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1. Principle:

This method involves the use of HS-GC-MS (single quad) for the determination of N-Nitrosodimethylamine (NDMA).

2. Scope:

This method is applicable to Active Pharmaceutical Ingredients (API) and Powdered Tablets. These samples are preliminarily screened in Scan mode, **Procedure A**. If NDMA is found to be present, samples undergo a NDMA Quantification method, outlined in **Procedure B**. If no NDMA is found samples follow **Procedure C**.

3. Apparatus:

3.1 **HS-GC-MS:** Shimadzu GC-2010Plus Gas Chromatograph (Item No. 1043B), Headspace HS-20 auto-sampler (Item No. 1043A), GC-MS QP2020 Mass Spectrometer (Item No. 1043C), or equivalent.

3.2 **Autopipettes, Gilson or equivalent**: (1,000 µL, 200 µL, 100 µL, 20 µL, 10 µL).

3.3 **Capillary GC Column:** Restek Rtx-624 (30m x 0.25 mm I.D, 1.4mm).

4. Reagents:

4.1 **Solvent:** Dimethyl sulfoxide (DMSO), (Merck SupraSolv, Dimethyl Sulfoxide for headspace gas chromatography), Catalogue no. 101900.

4.2 Helium gas (CP grade), BOC supplied or equivalent.

4.3 **Reference Standard:** NDMA (N-nitrosodimethyl amine), Restek Catalog no. 31427, Concentration **1000µg/mL** in Methanol (Reference Standard).

4.4 **Quality Control Standard:** NDMA (N-nitrosodimethyl amine), Supelco Catalog no. 48670, Concentration **200µg/mL** in Methanol (QC).

Prepared solutions are protected from light prior to analysis.

5. Calibration Standards:

5.1 **Stock standard Solution (NDMA) 200 µg/mL**

Dilute 200µL of NDMA Reference Standard (**4.3**) to 1mL in DMSO, in a volumetric flask.

5.2 **1µg/mL NDMA Standard:**

Dilute 10µL of stock standard (200µg/mL) (**5.1**) to 2mL with DMSO, in a volumetric flask.

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6. Quality Control Standard:

6.1 2µg/mL NDMA QC Solution:

Dispense 10µL of **Quality Control Standard, 200µg/mL** (**4.4**) into a headspace vial, add 1.0 mL of DMSO, cap and crimp.

7. Methods of Analyses:

7.1 Procedure A - Preliminary scan*

7.1.1 2µg/mL NDMA Check Standard:

Dispense 10µL of stock standard (200µg/mL) (**5.1**) into a headspace vial, add 1.0 mL DMSO, cap and crimp.

(A higher concentration standard may be used to achieve a greater response).

7.1.2 Sample Preparation

Place 0.20-0.25g of powdered tablet or API in a headspace vial, add 1.0 mL of DMSO, cap and crimp.

7.1.3 Analyses

Perform a full mass scan on the standard (**7.1.1**) and sample (**7.1.2**) using the GC and Headspace conditions specified in **Procedure A** (Annex I).

Establish the retention time, and confirm that the mass spectrum obtained from the eluted peak in the standard solution is that of NDMA.

Continue with Quantification of NDMA, **Procedure B (7.2)**, if NDMA is detected in the sample, or with **Procedure C (LOD/LOQ determination, 7.3)** if NDMA is not detected.

*This preliminary scan method can detect NDMA at a level of 10 µg/g. As LOD/LOQ determination (Procedure C) has a lower detection limit of 0.04 µg/g, if an appreciable amount of NDMA is observed when carrying out LOD/LOQ determination (Procedure C) (7.3) the sample in question will be reanalysed using Quantification of NDMA (Procedure B).

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7.2 Procedure B - Quantification of NDMA

7.2.1 Sample Preparation (Tablets)

Accurately weigh the same amount of sample (0.20-0.25g) into each of 4 Headspace vials.

7.2.1.1 Add 1.0mL of DMSO to Vial 1, cap and crimp.

7.2.1.2 Add 2µg (10µl of Stock standard solution (**5.1**)) of NDMA to Vial 2 and make up to 1.0mL with DMSO, cap and crimp.

7.2.1.3 Add 4µg (20µl of Stock standard solution (**5.1**)) of NDMA to Vial 3 and make up to 1.0mL with DMSO, cap and crimp.

