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DETERMINATION OF UNLABELED AND $^{13}C_6$-LABELED MORICIZINE IN HUMAN PLASMA USING THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY[a]

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SUMMARY

Moricizine hydrochloride is an orally effective antiarrhythmic agent currently marketed in the Soviet Union and undergoing clinical testing in the United States. To facilitate the simultaneous analysis of unlabeled and $^{13}C_6$-labeled moricizine in human plasma, a specific and sensitive method employing liquid–liquid extraction followed by thermospray liquid chromatography–mass spectrometry (LC–MS) was developed. Plasma samples, after addition of [$^2H_{11}$]moricizine as an

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internal standard, were extracted into methylene chloride under alkaline conditions. Extracts were evaporated, reconstituted with mobile phase, and chromatographed on an ODS column. The LC mobile phase consisted of methanol–0.1 *M* ammonium acetate containing 0.2% triethylamine (65 35) and it was used at a flow-rate of 1.5 ml/min. Under these conditions, moricizine and [$^{13}C_6$]moricizine coeluted at 1.2 min, while [$^{2}H_{11}$]moricizine eluted slightly earlier. The MS system consisted of a Finnigan 4600 TSQ and a Vestec thermospray interface. Selected ions at *m/z* 428, 434, and 439 were scanned at 0.2 s per ion. Over a plasma concentration range of 10–800 ng/ml, intra-day precision ($n=3$) ranged from 1.8 to 13.3% and intra-day accuracy ranged from 1.9 to 15.8%. This method was successfully used to assay human plasma samples from a pilot moricizine bioavailability study in which tablets and solution containing moricizine hydrochloride and [$^{13}C_6$]moricizine, respectively, were simultaneously administered.

INTRODUCTION

Moricizine·HCl (USAN; also moracizine·HCl, INN; or Ethmozine, the proprietary name frequently found in the existing literature), carbamic acid [10-[3-(4-morpholinyl)-1-oxopropyl]-10H-phenothiazin-2-yl] ethyl ester monohydrochloride, is a phenothiazine derivative with demonstrated clinical antiarrhythmic activity [1–3]. Three high-performance liquid chromatographic (HPLC) methods for the determination of moricizine in plasma have been reported [4–6]. These methods possess sufficient specificity and sensitivity for unlabeled, parent moricizine to conduct human pharmacokinetic studies. However, to facilitate the simultaneous analysis of unlabeled and $^{13}C_6$-labeled moricizine in human plasma, a method employing wet extraction followed by thermospray liquid chromatography–mass spectrometry (LC–MS) was developed. This method was required as part of a human moricizine bioavailability study in which tablets and solution containing moricizine·HCl and [$^{13}C_6$]moricizine, respectively, were simultaneously administered.

EXPERIMENTAL

Drug standards and chemicals

Unlabeled moricizine·HCl (Fig. 1) (monoisotopic mass of free base, 427) was synthesized by E.I. du Pont (Wilmington, DE, U.S.A.) and was 99.1% chemically pure. $^{13}C_6$-Labeled moricizine (Fig. 1) (monoisotopic mass of free base, 433) was synthesized by New England Nuclear (Billerica, MA, U.S.A.) under GMP conditions and was 98.0% chemically and 99.2% isotopically pure. $^{2}H_{11}$-Labeled moricizine (Fig. 1) (monoisotopic mass of free base, 438) was synthesized at E.I. du Pont by reacting [$^{2}H_3$]carbamic acid (10H-phenothiazin-2-yl) ethyl ester (New England Nuclear) with 3-chloropropionyl chloride and the resulting product was in turn reacted with [$^{2}H_8$]morpholine (Merck Sharp & Dohme Isotopes, Montreal, Canada) to give [$^{2}H_{11}$]moricizine free base. [$^{2}H_{11}$]Moricizine was 95.9% chemically and 99.1% isotopically pure. All moricizine amounts and concentrations are expressed in terms of the hydro-

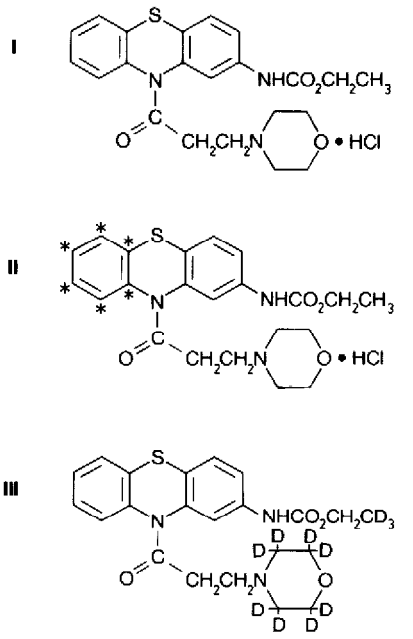

Fig. 1. Chemical structures for unlabeled moricizine·HCl (I), $^{13}C_6$-labeled moricizine·HCl (II), and $^{2}H_{11}$-labeled moricizine (III). The positions of the $^{13}C_6$ and $^{2}H_{11}$ atoms are denoted as * and D, respectively.

chloride salt except [$^{2}H_{11}$]moricizine which is expressed in terms of the free base.

