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Evaluation of capillary supercritical fluid chromatography with mass spectrometric detection for the analysis of a drug (mebeverine) in a dog plasma matrix

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ABSTRACT

Supercritical fluid chromatography with mass spectrometric detection was evaluated as a technique for the analysis of drugs in biological fluids. Dog plasma was spiked with a model drug, mebeverine, and with a deuterium-labeled analog of mebeverine. The spiked plasma was prepared for analysis by solid-phase extraction on octadecylsilane cartridges. Mebeverine levels in the spiked dog plasma samples were determined by interpolation from a standard curve. Accuracy and precision of the analysis were determined within and between days. In general, accuracy was found to be 100 ± 15% for plasma samples spiked with 6 to 60 ng mebeverine/ml. The relative standard deviation for replicate sample analysis over this concentration range was between 5 and 12.5%.

INTRODUCTION

The determination of drugs in plasma plays a central role in the development of modern pharmaceuticals. Data generated from these measurements aid in the interpretation of toxicology studies, provide insights into a drug's mechanism of action and are used to establish pharmacokinetic parameters. Historically, high resolution chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) have played a central role in the analysis of drugs in biological matrices. Gas chromatography coupled with mass spectrometric detection has been widely applied in ultratrace drug analysis. The general utility of gas chromatography, however, is limited by the requirement that the analyte or derivatized analyte be sufficiently volatile and thermally stable for gas-phase separation. Supercritical fluid chromatography (SFC) offers an alternative to GC for the analysis of nonvolatile and thermally labile compounds due to the solvating power of the supercritical fluid mobile phase. The use of SFC for the analysis of compounds that are difficult to analyze by GC has been documented [1–3]. However, the application of SFC for ultratrace analysis has been limited [3], particularly for the analysis of drugs in biological matrices [4–6]. In order to evaluate the potential of SFC–MS for ultratrace drug analysis, mebeverine was chosen as a model drug compound since it contains an array of functional group types often found in drug molecules (see Fig. 1). Dog plasma was selected as a representa-

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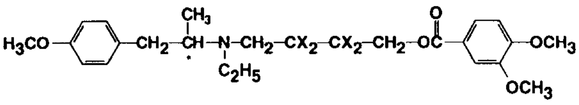

Fig. 1. Structure of mebeverine (X = H) and d_4-mebeverine (X = D). The * denotes the position of the ^{14}C-label.

tive biological matrix. Our attempts to analyze mebeverine by GC–MS were hindered due to thermal degradation at the temperatures required for elution from the GC column. An HPLC method with ultraviolet detection for mebeverine has been reported in the literature [6,7]. In this paper we report the evaluation of SFC–MS for the ultratrace analysis of mebeverine in dog plasma. The primary purpose of this work was not the development of an actual assay for mebeverine. Rather, the main focus of the work was centered on the evaluation of the potential strengths and limitations of SFC–MS as a technique for the analysis of drugs in biological fluids.

EXPERIMENTAL

Chemicals

Distilled, deionized water was from a Barnstead NANOpure II system (Dubuque, IA, USA). Methanol (HPLC grade) was from J.T. Baker (Phillipsburg, NJ, USA). Dog plasma was obtained from Pel-Freez (Rogers, AR, USA). Octadecylsilane (ODS) cartridges (500 mg) were obtained from J.T. Baker. Toluene (High Purity) was from Burdick and Jackson (Muskegon, MI, USA). Purum grade dimethylhexylamine (DMHA) and triethylamine (TEA) were purchased from Fluka (Ronkonkoma, NY, USA) and Aldrich (Milwaukee, WI, USA), respectively. Mebeverine (MEB) was obtained from Orgamol (Evionnaz, Switzerland). Mebeverine labeled with ^{14}C on the carbon alpha to the benzyl carbon (specific activity = 109 μCi/mg) and deuterium-labeled (d_4) mebeverine (d-MEB) were synthesized by Amersham (Arlington Heights, IL, USA) and Procter and Gamble, Miami Valley Laboratories, respectively.

Standard preparation

Stock solutions of MEB and d-MEB were prepared in methanol. MEB standards covering a mass range from 0.6 to 119 ng were prepared by transferring appropriate aliquots of the MEB stock solutions into small-volume vials (0.3 ml) containing 36 ng of the d-MEB internal standard. The methanol was removed under nitrogen and the residue was reconstituted in 0.025 ml of toluene–DMHA (3:1, v/v). A single set of standards was prepared each day of analysis and each standard was injected once.

