DIHYDROSTREPTOMYCIN and STREPTOMYCIN

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ADDENDUM

To the monograph and addenda prepared by the 43rd, 48th and 52nd meeting of the JECFA and published in FAO Food and Nutrition Papers 41/7, 41/10 and 41/12

INTRODUCTION

Dihydrostreptomycin and streptomycin were considered previously at the 43rd, 48th and 52nd meetings of the Committee. At its 43rd meeting, JECFA recommended temporary MRLs in cattle, pigs, sheep and chickens: 500 μg/kg for muscle, liver and fat, and 1000 μg/kg for kidney. A temporary MRL of 200 μg/kg also was recommended for milk. At the 48th meeting, a group ADI of 0-50 μg/kg body weight for the combined residues of dihydrostreptomycin and streptomycin was recommended. The compounds were again considered at the 52nd meeting where tissue residues and two analytical methods for tissues were reviewed. At that meeting the Committee recommended that the temporary status of the MRLs be removed, except for milk. Taking into account the higher limit of quantification of the bioassay relative to the HPLC assay, the MRLs were raised to 600 μg/kg for muscle, liver and fat of cattle, pigs, sheep and chickens. The recommended MRL for kidney was 1000 μg/kg for cattle, pigs, sheep and chickens. The temporary MRL for residues in milk was retained pending receipt of method validation information for both compounds at low concentrations found in cattle milk.

For evaluation at the 58th meeting, information has been provided on the performance of the method. Additionally, residue depletion data have been provided for dihydrostreptomycin (DHS) and streptomycin (STRP) in sheep milk with a request that the temporary MRL of 200 μg/kg for cattle milk be extended to sheep milk.

MILK RESIDUE DEPLETION STUDIES

Milk residue depletion studies compliant with Good Laboratory Practices were conducted in cattle and sheep.

Cattle

Eight lactating Friesian cows received an intramuscular administration of the test article at a dose of 10 mg active ingredients per kg body weight once daily for three consecutive days. Triplicate whole milk samples were collected prior to treatment and at approximately 12-hour intervals for eight consecutive milkings after the final treatment. Samples were stored below -20 °C until analyzed using an HPLC assay with post-column derivatisation and fluorescence detection (Norbrook, 2000). The results are summarized in Table 1.

Table 1. Concentrations of dihydrostreptomycin and streptomycin in cattle milk following intra-muscular administration of 10 mg active ingredients per kg bw once daily for three consecutive days

Time (hr)	Concentration (μg/mL)	
	Dihydrostreptomycin	Streptomycin
Pre-treatment	<0.05	<0.05
12	0.19±0.03	0.20±0.03
24	0.11±0.01	0.09±0.01
36	0.06±0.01	0.09±0.01
48	<0.05	<0.05
60	<0.05	<0.05
72	<0.05	<0.05
84	<0.05	<0.05
96	<0.05	<0.05

Each value is the mean ± SD for two animals. / The LOQ for the method is 0.05 μg/kg

The depletion of both analytes from cattle milk was fairly rapid, resulting in no detectable residues in the milk samples collected later than 36 hours after the final treatment.

Sheep

Eight lactating Suffolk and Suffolk/Cheviot sheep received an intramuscular administration of the test article at a dose of 10 mg active ingredients per kg body weight once daily for 3 consecutive days. Triplicate whole milk samples were collected prior to treatment and at approximately 12-hour intervals for 8 consecutive milkings after the final treatment. Samples were stored below -20°C until analyzed using an HPLC assay with post-column derivatisation and fluorescence detection (Norbrook, 2000). The results are summarized in Table 2.

Table 2. Concentrations of dihydrostreptomycin and streptomycin in sheep milk following intramuscular administration of 10 mg active ingredients per kg body weight once daily for 3 consecutive days

Time (hr)	Concentration (μg/mL)	
	Dihydrostreptomycin	Streptomycin
Pre-treatment	<0.05	<0.05
12	0.24±0.07	0.24±0.06
24	0.18±0.05	0.19±0.03
36	0.11±0.03	0.08±0.01
48	0.06±0.01	0.07±0.02
60	<0.05	<0.05
72	<0.05	<0.05
84	<0.05	<0.05
96	<0.05	<0.05

Each value is the mean ± SD for two animals.
The LOQ for the method is 0.05 μg/kg

The depletion of both analytes from sheep milk was fairly rapid, resulting in no detectable residues in the milk samples collected more than 48 hours after the final treatment.

