JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Immunochemical Screening and Liquid Chromatographic−Tandem Mass Spectrometric Confirmation of Drug Residues in Edible Tissues of Calves Injected with a Therapeutic Dose of the Synthetic Glucocorticoids Dexamethasone and Flumethasone

OLIVIA VAN DEN HAUWE,*,† MANUELA SCHNEIDER,‡ ALI SAHIN,‡ CARLOS H. VAN PETEGHEM,† AND HANSPETER NAEGELI‡

Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium, and Institute of Pharmacology and Toxicology, University of Zürich-Tierspital, August Forel-Strasse 1, CH-8008 Switzerland

A field study was performed to assess the drug residue level in edible tissues after a therapeutic application of the synthetic glucocorticoids dexamethasone and flumethasone. Three diseased calves were injected intramuscularly with a commercial batch of dexamethasone esters and slaughtered 72 h after treatment. Another three calves were injected intramuscularly with an aqueous flumethasone preparation and slaughtered 24 h later. Residues of synthetic glucocorticoids in liver, muscle, kidney, and urine were assessed by competitive enzyme immunoassay. All dexamethasone concentrations exceeded the maximal residue level of 0.75 μg/kg in muscle and kidney and 2 μg/kg in the liver. The presence of both dexamethasone and flumethasone in the liver was confirmed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). These results indicate that liver tissue provides a suitable matrix to monitor the presence of illegal residues of synthetic glucocorticoids in slaughtered animals.

KEYWORDS: Hormones; antiinflammatory agents; growth promoter; food safety

INTRODUCTION

Synthetic glucocorticoids belong to the group of drugs most frequently administered in livestock production. After the introduction of a 1,2 double bond and methylation at position C16 (**Figure 1**), fluorinated hydrocortisone derivatives such as dexamethasone (9α-fluoro-16α-methylprednisolone) or flumethasone (6α,9α-difluoro-16α-methylprednisolone) achieve an increased glucocorticoid potency with complete loss of mineralocorticoid activity (*1*, *2*). Therapeutic indications of these potent synthetic hormones include inflammatory diseases, disorders of the musculoskeletal system, skin diseases, and, in cows, primary ketosis (*3*−*5*). The same drugs may stimulate appetite and are able to induce parturition when administered in the last trimester of pregnancy. In conjunction with antimicrobial agents, synthetic glucocorticoids often serve as supportive therapy to shorten the period of clinical signs. High doses of synthetic glucocorticoids can be administered because these drugs are tolerated in amounts 10 times the normal therapeutic range or more without any risk of acute toxicity (*6*). In addition, various esters displaying limited water solubility have been developed for slow intramuscular resorption and prolonged times of action. Synthetic glucocorticoids also result in increased feed intake and weight gain (*7*, *8*), but the application of these synthetic hormones as growth-

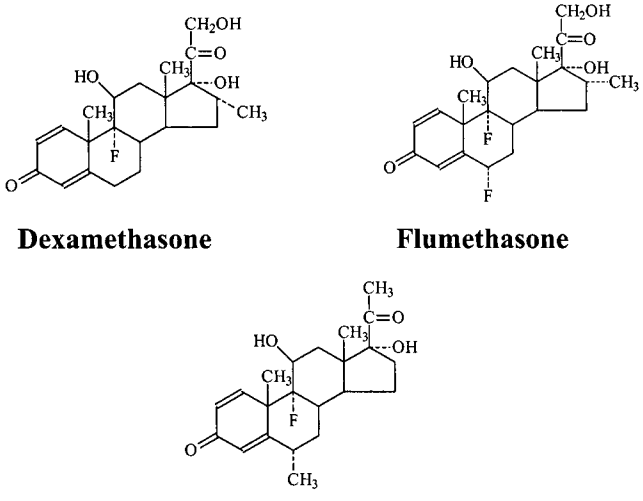

Figure 1. Structural formulas of dexamethasone, flumethasone, and fluorometholone.

