ELSEVIER

Journal of Chromatography B, 783 (2003) 411–423

JOURNAL OF
CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Simultaneous determination of six urinary porphyrins using liquid chromatography–tandem mass spectrometry

Wei Bu[a,*], Nichole Myers[a], Jane D. McCarty[b], Tom O'Neill[a], Shelly Hollar[a], Philip L. Stetson[a], Daniel W. Sved[a]

[a] *WIL Research Laboratories, Inc., Ashland, OH 44805, USA*
[b] *FMC Corporation, Princeton, NJ 08543, USA*

Received 9 April 2002; received in revised form 28 August 2002; accepted 11 September 2002

Abstract

A liquid chromatographic–tandem mass spectrometric (LC–MS–MS) method without sample pretreatment was developed and validated for determination of porphyrins in samples of canine urine. Acidified urine samples were directly injected into the LC–MS system and a gradient elution program was applied. The mass spectrometer was operated in the multi-reaction monitoring (MRM) mode and six porphyrins were detected with excellent sensitivity and selectivity. The lower limits of quantification were 0.014 nmol/mL for mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin and 7-carboxylporphyrin, and 0.029 nmol/mL for uroporphyrin I. Good ln-quadratic responses of calibration standards over the range 0.01 to 1.0 nmol/mL for mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin and 7-carboxylporphyrin, and 0.02 to 1.0 nmol/mL for uroporphyrin I were demonstrated. This method should be easily adapted through cross-validation for use in determining the effects of chemicals and pharmaceuticals on the urinary excretion profile of porphyrins in preclinical studies with other species, and in assisting the diagnosis of porphyria in clinical studies.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Porphyrins

1. Introduction

Porphyrins are a ubiquitous class of naturally occurring compounds with many important biological representatives, including hemes and chlorophylls. All porphyrin molecules have in common the porphyrin macrocyclic structure. The basic structure of the porphyrin macrocycle consists of four pyrrolic subunits linked by four methane bridges (Fig. 1).

Quantitative analysis of urinary porphyrins is important in the diagnosis of porphyria and for monitoring the effects of chemicals or drugs on porphyrin excretion. Porphyria is a series of related metabolic disorders in which there is accumulation of porphyrins or their precursors. These disorders are associated with defects in one of several enzymes catalyzing the synthesis of heme from porphyrins. These enzyme deficiencies can be recognized on the basis of the degree and characteristic pattern of urinary porphyrin excretion [1,2]. Several techniques have previously been developed for determining urinary porphyrins, e.g. spectrofluorometry [3], sec-

*Corresponding author. Tel.: +1-419-289-8700; fax: +1-419-289-3650.
E-mail address: wbu@wilresearch.com (W. Bu).

1570-0232/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1570-0232(02)00703-1

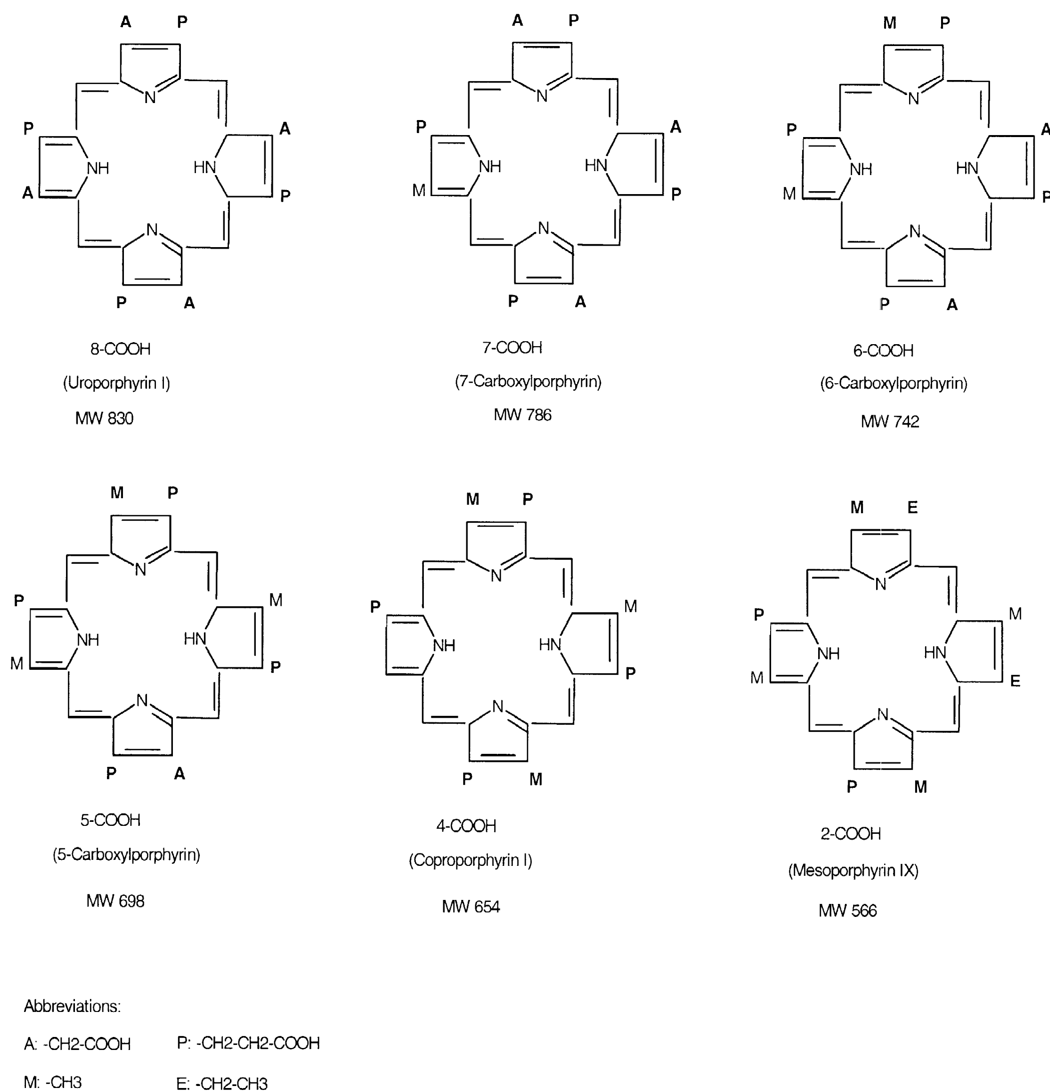

Fig. 1. Structures of porphyrins.

ond-derivative spectroscopy [4], high-performance liquid chromatography (HPLC) [5,6], thin-layer chromatography [7,8], ion-pair HPLC [9], capillary electrophoresis [10], laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF MS) [11], fast atom bombardment mass spectrometry (FAB-MS) [12], and LC–MS [13]. Most of these methods involve either sample pretreatment [3–6] or derivatization procedures that require a large sample size and are time-consuming [11–13].