Methanol, methylene chloride, and water were HPLC grade. Ammonium acetate, triethylamine, and sodium hydroxide were reagent grade.

Standard solutions

Separate primary stock solutions (100 μg/ml) of unlabeled and $^{13}C_6$-labeled moricizine were prepared by dissolving 10 mg of the solid materials in 100 ml of methanol. Secondary (50 μg/ml) and working (5, 0.5, and 0.05 μg/ml) stock solutions were prepared by further diluting the primary and secondary stock solutions, respectively, with methanol. Primary, secondary, and working (120, 60, and 3 μg/ml, respectively) stock solutions of [$^{2}H_{11}$]moricizine (internal standard) were prepared in a similar fashion.

Extraction procedure

Into 150 mm × 20 mm borosilicate culture tubes were placed 1.0 ml of control human plasma, 300 ng of [$^{2}H_{11}$]moricizine, two drops of 1.0 *M* sodium hydroxide, and 5.0 ml of Fisher (Pittsburgh, PA, U.S.A.) pH 9.0 buffer. The contents of these tubes were mixed for 30 s on an SMI (Emeryville, CA, U.S.A.)

multi-tube vortexer and then 12.0 ml methylene chloride was added. The tubes were rotated at full speed for 30 min on a Rugged Rotator™ (Kraft Apparatus RD-250, Mineola, NY, U.S.A.), then centrifuged for 10 min at ≥ 650 g. After discarding the upper aqueous phase, 8.0 ml of the remaining methylene chloride was pipetted into clean 125 mm × 20 mm conical borosilicate culture tubes and evaporated to near dryness under nitrogen at ≤ 40 °C. The remaining residue was reconstituted with 0.5 ml methanol and vortexed for 3 min. The methanol was pipetted into a 1.0-ml Reacti-Vial™ (Pierce Chemical, Rockford, IL, U.S.A.) and evaporated to near dryness under nitrogen at ≤ 40 °C using a Pierce Reacti-Therm™ heating–stirring module. The sides of the Reacti-Vial were then rinsed with ≃ 0.2 ml of methanol and evaporated to dryness. The final residue was dissolved in 15 μl of mobile phase.

Liquid chromatographic–mass spectrometric equipment and conditions

MS was performed using a Finnigan (San Jose, CA, U.S.A.) 4600 TSQ mass spectrometer and a Vestec (Houston, TX, U.S.A.) thermospray interface. The LC system was equipped with a Waters (Milford, MA, U.S.A.) Model 510 pump, a Waters Model 680 controller, and an ODS column (3.0 cm × 4.5 mm I.D., 3 μm particle size, Perkin Elmer, Norwalk, CT, U.S.A.) connected to a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector. The mobile phase consisted of methanol–0.1 *M* ammonium acetate containing 0.2% triethylamine (65:35, v/v) and it was filtered and degassed prior to use. The injection volume and mobile phase flow-rate were 4 μl and 1.5 ml/min, respectively. The thermospray conditions were as follows: T1, 110 °C; T2, 234 °C (probe tip temperature); T3, 241 °C (vapor temperature); T4, 253 °C (block temperature); and pump pressure, 0.5 Torr. Quantitative analysis of unlabeled, $^{13}C_6$-labeled, and $^{2}H_{11}$-labeled moricizine at *m/z* 428, 434, and 439, respectively, was performed utilizing selected-ion monitoring (SIM) with 0.5-a.m.u. windows. These *m/z* values were scanned at 0.2 s per ion (0.6 s total scan time). The peak areas for each moricizine *m/z* value were recorded by Finnigan Incos data system software (Rev. C). At the chromatographic retention time window for moricizine, a signal-to-noise ratio of ≥ 5 was the criterion to define a peak.

Calibration curves

To prepare calibration curves, [$^{2}H_{11}$]moricizine (300 ng) and unlabeled or $^{13}C_6$-labeled moricizine·HCl (10, 15, 75, and 800 ng) were added to plasma samples (1.0 ml). These were then extracted as previously described. Peak-area ratios (PAR) of unlabeled to $^{2}H_{11}$-labeled moricizine and $^{13}C_6$- to $^{2}H_{11}$-labeled moricizine were determined by division. Subsequently, separate calibration curves for unlabeled and $^{13}C_6$-labeled moricizine were constructed by an unweighted power curve regression analysis of PAR (*y*-axis) versus the amount of moricizine·HCl extracted (*x*-axis). Two separate calibration curves were sometimes required to better define the lower (usually 10–25 ng ex-

tracted) and upper (25–800 ng extracted) regions of the curve. Concentrations of unknown samples were calculated by the following equation: $C = [(\mathrm{PAR}/a)^{1/b}]/v$, where C is plasma concentration (μg/ml), a and b are regression coefficients, and v is the volume of plasma extracted.