* To whom correspondence should be addressed. Telephone: +32-9-264 81 16. Fax: +32-9-264 81 99. E-mail: olivia.vandenhauwe@rug.ac.be.
† Ghent University.
‡ University of Zürich-Tierspital.

10.1021/jf020533m CCC: $25.00
Published on Web 11/23/2002

promoting additives was banned by most regulatory agencies. Within the European Union, for example, the use of dexamethasone, betamethasone, prednisolone, and methylprednisolone is approved in livestock only for therapeutic indications, and, for that purpose, appropriate maximal residue limits (MRLs) in tissues and milk intended for human consumption have been determined (*9*−*13*). In the case of dexamethasone, toxicological studies in rats yielded a no-effect level of 1.5 μg/kg of body weight, while higher drug doses led to tyrosine aminotransferase induction in the liver (*14*). After correction with a safety factor of 100, this no-effect level has been converted to an acceptable daily intake (ADI) of 0−0.015 μg/kg of body weight, yielding the following MRLs for dexamethasone in cattle: 2 μg/kg in liver, 0.75 μg/kg in kidney and muscle, and 0.3 μg/kg in milk (*15*). Depending on the particular glucocorticoid formulation, withdrawal periods of up to several weeks are recommended to avoid illegal residues in animal products.

Previous reports demonstrated that sensitive immunological assays may be employed for high-throughput screening (*16*−*20*), while positive results could be confirmed by gas or liquid chromatography combined to mass spectrometry (*21*−*25*). The goal of this field study was to test the practicability of current protocols for the detection of synthetic glucocorticoid residues in edible tissues. Two groups of calves affected by respiratory infections were treated intramuscularly with a standard therapeutic dose of commercial preparations containing either a mixture of two dexamethasone esters or the biologically more potent flumethasone analogue. Residues of synthetic glucocorticoids in liver, muscle, kidney, and urine were assessed by competitive enzyme immunoassay. Dexamethasone and flumethasone were identified in the liver by liquid chromatography combined to tandem mass spectrometry (LC-MS/MS).

MATERIALS AND METHODS

Reagents and Materials. Radiolabeled [1,2,4-^{3}H]dexamethasone (60 Ci/mmol) was obtained from Amersham (Buckinghamshire, U.K.). Dexamethasone, flumethasone and fluorometholone reference standards were purchased from Sigma-Aldrich (Bornem, Belgium). *Helix pomatia* juice (containing glucuronidase and arylsulfatase) was from Boehringer (Mannheim, Germany, cat. no. 127698). Subtilisin A was from Sigma (Buchs, Switzerland). Acetonitrile, hexane, and methanol were obtained from BDH (Poole, Dorset, U.K.), ethyl acetate and acetone from Acros (Geel, Belgium), and formic acid and ethanol from Merck (Darmstadt, Germany). Baker C_{18} solid-phase extraction cartridges of 6-mL capacity were from Devos-François (Charleroi, Belgium). The Hypercarb column (100 × 2.1 mm) with a particle size of 5 μm and a protecting guard column (20 × 2 mm, 5 μm) were from Hypersil (Runcorn, U.K.). Dexafort was from Intervet International (Boxmeer, The Netherlands) and Flumilar from Veterinaria (Zürich, Switzerland). The glucocorticoid screening was performed with a microtiter enzyme immunoassay kit that uses antibodies raised against protein-conjugated dexamethasone (Neogen, Lexington, KY). According to the manufacturer, the antibodies display the following cross-reactivities with relevant structural analogues: dexamethasone, 100%, flumethasone, 49%, betamethasone, 1.5%, prednisolone, 0.25%, and hydrocortisone, 0.11%.

Instruments. The immunoassays were quantified by using a Bio-Tek microplate reader (Witec, Switzerland). Liquid chromatography analyses were carried out with a Waters Alliance 2690 HPLC system (Millford, MA) coupled to a Quattro LCZ triple quadrupole mass spectrometer (Micromass, U.K., Wythenshawe, Cheshire, U.K.).

