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Journal of Chromatography B, 754 (2001) 229–244

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Analysis of corticosteroids in equine urine by liquid chromatography–mass spectrometry

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Received 21 July 2000; received in revised form 23 November 2000; accepted 30 November 2000

Abstract

A liquid chromatography–mass spectrometry (LC–MS) method for the analysis of corticosteroids in equine urine was developed. Corticosteroid conjugates were hydrolysed with β-glucuronidase; free and enzyme-released corticosteroids were then extracted from the samples with ethyl acetate followed by a base wash. The isolated corticosteroids were detected by LC–MS and confirmed by LC–MS–MS in the positive atmospheric pressure chemical ionisation mode. Twenty-three corticosteroids (comprising hydrocortisone, deoxycorticosterone and 21 synthetic corticosteroids), each at 5 ng/ml in urine, could easily be analysed in 10 min.

Keywords: Corticosteroids; Equine urine; Liquid chromatography–mass spectrometry

1. Introduction

Synthetic corticosteroids possess anti-inflammatory activity and are commonly used to relieve inflammatory lesions in performance horses. Analyses of some of the corticosteroids are usually performed by immunoassays [1,2], liquid chromatography with ultraviolet detection (LC–UV) [3], gas chromatography–mass spectrometry (GC–MS) of the pyridium chlorochromate oxidation products [4–8], GC–MS of the methoxylamine-trimethylsilyl (MOX-TMS) or the trimethylsilyl (TMS) derivatives [9–11], or LC–MS [12–23].

Coverage by immunoassays is limited (at present only kits for dexamethasone, flumethasone, methylprednisolone and triamcinolone acetonide are available commercially). The screening by LC–UV is neither specific nor sensitive; matrix interference is also a problem. Corticosteroids are often analysed by negative chemical ionisation GC–MS after pyridium chlorochromate oxidation. The method is sensitive for some corticosteroids but insensitive for natural corticosteroids. Some corticosteroids are resistant to oxidation. Besides, due to the loss of the side chain and oxidation of hydroxyl groups, structural information is lost. Thus corticosteroids like prednisone and prednisolone cannot be differentiated after oxidation. Corticosteroids are also analysed by GC–MS as their TMS or MOX-TMS derivatives. It takes about 2 h for TMS derivatisation and an additional 45 min for MOX-TMS derivatisation. Despite the time involved, results are usually not satisfactory. Analysis by GC–MS requires another 20 min.

LC–MS has been used to analyse corticosteroids in various sample matrices, often for the quantification of hydrocortisone in equine urine [12,13]. But so

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0378-4347/01/$ – see front matter
PII: S0378-4347(00)00613-7

far only 12 synthetic corticosteroids, namely, beclomethasone [14], betamethasone [14–18], budesonide [19], dexamethasone [14,17–19], fludrocortisone [14,19], flumethasone [14,18,19], flunisolide [18], methylprednisolone [14,18,19], prednisolone [14,15,17–22], prednisone [14,15,18–22], triamcinolone [14,19,22] and triamcinolone acetonide [18,22,23] have been studied.

The majority of these LC–MS studies either involve only a few analytes or do not involve equine urine as the matrix. Equine urine, particularly that from racehorses on a high-energy diet, is a viscous and highly complex matrix; the reliable screening of a large number of analytes from such a matrix is not straightforward. Schubert et al. [15] reported the simultaneous LC–MS screening of just four synthetic or natural corticosteroids in equine urine. Savu et al. [18] reported the LC–MS analysis of eight synthetic and two natural corticosteroids in bovine urine – a matrix that may have some resemblance to equine urine. Both Fiori et al. [14] and Volmer and Hui [19] also worked with a similar number of corticosteroids but in less complicated matrices (i.e., milk replacer and human urine, respectively).

Equine urine is usually treated with β-glucuronidase to release corticosteroids from their glucuronide conjugates. β-Glucuronidase contains sulfatase activity but is, however, not effective in hydrolysing certain sulfate conjugates [24]. In a previous study [25], we have shown that ''methanolysis'' [26] could be used to release deoxycorticosterone (deoxycortone) from its conjugates. However, as ''methanolysis'' of equine urine generally gives dirtier products, and most reference standards of corticosteroid conjugates are unavailable, enzyme hydrolysis rather than ''methanolysis'' was used in the present study.

This paper describes a sensitive and quick method for the simultaneous screening of 21 synthetic corticosteroids, deoxycortone and hydrocortisone in equine urine by LC–MS. Derivatisation or oxidation is not required, and each LC–MS cycle takes only 20 min, resulting in a short overall analysis time. LC–MS–MS confirmatory analysis of some corticosteroids in urine samples will also be described. As full scan LC–MS data is available, this method is in fact applicable not only to corticosteroids but also to other extractable drugs.

2. Experimental

2.1. Materials

β-Glucuronidase type L-II from limpets, deoxycortone, flumethasone and Amberlite XAD-16 resin were purchased from Sigma (St. Louis, MO, USA). Acetic acid (Suprapur grade), chloroform, ethyl acetate and methanol (all of LiChrosolv grade), sodium chloride (reagent grade) and anhydrous sodium sulfate (granulated for organic trace analysis) were purchased from Merck (Darmstadt, Germany). Sodium acetate and sodium hydroxide (both of reagent grade) were purchased from Riedel-de Haën (Seelze, Germany). Beclomethasone, 21-deoxydexamethasone, desoximetasone, dichlorisone, fludrocortisone, flurandrenolide and isoflupredone were purchased from Steraloids (Wilton, NH, USA). Fluclorolone acetonide, fluocinolone acetonide, methylprednisolone, prednisone, triamcinolone acetonide, fluocortolone hexanoate were from British Pharmacopeia Commission (Middlesex, UK). Betamethasone, dexamethasone, fluocinonide, fluprednisolone, fluorometholone, prednisolone and triamcinolone were from United States Pharmacopeial Convention (Rockville, MD, USA). d_4-Hydrocortisone was from Cambridge Isotope Labs. (Andover, MA, USA). Hydrocortisone was from BDH (Poole, UK). Fluocortolone was prepared by heating fluocortolone hexanoate in a methanolic solution of potassium methoxide (10 mg in 10 ml) at 60°C for 20 min, followed by the removal of potassium ions using a cation-exchange resin AG50W-X8.

2.2. Instrumentation

LC–MS analyses of corticosteroids were performed on a Finnigan MAT LCQ Classic (ThermoQuest, San Jose, CA, USA) equipped with a Hewlett-Packard 1100 high-performance liquid chromatography (HPLC) system (Palo Alto, CA, USA).

2.3. Administration of proprietary preparations

Decort 20 (containing 100 mg of deoxycortone) was administered intramuscularly to a thoroughbred gelding. A urine sample was collected before drug administration, and then daily for 14 days.

Kenacort-A (containing 80 mg of triamcinolone acetonide) was administered intramuscularly to a thoroughbred gelding. A urine sample was collected before drug administration, and then daily for 3 days.

SOLU-MEDROL (containing 150 mg of methylprednisolone sodium succinate) was administered intramuscularly to a thoroughbred gelding. A urine sample was collected before drug administration, and then daily for 3 days.

Dexamethasone Weimer (containing 10 mg of dexamethasone sodium phosphate) was administered intramuscularly to a thoroughbred gelding. A urine sample was collected before drug administration, and then daily for 3 days.

