Journal of Chromatography, 146 (1978) 465—472
Biomedical Applications

CHROMBIO. 205

DETERMINATION OF MOLSIDOMINE IN PLASMA BY HIGH-PERFORMANCE LIQUID COLUMN CHROMATOGRAPHY

D. DELL and J. CHAMBERLAIN

Hoechst Pharmaceutical Research Laboratories, Walton Manor, Walton, Milton Keynes, Buckinghamshire (Great Britain)

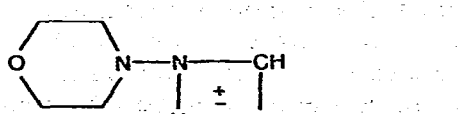
(Received February 24th, 1978)

SUMMARY

A high-performance liquid chromatographic method is described for the analysis of the anti-anginal compound 5-ethoxycarbonyl-3-morpholinosydnonimine (Molsidomine) in human and dog plasma. The drug was extracted from plasma into chloroform and the analysis was carried out on a reversed-phase column, the column effluent being monitored by UV absorption at 312 nm. The method is sensitive (2 ± 0.3 ng/ml) and specific. The method was applied to a study in which human volunteers received an aqueous solution of the drug and then, on a separate occasion, a tablet formulation. Peak plasma levels of 20—30 ng/ml (tablet) and 10—19 ng/ml (aqueous solution) were obtained following a 2-mg oral dose.

INTRODUCTION

Molsidomine (5-ethoxycarbonyl-3-morpholinosydnonimine, developed by Takeda Chemical Industries, Osaka, Japan, and licensed to Casella Farbwerke Mainkur, Frankfurt, G.F.R.) is a novel sydnonimine derivative with a mesionic ring which has been shown to possess a sustained anti-anginal effect following oral treatment [1].



Molsidomine

There are no reports of chromatographic methods for the analysis of Sydnones and, in view of the polarity of the mesionic ring system, it was considered unlikely that a gas chromatographic method would be applicable. It was

decided, therefore, to develop a high-performance liquid chromatographic method which would be sufficiently sensitive and specific to follow the concentration—time course of Molsidomine in plasma following therapeutic doses (1—2 mg) of the drug to human volunteers.

This paper describes the development of such a method, using reversed-phase chromatography, and its application to a human volunteer study involving two different formulations of Molsidomine.

EXPERIMENTAL

Apparatus and materials

The chromatograph consisted of a pump (constant flow syringe pump; Applied Chromatography Systems, Luton, Great Britain), an injector (modified hoke valve stop flow injector; H.S.C.P., Bourne End, Great Britain) and a UV detector (CE 212 UV variable wavelength monitor; Cecil Instr., Cambridge, Great Britain) operated at 312 nm, the $\lambda_{max.}$ for Molsidomine (ϵ = 1.5×10^4). The separation column was a 125 × 4.5 mm I.D. stainless-steel tube packed with ODS Hypersil (5 μm diameter, 200 m^2/g; Shandon Southern, Runcorn, Great Britain). The mobile phase consisted of a mixture of equal volumes of 0.1 *M* sodium acetate aqueous solution and methanol.

All chemicals and solvents were of analytical-reagent grade (Fison's Scientific Apparatus, Loughborough, Great Britain) and were used without further pre-treatment. Stock solutions of Molsidomine were prepared in distilled water and stored in the dark at 0—4°.

Preparation of columns

The separation column was packed by a slurry technique as follows: the packing material (2 g) was added to a solution of 80% methanol in 0.1% (w/v) aqueous sodium acetate trihydrate (10 ml) and the mixture was ultrasonicated for 5 min. The column was packed by forcing the slurry into the column tube by means of a constant-pressure pump (MCP 71; Olin Energy, Sunderland, Great Britain) set at 20,700 kPa.

Analysis of plasma samples

To 0.5 ml of plasma were added 2.0 ml of chloroform and the mixture was agitated using a Vortex mixer for 30 sec. After centrifuging (3000 *g* for 10 min) to separate the layers, 1.5 ml of the chloroform phase was transferred to a tapered tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 50 μl of the mobile phase under sonication and 20 μl was injected into the column. The above procedure was duplicated for each plasma sample. The Molsidomine peak height was measured and the plasma concentration calculated by reference to a standard curve obtained by processing control plasma containing known amounts of Molsidomine as described above. Peak height was plotted against plasma concentration over the range 0—10 ng/ml (human samples) and 0—10 μg/ml (dog samples). Plasma was stored at —20° until required for analysis.

