
A USP Global Public Health Program

4th Annual NOMCoL-Asia Pacific Quality Management Systems (QMS) Workshop | May 14-19, 2018 | Kuala Lumpur, Malaysia

WHO shared information on Common deficiencies noted during WHO-PQT inspections and their classification.



At the request of WHO, USP provided Technical Assistance to LNCM, Morocco to help address CAPA's and prepare the laboratory for WHO PQ inspection.

6th WHP Interregional Seminar for Quality Control Laboratories involved in the WHO Prequalification | 23 to 25 October 2018 | New Delhi, India

At the Seminar organized by WHO, USP provided an update on ISO 17025: 2017 and its implications and shared information on the Guidance document on Post Marketing Surveillance activities and role of QCLs in PMS

Summary

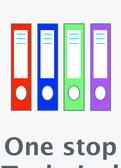
USP and WHO collaborate to support and build capacity of OMCLs by :

- Offering training and education
- Provide a forum for sharing challenges and discuss solutions
- Provide training on Quality Assurance topics
- Administering hands on laboratory training

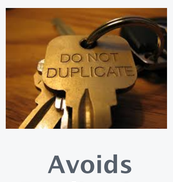
Collaboration ensures maximum utilization of resources and a robust approach towards helping ensure quality of essential medicines.

Partnership is an example of the type of multilateral cooperation that is needed to overcome the challenges of globalization.

Partnership potential benefits:

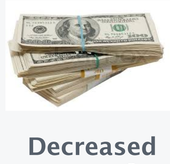


One stop Technical Assistance



Avoids Duplication

Resource Optimization



Decreased cost of regulation

Harmonized Practices

United States Pharmacopeia
www.usp.org

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

Faculty of Science – Pharmaceutical Institute

Three case studies of falsified medicines in Cameroon: WHO Medical Product Alerts N° 4/2017 (“Penicillin V”), N° 2/2018 (“Augmentin®”) and N° 6/2019 (“Hydrochlorothiazide”)

Simon Schaefermann[1], Dorothea Wistuba[2], Fidelis Nyaah[3], Manji Pattinora[3], Edward Ndze[4], Tambo Cletus[4], Pernette Bourdillon Esteve[5], Lutz Heide[1]

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[3] Presbyterian Church in Cameroon (PCC), Cameroon

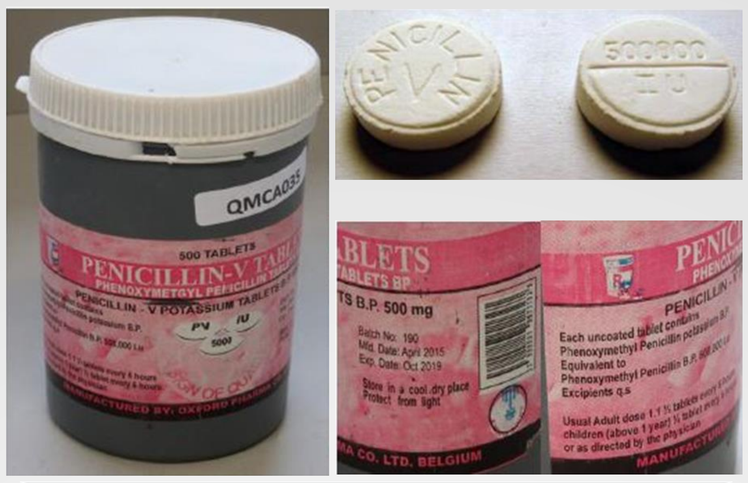
[4] Cameroon Baptist Convention Health Services (CBC), Cameroon

[5] World Health Organization (WHO), Switzerland

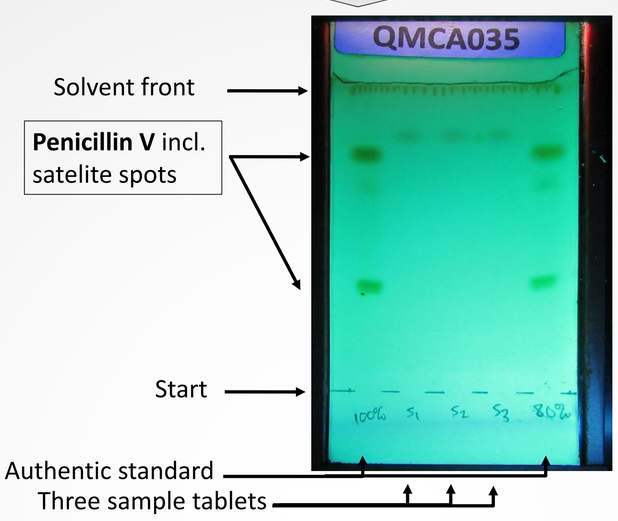
Introduction

Falsified medicines can pose a great risk to the health of patients and can undermine the trust in health professionals and systems [1]. In order to warn Member States and populations, the WHO publishes recently discovered cases of dangerous falsified medicines as „Medical Product Alerts“ [2]. N° 4/2017 and N°2/2018 were discovered at informal drug vendors in Cameroon during an ongoing suvey on the quality of medicines carried out by the University of Tübingen in collaboration with the two African church organisations PCC and CBC. N°6/2019 was discovered by PCC as part of their regular surveillance.

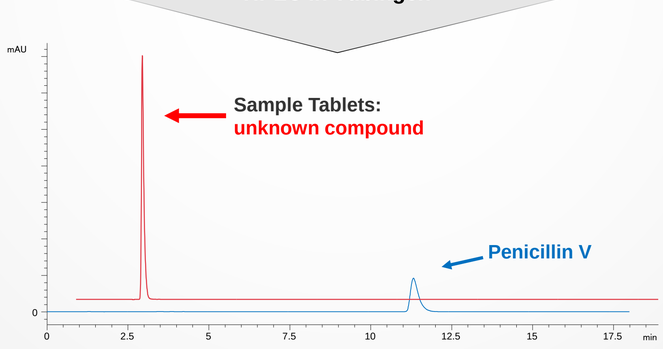
N° 4/2017



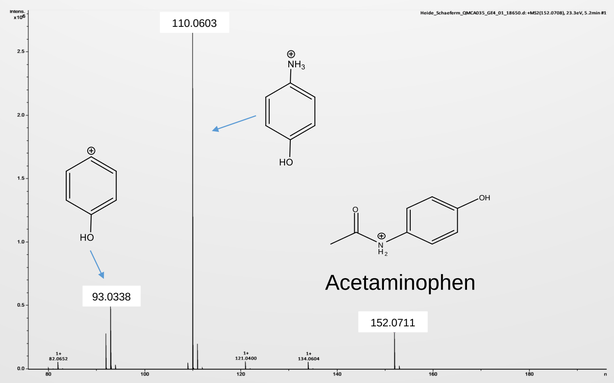
Thin layer chromatography in Cameroon



HPLC in Tübingen



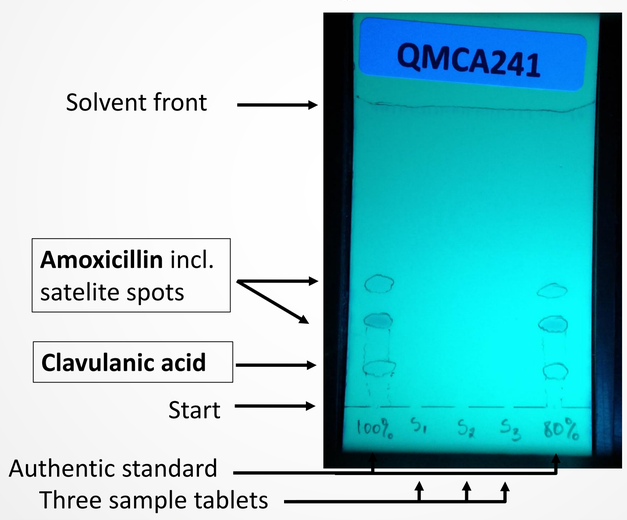
LC-HR-MS/MS in Tübingen



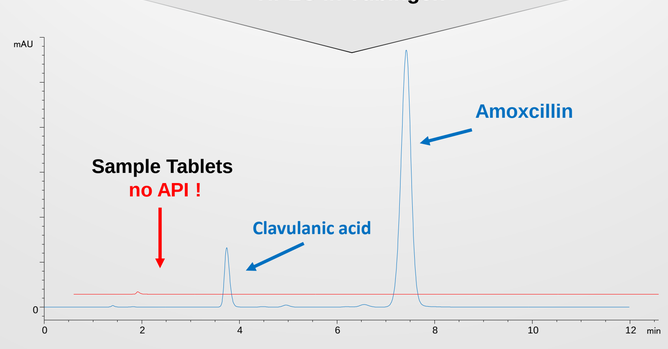
→ Falsified „Penicillin V“ actually contains paracetamol (50 mg/tablet)

