

## COMMENTS CONCERNING SOME REVISED/ CORRECTED TEXTS PUBLISHED IN SUPPLEMENT 4.7

*Here follows information concerning certain technical modifications to some revised/corrected texts adopted by the European Pharmacopoeia Commission at the March 2003 session. This information completes the modifications indicated by lines in the margin in the supplement. Hence, the information below is not necessarily exhaustive.*

### METHODS OF ANALYSIS

#### 2.7.8. Assay of tetanus vaccine (adsorbed)

The revised general method adds a serological model in guinea-pigs to the present methods using challenge with tetanus toxin in guinea-pigs or mice. The serological model is intended for use as an alternative to challenge methods in the circumstances described in the preamble and is, because of animal welfare, a preferred method potentially applicable to all combinations of tetanus vaccine (adsorbed) for routine analyses.

The development of the serological model has been carried out as an extensive project in the Biological Standardisation Programme of EDQM and was supported by the Council of Europe, the European Commission and the European Centre for the Validation of Alternative Methods of the European Commission (ECVAM/IHRC/JRC). The full report of the project is available in *Pharmeuropa Bio 2001-2*.

The serological model has advantages for animal welfare since it avoids challenge with toxin. It can also lead to reduction in the number of animals needed, particularly where a single-dilution model is used. In a separate project of the Biological Standardisation Programme, a serological model for the assay of diphtheria vaccine (adsorbed) is being developed. If

it proves possible to use the same guinea-pig serum for potency assays of both tetanus and diphtheria vaccines, this will further reduce the number of animals needed.

A number of reagents used in the assay will be available on a temporary basis from EDQM to facilitate the introduction of the method by different laboratories. The possibility of establishing BRPs for these reagents is under study. A reference guinea-pig tetanus antiserum for use as a positive control will be available on a permanent basis from EDQM.

Methods A and B at present allow for the use of a lethal end-point instead of a paralytic end-point in countries where the latter is not obligatory. In the interests of animal welfare, it was initially envisaged to phase out this provision for use of a lethal end-point in the near future and recognise only the paralytic end-point. It has been noted that this would hinder the introduction of the serological method. Laboratories not already using the paralytic end-point would be obliged to introduce the method and then demonstrate equivalence of the serological method. In order to facilitate the introduction of the serological method, it is now proposed to keep the provision for the use of the lethal end-point.

### GENERAL TEXTS

#### 5.3. Statistical analysis of results of biological assay and tests

The general chapter has been revised to add extended sigmoid dose-response curve models such as four-parameter logistic curve analysis to the chapter. The revision is required because of the wide-spread use of these models, and the explicit reference made to these models in some recent monographs. As these models

raise a number of statistical problems which may require different solutions for different types of assays, it is not possible to give a "recipe-like" description as is the case for the other models already included in the chapter. A general outline of the model, and a simple example are presented to offer users some guidance, but professional advice is nevertheless recommended for those wishing to use these models.

### MONOGRAPHS

#### Alkyl mesitates

An enquiry concerning alkyl mesilate impurities in mesilate salts was made via a note in *Pharmeuropa* 12.1. In the light of information and comments received, the European Pharmacopoeia Commission has decided that the following approach will be used in the monographs concerned.

The following production section will be included in all monographs on mesilate salts.

*The production method must be evaluated to determine the potential for formation of alkyl mesitates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesitates are not detectable in the final product.*

Tests for alkyl mesitates will not be included in monographs. The methods required to demonstrate

absence of alkyl mesitates at a suitable limit of detection are not suited for routine use and validation of the process is a better approach.

The monographs concerned, published in Supplement 4.7 are:

- Betahistine mesilate
- Bromocriptine mesilate
- Deferoxamine mesilate
- Dihydroergocristine mesilate
- Dihydroergotamine mesilate
- Pergolide mesilate
- Phentolamine mesilate

#### **Biperiden hydrochloride (1074)**

Since benzene is a potential impurity of synthesis, its control has been introduced in the monograph and the limit is that prescribed in general chapter 5.4. The limits in the test for related substances have been brought into line with current policy.

