DEXAMETHASONE

First draft prepared by
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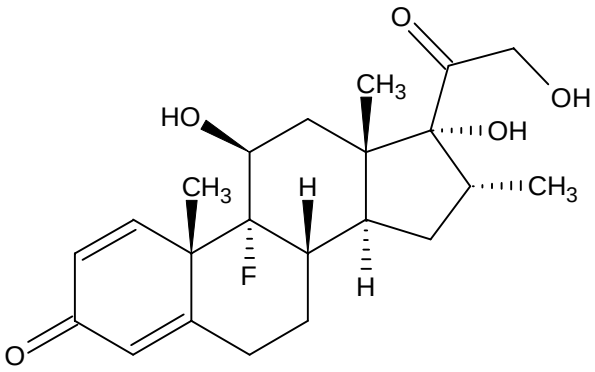
ADDENDUM
to the Dexamethasone monograph prepared by the 42nd , 43rd and 50th meetings of the Committee and published in FAO Food and Nutrition Paper 41/6, 41/7 and 41/11, respectively

IDENTITY

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

Systematic name: (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (IUPAC)

Structural formula:



Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.45

Pure active ingredient: Dexamethasone

INTRODUCTION

Dexamethasone is a fluorinated glucocorticosteroid 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 an anti-inflammatory agent.

Dexamethasone was evaluated at the forty-second and forty-third meetings of the committee (Wells, 1994a,b). At the forty-second meeting, the Committee established an ADI of 0-0.015 µg/kg of body weight for dexamethasone and recommended the following temporary MRLs: 0.5 µg/kg for muscle, 2.5 µg/kg for liver and 0.5 µg/kg for kidney, expressed as parent drug, in cattle and pigs; and 0.3 µg/l for cows' milk, expressed as parent drug. As its forty-second meeting, the Committee noted that dexamethasone undergoes extensive metabolism. However, it also noted that the metabolites did not exhibit any biological activity and consequently proposed dexamethasone as the marker residue. The MRLs were designated as temporary because an adequate method to determine compliance with the

MRL was not available. At the forty-third meeting, the same temporary MRLs were recommended for horses as were recommended at the forty-second meeting for cattle and pigs.
Performance data were requested on the analytical method for evaluation at the forty-eighth meeting of the Committee, but no data were provided. The temporary MRLs for dexamethasone were withdrawn at that meeting due to lack of an adequate analytical method allowing enforcement of the MRLs. At the 50th meeting, the Committee reviewed documentation on an HPLC-MS method (thermospray ionisation, selected ion monitoring) for the control of dexamethasone residues in tissue and milk (Cook and McCormack, 1996; Curl and McCormack, 1996). The chromatograms provided with the report showed some apparent retention time instabilities. Selectivity was not judged adequate because of the partial co-elution of betamethasone (16β-isomer); unambiguous identification of dexamethasone was considered as difficult. Large variation in detector response was reported to occur during analysis. Calculation of quantitative results in incurred samples may not be accurate because non-specific interferences are encountered occasionally. Even if the criteria recommended by the Codex Alimentarius Commission, Volume 3, Residues of Veterinary Drugs in Foods, for accuracy and precision were used and fulfilled, the Committee concluded that the method did not meet the required performance criteria for identification and quantification of incurred residues in tissues and milk.

ANALYTICAL METHODS

Two sponsors provided three methods for the quantification of dexamethasone in muscle/kidney, liver and milk.

Liquid chromatographic methods based on UV detection were considered unsuitable for residue analysis at sub-µg/kg (or sub-µg/l) concentrations. Methods based on GC-MS (negative chemical ionization) after dexamethasone oxidation are no longer in use. Liquid chromatography electrospray (positive or, better, negative mode) mass spectrometry methods are preferred because they provide improved sensitivity and specificity.

The analytical method for muscle and kidney from cattle consists of one common procedure using LC-MS/MS (ESI+). Sample preparation was performed using two solid-phase extraction (SPE) purification steps. The chromatographic method involves gradient elution using a reversed-phase column. No mention of the use of any internal standard was provided for muscle and kidney. The analytical method for liver and milk from cattle consists of two different procedures using LC-MS/MS (ESI−). Sample preparation was performed using one SPE purification step. The chromatographic method involves isocratic elution using a reversed-phase column. A chemical analogue (deltafludrocortisone) was utilized for dexamethasone identification and quantification.