Animals. Groups of three calves were selected from two different farms in Switzerland. These animals were diagnosed with respiratory infections on the basis of clinical signs (dyspnoea, nasal discharge, and coughing) and treated by repeated oral administration of sulfachlorpyrazidine and trimethoprim in combination with a single glucocorticoid injection. The animals were fed a milk replacer and kept with access to water and hay ad libitum.

Table 1. Animals, Glucocorticoid Preparation, Dosages, and Time of Slaughter after Treatment

calf no.	preparation	synthetic glucocorticoid	Dose, μg/kg	time of slaughter after treatment, h
1, 2, 3	Dexafort	dexamethasone sodium phosphate dexamethasone phenylpropionate	20 40	72
4, 5, 6	Flumilar	flumethasone	20	24

Drug Administration and Tissue Sampling. The glucocorticoid treatment consisted of a single dose injection of dexamethasone esters or of flumethasone (**Table 1**) into the neck muscles in amounts corresponding to the common therapeutic range. Three calves were treated with a conventional depot mixture, which contained the short-acting sodium phosphate ester in combination with the long-acting phenylpropionate ester of dexamethasone. The drug dose was 20 μg/kg of body weight for the sodium phosphate and 40 μg/kg of body weight for the phenylpropionate ester, yielding a total dose of 60 μg/kg (label dosage: 3−9 mg dexamethasone per calf). Because of its higher biological potency, flumethasone was injected at a lower dose of 20 μg/kg of body weight (label dosage: 1.25−2.5 mg per animal). A commercially available aqueous preparation of flumethasone intended for fast intramuscular absorption was tested. The animals treated with dexamethasone were slaughtered 72 h after drug administration, while the animals treated with flumethasone were slaughtered 24 h after injection. Several tissue samples of about 50 g were collected from the liver, kidney, and quadriceps femuralis. The accompanying urine could be obtained from five animals. All samples were stored in labeled plastic boxes at −80 °C until analyzed.

Immunochemical Screening. Previous extraction procedures (*16*, *19*) were optimized to obtain higher recoveries from tissues samples. Aliquots (5 g) of minced liver or kidney were homogenized in 10 mL of 3 M acetate buffer (pH 4.6) using a Polytron device (Kinematica GmbH, Kriens, Switzerland). Each sample was supplemented with 50 μL of *Helix pomatia* juice and incubated for 1 h at 60 °C. The hydrolyzate was mixed with 20 mL of acetonitrile. After shaking for 30 min, the acetonitrile layer was vortexed with 8 mL of hexane and 2 mL of dichloromethane. This yielded a three-phase liquid system, in which dexamethasone partitioned in the middle acetonitrile phase. The acetonitrile layer was recovered and evaporated to dryness. The final residue was dissolved in 0.5 mL of phosphate-buffered saline containing 0.01% (v/v) Tween 20. The muscle samples were processed as described by Daeseleire et al. (*26*) with the following modifications. Briefly, aliquots (1 g) of minced muscle were homogenized in 4 mL of 0.1 M Tris-HCl (pH 9.6) and digested with 1 mg of subtilisin A for 2 h at 37 °C. After addition of another 1 mg of subtilisin A, the digestion was continued for 2 h at 60 °C. The digest was then extracted with two 5-mL volumes of diethyl ether and the combined fractions were evaporated to dryness. The residue was taken up in 1 mL of methanol and 4 mL of water. This solution was immediately applied onto a C_{18} solid phase extraction column, previously conditioned with 2 × 5 mL of methanol and 2 × 5 mL of water. After washing the column with 2 × 5 mL of water, the analytes were eluted with 2 mL of methanol. The dried residue was dissolved in 0.25 mL of phosphate-buffered saline/0.01% Tween 20. Urine was processed without enzymatic hydrolysis as described by Derendorf et al. (*27*). Briefly, aliquots (1 mL) of urine were mixed with 1 g of ammonium sulfate and extracted twice with 3-mL portions of ethyl acetate. The organic phases were pooled and evaporated to dryness. The resulting residue was redissolved in 0.5 mL of phosphate-buffered saline/0.01% Tween 20. Before extraction, a trace amount of ^{3}H-labeled dexamethasone was added to all probes to determine the analytical recoveries. All sample cleanup procedures were performed in triplicate and the extracts were tested for synthetic glucocorticoids by a commercial ELISA kit, according to the manufacturer's instructions. Strongly positive samples were diluted to obtain quantitative results within the linear range of the assay. Dexamethasone and flumethasone concentrations were individually corrected for recovery, calculated by the radiotracer technique. The partial reaction with flumethasone (49% cross-reactivity) was taken

