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Detection of anti-diabetics in equine plasma and urine by liquid chromatography–tandem mass spectrometry

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Abstract

Aim: Anti-diabetics such as sulfonylurea and thiazolidinedione derivatives are hypoglycemic drugs used for the treatment of diabetes. However, they can also be used as a stopper in horseracing. This paper describes a convenient method for the separation and simultaneous detection of 10 anti-diabetic drugs (namely glipizide, glibenclamide, glimepiride, gliclazide, tolazamide, tolbutamide, nateglinide, repaglinide, rosiglitazone and pioglitazone) in equine plasma and urine by LC–MS-MS. *Method:* The anti-diabetics were isolated from equine plasma and urine by liquid–liquid extraction with 1,2-dichloroethane at acidic pH, and analysed by LC–MS-MS in the positive electrospray ionisation mode. Separation of 10 anti-diabetic drugs was achieved with a reversed phase C8 column using a mixture of aqueous ammonium formate (pH 3.0, 10 mM) and methanol as the mobile phase. *Results:* Detection and confirmation of the 10 anti-diabetic drugs at 10 ng/mL each in equine plasma and equine urine were achieved by full-scan MS-MS. All of these drugs were detected consistently at this concentration in spiked samples of different plasma and urine (n = 15 each). No significant matrix interferences were observed at the expected retention times of the targeted ions in blank urine samples (n = 30). This method has been used successfully in the analysis of drug-administration samples as well as official racing samples. *Conclusion:* An LC–MS-MS method has been developed for the simultaneous detection of 10 anti-diabetics in equine plasma and urine. This method can be used to detect the abuse of anti-diabetic drugs in racehorses.

Keyword: Anti-diabetics

1. Introduction

Anti-diabetics such as sulfonylurea and thiazolidinedione derivatives are commonly prescribed hypoglycemic drugs for the treatment of non-insulin-dependent Type II diabetes mellitus. However, they can also be used as a stopper in racehorses by reducing the blood glucose level.

The 10 anti-diabetic drugs chosen for this study were glipizide, glibenclamide, glimepiride, gliclazide, tolazamide, tolbutamide, nateglinide, repaglinide, rosiglitazone and pioglitazone. Structures of the 10 anti-diabetic drugs are shown in Fig. 1.

The first six of them are sulfonylurea drugs, which can increase the secretion of insulin by functioning islet β-cells. In the past few decades, several generations of sulfonylurea drugs have been developed for common use. Glipizide, glibenclamide, glimepiride and gliclazide are the principle representatives from the latest generation. This generation of hypoglycemic drugs are much more potent and are therefore effective at much lower dosages. Repaglinide acts also by stimulating insulin secretion from the β-cells, but it binds to sites distinct from the sulfonylurea binding sites [1]. Rosiglitazone and pioglitazone are members of the

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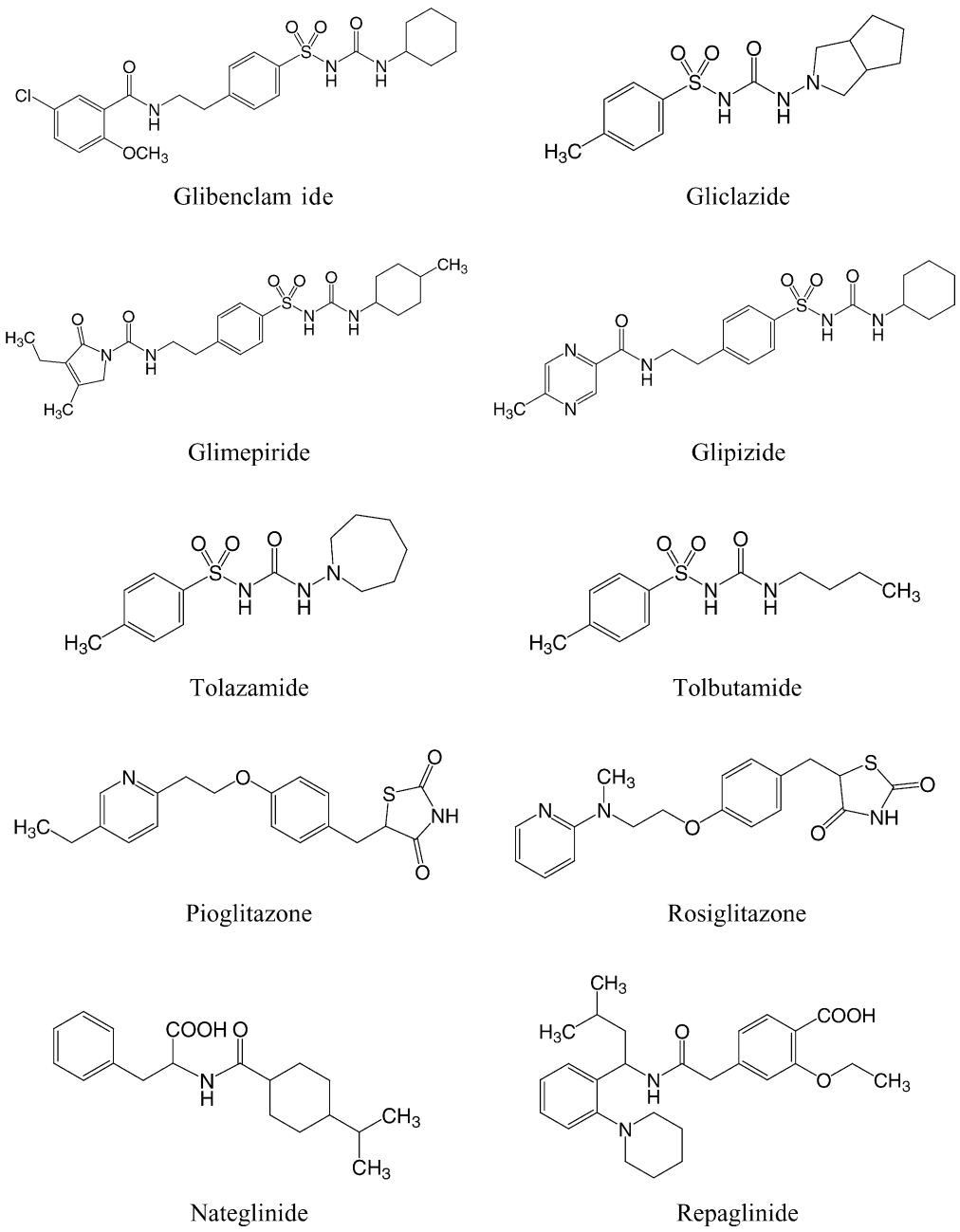

