Determination of Dexamethasone in Urine and Faeces of Treated Cattle With Negative Chemical Ionization–Mass Spectrometry*

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For several years, the misuse of dexamethasone and its esters in livestock production has been clearly demonstrated. The first part of the present study deals with the elaboration of a sensitive and specific method for the determination of residues of dexamethasone in excreta at the ppb level. Sample preparation for urine and faeces, including high-performance liquid chromatography (HPLC) fractionation, was carried out. The detection was based on established methodology employing negative chemical ionization–mass spectrometry (NCI–MS) after oxidation of the dexamethasone. In comparison with previous literature, the yield of oxidized dexamethasone was substantially improved and the oxidation procedure was made more simple and robust. In the second part of the study, the relationship between the dose of dexamethasone administered and the levels of the drug in excreta was investigated using this method, as was the ratio between drug levels in urine and faeces. Treatment was carried out for 7 d with an oral dose of 50 mg d^{-1}, the maximum levels found in urine and faeces were 980 and 744 ppb, respectively. While the elimination *via* faeces responded much slower at the start and the end of treatment, the final part of both excretion profiles were very similar and a level of 1 ppb was reached in both matrices 9 d after the end of treatment. Gas chromatography–mass spectrometry (GC–MS) results obtained for the urine samples were compared with those obtained with direct enzyme immunoassay.

Keywords: Dexamethasone; urine; faeces; excretion profile; gas chromatography–mass spectrometry; enzyme immunoassay; cattle

Introduction

Although it has long been recognized that large doses of synthetic glucocorticoids reduce growth rates and lead to muscle atrophy,[1] dexamethasone is frequently used as an illegal growth promoter in livestock production. The low dosages of 0.1–0.5 ppm found in feeds are in accordance with the results described by Istasse *et al.*,[2] according to whom low doses of dexamethasone resulted in increased live weight gain and a reduced feed conversion ratio.

For the control of feeds and premixes, different methods are available. Recently, we described the possibilities of enzyme immunoassay and high-performance thin-layer chromatography for screening purposes.[3] The subsequent confirmatory analysis can be done using high-performance liquid chromatography (HPLC) with diode array detection or electron impact-mass spectrometry (EI–MS) on trimethylsilyl (Tms) derivatives.[3] For the screening of urine samples for dexamethasone several sensitive radioimmunoassays and enzyme immunoassays are available. In order to overcome eventual problems of cross-reactivity with endogenous corticosteroids, HPLC fractionation was carried out.[4] A high-performance liquid chromatography–mass spectrometry (HPLC–MS) method has been reported for the detection of betamethasone in equine urine with a detection limit of 5 ng ml^{-1},[5] while gas chromatography–negative chemical ionization–mass spectrometry (GC–NCI–MS) would be able to detect oxidized dexamethasone at levels as low as 0.2 ng ml^{-1}.[6] For human plasma and bovine tissues, mass spectrometric methods were reported, based on the formation of the tetra-Tms derivative of dexamethasone, using mass spectrometry with chemical ionization (CI)[7] or electron impact ionization (EI)[8]

In the present study, the use of mass spectrometry after adequate clean-up of urine and faecal samples was examined in order to allow the detection of dexamethasone in both matrices at the ppb level. In the second part of the study, the newly developed mass spectrometric method was applied to follow the elimination of dexamethasone from an adult cow. Oral administration was preferred over injection, as it was found that dexamethasone is frequently mixed with the feed in livestock production. The concentrations of dexamethasone in urine and faeces were determined. In Belgium, the latter matrix received a fast growing interest, not only because of the ease of sampling, but also by the detectability of several anabolic substances (*e.g.*, gestagens) that were never found in urine.

Experimental

Reagents

All solvents used were of analytical-reagent or HPLC grade and were obtained from Merck (Darmstadt, Germany). De-mineralized water was prepared with the Elgastat UHQ system (Elga, High Wycombe, UK). *Helix pomatia* digestive juice (β-glucuronidase and sulfatase) were obtained from Industrie Biologique Française (Pasture, Brussels, Belgium). Chem Elut CE 1020 and 1010 columns were supplied by Analytichem International (Harbor City, CA, USA). Sodium acetate (analytical-reagent grade) was obtained from Merck and pyridinium chlorochromate was obtained from Sigma (St. Louis, MO, USA).

* Presented at The Second International Symposium on Hormone and Veterinary Drug Residue Analysis, Oud St-Jan, Bruges, Belgium, May 31–June 3, 1994.

An enzyme linked immunosorbent assay (ELISA) kit for dexamethasone was purchased from CER (Marloie, Belgium). Cross-reactivity data [dexamethasone (100%) for cortisol and cortisone, were 5.6% and 1% respectively]. A kit for corticosteroids with cross-reactivities of 1.1% for cortisol and 0.3% for cortisone, was obtained from the Horseracing Forensic Laboratory (HFL) (Newmarket, UK), which was originally developed for the detection of corticosteroids in equine urine.

Dexamethasone, 9α-fluoro-16α-methyl-11β, 17α, 21-triol-1,4-pregnadiene-3,20-dione, and flumethasone, 6α,9α-difluoro-16α-methyl-11β,17α, 21-triol-1,4-pregnadiene-3, 20-dione, were purchased from Serva Feinbiochemica (Heidelberg, Germany) and Sigma, respectively.

