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Analysis of taurine in feline plasma and whole blood by liquid chromatography with fluorimetric detection and confirmation by thermospray mass spectrometry

TERESA J AMISS*, KRYSTYNA L. TYCZKOWSKA and DAVID P. AUCOIN

Clinical Pharmacology Unit, Department of Anatomy, Physiological Sciences and Radiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 (U S A)

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SUMMARY

A liquid chromatographic method with fluorimetric detection was developed to measure taurine (2-aminoethanesulfonic acid) in feline plasma and whole blood. Plasma or lysed whole blood was diluted with a mixture of acetonitrile–methanol–triethylamine–water (25 22 3 50, v/v), filtered through a 10 000 dalton exclusion filter and derivatized with dansyl chloride for 30 min at room temperature. Dansyl taurine was separated from other compounds by reversed-phase liquid chromatography using an octadecyl column and a methanol–acetic acid–triethylamine (30 0.5 0.025, v/v) aqueous mobile phase. The effluent was monitored fluorimetrically at an excitation wavelength of 329 nm and an emission wavelength of 530 nm. The presence of mono-dansylated taurine in feline plasma was confirmed by thermospray mass spectrometry. The limit of detection was 16 nmol/ml and the detector response was linear from 40 to 4000 nmol/ml taurine.

INTRODUCTION

Taurine is a ubiquitous free β-amino acid found in most biological fluids and tissues. Its physiological role as a non-protein amino acid is still unclear; however, its involvement as an intracellular anti-oxidant has been the subject of much research [1,2]. Our goal was to develop a simple and rapid assay using an ultrafiltration technique developed in this laboratory. With ultrafiltration the sample is diluted, filtered, derivatized and injected. Sep-Pak extraction [3]

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or protein precipitation [4,5] steps which are needed with many assays are unnecessary. It is the first time that the ultrafiltration technique has been used in the analysis of an endogenous compound.

Ultrafiltration has been used to quantitate drug levels in plasma, serum, milk and tissue samples. The methods developed thus far include analysis of several β-lactam antibiotics, antineoplastics and antithyroid drugs [6–12]. When a plasma sample is diluted with solvent (the releasing agent), protein precipitation does not occur but the protein–protein and protein–analyte linkages dissociate. The solution is ultrafiltered to produce a clear and relatively clean ultrafiltrate which can be directly injected, or if needed derivatized.

This versatility and ease of sample preparation makes ultrafiltration highly suitable to automation using robotics.

EXPERIMENTAL

Reagents

HPLC-grade methanol, acetonitrile and acetic acid were supplied by Fisher Scientific (Raleigh, NC, U.S.A.). HPLC-grade water was obtained using a Model 1000 Hydro ultrapure water system (Hydro Services and Supplies, Raleigh, NC, U.S.A.). Ethylamine, triethylamine, taurine and dansyl chloride were supplied by Sigma (St. Louis, MO, U.S.A.). All reagents were of analytical grade.

Taurine standards and dansyl chloride solutions were prepared daily in water and acetonitrile, respectively. All solutions and samples were protected from photodegradation.

Materials

Centricon-10 microseparation filters were obtained from Amicon (Danvers, MA, U.S.A.). The Centricon-10 is a 10 000 M_r exclusion filter.

Instrumentation

The liquid chromatographic–fluorimetric detection (LC–FD) system consisted of a Waters high-performance pump and autoinjector Models 600 and 714, respectively (Waters Chromatography Division, Milford, MA, U.S.A.), a McPearson Model FL-749 fluorescence detector with a 150-W xenon short ARC lamp and power supply (S.L. McPherson, Acton, MA, U.S.A.) and a Flatron CH-30 column heater (Flatron Laboratory Systems, Oconomowoc, WI, U.S.A.). The liquid chromatographic–thermospray mass spectrometric (LC–MS) system consisted of a Waters Model 510 pump and U6K injector coupled to a Finnigan-MAT (San Jose, CA, U.S.A.) 4800 quadrupole mass spectrometer with Finnigan-MAT thermospray.

A Model IEC Centra-7R centrifuge equipped with a 45° fixed-angle rotor

(International Equipment, Needham Heights, MA, U.S.A.) was used during sample preparation.

