Simultaneous Determination of Isosorbide Dinitrate and Its Mononitrates in Human Plasma by Capillary Column GLC

M. T. ROSSEEL ˣ **and M. G. BOGAERT**

Received August 21, 1978, from the *J. F. & C. Heymans Institute of Pharmacology, University of Ghent, De Pintelaan 135, B-9000 Ghent, Belgium.* Accepted for publication October 25, 1978.

Abstract □ A previously described electron-capture GLC method for determination of isosorbide dinitrate in human plasma was adapted for the simultaneous determination of isosorbide dinitrate, isosorbide 2-mononitrate, and isosorbide 5-mononitrate using a capillary column. Quantitation was done with two internal standards. The lower limits of detection were approximately 0.5 ng/ml of plasma for isosorbide dinitrate, 2 ng/ml for isosorbide 2-mononitrate, and 20 ng/ml for isosorbide 5-mononitrate.

Keyphrases □ Isosorbide dinitrate—and mononitrates, simultaneous electron-capture GLC analysis, human plasma □ GLC, electron capture—analysis, isosorbide dinitrate and mononitrates, human plasma □ Isosorbide mononitrates—and dinitrate, simultaneous electron-capture GLC analysis, human plasma

The mononitrated metabolites of isosorbide dinitrate, isosorbide 2-mononitrate and isosorbide 5-mononitrate, are pharmacologically active and may contribute to the drug's therapeutic effect (1–4). Therefore, it is of interest to measure the levels of these metabolites together with the parent compound in plasma.

A method was described previously for the quantitative determination of isosorbide dinitrate in human plasma with electron-capture GLC (5), but quantitative mononitrate determination is sometimes difficult because of interfering peaks. This paper presents a modified method for simultaneous determination of isosorbide dinitrate and its two metabolites using a capillary column.

EXPERIMENTAL

Reagents and Materials—Ethyl acetate was pesticide grade[1]. Isosorbide 2-mononitrate, isosorbide 5-mononitrate, isoidide mononitrate, and isomannide dinitrate were prepared as previously described (6). Isosorbide dinitrate was available as a powder[2]. Charcoal, activated and neutralized, was washed three times with ethyl acetate[3].

Instrumental Conditions—The analysis was performed on a gas chromatograph[4] equipped with a linear ^{63}Ni-electron-capture detector[5]. The detector was operated with methane (5% v/v) in argon at a flow rate of 22.5 ml/min. The 25-m × 0.5-mm i.d. glass capillary column was deactivated and wall coated with OV-17[6]. The carrier gas was helium, prefiltered through a gas purifier[7] at a flow rate of 7.5 ml/min. Temperatures were: injector, 200°; column, 165°; and detector, 250°. Direct on-column injection without stream splitting was used. The peak areas were recorded on a recording integrator[8].

Procedure—All glassware was silanized as described previously (5).

The extraction procedure reported earlier (5) was slightly modified. To 2-ml of plasma in a glass-stoppered 10-ml centrifuge tube were added the internal standards isoidide mononitrate (25 ng) and isomannide dinitrate (200 ng). The plasma was extracted twice with 4 ml of ethyl acetate by shaking for 10 min. After separation from the aqueous phase by centrifugation (10 min, 6000 rpm), the organic phase was removed, avoiding the lipoprotein interface.

To the combined organic phase in another 10-ml tube, 55 mg of charcoal was added. The solution was shaken a few times by hand and centrifuged for 20 min at 7000 rpm. The organic phase was transferred into a 6-ml glass-stoppered conical tube and evaporated to dryness under a nitrogen flow at room temperature. The residue was immediately dissolved in 20 μl of ethyl acetate and stored at −18° until analysis. After evaporation of the ethyl acetate to approximately 5 μl under nitrogen, 0.3 μl was injected into the wide-bore glass capillary column.

Quantitation and Reproducibility—As the internal standard for the mononitrates, isoidide mononitrate was used; for isosorbide dinitrate, isomannide dinitrate was used. Human plasma samples spiked with increasing concentrations of isosorbide dinitrate (5–40 ng/2 ml), isosorbide 2-mononitrate (5–100 ng/2 ml), and isosorbide 5-mononitrate (50–1000 ng/2 ml) were carried through the whole procedure. The ratios of the test product peak areas to the internal standard peak areas were plotted *versus* the test product concentrations, and a least-squares linear regression analysis was performed. Values of unknown plasma drug concentrations were determined from this calibration graph.