Instrumentation

HPLC fractionation was performed using a Waters Millennium system (Millipore, Milford, MA, USA) consisting of a 600E pump, a 700 Satellite WISP automatic injector, a 996 photodiode array detector and a Waters fraction collector. The system was equipped with 2 automated switching valves and a Waters 501 backflush pump, enabling front-cutting on an RSIL C_{18} column of 5 cm × 10 mm i.d. with 10 μm particles (column I). Separation was further achieved on an Ultrasphere ODS-column, 25 cm × 10 mm i.d. with 5 μm particles from Beckman Instruments (column II, Fullerton, CA, USA).

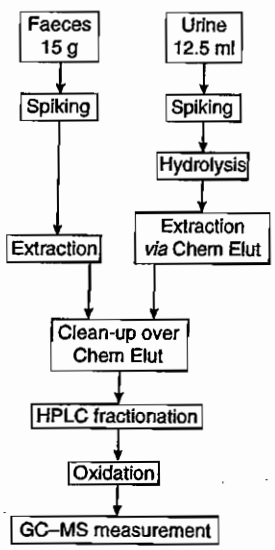
All GC–MS analyses were performed using a Hewlett-Packard Model 5989A MS Engine and a Model 5890 Series II gas chromatograph. The injector type used was split/splitless with a split ratio of 1:25. A Hewlett-Packard HP-5 MS fused-silica capillary column (25 m × 0.25 mm × 0.25 μm film thickness) was used. The carrier gas was high-purity helium (l'Air Liquide, Liège, Belgium) at a flow-rate of 0.8 ml min^{-1}. Methane was used as modifying gas at an ion-volume pressure of 133.3 Pa.

Microplates were measured with a microtitre plate reader from Eurogenétics (Tessenderlo, Belgium). Quantitative results were calculated with the available ELISA AID Software (Eurogenetics).

Experimental

Mass Spectrometry

Sample preparation for the mass spectrometric determinations included the following steps: spiking; hydrolysis (for the urine samples only); extraction; clean-up with HPLC fractionation; and chemical oxidation. (Scheme 1).



Spiking with internal standard

For urine samples, an aliquot of 12.5 ml was taken for analysis. Based on the levels of dexamethasone measured in the ELISA tests, the samples were divided into two series. To the high concentration samples (day 1 to day 8), 1.25 μg of flumethasone in 125 μl of methanol were added. The remaining urine samples were spiked with 125 ng of flumethasone in 12.5 μl of methanol.

As the faecal samples, in a first approximate mass spectrometric determination, showed a very large variation in the concentration of dexamethasone, further spiking was effected at two levels. To the samples (15 g) from day 2 to day 11, 6 μg of flumethasone in 60 μl of methanol were added, and to the second series 150 ng in 15 μl were added.

Hydrolysis of urine samples

After spiking of the urine samples, 5 ml of 0.2 mol l^{-1} acetate buffer (pH 5.2) were added, followed by 25 μl of *Helix pomatia* digestive juice and a few drops of chloroform. The mixture was incubated for 2 h at 62 °C.

Extraction

For the urine samples, the free steroids were extracted with diethyl ether (3 × 25 ml), over a Chem Elut CE 1020 column.

To the faecal samples, 10 ml of distilled water were added. This mixture was extracted twice with 50 ml of diethyl ether in a Teflon centrifuge bottle by mixing thoroughly for 2 h using a mechanical shaker.

Clean-up

About 15 min before application of the sample extracts, 1.5 ml of distilled water were applied to the underside of a Chem Elut CE 1010 column using a syringe. (In this way, an excessive loss of methanol can be prevented.) The combined ether extracts were evaporated under vacuum and the residue dissolved in 2.5 ml of methanol. The solution was applied onto the column. The flask was rinsed with 5 ml of a mixture of methanol–0.1 mol l^{-1} orthophosphoric acid (1 + 1, v/v), which was also brought on top of the column. The column was washed with 50 ml of petroleum spirit (boiling range 40–60 °C) and eluted with 50 ml of dichloromethane. The eluate, after washing with 10 ml of 1 mol l^{-1} carbonate buffer (pH 10.4) and 10 ml of distilled water, was evaporated to dryness.

HPLC fractionation

The residue was redissolved in 140 μl of a methanol–water mixture (80 + 20, v/v) and 100 μl were injected for fractionation over both columns in series. The eluent consisted of methanol–water (65 + 35, v/v) and the flow rate was held at 3 ml min^{-1}. After elution of the components of interest from column I (4.0 min), this column was back-flushed with pure methanol at a flow rate of 1.0 ml min^{-1}. After separation on column II, the eluent was changed to 100% methanol for 4 min. Collection started at the retention time of flumethasone minus 0.5 min and ended at the retention time of dexamethasone plus 0.5 min. Respective retention times, determined by injecting standards under the same conditions, were approximately 11.0 and 11.8 min.

