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Gas chromatographic analysis of isomeric organic mononitrates in plasma

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ABSTRACT

A specific, sensitive and precise capillary gas chromatographic method using electron-capture detection was developed for the determination of four isomeric vasodilating organic mononitrates, *viz.* L-isoidide mononitrate (L-IIMN), isosorbide-2-mononitrate (IS-2-MN), isomannide mononitrate (IMMN) and isosorbide-5-mononitrate (IS-5-MN), in rat plasma. With a sample size of 100 μl of rat plasma, the detection limits were found to be between 0.5 and 2 ng/ml for these mononitrates, and the absolute recovery was found to range from 83 to 90%. The within-day coefficients of variation for the assay of the four isomers were less than 5%, while the between-day coefficients of variation were less than 10%. Because of the short retention times of these isomers in this assay, routine analyses of about sixty plasma samples per day can be carried out. The possibility of *in vivo* interconversion among these four isomers in rats was investigated after individual administration of each isomer. No interconversion was found based on examination of plasma samples. The gas chromatographic method was applied to the pharmacokinetic studies of these four isomers in rats; at an intravenous dose of 2 mg/kg, the biological half-lives of L-IIMN, IMMN, IS-2-MN and IS-5-MN were found to be 13.2, 25.2, 54.6 and 112 min, respectively.

INTRODUCTION

Organic mononitrates are generated as metabolites of polynitrates such as isosorbide dinitrate (ISDN), and they also possess considerable pharmacologic activity as vasodilators. An advantage of some organic mononitrates is that they are well absorbed from the gastrointestinal tract with little first-pass hepatic metabolism [1]. Consequently, one of these compounds, *viz.* isosorbide-5-mononitrate (IS-5-MN), has been adopted for clinical use in angina pectoris [2,3] and congestive heart failure [4]. Studies of the coronary flow of Lagendorff heart preparations [5] and the perfusion pressure of dog hindleg preparations [6] indicated that other organic mononitrates such as L-isoidide mononitrate (L-IIMN) and isomannide mononitrate (IMMN) were also active vasodilators, with higher potency than IS-5-MN. The exact relationships between mononitrate structure and its pharmacokinetic/pharmacodynamic properties, however, have not been elucidated.

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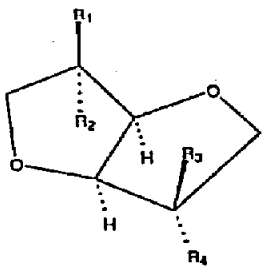

	L-IIMN	IS-2-MN	IMMN	IS-5-MN
R_1 (endo)	H	OH	OH	ONO_2
R_2 (exo)	ONO_2	H	H	H
R_3 (endo)	H	H	ONO_2	H
R_4 (exo)	OH	ONO_2	H	OH

Fig. 1. Structures of 1,4;3,6-dianhydrohexitol (*cis*-isohexide) mononitrates.

To initiate these investigations, we have developed a general gas chromatographic (GC) method for the determination of organic mononitrate concentrations in plasma. The structures of the four isomeric mononitrates studied, *viz.* L-IIMN, isosorbide-2-mononitrate (IS-2-MN), IMMN and IS-5-MN, are shown in Fig. 1. These four isomers are structurally identical to each other except for the spatial orientation of the hydroxy and nitrate groups.

GC with electron-capture detection (ECD) has been the most popular method of assay for organic mononitrates. However, several challenges remain in perfecting a GC–ECD assay for these compounds. The first problem involves the thermodynamic instability of organic mononitrates [7], which are well known to be labile in GC systems at temperatures above 150°C [8]. Furthermore, contact with catalytically active sites, column contaminants or oxygen in the GC systems, may also cause time-dependent decomposition even at lower temperatures. Therefore in order to preserve assay sensitivity, analytical conditions need to be optimized to minimize column degradation [9]. Secondly, several reports have indicated the presence of chromatographic interfering peaks, mainly in the assay of IS-5-MN [10–13], which arise from biological fluids, the extraction solvents or even from plastic receptacles [14]. Thus, extraction conditions need to be devised to reduce interference in the determination of these compounds. Thirdly, poor reproducibility and low sensitivity have been reported with the mononitrates due to irreversible adsorption of these compounds to the active surface of the inlet systems of the gas chromatograph, such as injector [9], or to the supports of packed columns [10,15,16]. Fortunately, this problem can be obviated by daily clean-up of the glass liner in the injector [17] and by using inert capillary columns [16,17].