N° 2/2018

Thin layer chromatography in Cameroon

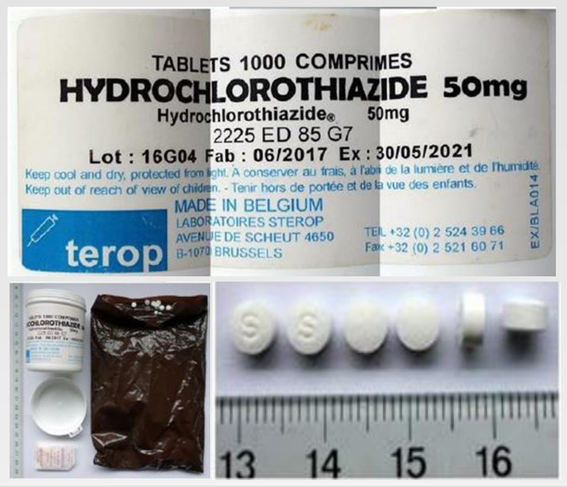


HPLC in Tübingen

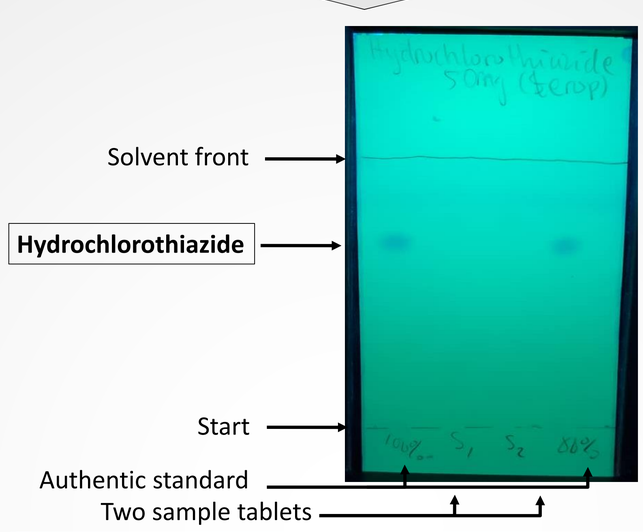


→ Falsified „Augmentin“ contains no API

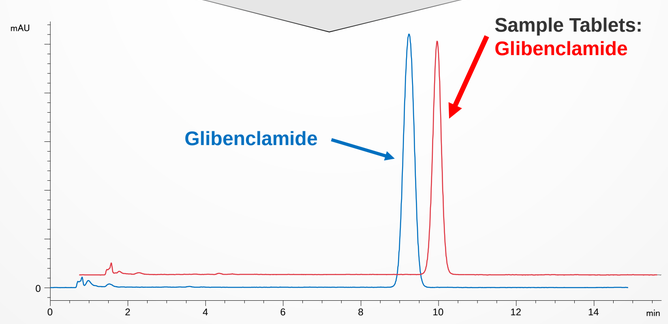
N° 6/2019



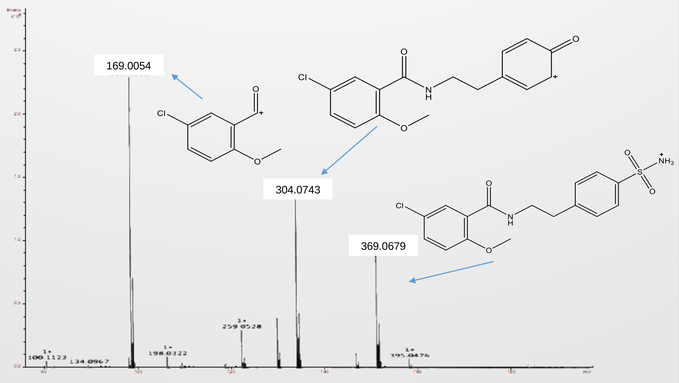
Thin layer chromatography in Cameroon



HPLC in Tübingen



LC-HR-MS/MS in Tübingen



→ Falsified „Hydrochlorthiazide“ actually contains glibenclamide (5 mg/tablet)

Conclusion

- Falsified medicines are in circulation in the Republic of Cameroon
- Some falsified medicines are produced by well-equipped criminal manufacturers
- The absence of the correct API could rapidly be detected by local reasearchers using thin layer chromatography
- Unequivocal identification of incorrect API’s requires a well equipped laboratory

1. WHO Global Surveillance and Monitoring System for substandard and falsified medical products. Geneva: World Health Organization; 2017. Available from: https://www.who.int/medicines/regulation/ssffc/publications/GSMS_Report_layout.pdf
2. WHO Medical Product Alerts - Background. Available from : https://www.who.int/medicines/regulation/ssffc/medical-products/en/

Quality of medicines in southern Togo: Investigation of antibiotics and of medicines for non-communicable diseases from pharmacies and informal vendors

Simon Schaefermann[1], Emmanuel Wemakor[1], Cathrin Hauk[1], Lutz Heide[1]

[1]Pharmaceutical Institute, University of Tübingen, 72076 Tübingen, Germany.

Email: simon.schaefermann@uni-tuebingen.de

Introduction

Access to essential medicines has been included in the Millennium Development Goals (MDG) of the United Nations and is now included in the Sustainable Development Goals as Goal No. 3.8 [1]. It comprises access to safe, effective, quality and affordable medicines for both non-communicable diseases (NCD) and communicable diseases [2,3]. Still the WHO estimated in 2017 that 10.5 % of medicines in low and middle income countries are substandard or falsified [4].
Only very few studies investigated substandard and falsified medicines in the Republic of Togo so far. The recently published quality evaluation of cardiac medicines in ten countries of Africa [5] also included 100 samples deriving from Togo. It concluded that 9% of the samples deriving from Togo where poor quality drugs. Also a study on the quality of veterinary medicines was carried out in the northern part of Togo. The objective of the present study was to contribute to the knowledge about the prevalence of substandard and falsified medicine in the Republic of Togo, including both anti-infective medicines and medicines for non-communicable diseases. Medicines were sampled from the private sector, i.e. licensed pharmacies, and informal vendors, in several towns in the southern part of Togo.

Sample Collection

The samples were collected in the southern part of Togo, in the regions Maritime and Plateaux from licensed pharmacies, and informal vendors. In Lomé the local investigator asked several citizens for well stocked informal drug vendors in the south of Togo. Figure 1 shows a map of the locations of this vendors. While visiting each of the six chosen informal drug outlets, the investigator identified the geographically nearest licensed pharmacy. A list of licensed pharmacies in Togo is available on the internet [6].

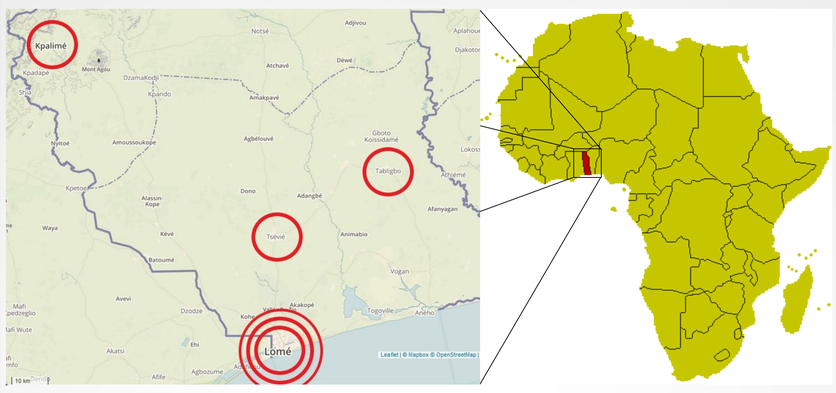


Figure 1: Map of the sampling sites in the regions Maritime and Plateaux of the Republic of Togo (www.openstreetmap.org)

In both types of sampling sites the investigator acted as a customer and purchased a quantity of 100 tablets or capsules for each of the 12 medicines, if available. If the quantity of 100 tablets or capsules per medicine was not available, a smaller quantity was purchased, but not less than 30 tablets or capsules to ensure a sufficient amount for chemical analysis.

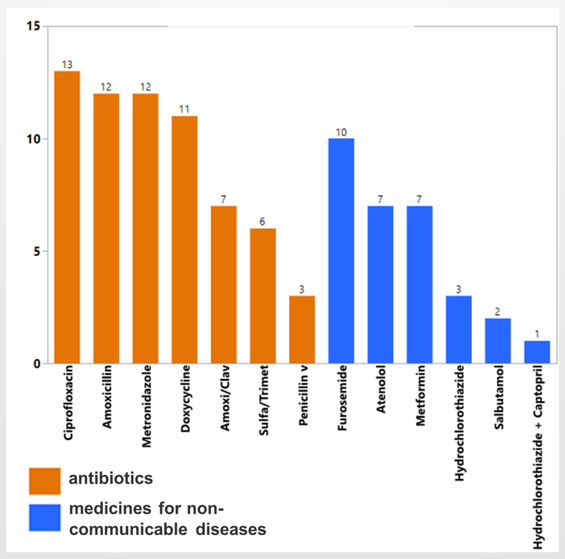


Figure 2: Numbers of medicines collected

Chemical Analysis

Chemical analysis was carried out according to the methods specified in the monographs of the United States Pharmacopoeia 2016 (USP 39) for the respective dosage forms for each of the 12 medicines. The chemical quality assessment included the determination of identity, assay (content of API) and dissolution (proportion of API dissolved from the dosage form over time). Following the respective monographs of the USP 39, the assay (quantification of the content of the API) was carried out by HPLC for all investigated medicines, and dissolution of the API was quantified by HPLC for all investigated medicines except metformin, which was quantified by UV spectroscopy.

As proposed by a study published by the WHO in 2011 [6], the non-compliant samples were further divided into those showing only moderate deviations from the USP 39 criteria, and those showing extreme deviations. As suggested by the mentioned WHO study, extreme deviation was defined as the content of API deviating more than 20% from the declared content and/or the average dissolution of the tested units falling more than 25% below the pharmacopoeial limit (i.e. below the pharmacopoeial Q-value minus 25%).

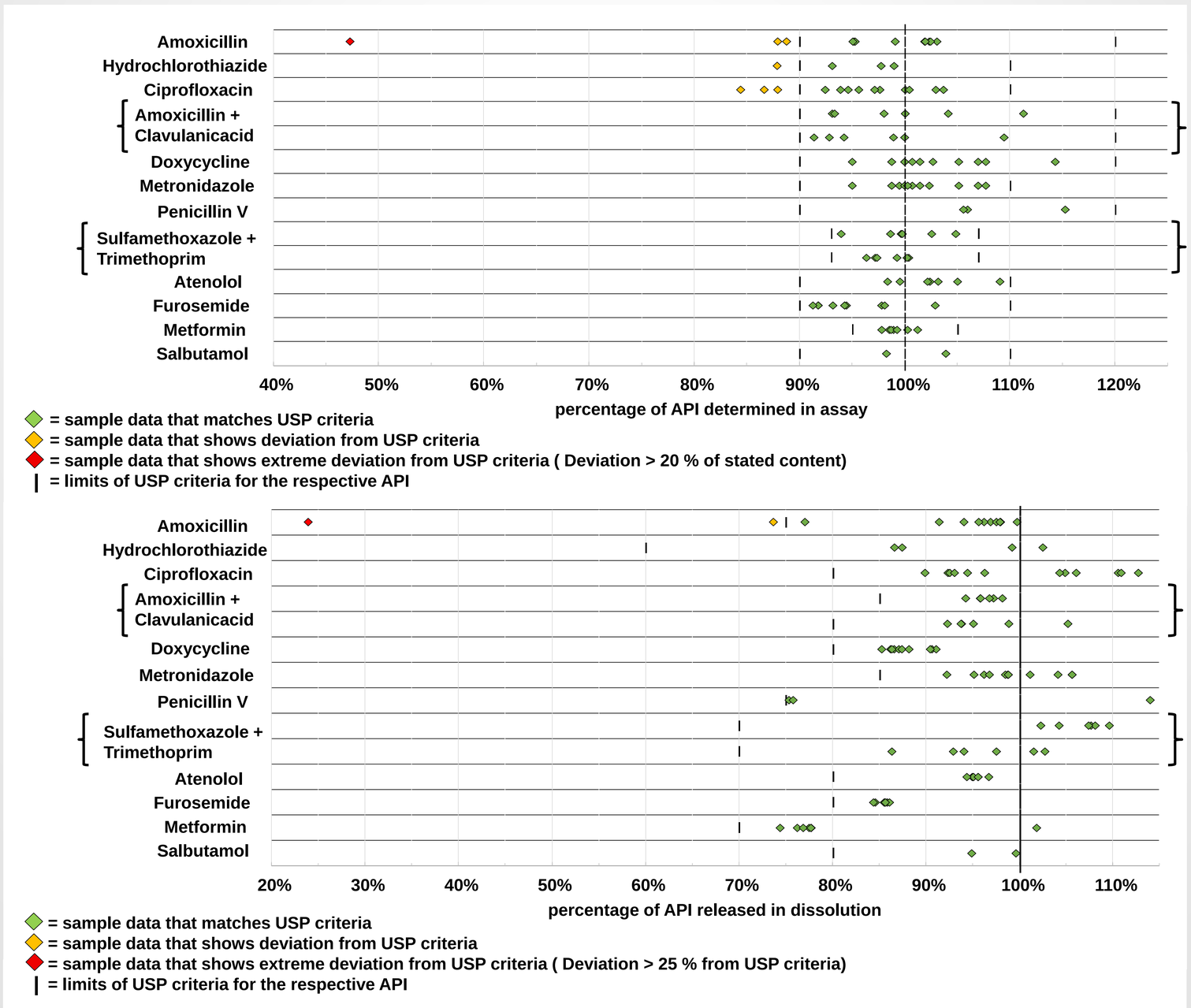
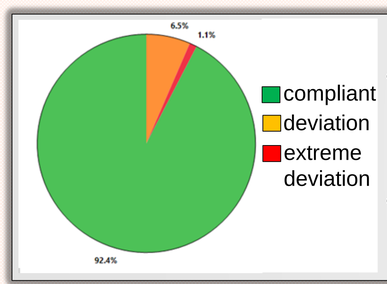