SUMMARY OF ANALYTICAL METHODS

Sampling

In all animal studies, samples were collected in triplicate and immediately placed on ice. Milk samples collected from cattle were at least 100 mL in volume while those collected from sheep were at least 20 mL in volume. Milk samples were stored at -20°C pending analysis.

HPLC assay

The standard operating procedure for an HPLC method (Norbrook, 1999; SOM: CRD/DSM/010) and its validation in sheep milk (Norbrook, 1999; VAL: CRD/DSM/020) and cattle milk (Norbrook, 1999; VAL: CRD/DSM/010) were provided. Additionally, revised standard operating procedures for the HPLC analysis of dihydrostreptomycin and streptomycin in tissues (Norbrook, 2001; SOM: CRD/DSS/011) and updated validations in cattle tissues (Norbrook, 2001; VAL: CRD/DSS/011), sheep tissues (Norbrook, 2001; VAL: CRD/DSS/021) and pig tissues (Norbrook, 2001; VAL: CRD/DSS/031) were included. The revised standard operating procedures supplement those evaluated at the 52nd meeting (JECFA, 1999).

An HPLC method has been developed for the measurement of dihydrostreptomycin and streptomycin in cattle and sheep milk. Milk samples from untreated animals were used as blanks. Fortified samples for the calibration curves and quality control (QC) samples were prepared by fortifying 200 mL of blank bulk milk with dihydrostreptomycin and streptomycin. Quality control samples are prepared to provide final concentrations of 0.05 μg/mL and 0.1 μg/mL for each analyte and the standard curve provided final concentrations from 0.05 to 5.0 μg/mL for each analyte. Ten millilitre aliquots of blank milk, fortified milk, QC samples and test samples were deproteinised with perchloric acid, vortexed and centrifuged. The process was repeated and the two supernatants were combined and centrifuged. The combined deproteinised supernatants were loaded onto prepared aromatic sulphonic acid solid phase extraction columns, washed with water, and the dihydrostreptomycin and streptomycin were eluted using phosphate buffer, pH 7.5. Water, perchloric acid and ion pair concentrate were added to the eluate, vortexed, filtered as needed, and retained for analysis. The eluate was analysed by HPLC with post-column derivatisation and fluorescent detection. Manual measurement of the peak heights was used to determine final analyte concentrations. Because the measurement resolution on the very small peaks was limited to integer values, the CV estimates for low concentration samples were correspondingly low and in some cases even mathematically zero.

Validation in cattle milk

The method was validated in cattle milk (VAL: CRD/DSM/010). For both analytes, the standard line was linear (r^2>98%) for each of the three analyses.

Recovery was determined by individually extracting six replicate samples of freshly fortified milk. Mean recoveries for the tested concentrations were 91.8-98.5% for dihydrostreptomycin and 94.0-95.5% for streptomycin.

Precision was determined using the fortified samples prepared for the accuracy determination. Acceptable precision, ≤12.2%, was demonstrated for all the tested concentrations for both analytes.

The limit of detection (LOD) was determined by preparing, extracting and chromatographing 24 blank samples. The LOD for the method for cattle milk was determined statistically to be 0.04 μg/mL for dihydrostreptomycin and 0.01 μg/mL for streptomycin.

The limit of quantification (LOQ) was determined by analysing replicate samples fortified at the 0.05 μg/mL concentration. Since all fortified samples at the 0.05 μg/mL concentration showed acceptable accuracy and precision, 0.05 μg/mL was determined to be the LOQ for both analytes in cattle milk. When calculated statistically, the LOQ was consistent with the value determined above, 0.07-0.11 μg/mL for dihydrostreptomycin and 0.02-0.03 μg/mL for streptomycin.