Liquid chromatography–fluorimetric detection

Reversed-phase LC was accomplished using a Brownlee ODS Spheri-5 analytical cartridge (220 mm × 4.6 mm I.D.) in a MPLC holder (220 mm) (Sci-Con, Winter Park, FL, U.S.A.). An initial aqueous mobile phase of methanol–acetic acid–triethylamine (33:0.5:0.025, v/v) [13] was run for 6 min followed by methanol–water (90:10, v/v) for 4 min. This was used to purge the column of the remaining endogenous compounds dansylated by the derivatization. The column was then re-equilibrated for 5 min with the initial mobile phase. With the mobile phase flow-rate at 1.5 ml/min and a column temperature of 50°C the retention time of dansyl taurine was approximately 6 min.

Liquid chromatography–mass spectroscopy

Thermospray spectra were acquired using the above method except the mobile phase was methanol–0.1 *M* ammonium acetate (30:70, v/v) with a flow-rate of 1.2 ml/min and a column temperature of 40°C. The thermospray interface source and the vaporizer were set at 270° and 105°C, respectively. The mass spectrometer was operated in the positive-ion–negative-ion detection mode with the filament on (0.2 mA at 1000 eV) to enhance chemical ionization under full-scan conditions for the initial acquisition of the spectra. For confirmation of dansyl taurine in feline plasma the instrument was operated in the multiple-ion detection mode monitoring *m/z* 357 (negative ion) and *m/z* 359 and 381 (positive ions), each for 0.2 s.

Sample preparation

Feline blood (3 ml) was drawn into chilled heparinized syringes and divided into two portions: one for plasma and the other for whole blood taurine analysis. The portion for the plasma taurine analysis was centrifuged immediately at 2677 *g* to separate the plasma from the taurine-enriched platelets and white blood cells. Care was taken during pipetting not to disturbed the buffy coat layer containing these cells since cell damage would falsely increase plasma concentrations of taurine. Normal plasma concentrations of taurine for domestic felines range from 49 to 115 nmol/ml [14] while platelet and white blood cells have concentrations approximately five times higher [15]. The plasma was stored at −100°C until analysis.

The whole blood portion of the original sample was subjected to two slow freeze–thaw cycles so that the cells would rupture and release their taurine content [16]. The blood was placed in a freezer at −10°C for 2 h and then removed and allowed to thaw at room temperature (24°C). After thawing was complete the blood was refrozen until analysis.

For the determination of taurine in plasma a 50-μl aliquot of plasma was

diluted with 200 μl of a releasing solution of acetonitrile–methanol–triethylamine–water (25:22:3:50, v/v). The sample was then vortex-mixed for 15 s, pipetted into a Centricon-10 microseparation filter and centrifuged for 15 min at 2677 g in a 45° fixed angle rotor. A 20-μl aliquot of the ultrafiltrate was placed in a small injection vial with 10 μl of a 10 mg/ml solution of dansyl chloride, vortex-mixed and placed in the dark at room temperature for 30 min. A 10-μl volume of a 3.5% (v/v) aqueous solution of ethylamine was added to terminate the dansylation reaction [17]. A 10-μl aliquot was injected for LC analysis.

Following two freeze–thaw cycles, the whole blood samples were treated similarly to plasma except that only 10 μl of whole blood was analyzed allowing for the higher concentration of taurine present.

RESULTS AND DISCUSSION

In several papers it has been suggested that dansyl chloride may not be a suitable derivatizing agent for amino acids [18–20]. Problems described include quenching, derivatizations which varied in reaction completeness depending upon the amount of dansyl chloride present, length of reaction times and reaction temperatures. To validate our assay we felt these questions needed to be addressed.