The present study describes a simple, fast, sensitive and specific assay for the determination of urinary porphyrins using HPLC with tandem mass spectrometry (LC–MS–MS) by direct injection without sample pretreatment. Compared to an HPLC–FLD (HPLC with fluorescence detector) assay, the LC–MS–MS method (with similar chromatographic conditions) demonstrated 10 times better sensitivity and far superior selectivity. The quantitation of porphyrin isomers by this system may be possible if the chromatography is optimized for this purpose or a chiral column is used to give

sufficient separation of isomers. Further research is needed to address the suitability of this method with porphyrin isomers.

2. Experimental

2.1. Materials and reagents

Porphyrin acid chromatographic marker kits containing 10 nmol of each of six porphyrins (mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin, 7-carboxylporphyrin and uroporphyrin I) were obtained from Porphyrin Products (Logan, UT, USA). HPLC-grade water and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analysis grade formic acid was a product of Sigma (St. Louis, MO, USA).

2.2. Instrumentation

A Micromass tandem quadrupole Quattro Ultima mass spectrometer (Micromass, Altrincham, UK), equipped with an electrospray ionization interface, a Hewlett-Packard (HP) (Wilmington, DE, USA) 1100 binary pump solvent delivery system, an HP 1100 degasser, an HP diode-array detector, and an HP 1100 auto-sampler, was used for LC–MS–MS analysis. Data acquisition and analysis were performed using MassLynx software version 3.4.

2.3. Chromatographic conditions

The chromatographic separation was performed on a 3×3 C_{18} cartridge (Perkin-Elmer, Wellesley, MA, USA) with a C_{18} guard column. A mobile phase gradient program with solvent A (0.1%, v/v, formic acid) and solvent B (90:9.9:0.1, v/v, acetonitrile–water–formic acid) was applied at a flow-rate of 1.0 mL/min. The gradient program started with 20% B for 1 min followed by a linear increase in B to 80% from 1 to 4 min and another linear increase in B to 95% from 4 to 4.1 min. Mobile phase B was held at 95% for another 2.9 min (from 4.1 to 7 min) and then reduced linearly to the initial condition (20% B) within 0.1 min. This condition was held until the end of the run. The total run time was 11.1 min.

2.4. LC–MS–MS conditions

A Micromass Quattro Ultima tandem mass spectrometer was interfaced via an electrospray ionization probe in the positive ion mode (ESI^+) with the HPLC system. Nitrogen was used as the desolvation gas at a flow-rate of 492 L/h and, also, as the nebulizing gas at a flow-rate of 100 L/h. The source block and desolvation temperatures were set at 100 and 350 °C, respectively. A 90:10 split was used between the HPLC and MS, which resulted in an approximate spray flux of 100 μL/min into the mass spectrometer. The capillary voltage was 4.0 kV. With continuous infusion of porphyrin standard (10 nmol/mL) at a flow-rate of 10 μL/min, the MS–MS parameters were optimized by determination of the best conditions for isolating precursor ions and creating product ions. Argon was used as the collision gas with an analyzer pressure of $5.5 \cdot 10^{-3}$ mbar. The cone voltage, collision energy and lens conditions were adjusted and the product ions were scanned to establish the optimal ions for quantitation of each porphyrin using the multiple reaction monitoring (MRM) mode. A dwell time of 0.2 s with an inter-channel delay of 0.03 s was used for each analyte. The electron multiplier setting was 650 V.

2.5. Calibration standards and quality control samples

A primary stock solution of six porphyrins was prepared by dissolving one vial of standard in 10.0 mL of 6 *M* formic acid followed by filtration with a 13-mm, 0.45-μm, syringe-driven filter. The final concentration of each porphyrin was 1.0 nmol/mL. Calibration standards were prepared by the dilution of the stock solution with 6 *M* formic acid to yield concentrations of 0.01 to 1.0 nmol of each porphyrin/mL. Quality control (QC) samples at 0.05, 0.2 and 0.8 nmol of each porphyrin/mL were prepared by the dilution of the primary stock with 6 *M* formic acid.

2.6. Sample preparation

Urine aliquots (700 μL) were transferred to auto-sampler vials. To each vial, 300 μL of formic acid (20 *M*) was added. The vials were capped and mixed

with vortex action. Samples with visible precipitation or cloudiness were centrifuged prior to analysis.

2.7. Calibration and quantitation

An external standard method of quantitation was used for determination of the six porphyrins in canine urine. Six calibration curves were constructed for each set of analyses. For mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin and 7-carboxylporphyrin, the calibration samples ranged in concentration from 0.01 to 1.0 nmol/mL. For uroporphyrin I, the calibration concentrations ranged from 0.02 to 1.0 nmol/mL. The porphyrin peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit to the ln-quadratic function using the least squares regression in Microsoft Excel. The results of the regression analysis were then used to back-calculate the concentration results from the peak area data, and the back-calculated concentrations and appropriate summary statistics [mean, standard deviation (SD), and percent relative standard deviation (RSD)] were calculated and presented in tabular form.

2.8. Application of the analytical method

The LC–MS–MS procedure developed was used to investigate porphyrin profiles in canine urine samples.