RESULTS AND DISCUSSION

Typical chromatograms of a blank plasma sample and of a spiked plasma sample are shown in Fig. 1. When the ratios of the peak area of isosorbide dinitrate to that of its internal standard were calculated and plotted *versus* the isosorbide dinitrate concentrations in spiked plasma

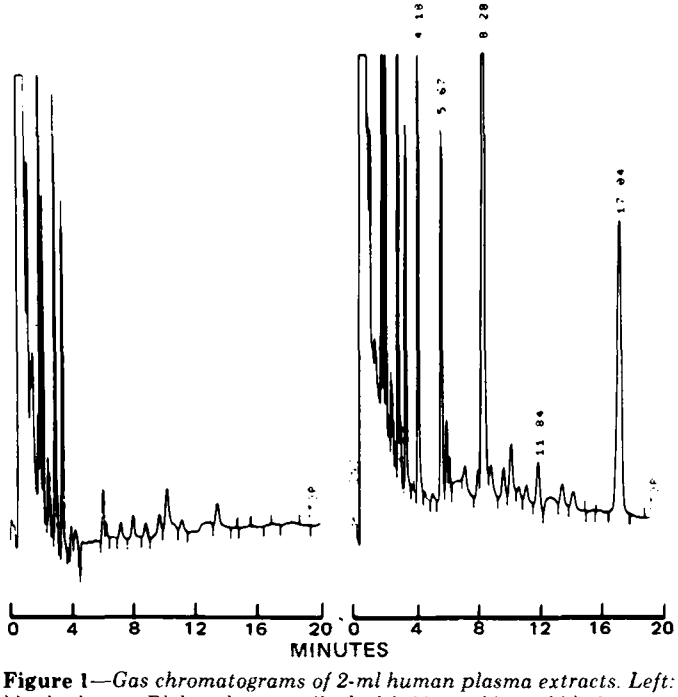

Figure 1—*Gas chromatograms of 2-ml human plasma extracts. Left: blank plasma. Right: plasma spiked with 20 ng of isosorbide 2-mononitrate (retention time of 4.18 min), 25 ng of isoidide mononitrate (5.67 min), 200 ng of isosorbide 5-mononitrate (8.28 min), 5 ng of isosorbide dinitrate (11.84 min), and 200 ng of isomannide dinitrate (17.04 min).*

[1] Carlo Erba, Italy.
[2] Cedona, The Netherlands.
[3] Sigma, St. Louis, Mo.
[4] Hewlett-Packard 5730 A series.
[5] Hewlett-Packard model 18713 A.
[6] RSL, Belgium.
[7] Alltech Associates.
[8] Hewlett-Packard 3380 A.

0022-3549/79/0500-0659$01.00/0

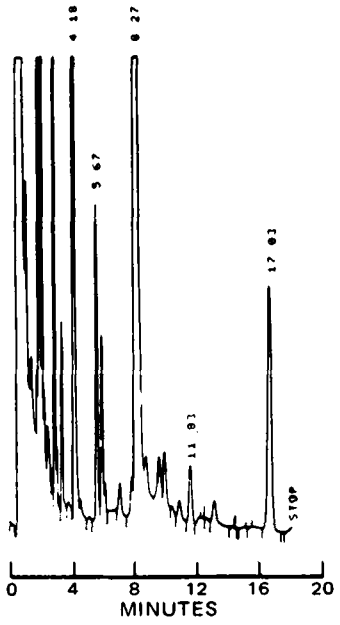

Figure 2—*Gas chromatogram of a 2-ml plasma sample extract obtained from a subject 6 hr after intake of 120 mg of isosorbide dinitrate slow-release preparation; 25 ng of isoidide mononitrate (retention time of 5.67 min) and 200 ng of isomannide dinitrate (17.03 min) were added as internal standards. Key (retention time): 4.18 min, isosorbide 2-mononitrate; 8.27 min, isosorbide 5-mononitrate; and 11.83 min, isosorbide dinitrate.*

samples, a linear relationship was found for the concentration range studied, and the intercept of the standard curve was negligible. The same was true for isosorbide 2-mononitrate and isosorbide 5-mononitrate.