Based on the recovery of dansyl taurine for both matrices (plasma and whole blood) an acetonitrile–methanol–triethylamine–water (25:22:3:50, v/v) solution was chosen as the releasing agent for both. Following dilution and ultrafiltration, the concentration of dansyl chloride needed for derivatization was evaluated by adding different molar amounts of dansyl chloride to standard solutions of taurine. Fig. 1 shows that higher concentrations of dansyl chloride

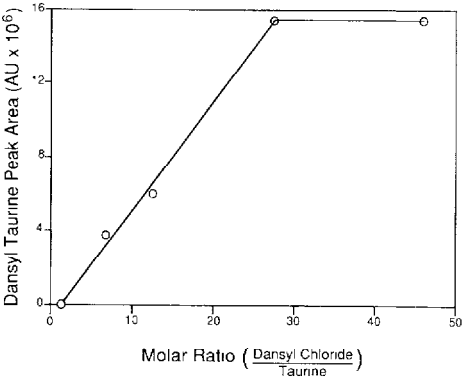

Fig. 1. Evaluation of excess dansyl chloride concentration needed to derivatize a constant molar amount (16 nM) of taurine in 30 min.

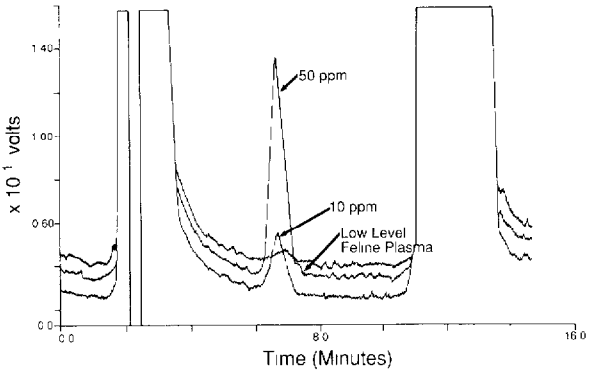

Fig. 2. Overlay of three separate chromatograms obtained from the LC–FD system: an endogenous taurine plasma extract, containing a low level of taurine (< 40 nmol/ml); the same plasma spiked at a 80 nmol/ml level and extracted; and the same plasma spiked at a 400 nmol/ml level and extracted

TABLE I

STATISTICAL SUMMARY OF LC-FD ANALYSES OF TAURINE IN PLASMA ($n=5$)

Concentration spiked (nmol/ml)	Concentration determined (mean ± S.D.) (nmol/ml)	Coefficient of variation (%)	Recovery (%)
0	24 ± 2.4	10	–
80	104 ± 6.4	6	100
400	392 ± 23.2	6	92

in solution produce the dansyl derivative more efficiently. Using a 30-min derivatization time at room temperature (24°C), mono-dansylation was rapid and complete. Increasing the reaction temperature did not improve the reaction efficiency and may increase its variability (data not shown). Spiked plasma samples were reliably stable overnight (≤ 18 h) at room temperature, if protected from light.

The linearity of response from the fluorescence detector and the uniformity of the derivatization reaction was evaluated over a 100-fold concentration range. Plasma samples containing small amounts of taurine (< 40 nmol/ml) and blank water samples were spiked with known amounts of taurine and assayed. Water samples not spiked with taurine were also assayed and showed no detectable concentration of taurine. The standard curve obtained for both the spiked plasma and water samples were essentially equivalent. Both the spiked plasma and water standard curves had a concentration range of 40–4000 nmol/ml taurine. A composite standard curve for $n=7$ replicates assayed on seven different days showed a linear response for the dansyl taurine area versus con-

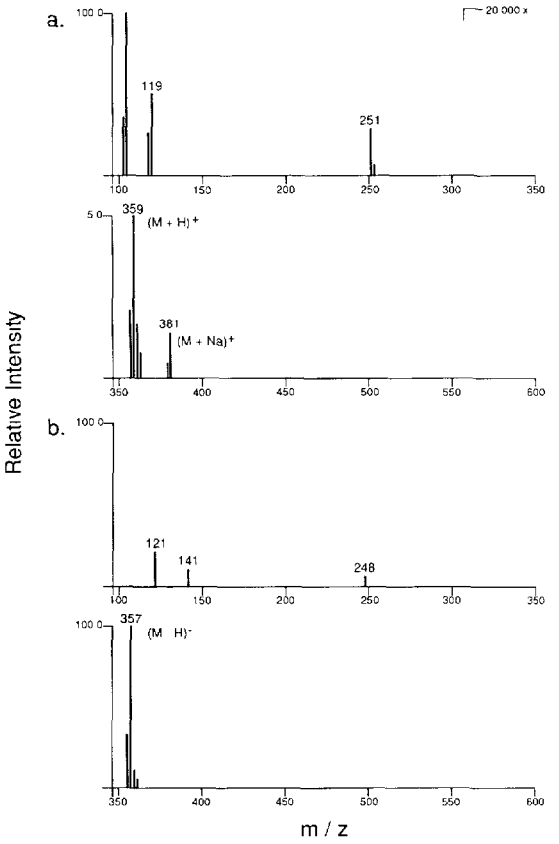

Fig. 3. Thermospray LC–MS spectra of the dansyl taurine. (a) Positive-ion detection; (b) negative-ion detection.