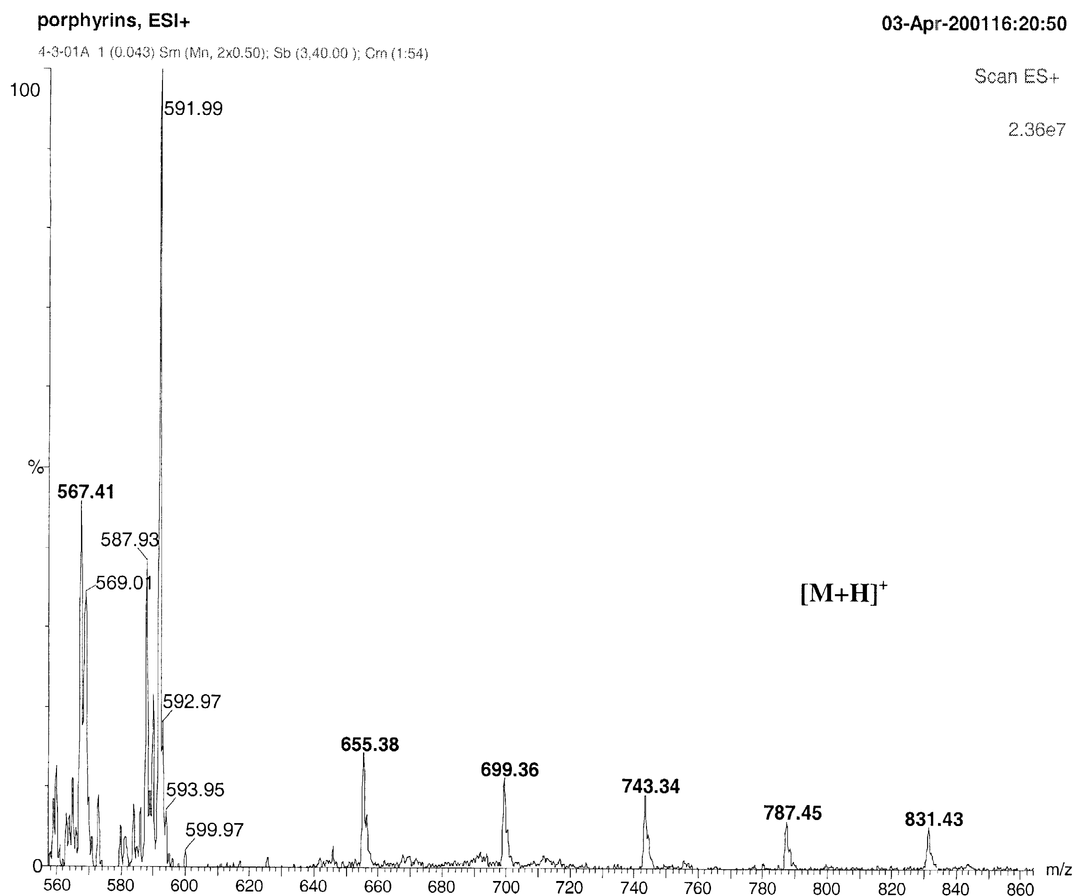

Fig. 2. Mass spectra of precursor ions of six porphyrins (10 nmol/mL, flow-rate 10 μL/mL): mesoporphyrin IX (*m/z* 567), coproporphyrin I (*m/z* 655), 5-carboxylporphyrin (*m/z* 699), 6-carboxylporphyrin (*m/z* 743), 7-carboxylporphyrin (*m/z* 787) and uroporphyrin I (*m/z* 831).

3. Results and discussion

3.1. Method development

The best LC–MS results can be achieved by optimization of both HPLC and MS conditions. In this study a gradient chromatographic program was developed to give sufficient separation of six porphyrins in a short period of time. Electrospray ionization in the positive mode was used based on the basic porphyrin structure (Fig. 1), in which the two pyrrolic nitrogen atoms bearing one pair of electrons (pK_b~9) can be protonated easily with acids [14]. A scan of a porphyrin standard solution dissolved in 6 *M* formic acid (10 nmol/mL) by MS1 displayed dominant pseudo-molecular ions $[M+H]^+$ for all six porphyrins (Fig. 2). These pseudo-molecular ions were chosen as precursor ions to undergo fragmentation. Argon gas was introduced into Q2 to collide with precursor ions and fragmentation was achieved with a cone voltage of 10 V and a collision energy of 55 eV for all precursor ions. A mixture of α- and β-cleavage was observed after fragmentation. For 5-carboxylporphyrin, 6-carboxylporphyrin, 7-carboxylporphyrin and uroporphyrin I significant product ions were obtained by the loss of an acetyl group and a carboxyl group (−104 *m/z*) from the precursor ions. A significant product ion of coproporphyrin I was observed at *m/z* 537 (Fig. 3), resulting from the loss of a single propionyl group and three methyl groups (−118 *m/z*) from the precursor ion. The precursor ion of mesoporphyrin IX gave a significant product ion (*m/z* 479) from the loss of a single propionyl group and a single methyl group (−88 *m/z*). The precursor ions, product ions and the transition conditions of all six porphyrins are listed in Table 1.

3.2. Assay validation

3.2.1. Specificity

Porphyrins are endogenous compounds and, therefore, there is no "blank urine" available and the specificity of the method was studied using the