The specificity of the method was demonstrated by extracting blank cattle milk from six different sources. Slight interferences at the retention time for dihydrostreptomycin and streptomycin that were less than 20% of the 0.05 μg/mL concentration response were deemed insignificant with result to the calculation of results. An additional test for method specificity was conducted to assess the effects of related compounds on the accuracy and precision of the extraction process. Milk samples were spiked with dihydrostreptomycin and streptomycin and then further spiked with gentamicin, neomycin, lincomycin and penicillin G. Mean recoveries for the examined concentrations of dihydrostreptomycin were between 83.2-98.8% with a %CV of ≤9.74%. Mean recoveries for the examined concentrations of streptomycin were between 95.9-105% with a %CV of ≤3.41%.

The stability of the analytes in cattle milk and a demonstration of method ruggedness were not provided.

Characteristics of the analytical method in cattle milk are summarized in Table 3.

Validation in sheep milk

The method was validated in sheep milk (VAL: CRD/DSM/020). Thirteen analyses were conducted to demonstrate linearity over the range 0.05-5.0 μg/mL. For both analytes, the standard line was linear (r^2>98%) for each of the thirteen analyses.

Accuracy was determined by individually extracting six replicate samples of freshly fortified milk. Mean accuracies for the tested concentrations were 77.5-102% for dihydrostreptomycin and 76.2-96.6% for streptomycin.

Precision was determined using the fortified samples prepared for the accuracy determination. Precision, expressed as percentage co-efficient of variation (%CV), should be ≤20% for concentrations at or below 0.1 μg/mL and ≤15% at concentrations above 0.1 μg/mL. Acceptable precision was demonstrated for all the tested concentrations.

The limit of detection (LOD) was determined by preparing, extracting and chromatographing 24 blank samples. The means and standard deviations of the blank sample responses for both analytes were calculated. The LOD for the method, defined as the mean blank value plus three times the standard deviation of the blank, was determined to be 0.04 0.02 μg/mL for dihydrostreptomycin and 0.02 μg/mL for streptomycin.

The limit of quantification (LOQ) was determined by analysing replicate samples fortified at the 0.05 μg/mL concentration. Since all fortified samples at the 0.05 μg/mL concentration showed acceptable accuracy and precision, 0.05 μg/mL was determined to be the LOQ for both analytes in sheep milk. When calculated statistically, the LOQ was consistent with the value determined above, 0.08-0.12 μg/mL for dihydrostreptomycin and 0.03-0.04 μg/mL for streptomycin.

The specificity of the method was demonstrated by extracting blank sheep milk from six different sources. Although the report indicates that slight interferences may occur at the retention time for dihydrostreptomycin and streptomycin, it concludes that if the interference is less than 20% of the lowest concentration spike response it should be deemed insignificant with result to the calculation of results. An additional test for method specificity was conducted to assess the effects of related compounds on the accuracy and precision of the extraction process. Milk samples were spiked with dihydrostreptomycin and streptomycin and then further spiked with gentamicin, neomycin, lincomycin and penicillin G. Mean accuracies for the examined concentrations of dihydrostreptomycin were between 88.2-100% with a %CV of ≤ 19.2%. Mean accuracies for the examined concentrations of streptomycin were between 95.7-97.2% with a %CV of ≤12.3%.

The stability of the analytes in sheep milk stored at < -20°C was demonstrated by preparing QC samples in bulk sheep milk at 0.05 and 0.1 μg/mL. Duplicate samples at each concentration were analysed over an eight-month period. To be

deemed acceptable, not more than one QC sample per day could exceed the 80-120% limits for accuracy. At the end of the 8-month storage period, mean accuracy was 97.4% (%CV=7.05) for samples containing 0.05 μg/mL dihydrostreptomycin and 96.8% (%CV=11.0) for samples containing 0.1 μg/mL dihydrostreptomycin. For samples containing streptomycin, mean accuracy at the end of the 8-month storage period was 97.5% (%CV=9.09) and 91.1% (%CV=7.55), for the 0.05 and 0.1 μg/mL concentrations, respectively.

