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Elimination kinetics of dexamethasone in bovine urine, hair and feces following single administration of dexamethasone acetate and phosphate esters

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ABSTRACT

Corticosteroids are hormonal substances widely used in human and veterinary medicine for their anti-inflammatory properties. Among the numerous existing artificial corticosteroids, dexamethasone remains the most commonly used, mainly throughout esterified forms such as acetate or phosphate. An experimental study was designed to assess its drug residue levels in urine and feces, as well as its fixation in bovine hair following a single administration of 0.15 mg/kg b.w. dexamethasone acetate and 0.12 mg/kg b.w. dexamethasone sodium phosphate. Different analytical methods based on GC–MS or LC–MS/MS were used for measuring dexamethasone and its esterified forms, which were implemented in 3 different European laboratories in the field that collaborated for this study. The obtained results confirmed the high and rapid urinary excretion rate of dexamethasone, with a maximal concentration (267 μg/L) measured one day after administration and 98% elimination within 3 days. The concentrations obtained with the GC–NCI-MS procedure (using chemical oxidation as derivatization) were found significantly higher than the ones obtained with LC–ESI-MS/MS, indicating a possible contribution of dexamethasone phase I and/or II metabolites to the monitored signal. Fecal elimination was also found rapid (95% elimination within 3 days) with a maximum concentration level (28.5 μg/kg) observed one day after administration. Detectable levels of dexamethasone in hair appeared on day 2 (11.5 μg/kg), reached a maximum around one week, and could be identified until 22 days upon treatment, establishing the suitability of hair as a biological matrix for medium to long-term residue controls of dexamethasone.

1. Introduction

It is well known that synthetic glucocorticosteroids, especially at low dosages, increase the weight gain and reduce the feed conversion ratio in cattle [1,2]. Synthetic corticosteroids, such as dexamethasone have for these reasons been used as growth promoting additives, although this abuse is banned in the European Union [3]. For therapeutic indications only, the use of some glucocorticosteroids is allowed and therefore maximum residue limits (MRLs) have been established for bovine edible tissues (0.75 μg/kg in kidney and muscle, and 2 μg/kg in liver) and milk (0.3 μg/kg) [3].

A variety of sample preparation and analytical methods have been proposed for the identification of corticosteroid residues in edible tissues or urine samples [4–6]. Historically, gas chromatography coupled to mass spectrometry (GC–MS) was the first confirmatory method used for measuring these compounds at trace levels in biological matrices [7]. The further development of LC–MS and LC–MS^n with atmospheric-pressure ionization (API) has been responsible for spectacular improvements in method performances for these moderately polar compounds [8,9]. Besides this analytical diversity, some fundamental questions arise especially in terms of quantification performances and methodological comparability, for instance revealed through the high variability sometimes observed in inter-laboratory studies. In this context, one objective of the present study was to combine the experiences of three European laboratories involved in hormone residue analysis to propose a comparative study involving various sample preparation and MS measurement strategies for measuring dexamethasone in biological samples.

In drug residue analysis, knowledge on the pharmacokinetics, and in particular elimination, of administered drugs, is of major interest. Indeed, the identification of more relevant biological matrices to collect and the characterization of the detectability time windows of drugs may be of valuable interest for improving efficiency in residue control. Although several studies describe the

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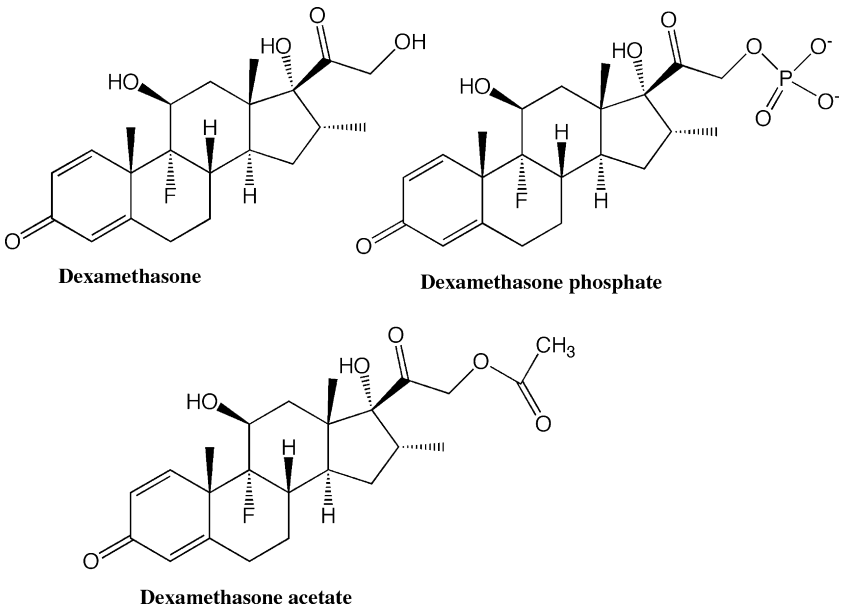