Fig. 1. Structures of the 10 anti-diabetic drugs under investigation.

thiazolidinedione class, which exert their glucose-lowering effects by binding to peroxisome proliferator-activated receptors gamma (PPARγ), thus increasing the receptor sensitivity to insulin [2–4]. The last target, nateglinide, functions by increasing pancreatic β-cell sensitivity to ambient glucose without increasing basal insulin secretion [5,6]. In addition to being the latest and most commonly prescribed drugs, the hypoglycemic drugs selected for this investigation are more pharmacologically potent, and subsequently, are prescribed at lower dosage. Because of the low drug levels in both urine and plasma, analytical methods for the detection of these drugs must be specific and sensitive in order to be able to adequately measure these anti-diabetics and their metabolites.

Several methods have been reported for the extraction and detection of anti-diabetic drugs in human plasma or urine, such as micellar electrokinetic capillary chromatography (MEKC) with diode-array detection (DAD) [7,8] and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [9–12]. However, most of them are tedious and time-consuming involving multiple extraction steps. Moreover, false positives may result from interferences due to basal components of sample matrices or concomitant therapies. Mass spectrometry, being a more definitive tech-

nique than both DAD and UV detection, may overcome these problems. In addition, tandem mass spectrometry can provide a further dimension of sample clean up which makes it well-suited in handling complex sample matrices. Application of liquid chromatography–mass spectrometry (LC–MS) for detection of anti-diabetics has been reported recently [13,14]. However, detection was optimised for only a few or just a single anti-diabetic drug in serum. Since urine samples are more readily available for drug testing in horseracing, methods capable of detecting multiple hypoglycemic drugs in urine as well as in plasma are desirable.

This paper describes a rapid and sensitive LC–MS method for the simultaneous determination of anti-diabetic drugs in equine plasma and urine after a simple single-step liquid–liquid extraction. Analytical quality criteria, including method sensitivity, specificity, precision and recovery, are discussed. The application of the method developed to profile glibenclamide and its metabolites after an oral administration of a single dose of Semi-Euglucon® (glibenclamide) to two geldings is demonstrated.

2. Experimental

2.1. Materials

Glibenclamide and glipizide were purchased from Sigma (St. Louis, MO, USA), glimepiride from Hoechst (Frankfurt, Germany), gliclazide from Servier (Gidy, France), tolazamide and diclofenac sodium from USP (Rockville, MD, USA), tolbutamide from EP (France), rosiglitazone maleate from Smithkline Beecham (Philadelphia, PA, USA) and repaglinide from Novo Nordisk (Bagsvaerd, Denmark). Pioglitazone was supplied by Takeda (Osaka, Japan), nateglinide by Novartis (Summit, NJ, USA) and 4-*trans*-hydroxyglibenclamide and 3-c*is*-hydroxyglibenclamide by Aventis Pharma (Frankfurt, Germany) as gifts. Semi-Euglucon® was purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Anhydrous sodium sulfate (granulated for organic trace analysis), potassium dihydrogen phosphate (KH_2PO_4, extra pure), citric acid (GR grade), acetonitrile (LiChrosolv®), 1,2-dichloroethane (GR grade) and methanol (LiChrosolv®) were obtained from Merck (Darmstadt, Germany). Ammonium formate was purchased from Fluka (Buchs, Switzerland). Formic acid was from Sigma. HPLC grade water was acquired from an in-house water purification system (Milli-Q, Molsheim, France). The HPLC mobile phases were filtered through a 0.45 μm Nylon-66 filter (Aligent Technologies, CA, USA) before use.

2.2. Instrumentation

LC–MS analyses of anti-diabetics were performed on a Thermo Finnigan LCQ Deca XP (Thermo Finnigan, San Jose, CA, USA) equipped with a Surveyor Autosampler and MS Pump system. The API source was operated in the positive ESI mode. A spray voltage of 5 kV and a capillary temperature of 250 °C was employed. The nitrogen sheath and auxiliary gas flow rates were set at 30 and 10 arbitrary LCQ units, respectively.