2.4. Isolation of corticosteroids from urine

Urine (5.0 ml) was transferred to a 15-ml graduated centrifuge tube. d_4-Hydrocortisone (100 ng) was added as an internal standard. Sodium acetate buffer (1 *M*, pH 4.1; 0.4 ml) was added and the solution adjusted to pH 5.0. A solution of β-glucuronidase (0.6 ml, 10 800 U) was added and the mixture incubated (65°C for 3.5 h or 37°C overnight). It was then extracted with 6.0 ml of ethyl acetate (rotation, 10 min). The organic extract was washed with 3.0 ml of base (1 *M* NaOH+0.15 *M* NaCl; rotation, 5 min), and then centrifuged (1900 *g*, 0.5 min). The organic layer was passed through an anhydrous sodium sulfate drying column into a 5-ml Reacti-vial, then evaporated to dryness (under nitrogen at 60°C). The residue was reconstituted in 30 μl of methanol and vortex-mixed. The content was then transferred to a conical insert in a Chrompack autosampler vial for LC–MS analyses.

2.5. LC–MS analyses of corticosteroids in equine urine

Samples (10 μl) were injected onto a reversed-phase DB-8 column (75 mm×4.6 mm I.D., 3 μm; Supelco) at 25°C. The mobile phase was composed of a solvent mixture of (A) 1.0% acetic acid and (B) methanol. Unless otherwise mentioned, gradient elution was performed from 100% solvent A to 100% solvent B in 15 min, and hold for another 3 min. The flow-rate was 1.0 ml/min. For the confirmation of triamcinolone acetonide in urine, 10 m*M* ammonium acetate was used instead of 1.0% acetic acid.

The atmospheric pressure chemical ionisation (APCI) source of the mass spectrometer was optimised at 450°C. The capillary temperature was maintained at 150°C. The source voltage was +5 kV (for positive ions) and −5 kV (for negative ions). The ''full maximum ionisation time'' was set at 50 ms. For screening, the mass spectrometer was set in the positive APCI full scan mode. The scan range was from m/z 300 to m/z 500. Ion traces for the protonated molecular ions of each corticosteroid were extracted from full-scan data. For confirmatory analysis, both positive and negative APCI modes were used, product-ion scans were performed on the APCI-generated quasimolecular ions. Helium was used as the collision gas.

3. Results and discussions

3.1. Detection of corticosteroids in spiked samples

Twenty-three corticosteroids and d_4-hydrocortisone were spiked at 10 ng/ml each in XAD-treated equine urine. The sample was processed (hydrolysis and extraction) as normal and analysed by LC–MS. Fig. 1 shows the extracted ion chromatograms from the LC–MS analysis. The separation was completed within 10 min. Three pairs of corticosteroids (betamethasone and dexamethasone, fluprednisolone and isoflupredone, 21-deoxydexamethasone and fluocortolone) could not be resolved under the conditions described above. Table 1 shows the ions selected for each corticosteroid and their retention times relative to d_4-hydrocortisone. Further analysis showed that all 24 corticosteroids could be easily detected at 5 ng/ml.

3.2. Confirmation of the corticosteroids in spiked samples

Confirmation of all 23 corticosteroids in urine was achieved by LC–MS–MS analysis. Although the unresolved isomers of betamethasone and dexamethasone gave similar MS–MS spectra, the two drugs could be resolved chromatographically (Fig. 2) if a slower gradient was used, namely, 1% acetic

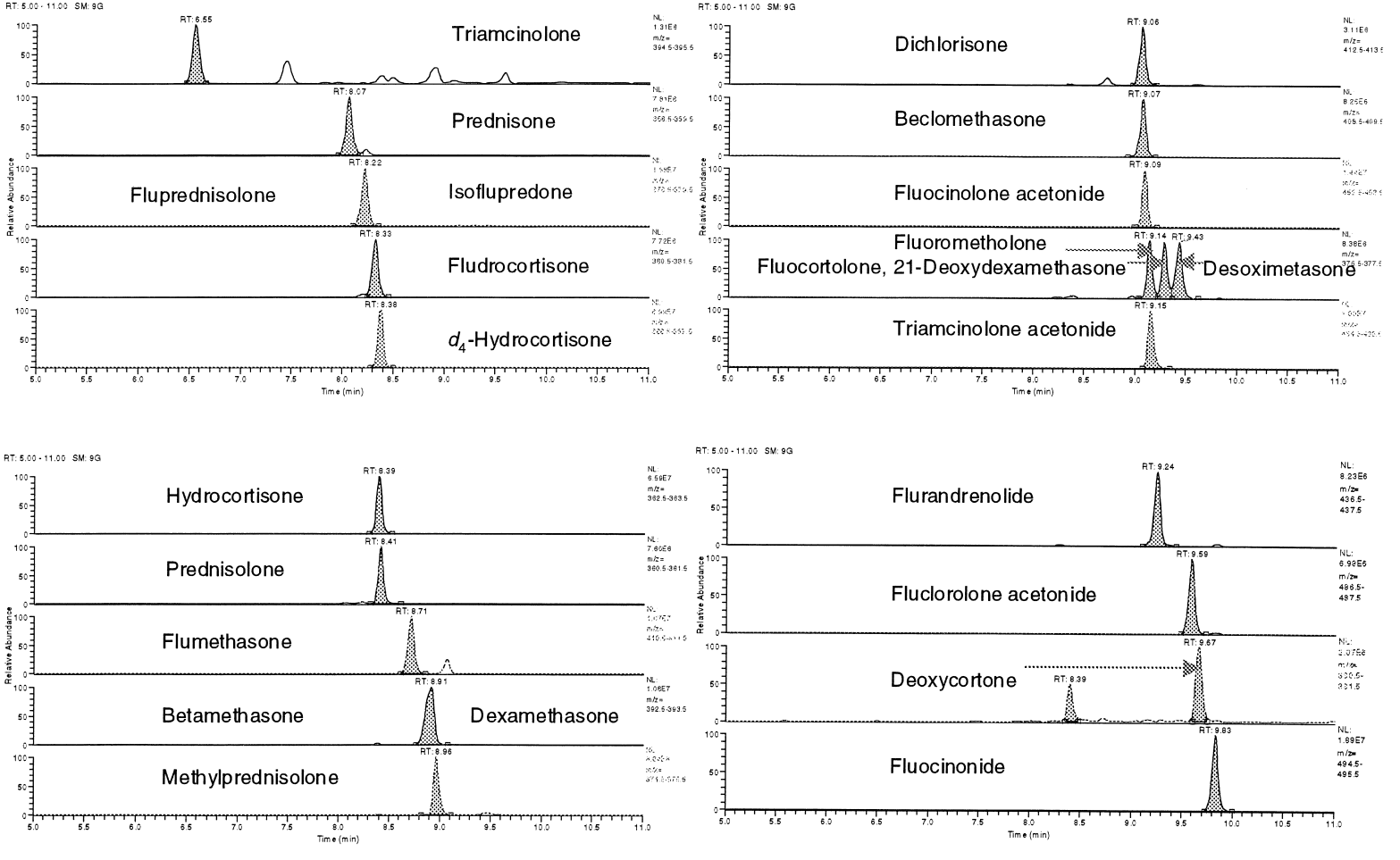


Fig. 1. LC–MS detection of corticosteroids in urine (10 ng/ml).