Fig. 1. Chemical structures of the corticosteroid of interest and its esters used in this study.

elimination rate of dexamethasone in cattle urine [10–12], previous work on the excretion kinetics in fecal samples is scarce. The same accounts for bovine hair. Although it has shown to be an attractive sample matrix for prolonged detectability of residues such as anabolic steroids and β-agonists [13], no previous reports are available on the incorporation of dexamethasone in bovine hair over time. In this context, the goal of the present study was to investigate the elimination kinetics of dexamethasone in cattle through non-invasive samples including hair, urine and feces, following a single dose administration. The influence of different administered esters (acetate or sodium phosphate) of dexamethasone on the analyte fixation was also investigated, since earlier studies provide evidence for potential differences depending on formulation [12,14] (Fig. 1).

2. Experimental

2.1. Reagents and chemicals

Methanol, cyclohexane, diethyl ether, ethyl acetate, glacial acetic acid and 1 M HCl were of analytical or HPLC grade and purchased from Solvents Documentation Syntheses (SDS, Peypin, France). Sodium acetate and sodium carbonate were purchased from Merck (Darmstadt, Germany). The enzyme for phase II metabolite deconjugation was a purified *Helix pomatia* preparation from Sigma–Aldrich (St. Louis, MO, USA). The derivatization reagents N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and trimethyliodosilane (TMIS), dithiothreitol (DTT) and the standard reference corticosteroids were purchased from Sigma–Aldrich. Fludrocortisone and dexamethasone-d_3 were used as internal standards. Standard solutions (1 mg/mL) were prepared in methanol. Working solutions were prepared by successive 10-fold dilution with methanol and stored in the dark at −20 °C.

2.2. Animal experiment

A healthy adult cow (600 kg) was simultaneously treated by intra-muscular injections of 25 mL Dexalone® Solution (equivalent to 90 mg of dexamethasone sodium phosphate) and 25 mL Dexalone® Suspension (equivalent to 75 mg of dexamethasone acetate). Simultaneous injection was preferred since this mimicks more closely the real life situation, where commercial preparations containing different dexamethasone forms are widely used. Urine, feces and hair samples were collected just prior to injection (D0) and on the following days upon injection (D1, D2, D3, D4, D6, D8, D10, D14, D17, D22, D27). All samples were stored at −20 °C before analysis.

2.3. LC–MS/MS analysis of urine and hair

2.3.1. Sample preparation

The analytical procedures used for extraction and purification of corticosteroids from urine and hair have been described earlier [15]. Briefly, a preliminary enzymatic hydrolysis of urine was carried out (*H. pomatia*, 50 °C, 4 h). For hair samples, acid hydrolysis (methanol/1 M HCl, 40/60, v/v, 50 °C, 4 h) of untreated hair was used for measurement of the parent drug (i.e. total dexamethasone), whereas direct liquid extraction of pulverized hair (methanol, ultrasonic bath, 30 min) was used for the measurement of potential ester form residues. All resulting extracts were then purified by reversed-phase SPE (C_{18}) with subsequent alkaline liquid–liquid clean-up (10% Na_2CO_3). A last purification stage by normal-phase SPE (SiOH) was applied for urine samples.

2.3.2. Liquid chromatography

HPLC separation was performed with an Alliance 2690 HPLC pump with automatic injector (Waters, Milford, MA, USA). Reversed phase liquid chromatography was performed on a 50 mm × 2 mm i.d., 5-μm particle, Nucleosil C_{18}AB octadecyl-grafted silica column (Macherey–Nagel, Düren, Germany) with guard column (10 mm × 2 mm i.d., 5-μm particle, Nucleosil C_{18}AB). The mobile phase was a gradient prepared from methanol (A) and 0.5% (v/v) acetic acid in water (B). Mobile phase composition (A/B; v/v) was: 0–10 min, 40/60; 10–20 min, 90/10; and 20–30 min, 40/60. The flow rate was 220 μL/min and the volume injected was 10 μL.