Oxidation

The residue obtained after evaporation of the HPLC fraction in a vacuum concentrator (45 °C) was taken up in 50 μl of acetonitrile and 200 μl of an aqueous solution, containing 50 mg ml^{-1} pyridiniumchlorochromate and 25 mg ml^{-1} sodium acetate. The mixture was vortexed and heated at 92 °C for 3 h. After cooling, the oxidized compounds were extracted with 3 ml of *tert*-butylmethylether–dichloromethane (2 + 1, v/v) under vortexing and ultrasonication. Complete separation of the layers was achieved by centrifugation at 3500*g* for 5 min. After freezing, the organic phase was decanted and evaporated in a vacuum concentrator (45 °C). The residue was reconstituted with 25 μl of toluene.

GC–MS Determination

The oven temperature was initially held at 100 °C for 2 min, then raised to 280 °C at a rate of 20 °C min^{-1}, then kept constant for 10 min and raised again at a rate of 10 °C min^{-1} to 300 °C. The final oven temperature was held for 3 min. The split valve was opened after 2 min.

Quantitative measurements were made in selected ion monitoring (SIM) mode. The mass peak width for the *m/z* values used was 0.5 u. The dwell time was 50 ms. The source temperature was held at 150 °C. The ionization current was 300 μA and the ionization voltage was 230 eV.

A 1 μl volume of each sample was injected. Each series of determinations was accompanied by one blank sample and two samples spiked with dexamethasone at the same level of the internal standard. The concentrations of dexamethasone in the samples were calculated on the base of the peak-area ratio of dexamethasone over the internal standard.

Ruggedness test

The above described oxidation procedure was effected 8 times with moderate variations in the 6 variables given in Table 1.

Validation

In order to test the linearity, a blank urine and faecal sample were spiked in triplicate with dexamethasone at concentrations of 1, 5, 20, 100 and 500 ppb, and 20 ppb of flumethasone. For repeatability, determinations ($n = 6$) were carried out for both matrices at the levels of 1, 5 and 100 ppb of dexamethasone.

Accuracy was checked with 25 samples each of urine and faeces. The levels of spiking were 1, 5, 20, 100 and 500 ppb, with $n = 5$.

In order to calculate the absolute recovery for extraction and clean-up, three samples were spiked with only 20 ppb of internal standard, and one with only 5 ppb of dexamethasone. Prior to oxidation, 1, 5 and 100 ppb of dexamethasone were added to the samples containing 20 ppb of internal standard, and 20 ppb of internal standard were added to the sample containing 5 ppb of dexamethasone.

Enzyme Immunoassay (EIA) Procedure

The test procedures were performed following the instructions provided with the ELISA kits. The sample pre-treatment was restricted to a simple dilution of the urine with the assay buffers delivered with the kits. All the samples were assayed in duplicate.

For the HFL kit, all urine samples were diluted ten times and 100 μl portions of these dilutions were pipetted into the microtitre plate wells. The concentrations were read on a standard graph, which was made in diluted (ten-fold dilution) blank calf urine.

For the CER dexamethasone kit a ten-fold dilution of the urine samples was also prescribed. However, for the urine samples that were taken during the treatment, a higher dilution (50 and 100 times) was required. From every dilution, 50 μl were taken into the assay.

Animal Administration and Collection of Excreta

A 5 year old black-pied cow, weighing about 600 kg, received dexamethasone for 7 d. The daily dose was 50 mg and administration was oral. Samples of urine and faeces were collected from 12 h onwards after the first administration until 23 d after the last administration. A blank sample was taken 3 d before the start of the experiment. Samples were stored at −20 °C until analysis.

Results and Discussion

Mass Spectrometric Methodology

Method development

For the confirmation of EIA-positive results for dexamethasone in excreta of cattle, various mass spectrometric techniques

Table 1 Combination of factors used in the ruggedness test for the oxidation procedure

	Factor						
Combination	1 Oxidation time/ min	2 Oxidation temperature /°C	3 Sodium acetate/ mg ml^{-1}	4 PCC* mg ml^{-1}	5 ACN† (%)	6 Age of reagent (months)	Result
1	165	92	200	48	19	3	37.6
2	165	92	240	48	21	Fresh	33.6
3	165	98	200	52	19	Fresh	47.5
4	165	98	240	52	21	3	38.8
5	195	92	200	52	21	3	39.6
6	195	92	240	52	19	Fresh	35.0
7	195	98	200	48	21	Fresh	40.1
8	195	98	240	48	19	3	36.9
Effect of factors	−1.5	4.4‡	−5.2‡	3.2	−1.2	0.8	

* = Pyridinium chlorochromate.
† = Acetonitrile.
‡ = Significant at the 5% level ($t_{0.05}$).