centration having a correlation coefficient of 0.9956. Fig. 2 shows a composite of three chromatograms obtained from a plasma sample containing a small amount of endogenous taurine and the chromatograms of the same plasma sample after spiking. The sensitivity of the method, defined as a greater than 2:1 signal-to-noise ratio, was 2.5 ng of taurine on-column. Table I shows that the average recoveries of taurine from plasma at two concentrations was greater than 95%.

LC–MS was performed on 125, 250 and 375 ng of dansyl taurine standards and dansyl taurine samples collected from feline plasma under the LC–FD conditions described in the Experimental section in order to verify the formation of mono-dansylated taurine.

The thermospray spectra for the dansyl taurine standard (M_r 358) exhibited an $[M+H]^+$ ion (*m/z* 359), $[M+Na]^+$ ion (*m/z* 381) and $[M-H]^-$ ion (*m/z* 357) as seen in Fig. 3a and b. Fig. 4 shows an outstanding signal-to-noise

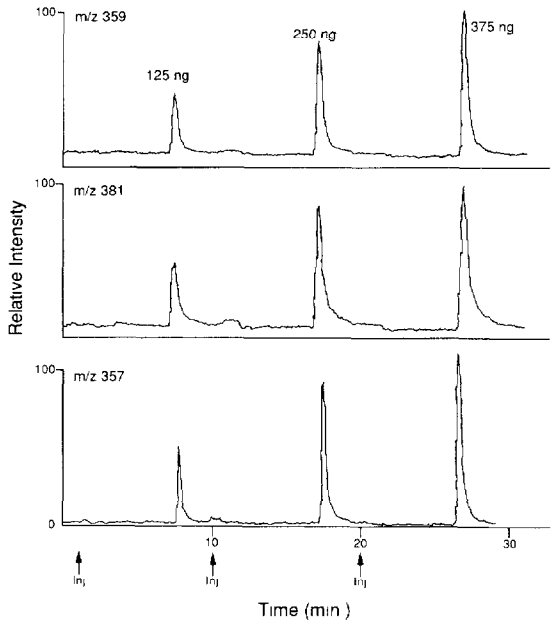

Fig. 4. Thermospray LC-MS chromatograms monitoring the $[M+H]^+$ ion (m/z 359), the $[M+Na]^+$ ion (m/z 381) and the $[M-H]^-$ ion (m/z 357) for 125, 250 and 375 ng of dansyl taurine injected.

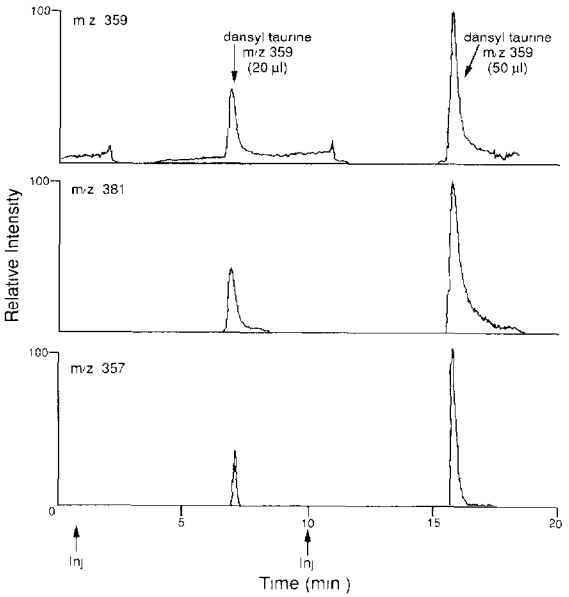

Fig. 5. Thermospray LC-MS chromatograms confirming the presence of dansyl taurine in feline plasma. The $[M+H]^+$ ion (m/z 359), the $[M+Na]^+$ ion (m/z 381) and the $[M-H]^-$ ion (m/z 357) maximize at the proper retention for dansyl taurine (20- and 50-μl injection volumes).