Table 1
LC–MS detection and recoveries of 23 corticosteroids spiked in equine urine at 10 ng/ml

Corticosteroid	Ion selected (m/z)	Relative retention time (min)	Mean recovery (%)	SD (n=14)
Triamcinolone	395	0.78	61	23[a]
Prednisone	359	0.96	77	8.7
Fluprednisolone	379	0.98	83	9.0
Isoflupredone	379	0.98	81	4.9
Fludrocortisone	381	0.99	83	8.4
d_4-Hydrocortisone	367	1.00	–	–
Hydrocortisone	363	1.00	82	8.2
Prednisolone	361	1.00	81	7.8
Flumethasone	411	1.04	86	9.7
Betamethasone	393	1.06	86	6.1
Dexamethasone	393	1.06	87	9.3
Methylprednisolone	375	1.07	84	8.7
Dichlorisone	413	1.08	36	3.5
Beclomethasone	409	1.08	76	7.1
Fluocinolone acetonide	453	1.08	99	9.0
Fluorometholone	377	1.09	88	8.1
Fluocortolone	377	1.10	90	8.2
21-Deoxydexamethasone	377	1.10	87	5.1
Desoximetasone	377	1.13	87	8.2
Triamcinolone acetonide	435	1.09	89	9.0
Flurandrenolide	437	1.10	88	7.5
Fluclorolone acetonide	487	1.14	86	9.7
Deoxycortone	331	1.15	89	7.8
Fluocinonide	495	1.17	78	8.9

[a] Unstable, subject to possible epimerisation.

acid–10% methanol (9:1) to 1% acetic acid–50% methanol (1:1) in 20 min, then hold for 5 min. On the other hand, fluprednisolone and isoflupredone could not be resolved even with a slow gradient, but their MS–MS spectra were clearly distinguishable from each other (Fig. 3A). Similarly, the MS–MS spectra of 21-deoxydexamethasone and fluocortolone were significantly different from each other (Fig. 3B).

3.3. Recovery of corticosteroids

Two sets of corticosteroids were spiked to XAD-treated urine at individual concentrations of 10 ng/ml. One set contained triamcinolone, prednisone, fluprednisolone, fludrocortisone, hydrocortisone, prednisolone, flumethasone, dexamethasone, methylprednisolone, dichlorisone, beclomethasone, fluocinolone acetonide, fluorometholone, fluocortolone, desoximetasone, triamcinolone acetonide, flurandrenolide, fluclorolone acetonide, deoxycortone and fluocinonide. The other set contained 21-deoxydexamethasone, betamethasone and isoflupredone. The two sets were processed (hydrolysis and extraction) as described above. Internal standard, d_4-hydrocortisone, was then added at 10 ng/ml prior to LC–MS analysis. Area ratios of the quasimolecular ions of individual corticosteroids to that of the internal standard were determined. Two sets of methanolic solutions containing the same mixture of corticosteroid standards and d_4-hydrocortisone at 10 ng/ml each were also prepared and analysed in the same manner. Table 1 shows the mean recovery of individual corticosteroids determined on 14 occasions. Of the 23 corticosteroids, dichlorisone and triamcinolone gave the lowest recoveries; the rest gave mean recoveries ranging from 76 to 99%.

3.4. Reproducibility

Table 2 summarises the precision data for injecting an extract twenty times each day for 4 consecu-

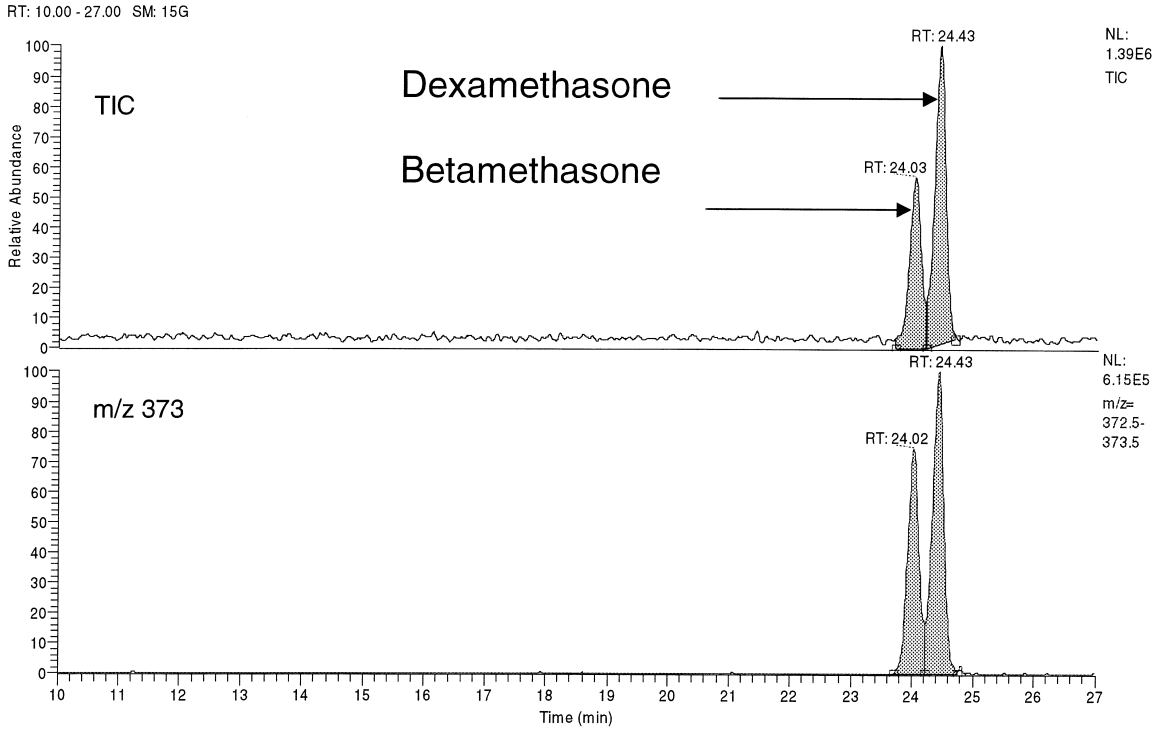


Fig. 2. LC–MS separation of betamethasone and dexamethasone. Gradient elution: 1% HOAc–10% MeOH (9:1) initially, changed to 1% HOAc–50% MeOH (1:1) in 20 min, hold for 5 min; 1 ml/min flow.

tive days. Fresh extracts were processed daily from a urine sample containing d_4-hydrocortisone at 10 ng/ml. The relative standard deviation (RSD) was less than 3%.

3.5. Screening of corticosteroids in post-race and administration samples

Two-hundred and eighty-nine different post-race urine samples have been screened with this method. As illustrated in Fig. 4, the samples were all negative, and only endogenous hydrocortisone together with the internal standard, d_4-hydrocortisone, were detected. The method has also been used for the detection of corticosteroid administrations. Fig. 5A–D shows the extracted ion chromatograms of the urine samples collected 1 day after the administration of, respectively, dexamethasone, deoxycortone, methylprednisolone and triamcinolone acetonide to four different geldings, together with those of the pre-administration samples. Peaks corresponding to the protonated molecular ions of these four corticosteroids were easily detected at the appropriate retention times. For the urine sample collected 1 day after the administration of triamcinolone acetonide, a peak at m/z 451, corresponding to the protonated molecular ion of the metabolite hydroxytriamcinolone acetonide, was also detected.