were evaluated. The first method was one described by McLaughin and Henion.[8] Here, a *tetra*-Tms derivative of dexamethasone was made with bovine serum albumin (BSA) and analysed with EI–MS. With the above described system in SIM, 2 ng of dexamethasone resulted in a signal-to-noise (S/N) ratio for the molecular ion at *m/z* 680 of about 8, and 27 for the most intense ion at *m/z* 345. These results did not allow the detection of dexamethasone as Tms derivatives in excreta at the ppb level. Therefore, the sensitive method described by Kayganish *et al.*[9] with chemical oxidation of dexamethasone followed by NCI–MS was tested. In chromatograms, two well-resolved peaks were obtained in order of elution (16α- and 16β-methyl epimers of the 11, 17-keto analogue of dexamethasone) (Fig. 1). In the spectra of both compounds, the most intense ion was at *m/z* 310 $(M\text{-}HF)^-$, while the second was the molecular anion. This ion at *m/z* 330, had an intensity of about 30% and 8% against the first, for the α- and β-epimers, respectively. This method showed a large day-to-day variation, and even within one series the repeatability was very bad. Furthermore, this method is time consuming, and the way of eliminating the excess of oxidation reagent *via* a solid phase extraction cartridge is relatively expensive and demands large quantities of chlorinated solvents. To eliminate these disadvantages we tried to adapt the oxidation procedure.

The following differences were gradually introduced into the procedure of Kayganich:

(*i*) After dissolving the residue with 50 μl of acetonitrile, the oxidation was carried out in aqueous solution with dissolved pyridinium chlorochromate and sodium acetate instead of in the anhydrous conditions described.

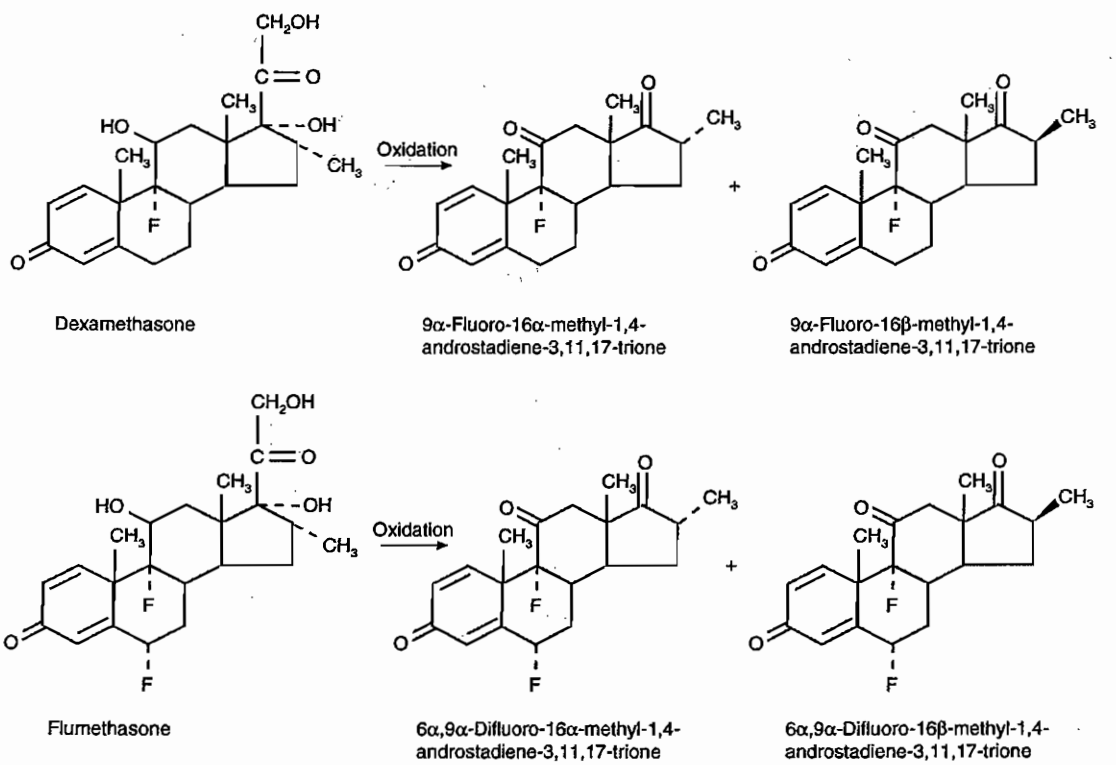
(*ii*) Reaction conditions applied were 92 °C for 3 h instead of 60 °C for 6.5 h.

(*iii*) The excess of oxidizing reagent was eliminated in a simple one-step extraction in the reaction vial instead of the more elaborate use of a solid phase extraction cartridge, needed to remove the undissolved oxidation reagent.

Temperature and time of oxidation were found to have a large influence on both the yield and on the relative intensity of the β-epimer to the α-epimer. Although higher temperatures and larger reaction times could still increase the yield on oxidized products by a few tenths of percent, conditions for temperature and time were chosen at 92 °C for 3 h. The reason was to obtain an optimal distinction between dexamethasone and its epimer betamethasone, which has a C-16 methyl group in the β-position instead of the α-position, and, thus, gives rise to the same oxidation products. Full details on the influence of the oxidation parameters on dexamethasone and betamethasone will be published later.

Because of the great similarity in structure between dexamethasone and flumethasone, the possibility of the use of flumethasone as an internal standard was examined. The structure of flumethasone, containing an extra fluorine atom on C-6 compared to dexamethasone, is shown in Fig. 1. As expected the oxidation of flumethasone also results in an α- and β-epimer, both with a molecular mass of *m/z* 348 and a base peak of *m/z* 328 $(M\text{-}HF)^-$. However, ions 310 and 330, typical for dexamethasone are also present. Therefore, the quantitative results obtained in the excretion experiment, based on the measurement of ion 310 of both epimers, were corrected for the contribution of the β-epimer of oxidized flumethasone.