Figure 3: Results of the assay and dissolution tests peformed

Conclusion



- 92% of the medicines samples from Togo complied with the USP 39 specifications, 7% showed moderate deviations and 1% showed even extreme deviations
- substandard medicines occured in antibiotics and medicines for non-communicable disease

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Development and optimization of an immunochemical assay particularly for potency testing of TBEV vaccine

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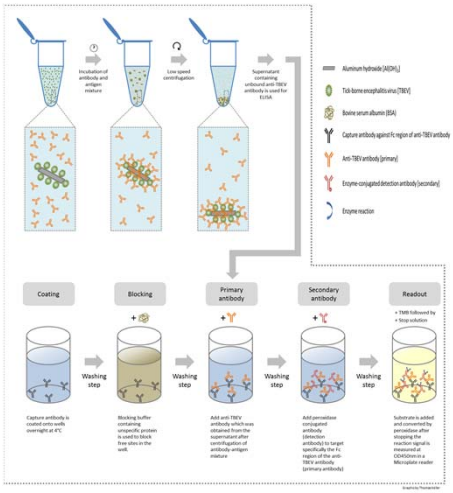
BACKGROUND and AIM

Tick-borne encephalitis virus (TBEV), a member of the family of Flaviviridae, causes tick-borne encephalitis (TBE). TBE is a human viral infectious disease involving the central nervous system (CNS). TBEV vaccines are used as protection against TBE. Each vaccine contains inactivated, whole TBE virus particles adjuvanted with aluminum hydroxide. Batch release testing of TBEV vaccines still depends on in vivo methods. Especially the potency assay includes a lethal challenge of vaccinated mice. To bypass the use of animals alternative methods are necessary. Vac2Vac/AGES will focus on development and optimization of an indirect Enzyme-linked Immunosorbent Assay (ELISA) particularly for potency testing of TBEV vaccine that can be used to measure consistency of antigen content and quality. An important and critical step is the selection of specific antibodies against the antigen (e.g. inactivated TBE virus) that recognizes relevant functional or structural epitopes on the antigen in the presence of aluminum hydroxide. The aim of this project is to develop an ELISA method to replace animal testing for TBEV vaccine batch release in the Official Control Authority Batch Release (OCABR) procedure and in the European Pharmacopoeia.

METHODS and RESULTS

1 DEVELOPMENT OF AN IMMUNOLOGICAL ASSAY FOR TBEV VACCINES



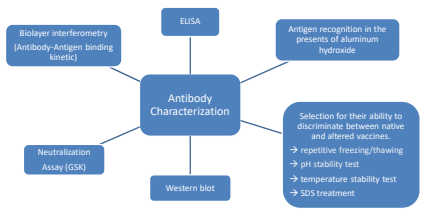
Current developed ELISA format was tested with 5 different monoclonal anti-TBEV antibodies (from GSK). Based on the experiences of GSK and on our own expertise the following format looks promising:

(1) Incubation of vaccine and antibody.

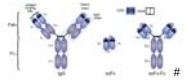
(2) Removal of vaccine and Antibody complexes by low speed centrifugation (aluminum hydroxide adjuvanted).

(3) Measurement of the unbound antibody content in the supernatant by ELISA: The more antibody (higher signal), the less/degraded antigen

2 CHARACTERIZATION OF 5 ANTI-TBEV ANTIBODIES

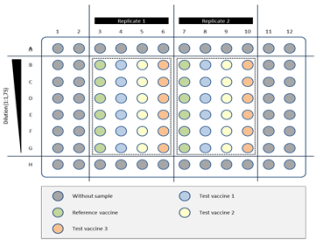


Nr.	Type	Antibody format	Species	
1	Hybridoma	IgG	FC mice	VH/VL mice
2	Phage display	scFv-IgG	FC mice	VH/VL human
3	Phage display	scFv-IgG	FC mice	VH/VL human
4	Phage display	scFv-IgG	FC mice	VH/VL human
5	Phage display	scFv-IgG	FC mice	VH/VL human



All 5 tested anti-TBEV antibodies bind to the antigen in the presence of aluminum adjuvant and have neutralizing abilities (GSK). Long- and short-term temperature stability studies as well as pH stability studies were used to analyze the capability of the antibodies to recognize artificial induced altered samples. Antibody 5 showed the most sensitive reaction on all tested induced alterations (structure indicating properties) and BioLayer interferometry (BLI) identifies antibody 5 as highly affine.

3 OVERVIEW OF THE CURRENT ELISA PLATE LAYOUT

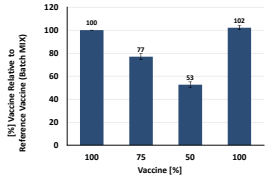


- 2 replicates of each sample /plate
- 6 vaccine serial dilutions
- 1 reference vaccine / plate
- 3 test vaccines / plate
- 2 independent plates

Analysis:
CombiStats™ (EDQM)

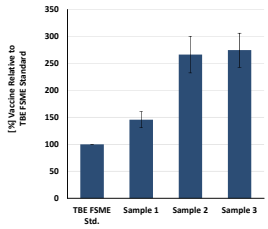
After analysis of several different plate layouts, the layout shown above was selected for evaluation specifically of linearity and accuracy. Six serial dilutions (1:1.75) of samples and references were prepared. For analyzing the relative potency of one batch of TBEV vaccine, 4 independent dilutions were prepared on two independent plates.

4 TESTING THE ACCURACY OF THE CURRENT ELISA FORMAT



Altered samples were artificially produced by diluting vaccine samples to 75% and 50% with TBEV excipient buffer to compare if the measured values correlate with the expected ones. Additionally, an undiluted vaccine was measured as third sample. The relative potency of a pre-diluted vaccine against the undiluted Vaccine (=100%) was then calculated with CombiStats.

5 BATCH COMPARISON: ANALYSIS OF DIFFERENT TBEV BATCHES



Verification of the current ELISA protocol by testing three different batches against the officially licensed reference standard. Consecutively, the relative potency was calculated.

TBEV reference standard was determined being lower than the consistently manufactured batches as observed by animal challenge test during routine batch release.

Fresh material (sample 2+3) showed a higher relative potency compared to older (expired, sample 1) material.

CONCLUSIONS and PERSPECTIVES

- Several immunochemical assays were tested to select the most suitable method for potency testing of TBEV vaccine e.g. DAFIA, Direct ELISA, Indirect ELISA, Flow cytometry.
- Antibody 5 was selected as most suitable antibody for an ELISA for potency testing of TBEV vaccine due to structure indicating properties.
- The ELISA is able to distinguish native vaccine and forced degraded (altered) material in a signal-dependent manner.
- Measurement of unbound antibodies allows quantification of the antigen.
- Evaluate repeatability, intermediate precision, accuracy and linearity.
- Method transfer is ongoing and an inter-laboratory study is planned for end of 2019.

supported by:

→ ***Anti-TBEV antibodies and technical support provided by GSK***

References (#):

Keller T, et al. (2015) Selection of scFv Antibody Fragments Binding to Human Blood versus Lymphatic Endothelial Surface Antigens by Direct Cell Phage Display. PLoS ONE 10(5): e0127169. https://doi.org/10.1371/journal.pone.0127169

The VAC2VAC project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement N- 115924. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA.

Protecting end-users of Reference Standards

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Introduction

Professionals handling very potent substances, especially cytotoxic products, may be exposed to a risk linked to their intrinsic properties [1].

Falck *and al* [2] were among the first to show an increase in the mutagenic activity of the urine of nurses in contact with these substances. This result has since been confirmed by several teams that have detected traces of anti-cancer drugs in the urine of professionals and/or external contamination of finished products [3] .

A wide variety of substances are used during the manufacture of reference standards. These can range from sorbitol, which does not have negative health effects, to methotrexate and other hazardous and/or carcinogenic, mutagenic and reprotoxic (CMR) active pharmaceutical substances.

Despite precautions taken during the manufacture of reference standards, it cannot be excluded that some residual quantity of the substance may be present on the outer surfaces of the vials. This places end-uses at potential risk, particularly since the main route of passage of cytotoxics into the body is through the skin [4].

The EDQM has conducted several studies to control and minimise the risk of external contamination and has implemented multiple measures in its manufacturing process to better protect end-users.

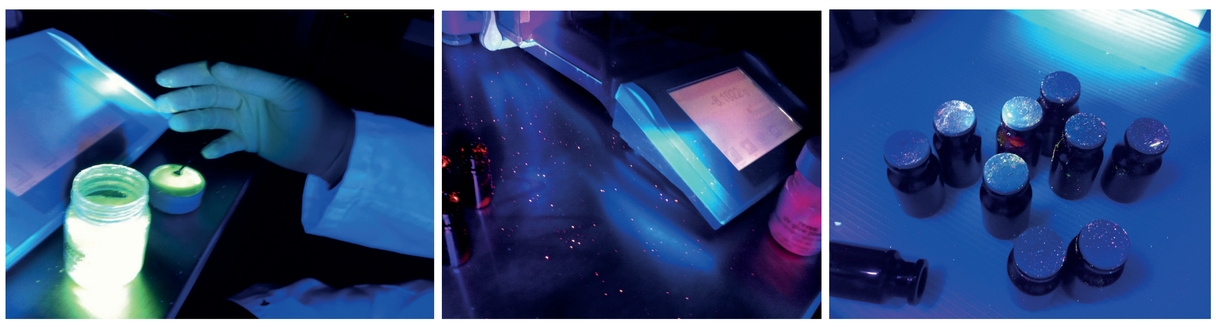
Several measures to reduce contamination

External contamination may be due to several factors. Each factor was assessed and appropriate measures were implemented in the manufacturing section to minimise risks of contamination:

- Specific design of premises to avoid cross-contamination
- General protective measures to reduce the risk for operators and environmental exposure during direct handling of products
- Personal protective equipment designed to protect the health of operators and avoid cross-contamination.
- Cleaning protocols for both premises and equipment, together with practical training and qualification of staff to guarantee that the procedures are correctly implemented and effective.
- A post-manufacturing vial decontamination protocol to eliminate any possible residues on the external surfaces of vials and ensure the safety of end-users.

