A sensitive LC-MS/MS method for the quantitative determination of dexamethasone in porcine plasma and porcine muscle, liver and kidney tissue

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Introduction

The synthetic glucocorticoid dexamethasone is approved within the EU for therapeutic indications in cattle. Cattle tissues intended for human consumption have to be analysed for their maximal residue limits (MRLs). The MRLs for dexamethasone have been established by the Committee for Veterinary Medicinal Products as follows[2]:

- 0.75 µg/kg in kidney and muscle
- 2 µg/kg in liver

As a result, sensitive analytical methods are required to determine MRLs in edible tissues derived from cattle.

The ultimate goal of our work was to develop and validate a sensitive and reliable bioanalytical LC-MS/MS method suitable for the determination of dexamethasone in porcine muscle, liver and kidney tissue and in porcine plasma.

A full validation of the method was performed according to Volume 8 of The Rules governing Medicinal Products in the EU[3].

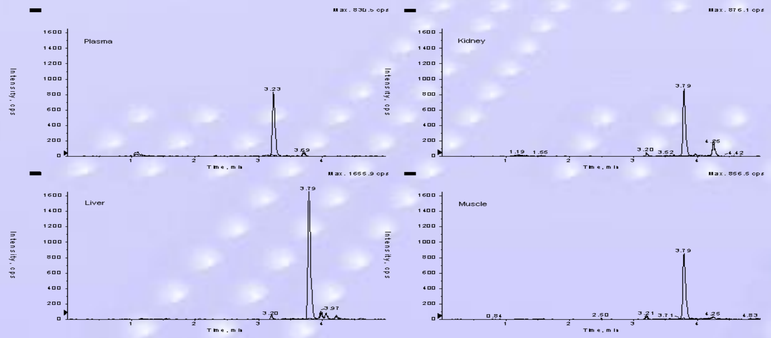

Figure 1: Chromatograms of dexamethasone in plasma (0.100 ng/mL), kidney (0.50 MRL), liver (0.50 MRL) and muscle (0.50 MRL). Data is obtained from a study performed at FRAH[1].

Materials and Methods

Porcine tissues were homogenised using a saline solution. After addition of the internal standard (prednisolone), the samples were subjected to liquid-liquid extraction using ACN. After dilution with H_2O and a washing step with pentane, the samples were extracted using TBME. The evaporated residues were dissolved in injection solvent and injected into the LC-MS/MS system for quantification.

The samples were chromatographed on a LUNA C18 LC column (3 µm, 150 x 3.0 mm). The mass-spectrometer consisted of a Sciex API 4000 equipped with a Turbo Ion Spray interface and was operating in the positive ion mode.

Results

Calibration: The assay was validated in the concentration range of:

- 0.0750 - 7.50 µg/kg (0.10 - 10.0 MRL) for kidney and muscle tissue
- 0.200 - 20.0 µg/kg (0.10 - 10.0 MRL) for liver tissue
- 0.100 - 25.0 ng/mL for plasma

A lower limit of quantitation (LLOQ) of 0.10 MRL and 0.100 ng/mL was achieved in porcine tissue and plasma, respectively.

Accuracy and precision: The method showed acceptable accuracies (expressed as bias) and precisions (expressed as CV) for all tissues (Table 1).

In plasma, biases of -5.8, -6.4, -10.7 and -8.7% and CVs of 11.9, 12.6, 9.1 and 6.5% were obtained at the QC levels LLOQ (0.100 ng/mL), Low (0.500 ng/mL), Medium (5.00 ng/mL) and High (20.0 ng/mL).

Recovery: The recovery of dexamethasone and the internal standard was essentially similar (>55% in tissue).