2.3. LC–MS screening for anti-diabetics

A reversed phase Supelcosil™ LC-8-DB column (10 cm × 2.1 mm i.d., 3 μm; Supelco, Bellefonte, PA, USA) was used for the analyses. The mobile phase was composed of water containing ammonium formate (pH 3.0, 10 mM) as solvent A and methanol as solvent B. A linear gradient was run at 0.2 mL/min, consisting of 30% solvent B at the start (t = 0 min), increased to 100% solvent B at t = 8 min, and then held at 100% solvent B for 5 min until t = 13 min. The gradient was subsequently decreased to 0% solvent B at t = 14 min, and held at 0% solvent B for 2 min. It was then returned to the initial mobile phase composition (30% solvent B) at t = 17 min, followed by an equilibration time of 3 min. The LC oven temperature was set at 30 °C and the injection volume was 5 μL each.

Screening and confirmation of the drugs were performed in MS-MS mode, with full-scan product-ion acquisition. The isolation width for MS-MS was set at 2 amu. Data acquisition for MS-MS was performed in two segments, covering the acquisition time before and after 9 min of the 20-min run. MS-MS data acquisition for pioglitazone, gliclazide, glipizide, tolazamide, tolbutamide, rosiglitazone was performed in segment 1, and glibenclamide, glimepiride, repaglinide, nateglinide, diclofenac acid in segment 2. The collision energy settings for these drugs ranged from 20 to 37% depending on the stability of the precursor ion towards collision-induced dissociation. The maximum ion injection time was set at 200 ms with 3 microscans for MS-MS analyses. In addition, a full-scan MS acquisition of the mass range between 150 and 550 was included in each of the two segments, with maximum ion injection time of 50 ms with 1 microscan, for the screening of any drugs or their metabolites that were not included in the target drug list. Signal-to-noise ratios were calculated using the Xcalibur S/N Calculator software, with the offset value set at 0.5 min after the base peak of interest, and background data points taken from 50 scans.

2.4. LC–MS quantification of glibenclamide and its metabolites

For the quantification of glibenclamide and its metabolites in equine urine and plasma, a slower gradient was applied with the same mobile phase mixture. The gradient was run at 0.2 mL/min, consisting of 30% solvent B at the beginning (t = 0 min), increased to 50% solvent B at t = 12 min, and then held at 50% solvent B for 7 min until t = 19 min. The gradient was then increased to 100% solvent B at t = 20 min, and held at 100% solvent B for 4 min. It was then returned to

the initial mobile phase composition (30% solvent B) at t = 25 min, followed by an equilibration time of 4 min. The LC oven temperature and the injection volume were the same as above.

For quantitative analysis of the administration samples, data acquisition was performed using MS-MS in the selected-reaction-monitoring (SRM) mode. The quantified ions selected were m/z 369 for glibenclamide, 4-*trans*-hydroxyglibenclamide (M1), 3-*cis*-hydroxyglibenclamide (M2) and m/z 321 for glipizide (internal standard for quantitative analyses). Helium was used as the collision gas.

2.5. Administration samples

Semi-Euglucon® (20 tablets, each tablet contains 2.5 mg glibenclamide) was administered orally by stomach tube to each of two thoroughbred geldings. One urine sample and one blood sample (50 mL) were collected from each horse before drug administration. Two urine samples were collected daily for 5 days and blood samples (50 mL) at 0, 0.5, 1, 2, 4, 8, 12, 24, 48 h after administration.

2.6. Extraction procedure for plasma samples

Blood samples were centrifuged at 3000 rpm for 30 min and plasma (2 mL) obtained. Diclofenac was used as the internal standard and was added to each sample to give a concentration of 100 ng/mL. Each sample was then diluted with citric acid/disodium hydrogenphosphate buffer (0.083 M/0.033 M, pH 2.8, 4 mL). The mixture was rotated with 1,2-dichloroethane (4.0 mL) for 3 min and centrifuged at 3000 rpm for 15 min. The organic layer was pipetted out and passed through a sodium sulfate drying tube. The solvent was evaporated to dryness under nitrogen. The dried residue was then reconstituted with H_2O/MeOH (1:1, v/v, 50 μL). The content was transferred to a conical insert in a Chrompack autosampler vial for LC–MS analysis.

2.7. Extraction procedure for urine samples

Aliquots (4 mL) of horse urine samples were used for the analysis. Diclofenac was used as the internal standard and was added to the samples to give a concentration of 100 ng/mL. Each sample was then diluted with potassium dihydrogen phosphate buffer (0.1 M, pH 2.3, 2 mL) and adjusted to pH 3.0. The mixture was rotated with 4.0 mL 1,2-dichloroethane for 3 min and centrifuged at 3000 rpm for 15 min. The organic layer was pipetted out and passed through a sodium sulfate drying tube. The solvent was evaporated to dryness under nitrogen. The dried residue was then reconstituted with H_2O/MeOH (1:1, v/v, 50 μL). The content was transferred to a conical insert in a Chrompack autosampler vial for LC–MS analysis.