The validation of the method for muscle and kidney was conducted with a target residue level set at 1 µg/kg, whereas the MRL recommended previously by the Committee was 0.5 µg/kg. The validation of the method for liver tissue was performed for a target residue level of 2 µg/kg, whereas the previous MRL in liver was 2.5 µg/kg. The validation of the method for milk was performed at a target residue level of 0.3 µg/l, equal to the previously recommended MRL for milk.

Sample preparation

Muscle, kidney. The sample is denatured in acid and then digested overnight with protease enzyme. After digestion, isopropanol is added to facilitate the extraction of the analytes. The mixture is diluted and passed through a C_{18} cartridge followed by further clean up a SPE anion exchange column and then onto a C18-SPE cartridge. The analytes are eluted from the SPE cartridges, evaporated to dryness, and the dried extracts are reconstituted in mobile phase for further determination by LC-MS/MS.

Milk. Sample preparation is based on protein precipitation using trichloroacetic acid. Clean-up is carried out using solid phase extraction. The final sample solution is analysed by liquid

chromatography (LC) with tandem mass spectrometric (MS/MS) detection using negative electrospray ionisation.

Liver. After enzymatic hydrolysis of the glucocorticosteroid conjugates, the free and aglycone residues are extracted using methanol. The extracts are then centrifuged, evaporated, dissolved in water and cleaned up by SPE. After SPE treatment, the methanol eluates are evaporated and the dry residue is dissolved in the mobile phase. The samples are analysed by liquid chromatography (LC) with tandem mass spectrometry (MS/MS) using negative electrospray ionisation.

Analytical measurement

Muscle, kidney. The chromatographic method (HPLC) was based on gradient elution using a C18 (2 x 150 mm; 4 µm) reversed phase column. The mobile phase consisted of acetonitrile and 50/50 - 0.01% formic acid/0.01M ammonium formate. Flow rate was set at 0.2 ml/min. Injection volume was 20 µl, column temperature was set at 40°C. Total run time was 40 min. The detection of dexamethasone was performed by electrospray (ESI, positive mode) ionisation tandem mass sepctrometry (triple quadrupole mass analyser). Capillary was set 2.5 kV, source temperature at 120°C. Selected Reaction Monitoring was employed and dexamethasone was monitored at 393>373 for quantification, and 393>355, 393>147, 393>337 for identification. No internal standard was used for identification (retention time criteria) nor quantification (calibration curve).

Liver, milk. The chromatographic method (HPLC) was based on an isocratic elution using a Hypercarb C18 column (2.1 x 100 mm; 5µm) reversed phase column. The mobile phase consisted of a mixture of acetonitrile - 0.1% formic acid (90/10; v/v). Flow rate was set at 0.6 ml/min for screening and 0.22 ml/min for confirmation. Injection volume was 20 µl, column was set at room temperature. The detection of dexamethasone was performed by electrospray (ESI, negative mode) ionisation tandem mass spectrometry (triple quadrupole mass analyser). Capillary was set 2.7 kV, source temperature at 120°C. Selected Reaction Monitoring was employed and dexamethasone was monitored at 437>361 for quantification (collision energy 18 eV), plus 437>345 (CE 25 eV) for identification. Deltafludrocortisone (DFUD) was used as an internal standard.

Method validation

The validation was conducted at 1 µg/kg for muscle and kidney, 2 µg/kg for liver and 0.3µg/kg for milk.

Stability

Muscle, kidney. Analytes obtained from tissue extracts are stable over the period of a typical analyses cycle. Analytes are stable under frozen conditions (-20°C) for up to 10 weeks. Standard solutions prepared in methanol using the analytes are stable for up to 1 year.

Liver, milk. The stock standard solutions have been found to be stable for at least 22 months for dexamethasone at –20°C. No information was given for the sample extract.

Specificity and selectivity

Muscle, kidney. The presented method demonstrated its ability to provide non-interfered signals. The technique of acquisition (SRM) used on the triple quadrupole instruments permitted it to eliminate most of the interferences. Betamethasone previously pointed out as a source of potential interfering signal (isobaric compound, close retention time when analysed on reverse phase liquid chromatography) was completely separated from dexamethasone because of the stationary phase. Betamethasone is eluted before dexamethasone and chromatographic peaks are fully separated (e.g. 19.5 min and 20.2 min, respectively). Finally, the method was able to detect truly negative samples uncontaminated with dexamethasone (Boison, et al., 2008).