Need for decontamination

Despite all the precautions taken during filling, work stations and vials may nonetheless be contaminated. This has been demonstrated with a fluorescent marker powder. The following pictures illustrate this contamination after filling of the marker.



Validation of washing

Several validation parameters and specific tests have been developed for the qualification of the equipment and validation of the washing cycles (e.g. control of temperature, volume of detergent used during the washing cycles, etc.).

Two tests have been used to assess the efficacy of the cleaning:

- **Soil test:** three sets of 35 vials of each type are stained with Soil Test (maintained in liquid phase) and then subjected to the cleaning process. These vials are used as visual evidence, in particular to check that the detergent solution is sufficiently diffused over the entire vial when it passes under the nozzles.
- **Naproxen test:** several series of vials are sprinkled with Naxopren and then subjected to the cleaning process. These tests are used to verify the reduction or even elimination of residual powder that can be present on the vials during the filling process.
  Conformity: the minimum basal value is the average of the blanks before contamination. The target criterion to be achieved is an average reduction of at least 90% in the average contamination level observed after filling and, if possible, to obtain an average contamination level of less than or equal to the minimum basal value.

Criterion	Expected value (ng/cm^2)	Mean measured value (ng/cm2)	Results
Minimum basal value		47.31	
Average contamination level after filling		782.74	
Average residual contamination level after washing	78.27	27.29	OK

Conclusion

A wide variety of substances are used during the manufacture of reference standards, many of which are considered to present a health hazard. It has been demonstrated that despite precautions taken during manufacturing, some contamination of the outer surfaces of vials may occur, leading to potential exposure of end-users.

The EDQM has therefore taken steps to ensure the safety of individuals coming into contact with its reference standards. One of the main steps has been the introduction of a post-manufacturing vial decontamination protocol to eliminate, insofar as is possible, any possible residues on the external surfaces of vials.

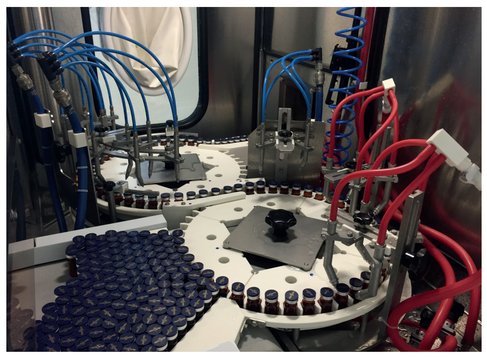
Both the qualification of the newly introduced equipment and the validation of cycles have been demonstrated to be efficient with regard to reducing the risk of exposure of end-users.

Decontamination of vials: washing the vials

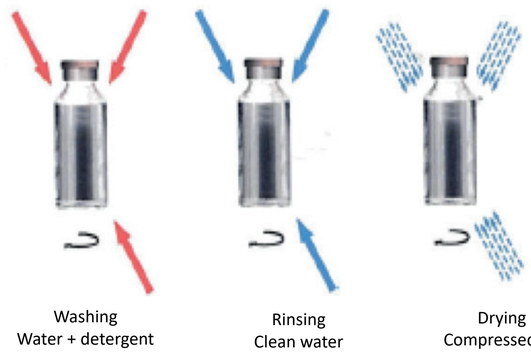
After filling, the closed vials are transferred to a special washing machine that is designed to remove any active pharmaceutical substance residues potentially present that could present a risk for end-users.

The equipment

Vials are transferred to a conveyer belt, then to star wheels. They are then washed with a detergent, rinsed with water and dried with cold compressed air.



View of the water nozzles



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[2] Falck K, Gröhn P, Sorsa M, Vainio H, Heinonen E, Holsti LR, Mutagenicity in urine of nurses handling cytostatic drugs, *Lancet*; 1, 1250-1, (1979)

[3] P. Monfort, S. Georget, J. Vigneron, B. Demore, A. Perrin, M.-A. Hoffman, M. Hoffman, Contamination chimique du conditionnement primaire de médicaments cytotoxiques, *Journal de Pharmacie Clinique*, Vol. 20, N°2, 87, (2001)

[4] Sessink PJ, VanDen Broek PH, Bos RP, Urinary cyclophosphamide excretion in rats after intratracheal, dermal, oral and intravenous administration of cyclophosphamide, *J. Appl Toxicol*; 11(2), 125-8, (1991)

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The European Paediatric Formulary (PaedForm) – first monographs launched

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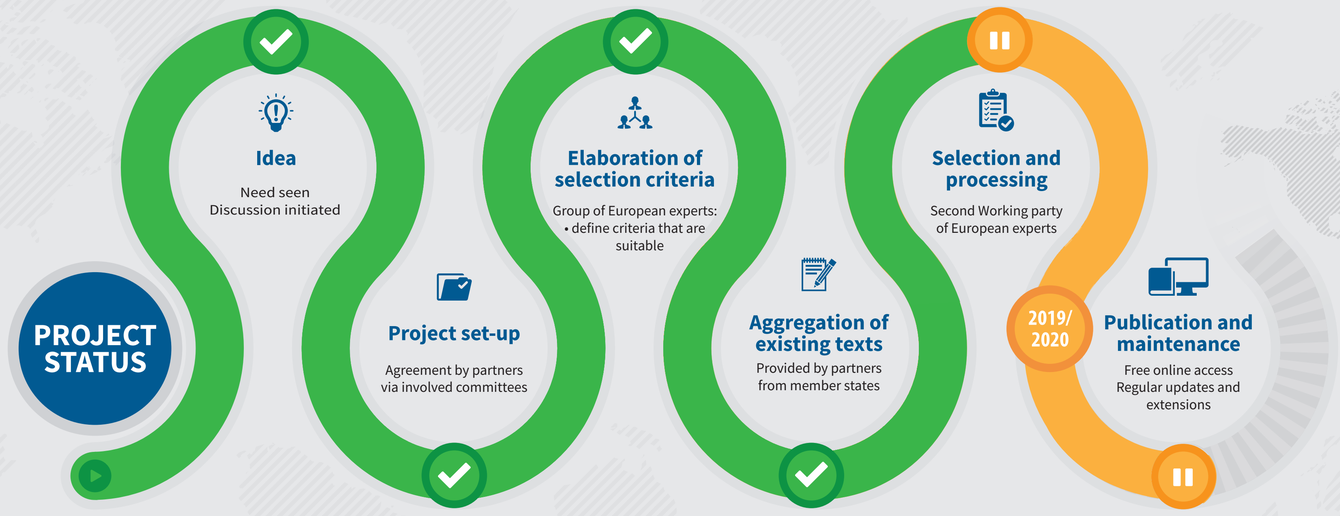
INTRODUCTION

The formulary will collect together on a pan-European level formulations for extemporaneous preparation currently described in national formularies, or those which are well-established in European countries, and make them freely available.
Pharmacists and clinicians will be provided with formulations of appropriate quality to allow preparation when no licensed alternative is available on the market.
The project has been launched by the European Committee on Pharmaceuticals and Pharmaceutical Care (CD-P-PH) and the European Pharmacopoeia Commission (for which the EDQM provides the Scientific Secretariat).



CRITERIA FOR SELECTION

Positive assessment for use of active substance including:

- is **of therapeutic benefit**
- relevant to current practice
- **no safety signals**

In addition:

- **no authorised products** with age-appropriate dosage form available
- **excipients not harmful**
- all **excipients** necessary and **suitable** for their intended use
- active substance and excipients **comply with Ph. Eur.**
- **evidence on stability**

SELECTED PARTS OF A PAEDFORM MONOGRAPH

Qualitative and quantitative composition

Supplementary information for the compounding pharmacist, e.g. on content of excipients with possible concerns, considerations during development.

Preparation **process** described in a way that ensures **reproducibility**

Suitable tests for **in-process controls**

All available quality control tests given: decision on which to apply for extemporaneous or stock preparation made by compounding pharmacist.

Expiry time: given as in-use and before first opening, if known. Storage conditions and container system are reported.

Source and list of **additional** scientific **references**

DRAFT OF FIRST PILOT PAEDFORM MONOGRAPH

Hydrochlorothiazide 0.5 mg/mL Oral Solution

Route of administration: oral

DEFINITION
1 mL of Hydrochlorothiazide 0.5 mg/mL Oral Solution contains 0.5 mg of Hydrochlorothiazide (Ph. Eur.).
Content: 90.0 to 110.0 (0.45 to 0.55 mg/mL) per cent of the hydrochlorothiazide label claim.
Content of methyl parahydroxybenzoate: 90.0 to 110.0 per cent of the nominal content. […]

QUALITATIVE AND QUANTITATIVE COMPOSITION

Composition per 107.7 g (corresponding to 100.0 mL):

Hydrochlorothiazide (Ph. Eur. 0394)	0.050 g
Methyl parahydroxybenzoate	0.077 g
Propylene glycol	0.275 g
Citric acid monohydrate	0.870 g
Disodium phosphate dodecahydrate	0.835 g
Natural orange flavour	0.052 g
Sucrose	20.16 g
Purified water	85.4 g

ADDITIONAL INFORMATION
The formulation contains at least 2.75 mg of propylene glycol per mL. Small additional quantities may be present in natural orange flavour. The formulation contains 0.77 mg of methyl parahydroxybenzoate per mL. The formulation contains 1.07 mg/mL of sodium, corresponding to 47 µmol/mL.
The concentration of methyl parahydroxybenzoate is lower than 0.1 per cent because of the risk of precipitation; the storage period is limited to 6 months. The concentration of 0.5 mg/mL hydrochlorothiazide is the maximum concentration that gives a clear liquid without additional co-solvent. Grinding hydrochlorothiazide powder before preparation may shorten the dissolution time during preparation.
Hydrochlorothiazide hydrolysis during storage is pH-dependent; it forms salamide and formaldehyde. The maximum concentration of salamide is 6 per cent. At this limit, the amount of formaldehyde formed is acceptable compared to the exposure from food intake. […] The pH value is set to the acidic target because of the more acceptable taste and reduced hydrolysis. […]

PRODUCTION
Ingredients
[…]
Production steps
1. Dissolve citric acid monohydrate and disodium phosphate in 70 g of purified water.
2. Mix sirupus simplex FNA with this solution.
3. Dissolve hydrochlorothiazide in this mixture by heating the solution to 60 °C and stirring.
4. Add methyl parahydroxybenzoate concentrated solution to the warm solution.
5. Cool to room temperature.
6. Add natural orange flavour.
7. Add purified water to reach a final mass of 107.7 g and mix.