As for isosorbide dinitrate (5), the recoveries of the mononitrates from plasma were quantitative. The relative standard deviations for analyses performed on different days were 7.01 for isosorbide dinitrate (11 samples, 5–20 ng), 9.13 for isosorbide 2-mononitrate (11 samples, 10–30 ng), and 10.96 for isosorbide 5-mononitrate (11 samples, 100–300 ng). The lower limits of detection were approximately 0.5 ng/ml of plasma for isosorbide dinitrate, 2 ng/ml for isosorbide 2-mononitrate, and 20 ng/ml for isosorbide 5-mononitrate.

After daily injections on the column, the glass injector adaptor was replaced weekly.

Figure 2 shows a typical plasma chromatogram 6 hr after intake of 120 mg of an isosorbide dinitrate slow-release preparation.

Several investigators reported difficulties with the quantitative mononitrate determination by conventional packed column GLC due to peak interference mainly with isosorbide 5-mononitrate (7–9). The wall-coated capillary column used here provides a suitable means of analyzing the isosorbide mononitrates with the required efficiency and specificity.

REFERENCES

(1) M. G. Bogaert and M. T. Rosseel, *Arch. Pharmacol.*, **275**, 339 (1972).

(2) R. L. Wendt, *J. Pharmacol. Exp. Ther.*, **180**, 732 (1972).

(3) M. Stauch, N. Grewe, and H. Nissen, *Verh. Dtsch. Ges. Kreislaufforschg.*, **41**, 182 (1975).

(4) M. Stauch, N. Grewe, and H. Nissen, *Herz.*, **3**, 223 (1978).

(5) M. T. Rosseel and M. G. Bogaert, *J. Pharm. Sci.*, **62**, 754 (1973).

(6) M. T. Rosseel and M. G. Bogaert, *Biochem. Pharmacol.*, **22**, 67 (1973).

(7) D. A. Chin, D. G. Prue, J. Michelucci, B. T. Kho, and C. R. Warner, *J. Pharm. Sci.*, **66**, 1143 (1977).

(8) L. Richard, G. Klein, and J. M. Orr, *Clin. Chem.*, **22**, 2060 (1976).

(9) J. O. Malbica, K. Monson, K. Neilson, and R. Sprissler, *J. Pharm. Sci.*, **66**, 384 (1977).

ACKNOWLEDGMENTS

Supported by Grant 3900171 from the National Fund for Medical Research, Belgium.

Improved Method for Morphine Extraction from Biological Samples

G. L. SPRAGUE and A. E. TAKEMORI ˣ

Received June 19, 1978, from the *Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.* Accepted for publication October 31, 1978.

Abstract □ Methadone, morphine, or naloxone extraction from brain homogenates, plasma, and urine is described. An aqueous sample was loaded on a surgical gauze support, which was washed with extracting solvents. Aqueous samples remained on the support, and nonpolar drugs partitioned into the lipophilic extracting solvent. The procedure recovered 80–100% of nanogram levels of methadone, morphine, or naloxone from biological samples. In addition, an approximate 10-fold timesaving capacity was demonstrated compared to standard liquid–liquid extraction techniques.

Keyphrases □ Methadone—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Morphine—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Naloxone—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Liquid–liquid extraction—analysis, methadone, morphine, naloxone, brain, blood, plasma, urine □ Narcotic analgesics—methadone, morphine, naloxone, liquid–liquid extraction, brain, blood, plasma, urine

Determination of tissue narcotic drug levels frequently involves radiolabeled drug administration. Following alkalinization, the lipophilic drugs are extracted from tissue preparations using standard liquid–liquid extraction techniques (1–5). These techniques typically involve partitioning the drug between two immiscible liquids by shaking, phase separation by centrifugation, and drug analysis in the nonpolar solvent phase or in the aqueous phase after back-extraction. Improved methods for morphine and related drug extraction have not altered the basic liquid–liquid extraction technique (4, 6).

A rapid, efficient extraction technique for tissue morphine and related drug determination is presented here. Extraction of tritiated methadone, morphine, and naloxone from brain, plasma, and urine samples is described. A polar support (gauze sponges) held an aqueous tissue ho-

0022-3549/79/0500-0660$01.00/0