Table 2. Concentration of Synthetic Glucocorticoids (μg/kg) Determined by Enzyme Immunoassay (Mean ± Standard Deviation; $n = 3$) and LC-MS/MS ($n = 2$)

calf no.	glucocorticoid injected	liver (immunoassay)	liver (LC-MS/MS)	muscle (immunoassay)	kidney (immunoassay)	urine (immunoassay)
1		32.8 ± 7.1	30.7 ± 0.7	4.5 ± 0.7	15.0 ± 1.8	14.8 ± 2.8
2	Dexafort	4.1 ± 1.1	3.1 ± 0.01	0.8 ± 0.2	2.4 ± 0.4	21.8 ± 4.2
3		21.2 ± 1.8	13.1 ± 1.0	2.0 ± 0.4	8.1 ± 1.7	42.5 ± 7.8
4		2.5 ± 0.5	5.87 ± 0.4	1.3 ± 0.2	2.9 ± 0.1	4.8 ± 1.1
5	Flumilar	31.9 ± 4.3	a	0.6 ± 0.2	16.0 ± 4.0	11.9 ± 2.6
6		2.5 ± 0.5	6.95 ± 0.3	0.4 ± 0.1	2.0 ± 0.2	not available

[a] Below limit of detection.

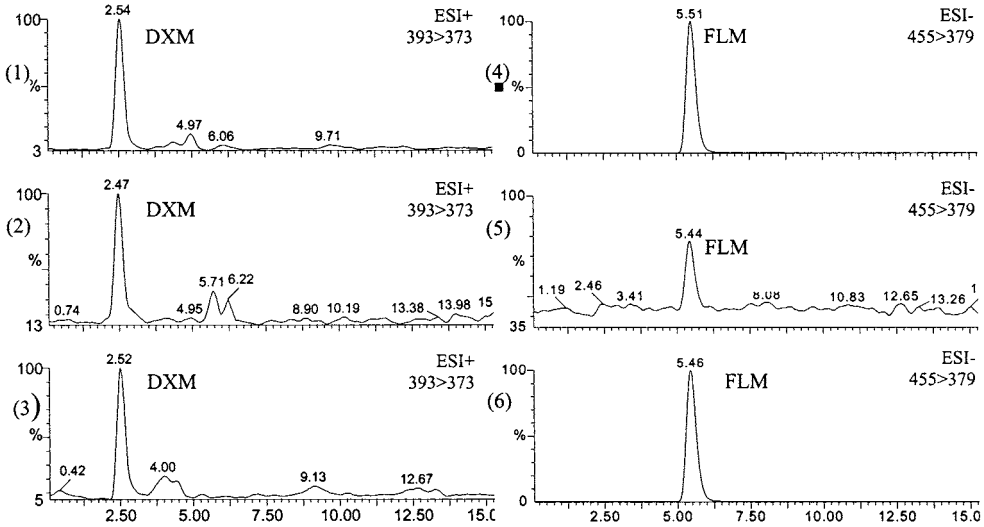

Figure 2. LC-MS/MS (MRM) chromatograms of liver sample extracts of calves nos. 1–3 showing the ESI+ (393 >S 373) transition of dexamethasone (DXM) and of calves nos. 4–6 showing the ESI– (455 > 379) transition of flumethasone (FLM)

into account when calculating the tissue flumethasone concentrations. For example, an apparent dexamethasone concentration of 1 μg/kg in the tissue of Flumilar-treated animals translates to a flumethasone concentration of 2.04 μg/kg.