At present, no literature method can provide adequate sensitivities for the

determination of the pharmacokinetics of these mononitrates in small animals. Most available methods required 1 ml of plasma per sample, with some requiring even up to 3–5 ml [10,11,18]. Other published methods involved time-consuming procedures for sample preparation, *e.g.*, derivatization of organic mononitrates to improve their chromatographic properties and reproducibilities [7,15], or using activated charcoal [19] or solid-phase column [16] for further purification of the plasma extract before analysis. Other methods either reported poor reproducibility in the lower concentration ranges [10,20], utilized no internal standards [10,11,21], or yielded variable reproducibility of the internal standard [13].

The only GC–ECD method for the analysis of IS-2-MN and IS-5-MN in rat plasma using 100 μl of plasma was published previously by this laboratory [22]. However, because of the use of a packed column, both the IS-2-MN and IS-5-MN peaks were not well separated from the solvent front, limiting the assay reliability of these mononitrates at low concentrations and possible extension of this method to L-IIMN and IMMN. Lutz *et al.* [17] reported a method for the simultaneous determination of IS-2-MN, IS-5-MN, IIMN, IMMN and ISDN in human plasma. However, this procedure required more than 30 min to finish a single run, and data on recovery, reproducibility, and precision of IIMN and IMMN were not given.

In the present report, we describe a rapid, specific, sensitive and reproducible capillary GC–ECD assay method for the determination of these four isomers in rat plasma. This method can also be adapted for the simultaneous determination of IS-2-MN, IS-5-MN and ISDN in plasma. Because of the usage of individual isomers as internal standards in the various pharmacokinetic studies, the possibility of *in vivo* interconversion among the four isomeric mononitrates in rats was also investigated.

EXPERIMENTAL

Chemicals and reagents

Unless specified, all chemicals were of analytical grade. Capillary GC/GC–MS grade ethyl acetate for plasma extraction was obtained from Burdick & Jackson Labs. (Muskegon, MI, USA). Toluene, methanol, ethyl acetate, *n*-hexane, glacial acetic acid and acetic anhydride were purchased from J. T. Baker (Phillipsburg, NJ, USA). Dichlorodimethylsilane was obtained from Eastman Kodak (Rochester, NY, USA). Concentrated ammonium hydroxide and silver nitrate (1 *M*) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Isomannide (98%) and fuming nitric acid (sp. gr. 1.49) were purchased from Alfa Products (Ward Hill, MA, USA) and Aldrich (Milwaukee, WI, USA), respectively.

IS-2-MN and IS-5-MN were provided by Schwarz Pharma (Monheim, Germany) as pure solids (> 99%) and were used without further purification. L-IIMN was kindly supplied by Dr. D. Hayward (D.H. Stereochemical Consulting, Vancouver, Canada). The reported purity, 98%, of L-IIMN was confirmed by GC using an authentic sample obtained from Schwarz Pharma.

IMMN was synthesized from isomannide according to the method reported by Jackson and Hayward [23]. Briefly, isomannide was nitrated with fuming nitric acid to form isomannide dinitrate (IMDN) in acetic acid–acetic anhydride (1:1, v/v) solution. The mixture was poured into ice water to yield crystallized IMDN, which then underwent controlled hydrolysis in 4 *M* hydrochloric acid at 37°C for 72 h. The solution was then neutralized with 5 *M* ammonium hydroxide and extracted with ethyl acetate twice. The ethyl acetate extract was then concentrated by vaporization and loaded on to a silica gel column (14 cm × 4 cm I.D.). IMMN was eluted with a gradient of 20–40% *n*-hexane in ethyl acetate. Melting point, ^{1}H nuclear magnetic resonance spectrum (Varian EM390, using tetramethylsilane as an internal standard) and infrared spectrum (Mattson Polaris FT-IR) were obtained to confirm the properties and structures of IMDN and IMMN against authentic samples obtained from Schwarz Pharma. The purity of IMMN was also confirmed by elemental analysis (Atlantic Microlab, Norcross, GA, USA) (theoretical: C 37.70%, N 7.32%, H. 4.71%; experimental: C 37.77%, N 7.31%, H. 4.72%) and by GC, which gave a value of 98.6%.