RESULTS AND DISCUSSION

Specificity

Full-scan mass spectra (using thermospray LC–MS) of unlabeled, $^{13}C_6$-labeled, and $^{2}H_{11}$-labeled moricizine are shown in Fig. 2. Very little fragmentation was observed for all three moricizine isotopes. The molecular ions at 428, 434, and 439 were monitored for quantification.

The specificity of the method was based both on the retention time and mass (m/z). Representative thermospray mass chromatograms using SIM mode of moricizine from human plasma are shown in Figs. 3–6. Blank plasma resulted

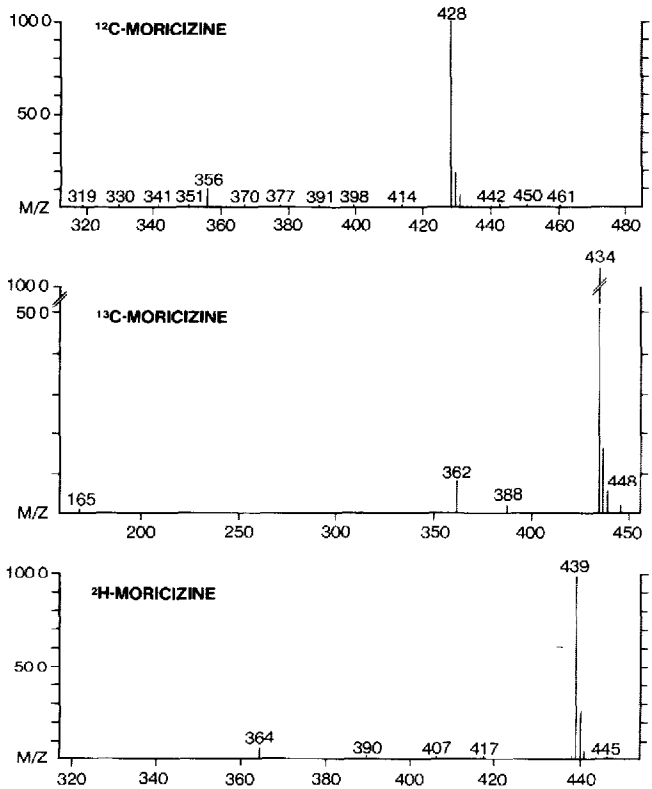

Fig. 2. Full-scan thermospray LC–MS mass spectra of unlabeled, $^{13}C_6$-labeled, and $^{2}H_{11}$-labeled moricizine (reconstituted from stock methanolic solutions).

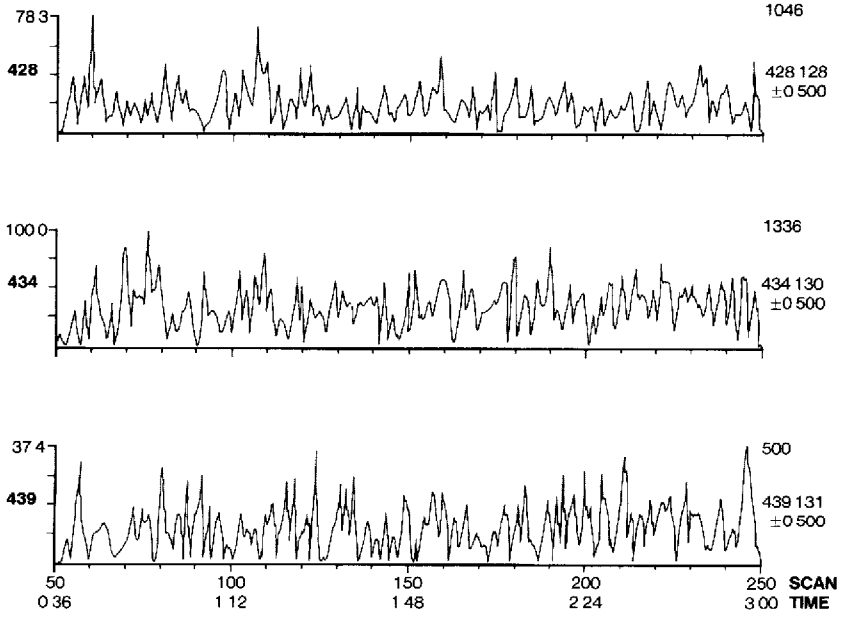

Fig. 3. Typical SIM mass chromatogram of a 'blank' human plasma extract.