In a ruggedness test on the new oxidation procedure the following factors were studied: oxidation time and temperature; concentration of sodium acetate and pyridinium chlorochromate; freshly prepared oxidation reagent; and finally, the percentage of acetonitrile in the oxidation medium. The test was performed according to Youden *et al.*,[10] with the combinations of factors given in Table 1. Further to the results the calculated effects of the variation of the factors are listed. Against the test value of 4.2, calculated according to Youden *et al.*,[10] only two factors had a significant influence on the result: temperature and sodium acetate concentration (a moderate increase of which led to lower results).



Dexamethasone — 9α-Fluoro-16α-methyl-1,4-androstadiene-3,11,17-trione — 9α-Fluoro-16β-methyl-1,4-androstadiene-3,11,17-trione

Flumethasone — 6α,9α-Difluoro-16α-methyl-1,4-androstadiene-3,11,17-trione — 6α,9α-Difluoro-16β-methyl-1,4-androstadiene-3,11,17-trione

Fig. 1 Oxidation of dexamethasone and flumethasone.

In a subsequent experiment the concentration of sodium acetate was varied from 10 to 220 mg ml^{-1}, with a constant amount of pyridinium chlorochromate (50 mg). The results obtained are shown in Fig. 2. An optimum yield of α-epimer is reached at about 25 mg ml^{-1}, which was further used in the procedure. In the absence of sodium acetate the oxidation of dexamethasone still takes place, which is not the case for the oxidation of betamethasone.

Method Validation

Specificity

The high specificity of the method is based on the combination of both the very efficient clean-up by HPLC fractionation (Fig. 3) and the highly selective detection achieved using NCI–MS. Typical GC–MS chromatograms obtained with urine and faeces are given in Figs. 4 and 5, respectively.

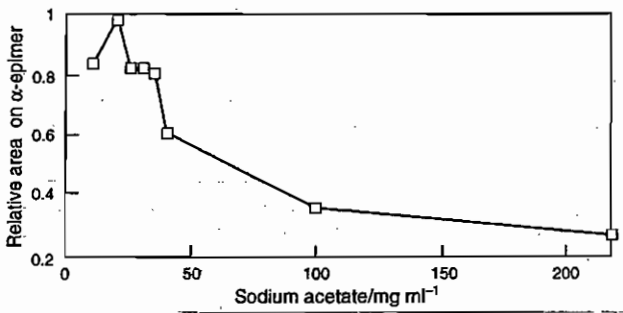


Fig. 2 Influence of the concentration of sodium acetate in the oxidation reagent on the yield of oxidized dexamethasone, expressed as area on α-epimer relative to the optimum of 25 mg ml^{-1}.

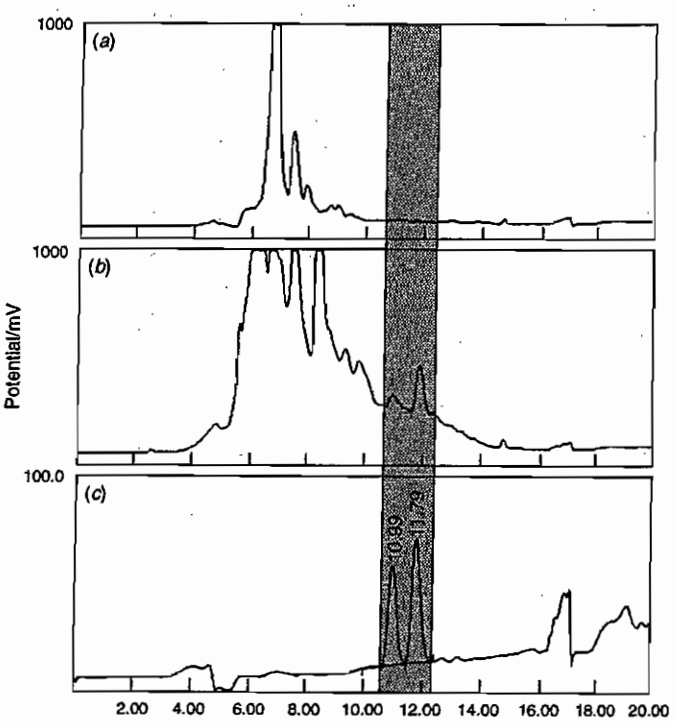


Fig. 3 Typical HPLC chromatograms. (*a*) Urine sample, and (*b*) faecal sample, with indication of the fraction collected corresponding to the elution of flumethasone (10.99 min) and dexamethasone (11.79 min) in the chromatogram of (*c*) the standards.

Linearity

The linearity was assessed by analysis of variance ($p = 0.05$). For urine samples a linear relationship between the concentration of dexamethasone and peak-area ratio of dexamethasone over the internal standard was found in the range of 1 to 500 ppb.