Table 1: Accuracy and precision in muscle, liver and kidney tissue

QC level	Porcine muscle		Porcine liver		Porcine kidney	
	Bias (%)	CV (%)	Bias (%)	CV (%)	Bias (%)	CV (%)
LLOQ (0.10 MRL)	3.7	13.4	20.2	7.9	-2.4	16.4
Low (1.00 MRL)	0.3	6.4	14.3	4.3	-3.8	5.3
Medium (2.00 MRL)	6.5	4.9	6.8	6.8	-3.9	2.7
High (5.00 MRL)	4.3	7.0	11.8	5.8	-7.6	2.9

Specificity: In kidney, muscle, liver and plasma from six different sources, no relevant interferences from endogenous compounds at the retention time of dexamethasone and IS were observed.

Dilution: The 10-fold dilution of kidney, liver, muscle and plasma samples with the blank tissue homogenate/plasma was considered valid.

Stability: 24 h storage of samples on the autosampler and one additional freeze/thaw cycle were successfully validated for all matrices.

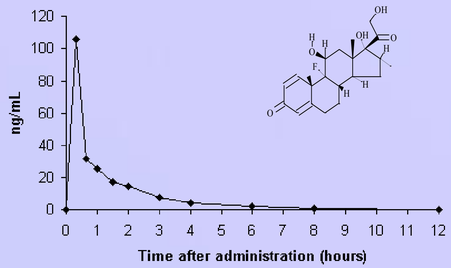

Figure 2: Mean dexamethasone plasma concentration in pigs (n=8) after a single intramuscular administration of 60 µg/kg dexamethasone

Discussion and Conclusions

- A sensitive bioanalytical LC-MS/MS method for the quantitative determination of dexamethasone in porcine plasma, muscle, liver and kidney tissue has been developed and validated successfully.
- Accurate and precise results and very low LLOQs were achieved (up to 0.10 MRL in porcine tissue) in comparison to other available methods (usually 0.50 MRL). Representative chromatograms are shown in Figure 1.
- The assays offer a competitive alternative to the methods available for the investigation of the pharmaco- and depletion kinetics of dexamethasone in porcine plasma, muscle, liver and kidney tissue. Examples are presented in Figure 2 and Table 2.
- The method can be used as a lead for the validation of dexamethasone in other species.

Table 2: Dexamethasone tissue concentrations in pigs after a single intramuscular administration of 60 µg/kg dexamethasone. Data is obtained from a residue study performed at FRAH[1].

Interval treatment-slaughter	Animal	Kidney	Liver	Muscle	Injection site
(days)	(number)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)
1	06	0.402	<0.200	0.206	<0.0750
	08	0.382	<0.200	0.101	0.383
	09	0.358	<0.200	<0.0750	0.450
	12	0.287	<0.200	<0.0750	0.583
3	02	<0.0750	<0.200	<0.0750	<0.0750
	03	<0.0750	<0.200	<0.0750	<0.0750
	07	<0.0750	<0.200	<0.0750	<0.0750
	16	<0.0750	<0.200	<0.0750	<0.0750
7	01	<0.0750	<0.200	<0.0750	<0.0750
	11	<0.0750	<0.200	<0.0750	<0.0750
	13	<0.0750	<0.200	<0.0750	<0.0750
	14	<0.0750	<0.200	<0.0750	<0.0750
14	04	<0.0750	<0.200	<0.0750	<0.0750
	10	<0.0750	<0.200	<0.0750	<0.0750
	17	<0.0750	<0.200	<0.0750	<0.0750
	18	<0.0750	<0.200	<0.0750	<0.0750
	LOQ	0.0750	0.200	0.0750	0.0750
	MRL	0.75	2.0	0.75	0.75

References

[1] Farma Research Animal Health (FRAH), Toernooiveld 300-H, Nijmegen, The Netherlands.

[2] EMEA 2001. Committee for Veterinary Products. Dexamethasone. Summary Report.

[3] Volume 8 of The Rules governing Medicinal Products in the European Union. Notice to Applicants and Guideline. Establishment of maximum residue limits for residues of veterinary medicinal products in foodstuffs of animal origin, October 2005.