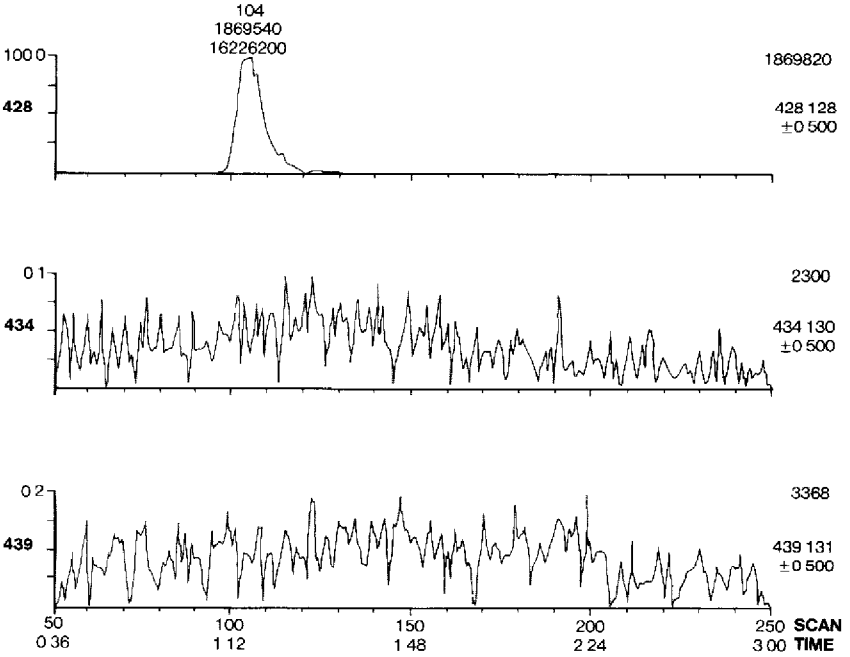

Fig. 4. Typical SIM mass chromatogram of a human plasma extract containing only unlabeled moricizine.

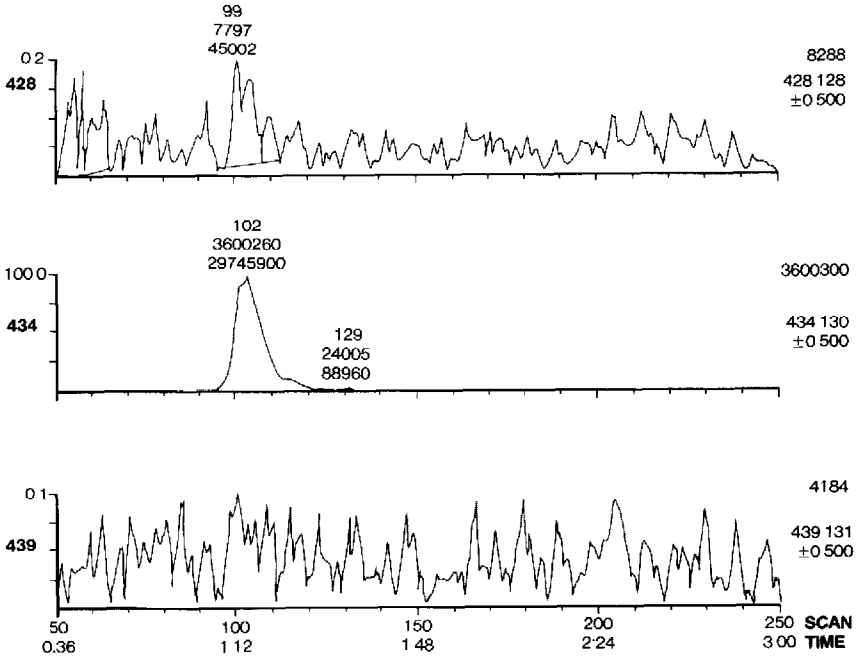

Fig. 5. Typical SIM mass chromatogram of a human plasma extract containing only [$^{13}C_6$]moricizine.