In-Process controls
Appearance: Clear, colourless liquid. Visual observation after steps 3 and 5. […]

PACKAGING […]

QUALITY CONTROL
Appearance: clear, colourless liquid.
Identification: see Assay.
pH (Ph. Eur. 2.2.3): 2.5-3.5.
Microbiological purity (Ph. Eur. 5.1.4). Complies.
Related substances. Liquid chromatography (Ph. Eur. 2.2.29). […]
Assay. Liquid chromatography (Ph. Eur. 2.2.29) as described in the test for related substances.
Methyl parahydroxybenzoate. Liquid chromatography (Ph. Eur. 2.2.29) as described […]

STORAGE […]

REFERENCES
Source: Formulary of Dutch Pharmacists (FNA). […]

METHODS AND ONGOING WORK

- Dedicated working party with **17 experts** from hospital pharmacies, academia and national authorities from **14 countries.**
- The experts started with **prioritisation** based on paediatric needs and criteria for the formulary adopted by the CD-P-PH.
- The **formulations** available for a specific preparation **of high priority will be screened** and a final selection will be made.
- **Draft texts** will be made available by the EDQM **for public consultation.**
- The **online formulary** will be **subsequently extended** and regularly reviewed.

RESULTS:

- The work on prioritisation is nearly complete.
- The first two draft monographs (**Hydrochlorothiazide** 0.5 mg/mL oral solution and **Sotalol** 20 mg/mL oral solution) have already been published for public enquiry at https://paedform.edqm.eu.
- An introduction and general principles are also published.
- 8 further monographs have been added to the work programme:
  - **Furosemide** 2 mg/mL oral solution,
  - **Azathioprine** oral suspension,
  - **Isoniazid** 10 mg/mL oral solution,
  - **Oxybutynin hydrochloride** 0.25 mg/mL intravesical solution,
  - **Ranitidine** 15 mg/mL oral solution,
  - **Omeprazole** oral suspension
  - **Chloral hydrate** oral solution
  - monograph on an **oral vehicle**.
- Future draft and finalised texts will be available at https://paedform.edqm.eu.

SUMMARY

While this project is still young, it reached a significant milestone with the public consultation for the first monographs at the end of 2018. With the input of all stakeholders, the formulary can in future fulfil its aims: to be an easily accessible, science-based online tool with a collection of child-appropriate formulations that supports its users by promoting the health of children in all countries where no licensed medicine is available. Publication of the first finalised texts is planned for the end of 2019 after adoption by the European Pharmacopoeia Commission and the CD-P-PH at **https://paedform.edqm.eu.**

ACKNOWLEDGEMENTS

The work on this project would not be possible without the support of the member states and the work of the nominated experts. Special thanks go to the Chair of the Working Party, Prof. Jörg Breitkreutz (University of Düsseldorf) and Filippo Capasso.

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COMPOUNDING, EXTEMPORANEOUS PREPARE OR RECONSTITUTION OF THE RADIOPHARMACEUTICALS

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[2] **Tallinn University of Technology, Estonia**

Background

In the recent developments regarding conceptually new medicines for human use, the manufacture of radiopharmaceuticals is regulated in a variety of ways even in the European Union member states. The applicable guidelines and directives view the preparing of such medicines in a large way similarly as manufacture or compounding.

Traditional compounding refers to the „pharmacy compounding“ or „extemporaneous compounding“, which is globally known as the practice of essential part of pharmacist' competency. The World Health Organisation (WHO) Technical Report Series refers that „Compounded preparations involve the preparation, mixing, assembling, altering, packaging and labelling of a medicine or drug-delivery device, in accordance with a licensed practitioner’s prescription, medication order or initiative based on the relationship between the practitioner, patient, pharmacist and compounder in the course of professional practice“.

Radiopharmaceuticals are unique medicinal formulations containing radioisotopes which are used in major clinical areas for diagnosis and/or therapy. Preparation of radiopharmaceuticals used as diagnostic investigational medicinal products is carried out in hospitals, health centres or clinics, by pharmacists or other persons legally authorised in the Member State concerned to carry out such process, and if the investigational medicinal products are intended to be used exclusively in hospitals. It follows that legal requirements for products that are created in accordance with the magistral formula and the officinal formula could be different of those that are imposed on medicinal products for human use which are prepared industrially or manufactured by a method involving an industrial process. Whereas The Magistral Formula is „any medicinal product prepared extemporaneously in a pharmacy (and dispensed immediately after preparation and not kept in stock) in accordance with a medical prescription for an individual patient.” and The Officinal Formula is “any medical product which is prepared in a pharmacy in accordance with the prescriptions of a pharmacopoeia. It is maintained in stock and it is intended to be supplied directly to the patients served by the pharmacy in question.”

In this respect, it is important, that the requirements for radiopharmaceutical preparation differ remarkably from the requirements for preparing biologicals, cytotoxic preparation for cancer treatments etc. due to radioactivity safety standards. Thus, it is suddenly interpreted as reconstitution of parental medicines done outside of hospital pharmacy.

The Resolution CM/Res(2016)2 on good reconstitution practices in health care establishments for medicinal products for parenteral use is remarkable work for the safety of patients done by the Council of Europe. The consensus at the level of regulations applying on the preparation techniques of new kind of medications is needed especially in regard to the increase of cross-border healthcare.

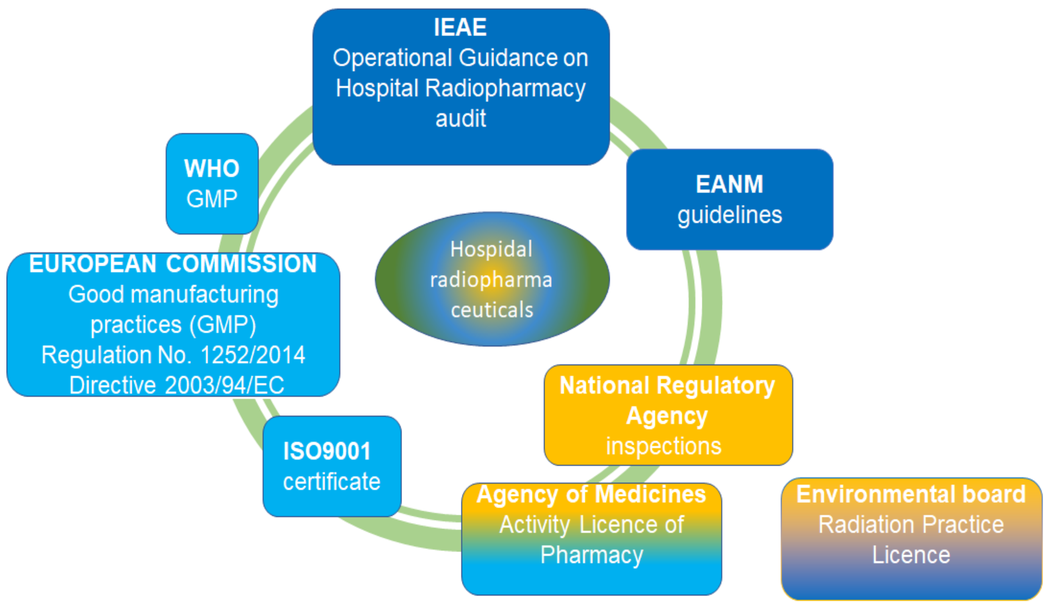

Figure 1. The international and national authorities guiding the handling of radiopharmaceuticals.

The European Statements of Hospital Pharmacy express commonly agreed on objectives and were subject of open consultation with national hospital pharmacy associations, European patient groups, doctors and nursing organisations. According to the provision 3.2. „ national hospital pharmacy associations, European patient groups, doctors and nursing organisations. According to provision 3.2. „Medicines that require manufacture or compounding must be produced by a hospital pharmacy, or outsourced under the responsibility of the hospital pharmacist.“

Depending on national regulations, extemporaneous preparations can be used in the hospital setting in case of an imminent shortage of risk, emergencies or high prices. A recent practice example (November 2018) comes from the Amsterdam Medical Centre in the Netherlands. In this instance, the Dutch government approved the legal possibility of large-scale production by hospital pharmacists, acknowledged as compounding experts, of medicines licensed nationally or by the European Medicines Agency (EMA) because of unreasonably high prices for a specific drug.

In Estonia, there is no specific regulation on radiopharmaceuticals. Estonian Medicinal Products Act refers that the „Act applies to radiopharmaceuticals in so far as legislation concerning radioactive substances does not provide otherwise“. In addition, it is provided „ A manufacturing authorisation is not mandatory where the activities … are carried out by the holder of a general pharmacy, hospital pharmacy or veterinary pharmacy authorisation (hereinafter *pharmacy service authorisation*) either for the preparation of medicinal products as magistral formulae in accordance with a medical prescription, officinal formulae or for dividing-up into retail packaging for dispensing. … Medicinal products prepared as magistral formulae are medicinal products prepared in a pharmacy in accordance with a medical prescription or order form.“

On the other hand, Estonian Radiation Act and its derivative regulative documents, do not contain any provisions about the preparation of radiopharmaceuticals, except for radiation safety.

Hospital unit handling radiopharmaceuticals should have Radiation Practice Licence. On the other hand, hospital pharmacy does not usually have the Radiation Practice Licence.

Is it reconstitution of the medicine preparation if the radiopharmaceutical is prepared for therapy outside of the hospital pharmacy?

A European Court of Justice (ECJ) ruling (C-544/13 and C-545/13 Abcur AB v Apoteket, 2015) found that it is the Member States’ prerogative to establish regulations vis-à-vis pharmacy preparations, including the need to obtain a national authorisation. Although there is a high degree of variation in the compounding policies across Member States, allowing extemporaneous pharmacy preparations when there is no licensed medicinal product available on the market is a common practice in order to ensure patient care.

***Compounding** should not be confused with the practice of **reconstitution** which involves “manipulation to enable the use or administration of a medicinal product for products with a marketing authorisation issued by any competent medicines regulatory authority [that] is carried out in accordance with the instructions given in the summary of product characteristics (SmPC) or the package leaflet” (Council of Europe Resolution CM/Res(2016)2 on good reconstitution practices in health care establishments for medicinal products for parenteral use). Reconstitution can neither be seen as being industrially manufactured, nor as an extemporaneous pharmacy preparation because the starting material is a licensed, market authorised medicinal product, not a raw material described in a pharmacopoeia monograph. Additionally, preparation occurs more often in clinical areas inside healthcare establishments, rather than in hospital pharmacies.*

Figure 2. There is a large variety of products in the field of nuclear radiopharmaceuticals.

According to the EU Directive, the radiopharmaceutical kit and the radionuclides of the generator (both mother and daughter radionuclides) are considered as active substances and detailed instructions for extemporaneous preparation and quality control of radiopharmaceutical should be included in the summary of the product characteristics. It should be mentioned, that those medicinal products provided by suppliers are not always authorised.

Figure 3. Aluminium breakthru kit for pertechnetate generator eluate.

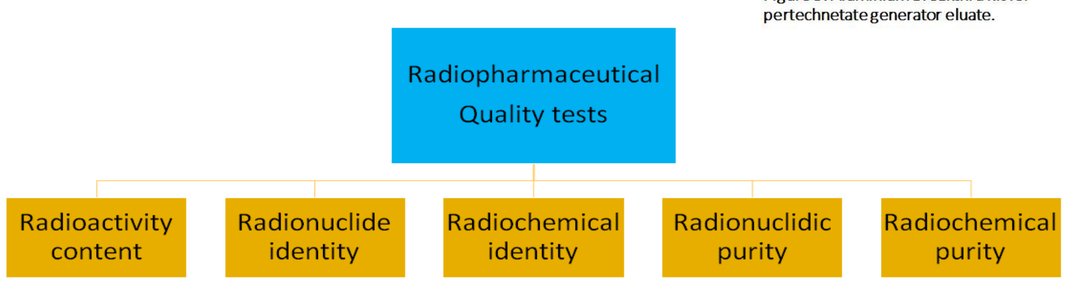

Figure 4. The Quality tests for radiopharmaceuticals. Several of them should be carried out in the hospital even the products are with a marketing authorisation.

