GENTAMICIN

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ADDENDUM
to the gentamicin residue monograph
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INTRODUCTION

The Committee, at its 43rd meeting, stated that a validated chemical analytical method with a limit of quantification at or preferably below the temporary MRL of 0.1 mg/L recommended for milk was required for evaluation. Additional information with respect to the toxicological evaluation was also requested before the temporary ADI recommended by the 43rd meeting of the Committee could be replaced by a permanent ADI. Establishment of a permanent ADI would permit the replacement of the temporary MRLs assigned by the 43rd Meeting of the Committee with a recommendation for permanent MRLs, with the exception of the MRL for milk, which required the validated methodology requested.

ANALYTICAL METHOD

A high performance liquid chromatographic (HPLC) method for the quantification of residues of gentamicin in cattle milk, as well as in muscle, liver, kidney and fat of cattle and pigs and skin of pigs was presented for review (Stilzebach, 1996a,b). The method for tissues, except fat, includes extraction of the analyte into buffer, deproteinization by heating, with subsequent clean-up using a Sephadex column. A further clean-up on an SAX column is required for muscle and liver. Gentamicin residues are detected by fluorescence after pre-column derivatization with 9-fluorenylmethyl chloroformate (Fmoc-Cl), with excitation at 261 nm and emission at 313 nm. HPLC separation is on a reversed phase C-18 column using gradient elution with a mixture of water and acetonitrile. The method, when applied to fat or skin from pigs, omits the deproteination step. For milk, the initial extraction into buffer is omitted.

The four major components of gentamicin are usually designated as C_1, C_{1a}, C_2, and C_{2a}, but are identified in the method as G1, G2, G3, and G4. The method sponsor did not have authentic standards of the 4 major gentamicin components, so the identification G1 to G4 relates to order of elution and does not relate to the order C_1, C_{1a}, C_2, C_{2a}. The difference between the components of gentamicin is the degree of methylation or, in the case of C_2 and C_{2a}, in the location of the methyl group. It is very important to recognize that gentamicin is not a single entity. The ratio between the four major components is not constant between different batches and producers of gentamicin. In the report on the analytical method provided for review, the proportions of the different components in the analytical standard used in the study were not available.

Laboratories using this method will require authentication of their standard and should obtain samples of the four major gentamicin components for calibration. However, the four major peaks are separated, with retention times of 20 to 25 minutes, and quantification is by external standard. Validation included testing to determine accuracy (as recovery) and precision on fortified samples, the determination of limits of quantification and detection and testing for interferences. Due to interfering co-extractives, the gentamicin C_1 peak could not be used in the analysis of cattle muscle, liver and fat, while the gentamicin C_1 and C_2 peaks could not be used for the analysis of pig muscle and liver samples. Quantification was based on the remaining components. Limits of detection were based on the analysis of blank matrix and varied for the individual components. Recovery was determined at the limit of quantification (LOQ), and twice and 10 times the LOQ. The results of the validation are summarized in Table 1.

Table 1. Performance characteristics of liquid chromatographic method of analysis for gentamicin residues in edible tissues of cattle and pigs and in cows' milk.

Species	Tissue	LOQ* (mg/kg)	Lowest fortification level (mg/kg)	Recovery (%)	Coefficient of Variation (%)
cattle	muscle	0.10	0.10	90	13
	liver	0.20	0.20	95	7
	kidney	1.0	1.0	85	7
	fat	0.10	0.10	77	4
	milk	0.10	0.10	71	10
pig	muscle	0.10	0.10	81	4
	liver	0.20	0.20	72	5
	kidney	1.0	1.0	94	15
	fat	0.10	0.10	85	6
	skin	0.10	0.10	97	7

* LOQ = Limit of quantification, determined with respect to CVMP criteria, based on the lowest fortification level which meets the requirements for accuracy (as recovery) and precision.

APPRAISAL

Gentamicin was previously evaluated at the 43rd Meeting of the Committee. A temporary ADI of 0-4 μg/kg of body weight was established using a microbiological end-point and temporary MRLs were recommended of 100 μg/kg for muscle and fat, 200 μg/kg for liver and 1000 μg/kg for kidney in both cattle and pigs, as well as 100 μg/L for cows' milk, expressed as parent drug. The Committee requested the following information for evaluation in 1997:

1. Results of studies on the effects of gentamicin on specific genera of microorganisms obtained from the human intestine.