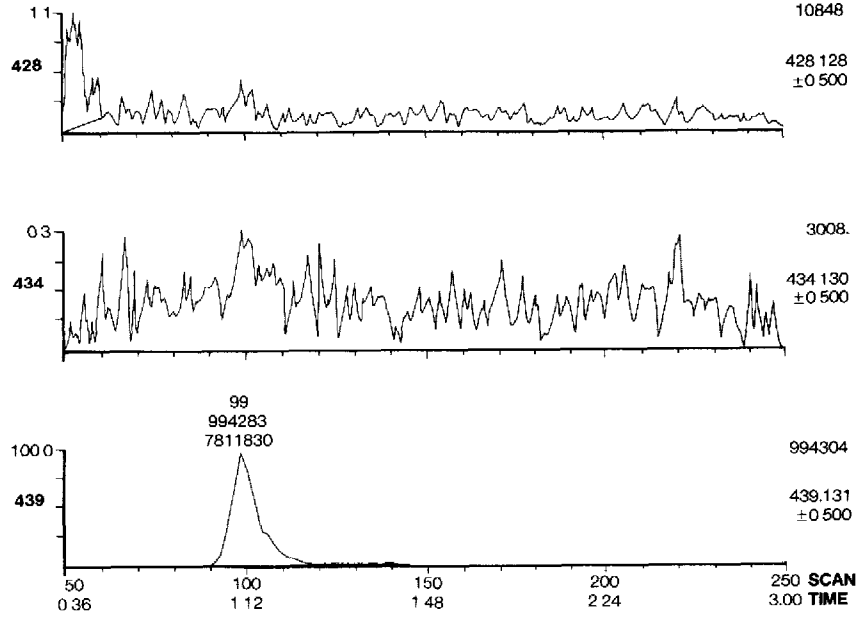

Fig. 6. Typical SIM mass chromatogram of a human plasma extract containing only [$^{2}H_{11}$]moricizine.

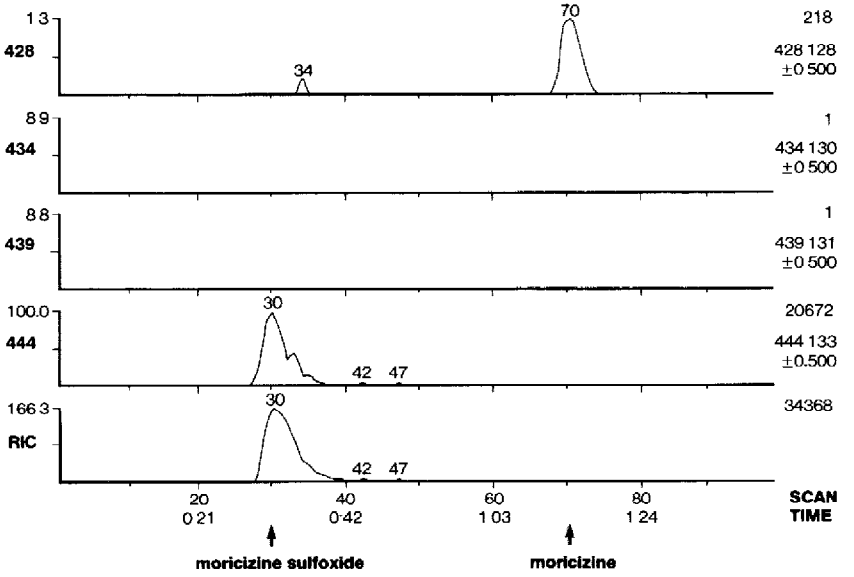

Fig. 7. SIM mass chromatogram of a reconstituted stock solution containing moricizine sulfoxide and a trace amount of unlabeled moricizine.

in no quantitative interference at any of the *m/z* values monitored. Sometimes a small signal was observed at *m/z* 428 (presumably due to incomplete isotopic labeling) when SIM mass chromatograms were run on plasma 'spiked' with only [$^{13}C_6$]moricizine (see Fig. 5). This signal, however, was always very minor ($< 0.6\%$) and was not considered significant for calculation purposes. The chromatographic retention times of moricizine and [$^{13}C_6$]moricizine were both $\simeq 1.2$ min. [$^{2}H_{11}$]Moricizine eluted slightly earlier than the other two isotopes.

The potential assay interference of a known moricizine metabolite, moricizine sulfoxide [7], was evaluated. This metabolite has a higher molecular mass than moricizine and also is found in plasma after moricizine administration [8]. Although moricizine sulfoxide gave a fragment of *m/z* 428 (1.0% of *m/z* 444), it was chromatographically separated (retention time, $\simeq 0.5$ min) from the three moricizine isotopes (Fig. 7).

Precision

Intra-day precision (Table I) was estimated from three replicate assays of 'spiked' human plasma (1.0 ml) analyzed on three separate days. Plasma contained both moricizine and [$^{13}C_6$]moricizine. The added amounts were unknown to the analyst and results were generated from independent standard calibration curves.