Apparatus and assay conditions

Analysis was carried out with a Hewlett-Packard Model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a ^{63}Ni electron-capture detector and a split–splitless injector, containing a 3.1 cm × 2 mm glass liner. Injections were made with a Hewlett-Packard Model 7673A automatic sampler. A SE-54 fused-silica capillary column (15 m × 0.25 mm I.D., 0.25 μm film thickness, Supelco, Bellefonte, PA, USA) held up with a meter of deactivated pre-column (Supelco) was used. The pre-column was changed approximately after every 100 injections. The carrier gas was helium (ultra-high-purity grade, Cryogenic Supply, Hamburg, NY, USA), the flow-rate of which was set at the maximum allowed in this inlet system, 2 ml/min. The detector make-up gas, argon–methane (95:5) (Cryogenic Supply) was set at 40 ml/min. The carrier and make-up gases were pre-purified with a carrier gas purifier (Supelco) and moisture trap (Hewlett-Packard), respectively. The splitless injection mode was used. Carrier gas flow-rate at the split vent and septum purge were 40 and 4 ml/min, respectively. The injection port temperature was 150°C. For assay reproducibility, it was critical to use a clean (and silanized) injector glass-liner every day (about every 60 samples). Since the electron-capture detector had been shown to exhibit an excellent sensitivity for nitrates at temperatures near 200°C or lower [24], the detector temperature was set at 225°C, as a compromise between detector sensitivity and avoidance of contamination due to condensation from column eluates. For analysis, the column temperature was initially set at 100°C for 0.8 min, then the oven was increased at 25°C/min to 110°C for 1 min and at 5°C/min to 130°C for 1 min. After each analysis, the column temperature was increased at 35°C/min to 200°C and maintained for 3 min to purge residual materials. Measurement of peak height was made with a Hewlett-Packard Model 3393A integrator.

Sample preparation

Because adsorption of mononitrates from solution onto active surfaces has been suggested [15], all glassware was silanized with 10% (v/v) dimethyldichlorosilane solution in toluene to prevent drug adsorption. After being soaked for at least 1 h in the silanization reagent, the glassware was immediately rinsed with toluene and methanol before being dried.

Mononitrates, in 0.1 ml of rat plasma, were stabilized with 10 μl of 1 *M* silver nitrate [25] and extracted once in a 8-ml tube with 1 ml of ethyl acetate containing 20 ng of internal standard. This tube was sealed with a PTFE-lined screw cap and shaken at low speed for 30 min. After centrifugation for 10 min at a speed of 3000 rpm ($\sim$1000 *g*), the upper layer was transferred to a 3-ml centrifuge tube for evaporation to dryness under pre-purified nitrogen in an ice bath (4°C). Once dry, the tube was removed from nitrogen right away to avoid evaporation of the mononitrates. The residues were then reconstituted with 1 ml of ethyl acetate, transferred into an autosampler vial, and 1-μl samples were injected into the chromatograph.

Because of its short retention time in chromatography, IS-2-MN was chosen as an internal standard for the assay of the other three isomers. Alternatively, the internal standard for the analysis of IS-2-MN was IS-5-MN.

Standard curves and analyses

Stock solutions of mononitrates were prepared by dissolving 100 mg of each mononitrate in 100 ml of ethyl acetate. Standard solutions in ethyl acetate were made by serial dilution of the stock solutions to final concentrations of 0.05, 0.1, 0.2, 0.5, 1, 1.5, 2, 2.5 and 3 μg/ml. Calibration standards were obtained by spiking 100 μl of each of these standard solutions into tubes, and evaporating the ethyl acetate to dryness in an ice bath under nitrogen. A 100-μl aliquot of blank rat plasma was added to each tube, mixed an then processed as described previously. Calibration curves were obtained daily. The ratio of the peak height of the mononitrate to that of the internal standard was plotted *versus* the concentration of the test compound in the calibration standard, and a least-squares linear regression analysis was performed. Values of unknown plasma concentrations were determined from the regression line of this calibration curve.