Sample preparation

Plasma samples were prepared for analysis by solid-phase extraction (SPE) using a Speed Wiz instrument (Applied Separations, Bethlehem, PA, USA). Aliquots (1.0 ml) of blank dog plasma and dog plasma spiked with MEB (5.95, 23.8 and 59.3 ng/ml) were placed in individual glass test tubes (13 mm × 100 mm) containing 36 ng of the d-MEB internal standard and 1.0 ml of 1.0 *M* potassium phosphate buffer (pH 7.7). The tubes were mixed on a vortex-mixer. The contents of the test tubes were then applied to ODS cartridges which had previously been conditioned with 5.0 ml of methanol followed by 5.0 ml of water–methanol (97:3, v/v). The cartridges were then successively washed (5.0 ml) with water–methanol (97:3) and water–methanol (70:30, v/v). The MEB and d-MEB were eluted from the cartridges using 5 ml of water–methanol–TEA–formic acid (25:75:0.32:0.5, v/v) and collected into glass test tubes (16 mm × 100 mm). The collected eluent was taken to dryness under nitrogen at 40°C on a TurboVap (Zymark, Hopkinton, MA, USA) and reconstituted in 0.025 ml of toluene–DMHA (3:1, v/v).

Sample analysis

The SFC system was similar to that previously described [8]. The column was a 10 m × 50 μm I.D. SB-Methyl 100 (Dionex/Lee Scientific, Salt Lake City, UT, USA) with a 0.25-μm film thickness. The tapered flow-restrictor was fashioned from a 70 cm × 50 μm I.D., 180-μm O.D. fused-silica tube (Polymicro Technologies, Phoenix, AZ, USA) in the manner previously described [9]. The flow restrictor tapered to an aperture of approximately 6 μm over a 2.8-cm length. The commercially available SFC–MS (Finnigan-MAT, San Jose, CA, USA) interface probe housed the flow restrictor and was similar to the one previously described [10]. The tip of the interface probe mated with the side of the standard electron ionization/chemical ionization ion source of the mass spectrometer, a Finnigan-MAT TSQ-70 triple quadruple instrument. The aperture of the flow restrictor was positioned inside the interface probe. The probe stem was held at 140°C and the probe tip was held at 450°C. The mass spectrometer was tuned and calibrated using perfluorotributylamine with automatic tuning software in the customary manner. The ion source temperature was held at 150°C and the analyzer manifold was held at 70°C. Full-scan and selected-ion monitoring (SIM) data were acquired in the ammonia chemical ionization (CI) mode with 1% ammonia in methane (Matheson purity, 99.99% minimum, Matheson Gas Products, Dayton, OH, USA). The indicated ion source pressure used for CI was approximately 1.3 kPa using an uncalibrated Pirani gauge in the CI reagent gas supply line. The electron multiplier was operated at − 1500 V with a conversion dynode voltage of − 20 kV. The first and second quadruples (Q1 and Q2) were operated in the non-mass-selective, rf-only mode, while Q3 was used as the mass filter in these experiments. The column was held at 140°C and the mobile phase was SFC-grade carbon dioxide (Scott Speciality Gases, Plumsteadville, PA, USA). Standards and samples were injected (0.1 μl) and the column pressure was ramped from 10.1 MPa (100 atm) to 40.5 MPa (400 atm) at a rate of 10.1 MPa/min (100 atm/min). For quantification of MEB the column effluent was monitored in the SIM mode at m/z 430 (MEB) and m/z 434 (d-MEB) with a dwell time of 0.2 s for each ion. Standard curves were constructed using the peak-area ratio (integrated area MEB peak/integrated area d-MEB peak) for the standards. Sample concentrations were obtained by interpolation from the standard curve using the peak-area ratio obtained for each sample.

RESULTS AND DISCUSSION

Standards

The structures of MEB and d-MEB are shown in Fig. 1 and the full scan CI spectrum for each compound is shown in Fig. 2. The major ion for MEB and d-MEB was the protonated molecular ion [M + 1] occurring at m/z 430 and 434, respectively. These ions were used for SIM in all experiments. A typical chromatogram obtained for a 0.6-ng MEB standard containing 36.2 ng d-MEB is shown in Fig. 3. MEB and d-MEB were quickly eluted from the column using the rapid pressure program (10.1 MPa/min). The use of a rapid pressure ramp relative to the conventional ramp rates has been shown to give peaks with narrower widths and improved signal-to-noise ratio [11].