2.8. Preparation of calibrators for quantitative analyses of glibenclamide and its metabolites

Calibration curves were prepared for the quantitative analyses of the samples obtained from the glibenclamide administrations. The calibration ranges were determined based on the initial semi-quantification of the administration samples using the screening method. For glibenclamide, the calibrators were prepared at 0, 10, 20, 30 and 50 ng/mL in plasma, and at 0, 1, 2, 3 and 5 ng/mL in urine. Glipizide was used as the internal standard and was spiked at 20 ng/mL in plasma samples and at 5 ng/mL in urine samples. For both 4-*trans*-hydroxyglibenclamide and 3-*cis*-hydroxyglibenclamide, the calibrators were prepared at 0, 10, 20, 30 and 50 ng/mL in urine. Glipizide was spiked at 20 ng/mL in urine samples as the internal standard. The quality control samples, spiked at 20 ng/mL glibenclamide in plasma, 2 ng/mL glibenclamide, and 20 ng/mL each of 4-*trans*-hydroxyglibenclamide and 3-*cis*-hydroxyglibenclamide in urine, were also prepared. Calibrators and quality controls were prepared independently from separate weighing of the drug standards. For each batch of samples, the calibrators and quality controls were prepared freshly prior to assay and analysed in duplicate with the test samples. Samples with drug concentrations outside the calibration range were diluted with the corresponding blank matrix before sample extraction. The peak area ratios of glibenclamide, 4-*trans*-hydroxyglibenclamide or 3-*cis*-hydroxyglibenclamide to glipizide (internal standard) versus the concentration of the calibrators were fitted using linear regression to obtain the calibration curve. The response was linear throughout the concentration ranges of this study, with correlation coefficients (r) greater than 0.99 in all cases. The measured concentrations of the quality control samples were found to give a deviation of less than ±10% from their target values.

3. Results and discussion

3.1. Method sensitivity and specificity

Within the same 20-min LC run, all the 10 targeted anti-diabetic drugs at 10 ng/mL each under investigation could be easily detected from equine plasma and urine matrices (n = 15 each). Confirmation of these drugs could be readily achieved by comparing the product-ion mass spectra as well as the retention times obtained from the sample with those of their corresponding drug standards.

The limit of detection (LOD) was defined as the lowest concentration that could consistently produce an extracted-ion chromatogram of the target product-ion with signal-to-noise ratio greater than or equal to 3. Fig. 2 shows the extracted-ion chromatograms of the 10 anti-diabetics obtained from plasma and urine samples spiked with the drugs at 1 ng/mL. The signal-to-noise ratios larger than 10 were obtained for all the drugs investigated indicate

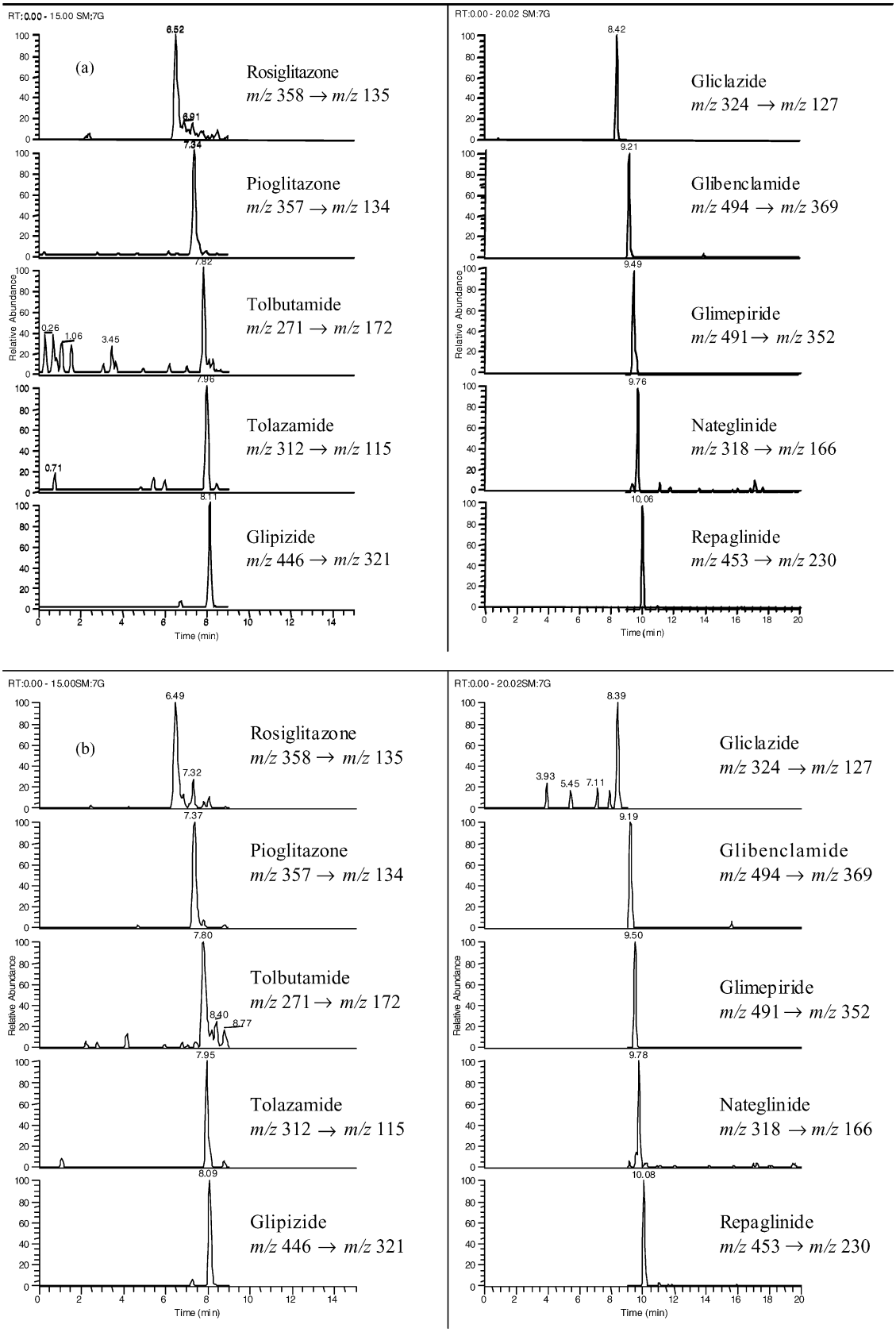

Fig. 2. Extracted-ion chromatograms from (a) a plasma and (b) a urine sample spiked with the 10 anti-diabetic drugs at 1 ng/mL. (Note: The typical retention time of diclofenac (internal standard) is 9.6 min.)