Inter-day precision (Table I) was estimated from the mean concentrations

TABLE I

INTRA- AND INTER-DAY PRECISION FOR UNLABELED AND $^{13}C_6$-LABELED MORICIZINE (MRZ)

Concentration added[a] (ng/ml)		Intra-day precision (C.V., %)						Inter-day precision (C.V., %)	
MRZ	[$^{13}C_6$]MRZ	Day 1		Day 2		Day 3		MRZ	[$^{13}C_6$]MRZ
		MRZ	[$^{13}C_6$]MRZ	MRZ	[$^{13}C_6$]MRZ	MRZ	[$^{13}C_6$]MRZ		
5.0	5.0	7.8	8.7	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]
10.1	10.1	2.4	13 3	5.5	5.6	3.3	3.4	2 8	6 5
15.1	15.2	1.8	2 9	6.8	5 2	5.6	8.2	6.4	12 0
74.4	74.7	2 7	2 0	1.5	2.6	3.3	4.7	4.5	5.1
805	808	4 0	1 8	4.8	7.4	4.4	3.8	3.0	1.9

[a]In terms of the hydrochloride salt.
[b]Not assayed or determined.

of the intra-day replicates for the three assay days. Over a plasma concentration range of 10–800 ng/ml, intra- and inter-day precision ranged from 1.5 to 13.3% and 1.9 to 12.0%, respectively.

Accuracy

Three separate methods were used in estimating accuracy of the LC–MS assay. One method was to use the results generated from the intra-day precision samples since the samples were assayed blind and their concentrations generated from an independent calibration curve (Table II). Over a plasma concentration range of 10–800 ng/ml for either unlabeled or $^{13}C_6$-labeled moricizine·HCl, the differences between measured and 'spiked' concentrations were all less than 20% except in two instances. Over 75% of the calculated differences were less than 10%.

A second accuracy assessment was a comparison of the results generated by the present LC–MS method and an HPLC method employing UV detection [4]. On two days blank human plasma was 'spiked' with unlabeled and $^{13}C_6$-labeled moricizine·HCl and assayed by both methods. All sample concentrations were unknown to both the LC–MS and HPLC analysts. The differences between the measured (by LC–MS) and 'spiked' concentrations were always less than 10% when moricizine·HCl concentrations were higher than 12 ng/ml (Table III). A high concentration of one isotope in the presence of a low concentration of the other had no effect on accuracy. There was good agreement between the HPLC and LC–MS results for total moricizine·HCl (i.e., unlabeled + $^{13}C_6$-labeled moricizine·HCl) concentration.

The third method for accuracy determination also was a comparison of the LC–MS and HPLC–UV method of Whitney et al. [4]. However, plasma samples from patients receiving moricizine·HCl therapy were assayed this time. These results are in Table IV.

TABLE II

ACCURACY ASSESSMENT FROM UNLABELED AND $^{13}C_6$-LABELED MORICIZINE (MRZ) 'SPIKED' HUMAN PLASMA SAMPLES

Concentration added[a] (ng/ml)		Percentage difference[b]					
		Day 1		Day 2		Day 3	
MRZ	[$^{13}C_6$]MRZ	MRZ	[$^{13}C_6$]MRZ	MRZ	[$^{13}C_6$]MRZ	MRZ	[$^{13}C_6$]MRZ
5.0	5.0	18.8	5.2	-[c]	-[c]	-[c]	-[c]
		23.2	5.2				
		5.8	−10.0				
10.1	10.1	7.9	22.8	1.0	1.0	5.0	6.9
		11.9	−1.0	10.9	−4.2	11.9	3.0
		6.9	−2.6	1.0	−9.7	6.9	0
15.1	15.2	12.6	17.8	17.2	10.5	6.0	1.3
		8.6	14.5	19.9	22.4	2.0	−9.9
		10.6	11 2	5.3	14.5	−5.3	−13.2
74.4	74.7	0.3	3.2	2.2	4.0	−10.5	−12.2
		−1.7	−0.7	1.7	−0.7	−4.8	−3 7
		3.6	2.3	−0.7	−0.4	−5.4	−6.3
805	808	2.7	1.4	3.0	2.2	−6.8	−4 1
		−5.2	−1.4	−2.1	−3.0	−3.5	2.1
		−1.5	−2.1	7.8	12.1	1.6	3.0

[a]In terms of the hydrochloride salt.

[b]Percentage difference $= \left(\frac{\text{measured concentration} - \text{actual concentration}}{\text{actual concentration}} \right) \times 100.$

[c]Not determined.

Excellent agreement was observed between the two methods. Differences (except for one sample) were less than 20% when moricizine·HCl concentrations were greater than 10 ng/ml; many of the values were less than 10%. The LC–MS plasma concentration results for the 0.5- and 1.0-ml samples from the same patient were similar which supports the upper quantitation limit of the assay (i.e., 800 ng extracted).

These results further confirm the specificity of the LC–MS method. If any metabolites eluted from the LC column at the same time as moricizine, and gave a mass fragment of *m/z* 428, then the moricizine·HCl concentration would have been overestimated.