Conclusions

- There is no harmonisation how the new generation pharmaceutical product is being handled.
- The products could be prepared following the regulations, recommendations, guidelines for compounding, manufacturing, extemporaneous preparation as a magistral formula or the officinal formula or even reconstitution.
- Cross-border healthcare and medical tourism is increasing.
- Those circumstances could pose the risk for patients, doctors, medical professionals and governing bodies.
- Harmonisation of the standards guiding the medicinal products and therapy bears of great advantage for patients as well as for whole social care segment.

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The MSD Analytical Monitoring Tool: Potentials and Challenges

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Steven Kok; Dion Luykx; Ton Swolfs

MSD Netherlands, Oss, Netherlands

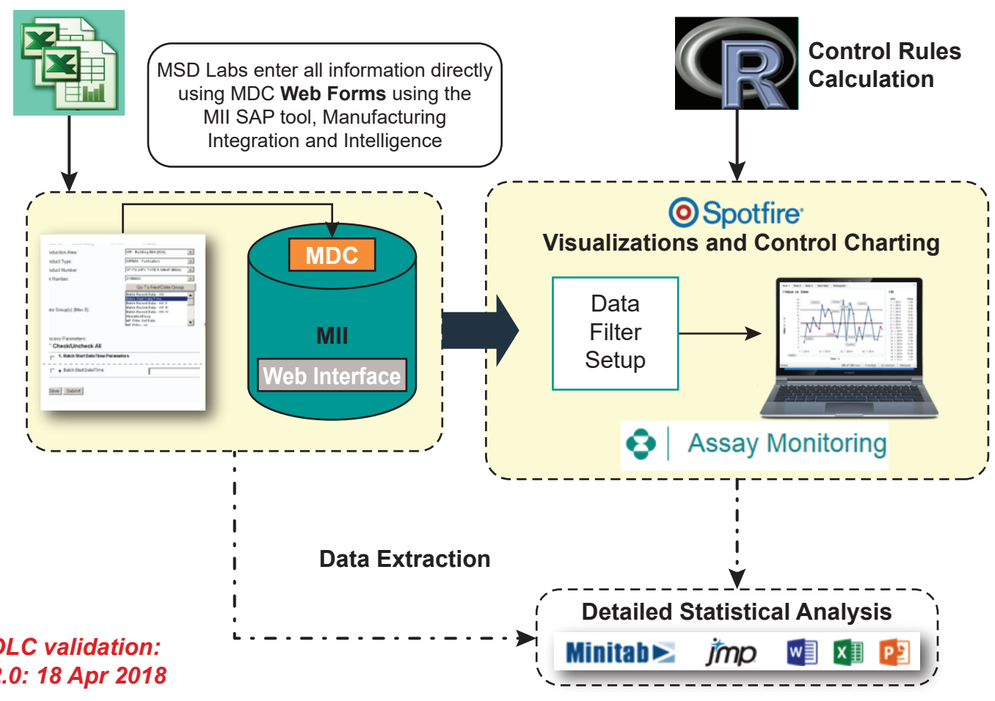
SUMMARY

The Analytical Assay Monitoring Tool (AMT) is a powerful process developed by MSD to monitor control of release methods performance with the support of a network of analytical experts. It is applied to the analytical lifecycle management of biological monoclonal antibody drug assays.

The tool consists of two parts: the software package and the experts team. It involves 11 laboratories and monitors 16 methods for each product worldwide, based on the results of Reference Standards and Control samples.

The software setup is cGMP- and SDLC-validated. It allows data entry of test results and testing parameters in real time, together with their control limits. Visual inspection using filters and statistical evaluation of the data gives valuable information to support laboratory comparisons, analytical investigation method transfers, and reference standards stability monitoring. A periodic review reveals many potentials of the AMT for analytical lifecycle management. A team of 15 analytical "method champions" supervises routinely the data input, trends, and follow-up actions. Examples are presented in the poster together with some of the challenges of the tool, such as the validation of automated data entry and the capturing of all lessons learned.

Software System Overview

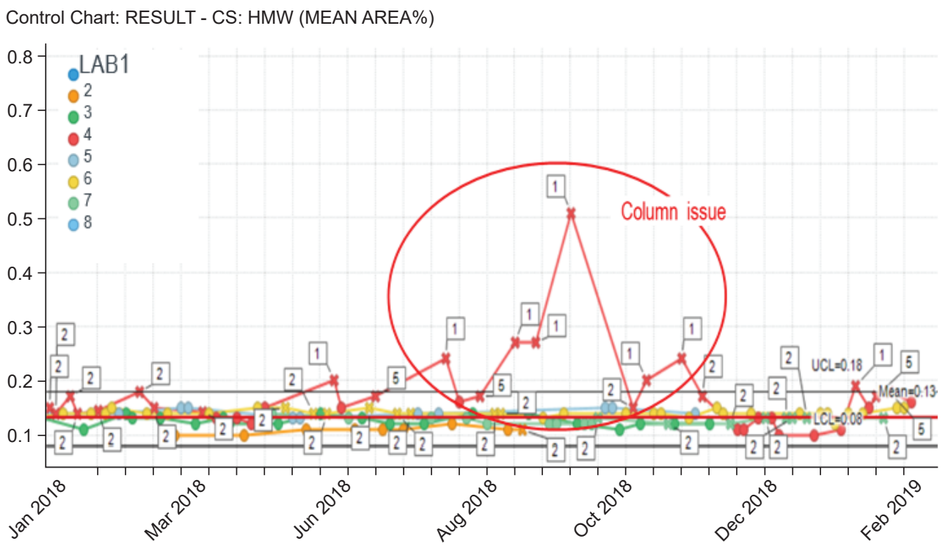


The Assay Monitoring Tool software (AMT) is a web-based intranet application based on Spotfire which extracts, visualizes, and performs basic statistical analysis on data using filters preset by the user.

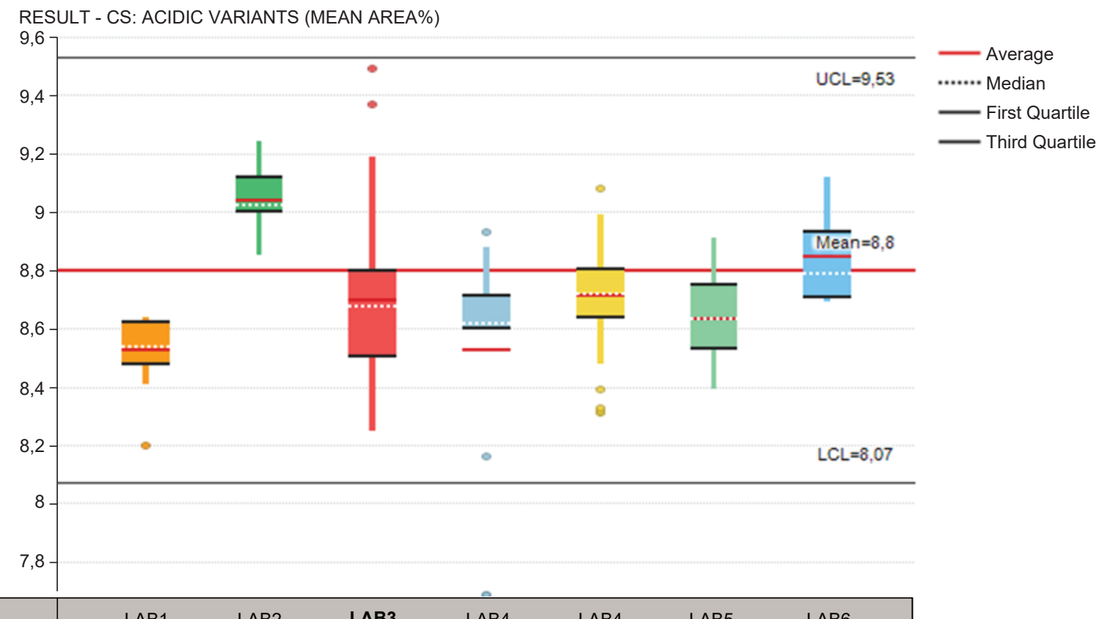
The data can be entered through a web form and extracted for further detailed statistical analysis. The layout of AMT consists of several tabs as stand-alone with predefined functionality.

AMT Functionality

A. Analytical Investigations

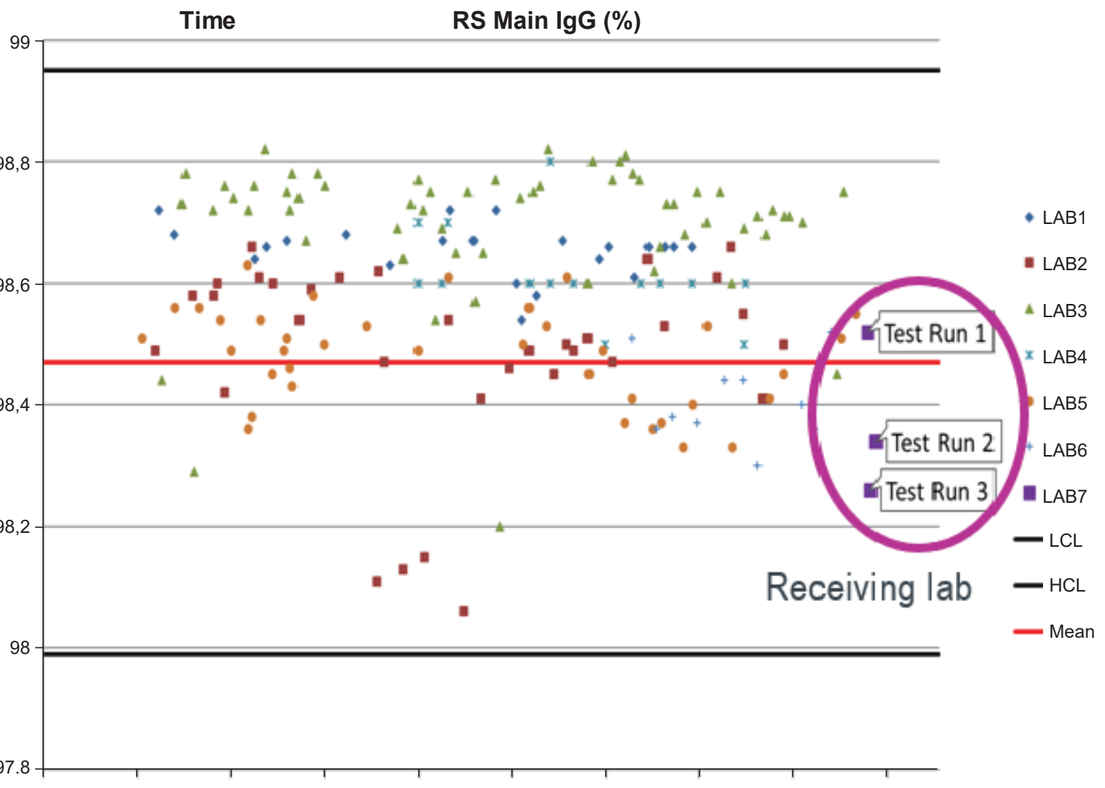


B. Studying Inter-Lab Precision



	LAB1	LAB2	LAB3	LAB4	LAB4	LAB5	LAB6
Count	24	45	66	14	48	17	4
Median	8,535	9,02	8,675	8,6175	8,72	8,63	8,79
Avg	8,5275	9,03667	8,69471	8,52436	8,71333	8,63471	8,8475
Outliers	1	0	2	4	5	0	0