Confirmatory Method. Liver samples were pretreated in duplicate and subjected to LC-MS/MS as outlined before (*28*). Briefly, the homogenized tissue (5 g) was supplemented with an internal standard (fluorometholone), hydrolyzed as described in the previous section, extracted using 2 × 15 mL of acetronitrile, and washed with 8 mL of hexane. The acetonitrile layer was evaporated and the tissue residue extracts were taken up in 1 mL of ethanol and 10 mL of water before loading onto pre-conditioned C_{18} extraction cartridges, washed with an acetone/water (20/80, v/v) mixture, and further eluted with 2 mL of ethyl acetate. The eluates were evaporated and dissolved in 50 μL of acetonitrile/water (90:10) containing 0.3% (v/v) formic acid and finally 10 μL was injected into the Hypercarb column. The chromatography was performed under isocratic conditions at a flow rate of 0.22 mL/min. MS/MS analyses were carried out in both the electrospray positive and negative ionization modes with the mass spectrometer operating in the multiple reaction monitoring option. For the detection of dexamethasone, the electospray interface was operated in the positive ionization mode and $[MH]^+$ was chosen as the parent ion at *m*/*z* 393. The product ions followed were $[M + H - HF]^+$ and $[M + H - HF - H_2O]^+$ at *m*/*z* 373 and 355, respectively. The *m*/*z* 393 > 373 transition was used for quantification. The detection of flumethasone was performed in the negative ionization mode. The transitions followed for flumethasone detection were 455 [M + formate] > 379 $[M - H - CH_2O]^-$ and 379 > 305 $[M - H - CH_2O - H_2O - CH_4 - 2HF]^-$, with the former transition representing the most abundant one and therefore used for the quantification. The complete method was validated according to SANCO/1085/2000 (*29*). Calibration curves were obtained in order to calculate the decision limits (CCα) and the detection capabilities (CCβ). The repeatabilities and the approximations of the trueness were determined at 1, 2, and 4 μg/kg. As mentioned above one precursor ion and two product ions were monitored for the mass spectrometric detection, giving four identification points as required by SANCO/1085/2000. Signal-to-noise ratios (S/N) were checked for all transitions as well as the relative ion intensities.

RESULTS AND DISCUSSION

Immunological Detection of Synthetic Glucocorticoids in Bovine Tissues. The limit of detection of the commercially available immunoassay was reexamined under the conditions used in our study. Analysis of samples from untreated animals ($n = 6$) yielded apparent dexamethasone concentrations of 0.16 ± 0.11 μg/kg (mean value ± standard deviation) in liver, 0.07 ± 0.01 μg/kg in kidney, 0.24 ± 0.05 μg/kg in muscle, and 0.32 ± 0.17 μg/L in urine. After addition of three standard deviations (*19*), these results translated to a limit of detection of 0.49 μg of dexamethasone/kg in liver, 0.10 μg/kg in kidney, 0.39 μg/kg in muscle, and 0.83 μg/L in urine.