Table 1
Precision data (%R.S.D.) for area ratios and relative retention times for the analysis of sample extracts ($n = 6$) from plasma and urine spiked with 10 anti-diabetic drugs using diclofenac as internal standard

	Plasma (% R.S.D.)					Urine (%R.S.D.)				
	Day 1	Day 2	Day 3	Day 4	Inter-day	Day 1	Day 2	Day 3	Day 4	Inter-day
Peak area ratio										
Glibenclamide	8	15	22	20	17	27	15	22	31	25
Gliclazide	25	22	20	9	20	32	16	19	36	27
Glimepiride	24	20	15	13	19	26	19	21	33	25
Glipizide	25	19	19	13	19	32	21	17	22	24
Nateglinide	24	18	32	24	25	24	9	18	27	21
Pioglitazone	19	27	25	37	28	29	11	39	30	29
Repaglinide	15	20	14	22	18	26	18	21	25	23
Rosiglitazone	31	27	29	43	33	28	11	48	29	32
Tolazamide	38	22	22	15	26	36	23	22	27	28
Tolbutamide	22	18	17	13	18	35	17	11	33	26
Relative retention time										
Glibenclamide	0.15	0.18	0.02	0.22	0.16	0.17	0.02	0.02	0.02	0.09
Gliclazide	0.14	0.18	0.22	0.23	0.20	0.16	0.02	0.05	0.04	0.09
Glimepiride	0.20	0.01	0.17	0.20	0.17	0.22	0.16	0.20	0.22	0.20
Glipizide	0.24	0.15	0.20	0.20	0.20	0.25	0.17	0.05	0.18	0.18
Nateglinide	0.21	0.02	0.20	0.20	0.18	0.21	0.21	0.16	0.19	0.19
Pioglitazone	0.47	0.20	0.27	0.22	0.31	0.15	0.01	0.23	0.20	0.17
Repaglinide	0.18	0.03	0.20	0.20	0.17	0.20	0.21	0.20	0.18	0.20
Rosiglitazone	1.06	0.82	0.33	0.68	0.77	0.26	0.36	0.82	0.81	0.62
Tolazamide	0.14	0.06	0.17	0.24	0.16	0.14	0.03	0.19	0.22	0.16
Tolbutamide	0.30	0.07	0.04	0.20	0.18	0.23	0.24	0.23	0.22	0.23

that the LODs for all 10 anti-diabetics were well below 1 ng/mL.

The method specificity was assessed with different post-race equine plasma and urine samples ($n = 30$ each) analysed with the described method. Interferences from the matrices at the targeted masses and retention times were not observed.

3.2. Method precision

Method precision and the reproducibility of the LC–MS relative retention times were evaluated by replicate analyses ($n = 6$) of a sample containing 100 ng/mL diclofenac (internal standard) and 50 ng/mL of each anti-diabetic drug on 4 consecutive days. Method precision was calculated as percentage relative standard deviation (%R.S.D.) of the peak area ratio of anti-diabetics to the internal standard. Table 1 summarises the precision data. The inter-day precision of the peak area ratios were about 20–30% and those of the relative retention times were less than 0.31% for all targeted drugs, except for rosiglitazone which gave %R.S.D.s of 33% for the peak area ratios and 0.77% for the relative retention time.

Table 2
Recovery of 10 anti-diabetic drugs from different plasma and urine samples, with each drug spiked at 10 ng/mL

Drug	Plasma samples ($n = 6$)		Urine samples ($n = 6$)	
	Recovery (%)	%R.S.D.	Recovery (%)	%R.S.D.
Glibenclamide	85.8	21.1	46.1	33.5
Gliclazide	82.3	24.3	55.8	65.7
Glimepiride	70.7	11.7	49.3	23.9
Glipizide	87.5	20.1	61.5	42.2
Nateglinide	79.1	15.3	46.3	32.9
Pioglitazone	44.4	18.8	53.5	28.9
Repaglinide	78.0	16.0	53.6	32.9
Rosiglitazone	58.2	14.9	29.6	35.5
Tolazamide	66.8	46.8	55.0	39.7
Tolbutamide	89.1	37.7	57.2	54.7

3.3. Extraction recovery

Plasma and urine samples from different horses ($n = 6$) spiked with the 10 anti-diabetic drugs at 10 ng/mL each were

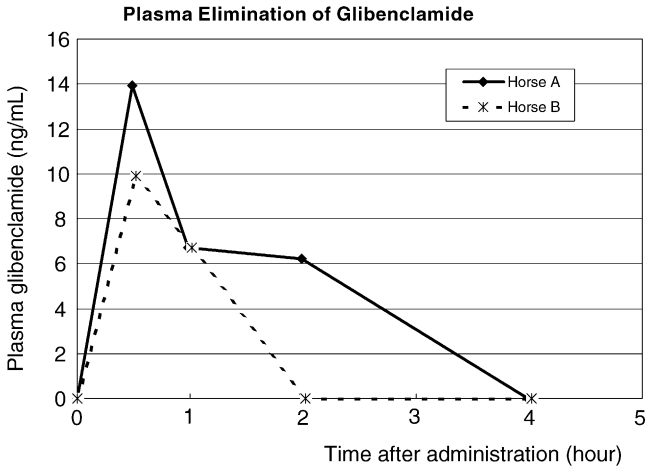

Fig. 3. Plasma elimination of glibenclamide from two horses after an oral administration of Semi-Euglucon®. Each horse was given 50 mg of glibenclamide by stomach tube.