In vivo interconversion

A total of eight rats was used in this study. Two rats each received separate intravenous doses of 22 mg/kg L-IIMN, 100 mg/kg IS-2-MN, 2 mg/kg IMMN or 300 mg/kg IS-5-MN. These doses of L-IIMN, IS-2-MN and IS-5-MN have been shown to be equipotent in producing blood pressure-lowering effect [26]. After dosing, blood samples (about 0.25 ml each) were taken through the jugular vein using the following regimens: IS-2-MN: 30, 60, 90, 140, 190, 290, 390, 490, 590, 690 and 790 min; L-IIMN: 5, 10, 15, 25, 35, 45 and 60 min; IMMN: 5, 10, 15, 30, 60, 80 and 100 min; IS-5-MN: 90, 180, 270, 405, 540, 810, 1080, 1350 and 1620

min. These regimens took into account the different elimination characteristics of the four mononitrates. Plasma samples (100 μl each) were extracted and analyzed as described above without the addition of an internal standard.

Pharmacokinetic studies

Each mononitrate was dosed intravenously to a group of six rats (for IS-2-MN group, $n = 4$) at 2 mg/kg through a cannula implanted at the right jugular vein. The drug was dissolved in 0.5 ml of normal saline. The cannula was flushed with normal saline immediately after dosing to ensure delivery of the entire dose. Blood samples (about 0.25 ml) were taken through the same cannula before and after dosing using the following protocols: IS-2-MN: 15, 30, 45, 60, 80, 120, 160, 200, 240 and 280 min; L-IIMN: 5, 10, 15, 25, 35, 45, 60 and 75 min; IMMN: 5, 10, 15, 20, 30, 40, 60, 80, 100 and 120 min; and IS-5-MN: 30, 60, 90, 135, 180, 270, 360, 450 and 540 min. Plasma (100 μl) was immediately separated by centrifugation and stored at −20°C until analysis. Plasma analyses were performed as described above.

RESULTS AND DISCUSSION

Performance of chromatographic system

Fig. 2A and B show, respectively, a representative chromatogram of blank plasma and plasma containing all four mononitrate isomers. It is quite evident from Fig. 2A that no significant interfering peaks were observed. The retention times of IS-2-MN, IMMN, L-IIMN and IS-5-MN were 3.2, 4.1, 4.3 and 4.9 min, respectively (Fig. 2B). The four isomers were well separated except for a non-

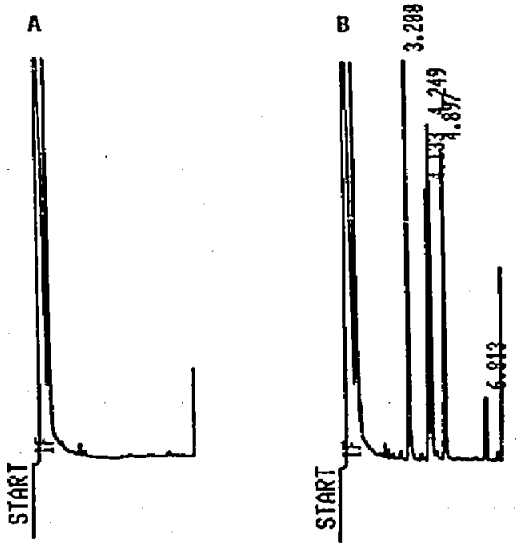

Fig. 2. Gas chromatograms of blank and spiked plasma extract. (A) Blank rat plasma extract; (B) plasma extract spiked with 0.5 μg/ml IS-2-MN (3.20 min), IMMN (4.13 min), L-IIMN (4.25 min) and IS-5-MN (4.90 min) as well as 0.2 μg/ml ISDN (6.81 min).

baseline separation between L-IIMN and IMMN. Further refinement of the chromatographic system was not attempted since each of the mononitrate isomers can be individually determined without ambiguity for the studies described here.