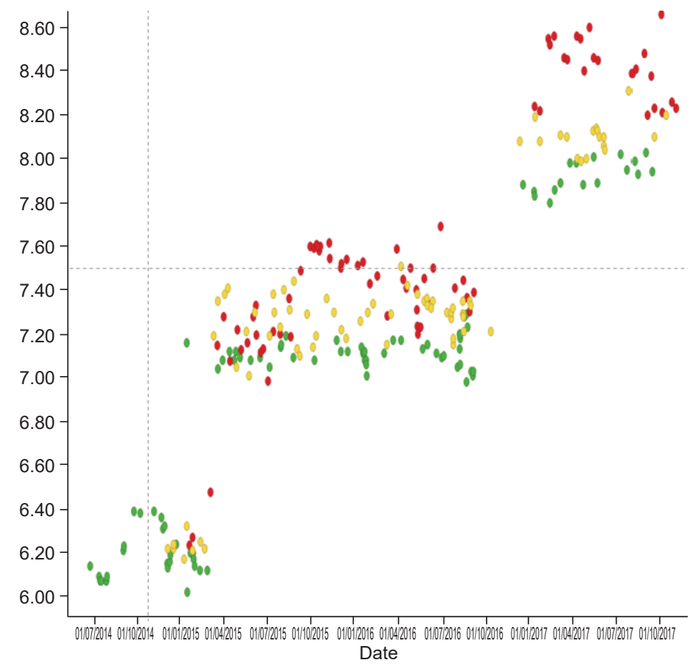
C. Evaluate Method Transfers



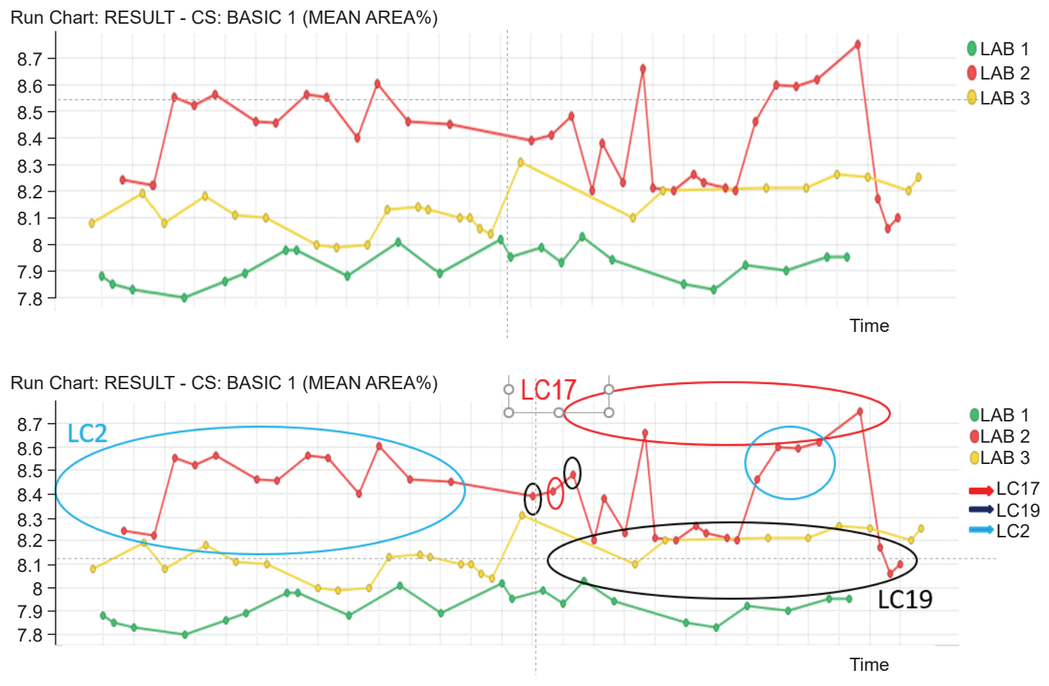
- Detect and evaluate method variability
- Help understand the one or many sources of variation
- Evaluate the impact of the variation on the results
- Control and remediate the variation and related risks

Example of a Root Cause Investigation-HPLC Assay

- Systematically higher values at an RS result are investigated, still within control limits. Information on instruments, columns, sites, and reagent lots can be retrieved easily using filters. The correlation with the data assists the investigation and reveals the root cause of an observation
- Chromatographic parameters in lab 2 are correlated with the HPLC system 17, equipped with a different fitting in the pump. All other causes were excluded. This system was put aside and the high values were eliminated

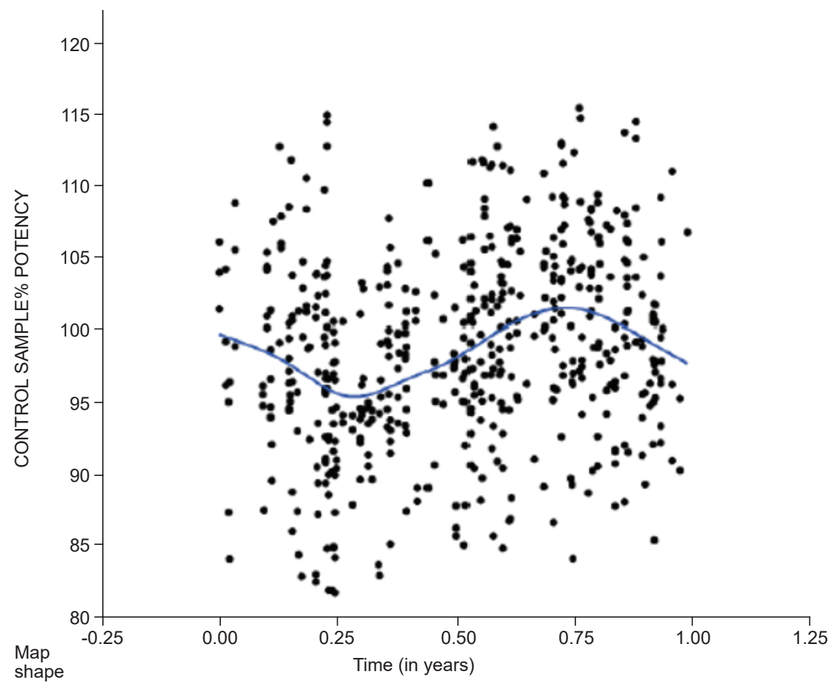


- The data from three different reference standards lots tested at three labs over time. Lab2 reports consistently higher results

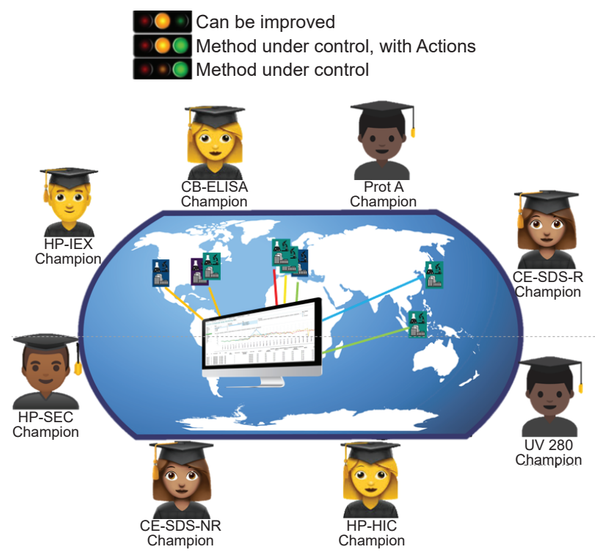


Monitoring the Reference Standard Stability

- AMT is key for Secondary Reference Standard (SRS) Lifecycle Management
- Data on >30 parameters are monitored in the intervals of the Secondary Reference Standards requalification dates
- All data are reviewed routinely for any instability trends preventing out-of-specs
- Absence of trends in the results of the quality control samples confirms indirectly the reference standard stability



- Global network of method champions reflects inclusion culture of MSD. AMT connects users and creates a platform for exchange of knowledge and for continuous improvement. Periodical AMT data review is performed based on protocol. Actions are highlighted with a traffic light code



OUR CONCLUSIONS TO DISCUSS

- The AMT is a user friendly, simple tool to evaluate analytical information. The amount of data is sufficient for the method performance evaluation without increase in complexity
- Data entry is still labor-intensive as test results are in various formats and the automated data transfer from the analytical software is not easy to validate under GMP
- Method improvements recommended by AMT are often difficult to implement worldwide due to different product filings and the complexity of change controls required
- Users wishes for improvement of the AMT in the upcoming versions: improved graphs for better presentation, all older results are captured for extended comparisons, additional statistics in selected groups of data, ie, per lab, per instrument

Presented at the International Conference on the EDQM & European Pharmacopoeia; Strasbourg, France; 19-20 June 2019.

Batch release of vaccines in Europe: from method transfer to first commercial batch available on the market. Straightforward approach that requires a proactive mind-set of the manufacturer.

Wim Van Molle, Lorenzo Tesolin, Geneviève Waeterloos, and the whole team of QVBP[1]

1. Quality of Vaccines and Blood Products, Sciensano, Brussels, Belgium

1. The principle of batch release in Europe

- ✓ Directive 2001/83/EC
- ✓ Directive 2004/27/EC
- ✓ Official Control Authority Batch Release (OCABR)
- ✓ OCABR is coordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM)
- ✓ Each batch must be released independently from manufacturer by an Official Medicines Control Laboratory (OMCL)
- ✓ Release based on analytical testing and critical document review by the OMCL in addition to release testing done by manufacturer
- ✓ Mutual recognition of OMCL batch release by all member states

Vaccine manufacturer

- ✓ Choice of OMCL (free)
- ✓ More than 1 OMCL is recommended (back-up)
- ✓ Needs to be done before submission of MA dossier
- ✓ EDQM should be notified

- ✓ **OCABR guidelines (vaccine type specific)**
- ✓ Section 1: legal framework and reference to Ph. Eur. Monographs
- ✓ Section 2: samples needed by the OMCL and tests to be performed
- ✓ Section 3: protocol submission (manufacturer's data)

Guideline exists for vaccine → Modification needed or not ?