Ruggedness was determined by calculating the interassay accuracy and precision at three concentrations: 0.05, 0.1, and 0.2 μg/mL. For dihydrostreptomycin, the interassay accuracy was 82.9-101% with an interassay precision ≤9.13%. For streptomycin, the interassay accuracy was 86.7-96.2 with an interassay precision ≤17.1%.

Characteristics of the analytical method in sheep milk are summarized in Table 3.

Table 3. Characteristics of the analytical method for dihydrostreptomycin and streptomycin in cattle and sheep milk

Criteria	Cattle milk	Sheep milk
Linearity	r^2>0.98	r^2>0.98
Recovery (fortified standard curve)	91.8-98.5% DHS 94.0-95.5% STRP	77.5-102% DHS 76.2-96.6% STRP
LOD	0.04 μg/mL DHS 0.01 μg/mL STRP	0.04 μg/mL DHS 0.02 μg/mL STRP
LOQ (recovery of fortified samples)	0.05 μg/mL DHS and STRP	0.05 μg/mL DHS and STRP
LOQ (determined statistically)	0.07-0.11 μg/mL DHS 0.02-0.03 μg/mL STRP	0.08-0.12 μg/mL DHS 0.03-0.04 μg/mL STRP
Specificity from blank (6 different sources of milk)	slight interference ≤20% of 0.05 μg/mL concentration response	slight interference ≤20% of 0.05 μg/mL concentration response
Specificity from related compounds	83.2-98.8%;%CV ≤9.7% DHS 95.9-105%;%CV ≤3.4% STRP	88.2-100%;%CV ≤19.2% DHS 95.7-97.2%;%CV ≤12.3% STRP
Ruggedness	Not provided	Interassay accuracy: 82.9-101% DHS 86.7-96.2% STRP Interassay precision: ≤9.1% DHS ≤17.1% STRP

Validation in tissues

Revised standard operating procedures for the analysis of residues in tissues, CRD/DSS/011 (Method of analysis for dihydrostreptomycin and streptomycin in tissue, Norbrook, 2001), as well as validation reports for cattle (VAL: CRD/DSS/010), sheep (VAL: CRD/DSS/020), and pig (VAL: CRD/DSS/030) tissues were provided. Each of the validation reports was followed by an addendum (VAL: CRD/DSS/011, VAL: CRD/DSS/021, and VAL: CRD/DSS/031, for cattle, sheep and pig tissues, respectively). For all three species, the concentration range of 0.25 to 5.0 μg/mL was selected for both analytes. The method can still be used for streptomycin concentrations as low as 0.2 μg/mL (previous validation level) and for dihydrostreptomycin concentrations as high as 6 μg/mL (previous validation level). The revised validation range now covers the expected sample concentrations and lowers the LOQ for dihydrostreptomycin to one half the MRL.

For each species, revised mean accuracies and precision determinations were provided. Data were provided to support the lower LOQ for dihydrostreptomycin and ruggedness determinations in the presence of gentamicin, neomycin, lincomycin and penicillin G are included. All of the validation parameters were judged to be acceptable and, as a result, the revised method was considered rugged.

APPRAISAL

The Committee considered that the sponsor had provided satisfactory answers to the requests made by the 52nd meeting and, therefore, decided to delete the temporary status of the MRL for the combined residues of dihydrostreptomycin and streptomycin for cattle milk.

After reviewing the residue depletion study in sheep milk, the Committee concluded that the sponsor had provided the data needed to recommend an MRL for the combined residues of dihydrostreptomycin and streptomycin for sheep milk.

Therefore, the Committee recommended an MRL of 200 μg/kg for the combined residues of dihydrostreptomycin and streptomycin for cattle and sheep milk

The ADI is equivalent to 0-3000 μg per 60 kg person. The theoretical maximum daily intake for all tissues and milk using the recommended MRLs is 620 μg.

REFERENCES

Norbrook. (1999). Streptomycin/Dihydrostreptomycin, Analytical method standard operating procedures and validation reports for cattle and sheep milk for consideration at the 58th JECFA meeting. Norbrook Laboratories Ltd.

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