Following hydrolysis and extraction, dexamethasone recoveries of 82 ± 14% from liver (mean value ± standard deviation; $n = 24$) and 89 ± 13% from kidney ($n = 24$) were achieved. Digestion and extraction of the muscle tissue yielded dexamethasone recoveries of 71 ± 12% ($n = 24$). From urine, 91 ± 6% ($n = 21$) of dexamethasone was recovered. The initial immunochemical screening revealed that the dexamethasone concentration in liver, muscle, and kidney was higher than the detection limits determined for these different tissues. There was a striking variability of the dexamethasone levels, although all animals were dosed strictly according to their body weight. In fact, we observed a 5- to 8-fold difference between animal no. 1, which had the highest dexamethasone levels, and animal no. 2 that contained the lowest dexamethasone concentrations (**Table 2**). The highest value in absolute terms was detected in

the liver of animal no. 1 (32.8 μg/kg) and the lowest dexamethasone concentration was found in the muscle of animal no. 2 (0.8 μg/kg). There are several possible sources of variability that may account for these differences. As the animals were affected by an infectious disease, their different pathologic conditions are likely to influence pharmacokinetic parameters such as uptake, distribution, metabolism, and elimination of the drug. Also, the varying efficiency of hydrolysis by *Helix pomatia* juice and the subsequent extraction of crude tissue homogenates could explain part of the observed differences between individual animals. Nevertheless, all values exceeded the recommended MRLs for dexamethasone, i.e., 2 μg/kg in liver and 0.75 μg/kg in kidney or muscle. This result was expected in view of the long withdrawal periods imposed on the tested drug (Dexafort), ranging in Europe from a minimum of 14 days up to 2 months. We also analyzed the accompanying urine samples, where dexamethasone concentrations between 14.8 and 42.5 μg/kg were found.

Using the same immunological screening assay, we were also able to detect flumethasone residues in liver, muscle, and kidney. In fact, the flumethasone level in the treated animals was generally above the detection limit although, in most cases, the concentration of flumethasone was lower than the corresponding glucocorticoid level in the dexamethasone-exposed animals (**Table 2**). Flumethasone was also detectable in the accompanying urine samples.

LC-MS/MS Confirmatory Analysis. The type of synthetic glucocorticoid contained in the samples resulting positive in the screening assay was identified by using LC-MS/MS. The liver was chosen as the matrix for drug identification because it generally contained higher drug residue levels than kidney or muscle. The decision limit and detection capability of dexamethasone were 2.06 and 2.13 μg/kg, respectively, while for the banned flumethasone they were calculated as 0.09 and 0.19 μg/kg, respectively. The repeatabilities, expressed as the coefficients of variation, were not higher than 10% for both compounds, while the percent bias, expressing the approximation of the trueness, were between −2% and + 2%. Dexamethasone concentrations in liver samples no. 1 and no. 3 were quantified by using a standard curve ranging from 0 to 50 μg/kg. The drug concentrations found were 30.7 ± 0.7 μg/kg for liver no. 1 and 13.1 ± 1.0 μg/kg for liver no. 3 (**Table 2**). For the quantification of dexamethasone concentration in liver sample no. 2, the standard curve ranged from 0 to 8 μg/kg and a dexamethasone concentration of 3.1 ± 0.01 μg/kg was found. For all three liver samples the two diagnostic MRM traces gave a S/N > 3 and the relative ion intensities were within the permitted tolerances as required by SANCO/1085/2000. Thus, the quantitative LC-MS/MS analysis confirmed that all dexamethasone concentrations in liver exceeded the MRL value of 2 μg/kg.

The flumethasone detection, did not give satisfying results in the positive electrospray ionization and was therefore replaced by the negative ionization mode. A standard curve ranging from 0 to 25 μg/kg was applied for the quantification and flumethasone concentrations of 5.87 ± 0.4 and 6.95 ± 0.3 μg/kg were found for liver samples 4 and 6, respectively. The relative ion intensities were within the required standards, and S/N > 3. However, for sample no 5, the second MRM trace (379 > 305) did not give a S/N > 3, and therefore the presence of flumethasone could not be confirmed. **Figure 2** shows the MRM chromatograms all six liver sample extracts.