Linearity and minimum quantifiable level

Seven 1.0-ml plasma standards containing 10, 15, 25, 101, 252, 504, and 806 ng of both unlabeled and $^{13}C_6$-labeled moricizine·HCl were prepared and assayed on seven different days. The resulting LC–MS calibration curves for

TABLE III

MORICIZINE (MRZ) CONCENTRATIONS OF 'SPIKED' HUMAN PLASMA SAMPLES: HPLC VERSUS LC–MS COMPARISON

All concentrations are in terms of the hydrochloride salt.

Concentration added (ng/ml)		HPLC concentration MRZ + [$^{13}C_6$]MRZ (ng/ml)		LC–MS concentration (ng/ml)					
MRZ	[$^{13}C_6$]MRZ	Day 1	Day 2	Day 1			Day 2		
				MRZ	[$^{13}C_6$]MRZ	Total	MRZ	[$^{13}C_6$]MRZ	Total
5.0	5.0	8.2	10.4	4.0	3.4	7.4	5.6	5.5	11.1
12.5	12.6	22.2	21.7	11.9	13.7	25.6	11.6	12.7	24.3
40.0	40.2	78.3	98.1	42.6	40.4	83.0	41.2	40.4	81.6
300	301	604	597	317	316	633	284	289	573
1000	1005	2035	2028	1048	1020	2068	934	958	1882
0	100	102	95.6	0	96.2	96.2	0	100	100
100	0	104	97.3	110	0	110	92.2	0	92.2
100	10.0	117	107	109	8.8	118	91.8	9.9	102
10.0	100	107	111	7.6	92.5	100	10.3	93.7	104

TABLE IV

MORICIZINE·HCl PLASMA CONCENTRATIONS IN PATIENTS RECEIVING MORICIZINE·HCl: HPLC VERSUS LC–MS COMPARISON

HPLC (ng/ml)	LC–MS[a] (ng/ml)	Percentage difference
5.0	9.1	+82.2
9.8	15.1	+54 1
11.8	11.2	−5.1
15.2	18.4	+21.0
16.0	13.5	−15.6
31.0	30.0	−3.2
57.0	55.6	−2.4
64.0	61.0	−4.7
82.0	83.1	+1.3
102	85.9	−15 8
157	150	−4.4
188	167	−11.2
270	258	−4.4
302	249	−17.5
306	249	−18.6
839	777 (786[b])	−7.4 (−6.3)
1041	957 (916[b])	−8.1 (−12.0)

[a] 1.0 ml assayed.
[b] 0.5 ml assayed.

unlabeled and $^{13}C_6$-labeled moricizine·HCl were linear and reproducible over this concentration range (i.e., 10–800 ng/ml) (Fig. 8). The coefficient of determination for seven assay runs was always > 0.997. Based on these results and the precision and accuracy findings, the minimum quantifiable level of this method is 10 ng/ml of plasma for either moricizine·HCl isotope.

Stability

All moricizine (unlabeled, $^{13}C_6$-labeled and $^{2}H_{11}$-labeled) methanolic stock solutions were stable indefinitely when stored at −20°C in the absence of light.

The stability of moricizine and [$^{13}C_6$]moricizine in frozen (−20°C) human plasma was determined to be at least six months based on the accuracy results presented in Table III. The day 1 and day 2 HPLC results were generated three days apart and six months prior to the LC–MS results. The plasma was kept frozen during this time interval.

Method applicability

The results presented in Table IV illustrate the ability of the present thermospray LC–MS method to assay moricizine plasma samples from patients. However, this method offers no advantages over HPLC in this situation. The strength of LC–MS analysis is its ability to quantitatively discriminate unla-

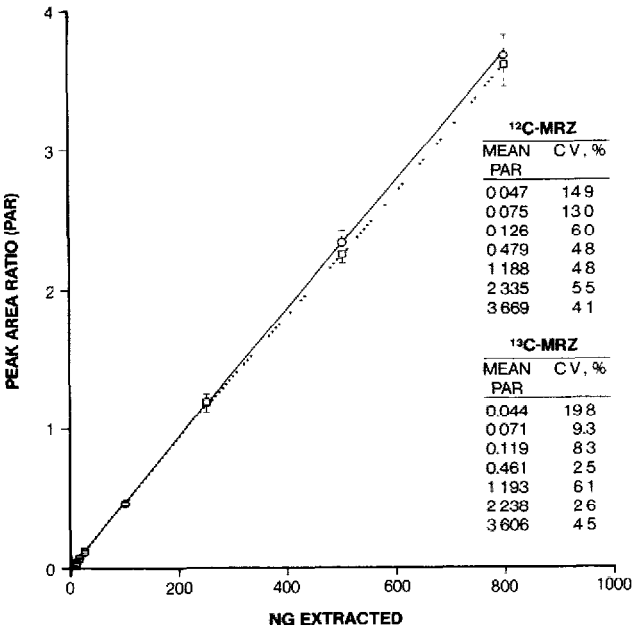

Fig. 8. HPLC-MS calibration curves (mean ± S.D.) for moricizine·HCl (MRZ) from human plasma extracts: (○) MRZ; (□) [$^{13}C_6$]MRZ.