2. Additional data to assist in the assessment of carcinogenic potential, which should include:
   (a) results of genotoxicity assays for gene mutations in mammalian cells and chromosomal aberrations *in vitro* and *in vivo*; and
   (b) details of an investigation on possible structural similarities between gentamicin and known carcinogens.

3. A validated chemical analytical method with a limit of quantification below the MRL recommended for milk.

Pharmacokinetic data

No additional data were requested or provided.

Residue data

No additional data were requested or provided.

Analytical methods

Residue studies considered by the 43rd Meeting of the Committee primarily relied on microbial growth inhibition assays. Given the non-specificity of microbial growth inhibition assays and the apparent availability of liquid chromatographic assays for gentamicin residues in edible tissues, the Committee requested that a method based on a chemical assay be provided for the analysis of gentamicin residues in milk, with a limit of quantification below the MRL.

It was noted by the 43rd meeting of the Committee that while no analytical methods were available that met the multi-laboratory validation criteria described in Codex Alimentarius, Volume 3 (1993), there were published methods in the current scientific literature for gentamicin residue analysis in edible tissues based on high performance liquid chromatography. Several such methods were included in method compilations prepared for regulatory authorities.

An HPLC method for the quantification of residues of gentamicin in cattle milk, as well as muscle, liver, kidney and fat of cattle and pigs and skin of pigs was presented for review. The method for tissues, except fat, includes solvent extraction of the analyte into buffer, deproteination by heating, clean-up using solid-phase extraction and analysis by liquid chromatography. Detection is by fluorescence after pre-column derivatization. Deproteination is not required for the analysis of fat or skin from pigs, while the initial extraction into buffer is omitted for the analysis of cows' milk. The four major components of gentamicin (C_1, C_{1a}, C_2, C_{2a}) are separated, with retention times of 20 to 25 minutes, and quantification is by external standard. Interfering co-extractives prevent the use of all four components for quantitative analysis in some tissue matrices. A standard containing the four major components was used in this study. For quantification, the Committee considered that well-characterized standards, preferably of the 4 individual major gentamicin components, should be used for calibration in the analysis. The method was tested using samples fortified at the MRLs recommended by the Committee at its 43rd Meeting. Analytical recoveries were 72-97% for tissues and 71% at 0.1 mg/kg for cows' milk. The method appears suitable for use in a regulatory program to determine compliance with recommended MRL's for residues in edible tissues from cattle or pigs, or in cows' milk.

Maximum Residue Limits

In recommending MRLs, the Committee took into account the following factors:

- An established ADI of 0-20 μg per kg of body weight based on a microbiological endpoint derived from data provided for review by the present Committee. This would result in a maximum ADI of 1200 μg for a 60-kg person.
- Gentamicin residues are persistent in kidney and liver, but deplete rapidly in muscle, fat and milk.
- A suitable analytical method is available for analysis of gentamicin residues in edible tissues and milk. The LOQ for milk is 100 μg/L.
- The marker residue is parent drug.

On the basis of the maximum observed residues in studies with gentamicin in food animals presented for review by the 43rd Meeting of the Committee, the following permanent MRLs are recommended for edible tissues of cattle and pigs, expressed as parent drug:

Muscle	100 μg/kg
Liver	2000 μg/kg
Kidney	5000 μg/kg
Fat	100 μg/kg

The Committee also recommended a permanent MRL of 200 μg/L for gentamicin in milk from cattle.

The MRL's recommended above would result in a theoretical daily maximum intake of 785 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 L of milk.

REFERENCES

Codex Alimentarius, Volume 3 (1993). Residues of Veterinary Drugs in Foods, 2nd ed., Food and Agriculture Organisation of the United Nations, Rome, Italy.

Stilzebach, D. (1996a). Validation of a method for the quantification of gentamicin residues in matrices of cattle. ECON Project EF 95-12-01 Final Report, August 21, 1996, Sponsor Submitted.

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