In summary, the results of this field study confirm that the conventional therapeutic use of dexamethasone causes drug concentrations in edible tissues that may exceed the MRLs by more than 1 order of magnitude. In view of the frequent use of dexamethasone, flumethasone, and other similar glucocorticoids in food producing animals, and considering the pharmacological potency of these synthetic hormones, a large margin of safety has to be maintained to protect the consumers from possible adverse effects. This study demonstrates the practicability of a sensitive drug screening combined with reliable identification methods monitoring residues of synthetic glucocorticoids in animal tissues intended for human consumption. In particular, the liver of slaughtered animals provides a suitable matrix to control the enforcement of such regulatory actions using both immunological screening and physicochemical identification. There was good correspondence between the dexamethasone concentrations found by immunoassay and the LC-MS/MS follow-up analysis. It is not clear why there was much less agreement in the flumethasone values determined by the two different techniques. Possibly, a more specific antibody raised against flumethasone may yield more reliable quantitative data in the immunoassay screening.

LITERATURE CITED

(1) Ferguson, D. C.; Hoenig, M. Glucocorticoids, mineralocorticoids, and steroid synthesis inhibitors. In *Veterinary Pharmacology and Therapeutics*, 7th ed.; Adams, H. R., Ed.; Iowa State University Press: Ames, Iowa, 1995; pp 622−643.

(2) Pugh, D. M. The trophic hormones and the endocrine regulators of metabolism. In *Veterinary Applied Pharmacology and Therapeutics*, 5th ed.; Brander, C. G., Pugh, D. M., Bywater, R. J., Jenkins, W. L., Eds.; Bailliere Tindall: London, U.K., 1991; pp 259−278.

(3) Blood, D. C.; Radostis, O. M. *Veterinary Medicine*, 7th ed.; Baillière Tindall: London, U.K., 1989; pp 1128−1130.

(4) Phillippe, H.; Goossens, L.; Limper, J.; Quirke, J. F. Effect of dexamethasone isonicotinate on milk yield in ketotic cows. *Vet. Rec*. **1991**, *128*, 427.

(5) Schimmer, B. P.; Parker, K. L. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogues; inhibitors of the synthesis and actions of adrenocortical hormones. In *The Pharmacological Basis of Therapeutics*, 9th ed.; Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Gilman, A. G., Eds.; McGraw-Hill: New York, 1996; pp 1459−1485.

(6) Plumb, D. C. *Veterinary Drug Handbook*, 3rd ed.; Iowa State University Press: Ames, Iowa, 1995; pp 355−358.

(7) Rijckaert, M. L.; Vlemmix, H. P. *The Growth Promoting Effect of Glucocorticoids*; Department of Chemical Engineering, Eindhoven University of Technology: Eindhoven, The Netherlands, 1992.

(8) Istasse, L.; De Haan, V.; Van Eenaeme, C.; Buts, B.; Baldwin, P.; Gielen, M.; Demeyer, D.; Bienfait, J. M. Effects of dexamethasone injections on performances in a pair of monozygotic cattle twins. *J. Anim. Physiol. Anim. Nutr.* **1984**, *62*, 150−158.

(9) EEC Council Directive No 22/96. *Off. J. Eur. Commun.* 1996, No. L 125.

(10) EEC Council Regulation No 508/99. *Off. J. Eur. Commun.* 1999, No. L 060.

(11) EEC Council Regulation No 2593/99. *Off. J. Eur. Commun.* 1999, No. L 315.

(12) EEC Council Regulation No 2535/00. *Off. J. Eur. Commun.* 2000, No. L 291.

(13) EEC Council Regulation No 77/02. *Off. J. Eur. Commun.* 2002, No L16.

(14) Bette, P.; Kietzmann, M. Effect of dexamethasone on tyrosine aminotransferase activity in rat liver−a sensitive test to define its hormonal no-effect-level. *Acta Vet. Scand*. **1991**, *87*, 200−204.

(15) Leeuwen, F. X. Toxicological evaluation of certain veterinary drug residues in food. The forty-second meeting of the joint FAO/WHO Expert Committee on food additives. *WHO Food Addit.* **1994**, *33*, 111−126.