Because of the relatively short retention times of the mononitrates, each chromatographic run required about 13 min to complete, including conditioning of the column after analysis. This made it possible to analyze 60 samples by duplicate injection per day. This method represents, so far, the most rapid assay procedure reported for these mononitrates in the literature.

Reproducibility of this assay was critically dependent on replacement of the silanized injection glass liner after every 60 injections or so. This phenomenon, also observed by Rosseel and Bogaert [19] and Lutz *et al.* [17], was probably due to accumulation of particles on the inner surface of the glass liner carried by the syringe needle from the injection septum. These particles could cause severe peak tailing and decreased sensitivity, especially for IS-5-MN.

In vivo interconversion of mononitrates

Positional isomers are usually ideal internal standards for assay because they possess similar physico-chemical properties to the compounds being assayed. However, the possibility of inter-conversion *in vivo* or during analysis has to be ruled out. Among the mononitrates, IIMN and IMMN have been used as internal standards for the assay of IS-2-MN and IS-5-MN [16,19], and they have been shown to be metabolites of ISDN in rats [18], dogs [27] and man [17]. IS-5-MN was also found in rat urine after administration of IMDN [18]. This study, however, found no evidence of *in vivo* interconversion among the four mononitrates in any of plasma samples collected after intravenous dosing of each of the four isomers, even at the relatively high doses administered. This finding was consistent with the observation by Rosseel and Bogaert [18] who reported that no IS-5-MN was found in the urine of rats after the administration of IMMN (4 mg/kg). Artefactual interconversion during GC analysis was also not found. Therefore, it was appropriate to use one of the isomers as the internal standard of the others in the bioanalysis of organic mononitrates.

Extraction and internal standard

Ethyl acetate was an efficient extraction solvent for these four mononitrate isomers. Both dichloromethane and chloroform gave clean chromatograms and provided recoveries of about 80% for all isomers, consistent with literature values [17,22]. However, these chlorinated solvents were not chosen because (1) they are highly electron-capturing and their complete removal during evaporation would be essential and (2) a relatively tedious prepurification process, with activated charcoal, was required because commercially available products contained interference peaks which would co-chromatograph with IS-5-MN. Ethyl acetate, on the other hand, provided good recoveries of mononitrates with minimal ex-

traction of interfering substances, coupled with ease of evaporation. It was suggested that ethyl acetate might co-extract other plasma components which could interfere with the compounds of interest [28]. However, this problem is non-existent here, probably due to the usage of extraction solvent of high purity as well as a high-resolution capillary column.

Moisture in the extract has been reported to cause progessive loss of resolution by the column accompanied by peak tailing [29]. This problem was avoided by complete evaporation of the extraction solvent down to dryness, prior to reconstitution. Use of potassium carbonate and other drying agents was attempted, but was abandoned because of occurrence of interfering peaks.

Calibration curves and sensitivity

In general, our pharmacokinetic studies did not require the most sensitive settings of the detector current and attenuation, and attempts were not made to attain the best sensitivity possible under these conditions. Using a peak-to-noise ratio of 3 as a criterion, the following assay sensitivities can be readily obtained, *viz.*, about 1, 0.5, 1 and 2 ng/ml for L-IIMN, IS-2-MN, IMMN and IS-5-MN, respectively. The sample size was 100 μl of rat plasma, without sample concentration (*i.e.*, the extracted residues were reconstituted with more than 100 μl of ethyl acetate). The satisfactory sensitivity provided by this method might, in part, be due to the short retention of all nitrates which reduced on-column degradation. The high sensitivity obtained also made it possible to inject highly diluted samples to save the instrument from heavy contamination by the biological samples, thus making the method suitable for use with autosamplers on a routine basis and allowing up to about 300 injections before it was necessary to change the guard column.