#### **Bismuth subgallate (1493)**

Since it was not possible to dissolve the substance entirely to prepare Solution S, the preparation of this solution has been revised in order to proceed by ignition of the substance.

#### **Bismuth subsalicylate (1495)**

Since it was not possible to dissolve the substance entirely to prepare Solution S, the preparation of this solution has been revised in order to proceed by ignition of the substance. Based on batch data it was decided to re-centre the limits on the average bismuth content of 57.4 per cent.

#### **Bumetanide (1076)**

The existing TLC method for the test for related substances has been replaced by a LC method based on an isocratic ion-pair reversed-phase separation. This technique avoids either 2 LC separations or the use of very steep gradients, that otherwise would have been necessary due to the large differences in polarity of the impurities.

The test for heavy metals has been deleted due to the low posology of the compound.

#### **Carmellose calcium (0886)**

This monograph has been revised within the scope of international harmonisation with JP and USP. In particular, the possible presence of anticaking agent is no longer mentioned in the definition; consequently, the test for silica has been deleted and the conditions of the test for sulphated ash have been modified accordingly.

#### **Castor oil, virgin (0051)**

The limit for absorbance is lowered in order to exclude technical grades of virgin castor oil.

The test for iodine value is considered redundant in comparison with that for composition of fatty acids. It is therefore deleted.

#### **Cellulose acetate (0887)**

#### **Cellulose, microcrystalline (0316)**

#### **Cellulose, powdered (0315)**

These monographs have been revised within the scope of international harmonisation with JP and USP.

#### **Cetirizine dihydrochloride (1084)**

The test for heavy metals has been deleted due to the low posology of the substance.

#### **Chlorobutanol, anhydrous (0382)**

#### **Chlorobutanol hemihydrate (0383)**

As no stability data are available to support the storage temperature recommended in the storage section, this indication has been deleted from these monographs. The reference to the odour of iodoform has been deleted in identification C, in accordance with general policy.

#### **Dihydralazine sulphate, hydrated (1310)**

In the test for related substances, dihydralazine impurity A CRS is no longer available, it is therefore proposed to use a sample of dihydralazine for system suitability CRS containing this impurity.

#### **Dipotassium clorazepate (0898)**

The preparation of the test solution in the test for related substances has been modified due to the possible degradation of dipotassium clorazepate in solution and the limit for "any other impurity" has been modified.

The limit for the loss on drying has been increased in view of batch analysis data.

#### **Estrogens, conjugated (1512)**

The storage section has been deleted because this compound is stored at -20 °C by some producers whereas others keep it at room temperature. In any case, the statement "do not freeze" is not suitable.

#### **Ethylmorphine hydrochloride (0491)**

This monograph has been revised, notably to amend the assay by removing the use of mercuric acetate.

#### **Etilefrine hydrochloride (1205)**

Following the addition of impurities E, D and F to the transparency list, the test for related substances has been revised to introduce the relative retention of the impurities and to increase the run time to cover impurity D. The limit for "any other impurity" has been modified in line with current policy.

#### **Glycerol (0496)**

#### **Glycerol (85 per cent) (0497)**

The monographs published in Supplement 4.5 contain 2 errors in the expression of the limits in the test for Impurity A and related substances.

Although the limit indicated in parenthesis for "any other impurity with a retention time less than glycerol" is 0.1 per cent and that for "total of all impurities with retention times greater than the retention time of

glycerol" is 0.5 per cent, the limits imposed by the monograph correspond to these values only where the glycerol being examined contains the maximum allowable amount of diethylene glycol. If the glycerol being examined contains no diethylene glycol, the limits for other impurities are halved (0.05 per cent and 0.25 per cent, respectively).

