MONITORING OF CORTICOSTEROIDS IN THE NATIONAL PLAN FOR CONTROL OF RESIDUES IN RUSSIA

M.A. Yunin, P.S. Metalnikov, A.A. Komarov
Russian State Center for Quality and Standardization of Veterinary Drugs and Feeds (VGNKI), Moscow, Russia
E-mail: yuninma@gmail.com

Introduction

In accordance with the Russian Federation regulations the residues of synthetic steroids, β-agonists, sedatives, thyreostats and some groups of antibiotics are not allowed in food (*Customs Union 2010; Customs Union 2013*). Therefore, the monitoring of these residues is necessary to ensure that food products are meeting the Russian Federation regulations. The aim of this study was to develop a rapid and sensitive method for the analysis of 4 corticosteroids (dexamethasone, prednisolone, methylprednisolone, and triamcinolone acetonide) in meat, milk and liver using LC-MS/MS.

Prednisolone, dexamethasone, triamcinolone acetonide, and methylprednisolone are synthetic corticosteroids, structurally based on endogenous corticosteroid – cortisol (Figure 1).

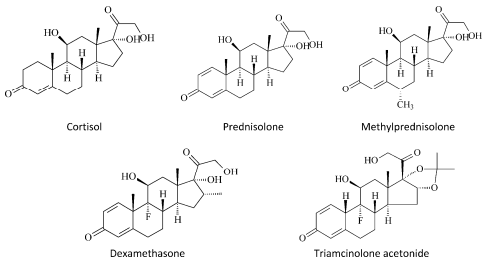

Figure 1. Chemical structures of corticosteroids.

However recent studies reported on a potential endogenous origin of prednisolone. It is suggested that unlike other analogues, such as dexamethasone, prednisolone does not contain any halogen atom and its structure closely resembles that of other endogenous steroids, including cortisol. From this structural similarity arises the hypothesis that in meat cattle prednisolone could be generated by physiological metabolic processes, possibly under extremely stressful conditions, such as transport and slaughtering, or by faecal contamination (*Leporati et al., 2013*).

Prednisolone, methylprednisolone, dexamethasone and its epimer betamethasone are authorized for therapy in both human and veterinary practices. They affect glucose utilization, fat metabolism, and bone development and commonly used in the treatment of allergic reactions. They are also used to reduce inflammation (*Tolgyesi et al., 2012*).

Corticosteroids are also known to exert growth-promoting effects in cattle fattening and to show synergetic effects in combination with β-agonists and other anabolic steroids in cattle. Therefore, they might be used illegally as growth promoting agents (*Schmidt et al., 2009*).

Sample preparation

5 g of meat, milk or lver,10 ml of sodium acetate buffer pH 5.2, homogenization

↓

Enzymatic hydrolysis with Helix Pomatia 4 h, 52˚C
(only for liver samples)

↓

LLE with 15 ml of TBME

↓

Evaporation of TBME, reconstitution in 1.5 mL of methanol/ water (80/20, v/v)

↓

Defatting with 3 ml of hexane

↓

LC-MS/MS

MS/MS parameters

A triple quadrupole mass spectrometer **(QTRAP 5500, AB SCIEX, Toronto, Canada)** was used with electrospray ionization source. The transitions for each analyte, as well as the corresponding collision energies, are shown in Table 1. Declustering potential and collision cell exit potential were -110 V and -20 V in negative mode, correspondingly. The resolution for quadrupole 1 (Q1) and quadrupole 3 (Q3) was set to “unit”.

Table 1. HPLC-MS/MS parameters of investigated analytes.

