DEXAMETHASONE

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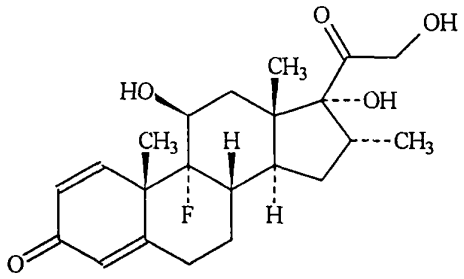
ADDENDUM

to the Dexamethasone monographs prepared by the forty-second and forty-third meetings of the Committee and published in FAO Food and Nutrition Paper 41/6, Rome 1994, and 41/7, Rome 1995, respectively

IDENTITY

Chemical Name: (11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

Structural formula:



Active ingredient: Dexamethasone

INTRODUCTION

Dexamethasone is a fluorinated glucocorticoid and a potent anti-inflammatory agent used frequently for treatment of inflammatory processes and primary ketosis in domestic food producing animals. Dexamethasone lacks effects on electrolyte balance but is 30-35 times more potent than cortisol as a anti-inflammatory agent.

At its 42nd and 43rd meetings the Committee reviewed dexamethasone (Wells, 1994a,b) and set temporary maximum residue levels (MRLs) of 0.5 μg/kg in muscle, 0.5 μg/kg in kidney and 2.5 μg/kg in liver of cattle, horses and pigs and 0.3 μg/L in cattle milk based on an ADI of 0 – 0.015 μg/kg. Dexamethasone can be administered to animals as the parent drug, or as one of several commercially available esters. Large variations in the absorption of the ester derivatives were reported. However, the esters are rapidly hydrolyzed in the blood to dexamethasone and dexamethasone was, therefore, considered the marker residue in terms of tissue residues. Considerable metabolism of dexamethasone was demonstrated. Apparently the metabolites do not carry any biological activity and, consequently, the parent drug, dexamethasone, was proposed as a marker residue.

In the JECFA review analytical methods for detection of dexamethasone at the set MRLs were thoroughly reviewed. It appeared that only the HPLC-MS method of the sponsor appeared to meet the criteria required for a residue control method at the allocated MRLs. However, the sponsor failed to provide full documentation of this method at that time. The dexamethasone analytical method was scheduled for review at the 48th meeting of the Committee but no data were received for evaluation. The Committee decided to withdraw the temporary MRL values set for dexamethasone due to lack of analytical method allowing enforcement of the set MRLs.

The present evaluation concerns only the documentation for the HPLC-MS method for control of dexamethasone residues in tissues and milk (Cook and McCormack,1996; Curl and McCormack,1996), provided by the sponsors Boeringer Ingelheim Vetmedica GmbH and Intervet International B.V.

General

The essential studies were performed in accordance to GLP. The appropriate references and statements were provided.

Sample preparation

Tissue, milk and serum sample preparation was performed using liquid/liquid extraction. The procedure appeared relatively simple and not too time consuming. The sample (5 g) was homogenized in 10 mL of Sorensen buffer. After centrifugation, the supernatant was extracted against hexane. Sodium hydrogen carbonate was added to the aqueous phase which was then extracted with 70% ethyl acetate in hexane. The extraction was repeated and the organic phases combined, evaporated to dryness and reconstituted in 0.5 N sulfuric acid. After hexane wash of the sulfuric acid fraction, sodium hydrogen carbonate was added to the aqueous phase. Extraction with 70% ethyl acetate in hexane was performed and the organic phase collected, evaporated to dryness and reconstituted in 50% acetonitrile in water. The sample was now ready to be injected to the chromatographic system.

Chromatographic method

The chromatographic method was based on gradient elution using an ODS2 (5 micron, 15 cm x 4.6 mm) reversed phase column. The mobile phase consisted of acetonitrile and 0.1 M ammonium acetate in ratio changing from 10:90 to 80:20 during the 10 minute chromatographic run. The injection volume was 100 µL.

Mass spectrometry

The Detection of dexamethasone was performed by thermospray mass spectrometry utilizing filament ionization. The ion source was adjusted at 230 °C and the initial probe temperature of 105 °C decreasing to 86 °C at 10 min was used. Single ion detection was employed and dexamethasone was monitored at 333 m/z and the internal standard (methyl prednisolone) at 315 m/z.

Quantitative calculations

Dexamethasone concentration in the sample was calculated by applying the detector response to a linear regression curve. The method uses internal standard (I.S.) for quantification because of large variation in detector response. However, it is not clear how peak area ratios (sample/I.S.) should be applied to the regression equation (Curl and McCormack, 1996). The amended report (Curl and McCormack, 1996) indicates that a subtraction of concentrations found in control samples should be done to the fortified samples. It is not clear how this procedure should be applied to incurred residue samples. The original report (Cook and McCormack, 1996) does not indicate how quantitative results were calculated.

Specificity

The presence of dexamethasone in the sample was determined on basis of retention time and typical MS ions. The ion 333 m/z in dexamethasone spectrum was observed to have highest abundance and was chosen as the ion to be monitored. Accordingly, the ion 315 m/z was chosen for the internal standard, methyl prednisolone. Of the compounds tested *prednisolone, cortisone, methylprednisolone, triamcinolone, flumethasone,* and *isoflupredone did not interfere* the dexamethasone analysis. However, betamethasone was found to elute together with dexamethasone and possessed the same 333 m/z ion and, consequently, it was not possible to distinguish between these compounds. The chromatograms provided with the amended report (Curl and McCormack, 1996) showed some apparent retention time instabilities. However, the report did not offer any explanation for this phenomenon.