The regression equation was:

$$y = 0.20 + 0.23\,x, \text{ with } r^2 = 0.9998.$$

With faecal samples in the same range the analysis of variance led to a quadratic fit with the following regression:

$$y = -0.016 + 0.23\,x - 0.00004\,x^2, \text{ with } r^2 = 0.9991.$$

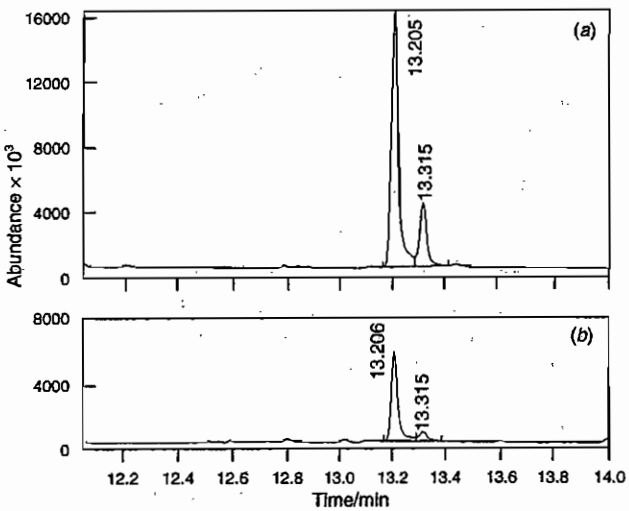


Fig. 4 Typical selected ion chromatograms of (*a*) ion 310 and (*b*) ion 330 under negative chemical ionization conditions of a urine sample spiked with dexamethasone at 1 ppb, with the α-epimer and the β-epimer of 'oxidized dexamethasone' at about 13.2 and 13.3 min, respectively.

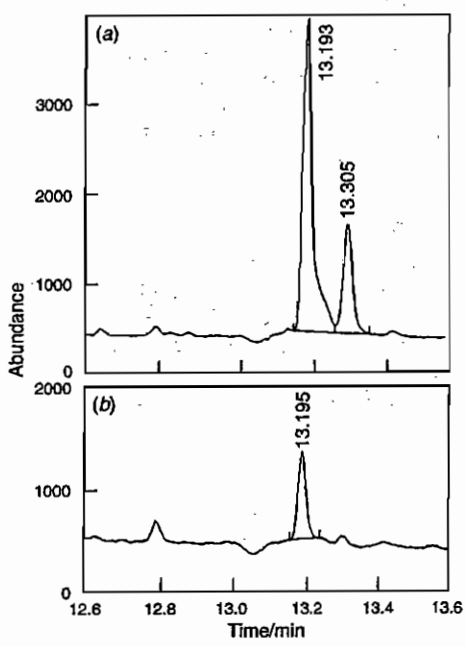


Fig. 5 Typical selected ion chromatograms of (*a*) ion *m/z* 310 and (*b*) ion *m/z* 330 under negative chemical ionization conditions of a faecal sample spiked with dexamethasone at 1 ppb, with the α-epimer and the β-epimer of 'oxidized dexamethasone' at about 13.2 and 13.3 min, respectively.

In the range of 1 to 100 ppb the relationship was found to be linear:

$$y = 0.0041 + 0.22\,x, \text{ with } r^2 = 0.9975.$$

Recovery

The absolute recovery in the range of 1 to 100 ppb for both dexamethasone and the internal standard, was 48% for faecal samples and 52% for urine samples.

Repeatability and accuracy

In Table 2, the means and relative standard deviations (s_r) of the concentrations found for six replicate determinations of dexamethasone, spiked at three levels, are listed for urine and faeces. All s_r values are within EU requirements[11] for repeatability.

The accuracy of the procedure was evaluated by means of the graphs of the concentrations found *versus* the concentrations added. The equation of the linear regression found for urine was:

$$y = (1.23 \pm 4.91) + (0.997 \pm 0.022)\,x, \text{ with } r^2 = 0.99986.$$

The equation for faeces was

$$y = (-0.0368 \pm 5.27) + (1.0002 \pm 0.023)\,x, \text{ with } r^2 = 0.99984.$$

Both graphs have a slope of unity and pass through the origin, indicating that there is no constant systematic error, nor a proportional systematic error.

Detection limit

The detection limit, obtained according to Mücke and Ebel,[12] for pure dexamethasone standard in concentrations varying from 1 to 32 pg μl^{-1}, was 0.25 pg μl^{-1}. This was based on the measurement of the molecular ion (*m/z* 330) of the α-epimer. For urine and faeces samples spiked at the level of 0.1 ppb, S/N ratios for the ion at *m/z* 310 $(M\text{-}HF)^-$ of the α- and β-epimers were about 250 and 50, respectively. The other important ions of the α-epimer at *m/z* 330 and 295 gave S/N ratios of about 100 for both.

This illustrates that the possibilities of the described procedure exceed the practical level of interest, *i.e.*, above 0.1 ppb. As the S/N ratios at this level are much larger than 30, according to Mass *et al.*,[13] a real quantification of the limit of detection is less important.