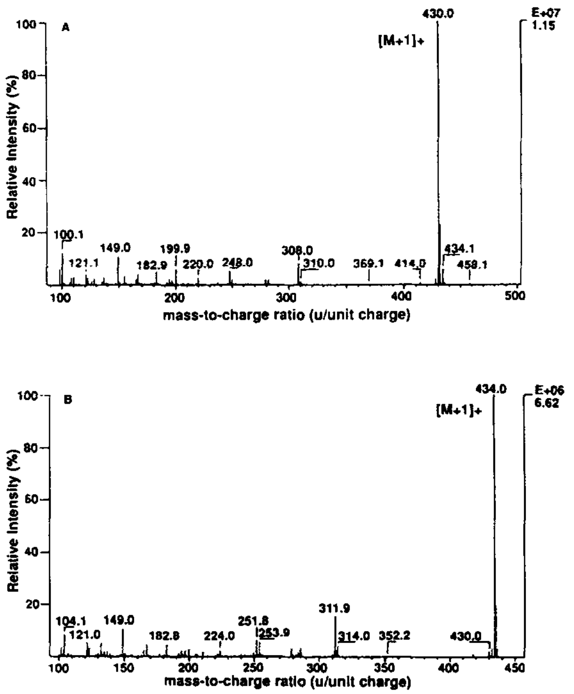

Fig. 2. Full-scan ammonia chemical ionization mass spectrum for: (A) mebeverine and (B) d_4- mebeverine.

The MEB and d-MEB peaks were separated from system peaks arising from the SFC–MS instrumentation. The cause of the system peaks is unknown at the present time but is likely due to contamination in the SFC pump. A linear response was obtained for the peak-area ratio (MEB peak area/d-MEB peak area) for the MEB standards over a range from 0.3 to 119 ng ($r = 0.998$). The average slope and intercept obtained for freshly prepared standards over three days of analysis were 0.0282 ± 0.0011 and 0.0160 ± 0.0148, respectively. The reproducibility of the peak-area ratio for replicate ($n = 3$)

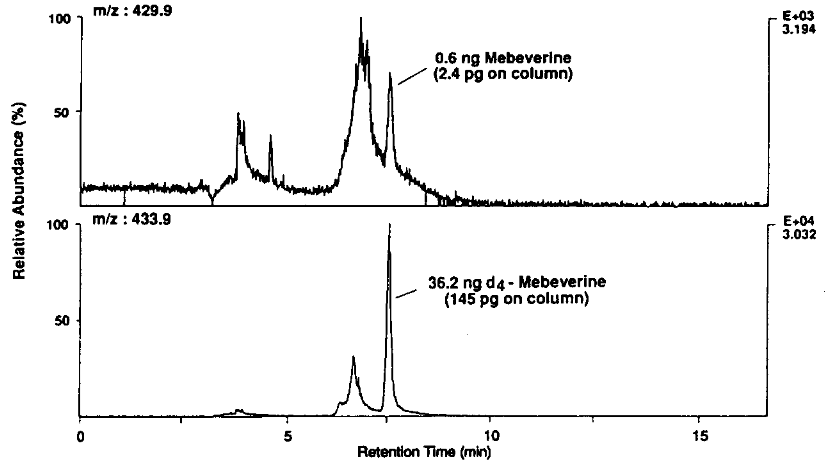

Fig. 3. SFC–MS chromatographic profile for a 0.6-ng mebeverine standard and 36 ng of d_4-mebeverine.

injections of MEB standards at the 3.0, 30 and 300 ng level was 3.0, 4.2 and 2.6%, respectively.

Solid-phase extraction (SPE)

Plasma samples were prepared for analysis by SPE using ODS cartridges. The absolute recovery of ^{14}C-MEB from the ODS cartridges was essentially quantitative over a concentration range from 5 to 1000 ng/ml (Table I). The %R.S.D. values for replicates ($n = 3$) were less than 7%. It should be noted that the presence of TEA was required to elute MEB from the ODS cartridges. The TEA serves to mask interactions between MEB and residual silanols of the ODS packing.

TABLE I

ABSOLUTE RECOVERY OF [^{14}C]MEBEVERINE FROM SOLID-PHASE EXTRACTION

$n = 3$ for each concentration.