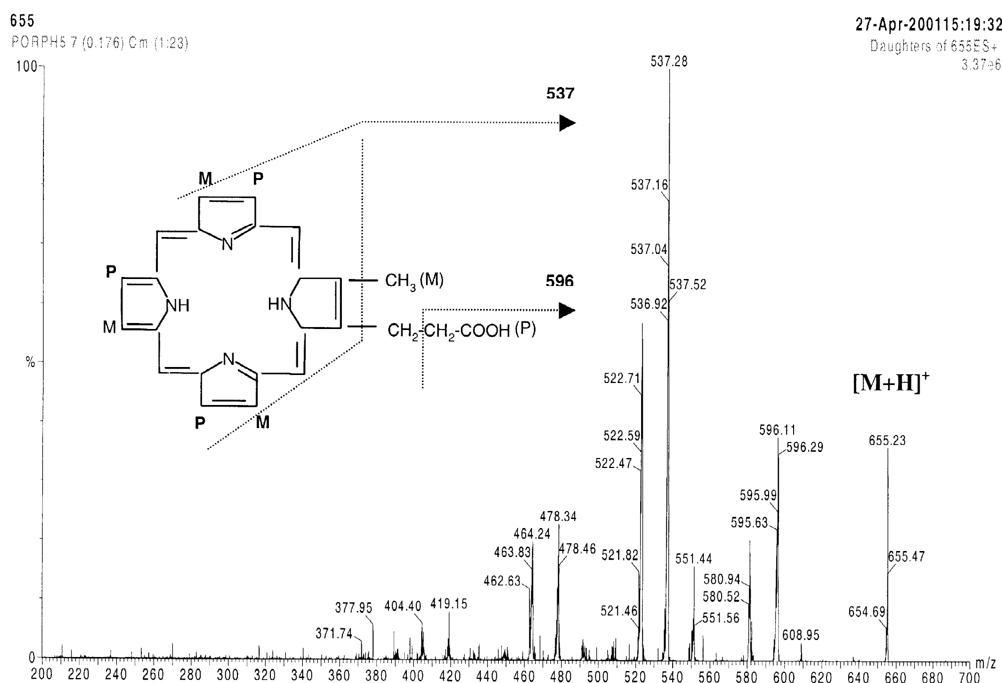

Fig. 3. Representative product ion spectrum of coproporphyrin I (655>537). The significant product ion is produced by losing a single propionyl group and three methyl groups (−118 *m/z*). Another product ion is obtained from α-cleavage (−59 *m/z*).

Table 1
Precursor ions and product ions of porphyrins used for quantitation in MS–MS with positive electrospray mode (ESI+)

Porphyrin	Precursor ion (M+1) (*m*/*z*)	Major product ion (*m*/*z*)	Mass loss (Da)	Functional group lost	Other product ions (*m*/*z*)	Cone voltage (V)	Collision energy (eV)	Mass monitor window (min)
Mesoporphyrin IX	567	479	88	1P, 1M	493, 449, 420, 405	10	55	5.5–6.9
Coproporphyrin I	655	537	118	1P, 3M	596, 551, 478	10	55	3.2–4.5
5-Carboxylporphyrin	699	595	104	1A, 1(–COOH)	639, 581, 566, 522, 463	10	55	3.0–4.3
6-Carboxylporphyrin	743	639	104	1A, 1(–COOH)	683, 625, 579, 506	10	55	2.9–4.2
7-Carboxylporphyrin	787	683	104	1A, 1(–COOH)	728, 699, 656, 622, 577	10	55	2.7–4.1
Uroporphyrin I	831	727	104	1A, 1(–COOH)	785, 749, 714, 667, 590	10	55	2.5–4

P, propionyl group (–CH_2–CH_2–COOH); M, methyl group (–CH_3); A, acetyl group (–CH_2–COOH).

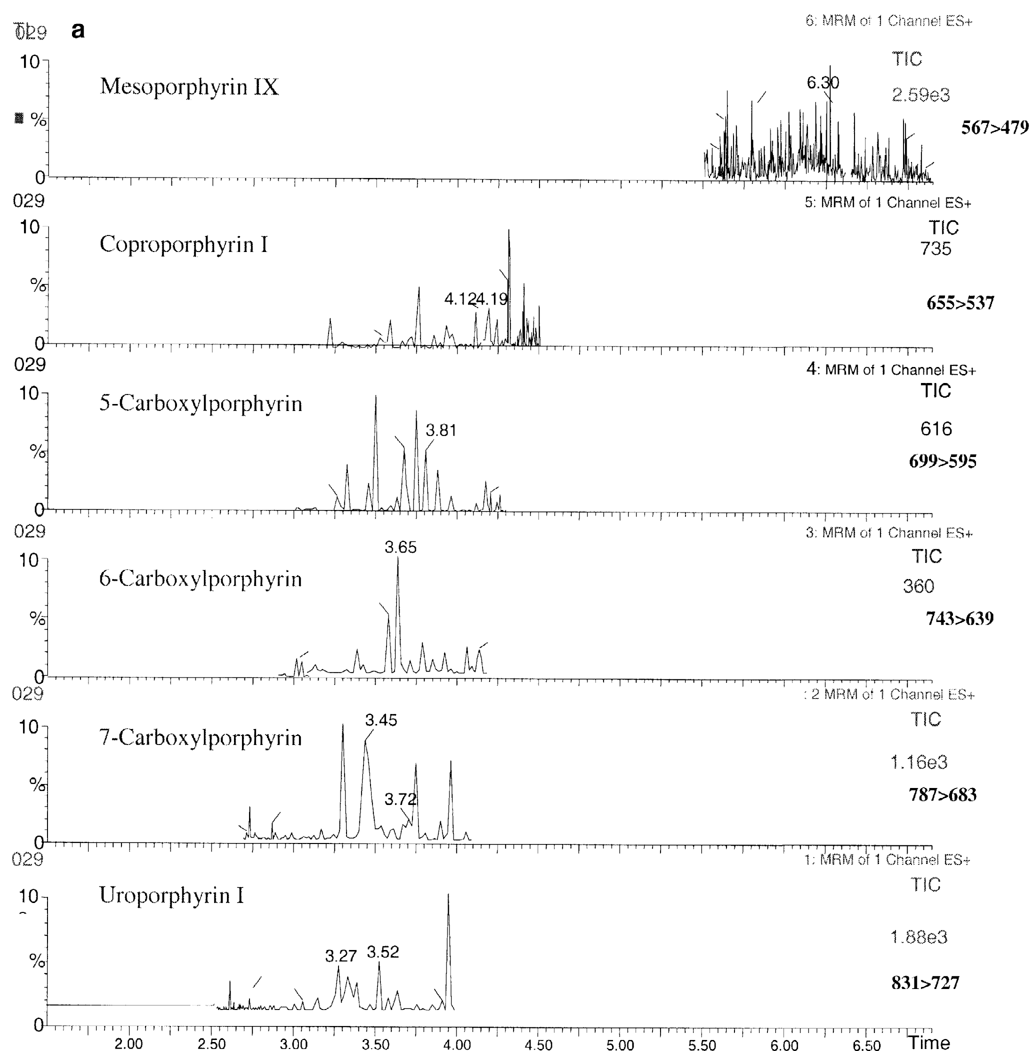

Fig. 4. (a) Chromatogram (MRM) of 6 *M* formic acid (blank solvent control). (b) Chromatogram (MRM) of 0.01 nmol/mL calibration standard in 6 *M* formic acid. (c) Chromatogram (MRM) of an unknown urine sample. (d) Representative chromatograms of a processed, fortified (0.1 nm each porphyrin/mL) canine urine sample.