(16) Brunn, H.; Georgii, S. Identifizierung und Quantifizierung von Dexamethason und verwandter xenobiotischer Corticosteroide im Rinderurin mittels ELISA und HPLC/ELISA. *Arch. Lebensmittelhyg*. **1994**, *45*, 49−72.
(17) Chen, C. L.; Zhu, D.; Gillis, D.; Meleka-Boules, M. Use of enzyme-linked immunosorbent assay and radioimmunoassay to determine serum and urine dexamethasone concentrations in Thoroughbreds after intravenous administration of the steroid. *Am J. Vet. Res*. **1996**, *57*, 182−186.
(18) Friedrich, A.; Schulz, R.; Meyer, H. H. Use of enzyme immunoassay and reverse-phase high-performance liquid chromatography to detect and confirm identity of dexamethasone in equine blood. *Am. J. Vet. Res*. **1990**, *53*, 2213−2220.
(19) Meyer, H. H.; Dürsch, I. Dexamethasone: an enzyme immunoassay for residue analysis and pharmacokinetics. *Arch. Lebensmittelhyg.* **1996**, *47*, 22−24.
(20) Delahaut, Ph.; Jacquemin, P.; Colemonts, Y.; Dubois, M.; De Graeve, J.; Deluyker, H. Quantitative determination of several synthetic corticosteroids by gas chromatography−mass spectrometry after purification by immunoaffinity chromatography. *J. Chromatogr. B* **1997**, *2*, 203−215.
(21) Mallinson, E.; Dreas, J.; Wilson, R.; Henry, A. Determination of dexamethasone in liver and muscle by liquid chromatography and gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **1995**, *43*, 140−145.
(22) Rizea Savu, S.; Silvestro, L.; Haag, A.; Sörgel, F. A confirmatory HPLC-MS/MS method for 10 synthetic corticosteroids in bovine urines. *J. Mass Spectrom.* **1996**, *31*, 1351−1363.
(23) De Wasch, K.; De Brabander, H.; Courtheyn, D.; Van Peteghem, C. Detection of corticosteroids in injection sites and cocktails by MS^n. *Analyst* **1998**, *129*, 2415−2422.
(24) Antignac, J. P.; Le Bizec, B.; Monteau, F.; Poulain, F.; Andre, F. Multi-residue extraction-purification procedure for corticosteroids in biological samples for efficient control of their misuse in livestock production. *J. Chromatogr. B* **2001**, *757*, 11−19.
(25) Draisci, R.; Marchiafava, C.; Palleschi, L.; Cammarata, P.; Cavalli, S. Accelerated solvent extraction and liquid chromatography-tandem mass spectrometry quantitation of corticosteroid residues in bovine liver. *J. Chromatogr. B* **2001**, *753*, 217−223.
(26) Daeseleire, E.; De Guesquière, A.; Van Peteghem, C. Combined high-performance liquid chromatography and radioimmunoassay for the screening of 19-nortestosterone and methyltestosterone residues in meat samples. *J. Chromatogr*. **1991**, *564*, 445−449.
(27) Derendorf, H.; Rohdewald, P.; Hochhaus, G.; Möllmann, H. HPLC determination of glucocorticoid alcohols, their phosphates and hydrocortisone in aqueous solutions and biological fluids. *J. Pharm. Biomed. Anal*. **1986**, *4*, 197−206.
(28) Van den hauwe, O.; Castro Perez, J.; Claereboudt, J.; Van Peteghem, C. Simultaneous determination of betamethasone and dexamethasone residues in bovine liver by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 857−861.
(29) SANCO/1085/2000 Revision 7 (Draft), European Commission.

Received for review May 7, 2002. Revised manuscript received September 21, 2002. Accepted September 23, 2002. This work is supported by the EC Grant QLK1-CT-1999-00122 and Grant 012.96.1 from the Swiss Bundesamt für Veterinärwesen.

JF020533M