2.3.3. Mass spectrometry

Mass spectrometric analysis was performed on a QuattroLC triple-quadrupole analyzer (Micromass, Manchester, UK) operated in negative electrospray ionization (ESI) mode. Nitrogen was used as nebulization and desolvation gas, at flow rates of 90 and 600 L/h,

respectively. Source and desolvation temperatures were 130 and 400 °C, respectively. Potentials applied to the capillary (from 3.0 to 4.0 kV) and cone (from 15 to 35 V) were optimized for dexamethasone and its esters, as was the energy applied in the collision cell (from 5 to 30 eV). Four and two diagnostic signals were recorded in the multiple reaction acquisition mode (MRM) for dexamethasone and dexamethasone esters, respectively.

2.4. GC–MS analysis of urine

2.4.1. Sample preparation

The analytical procedure used for extraction of urine was described earlier [16] and involved a preliminary filtration step, an enzymatic hydrolysis (β-glucuronidase, 2 h, 62 °C), an HPLC fractionation, a chemical oxidation/derivatization reaction with potassium dichromate and a liquid/liquid extraction with a hexane/dichloromethane 2/1 mixture.

2.4.2. Gas chromatography–mass spectrometry

GC separation was performed on a BPX-35 GC column (25 m × 0.22 mm i.d., 0.25 μm). Gas chromatographic parameters were as follows: split/splitless injector temperature of 250 °C with a split vent flow of 20 ml/min; injection in splitless mode (split valve closed at −0.10 min, open at 1.00 min); ion source at 200 °C, damping gas flow of 0.3 ml/min (default). The MS acquisitions were performed in negative chemical ionization mode (NCI) with ammonia as reagent gas at a flow of 1 ml/min.

2.5. LC–MS/MS analysis of feces

2.5.1. Sample preparation

The analytical procedure for extracting dexamethasone from feces samples consisted out of an enzymatic hydrolysis step (β-glucuronidase, 2 h, 52 °C) with 20 μL *H. pomatia* juice after correcting the pH of the sample with acetic acid buffer (0.2 M). Next, 4 g of Tube hydromatrix granules (Varian Inc., Palo Alto, CA, USA) were added and the analytes extracted with 40 mL diethylether while shaking for 30 min. Subsequently, the sample was frozen till the underlayer was solid and the upper layer containing the analyte decanted in a round bottom flask using a funnel with filter. Finally, the ether phase was evaporated and 2 mL methanol/ethanol/isooctane (40/40/10) were added to solve the evaporated extract. The extract was transferred into a pyrex tube. After addition of 400 μL 0.2 M acetic acid, the extract was vortexed and centrifuged at 3400 rpm for 15 min. Extraction of the unconjugated dexamethasone fraction was performed by omitting the addition of *H. pomatia* from the above-mentioned procedure.

Clean up was performed by applying a double SPE extraction, i.e. Oasis HLB (Waters) and Bond Elut NH_2 (Varian Inc.) on the extracts. The eluent of the SPE columns was fractionated using a reversed phase C_{18} column (25 cm × 3.2 mm i.d., 5-μm particle, Alltima). The amount of dexamethasone present in the collected fraction was quantified with LC–MS.

2.5.2. Liquid chromatography–mass spectrometry

Chromatographic separation was achieved using a C_{18} column (25 cm × 3.2 mm i.d., 5-μm particle, Alltima) on a 1100 series quaternary pump and autosampler from Agilent Technologies (Santa Clara, CA, USA). The mobile phase consisted of (A) methanol/1% acetic acid in water (50/50) and (B) methanol and was pumped at a gradient flow. Mobile phase composition (A/B; v/v) was at 0 min: 80/20 to 12 min: 60/40 to 18 min: 30/70 to 18.1 min: 0/100, holding this for 2 min and then back to the initial conditions for 3 min. The flow rate was 500 μL/min and the volume injected was 20 μL.

The MS detector was a Thermo LCQ Deca ion trap equipped with an atmospheric pressure chemical ionization (APCI) probe in negative ion mode (Thermo Fischer Scientific, San Jose, CA, USA). To perform MS^2 and MS^3, the precursor isolation width was set to 3 Da, the activation *Q* to 0.5 and the collision energy to 22%.