RESULTS AND DISCUSSION

Recovery from aqueous systems

Aqueous buffer systems to which had been added Molsidomine (10 μg/ml) were extracted and analysed as described above for plasma (Table I). The percentage extracted was calculated by comparing the peak heights obtained following extraction of these buffered solutions with the peak heights of corresponding aqueous standard solutions. From pH 4 to 11 the recovery of the drug was virtually quantitative.

TABLE I

RECOVERY OF MOLSIDOMINE FROM AQUEOUS BUFFER SOLUTIONS

pH	Molsidomine extracted (%)
2.0	65
3.0	94
4.0	96
4.9	97
6.3	97
7.1	97
7.9	97
8.9	97
10.2	97
11.3	96
12.4	94

Recovery from plasma

Molsidomine was added to control plasma to give six replicate samples at five concentrations (2—10 ng/ml). This is the concentration range typically found in the plasma of human subjects following a therapeutic oral dose of 2 mg. The samples were processed as described and the recovery determined by referring the peak heights to a calibration curve obtained by analysing standard aqueous solutions of the drug (Table II). Single samples were analysed over a much wider concentration range (0.02—2.0 μg/ml) and the results indicate no significant dependence of recovery on concentration (Table II).

TABLE II

RECOVERY OF MOLSIDOMINE FROM PLASMA AT pH 7.4

C.V. = Coefficient of variation.

Molsidomine concn. (ng/ml)	Percent extracted ± C.V.	Molsidomine concn. (μg/ml)	Percent extracted
10	79± 6	2.0	87
8	79± 7	1.0	88
6	83± 5	0.5	88
4	85±24	0.2	84
2	85±14	0.1	85
		0.05	84
		0.02	83

Chromatography

Normal-phase chromatography on LiChrosorb SI 100 using 1,2-dichloroethane—methanol (95:5) as mobile phase was unsatisfactory, the Molsidomine peak eluting on the tail of a large plasma component [Fig. 1a]. Good separation of Molsidomine from the endogenous matrix was obtained on an octadecylsilyl reversed-phase column (Fig. 1b), eluting with an acetate buffer—methanol mobile phase.