Table 2
Precision data for injecting a urine extract 20 times daily

	Area of d_4-hydrocortisone			
	Day 1	Day 2	Day 3	Day 4
Mean	$79.2 \cdot 10^6$	$79.1 \cdot 10^6$	$80.7 \cdot 10^6$	$82.8 \cdot 10^6$
SD	$1.22 \cdot 10^6$	$1.66 \cdot 10^6$	$1.46 \cdot 10^6$	$2.18 \cdot 10^6$
RSD (%)	1.5	2.1	1.8	2.6

3.6. Confirmatory analysis of corticosteroids in administration and referee samples

Fig. 6A–C shows the results of LC–MS–MS

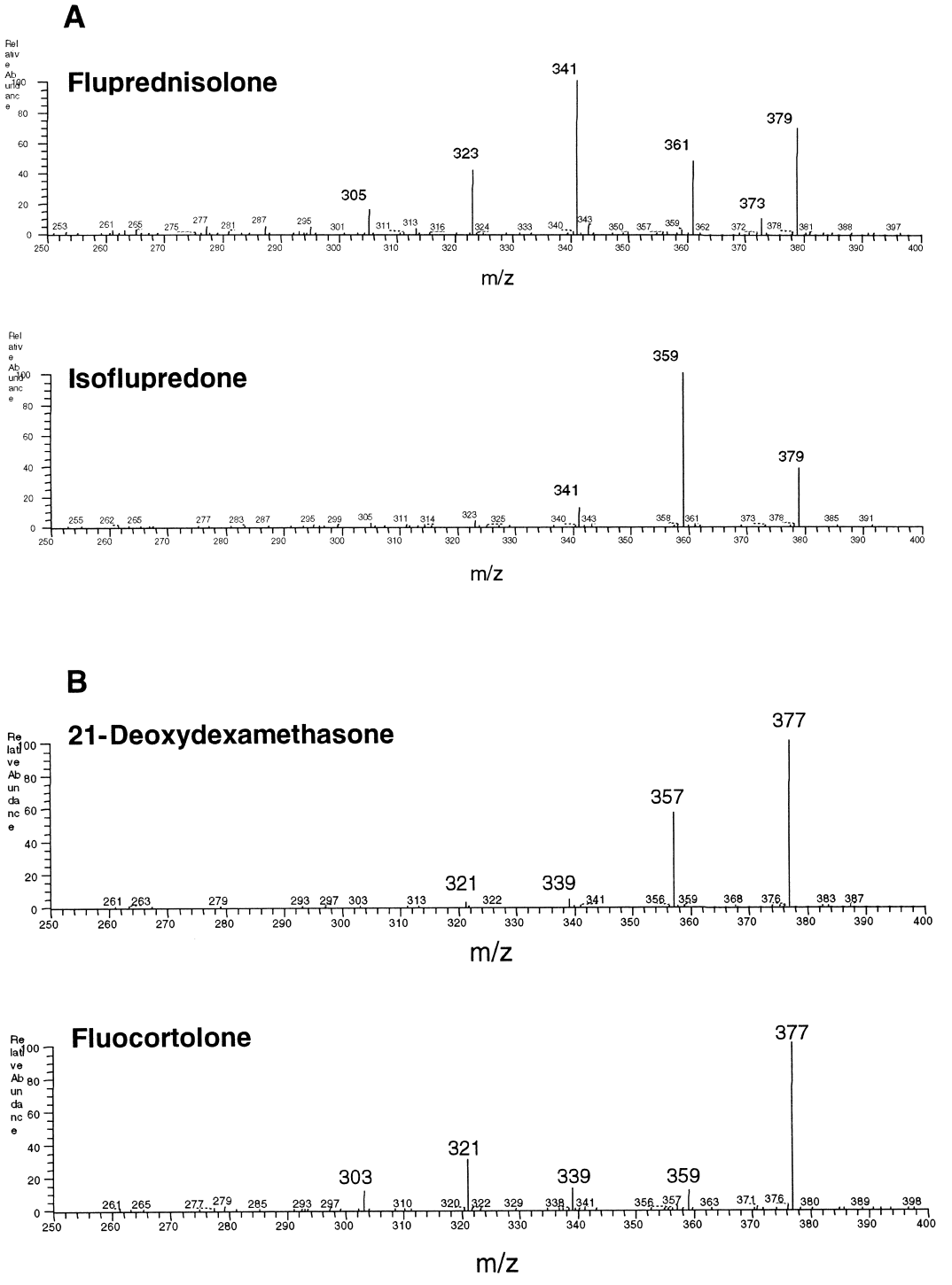


Fig. 3. (A) LC–MS–MS product-ion scans of m/z 379 from fluprednisolone and isoflupredone; (B) LC–MS–MS product-ion scans of m/z 377 from 21-deoxydexamethasone and fluocortolone.

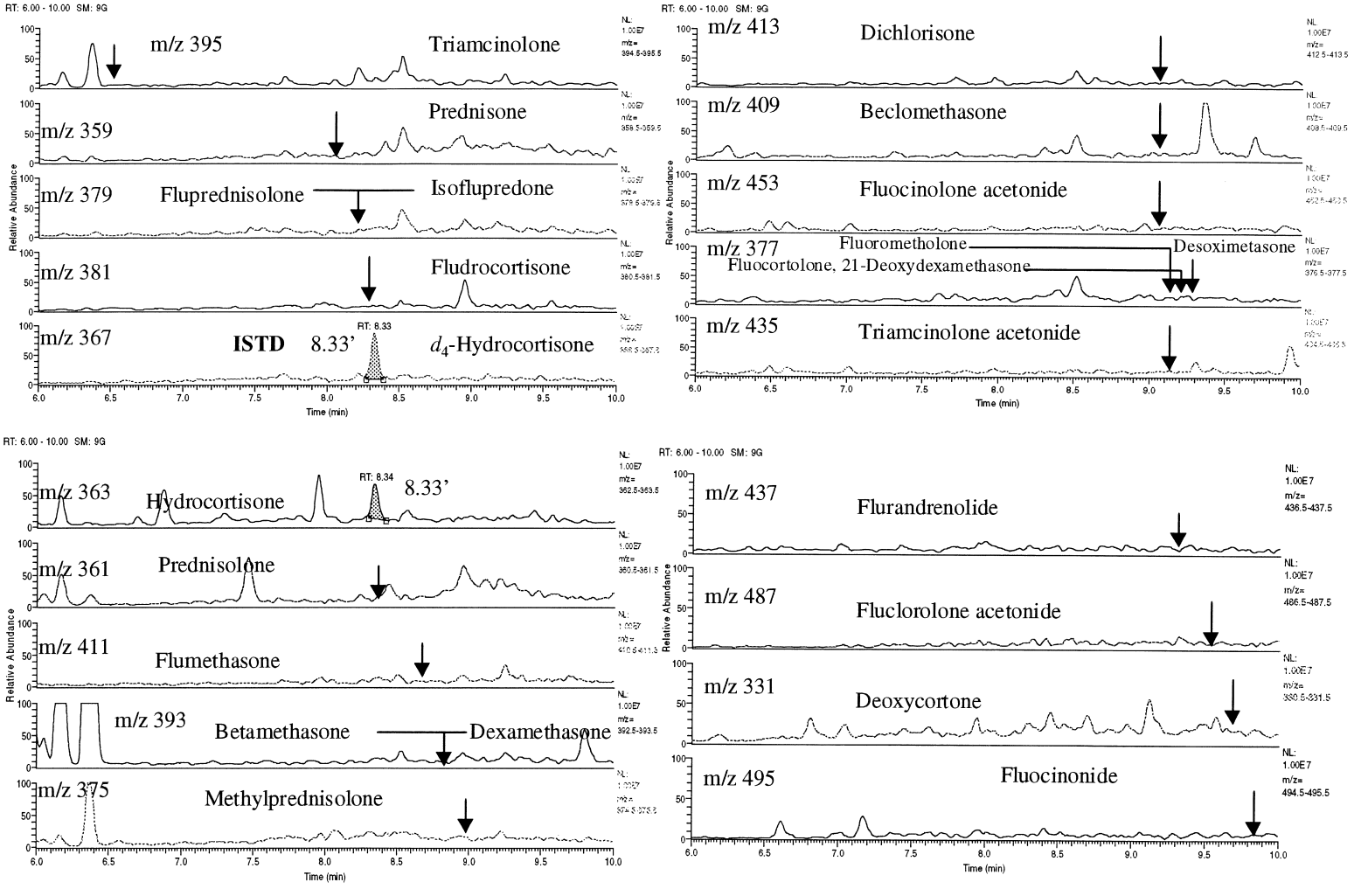


Fig. 4. LC–MS screening of 23 corticosteroids in a post-race equine urine sample. Individual chromatograms are normalised to the abundance of d_4-hydrocortisone at m/z 367. Arrows indicate the positions of target corticosteroids.