2.6. Quality control

Before and after each analyzed series of samples, a standard mixture of the targeted corticosteroids and internal standards was injected to check the instrument parameters of the LC–MS/MS or $GC–MS^n$ devices. Quality control of the methods was performed by analysis of blank and spiked samples, together with every series of samples. Linear calibration curves ($R^2 = 0.99$) were constructed for dexamethasone by fortifying different blank urine aliquots, hair or feces samples (D0) at six different concentration levels. The concentrations ranged from 10 to 500 ng/mL in urine and from 1 to 500 ng/g in hair and feces samples. All methods were individually validated according to CD 2002/657/EC and have been routinely employed in the respective labs for over 10 years. For the urine and hair analysis by LC–MS/MS, quality control procedures and validation parameters have been published earlier [7,15,17,18]. The precision in terms of intra-laboratory reproducibility of the LC–MS/MS assay for dexamethasone in urine and hair, respectively, were 33.3 and 7.6% ($n = 72$). Optimization and validation of the GC–MS analysis procedure for urine is reported in Courtheyn et al. [16]. The precision in terms of repeatability of the GC–MS analysis in urine amounted 12.5% ($n = 18$). The LC–MS/MS method for dexamethasone in feces is an in house validated method of the Federal Laboratory for the Safety of the Food Chain in Belgium. The precision in terms of repeatability upon validation according to CD 2002/657/EC for this LC–MS/MS assay was 4.4% ($n = 18$).

3. Results and discussion

The information available regarding the pharmacokinetics of steroid esters of dexamethasone remains extremely scarce. In particular, the characterization of the incorporation of dexamethasone and its steroid esters in bovine hair, as well as the excretion profile of dexamethasone in cattle feces has to the best of our knowledge never been reported before. To provide specific information on the distribution of these steroid esters and their liberated active forms, it was decided to analyze all urine, feces and hair samples collected during the treatment period at least in duplicate. The use of different analysis techniques, i.e. GC–MS and LC–MS/MS and different extraction or sample preparation methodologies, i.e. with and without hydrolysis or derivatization, will allow a more accurate elucidation of the kinetic profile, and include some important mechanistic aspects on dexamethasone pharmacokinetics.

3.1. Dexamethasone excretion kinetics in urine

The kinetics of dexamethasone excretion in urine were determined by measuring the dexamethasone concentration in the successive urine samples collected from the treated animal by two different analytical techniques, i.e. GC–NCI-MS and LC–ESI-MS/MS, the latter with and without enzymatic hydrolysis (Fig. 2). For both analytical methods, the maximal concentrations were observed the day following the injection (day 1), and a tremendous decrease was noticed at day 3. Afterwards, the dexamethasone elimination rate decreased steadily, with values approaching the limit of detection in case of the more sensitive LC–MS/MS analysis (LOQ = 0.1 ng/mL) and below the detection limit for the GC–MS analysis (LOQ = 1 ng/mL) on day 8 after the treatment. Taken into account some differences in experimental protocol in comparison with previously published studies, our results confirm the rapid urinary excretion rate of dexamethasone in cattle, being rarely detectable after one week [10–12]. Calvarese et al. [10] indicated a

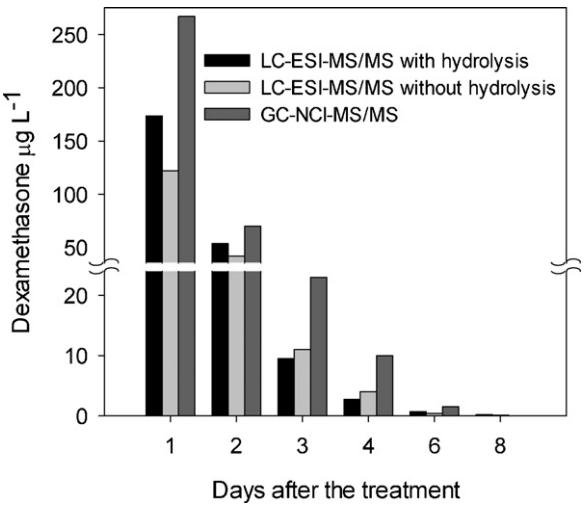
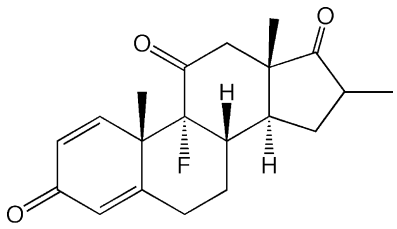