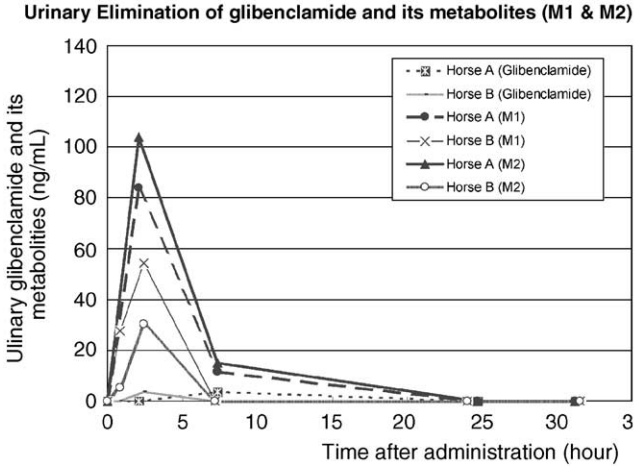

Fig. 4. Urinary elimination of glibenclamide and its metabolites, 4-*trans*-hydroxyglibenclamide (M1) and 3-*cis*-hydroxyglibenclamide (M2), from two horses after an oral administration of Semi-Euglucon®. Each horse was given 50 mg of glibenclamide by stomach tube.

analysed. Internal standard diclofenac was added after extraction. The area ratios of the targeted drugs to the internal standard were compared with those obtained from blank extracts spiked with the 10 target drugs after extraction (taken as 100% recovery of the drug from that particular matrix). Recoveries of the 10 anti-diabetic drugs are summarised in Table 2. The mean recoveries were found to range from 44 to 89% in plasma and 30 to 62% in urine.

3.4. Application of the method in administration studies of glibenclamide

Glibenclamide is one of the most potent anti-diabetic drugs for which much lower dosages are required. Glibenclamide has been reported to be cleared quickly within 5 h in plasma for humans [14]. Fig. 3 shows the plasma elimination profile of glibenclamide in two horses following an oral dose of Semi-Euglucon® (50 mg of glibenclamide). The highest concentration of glibenclamide in plasma (at about 14 ng/mL) was observed at 0.5 h after administration. Plasma glibenclamide was rapidly eliminated from both horses, being quantifiable up to only 2 h after dosing. Metabolites were not detected in the post-administration plasma samples.

Fig. 4 shows the urinary elimination profile in two horses. The results indicated that only a small amount of the administered dose was recovered as parent drug with peak urinary concentrations at about 3.5 ng/mL.