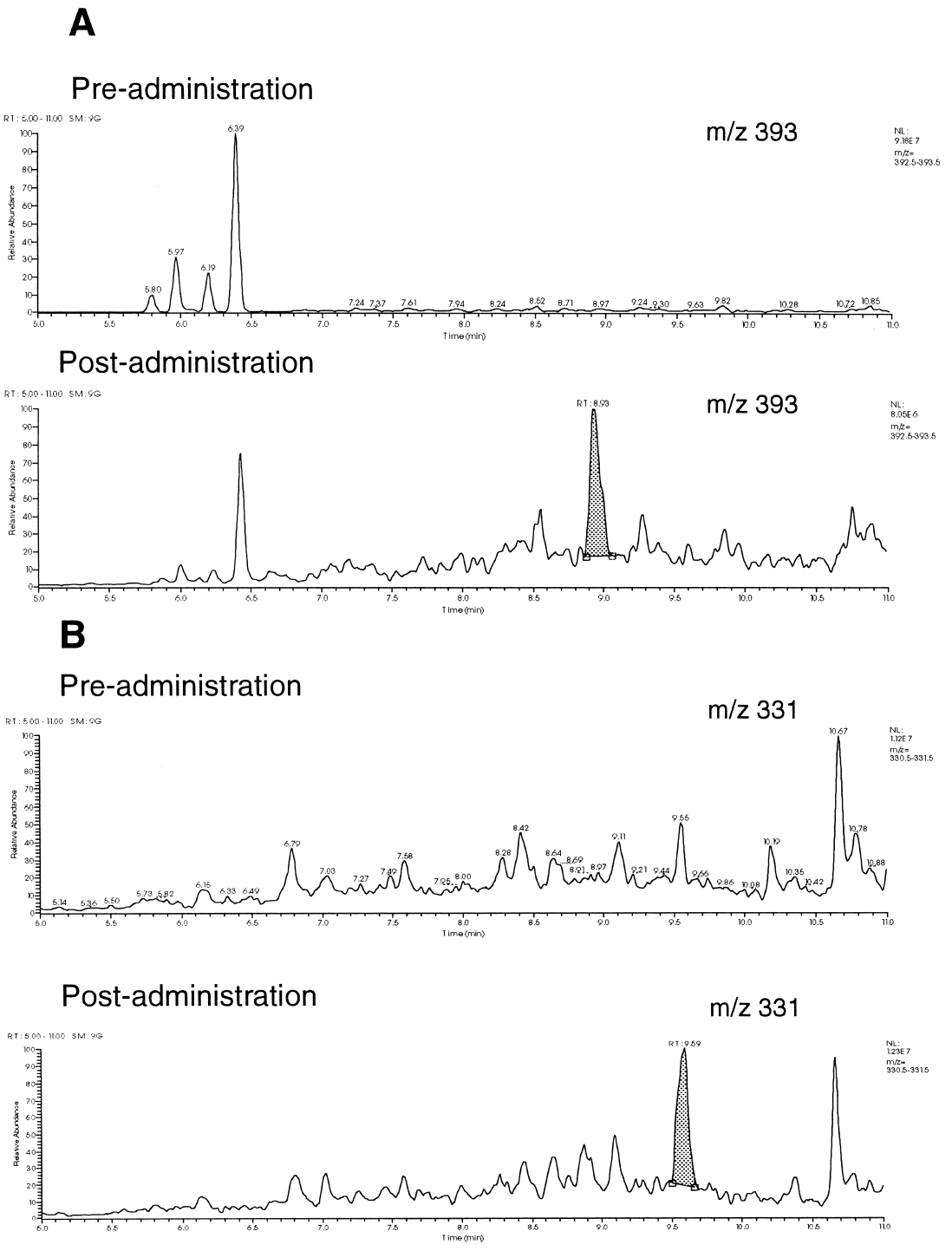
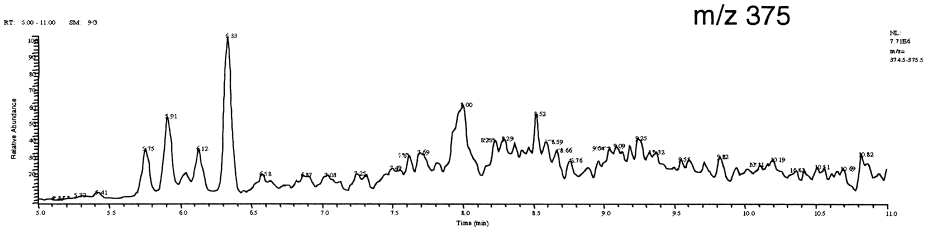


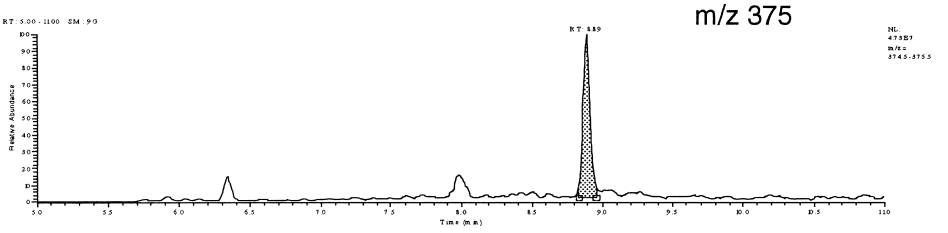
Fig. 5. LC–MS screening of equine urine obtained before and after the administration of (A) dexamethasone, (B) deoxycortone, (C) methylprednisolone and (D) triamcinolone acetonide.

C

Pre-administration

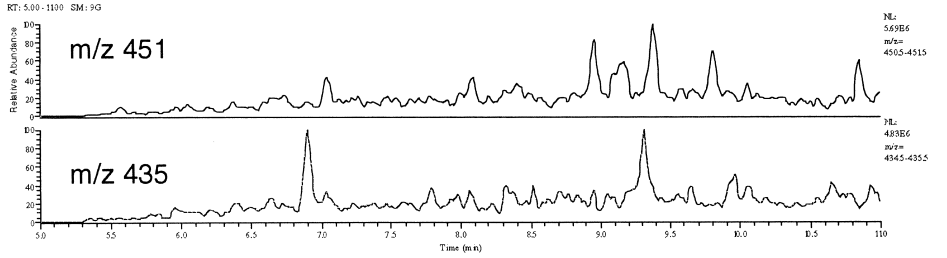


Post-administration



D

Pre-administration



Post-administration

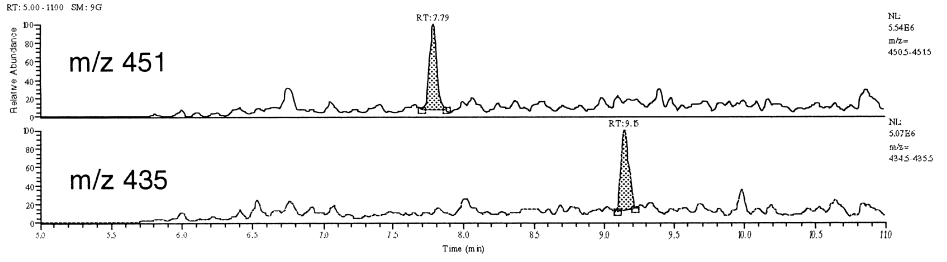


Fig. 5. (*continued*)