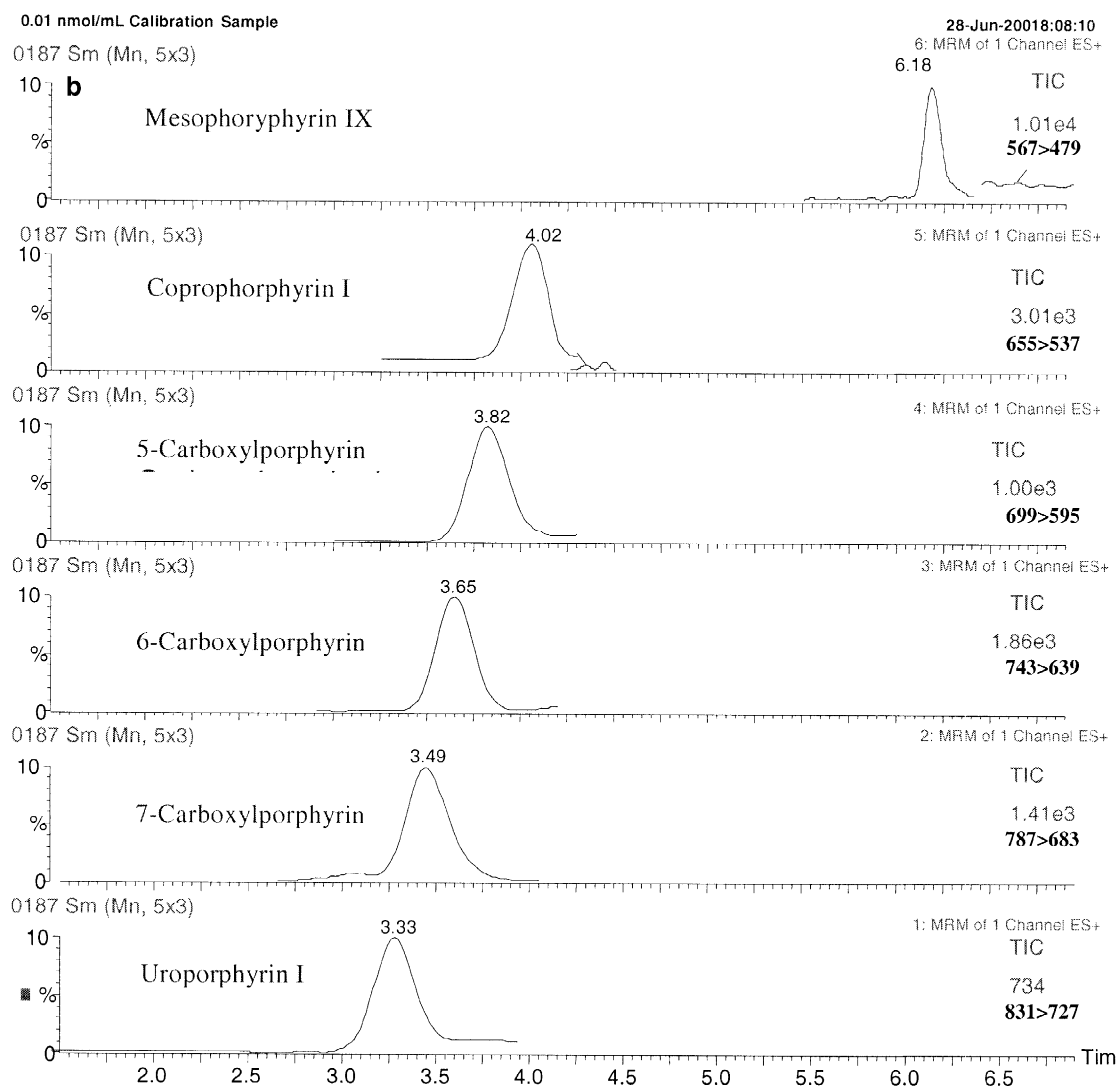

Fig. 4. (*continued*)

solvent as a blank control. Fig. 4a–d show the MRM chromatograms of a solvent blank (6 *M* formic acid), a porphyrin standard (0.01 nmol/mL), an unknown urine sample and a urine sample fortified with 0.1 nmol/mL of standard, respectively. The chromatograms demonstrate no interference at each porphyrin retention time window.

3.2.2. Calibration reproducibility

During each validation session, triplicate calibration samples at each of seven concentrations were prepared and analyzed as described above. A single injection was made for each processed calibration sample. The resulting porphyrin peak area versus theoretical concentration data were fit to the ln-quadratic function using the least-squares regression analysis over the range of 0.01 to 1.0 nmol/mL for mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin and 7-carboxylporphyrin. For uroporphyrin I the calibration range was