Excretion Study

The results from the excretion study obtained by GC–MS, using flumethasone as an internal standard, are given in Table 3. Comparison between GC–MS and EIA results in urine are presented in Table 4. The EIAs used were direct procedures, without extraction of HPLC purification. For control purposes HPLC-EIA is sometimes preferred in order to avoid some low false-positive results caused by endogeneous cortico-steroids. In this study, zero value obtained with both kits

Table 3 Dexamethasone concentrations measured by GC–MS in faeces and urine of a cow treated orally with 50 mg of the drug for 7 d. Times given in 24 h clock

			GC–MS	
Day	Treatment with 50 mg dexamethasone at	Sample collection	Dexamethasone/ in faeces/ ng g^{-1}	Dexamethasone/ in urine/ ng ml^{-1}
1	0800	2000	15.1	706
2	0800	0800	146	851
3	0800	2000	269	980
4	0800	0800	636	669
5	0800	2000	744	458
6	0800	0800	468	343
7	0800	2000	422	451
8		0800	596	287
9		0800	473	36.6
10		0800	231	34.7
11		0800	132	29.3
12		0800	18.3	17.6
13		0800	12.5	11.7
14		0800	4.89	7.24
15		0800	3.41	1.85
16		0800	1.05	1.16
17		0800	0.81	0.77
18		0800	0.65	0.98
19		0800	0.48	0.44

Table 4 Results from direct enzyme immunoassays for dexamethasone in urine compared with GC–MS results (concentrations are expressed in ng ml^{-1})

		Enzyme immunoassay kit used				
		HFL	Marloie			
Day	Dilution:	10×	10×	50×	100×	GC–NCI–MS
1		*	—	321	316	706
2		*	—	252	283	851
3		*	—	613	680	980
4		*	—	282	314	669
5		*	—	241	256	458
6		*	—	154	163	343
7		*	—	299	302	451
8		174	—	144	164	287
9		41	10	19	32	37
10		11	14	16	34	35
11		34	13	12	40	29
12		21	9.6	17	—	18
13		11	6.9	—	—	12
14		8.7	4.9	—	—	7.2
15		0	0.1	—	—	1.9
16		1.6	0	—	—	1.2
17		—	0	—	—	0.8
18		—	0	—	—	1.0
19		—	0	—	—	0.4

* Out of working range of the calibration curve.

Table 2 Repeatability data for dexamethasone in urine and faeces

		Urine		Faeces	
Concentration added (ppb)	*n*	Mean concentration found (ppb)	s_r (%)	Mean concentration found (ppb)	s_r (%)
1	6	0.92	18	1.03	4.6
5	6	4.77	17	5.03	4.5
100	6	102.1	2.5	100.7	4.2

about 8 d after withdrawal, indicates that the influence of cortisone is negligible. The results obtained with the HFL kit from day 8 until day 16 are in good accordance with those of the GC–MS measurements. The values obtained during treatment were out of the working range of the calibration graph. Hence, by further diluting the urine samples, with the CER kit, it was possible to make a better estimation of the concentration. The values obtained with this kit are in general somewhat lower than those obtained with GC–MS. A possible explanation that the cross-reaction with the dexamethasone conjugates present in the urine is less than 100%.

The excretion profiles, based on GC–MS measurements, are shown in Fig. 6. Excretion of dexamethasone *via* urine started very soon after ingestion. The first sampling after 12 h, reached a value that was already as high as the mean level observed during the 7 d of treatment. However elimination of the drug after the last treatment also occurred very rapidly: within 48 h the level was reduced by more than a factor of 10. From then on, the elimination rate was much slower: taking 6 d to see a further decrease by a factor of 10. The total period over which results were obtained that were above the blank value was 12 d after the end of the treatment. The urinary dexamethasone excretion profile obtained (after injection) is compared in Fig. 7 with a profile described by Delahaut *et al.*[4] As the dose in the previous experiment was only 30 mg, the values in the first period were much lower. A few days after treatment, however, the similarity in the rate of elimination was remarkable.

Although the maximum values in both profiles of urine and faeces were comparable, the elimination *via* faeces responsed much slower at the start and the end of treatment. The ratio of concentration in faeces to that in urine was only 2% 12 h after the first treatment and increased to about 25% after 60 h. From 3 d after the start until the last day of treatment, concentrations were very similar in both matrices. The first 4 d after ceasing administration concentrations were clearly higher in faecal samples: the mean ratio of concentrations in faeces over urine was more than 5. After this period, the concentrations in faeces were nearly identical to those in urine. Although the quantities of urine and faeces were not measured, it is clear that the proportion of total amount of dexamethasone excreted *via* urine and faeces was comparable, with perhaps a slight surplus being excreted *via* faeces. Indeed, the amount of faeces produced over urine is generally about 1.7 times higher. This appears to be very similar to the situation found in treated horses. According to Dumasia *et al.*[14] the horse, excreting 50–60% of dexamethasone and its metabolites *via* urine, takes an intermediate position between man and rat, with a predominantly urinary route (in man) and a mainly faecal route (in the rat), respectively.