Fig. 2. Daily excretion profile of dexamethasone in cattle urine treated with a single dose of 0.15 μg/kg dexamethasone acetate and 0.12 μg/kg dexamethasone sodium phosphate, measured with three different analytical protocols. Each value is the average of two determinations.

urinary concentration of 2 ng/mL after 11 days following the last (of four) administrations of 0.15 mg/kg b.w. dexamethasone sodium phosphate in combination with 1 mg/kg b.w. of 19-nortestosterone. A possible explanation for this extended duration in excretion as compared to our results might be the different treatment schedule comprising four treatments on four successive days. In addition, their concomitant administration of 19-nortesterone may have prolonged the elimination rate of dexamethasone as well. Vincenti et al. [11] on the other hand reported the urinary dexamethasone level to drop below 2 ng/mL as early as 3 days after the last (of three) treatments. This discrepancy with our study can be explained by the lower dosage used during this study, which only amounted 0.06 mg/kg b.w. dexamethasone sodium phosphate. Another study, in which a similar relatively low therapeutic dose of dexamethasone sodium phosphate (0.02 mg/kg b.w.) and dexamethasone phenyl propionate (0.04 mg/kg b.w.) were administered as a single dose, did however report an average concentration of 26 ng/mL after 3 days of treatment, more in accordance with our results [12]. This long-acting phenyl propionate ester has a similar activity than the acetate ester used during our study. The relative higher hydrophobicity of phenyl propionate esters and acetate esters has indeed been reported to reduce the urinary elimination rate and as a result prolong the elimination duration of dexamethasone upon administration [7,11].

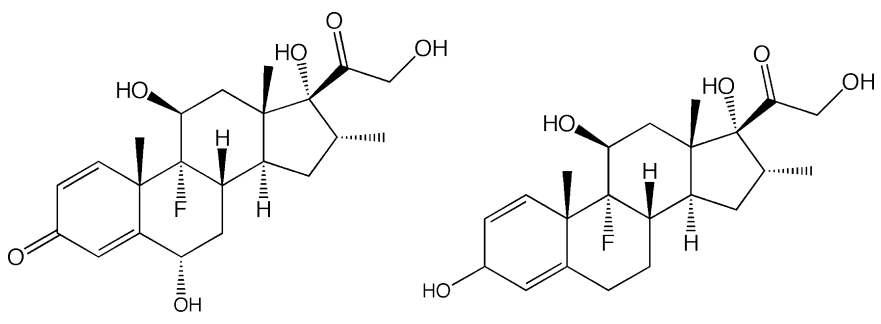
Besides the provided information regarding the pharmacokinetics of dexamethasone, a second important aspect of this study lies in the different analytical techniques applied to the urine



9a-Fluoro-16α/β-methyl-1,4-androstadiene-3,11,17-trione

Fig. 3. Derivatization products upon oxidation of dexamethasone.

aliquots. As mentioned earlier the GC–NCI-MS analysis is preceded by a derivatization through chemical oxidation. This derivatization comprises of an oxidation of the acidic side chain and free hydroxyl substituents of dexamethasone into ketogroups [16], resulting in the structure depicted in Fig. 3. As demonstrated in Fig. 2, the determined concentrations in the successive urine aliquots were significantly higher for the GC–NCI-MS analysis as compared to the LC–ESI-MS/MS analysis. The same, but to a lesser extent could be observed between the non-hydrolyzed and hydrolyzed urine aliquots within the LC–ESI-MS/MS analysis. These differences in concentration between the analysis techniques may be attributed to phase I and II reaction products, which may account for a certain fraction of the dexamethasone signal monitored in urine. To discriminate between these different measured fractions and the resulting concentrations reported, it is important to gain a better understanding of the nature of these phase I and II metabolites. Despite its extensive use, little work is however available about the dexamethasone biotransformation in cattle. *In vivo* studies performed in camels revealed two phase I metabolites, the major one arising from reduction of the 3-carbonyl group in ring A (reduced dexamethasone), mediated by hydroxysteroid dehydrogenases, the minor one resulting from the cytochrome P450-dependant hydroxylation of the same ring (6-OH-dexamethasone) (Fig. 4). Phase II metabolism on the other hand, primarily involves conjugation through glucuronidation or sulfatation and this at the level of both the phase I metabolites and the parent compound [19]. Antignac et al. [20] measured the proportion of total conjugated (sulfate + glucuronide) forms of dexamethasone in cattle urine upon administration, and recorded an average of 4–27% of the urinary excreted dexamethasone as mono-conjugated, with the major conjugate being sulfated dexamethasone (2.9–17.2%). The differences in concentrations noted within the LC–ESI-MS/MS analysis between the sample preparations with and without hydrolysis may thus be explained by phase II conjugates (glucuronides and sulfates) of the parent compound. The discrepancy in concentrations between the LC–MS/MS and chemical oxidation GC–NCI-MS procedure on the other hand, may be accounted for by phase I metabolites