TABLE I

REPRESENTATIVE CALIBRATION CURVES FOR L-IIMN, IS-2-MN, IMMN AND IS-5-MN IN SPIKED BLANK PLASMA

Compound	Concentration range (μg/ml)	Internal standard (μg/ml)	Equation of linear regression[a]	Correlation coefficient (r)
L-IIMN	0.01–0.5	IS-2-MN (0.2)	$y = 2.78x - 0.0095$	0.9997
	0.5 –3.0	IS-2-MN (0.2)	$y = 3.18x + 0.0565$	0.9991
IS-2-MN	0.01–1.0	IS-5-MN (1.0)	$y = 1.39x - 0.0301$	0.9981
	0.5 –3.0	IS-5-MN (1.0)	$y = 2.76x - 0.973$	0.9954
IMMN	0.01–0.5	IS-2-MN (0.2)	$y = 3.88x + 0.0505$	0.9990
	0.5 –3.0	IS-2-MN (0.2)	$y = 5.88x - 1.152$	0.9965
IS-5-MN	0.01–0.5	IS-2-MN (0.2)	$y = 2.58x - 0.0100$	0.9994
	0.5 –3.0	IS-2-MN (0.2)	$y = 3.57x - 0.601$	0.9940

[a] y = peak-height ratio; x = mononitrate concentration (μg/ml).

The standard curves, however, were not linear over the entire concentration range of 0.01–3 μg/ml. As indicated in Table I, at low concentrations (0.01–0.5 μg/ml), the slope of the standard curve was generally 35–50% lower than that obtained with higher concentrations. This was not due to a change in the recovery (see later). The break in the standard curves was quite constant among the four mononitrates and was observed at about 0.5 μg/ml. The same phenomenon has been observed in the assay of nitroglycerin and its dinitrate metabolites [30,31]. However, in these cases, the slope of the standard curve at low concentration ranges was higher than that found with high concentrations. The reasons for these non-linearities are not known at present.

Recovery and precision

Table II shows that the average recovery for all four mononitrates was between 85 and 90% for all four isomers, over a 100-fold concentration range. Recovery was not apparently concentration-dependent.

The within-day reproducibility was determined on three identical plasma samples at concentrations of 0.1, 1 and 10 μg/ml. The between-day reproducibility was determined on plasma samples at the same concentrations during three consecutive days. Data shown in Tables III and IV indicated that the coefficients of variation were all below 10%.

Assay application

The assay method was used to analyze rat plasma samples obtained after 2 mg/kg intravenous bolus doses of each of the four mononitrate isomers. Fig. 3 shows the plasma concentrations obtained. The mean apparent plasma half-lives (± 1 S.D.) of L-IIMN, IMMN, IS-2-MN and IS-5-MN were 13.2 ± 0.9, 25.2 ± 8.2, 54.6 ± 13.7 and 112 ± 20 min, respectively. These half life values of IS-2-MN and IS-5-MN were similar to those reported previously at the same dose [32]. A detailed analysis of the pharmacokinetics of these mononitrates will be presented elsewhere.

TABLE II

ABSOLUTE RECOVERIES OF ISOMERIC MONONITRATES FROM RAT PLASMA WITH ETHYL ACETATE AS SOLVENT

Spiked concentration (μg/ml)	Recovery (mean ± S.D., n = 3) (% of spiked concentration)			
	L-IIMN	IS-2-MN	IMMN	IS-5-MN
0.1	86.6 ± 5.5	85.8 ± 4.8	88.9 ± 3.1	98.8 ± 6.8
1	86.3 ± 3.4	83.7 ± 2.6	87.3 ± 7.0	89.8 ± 1.8
10	87.9 ± 3.8	86.2 ± 3.0	91.2 ± 2.1	93.0 ± 3.8

TABLE III

WITHIN-DAY REPRODUCIBILITY AND ACCURACY OF MONONITRATE ASSAY

Values in parentheses are coefficients of variation (%); $n = 3$.