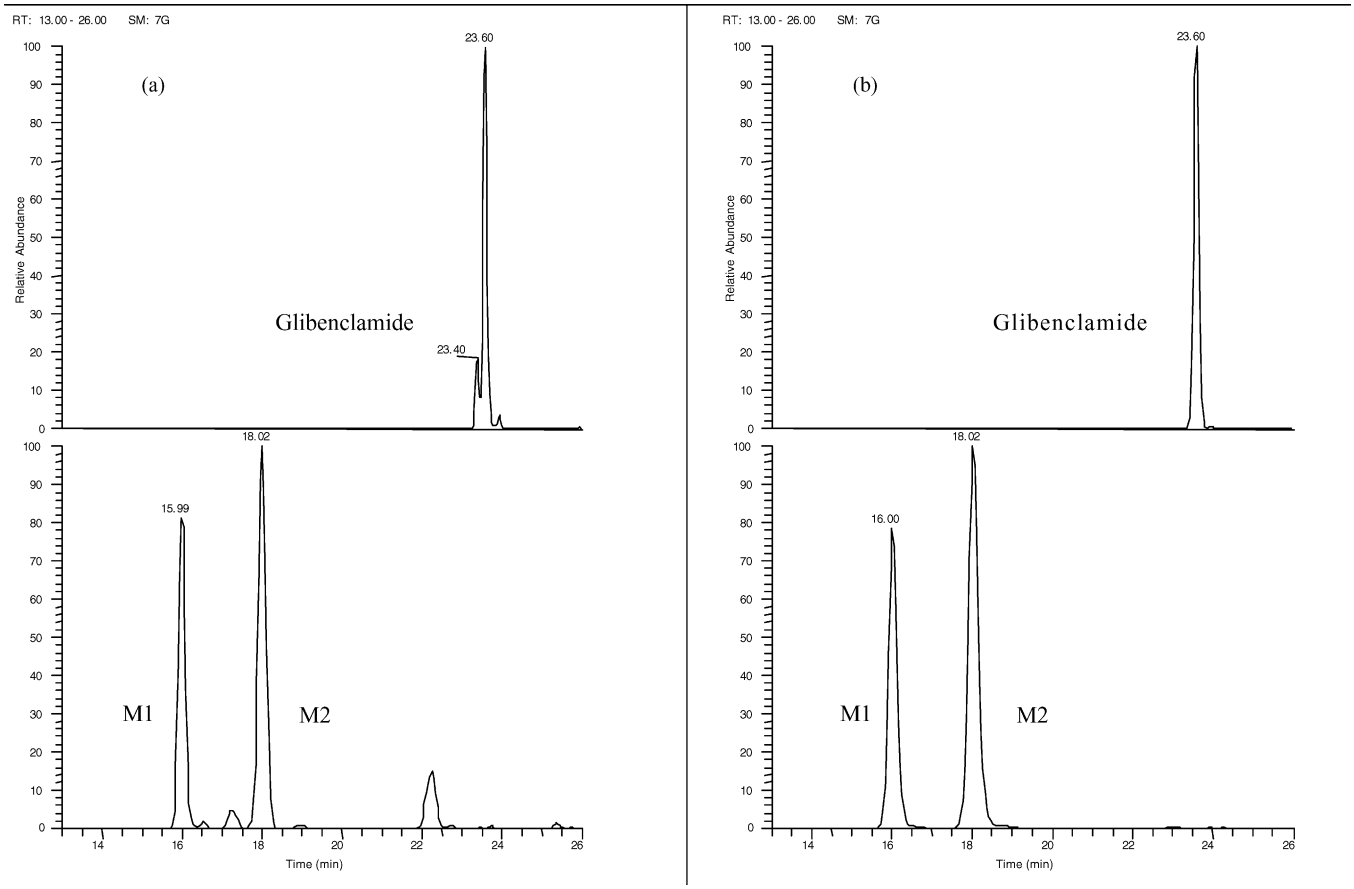

Fig. 5. Extracted-ion chromatograms for glibenclamide, M1 and M2 from (a) the urine sample collected 7.5 h after a single dose oral administration of glibenclamide and (b) the authentic standards.

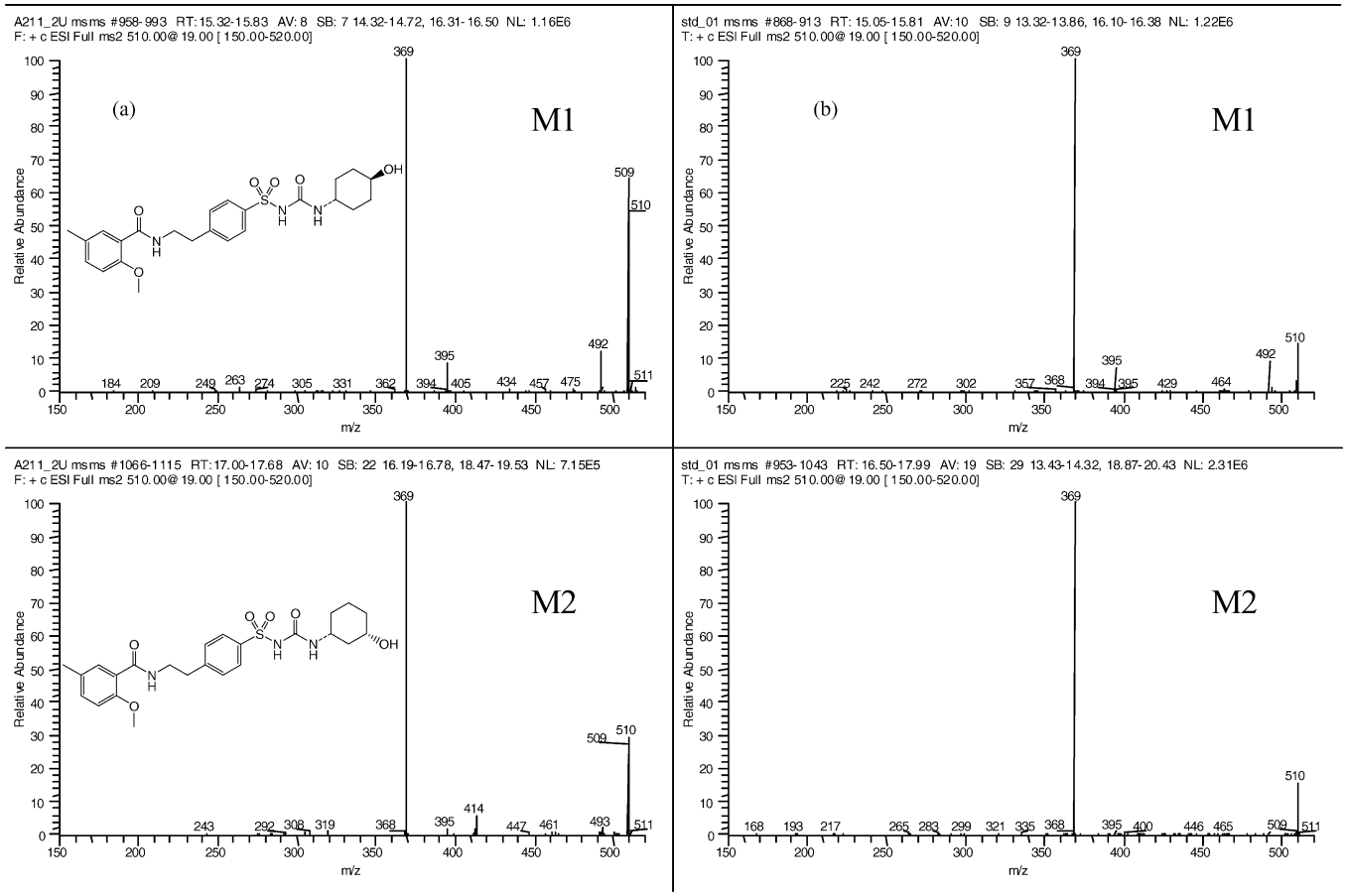

Fig. 6. Product-ion scan of precursor ion *m*/*z* 510 of M1 and M2 from (a) the urine sample collected 7.5 h after a single dose oral administration of glibenclamide and (b) the authentic standards.