6-OH-dexamethasone **Reduced dexamethasone**

Fig. 4. Suggested major phase I metabolites of dexamethasone in cattle.

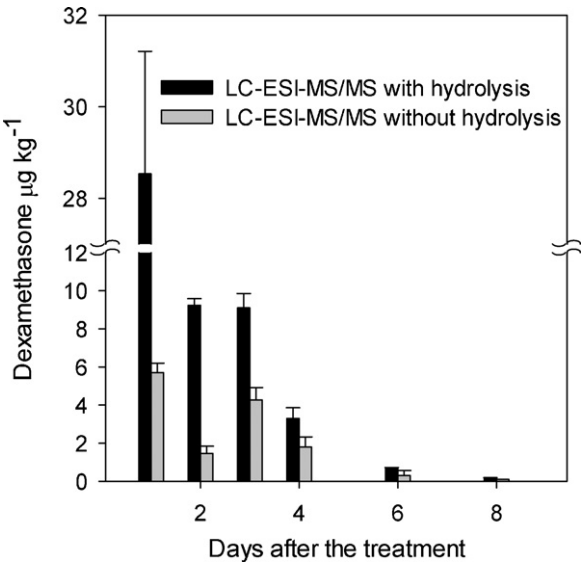


Fig. 5. Daily excretion of dexamethasone in cattle feces treated with a single dose of 0.15 μg/kg dexamethasone acetate and 0.12 μg/kg dexamethasone sodium phosphate, upon measurement with different analytical protocols. Results are presented as means ± SD ($n = 3$).

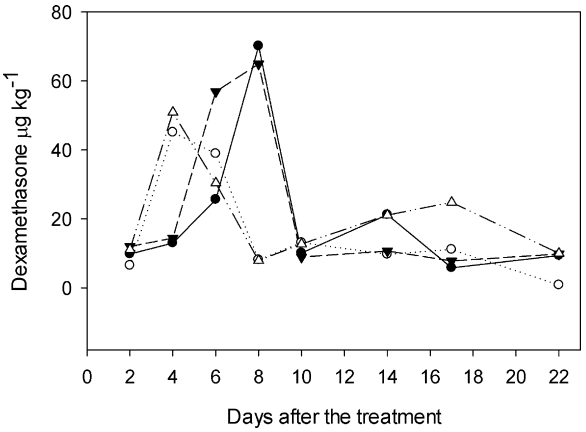


Fig. 6. Concentrations of dexamethasone retrieved in hair from a cow treated with a single dose of 0.15 μg/kg dexamethasone acetate and 0.12 μg/kg dexamethasone sodium phosphate. LC–ESI-MS/MS analysis of hair of the (○) left flank without hydrolysis, (●) right flank without hydrolysis, (△) left flank with hydrolysis and (▼) right flank with hydrolysis.

or phase I metabolite conjugates. In particular the reduced dexamethasone and its glucuronide or sulfate conjugates may undergo a similar oxidation during derivatization as dexamethasone itself, likewise resulting in the molecule presented in Fig. 3 and an identical signal upon GC–MS analysis. Further research is warranted to identify the nature and relative abundance of phase I metabolites in cattle urine. These results however highlight the relative significance of phase I and II metabolism in the urinary disposition of dexamethasone. Phase I metabolites may be accounted for by the difference in concentration measured between the GC–MS analysis and the LC–MS/MS analysis with hydrolysis and seem particularly abundant. Parent compound phase II metabolites are represented by the difference in concentration between the LC–MS/MS analysis with and without hydrolysis and seem of inferior importance in urine.