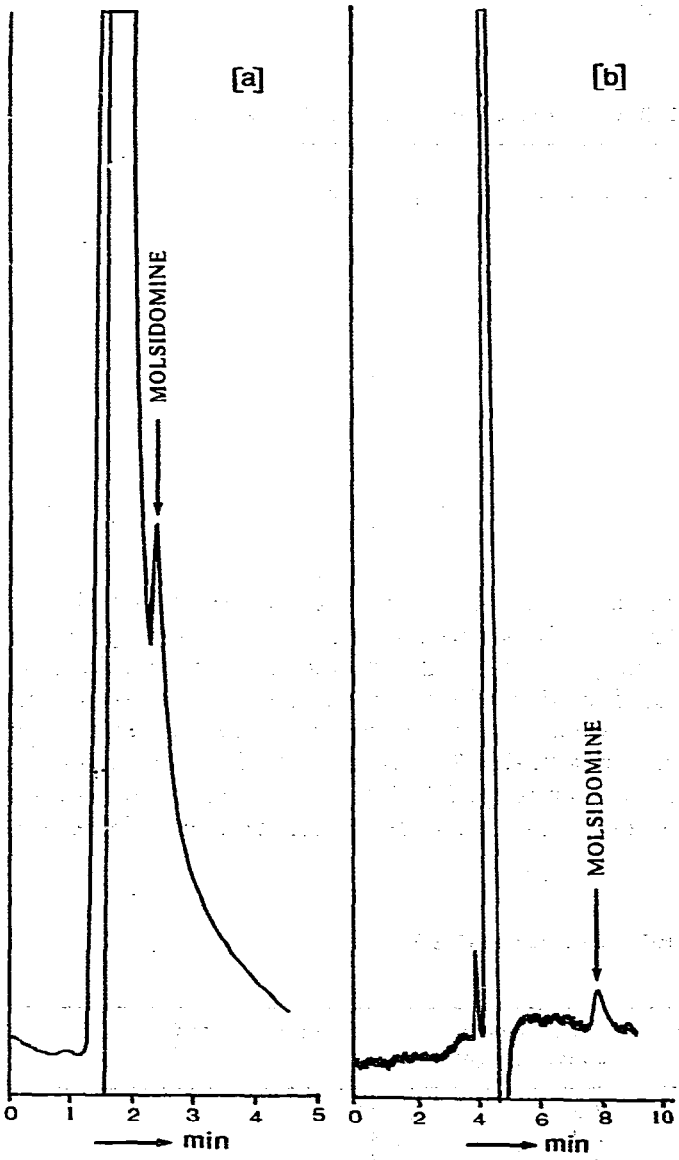


Fig. 1. Chromatogram of an extract of human control plasma to which had been added Molsidomine to give a plasma concentration of 10 ng/ml, a, LiChrosorb SI 100 column (0.005 a.u.f.s.); b, ODS Hypersil column (0.01 a.u.f.s.).

Limit of detection

A plasma concentration of 3.6 ng/ml gave a signal-to-noise ratio of 8 (Fig. 2). Extrapolating to a signal-to-noise ratio of 3 would yield a limit of detection of approximately 1.5 ng/ml. In practice, the precision of the method at a plasma concentration of 2 ng/ml (six replicates) was ± 16% (Table III).

Specificity

The specificity of the method was imparted by the chromatographic system and the ultraviolet detection wavelength. Evidence for the specificity was obtained from an experiment in which two beagle dogs were given an intravenous dose of 6 mg/kg of [^{14}C]Molsidomine. Blood samples were collected and total plasma radioactivity was determined by scintillation counting. Molsidomine concentrations were determined as described (Table IV). The peak corresponding to Molsidomine in the chromatogram was collected and the radioactivity content determined. Specific activity was obtained by dividing the micro-

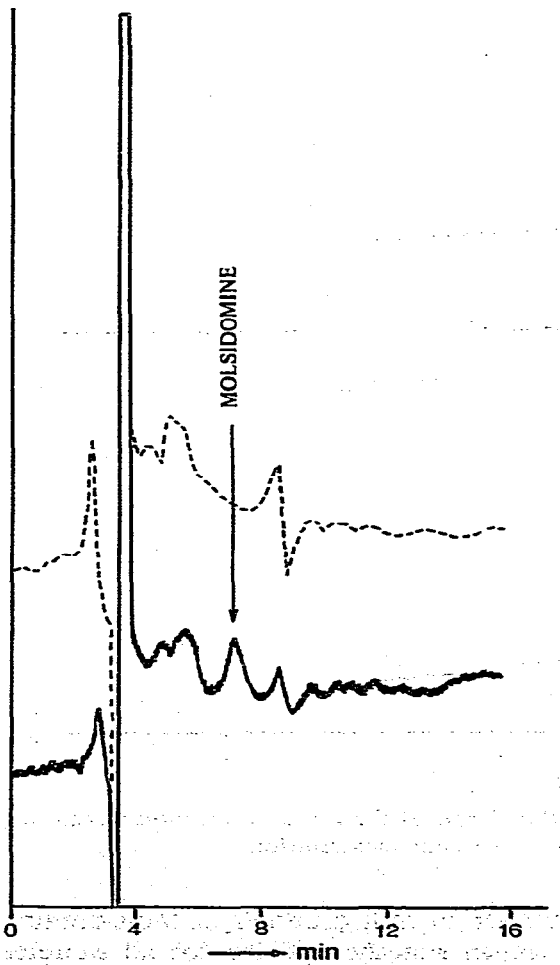


Fig. 2. Chromatogram of a plasma extract obtained from a human volunteer 45 min after receiving a p.o. dose of 2 mg of Molsidomine. The Molsidomine peak corresponds to a plasma concentration of 3.6 ng/ml (0.01 a.u.f.s.). - - - -, Control plasma extract; ——, plasma extract from volunteer receiving Molsidomine.