7.2.1.4 Add 6µg (30µl of Stock standard solution (**5.1**)) of NDMA to Vial 4 and make up to 1.0mL with DMSO, cap and crimp.

7.2.1.5 Run all solutions using the GC and Headspace conditions described in **Procedure B** (Annex 1). Plot the standard addition curve and obtain the sample NDMA concentration (µg/mL) from the intercept. See calculated example in Annex 2.

7.2.2 Sample Preparation (API)

7.2.2.1 Accurately weigh the same amount of sample (0.05-0.2g) into each of 4 vials.

7.2.2.2 Add 1.0mL of DMSO to Vial 1, cap and crimp.

7.2.2.3 Add 2µg (10µl of Stock standard solution (**5.1**)) of NDMA to Vial 2 and make up to 1.0mL with DMSO, cap and crimp.

7.2.2.4 Add 4µg (20µl of Stock standard solution (**5.1**)) of NDMA to Vial 3 and make up to 1.0mL with DMSO, cap and crimp.

7.2.2.5 Add 6µg (30µl of Stock standard solution (**5.1**)) of NDMA to Vial 4 and make up to 1.0mL with DMSO, cap and crimp.

7.2.2.6 Run all solutions using the GC and Headspace conditions described in **Procedure B** (Annex 1). Plot the standard addition curve and obtain the sample NDMA concentration (µg/mL) from the intercept. See calculated example in Annex 2.

7.2.3 Include a 2.0µg/mL NDMA Check Standard **(7.1.1**), and a 2.0µg/mL NDMA QC Solution (**6.1**) with each analytical run.
The peak areas of the 2.0µg/mL standard and QC solution must correspond within ±10%.

7.2.4 The correlation coefficient (r) of the calibration curve must be ≥0.990.

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7.3 Procedure C - LOD/LOQ determination

7.3.1 Sample Preparation (API/ Finished products)

Accurately weigh in duplicate 0.5 g of each sample (powdered) into a headspace vial.

7.3.1.1 Add 1.0mL of DMSO to Vial 1, cap and crimp.

7.3.1.2 Add 20µl (0.02µg NDMA) of 1µg/mL NDMA Standard (**5.2**), and 1.0mL of DMSO to Vial 2, cap and crimp.

7.3.1.3 Run all solutions using the conditions described in **Procedure C** (Annex 1). Ensure that a peak with a S/N of at least 3 is obtained for the 0.02µg/mL NDMA standard. Examine all chromatograms and if no NDMA peak is observed, or a peak area less than that of the standard (0.02µg/mL) is observed in the samples, results are reported as <0.04µg/g.

If the sample peak area is significantly greater than that of the standard, proceed with **Procedure B (7.2).**

Annex I – GC-MS conditions – Procedures A, B and C

Annex 2 – Standard Addition Plot

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Procedure A

[Comment]

═══ Analytical Line 1 ═══
[HS-20]

Oven Temp.	:120.0 °C
Sample Line Temp.	:150.0 °C
Transfer Line Temp.	:150.0 °C
Shaking Level	:2
Multi Injection Count	:1
Pressurizing Gas Pressure	:80.0 kPa
Equilibrating Time.	:10.00 min
Pressurizing Time.	:3.00 min
Pressure Equilib.Time	:0.10 min
Load Time	:0.50 min
Load Equilib. Time	:0.10 min
Injection Time	:0.08 min
Needle Flush Time	:5.00 min
GC Cycle Time	:35.00 min
Check System Ready	:ON
Extended System Ready Check Limit	:45 min
Check GC Ready	:ON
Extended GC Ready Check Limit	:10 min
Analysis Mode	:Constant
Needle Check	:Yes
Action on Leak Check Error	:Stop
Action with No Vial on Tray	:Stop

[GC-2010]

Column Oven Temp.	:70.0 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:61.8 kPa
Total Flow	:23.4 mL/min
Column Flow	:0.97 mL/min
Linear Velocity	:36.1 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:20.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	70.0	12.00
3.00	100.0	2.00
60.00	240.0	2.00

< Ready Check Heat Unit >	
Column Oven	: Yes
HS Flow	: Yes
MS	: Yes
< Ready Check Detector(FTD/BID) >	
< Ready Check Baseline Drift >	
< Ready Check Injection Flow >	
HS Flow Carrier	: Yes
HS Flow Purge	: Yes
< Ready Check APC Flow >	
APC1	: Yes
< Ready Check Detector APC Flow >	
External Wait	:No
Equilibrium Time	:3.0 min
< Additional Flow >	
APC1 Pressure	:80.0 kPa