3.2. Dexamethasone excretion kinetics in feces

The elimination rate of dexamethasone in feces was determined by analyzing its concentration in the series of feces samples collected from the treated animal by LC–ESI-MS/MS (Fig. 5). To this purpose, two different extraction protocols, namely with and without enzymatic hydrolysis, were employed. In both cases, the maximum concentration of dexamethasone was reached on the first day (D1) after treatment. Ten days (D10) after treatment dexamethasone could not be detected above the LC–ESI-MS/MS quantification limit (LOQ = 0.1 μg/kg). These results support the hypothesis that dexamethasone is not only rapidly excreted in urine, but fairly rapid in feces as well. To the best of our knowledge, only one previously published study reports the analysis of dexamethasone in feces upon therapeutical administration [16]. During this study, the treatment regime differed significantly from ours since administration of dexamethasone was performed during 7 days with an oral dose of 0.08 mg/kg b.w. per day. In addition, analysis was carried out by GC–NCI-MS preceded by oxidative derivatization as opposed to the LC–ESI-MS/MS used in our study. One day after the first administration, a concentration of 15.1 μg/kg was noted by Courtheyn et al. [16], which is within the same order of magnitude than the 28.5 μg/kg that was obtained by LC–MS/MS analysis upon acid hydrolysis during our study. The remaining discrepancy in concentration may be explained by the higher dosage used during our study. Complete elimination of dexamethasone from feces, was however not obtained during the length of study by Courtheyn et al. [16], since 8 days after the last administration a concentration of 1.9 μg/kg was still recorded, which is a factor 10 higher than the concentrations we observed at that time. The different dosing regiment (i.e. during seven consecutive days) probably lies at the origin of this phenomenon. Successive lower doses are as a result more likely to cause longer fecal elimination profiles, as compared to one-time high doses.

Remarkable with regard to the fecal excretion results, were the large differences in concentration noted between the samples with and without hydrolysis. In line with previous arguments, this 5-fold difference may be explained by phase II conjugates (glucuronides and sulfates) of the parent compound, measured upon hydrolysis. This implies that a large fraction of the parent compound phase II metabolites is excreted fecally.

3.3. Dexamethasone disposition in hair

Dexamethasone disposition in hair was determined by analyzing the successive hair samples collected from the right and left flank of the treated animal. Two different extraction procedures were applied to the hair samples, namely with and without acid hydrolysis (Fig. 6). Remarkably, dexamethasone was detected in hair relatively fast after the treatment (i.e. 2 days) at an average concentration of 11.5 μg/kg, which tends to confirm a rapid diffusion. This rapid diffusion may be attributed to the keratin binding mechanism of hair, which has been already reported for other xenobiotics such as β-agonists and anabolic steroids [13,21,22]. Drug substances, which are present in systemic circulation are not only taken up during histogenesis directly from the blood, but may be absorbed or transferred to the keratinized hair from perspiration (sweat, sebum and transdermal excretion) as well [23]. Afterwards, the dexamethasone elimination rate increased steeply and reached a maximum on day 4 and day 8 for the left and right flanks, respectively. Similar concentration profiles for dexamethasone were obtained with the two tested methods, implying that only free and no conjugated dexamethasone was allocated to the hair. Finally, no traces of acetate or phosphate esters were detected in these hair samples, indicating complete liberation from the esterified forms before diffusion to the hair. These results are in line with previous research, demonstrating the presence of hydrophilic esters (hemisuccinate) but not of lipophilic esters (acetate) of