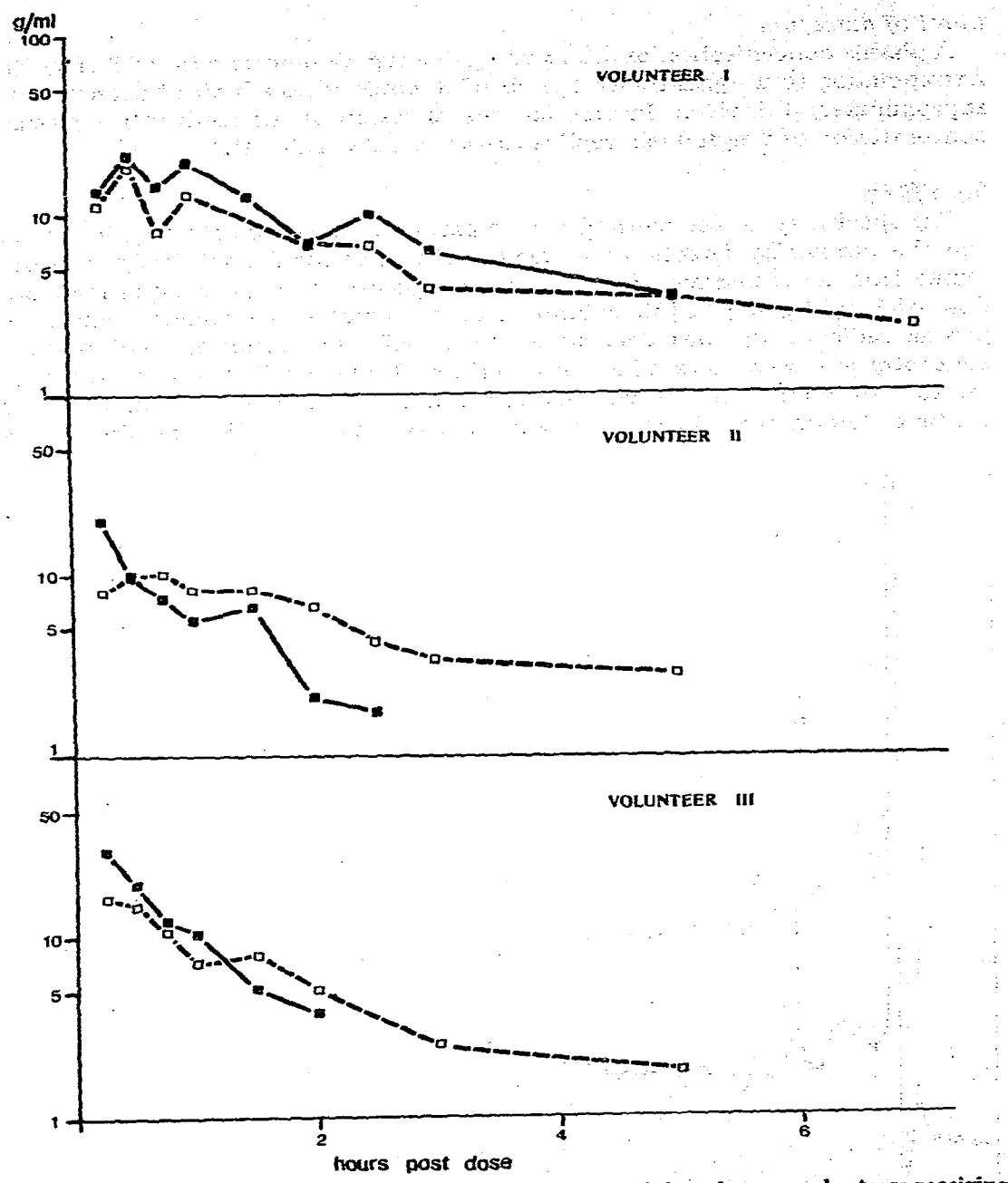


Fig. 3. The elimination of Molsidomine from the plasma of three human volunteers receiving a p.o. dose of 2 mg. ▪——▪, Tablet formulation; □- - -□, aqueous solution..

curies in the collected peak by the number of milligrammes of Molsidomino to which this peak corresponded. The mean specific activity for all sample was 0.65 ± 0.069 (10.6%) μCi/mg; this is in good agreement with the specific activity of the Molsidomine used in the experiment (0.60 μCi/mg). The total radioactivity in the plasma samples, in terms of the equivalent weight of Mols

TABLE III

PRECISION OF THE METHOD AND REGRESSION ANALYSIS (STANDARD CURVE)

s = standard deviation.