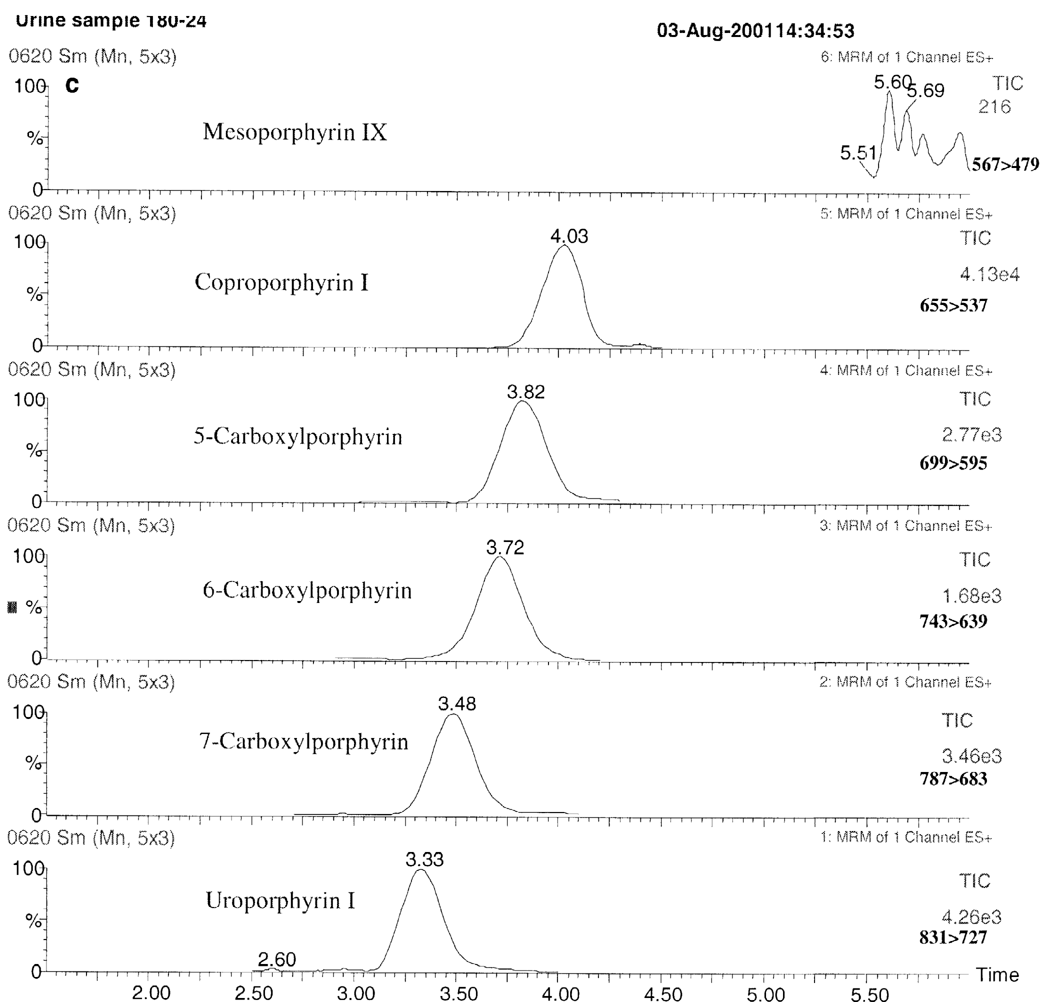

Fig. 4. (*continued*)

0.02 to 1.0 nmol/mL. A representative calibration curve is shown in Fig. 5. The calibration equation, mean values and standard deviations (SD) of the calibration parameters, including correlation coefficients, are listed in Table 2. The correlation coefficient of the regression line for each porphyrin was greater than 0.9925. The results of the regression analyses were used to back-calculate the corresponding concentrations from the peak area data. The reproducibility of the calibration curve data was considered valid when: (1) the inter-session relative standard deviation (RSD) of the back-calculated concentrations at each concentration level was ≤15%, except at the lowest concentration where RSD ≤20% was acceptable; and (2) the inter-session mean back-calculated concentrations at each concentration level were within 15% of the theoretical values, that is the percent relative errors (%REs) were within ±15% except at the lowest concentration level where %RE within ±20% was acceptable.

The back-calculated concentration values and the

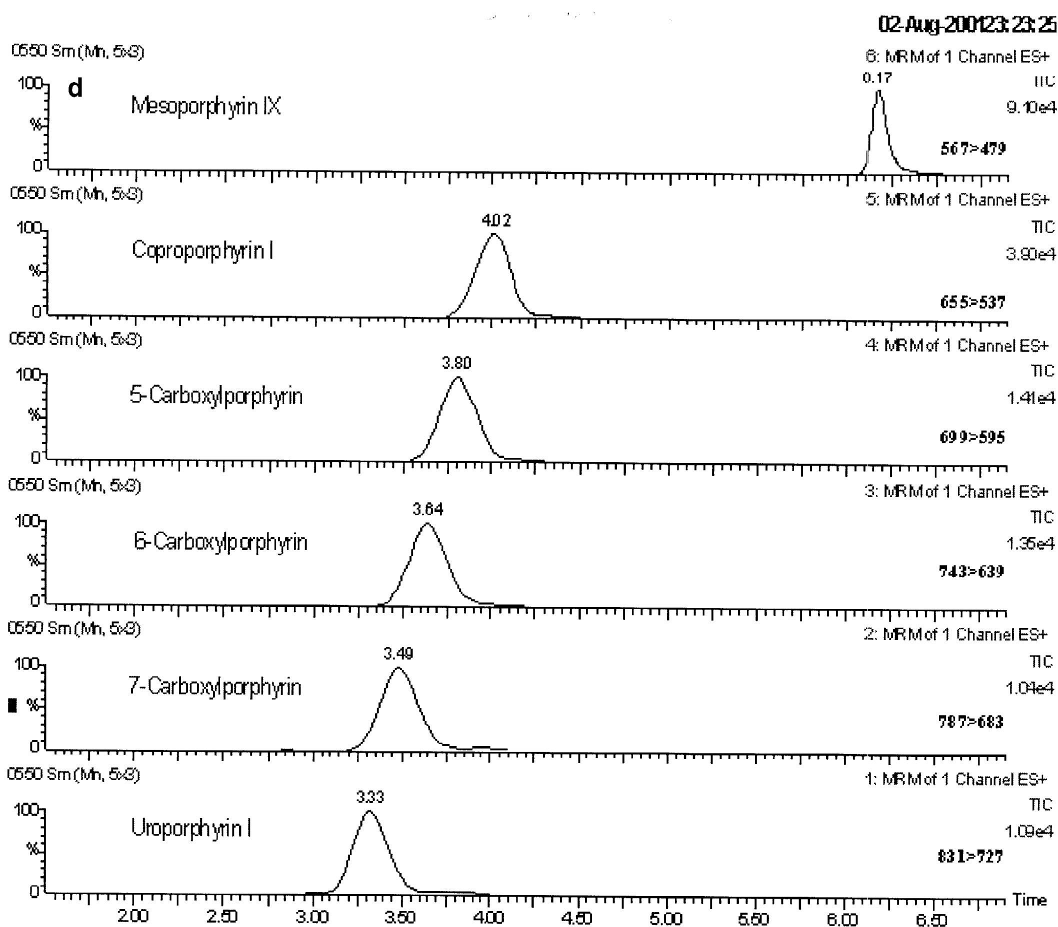

Fig. 4. (*continued*)

associated intra- and inter-session statistics for the porphyrin assay calibration samples are summarized in Table 3. The inter-session variability (RSD) of the back-calculated concentrations at each concentration level ranged from 1.647 to 15.67% (at the lowest concentration level). The inter-session concentration means had %RE values ranging from −2.6 to 6.2%.