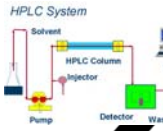
New vaccine, no guideline → CVs can propose chapter 2; but guideline will be drafted and approved by EDQM network (based on MAA file)

2. The method(s) transfer process

- ✓ Should be done in parallel with the registration procedure

Method Transfer Evaluation

- ✓ Availability of key equipment in the OMCL
- ✓ Structural differences in equipment between OMCL and manufacturer: **specifications depend on the method used !**
- ✓ New methods or adaptation of existing methods in the OMCL
- ✓ Need for extended training and technical support
- ✓ Guarantee supply of test samples (bulks, intermediates, final lot) and (non-commercial) reagents
- ✓ Shelf life of material (samples and reagents)
- ✓ Import licences for animal-derived material from outside EU
- ✓ ISO17025 at OMCLs



ISO 17025 Accredited

Vaccine manufacturer

edqm — European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE

EUROPEAN MEDICINES AGENCY — SCIENCE · MEDICINES · HEALTH

- ✓ Feedback from EDQM drafting group on OCABR guidelines
- ✓ Tests added, tests deleted
- ✓ Intermediates, bulks, final lot

- ✓ Feedback from registration process D120-D180-D210
- ✓ Impact on methods and **specifications**

3. The routine batch release

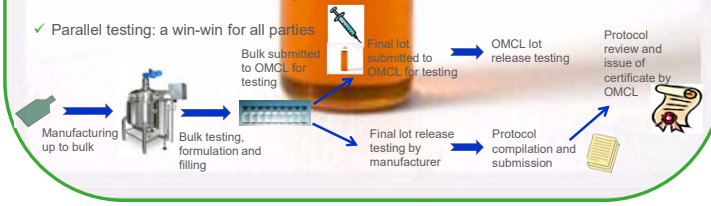
- ✓ Routine batch release for each batch can start upon MA approval
- ✓ Samples should be sent (intermediates, bulks, final container) upon availability
- ✓ OMCL release tests (depending on OCABR guidelines) are executed in parallel with manufacturer's release testing: principle of parallel testing (advantage for company: reduction in lead time and OOS risk)
- ✓ Protocol is submitted when batch is released by the manufacturer
- ✓ Batch is released by OMCL based on test results and protocol review: **procedure of 60 calendar days**.
- ✓ Compliant batch: release certificate
- ✓ Non-compliant batch: certificate of non compliance
- ✓ Withdrawal-rejection-recall

- ✓ Release for WHO market is based on protocol review only: **procedure of 21 calendar days at QVBP**
- ✓ To put a batch on the market in an EU country other than that of the releasing OMCL: release by Marketing information form (MIF): **procedure of 7 calendar days**.

Vaccine manufacturer

- ✓ A good management for sample and reagent shipment must be maintained: change in reagents, standards, controls must be anticipated in time **(validation and bridging studies)**
- ✓ Specifications are influenced by the analytical method chosen: differences between manufacturer and OMCL should be avoided but are possible
- ✓ Any approved variations having impact on testing and specifications must be communicated to the releasing OMCL.

✓ Parallel testing: a win-win for all parties

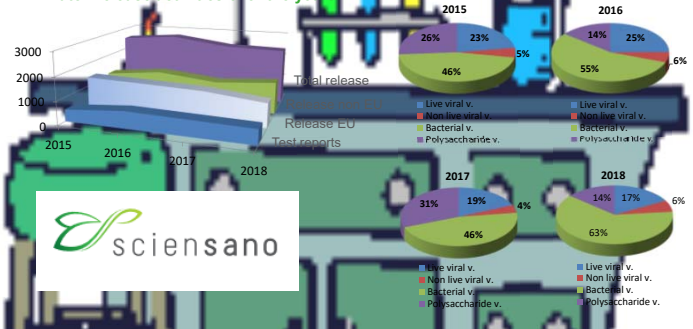


4. The batch release activities of Quality of Vaccines and Blood Products at Sciensano

Types of vaccines routinely tested by QVBP (Sciensano)

- ✓ Live attenuated viral vaccines: M(umps) M(easles) R(ubella), MMR-V(aricella), Oral poliovirus (OPV), Rotavirus, Varicella
- ✓ Inactivated/recombinant viral vaccines: Hepatitis A/B, Hepatitis A+B, Inactivated polio (IPV), Papilloma, Plasmodium falciparum and hepatitis B, herpes zoster
- ✓ Bacterial vaccines: D(iphteria) –T(etanus), BT-a(cellular)P(ertussis), DTPa-HepB, DTPa-IPV, DTPa-[illegible]TPa-IPV + Hib, DTPa-IPV-HepB, DTPa-IPV-HepB + Hib, DTpA-IPV-HepB-Hib, [illegible]WY, conjugated M[illegible]ococcal, Typhoid, Conjugated Streptococcal

Batch release activities over the years



sciensano

Technical competence at Quality of Vaccines and Blood Products

Activity	Test and method
Cell culture	Potency by titration, identity by antibody neutralization
Biochemistry	Appearance by visual inspection, pH, purity by SDS-PAGE, identity by immuno-diffusion tests, (free)- PS content by resorcinol, HPLC, UPLC, ICP, DMAB, MSD by HPLC, endotoxin content by LAL, total protein content by Lowry, total protein content by Biuret, total protein content by UV, protein composition by electrophoresis
ELISA	Antigen content, potency by serology AB response
In Vivo	Potency by vaccination and challenge, immunization for serology testing
Molecular Biology	PCR, RT-PCR

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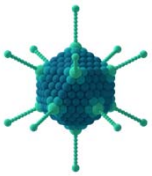
.be

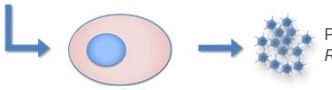
platform: Ad26/PER.C6®

Mark van Ooij PhD, Michel Duchene PhD, Paul Ives PhD, Harold Backus PhD, Dirk Redlich PhD

1. Wild-type Adenovirus

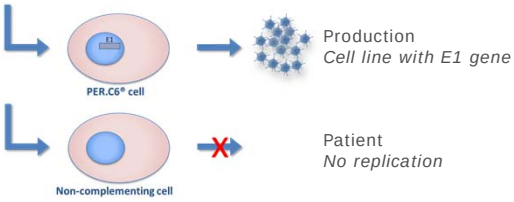


- DNA containing virus
- Relatively harmless (healthy individuals)
  - Symptoms such as common cold[1]
  - Rarely serious illness[1]

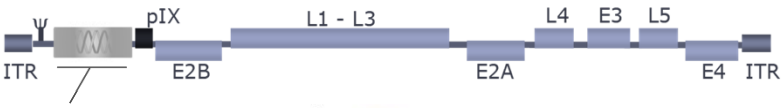


2. Janssen's AdVac®

- Adenovirus as *vector* to deliver transgene
- E1 and E3 genes removed
  - Space for transgene
  - Non-replicating (= Increase product safety)



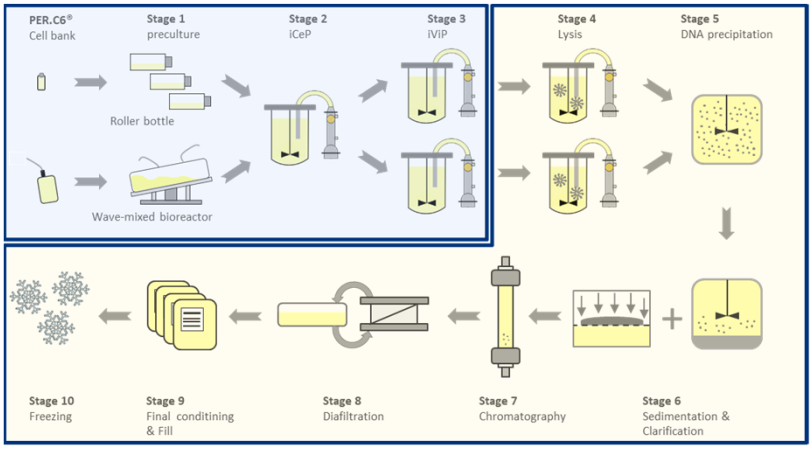
3. Vector construction



Insert coding for disease specific antigen; insert size ~3kb

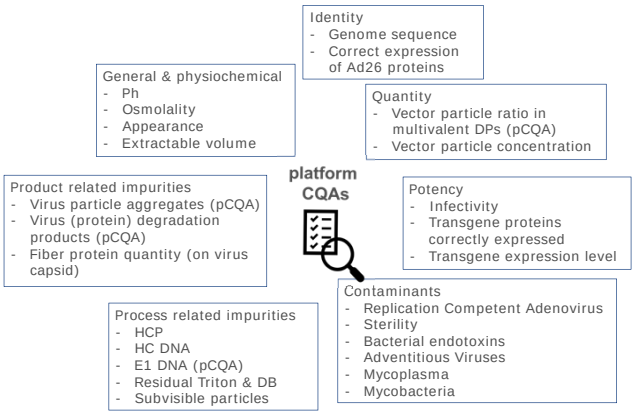
- Defined platform critical quality attributes
- Harmonized platform control strategies, including specifications
- Use platform / generic analytical methods
- Product understanding increased, including definition of routes of degradation
- Detailed assessment of all product and process differences, or define **proven acceptable ranges**

4. Generic Drug Substance production platform

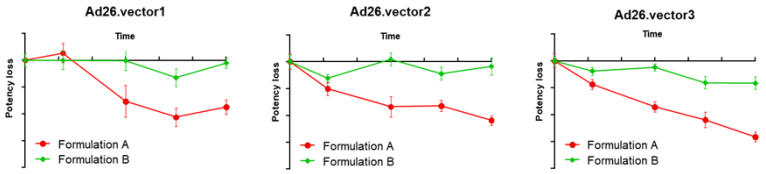


- High yields production system
- From sequence selection to First in Human (FIH) in less than 12 months

5. Platform Critical Quality Attributes



6. Drug Product Platform Stability (2-8°C)



✓ DS and DP stable for multiple years at deep-frozen conditions

✓ DP shelf life of ~2 years at 2-8°C

✓ DP robust during freeze/thaw, agitation and temperature cycling stress, and during use in the clinic (Compatible with the intended supply chain)

7. Janssen Vaccines' Adenovirus pipeline

Disease	# of inserts	Clinical phase
HIV		3
Ebola (multi)		1
Ebola (mono)		3
RSV		2(Sr); 1(Jr)
Zika		1

8. Advantages/ Benefits

- Vaccination creates an immune response without disease
- Adenovirus vectors (AdVac®)
  - Recombinant: Carrying transgene; not actual pathogen
  - Replication incompetent
- AdVac® Technology that allows " Family approach" with Platform Critical Quality Attributes
- Production platform from Sequence selection to First in Human in 12 months
- DP shelf life ~2 years at 2-8°C
- Janssen Vaccines has a full pipeline

Above illustrations are an oversimplification of reality

1. https://www.cdc.gov/adenovirus/about/symptoms.html Checked July 28th 2017

janssen | PHARMACEUTICAL COMPANIES of Johnson & Johnson