[GC Program]

[GCMS-QP2020]

IonSourceTemp	:250.00 °C
Interface Temp.	:250.00 °C
Solvent Cut Time	:4.00 min
Detector Gain Mode	:Absolute
Detector Gain	:0.93 kV
Threshold	:0

[MS Table]
--Group 1 - Event 1--

Start Time	:6.00min
End Time	:22.00min
ACQ Mode	:Scan
Event Time	:0.30sec
Scan Speed	:3333
Start m/z	:30.00
End m/z	:800.00

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Procedure B

[Comment]

═══ Analytical Line 1 ═══
[HS-20]

Oven Temp.	:120.0 °C
Sample Line Temp.	:150.0 °C
Transfer Line Temp.	:150.0 °C
Shaking Level	:2
Multi Injection Count	:1
Pressurizing Gas Pressure	:80.0 kPa
Equilibrating Time.	:10.00 min
Pressurizing Time.	:3.00 min
Pressure Equilib.Time	:0.10 min
Load Time	:0.50 min
Load Equilib. Time	:0.10 min
Injection Time	:0.08 min
Needle Flush Time	:5.00 min
GC Cycle Time	:25.00 min
Check System Ready	:ON
Extended System Ready Check Limit	:45 min
Check GC Ready	:ON
Extended GC Ready Check Limit	:10 min
Analysis Mode	:Constant
Needle Check	:Yes
Action on Leak Check Error	:Stop
Action with No Vial on Tray	:Stop

[GC-2010]

Column Oven Temp.	:70.0 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:61.8 kPa
Total Flow	:23.4 mL/min
Column Flow	:0.97 mL/min
Linear Velocity	:36.1 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:20.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	70.0	12.00
40.00	240.0	2.00

< Ready Check Heat Unit >	
Column Oven	: Yes
HS Flow	: Yes
MS	: Yes
< Ready Check Detector(FTD/BID) >	
< Ready Check Baseline Drift >	
< Ready Check Injection Flow >	
HS Flow Carrier	: Yes
HS Flow Purge	: Yes
< Ready Check APC Flow >	
APC1	: Yes
< Ready Check Detector APC Flow >	
External Wait	:No
Equilibrium Time	:3.0 min
< Additional Flow >	
APC1 Pressure	:80.0 kPa

[GC Program]

[GCMS-QP2020]

Micro Scan Width	:0.00 amu
IonSourceTemp	:250.00 °C
Interface Temp.	:250.00 °C
Solvent Cut Time	:4.00 min
Detector Gain Mode	:Absolute
Detector Gain	:0.93 kV

[MS Table]

--Group 1 - Event 1--	
Start Time	:8.00min
End Time	:12.00min
ACQ Mode	:SIM
Event Time	:0.30sec
Ch1-m/z	:74.00
Ch2-m/z	:42.00
Ch3-m/z	:43.00
Sample Inlet Unit	:GC