Liver, milk. The method demonstrated its ability to provide non-interference signals for dexamethasone and its internal standard (i.e. deltafludrocortisone) especially when an HPLC column Hypercard 100 x 2.1 mm is used. The SRM acquisition of the signals provided high specificity. Betamethasone a 16-stereoisomer of dexamethasone was fully chromatographically separated eluting after dexamethasone (e.g. 3.5 min and 4.6 min respectively). Finally, the method was able to detect true negative samples uncontaminated with dexamethasone. Deltafludrocortisone eluted at 2.8 min.

Accuracy-Trueness

Muscle, kidney. Within-day and between day accuracy data generated from the method showed that quantitation can be performed with a trueness below 10% (%RSD).

Liver, milk. Trueness was determined by spiking a pooled sample of bovine liver at 0.5, 1.0 and 1.5 times the MRL for dexamethasone. The matrix matched standard curve was prepared from the same pooled sample as the other spiked samples. Trueness was 15% or better at the three levels either in the screening or confirmatory methods both for milk and liver.

Accuracy-Precision

Muscle, kidney. Within-day and between day accuracy data generated from the method showed that quantitation can be performed with a precision below 10% (%RSD).

Liver. The repeatability (within day) for the confirmation set-up was determined by analysing 6 replicates of a pooled bovine liver sample on 3 occasions spiked at 0.5, 1.0 and 1.5 times MRL. In addition, the repeatability (within day) for the confirmation set-up was determined by analysing a total of 20 different samples on three occasions (spike level at 1 MRL). Repeatability was better than 10.3% (CV) in the pool samples and 12.6% (CV) for the different samples. Reproducibility (within-day and inter-day) was better than 20.4% (CV).

Milk. The repeatability was determined for 20 different samples spiked at the 1-MRL level on three different occasions. Repeatability was better than 10.7% (CV). Reproducibility (within-day and inter-day) was better than 24.6% (CV).

Accuracy-Recovery

Muscle, kidney. The mean absolute recovery was calculated at each of the six calibration points in one of two ways; by either comparing the slope of the calibration curve obtained from the matrix fortified sample to the slope of the chemical standard or matrix matched standard curve, or comparing the interpolated concentrations at each of the six calibration points, pooling them together and calculating the mean absolute recovery over the calibration range. Dexamethasone mean recovery was 66 ± 4 %.

Liver, milk. The absolute recoveries were determined by comparing the absolute peak area response for six individual blank samples spiked at the MRL before sample preparation with the same six blank samples spiked after sample preparation. In this case no internal standard was used, and quantification was carried out using external absolute response. When diluting the extracts, care was taken to ensure that the suppression/enhancement effects were the same in samples spiked before and samples spiked after the extraction. In liver recovery values were 70.0 ± 10.6 % for the screening method and 69.0 ± 15.5 % for the confirmation process. For milk, lower values have been found, i.e. below 25% for dexamethasone.

Limit of detection, quantitation, decision limit, detection capability

Muscle, kidney. Linear regression analysis was performed on three sets of calibration standards (i.e., chemical standards, matrix matched standards, and matrix fortified standards). Calibration curves from

a minimum of three different days were pooled and analysed. Calibration curves generated from the chemical standards were linear over the calibration range of 0.5 – 10.0 ng g^{-1} with a correlation coefficient better than 0.996. Similarly, calibration curves generated from matrix fortified and matrix matched standards were also linear over the same analytical range and had a correlation coefficient better than 0.992. The claimed limits of quantification and identification were both 0.4 µg/kg. Limit of decision (CCα, risk α=5%) and detection capability (CCβ, risk β=5%) were 1.2 µg/kg and 1.5 µg/kg. The validation has been conducted with a MRL set at 1 µg/kg wheras the recommended MRL by the JECFA was 0.5 µg/kg (the EMEA fixed the MRL at 0.75 µg/kg).

Milk. The calibration curve was tested in the range 0-0.6 µg/L. The correlation coefficient (R^2) was found to be better than 0.95. The claimed limit of quantification was defined as the lowest validated level which was 0.15 µg/L. Limit of decision (CCα) was 0.45 µg/L. Detection capability (CCβ) was 0.57 µg/l.

Liver. The calibration curve in bovine liver was tested in the range of 0-4 µg/kg. The correlation coefficient (R^2) was found to be better than 0.95. The claimed limit of quantification was defined as the lowest validated level which was 1µg/kg. Limit of decision (CCα) was 2.9 µg/kg. Detection capability (CCβ) was 3.7 µg/kg.