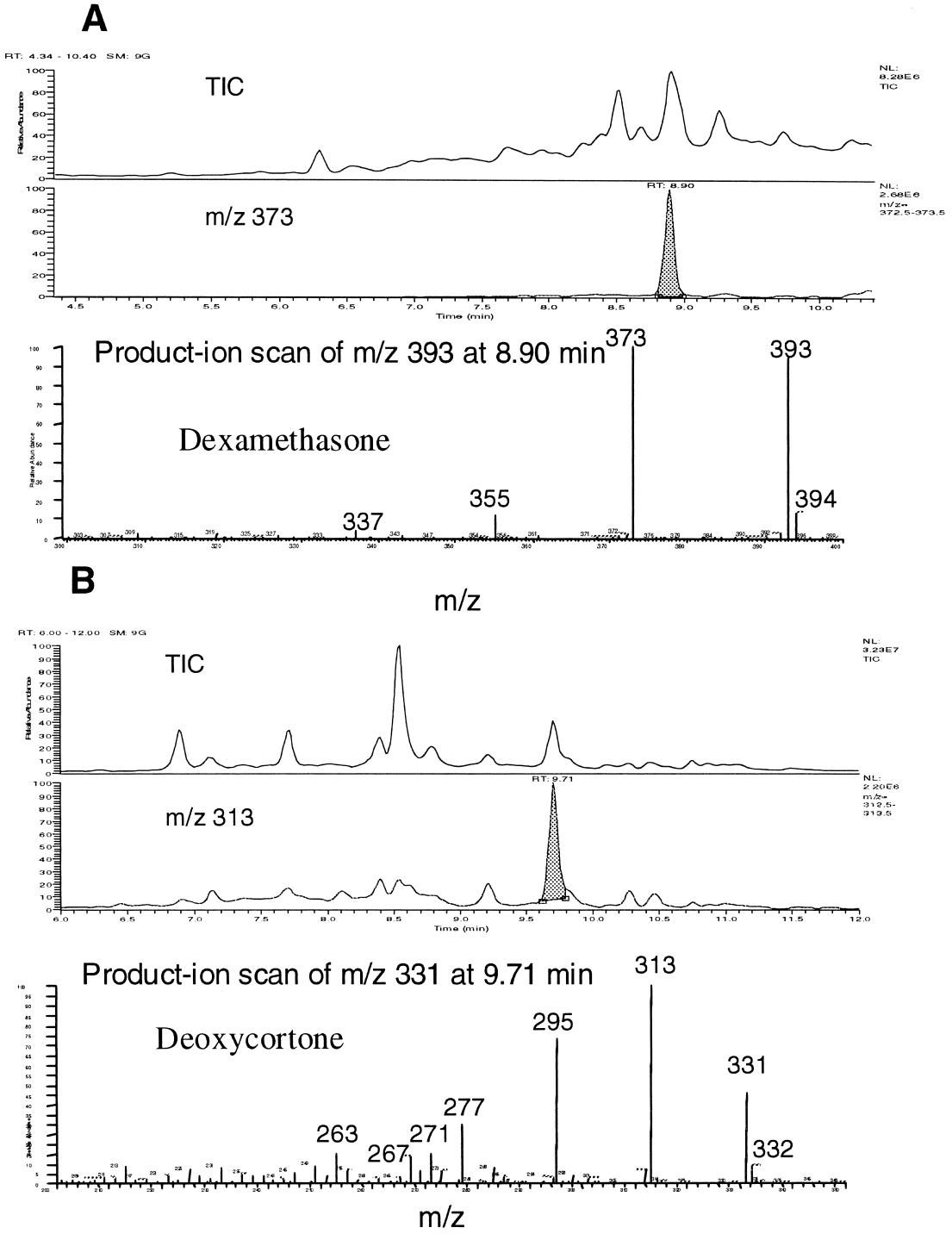


Fig. 6. LC–MS–MS confirmation of the administration of (A) dexamethasone, (B) deoxycortone, and (C) methylprednisolone.

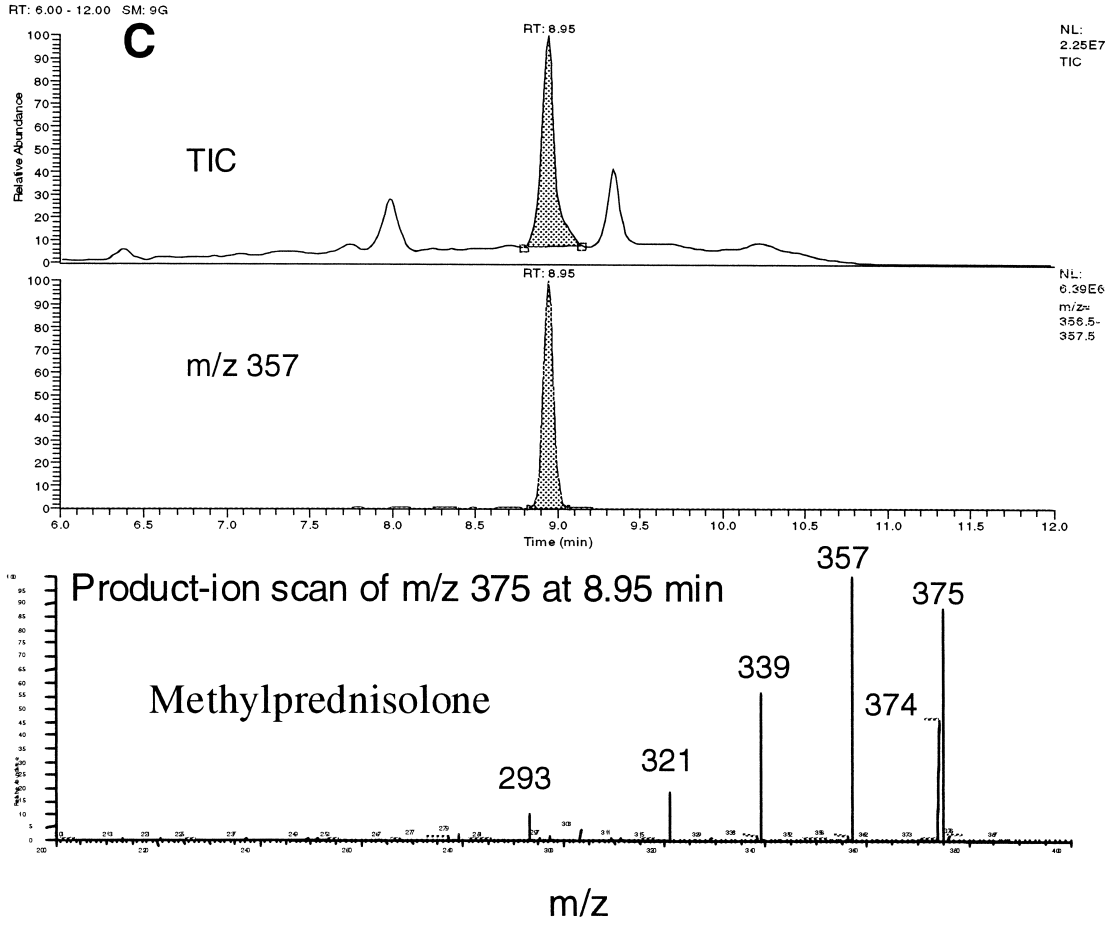


Fig. 6. (*continued*)

analyses of urine samples collected 1 day after the administrations of, respectively, dexamethasone, deoxycortone and methylprednisolone. The parent drugs were easily confirmed by positive APCI LC–MS–MS. Hydroxylated metabolites were not detected in these samples.

Fig. 7 shows the results of LC–MS–MS confirmation of the administration of triamcinolone acetonide. The urine sample was collected 1 day after administration. Negative APCI was used in here, and 10 m*M* ammonium acetate was used in place of 1% acetic acid as one of the mobile phase components. Two major peaks were observed in the total ion chromatogram. The MS–MS spectrum of the late-eluting component corresponded to triamcinolone acetonide (with its acetate adduct at m/z 493), and that of the early-eluting one could be assigned to its metabolite, hydroxytriamcinolone acetonide (with its acetate adduct at m/z 509). Positive APCI mode could also be used, but in this case the negative ion mode resulted in better sensitivity and reduced matrix interference.