Compound	Retention time (min)	Parent ion (*m/z*)	Daughter ion (*m/z*)	Collision energy (eV)
Prednisolone	18.0	405.2	280.1[a], 295.1	-45, -50
Methylprednisolone	20.3	419.2	309.2, 343.2[a]	-43, -23
Dexamethasone	20.0	437.1	345.2, 361.2[a]	-34, -24
Triamcinolone acetonide	20.4	479.2	337.2, 413.2[a]	-32, -26
Triamcinolone acetonide-D6	20.4	485.2	337.2	-65

[a] *The ion used for quantitative analysis.*

LC conditions

A binary solvent delivery system (**Eksigent UltraLC-100, Eksigent, USA**), including a binary pump and a degasser, was used. Chromatographic separation was achieved by reversed phase chromatography and gradient elution. Separation of the analytes was carried out on a **ACE Excel 3 Super C18 column** (150 mm× 2.1 mm, particle size 3 µm, ACE), maintained at 40 °C. The autosampler temperature was set to 4 °C and the injection volume was 20 µL. Samples were analyzed in negative polarity mode. The mobile phases were **water (phase A)** and **methanol (phase B)**. A linear gradient was applied at a flow rate of 150 µL min^{-1} starting at 20 % B, increasing to 70 % B within 20 min, and keeping 70 % B for 10 min. Subsequently, the column was re-equilibrated for 10 min at 20 % B.

Validation

The validation experiment was based on full factorial design for two factors and consisted of 4 runs. A run contains blank samples of meat, milk and liver fortified at 0.1, 0.5, 2, 8, 30 µg kg^{-1}, which were analyzed in two replicates and matrix-matched calibration samples fortified at the same levels. Operator and storage of extract after sample preparation were selected as factors which cannot be controlled in routine analysis. The method was validated in the range of 0.1 – 30 µg kg^{-1} for all analytes. The recovery lies in the range of 75 – 110 %.

From the data obtained during validation experiment the combined uncertainties (u_c) were calculated in accordance with Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results (EUROLAB, 2006) using equation:

$$u_c = \sqrt{\frac{s_r^2}{m} + \frac{S_{wR}^2}{n} + u_{ref}^2 + \Delta^2}$$

where s_r - repeatability standard deviation; S_{wR} - within-laboratory reproducibility standard deviation; u_{ref} - the uncertainty of analyte concentration in fortified sample (uncertainty of the reference value); Δ - mean deviation from the reference value (bias), m - number of replicates; n - number of experiments (runs).

The combined uncertainty was below 40 % for all analytes.

The specificity of the method was demonstrated as no interfering peaks were observed at the retention time of analytes in a variety of blanks (Figure 2).

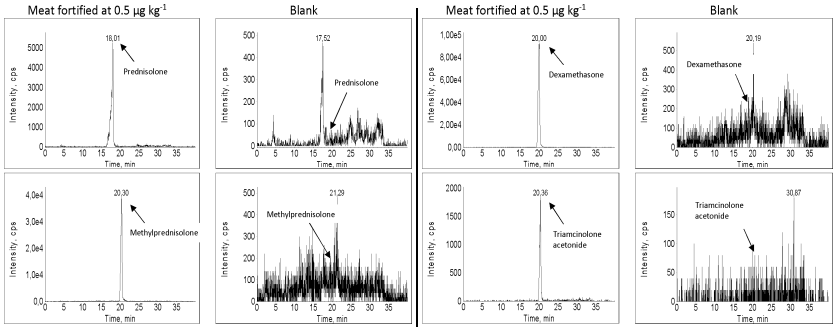

Figure 2. Selected reaction monitoring chromatograms obtained from a meat fortified at 0.5 µg kg^{-1} and a meat blank (SRM transitions used for quantitative analysis are shown).

Results and Discussion

Time-consuming procedures based on GC-MS with derivatisation have been mainly used in the past for the analysis of corticosteroids. More recent the LC-MS/MS procedures without derivatisation step have been developed. The sample preparation of LC-MS/MS procedures normally used two solid phase extractions on reversed and normal phases. In our study we compare two sample preparation schemes: one based on liquid-liquid extraction with methyl tert-butyl ether and solid phase extraction on silica cartridges, and the other, on liquid-liquid extraction with methyl tert-butyl ether and deffating with hexane. Both schemes have resulted in high recoveries and low detection limits. As a result the scheme with the defatting step was chosen as less time-consuming.

41 bovine liver samples and 78 beef samples from Russia and South America were analysed in the frame of National Residue Programm.

Conclusions

A rapid and sensitive method with simple sample preparation for the determination of 4 corticosteroids has been developed. The method was validated and applied for the analyses of 119 samples in the National Residue Program.

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