ratio for the analysis of the three standards of dansyl taurine used for calibration. Calibration curves for dansyl taurine using either *m/z* 358, *m/z* 381 or *m/z* 357 ions were linear over the range from 125 to 375 ng and had an average correlation coefficient of 0.9966.

Fig. 5 shows the LC–MS profile of a collected feline plasma sample analyzed at injection volumes of 20 and 50 μl. The dansyl taurine peak occurred at the same retention time as the ions *m/z* 359, 381 and 357, confirming the presence of mono-dansylated taurine in feline plasma.

CONCLUSION

The increasing interest in taurine as a general detoxifier eliminating excessive cholates, removing xenobiotics and scavenging chlorine oxidants [21] has given way to escalating research into the quantitation of taurine in various biological matrices. Because of the simplicity and the ruggedness of this assay we feel that it will be beneficial to this research.

REFERENCES

1 S.S Oja, L. Ahtee, P. Kontro and M.K. Paasonen, Taurine: Biological Actions and Clinical Perspectives, Progress in Clinical and Biological Research, Vol. 179, Alan R. Liss, New York, 1985.
2 R.J. Huxtable, F. Franconi and A Giotti, The Biology of Taurine, Medicine and Biology, Vol. 217, Plenum Press, New York, 1987.
3 C.A. Palmerini, C. Fini, M.G. Cantelmi and A. Floridi, J. Chromatogr., 423 (1987) 292.
4 D.W. Porter, M.A. Banks, V Castranova and W.G. Martin, J. Chromatogr., 454 (1988) 311.
5 L.L. Hirschberger, J. De La Rosa and M.H. Stipanuk, J. Chromatogr., 343 (1985) 303.
6 K. Tyczkowska and A.L. Aronson, J. Assoc Off Anal. Chem., 69 (1986) 760
7 T. Tyczkowska, D.P Aucoin, D C. Richardson and A L. Aronson, J. Liq. Chromatogr., 10 (1987) 2613.
8 K. Tyczkowska, K.M. Heeden, D.P. Aucoin and A.L. Aronson, J. Chromatogr. Sci., 26 (1988) 533.
9 K. Tyczkowska and A.L. Aronson, J. Chromatogr., 427 (1988) 103
10 K. Tyczkowska and A.L. Aronson, J. Assoc. Off Anal. Chem., 71 (1988) 773.
11 K. Tyczkowska, J. Chromatogr., 490 (1989) 101.
12 K. Tyczkowska, K.M. Heeden, D.P. Aucoin and A.L. Aronson, J. Chromatogr., 493 (1989) 337.
13 F.J. Martinez, A R. Quesaba, F. Sanchez-Jiminez and I. Nuzez de Castro, J. Chromatogr., 380 (1986) 275.
14 P.D. Pion, M.D. Kittleson, Q.D. Rogers and J D. Morris, Science, 237 (1987) 764.
15 P.D. Pion, K. Greene, J Lewis and M.D. Kittleson, Presentation at the 7th Annual Veterinary Medical Forum, San Diego, CA, 1989.
16 Q. Rogers, J. Immunol., in press.
17 Y. Tapuhi, D E. Schmidt, W. Lindner and B.L. Karger, Anal. Biochem., 115 (1981) 123
18 G. McClung and W.T. Frankenberger, Jr., J. Liq. Chromatogr , 11 (1988) 613.
19 J.M. Wilkinson, J. Chromatogr. Sci., 16 (1978) 547.
20 D.J. Neadle and R.S. Pollit, J. Biochem., 97 (1965) 607.
21 C.E. Wright, T.T. Lin, Y.Y. Lin, J.A. Sturman and G.E. Gaull, Taurine: Biological Actions and Clinical Perspectives, Progress in Clinical and Biological Research Vol 179, Alan R. Liss, New York, 1985, pp. 137-147.