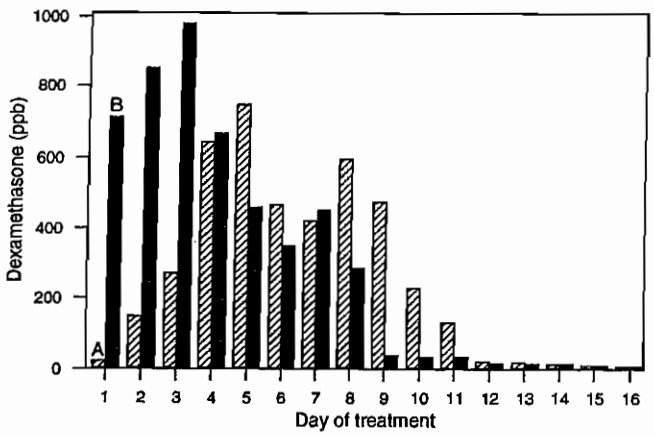


Fig. 6 Dexamethasone excretion profiles in A, faeces and B, urine of a cow treated orally with 50 mg of the drug for 7 d.

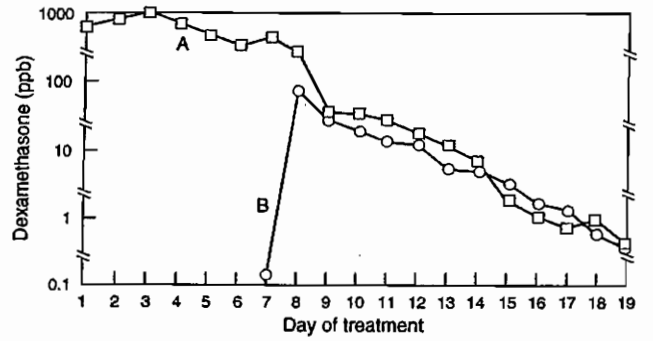


Fig. 7 Comparison of urinary dexamethasone excretion profiles obtained by A, oral administration (7 × 50 mg) and B, injection[4] (30 mg) of cows.

Conclusion

The elaborated mass spectrometric method for the determination of dexamethasone in urine and faecal samples proved to be very well suited for confirmatory purposes at the low concentrations that are often encountered. The superior sensitivity and selectivity towards other MS methods is obtained by the application of NCI–MS on oxidized dexamethasone. The high sensitivity and ruggedness also results from the far-reaching adaptation of earlier reported oxidation procedures.

The excretion experiment with an adult cow treated orally with dexamethasone showed that residues can be determined in both faeces and urine, even a considerable time after withdrawal. Although shifted in time, excretion profiles in these matrices were comparable in concentrations. Therefore, faeces, as well as urine, are well suited for the control on the misuse of dexamethasone in livestock production.

References

1 Sharpe, P. M., Haynes, N. B., and Buttery, J. P., in *Control and Manipulation of Animal Growth*, ed. Buttery, J. P., Lindsay, D. B., and, Haynes, N. B., Butterworths, 1986, p. 201.
2 Istasse, L., De Haan, V., Van Eenaeme, C., Buts, B., Baldwin, P., Gielen, M., Demeyer, D., and Bienfait, J. M., *J. Anim. Physiol. Anim. Nutr.*, 1989, **62**, 150.
3 Courtheyn, D., Verheye, N., Bakeroot, V., Dal, V., Schilt, R., Hooijerinck, H., Van Bennekom, E. O., Haasnoot, W., Stouten, P., and Huf, F. A., *Proceedings of the Euroresidue II Conference on Residues of Veterinary Drugs in Food, Veldhoven, The Netherlands*, ed. Haagsma, N., Ruiter, A., and Czedik-Eysenberg, P. B., 1993, p. 251.
4 Delahaut, Ph., Colemonts, Y., and Dubois, M., *Proceedings of the Euroresidue II Conference on residues of Veterinary Drugs in Food, Veldhoven, The Netherlands*, ed. Haagsma, N., Ruiter, A., and Czedik-Eysenberg, P. B., 1993, p. 262.
5 Skrabalak, D. S., Cuddy, K. S., and Henion, J. D., *J. Chromatogr.*, 1985, **341**, 261.
6 Her, G. R., and Watson, J. T., *Anal. Biochem.*, 1985, **151**, 292.
7 Kasuya, Y., Althaus, J. R., Freeman, J. P., Mitchum, R. K., and Skelly, J. P., *J. Pharm. Sci.*, 1984, **73**, 446.
8 McLaughlin, L. G., and Henion, J. D., *J. Chromatogr.*, 1990, **529**, 1.
9 Kayganich, K., Watson, J. T., Kilts, C., and Ritchie, J., *Biomed. Environ. Mass Spectrom.*, 1990, **19**, 341.
10 Youden, W. J., and Steiner, E. H., in *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Washington, D.C., 1975, p. 33 and p. 82.

11 Commision of the European Communities, 1989, *Off. J. Eur. Communities* **L351**, 39.
12 Mücke, G., and Ebel, S., *Fresenius' Z. Anal. Chem.*, 1985, **320**, 639.
13 Maas, C., Bravenboer, A., Van de Putten, A., and Salm, M., *De Ware(n)-Chemicus*, 1993, **23**, 90.
14 Dumasia, M. C., Houghton, E., Moss, M. S., Chakraborty, J., and Marks, V., *J. Steroid Biochem.*, 1986, **25**, 547.

Paper 4/05627B
Received September 15, 1994
Accepted October 10, 1994