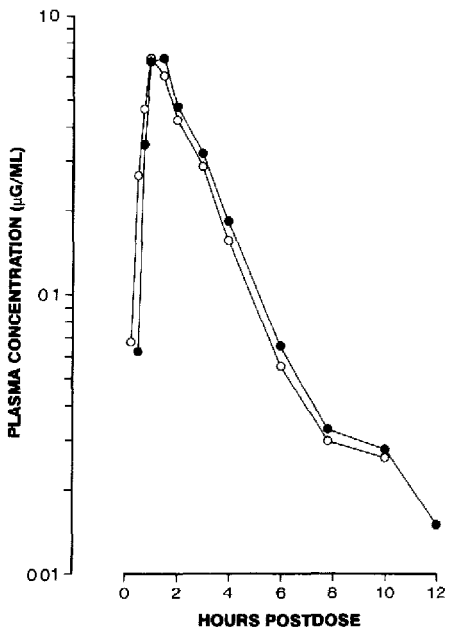

Fig. 9. Concentration-time profile after simultaneous administration of a 200-mg moricizine·HCl tablet (●) and a 200-mg [$^{13}C_6$]moricizine·HCl solution (○) to a human subject.

beled and $^{13}C_6$-labeled moricizine when both are present. Fig. 9 depicts such a situation for a normal volunteer concomitantly administered a moricizine·HCl tablet and a [$^{13}C_6$]moricizine aqueous solution (200 mg hydrochloride salt equivalents of both). In this subject, the absorption rate for solution appeared to be slightly more rapid than tablet (time of peak concentration, 1.0 versus 1.5 h), but peak levels (0.702 versus 0.707 μg/ml) and areas under the plasma concentration–time curve were comparable (relative bioavailability, 105%). It should be noted that moricizine is thermally labile and, as such, is not amenable to either gas chromatography–MS or direct-probe MS for quantitative analysis.

Concluding remarks

The use of LC–MS as an analytical tool was first reported over fifteen years ago [9]. However, its use for routine and robust quantification of drugs and/or metabolites in biological fluids has been limited [10–17]. We report such an application and suspect that as the need for more sensitive and specific assays of polar, non-volatile, or thermally labile compounds increase, LC–MS will emerge as a most powerful, and perhaps, routine analytical technique.

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REFERENCES

1 J. Morganroth, A.S. Pearlman, W.B. Dunkman, L.N. Horowitz, M.E. Josephson and E.L. Michelson, Am. Heart J., 98 (1979) 621.
2 P.J. Podrid, A. Lyakishev, B. Lown and N. Mazur, Circulation, 61 (1980) 450.
3 C.M. Pratt, S.C. Yepsen, A.A. Taylor, D.T. Mason, R.R. Miller, M.A. Quinones and R.A. Lewis, Am. Heart J., 106 (1983) 85.
4 C.C. Whitney, S.H. Weinstein and J.C. Gaylord, J. Pharm. Sci., 70 (1981) 462.
5 V.K. Piotrovskii and V.I. Metelitsa, J. Chromatogr., 231 (1982) 205.
6 J.M. Poirier, Ther. Drug Monit., 7 (1985) 439.
7 Y I. Vikhlyaev, V.P. Zherdev, A.N. Gritsenko and Z.I. Ermakova, Farmakol. Toksikol., 40 (1977) 19.
8 H.J. Pieniaszek, Jr., unpublished results.
9 M.A. Baldwin and F.W. Mc Lafferty, Org. Mass Spectrom., 7 (1973) 1111.
10 T. Coney and J. Henion, Anal. Chem., 55 (1983) 2275.
11 A.L. Yergey, D.J. Liberato and D.S. Millington, Anal. Biochem., 139 (1984) 278.
12 M.S. Lant, L.E. Martin and J. Oxford, J. Chromatogr., 323 (1985) 143.
13 J. van der Greef, A.C. Tas, M.A.H. Rijk and M.C. ten Noever de Breuw, J. Chromatogr., 343 (1985) 397.
14 S.J. Gaskell, K. Rollins, R.W. Smith and C.E. Parker, Biomed. Environ. Mass Spectrom., 14 (1987) 717.
15 L. Butfering, G. Schmelzeisen-Redeker and F.W. Rollgen, J. Chromatogr., 394 (1987) 109.
16 K.H. Schellenberg, M. Linder, A. Groeppelin and F. Erni, J. Chromatogr., 394 (1987) 239.
17 M.S. Lant, J. Oxford and L.E. Martin, J. Chromatogr., 394 (1987) 223.