This observation is consistent with reported human studies on glibenclamide [15,16], showing <1% excretion of parent glibenclamide in urine. Conversely, its two metabolites, 4-*trans*-hydroxyglibenclamide (M1) and 3-*cis*-hydroxyglibenclamide (M2), were readily identified in urine. Both metabolites are the result of hydroxylation of the cyclohexyl group and had been previously reported in humans [16]. Both M1 and M2 had peak concentrations (M1: 84 ng/mL for Horse A, 55 ng/mL for Horse B; M2: 104 ng/mL for Horse A, 30 ng/mL for Horse B) at 2 h post-administration and could be detected for up to 7.5 h, suggesting that urine may provide a longer detection duration than plasma. The result also shows that M1 and M2 are the analytes of choice for the detection of glibenclamide administration, as they have higher urinary concentrations and could be detected longer than the parent drug.

The extraction procedures described allow simultaneous extraction of glibenclamide together with M1 and M2, from both equine plasma and urine. Both the hydroxylated metabolites (M1 and M2) are more hydrophilic than their parent and elute much earlier on a reversed phase column. A slower solvent gradient was found to be more suitable for the quantification of the metabolites. Fig. 5 shows the extracted-ion chromatograms from a urine sample collected 7.5 h post-administration. Glibenclamide, M1 and M2 could all be easily detected. Both the retention times and the product-ion spectra from M1 and M2 in the post-administration sample match well with those from the corresponding authentic standards. Fig. 6 shows the product-ion mass spectra of *m*/*z* 510 (MH^+ of M1 and M2) from a post-administration urine sample and the corresponding authentic standards. Glibenclamide and its metabolites M1 and M2 all produce the same major MS-MS fragment ion at *m*/*z* 369, which originates from the cleavage of the amide bond of the urea moiety with the loss of the cyclohexylamine group.

4. Conclusion

This study demonstrated the application of LC–MS-MS for the detection of anti-diabetic drugs in equine plasma and urine. The method allows the simultaneous identification of 10 commonly used hypoglycemic drugs in both plasma and

urine after a simple liquid–liquid extraction step. The detection of the anti-diabetics at 1 ng/mL in equine plasma and urine could be achieved. Validation experiments have shown that the method has adequate precision and no interference from sample matrix was observed. This method was also validated through its application to the analysis of plasma and urine samples obtained from drug administration studies. After an oral dosing with 50 mg of glibenclamide, the peak plasma concentration of glibenclamide was rapidly attained at less than an hour. Thereafter, plasma concentrations decreased rapidly and remained detectable in plasma for about 2 h. Both glibenclamide and its metabolites, 4-*trans*-hydroxyglibenclamide (M1) and 3-*cis*-hydroxyglibenclamide (M2), were detected in urine for up to 7.5 h. This method is simple, rapid and robust, and has been used successfully in the analysis of drug-administration samples as well as a large number of official racing samples.

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References

[1] J. Fuhlendroff, P. Rorsman, H. Kofod, C.L. Brand, B. Rolin, P. Mackay, R. Shymko, R.D. Carr, Diabetes 47 (1998) 345.
[2] J.M. Lehmann, L.B. Moore, T.A. Smith-Oliver, W.O. Wilkison, T.M. Willson, S.A. Kliewer, J. Biol. Chem. 270 (1995) 12953.
[3] T.M. Willson, J.E. Cobb, D.J. Cowan, R.W. Wiethe, I.D. Correa, S.R. Prakash, K.D. Beck, L.B. Moore, S.A. Kliewer, J.M. Lehmann, J. Med. Chem. 39 (1996) 665.
[4] P.W. Young, D.R. Buckle, B.C.C. Cantello, H. Chapman, J.C. Clapham, P.J. Coyle, D. Haigh, R.M. Hindley, J.C. Holder, H. Kallender, A.J. Latter, K.W.M. Lawrie, D. Mossakowska, G.J. Murphy, L. Roxbee Cox, S.A. Smith, J. Pharmacol. Exp. Ther. 284 (1998) 751.
[5] A.H. Karara, B.E. Dunning, J.F. McLeod, J. Clin. Pharmacol. 39 (1999) 172.
[6] L. Keilson, S. Mather, Y.H. Walter, S. Subramanian, J.F. McLeod, J. Clin. Endrocrinol. Metab. 85 (2000) 1081.
[7] M. Núñez, J.E. Ferguson, D. Machacek, G. Jocab, R.P. Oda, G.M. Lawson, J.P. Landers, Anal. Chem. 67 (1995) 3668.
[8] M.E. Roche, R.P. Oda, G.M. Lawson, J.P. Landers, Electrophoresis 18 (1997) 1865.
[9] L.L. Hansen, K. Brosen, Ther. Drug Monit. 21 (1999) 664.
[10] J.R.V. Santurio, E.G. Porto, J. Chromatogr. B 682 (1996) 364.
[11] I. Ono, K. Matsuda, S. Kanno, J. Chromatogr. B Biomed. Sci. Appl. 692 (1997) 397.
[12] K. Yamashita, H. Murakami, T. Okuda, M. Motohashi, J. Chromatogr. B Biomed. Sci. Appl. 677 (1996) 141.
[13] F. Magni, L. Marazzini, S. Pereira, L. Monti, M. Galli Kienle, Anal. Biochem. 282 (2000) 136.
[14] S. Hsieh, K. Selinger, J. Chromatogr. B 772 (2002) 347.
[15] M.E. Roche, R.P. Oda, G.M. Lawson, J.P. Landers, Electrophoresis 18 (1997) 1865.
[16] T. Rydberg, E. Wahlin-Boll, A. Melander, J. Chromatogr. 564 (1991) 223.