Method validation

Linearity of the detector response was determined using concentration standards in the range from 0.25 to 10 ng/ml for milk and 0.5 to 20 ng/g for tissues and plasma. Linearity was considered to be acceptable when correlation coefficient exceeded 0.98. The recovery and accuracy of the method were determined by fortifying 5 replicates at concentration

Amended protocol

An amendment to the original report has been released (Cuel and MacCoemack, 1996). The purpose of the amendment was to comply with the EU guidelines and to the ISO 78/2 format. No essential changes concerning the method performance were added.

APPRAISAL

At its 42nd and 43rd meetings the Committee reviewed dexamethasone and recommended temporary maximum residue levels (MRL) of 0.5 μg/kg in muscle, 0.5 μg/kg in kidney and 2.5 μg/kg in liver of cattle, horses and pigs and 0.3 μg/L in cattle milk based on an ADI of 0–0.015 μg/kg of body weight. Considerable metabolism of dexamethasone was noted. However, the metabolites do not have any biological activity and, consequently dexamethasone, was proposed as a marker residue. MRLs were designated as temporary because there was no adequate method to determine compliance with the MRL.

Performance data was requested on the dexamethasone analytical method for evaluation at the 48th meeting of the Committee but no data were received for evaluation. The Committee decided to withdraw the temporary MRLs for dexamethasone due to lack of an adequate analytical method allowing enforcement of the MRLs. At the present meeting the Committee reviewed documentation for the HPLC-MS method for control of dexamethasone residues in tissues and milk.

General

Liquid chromatographic methods based on UV detection were considered unsuitable for residue analysis at sub-μg/kg concentrations. Although a method for analysis of dexamethasone in samples at 0.1 μg/L by gas chromatography/mass spectrometry using negative chemical ion monitoring has been described, attempts to apply this method for food commodities failed. Immunoassays were considered to meet the required detection levels but other technical problems were encountered. A liquid chromatography thermospray mass spectrometry (TS-LS/MS) method was developed. The essential studies were performed in accordance to GLP. The appropriate references and statements were provided.

TS-LC/MS based methods require high quality laboratories to maintain the complex and expensive equipment and skilled operating personnel. Failure to maintain instrument performance may adversely affect method reproducibility. The transferability of such a method is questionable and this limits its use as a regulatory method.

Analytical Method

Tissue and milk sample preparation was performed using liquid/liquid extraction. The sample is homogenized in buffer, extracted and purified and transferred to the chromatographic system.

The chromatographic method involves gradient elution using a reversed phase column. The chromatograms provided with the report showed some apparent retention time instabilities. The report did not offer any explanation for this phenomenon.

Large variation in detector response was reported to occur during analysis. Non-specific interferences are encountered occasionally, requiring adjustments to the concentrations found. While this can be done in fortified samples, such correction can not be done accurately in incurred residue samples. Therefore, calculation of quantitative results in incurred samples may not be accurate.

Prednisolone, cortisone, methylprednisolone, triamcinolone, flumethasone, and isoflupredone did not interfere with the dexamethasone analysis. However, betamethasone, an isomer of dexamethasone, was found to elute together with dexamethasone. Consequently, the method does not make an unambiguous identification of dexamethasone .

Method validation

Linearity was considered acceptable when the correlation coefficient exceeded 0.98. The recovery and accuracy of the method were determined by fortifying 5 replicates at the LOQ, twice the LOQ, 10 times the LOQ, and by use of blank tissues. The method failed to report absolute recoveries. Precision was calculated as coefficient of variation of the concentrations of the fortified samples. The criteria set by the Codex Alimentarius, Residues of Veterinary Drugs in Foods, Vol 3 for accuracy and precision were used and fulfilled. The limit of detection (LOD) was determined as the concentration that gave a signal to noise ratio greater than 3 and limit of quantification (LOQ) as the lowest concentration at which acceptable precision and accuracy

were recorded. The claimed LOQs were 0.5 μg/kg for muscle, kidney and fat tissues in bovine, porcine and equine species and 0.5, 1.0 and 1.0 μg/kg in the liver tissue of the respective species. The LOQ for porcine skin was 0.5 μg/kg and for bovine milk 0.25 μg/kg.

Conclusion

The method did not meet the required performance criteria for identification and quantification of incurred residues in tissues. Therefore, the method was not considered to be suitable for regulatory dexamethasone residue analysis. The Committee agreed that the MRL showed remain withdrawn in absence of an acceptable analytical method for regulatory purposes.

REFERENCES

Wells, R. (1994a). Dexamethasone in JECFA Monograph "Residues of some veterinary drugs in animals . and foods". FAO Food and Nutrition Paper 41/6 pp. 13-31.

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Cook, J. and McCormack, A. (1996). Determination of specificity of a dexamethasone assay with respect to other corticosteroids. Final Report (1043/14-1012). Corning Hazleton Europe, North Yorkshire, England.

Curl, M.G. and McCormack, A. (1996). Development and validation of an analytical method for the determination of dexamethasone in tissues and plasma of cattle, pigs and horses and in milk of cattle. Amended Final Report (7309-806/3). Corning Hazleton Europe, North Yorkshire, England