Spiked concentration (μg/ml)	Assay concentration (mean ± S.D.) (μg/ml)			
	L-IIMN	IS-2-MN	IMMN	IS-5-MN
0.1	0.100 ± 0.000 (0.0)	0.099 ± 0.004 (3.7)	0.096 ± 0.004 (4.7)	0.099 ± 0.008 (8.1)
1	0.997 ± 0.011 (1.2)	1.01 ± 0.028 (2.7)	1.00 ± 0.034 (3.4)	0.950 ± 0.017 (1.8)
10	10.0 ± 0.40 (4.0)	10.4 ± 0.100 (1.0)	10.0 ± 0.078 (0.8)	9.85 ± 0.357 (3.6)

TABLE IV

DAY-TO-DAY REPRODUCIBILITY AND ACCURACY OF MONONITRATE ASSAY

Values in parentheses are coefficients of variation (%); $n = 3$.

Spiked concentration (μg/ml)	Assay concentration (mean ± S.D.) (μg/ml)			
	L-IIMN	IS-2-MN	IMMN	IS-5-MN
0.1	0.100 ± 0.005 (5.4)	0.101 ± 0.004 (3.7)	0.101 ± 0.005 (4.5)	0.103 ± 0.006 (5.7)
1	1.03 ± 0.030 (2.9)	0.978 ± 0.057 (5.8)	1.01 ± 0.030 (3.0)	1.03 ± 0.048 (4.7)
10	10.1 ± 0.557 (5.5)	9.94 ± 0.576 (5.8)	10.1 ± 0.100 (1.0)	9.56 ± 0.380 (4.0)

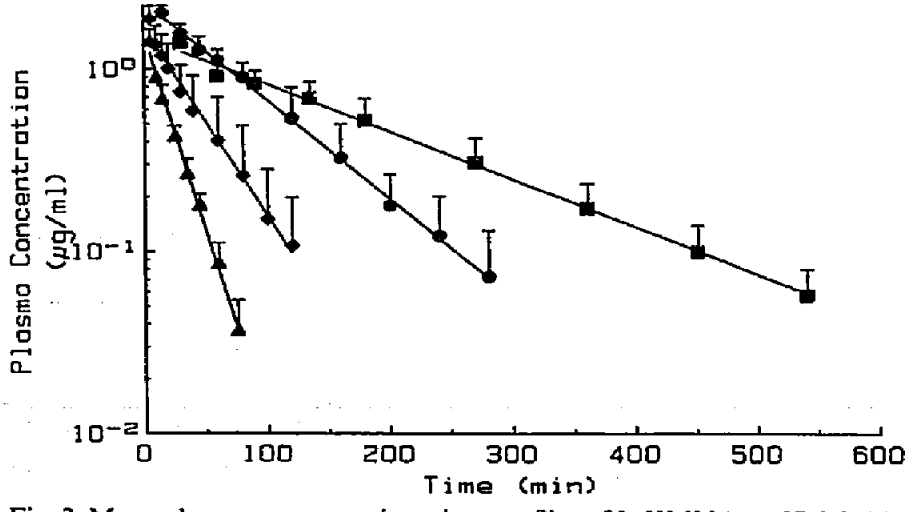

Fig. 3. Mean plasma concentration–time profiles of L-IIMN (▲), IS-2-MN (●), IMMN (◆) and IS-5-MN (■) in rats after intravenous administration of 2 mg/kg doses. Bars are standard deviations.

The GC–ECD method reported here is not only suitable for animal studies but also for human studies. Chromtograms of blank human plasma showed no interfering peaks at the retention times of interest. In addition, the present method can also be employed for the simultaneous determination of ISDN and its major metabolites, IS-2-MN and IS-5-MN. The retention time of ISDN was about 6.8 min (see Fig. 2B) which was well separated from all the mononitrates.

CONCLUSION

This paper describes a simple capillary GC–ECD method for the determination of L-IIMN, IMMN, IS-2-MN and IS-5-MN in rat plasma. This method allowed the use of small sample sizes, and is sensitive, specific and reproducible. The through-put of this assay is excellent, permitting ready analysis of up to 60 plasma samples on a daily basis.