Because of matrix effects, it is necessary to prepare the reference solution with an amount of glycerol equivalent to that of the test solution and this has made the setting of limits rather complex.

Production batches of glycerol rarely contain detectable amounts of diethylene glycol so that the limits will in many cases be difficult to meet.

In order to find a solution to the problem rapidly, it has been decided to correct the expression of limits without changing the test conditions, by preparing the reference solution with a sample of glycerol free from diethylene glycol. Ideally, this should be supplied as a CRS but this cannot be done immediately. However, analysts can readily obtain a suitable batch and check the diethylene glycol content by the chromatographic method in the monograph.

#### **Hawthorn leaf and flower (1432)**

The TLC identification has been revised to harmonise with that proposed for the dry and liquid extracts. Rutin is no longer used in the reference solution.

#### **Hydroxyethylcellulose (0336)**

This monograph figures on the International Harmonisation programme. This monograph, as well as the other monographs on cellulose derivatives, will be further revised to introduce a section on functionality-related characteristics. The test for viscosity will be moved to this section.

The test for appearance of solution has been deleted because it is not significant for this substance.

#### **Macrogol lauryl ether (1124)**

The current monograph only refers to mixtures of ethers of mixed macrogols with linear fatty alcohols. Products obtained with branched fatty alcohols, and those complying with the specifications of this monograph, are now available on the market.

#### **Naftidrofuryl hydrogen oxalate (1594)**

The revision includes a modification of the LC in the test for related substances and the introduction of a GC.

#### **Potassium sulphate (1622)**

Since the use of potassium sulphate is not limited to homoeopathy, the monograph Potassium sulphate for homoeopathic preparations has been revised to extend its application.

#### **Povidone (0685)**

The symmetry factor in the test for Impurity B is about 2.0 which is out of the limits prescribed in Chapter 2.2.46. *Chromatographic Separation Techniques*. It is therefore necessary to add the statement in the monograph.

#### **Theophylline (0299)**

#### **Theophylline monohydrate (0302)**

The revision includes the replacement of the TLC for the test for related substances by an LC in order to give improved control of impurities.

#### **all-*rac*- $\alpha$ -Tocopherol (0692)**

The revised monograph on all-*rac*- $\alpha$ -tocopheryl acetate was taken as a model.

#### **all-*rac*- $\alpha$ -Tocopheryl acetate (0439)**

The limits for content have been tightened to 96.5 per cent to 101.0 per cent, based on improved precision of the new method.

The only way to achieve the required purity is to distill the substance. Therefore, the tests for acid value, heavy metals and sulphated ash are no longer relevant.

The test for absorbance does not give any information on the purity of the substance and has therefore been deleted.

The test for free tocopherol has been deleted since  $\alpha$ -tocopherol is limited by the GC method in the test for related substances.

#### **Urea (0743)**

The revision of the assay allows an improvement of the accuracy and precision of the method. The limits of content have been changed to take into account the manipulations before titration.

## VACCINES FOR HUMAN USE

#### **Hepatitis B vaccine (rDNA) (1056)**

The test for extraneous agents refers to general chapter 2.6.16 which requires the use of an amount of harvest equivalent to 500 doses. Whereas this is suitable for classical viral vaccines prepared in cell culture, it leads to the use of extremely large volumes of harvest for a recombinant DNA product, which is virtually impossible in practice. Therefore, a volume of 200 ml has been specified.

#### **Influenza vaccine (surface antigen, inactivated) (0869)**

The monograph has been revised to extend the scope to cover adjuvanted vaccines which have recently become available.

#### **Tetanus vaccine (adsorbed) (0452)**

The test for residual toxin and irreversibility of toxoid was revised to remove the requirement for testing of a sample maintained at  $5 \pm 3$  °C, so that reversibility is tested only at 37 °C. It has been shown experimentally that toxin can degrade at this temperature suggesting the possibility of a false negative test. Consequently the test at  $5 \pm 3$  °C has been reinstated as a precaution.