Based on these criteria and the resulting validation data, the reproducibility of the calibration data was acceptable.

3.2.3. Sensitivity

The lower limit of quantitation (LLOQ) for mesoporphyrin IX, coproporphyrin I, 5-carboxylporphyrin, 6-carboxylporphyrin and 7-carboxylporphyrin using 700 μL of canine urine was 0.014 nmol/mL, with a signal-to-noise ratio of approximately 13.4. The lower limit of detection (LLOD), therefore, was 0.0031 nmol/mL based on a signal-to-noise ratio of 3. The LLOQ for uroporphyrin I using 700 μL of canine urine was 0.029 nmol/mL. This method demonstrated 10 times better sensitivity than an HPLC–FLD method utilized prior to developing the LC–MS–MS method.

3.2.4. Accuracy and precision

During each validation session, triplicate QC samples at three concentration levels were prepared and analyzed as described above. Single injections were made of each processed QC sample. The results

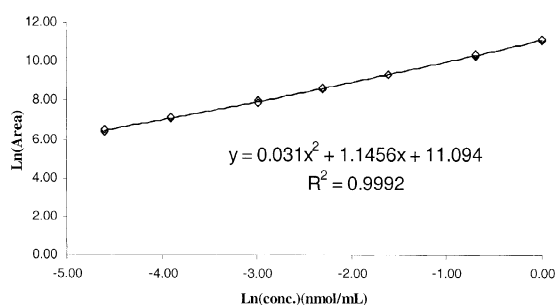

Fig. 5. Representative calibration curve for one of the porphyrins—mesoporphyrin XI.

of the regression analyses were used to calculate the corresponding concentrations from these QC peak area data. The variability (RSD) of these calculated QC concentration data was used as a measure of assay precision. The precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC concentration level was $\leq$15%. The difference from theoretical of the calculated QC concentration means (%RE) was used as a measure of assay accuracy. The accuracy of the method was considered acceptable when the inter-session concentration means of the calculated concentrations at each QC concentration level had %RE values $\leq$15%.

The calculated concentration values and the associated intra- and inter-session statistics for QC samples are summarized in Table 4. The inter-session variability (RSD) of the calculated concentrations at each level for six porphyrins ranged from 3.063 to 11.72%. The inter-session concentration means of the QC samples had %RE values ranging from −5.92 to 8.72%, all within ±15%.

3.2.5. Recovery

The purpose of assessing the recovery of the method was to demonstrate the validity of the assay procedure in determining the concentration of each of the six porphyrins in urine while using formic acid solutions of the six porphyrins as calibration standards. The study was conducted with four canine urine samples (two male and two female). The urine samples were analyzed (in triplicate) to establish the endogenous levels of each of the six porphyrins. In addition, these four dog urine samples were analyzed (in triplicate) after being fortified with the appro-

Table 2
Summary of calibration regression results

Porphyrin	Number of Assays (n)	a Mean±SD	b Mean±SD	c Mean±SD	r^2 Mean±SD
Mesoporphyrin IX	5	0.013±0.012	1.07±0.049	11.289±0.209	0.9987±0.0010
Coproporphyrin I	5	0.0015±0.015	1.02±0.053	10.998±0.12	0.9986±0.00059
5-Carboxylporphyrin	5	0.0045±0.017	1.03±0.066	10.423±0.061	0.9960±0.0017
6-Carboxylporphyrin	5	0.0039±0.015	1.02±0.060	10.453±0.10	0.9964±0.0012
7-Carboxylporphyrin	5	−0.0068±0.014	0.98±0.0381	10.083±0.14	0.9953±0.0018
Uroporphyrin I	5	−0.0039±0.030	0.97±0.084	9.6825±0.15	0.9934±0.0012

Calibration equation: $\ln y = a(\ln x)^2 + b(\ln x) + c$.

Table 3
RSD and %RE of back-calculated concentrations of standards in five sets of validation studies

Conc. (nmol/mL)	Mesoporphyrin IX		Coproporphyrin I		5-Carboxylporphyrin		6-Carboxylporphyrin		7-Carboxylporphyrin		Uroporphyrin I	
	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE
0.01	9.27	0.93	7.98	1.7	14.0	−0.27	14.9	0.93	15.7	2.6		
0.02	6.92	−0.20	5.93	−1.7	12.9	−0.35	9.97	1.6	14.6	−1.9	15.2	0.20
0.05	4.77	−0.65	5.41	−0.96	8.41	−1.8	8.61	−1.1	11.0	−0.63	12.5	3.8
0.1	3.56	−0.070	5.16	1.1	6.46	−1.7	6.44	−0.43	6.66	2.7	11.1	−2.2
0.2	9.56	1.9	9.08	2.8	9.64	3.4	10.6	2.2	11.9	1.7	11.5	6.2
0.5	2.62	−0.51	2.95	−2.3	4.77	−1.2	3.58	−0.35	4.59	−2.6	3.91	0.070
1.0	1.65	−0.060	2.54	0.74	3.91	0.54	2.35	−0.11	3.53	1.1	3.06	−1.7

Table 4
RSD and %RE of back-calculated concentrations of QC samples in five sets of validation studies

Conc. (nmol/mL)	Mesoporphyrin IX		Coproporphyrin I		5-Carboxylporphyrin		6-Carboxylporphyrin		7-Carboxylporphyrin		Uroporphyrin I	
	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE	RSD (%)	%RE
0.05	9.282	3.17	7.734	−5.92	9.700	−4.19	9.743	2.55	11.72	−4.41	10.30	8.72
0.2	8.818	4.90	5.048	−5.56	6.873	−1.15	7.750	−0.960	7.518	−0.900	8.279	0.820
0.8	4.574	1.05	3.063	−2.99	3.204	−0.690	5.068	0.270	3.658	1.61	4.109	−1.90

priate amounts of the six porphyrins to result in a 0.1 nmol/mL increase in their endogenous concentrations. Recovery was calculated by comparing the analyzed concentrations of fortified urine with their theoretical target concentrations (endogenous level + 0.1 nmol/mL). Table 5 presents the recoveries of each of the six porphyrins with the results ranging from 90.0 to 111.9%. All endogenous levels of mesoporphyrin IX (*m/z* 567) and 6-carboxylporphyrin, (*m/z* 743) were less than the assay's LLOQ (0.014 nmol/mL each porphyrin). However, assuming their levels in these urine samples were zero, their mean recoveries, after addition of 0.1 nmol each porphyrin/mL to the samples, proved quantitative.