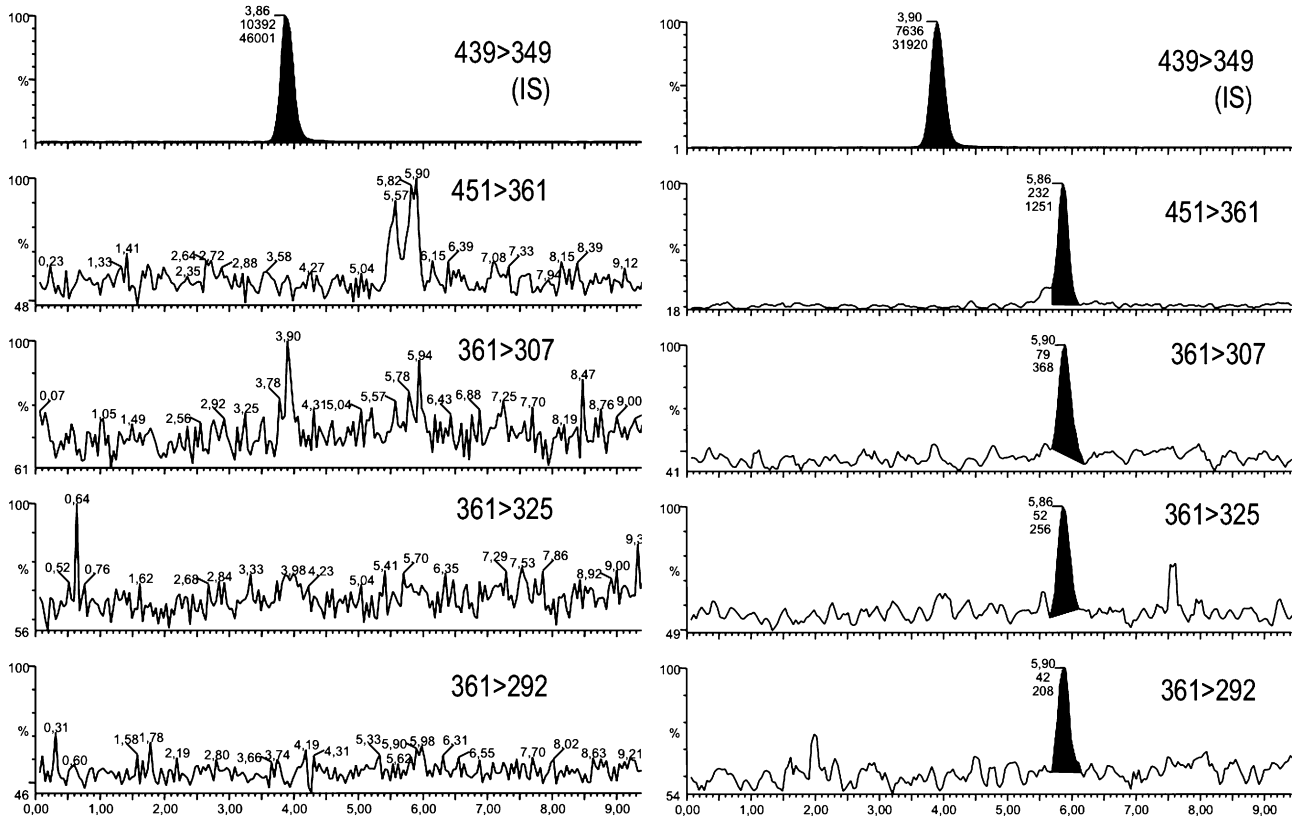


Fig. 7. Diagnostic ion chromatograms obtained for fludrocortisone (ISTD) and dexamethasone in bovine hair samples collected before (D0) and 22 days after (D22) a therapeutic treatment with 0.15 μg/kg dexamethasone acetate and 0.12 μg/kg dexamethasone sodium phosphate.

methylprednisolone in hair of an adult cow upon administration with 120 mg methylprednisolone hemisuccinate [7]. Although the presence of ester forms in hair has been reported for other groups of compounds including anabolic steroids [24], the fixation of such esterified forms appears as a complex issue, with non-homogenous results depending on the considered parent drug and the exact nature of the considered ester (i.e. relatively hydrophilic or at the contrary more lipophilic). Finally, dexamethasone could be properly identified in hair until day 22 after treatment (Fig. 7), confirming the previously demonstrated prolonged detectability of residues in hair as opposed to urine and feces [13,21,22] for dexamethasone as well. This finding underpins the attractiveness of hair as a biological matrix for medium to long term screening in residue control of dexamethasone.

4. Conclusions

For the first time the elimination kinetics of the main representative of the glucocorticoid family, i.e. dexamethasone, was determined in urine, feces and hair, upon administration of a therapeutic dose to a cow. Urinary excretion profiles were in line with previously reported studies, both in terms of elimination rate and concentration, when taken into account the differences in experimental protocol. Remarkable were the significant higher concentrations yielded when applying the GC–NCI-MS method, preceded by an oxidative derivatization. This finding may be explained by a contribution of reduced dexamethasone (phase I metabolite) and its phase II glucuronide or sulfate conjugates to the signal monitored, since these metabolites result in the same final oxidated molecule upon derivatization. Fecal elimination proved also to be quite rapid, but notably lower than urine excretion. Phase II metabolites and/or esterified derivates were more abundant than free dexamethasone in feces, since hydrolysis yielded 5-times higher concentrations. Fixation in hair appeared to be detectable quite rapidly (2 days after treatment). Finally, dexamethasone could be still properly identified around 3 weeks after treatment, demonstrating the attractiveness of hair as a biological matrix in residue control of dexamethasone.

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