The method has already been used in the referee analysis of ‘‘B-samples’’ from overseas racing jurisdictions. The corticosteroids confirmed include triamcinolone acetonide in urine, isoflupredone in urine, methylprednisolone in urine and dexamethasone in urine as well as in blood. Fig. 8 shows the LC–MS–MS confirmation of dexamethasone in the blood sample.

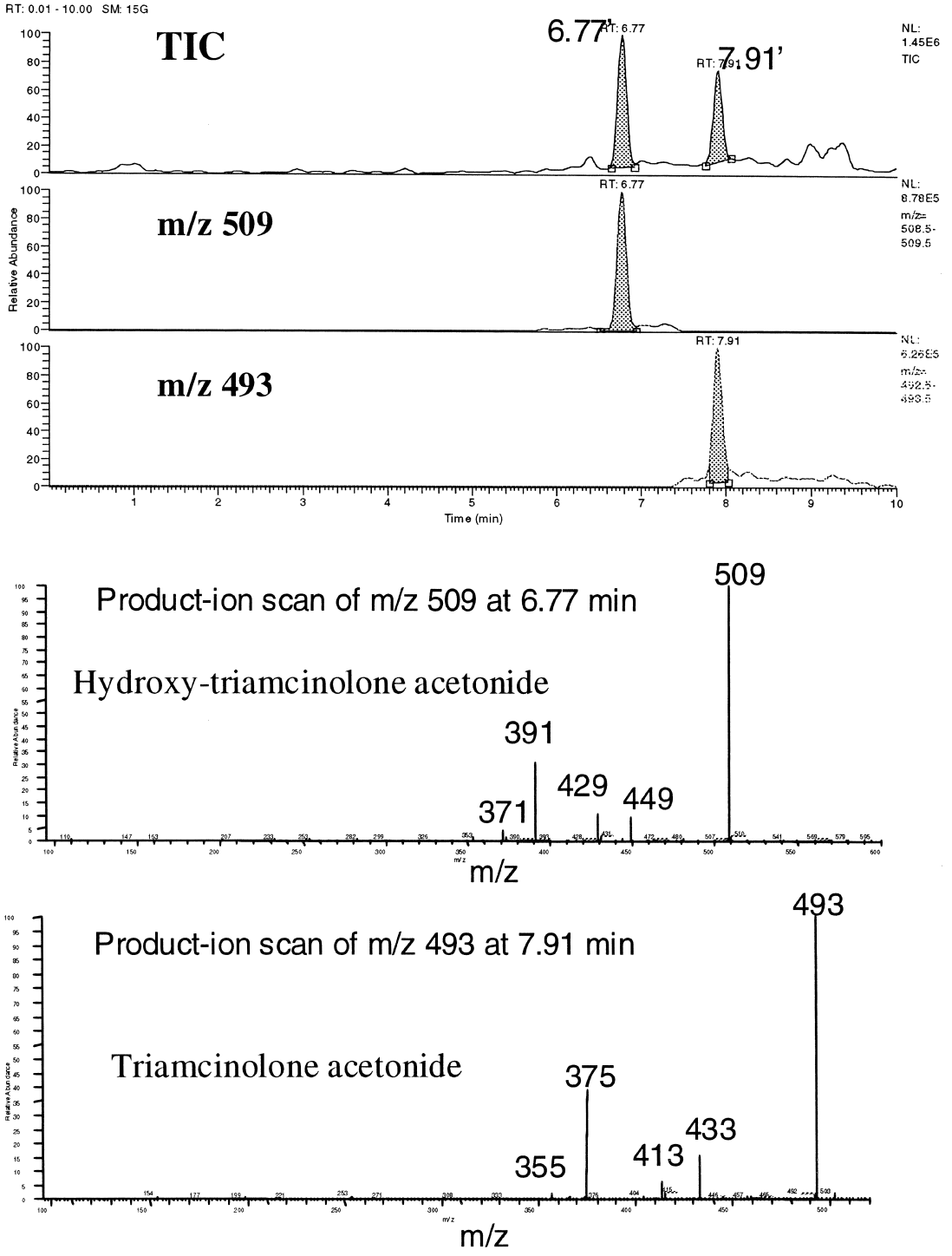


Fig. 7. Negative APCI LC–MS–MS confirmation of the administration of triamcinolone acetonide.

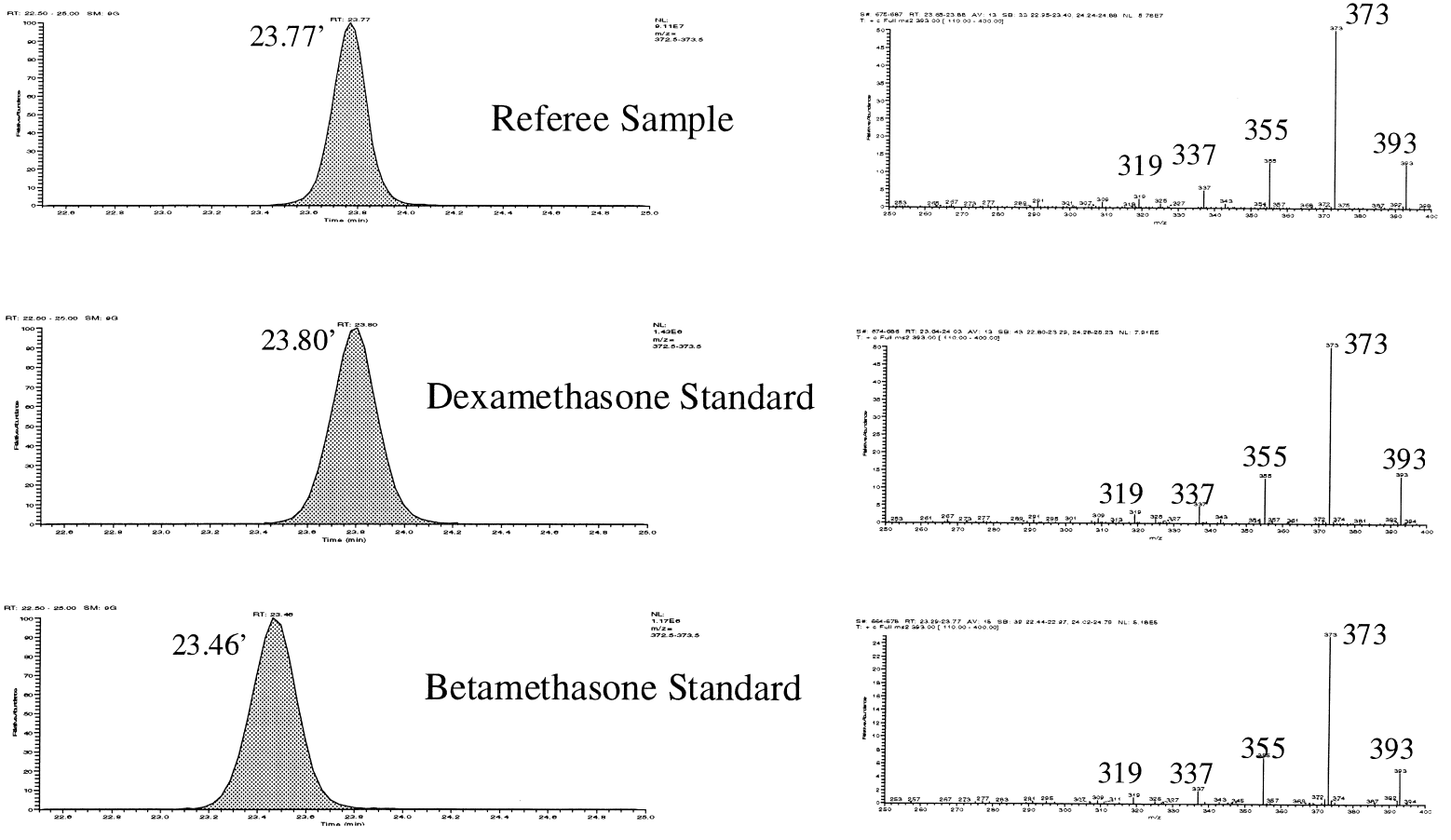


Fig. 8. LC–MS–MS confirmation of dexamethasone in a referee blood sample (0.5 ml whole blood diluted to 5 ml, EtOAc extraction, base wash).