Table 5
Mean recovery of porphyrins in canine urine

Porphyrin	Spiked (nmol/mL)	Mean recovery (%)	
		Male	Female
Mesoporphyrin IX	0.1	111.9	108.8
Corpoporphyrin I	0.1	105.6	107.4
5-Carboxylporphyrin	0.1	103.0	97.63
6-Carboxylporphyrin	0.1	113.8	108.5
7-Carboxylporphyrin	0.1	98.04	90.00
Uroporphyrin	0.1	100.9	98.1

3.2.6. Stability

Four urine samples (two male, two female) were used for stability studies. The stability of porphyrins in processed urine samples at 4 °C was examined and no changes were found for at least 48 h. After three freeze–thaw cycles, porphyrins in urine demonstrated acceptable stability (Table 6). The long-term storage stability of porphyrins in urine at −20 °C was reported previously [15]. The authors reported that there was no detectable loss of porphyrins in urine after 4 weeks of storage at −20 °C.

3.3. Method application

Using the developed method, a total of eight canine urine samples (four male, four female) were analyzed after 2 weeks of storage at −70 °C. The animals were not treated with, or exposed to, any drugs or chemicals before the urine was collected and, therefore, the concentrations of porphyrins measured in this study represent the normal levels of porphyrins in canine urine. Each urine sample was analyzed in triplicate and the results are summarized in Table 7. Since mesoporphyrin IX was not detected in any urine sample (see representative MRM chromatogram of urine sample in Fig. 4c), and all

Table 6
Freeze–thaw stability of porphyrins in canine urine

Porphyrin	Nominal conc. (nmol/mL)		Found (% of initial)	
	Male	Female	Male	Female
Coproporphyrin I	0.172	0.126	105	103
5-Carboxylporphyrin	0.0209	0.0163	92.1	94.6
7-Carboxylporphyrin	0.0360	0.0219	104	124
Uroporphyrin I	0.0553	0.0424	86.2	99.2

Table 7
Porphyrin concentrations in canine urine determined by LC–MS–MS

Urine source	Porphyrine conc. (nmol/mL)±SD					
	Mesoporphyrin IX	Coproporphyrin I	5-Carboxyl-porphyrin	6-Carboxyl-porphyrin	7-Carboxyl-porphyrin	Uroporphyrin I
Male	ND	0.126±0.0327	0.0187±0.00460	<LLOQ	0.0324±0.0114	0.0581±0.0132
Female	ND	0.140±0.0365	0.0207±0.00340	<LLOQ	0.0332±0.0177	0.0646±0.0401

ND, not detected; <LLOQ, lower than the LLOQ.

extrapolated concentrations of 6-carboxylporphyrin were below the LLOQ, they are not listed in the table.

4. Conclusion

An HPLC–MS–MS method for the determination of porphyrins in canine urine was developed and validated. The advantages of this novel method are its simplicity, rapid sample throughput, minimal sample pretreatment and superior specificity/selectivity due to use of the MRM mode in the mass detection system. Because of the great selectivity/specificity, the utility of this LC–MS–MS technique should not be species limited, and could possibly be used clinically for the determination of urine porphyrin levels in porphyria patients as well. Further investigation of the applicability of the method to the quantitation of porphyrin isomers would be necessary.

Acknowledgements

Financial support of this project from FMC Corporation is gratefully acknowledged. The authors thank Nancy Jordan, Robert Wally and Cathy Shay for their assistance in the preparation of the electronic manuscript.

References

[1] C. Herrero, M.D. Ozalla, M. Sala, R. Otero, M. Santiago-Silva, M. Lecha, J. To-Figueras, R. Deulofeu, J.M. Mascaro, J.O. Grimalt, J. Sunyer, Arch. Dermatol. 135 (1999) 400.
[2] C.K. Lim, T.J. Peters, J. Clin. Chem. Acta 139 (1984) 55.
[3] J. Westerlund, M. Pudek, W.E. Schreiber, Clin. Chem. 34 (1988) 345.
[4] A.W. van de Giessen, E.M. van Wijk, J. Clin. Chem. Clin. Biochem. 28 (1990) 605.
[5] M.E. Hahn, T.A. Gasiewicz, J. Chromatogr. 563 (1991) 363.
[6] M.A. Bowers, L.D. Aicher, H.A. Davis, J.S. Woods, J. Lab. Clin. Med. 120 (1992) 272.
[7] S.G. Smith, Br. J. Dermatol. 93 (1975) 291.
[8] M.J. Heuderson, J. Clin. Chem. 35 (1989) 1043.
[9] K. Jocab, E. Egeler, D. Neumeier, M. Knedel, J. Chromatogr. 468 (1989) 329.
[10] R. Weinberger, E. Sapp, S. Moring, J. Chromatogr. 516 (1990) 271.
[11] R.M. Jones, J.H. Lamb, C.K. Lim, Rapid Commun. Mass Spectrom. 9 (1995) 921.
[12] J. Luo, J.H. Lamb, C.K. Lim, J. Pharm. Biomed. Anal. 15 (1997) 1289.
[13] X. Ausió, J.O. Grimalt, D. Ozalla, C. Herrero, Anal. Chem. 72 (2000) 4874.
[14] K.M. Smith (Ed.), Porphyrins and Metalloporphyrins, Elsevier, Amsterdam, 1975.
[15] K.R. Allen, P.A. Rushworth, T.J. Degg, J.H. Barth, Ann. Clin. Biochem. 34 (1997) 553.