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REFERENCES

1 U. Abshagen, in J. N. Cohn and R. Rittinghausen (Editors), *Mononitrates*, Springer-Verlag, Berlin, 1985, p.3.
2 A. Distante, M. Lombardi, E. Moscarelli, M. A. Morales and A. L'Abbate, *Z. Kardiol.*, 75 (Suppl. 3) (1986) 38.
3 M. A. D. Belder, A. Schneeweiss and A. J. Camm, *Am. J. Cardiol.*, 65 (1990) 6J.
4 G. Schiavoni, M. Mazzari, A. S. Montenero, R. Mongiardo, E. Scabbia and U. Manzoli, in J. N. Cohn and R. Rittinghausen (Editors), *Mononitrates*, Springer-Verlag, Berlin, 1985, p. 311.
5 E. Noack, *Methods Find. Exp. Clin. Pharmacol.*, 6 (1984) 583.
6 M. G. Bogaert and M. T. Rosseel, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 275 (1972) 339.
7 A. Marzo and E. Treffner, *J. Chromatogr.*, 345 (1985) 390.
8 J. A. Settlage, W. Gielsdorf and H. Jaeger, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 68.
9 M. Ahnoff and L. Johansson, *J. Chromatogr.*, 229 (1983) 327.
10 D. A. Chin, D. G. Prue, J. Michelucci, B. T. Kho and C. R. Warner, *J. Pharm. Sci.*, 66 (1977) 1143.
11 L. Richard, G. Klein and J. M. Orr, *Clin. Chem.*, 22 (1976) 2060.
12 J. O. Malbica, K. Monson, K. Neilson and R. Sprissler, *J. Pharm. Sci.*, 66 (1977) 384.
13 G. S. Tam, K. Nakatsu, J. F. Brien and G. S. Marks, *Biopharm. Drug Dispos.*, 8 (1987) 37.
14 M. T. Rosseel and M. G. Bogaert, *J. Pharm. Pharmacol.*, 28 (1976) 942.
15 R. V. Smith and J. Besic, *Microchem. J.*, 23 (1978) 185.
16 Y. Santoni, P. H. Rolland and J.-P. Cano, *J. Chromatogr.*, 306 (1984) 165.
17 D. Lutz, J. Rapser, W. Gielsdorf, J. A. Settlage and H. Jaeger, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 58.
18 M. T. Rosseel and M. G. Bogaert, *Biochem. Pharmacol.*, 22 (1973) 67.
19 M. T. Rosseel and M. G. Bogaert, *J. Pharm. Sci.*, 68 (1979) 659.
20 T. Taylor, L. F. Chasseaud, R. Major and E. Doyle, *Biopharm. Drug Dispos.*, 2 (1981) 255.
21 A. Sioufi and F. Pommier, *J. Chromatogr.*, 305 (1984) 95.

22 R. A. Morrison and H.-L. Fung, *J. Chromatogr.*, 308 (1984) 153.
23 M. Jackson and L. D. Hayward, *Can. J. Chem.*, 38 (1960) 496.
24 M. Ahnoff, M. Ervik, P. Lagerstrom, B. Persson and J. Vessman, *J. Chromatogr.*, 340 (1985) 73.
25 F. J. Dicarlo and M. D. Melgar, *Proc. Soc. Exp. Biol. Med.*, 131 (1969) 406.
26 T. -B. Tzeng, *Ph. D. Dissertation*, State University of New York at Buffalo, Buffalo, NY, 1991.
27 D. E. Reed, J. F. May, L. G. Hart and D. H. McCurdy, *Arch. Int. Pharmacodyn. Ther.*, 191 (1971) 318.
28 C. C. Wu, T. Sokoloski, A. M. Burkman, M. F. Blanford and L. S. Wu, *J. Chromatogr.*, 228 (1982) 333.
29 A. S. Carlin, J. E. Simmons, G. K. Shiu, A. O. Sager, V. K. Prasad and J. P. Skelly, *Pharm. Res.*, 5 (1988) 99.
30 P. K. Noonan, I. Kanfer, S. Riegelman and L. Z. Benet, *J. Pharm. Sci.*, 73 (1984) 923.
31 F. W. Lee, N. Watari, J. Rigod and L. Z. Benet, *J. Chromatogr.*, 426 (1988) 259.
32 R. A. Morrison and H.-L. Fung, *J. Pharmacol. Exp. Ther.*, 231 (1984) 124.