4. Conclusion

Free and enzyme-released corticosteroids were extracted from equine urine samples using liquid–liquid extraction. The ethyl acetate extract was washed with base to remove the acidic and highly polar matrix components. The isolation of free and enzyme-released corticosteroids using solid-phase $C_{18,}$ Chem Elute and normal-phase LC-Si cartridges, or a combination of C_{18} and LC-Si cartridges as for the isolation of anabolic steroids [27–30] were also tried, but these did not improve significantly the recovery of corticosteroids or the removal of matrix components.

The present study demonstrated good sensitivity in the screening of 21 synthetic and two natural corticosteroids in equine urine after a simple liquid–liquid extraction. As shown in Table 2, the inter-day precision data is excellent and the LC–MS system is robust. When a larger volume (20 ml) of sample was used, it was possible to detect and confirm the presence of triamcinolone acetonide in equine urine at 0.5 ng/ml, and similarly dexamethasone, isoflupredone and methylprednisolone at 0.2 ng/ml each. Using a neutral or slightly basic mobile phase, the negative APCI mode may offer additional improvement in sensitivity. Further enhancement in selectivity and sensitivity might be achieved by performing the screening in multiple reaction monitoring mode. When a corticosteroid is flagged by LC–MS screening, LC–MS–MS confirmation can immediately be performed on the same instrument.

With d_4-hydrocortisone as the internal standard, the concentration of hydrocortisone in urine can also be estimated. Only urine samples with a hydrocortisone level close to or above the 1.0 μg/ml international threshold will need to be accurately quantified. With minor modification, the present LC–MS method has in fact been used for such quantification.

Acknowledgements

The authors wish to thank the veterinary surgeons of the Hong Kong Jockey Club for their assistance in performing corticosteroid administration experiments and for arranging sample collection. We are also grateful to Mr. Ivan Cheung and Mr. M.Y. Lau for technical assistance.

References

[1] D. Zhu, K.D. Gillis, C.L.D. Chen, in: C.R. Short (Ed.), Proceedings of the 9th International Conference of Racing Analysts and Veterinarians, New Orleans, LA, 1992, p. 393, Vol. II.
[2] M.S. Moss, H.J. Rylance, J. Pharm. Pharmacol. 18 (1966) 13.
[3] A. Gotzmann, W. Schänzer, M. Donike, in: P. Kallings, U. Bondesson, E. Houghton (Eds.), Proceedings of the 10th International Conference of Racing Analysts and Veterinarians, Stockholm, 1994, p. 307.
[4] J.H. Vine, D.C. Batty, K.E. Mason, A.M. Stenhouse, J.M. Ralston, C.L. Russo, in: C.R. Short (Ed.), Proceedings of the 9th International Conference of Racing Analysts and Veterinarians, New Orleans, LA, 1992, p. 103, Vol. II.
[5] A.R. McKinney, S.D. Wise, A.M. Duffield, in: D.E. Auer, E. Houghton (Eds.), Proceedings of the 11th International Conference of Racing Analysts and Veterinarians, Queensland, 1996, p. 97.
[6] G.R. Her, J.T. Watson, Biomed. Mass Spectrom. 13 (1986) 57.
[7] G.R. Her, J.T. Watson, Anal. Biochem. 151 (1985) 292.
[8] K. Kayganich, J.T. Watson, C. Kitts, J. Ritchie, Biomed. Mass Spectrom. 19 (1990) 341.
[9] W. Schänzer, A. Gotzmann, S. Horning, M. Donike, in: P. Kallings, U. Bondesson, E. Houghton (Eds.), Proceedings of the 10th International Conference of Racing Analysts and Veterinarians, Stockholm, 1994, p. 140.
[10] E. Houghton, P. Teale, M.C. Dumasia, J.K. Wellby, Biomed. Mass Spectrom. 9 (1982) 459.
[11] E. Houghton, P. Teale, M.C. Dumasia, Analyst 109 (1984) 273.
[12] T. Samuels, P. Teale, E. Houghton, in: P. Kallings, U. Bondesson, E. Houghton (Eds.), Proceedings of the 10th International Conference of Racing Analysts and Veterinarians, Stockholm, 1994, p. 115.
[13] M.A. Popot, V. Laroute, E. Lacabaratz, P. Garcia, P.L. Toutain, D.A. Cowan, in: D.E. Auer, E. Houghton (Eds.), Proceedings of the 11th International Conference of Racing Analysts and Veterinarians, Queensland, 1996, p. 62.
[14] M.F. Fiori, E. Pierdominici, F. Longo, G. Brabilla, J. Chromatogr. A 807 (1998) 219.
[15] B. Schubert, A. Ingvast, P.E. Andrén, U. Bondesson, in: D.E. Auer, E. Houghton (Eds.), Proceedings of the 11th International Conference of Racing Analysts and Veterinarians, Queensland, 1996, p. 203.
[16] G.M. Rodchenkov, V.D. Uralets, V.A. Semenov, P.A. Leclercq, J. High Resolut. Chromatogr., Chromatogr. Commun. 11 (1988) 283.
[17] E. Houghton, M.C. Dumasia, P. Teale, Analyst 113 (1988) 1179.

[18] S.R. Savu, L. Silvestro, A. Haag, F. Sörgel, J. Mass Spectrom. 31 (1996) 1351.
[19] D.A. Volmer, J.P.M. Hui, Rapid. Commun. Mass Spectrom. 11 (1997) 1926.
[20] S. Steffenrud, G. Maylin, J. Chromatogr. 577 (1992) 221.
[21] H. Shibasaki, T. Furuta, Y. Kasuya, J. Chromatogr. B 692 (1997) 7.
[22] S.J. Park, Y.J. Kim, H.S. Pyo, J. Park, J. Anal. Toxicol. 14 (1990) 102.
[23] M.R. Koupai-Abyazani, N. Yu, B. Esaw, B. Laviolette, J. Anal. Toxicol. 19 (1995) 182.
[24] E. Houghton, in: C.R. Short (Ed.), Proceedings of the 9th International Conference of Racing Analysts and Veterinarians, New Orleans, LA, 1992, p. 3, Vol. II.
[25] P.W. Tang, D.L. Crone, in: G.H. Johnston (Ed.), Proceedings of the 50th Meeting of Association of Official Racing Chemists, Boston, MA, 1997, p. 89.
[26] P.W. Tang, D.L. Crone, Anal. Biochem. 182 (1989) 289.
[27] C. Ayotte, D. Goudreaut, A. Charlebois, J. Chromatogr. B 687 (1996) 3.
[28] P.W. Tang, D.L. Crone, in: G.H. Johnston (Ed.), Proceedings of the 48th Meeting of Association of Official Racing Chemists, Oklahoma City, OA, 1995, p. 205.
[29] L.D. Bowers, D.J. Borts, J. Chromatogr. B 687 (1996) 69.
[30] T. Kim, J.W. Suh, J.C. Ryu, B.C. Chung, J. Park, J. Chromatogr. B 687 (1996) 79.