Molsidomine concn. (ng/ml)	Peak height (cm) at 0.01 a.u.f.s. deflection	Regression analysis		s	Precision (%)
		Fitted values	Departures (95% confidence)		
10.0	2.36 1.98 2.20 2.20 2.10 2.38	2.20	0.099	0.15	6.9
8.0	2.00 1.57 1.77 1.71 1.66 1.83	1.78	0.071	0.15	8.5
6.0	1.34 1.43 1.29 1.48 1.34	1.35	0.060	0.08	5.7
4.0	1.14 0.81 0.96 0.67 1.36 0.78	0.93	0.074	0.25	28.0
2.0	0.54 0.54 0.45 0.45 0.36	0.50	0.104	0.08	16.0

domine, was greater than the Molsidomine concentration as determined by the method, indicating the presence of metabolites in the plasma extract, which did not, however, interfere in the assay of Molsidomine.

Precision

This was evaluated from the data obtained from the samples used for the determination of recovery. The precision was calculated for each concentration (Table III). The calibration line was described by the regression equation: $y = 0.2132\ x + 0.0726$, where y is the Molsidomine peak height and x is the plasma concentration. The correlation coefficient was 0.985; this describes the "goodness of fit" of the experimental data with the regression line.

Calibration

Due to variations in response of the column and detector system, calibration

TABLE IV

MOLSIDOMINE AND ^{14}C CONCENTRATIONS IN DOG PLASMA FOLLOWING AN INTRAVENOUS DOSE OF 6 mg/kg

ND = not detectable (< ng/ml). For both dogs, mean specific activity = 0.65 (s = 0.069); specific activity of specific activity Molsidomine dosed = 0.60.

Time after dose	Dog A			Dog B		
	Molsidomine (μg/ml)	^{14}C (μg equiv. of Molsidomine per ml plasma)	Calc. specific activity (μCi/mg)	Molsidomine (μg/ml)	^{14}C (μg equiv. of Molsidomine per ml plasma)	Calc. specific activity (μCi/mg)
5 min	5.5	12.1	0.69	7.9	11.2	0.71
10 min	8.4	15.1	0.68	6.0	9.5	0.71
15 min	5.1	11.5	0.69	5.2	9.0	0.59
30 min	3.7	10.4	0.60	4.0	8.1	0.67
45 min	2.4	9.4	0.65	3.2	7.4	0.64
1 h	1.6	9.0	0.72	2.2	7.0	0.70
1.5 h	0.8	8.1	0.60	1.2	6.4	0.62
2 h	0.3	7.3	0.45	0.6	6.1	0.73
3 h	0.07	6.3	0.66	0.1	4.8	
4 h	ND	4.8		0.07	3.8	
6 h	ND	3.8		0.02	2.6	
24 h	ND	2.5		ND	2.0	
54 h		2.3		ND	1.9	
4 days		2.4			1.8	
8 days		1.6			1.0	

samples were always analysed on the same day as the unknown samples. The data presented in Tables II and III were obtained during one working day. Over a period of two weeks, during which time calibration samples were analysed daily, it was found that the variation of the slope of the standard curve about a mean line was ± 4%. The response of the column/detector system was linear from 2 ng to (at least) 2 μg injected.

Application

The method has been applied to the analysis of Molsidomine in the plasma of dogs receiving an intravenous dose of 6 mg/kg (Table IV), and to the analysis of the drug in human plasma following (a) a 2-mg p.o. dose of a tablet formulation, and (b) a 2-mg p.o. dose of an aqueous solution to three volunteers (Fig. 3).

ACKNOWLEDGEMENTS

The authors thank Mrs. J. Gibson for technical assistance and Dr. R.M.J. Ings for carrying out the pharmacokinetic analysis. Dr. J. McEwen conducted the bioavailability study.

REFERENCES

1 A. Takeshita, M. Nakamura, T. Tajimi, N. Matsuguchi, A. Kuroiwa, S. Tanaka and Y. Kikuchi, Circulation, 55 (1977) 401.
2 J. Ostrowski, Cassella Internal Report, 1976.