Sample concentration (ng/ml)	Absolute recovery (%)	
	Mean ± S.D.	R.S.D.
5.0	92.3 ± 4.5	4.9
100	95.5 ± 3.4	3.6
1000	99.4 ± 6.2	6.2

Analysis of plasma samples

Typical chromatograms for blank plasma and blank plasma spiked with 5.95 ng MEB/ml and 36 ng d-MEB/ml are shown in Fig. 4. The chromatogram for the blank plasma is free of interfering peaks at the retention time of MEB and d-MEB, allowing selective quantification of MEB. Good peak shapes were obtained for MEB and d-MEB for the spiked plasma sample and the MEB peak (5.95 ng MEB/ml) had a signal-to-noise ratio of at least 10. The limit of quantitation (LOQ) of the analysis was hampered by the presence of background peaks due to the SFC–MS system itself. Spiked plasma samples were analyzed in triplicate on three separate days to evaluate accuracy and precision (Table II). The average relative recoveries for MEB were 101, 104 and 91% for plasma spiked at the 5.95, 23.8 and 59.5 ng/ml levels, respectively. The %R.S.D. for the replicate analysis were, in general, less than 10% across the spiked plasma concentration range examined in this study. One area of difficulty uncovered during the analysis of the spiked dog plasma samples and standards by SFC–MS was adsorption of the drug by active silanol sites with some columns. Certain columns provided satisfactory peak shape, while other columns did not allow elution of the drug. It should be noted that similar silanol interactions are commonly encountered in

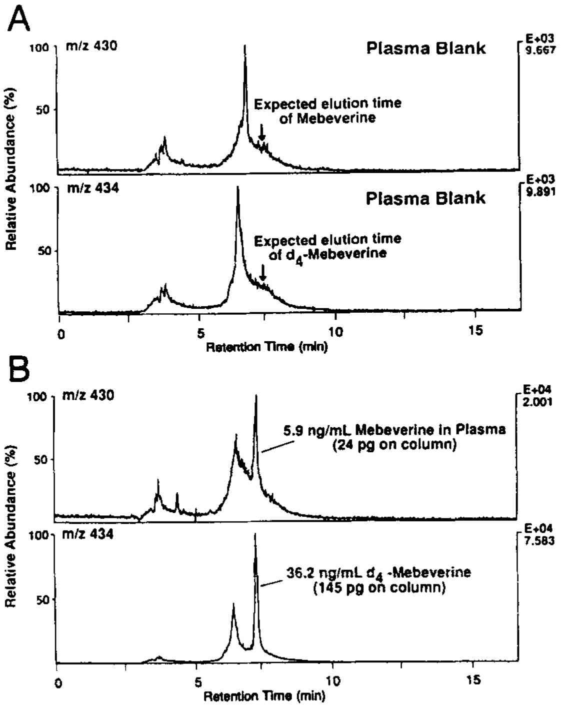

Fig. 4. SFC–MS chromatographic profiles for: (A) blank dog plasma and (B) blank dog plasma spiked to contain 5.9 ng mebeverine/ml and 36.2 ng d_4-mebeverine/ml.

both HPLC and GC. Therefore, it is not surprising that such interactions occur in SFC.

CONCLUSION

The applicability of SFC–MS for the ultratrace analysis of drugs has been demonstrated using mebeverine as a model compound and dog plasma as a model biological matrix. The combination of SFC–MS allowed the interference-free quantification of mebeverine at ultratrace levels (6 to 60 ng/ml). Although the LOQ obtained by the present SFC–MS approach was not significantly greater than that achievable by HPLC the potential utility of the SFC–MS technique was demonstrated. The accuracy and precision of the mebeverine SFC–MS analysis were proven over a three day period. SFC–MS may offer an attractive alternative to conventional GC–MS and HPLC–MS for ultratrace drug analysis. However, since most drugs contain surface-active functional groups, such as carboxylic acids, alcohols and amines, improvement in column inertness is a must. Additionally, an increase in the allowable injection volume would aid in

TABLE II

SFC–MS ANALYSIS OF SPIKED MEB PLASMA SAMPLES

All mean values represent triplicate sample preparations and analysis.

Day	Spiked concentration (ng/ml)	Found concentration (Mean ± S.D.) (ng/ml)	Relative recovery (%)	
			Mean	R.S.D.
1	5.95	6.05 ± 0.35	101.7	5.8
2	5.95	6.09 ± 0.05	102.4	0.8
3	5.95	5.93 ± 0.39	99.7	6.6
1	23.8	26.3 ± 2.62	110.5	10.0
2	23.8	24.2 ± 1.69	101.7	7.0
3	23.8	23.8 ± 0.97	100.0	4.1
1	59.5	51.9 ± 6.46	87.2	12.5
2	59.5	55.5 ± 0.48	92.8	0.5
3	59.5	55.0 ± 4.00	92.4	7.3

lowering the LOQ for SFC–MS analysis below the values reported in this work.

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