Procedure C

```
═══ Analytical Line 1 ═══
[HS-20]
Oven Temp.                                  :145.0 °C
Sample Line Temp.                           :150.0 °C
Transfer Line Temp.                         :150.0 °C
Shaking Level                               :2
Multi Injection Count                       :1
Pressurizing Gas Pressure                   :80.0 kPa
Equilibrating Time.                         :15.00 min
Pressurizing Time.                          :3.00 min
Pressure Equilib.Time                       :0.10 min
Load Time                                   :0.50 min
Load Equilib. Time                          :0.10 min
Injection Time                              :0.08 min
Needle Flush Time                           :5.00 min
GC Cycle Time                               :15.00 min
Check System Ready                          :ON
Extended System Ready Check Limit           :45 min
Check GC Ready                              :ON
Extended GC Ready Check Limit               :10 min
Analysis Mode                               :Constant
Needle Check                                :Yes
Action on Leak Check Error                  :Stop
Action with No Vial on Tray                 :Stop

[GC-2010]
Column Oven Temp.           :80.0 °C
Injection Mode              :Split
Flow Control Mode           :Linear Velocity
Pressure                    :64.9 kPa
Total Flow                  :13.6 mL/min
Column Flow                 :0.96 mL/min
Linear Velocity             :36.1 cm/sec
Purge Flow                  :3.0 mL/min
Split Ratio                 :10.0
High Pressure Injection     :OFF
Carrier Gas Saver           :OFF
Splitter Hold               :OFF
Oven Temp. Program
  Rate                      Temperature(°C)          Hold Time(min)
  -                         80.0                     8.50
  50.00                     240.0                    1.00

< Ready Check Heat Unit >
    Column Oven             : Yes
    HS Flow                 : Yes
    MS                      : Yes
< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
    HS Flow Carrier         : Yes
    HS Flow Purge           : Yes
< Ready Check APC Flow >
    APC1                    : Yes
< Ready Check Detector APC Flow >
External Wait               :No
Equilibrium Time            :3.0 min
< Additional Flow >
APC1 Pressure               :80.0 kPa

[GC Program]

[GCMS-QP2020]
Micro Scan Width                        :0.00 amu
IonSourceTemp                           :250.00 °C
Interface Temp.                         :250.00 °C
Solvent Cut Time                        :4.00 min
Detector Gain Mode                      :Absolute
Detector Gain                           :0.93 kV

[MS Table]
 --Group  1 - Event  1--
Start Time                              :6.00min
End Time                                :9.00min
ACQ Mode                                :SIM
Event Time                              :0.30sec
Ch1-m/z                                 :74.00
Ch2-m/z                                 :42.00
Ch3-m/z                                 :43.00

Sample Inlet Unit                       :GC
```

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Determination of NDMA (HS-GC-MS)
Method 3/30-Annex 2 GC-MS Standard Addition Plot
Issue No. 1

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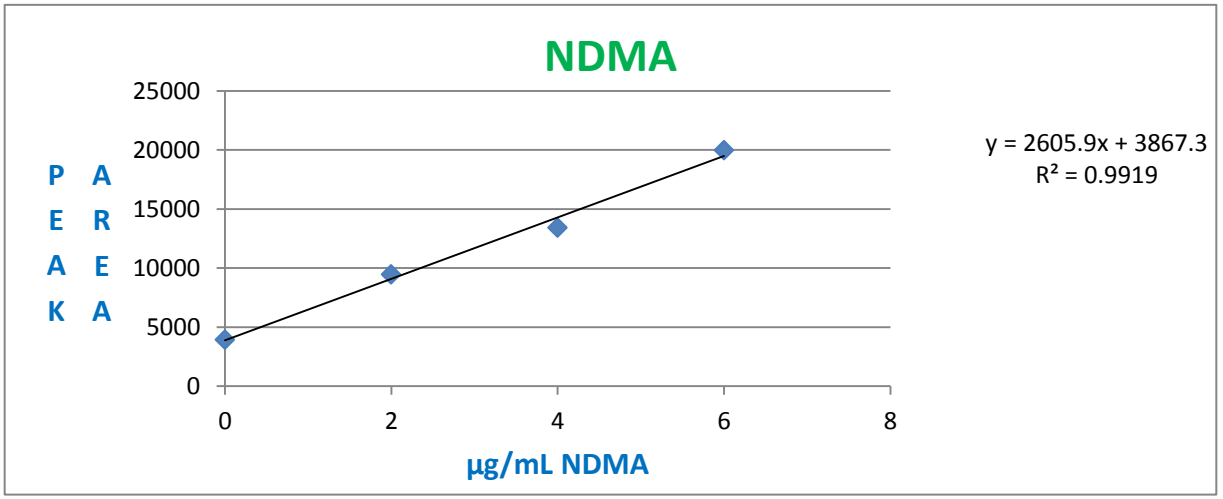
Standard Addition Plot of Peak Area Vs µg/mL of added NDMA

Date:		Sample		Batch:		Data Folder: GCMSsolutions\NDMA

Added NDMA (µg/L)	Area	sample (g)
0	3918	0.19908
2.0	9441	0.20165
4.0	13415	0.20004
6.0	19966	0.19946
	mean =	0.20006



Average tablets mass (g) =	0.083

API content of tablet (g) =	0.040

X Intercept =	**-1.4475 µg/mL**

Actual value of the intercept.

→ **7.24** **µg NDMA/g**

Absolute value of the intercept divided by average mass (g) of sample used per vial.

→ **0.60** **µg NDMA/tablet**

µg NDMA/g multiplied by average tablet mass.

→ **15.0** **µg NDMA/g Valsartan**

µg NDMA/tablet divided by API content (g) of tablet.