APPRAISAL

General

Muscle, kidney, liver, milk. Liquid chromatographic methods based on UV detection were considered unsuitable for residue analysis at sub µg/kg (or l^{-1}) concentrations. Methods based on GC-MS (negative chemical ionisation) after dexamethasone oxidation are no longer used. Liquid chromatography electrospray (positive or better negative mode) mass spectrometry methods were developed, validated and used. This technology is now available is most laboratories worldwide.

Analytical method

Muscle, kidney, liver, milk. Tissue sample preparation was performed using at least one (milk, liver) or two (muscle, kidney) SPE purification steps. The chromatographic method involves gradient (liver, kidney) or isocratic (milk, liver) elution using a reversed phase column. Electrospray ionisation was used to produce ions further characterised by selective reaction monitoring. No mention regarding the use of any internal standard is provided for muscle and kidney, whereas a chemical analogue (deltafludrocortisone) was utilised for dexamethasone identification and quantification in liver and milk samples.

Method validation

Muscle, kidney, liver, milk. Stability of the target analyte was demonstrated in standard solution (all matrices), and biological extract (muscle, kidney). Selectivity was proved as fitting with the needs, especially the efficient chromatographic separation of betamethasone and dexamethasone was made possible. The validation for muscle and kidney was conducted with a target residue level of 1µg/kg wheras the MRL recommended previously by the Committee was 0.5 µg/kg. The validation for liver has been performed with a target residue level of 2 µg/kg wheras the MRL recommended previously by the Committee was 2.5 µg/kg. The validation for milk was performed with a target residue level of 0.3 µg/l equal to the previously recommended MRL for milk. The claimed limits of quantification and identification were 0.4 µg/kg both for muscle and kidney. The claimed limits of quantification were 0.15 µg/kg for milk and 1 µg/kg for liver (LOQ defined as lowest validated level).

Limit of decision (CCα, risk α=5%) and detection capability (CCβ, risk β=5%) were 1.2 µg/kg and 1.5 µg/kg for muscle and kidney. These performance values fit the expectations of a MRL method at 1.0 µg/kg, but are insufficient considering the MRL as recommended by the Committee at its forty-second and forty-third meetings.

Limits of decision and detection capabilities were 0.45 µg/L and 0.57 µg/l for milk (MRL recommended by JECFA at 0.3 µg/l), and 2.9 µg/kg and 3.4 µg/kg for liver (recommended MRL by JECFA at 2.0 µg/kg). These performance values have been calculated in reproducibility conditions, which can be considered as conservative. Any laboratory implementing the methods will characterize an "in-house" decision limit in repeatability conditions. The CCα would then be significantly closer to the MRL. The same applies to the CCβ. In summary, the performances of the methods fulfil the minimum performance criteria corresponding to dexamethasone residues in milk and liver at the MRL as recommended by the Committee in its forty-second and forty-third meetings.

Conclusion

A suitable validated routine method was available for monitoring dexamethasone in bovine milk and liver at 0.3 µg/l and 2.0 µg/kg, respectively. A suitable validated routine method was available for monitoring dexamethasone in bovine muscle and kidney at 1.0 µg/kg, but not at 0.5 µg/kg. No validated method for horses and pigs was provided or could be found, but the method provided for cattle tissue is adequate to be extended to pig and horse tissues.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for dexamethasone, the Committee considered the following factors:

- The established ADI is 0–0.015 µg/kg-bw, equivalent to 0–0.9 µg for a 60-kg person.
- The marker residue is dexamethasone.
- The appropriate target tissues are liver or kidney and milk.
- A suitable validated routine method was available for monitoring dexamethasone in bovine milk and liver at 0.3 µg/l and 2.0 µg/kg, respectively.
- A suitable validated routine method was available for monitoring dexamethasone in bovine muscle and kidney at 1.0 µg/kg, but not at 0.5 µg/kg.
- No validated method for horses and pigs was provided or could be found, but the method provided for cattle tissue is adequate to be extended to pig and horse tissues.
- The recommended MRLs are based on the performances of the analytical methods at twice the LOQ.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, pigs and horses, expressed as the marker residue dexamethasone: muscle/kidney, 1.0 µg/kg; liver, 2.0 µg/kg; cow's milk, 0.3 µg/l. Based on these values for the MRLs, the maximum theoretical intake would be 1µg/day per person. This would be compatible with a maximum ADI of 0.9 µg for a 60-kg person. The Committee noted that at its forty-second meeting it was concluded that dexamethasone is rapidly eliminated from muscle and milk, and that the probability of exposure to residues from these tissues is low.

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