Journal of Chromatography, 349 (1985) 421–424
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 645

Note

Rapid, isothermal gas–liquid chromatographic determination of nitroglycerine in plasma using an electron-capture detector. II.

KARINA LANGSETH-MANRIQUE* and JAN E. BREDESEN
Division of Clinical Pharmacology and Toxicology, The Central Laboratory, Ullevaal Hospital, 0407 Oslo 4 (Norway)
and
TYGE GREIBROKK
Department of Chemistry, University of Oslo, Oslo (Norway)

A previous publication from this laboratory[1] described a routine gas chromatographic (GC) method for the determination of nitroglycerine in plasma with SP-2401 as the stationary phase and an electron-capture detector. The sensitivity of the method was 0.2 nmol/l. In connection with a pharmacological study to be performed in cooperation with our laboratory, we modified our earlier method. This paper describes the modifications performed with the intention of lowering the sensitivity limit of nitroglycerine detection.

EXPERIMENTAL

Apparatus

A gas chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a ^{63}Ni electron-capture detector was used. The stationary phase was a mixture (37:63, w/w) of the following liquid phases: 3% SP-2550 on Supelcoport (100–120 mesh) and 3% OV-225 on Gas-Chrom Q (80–100 mesh) (both from Supelco, Bellefonte, PA, U.S.A.). The operating conditions were as follows: injection temperature, 150°C; column temperature, 135°C; detector temperature, 200°C; carrier gas (argon–methane, 90:10) flow-rate, 60 ml/min.

Blood sampling

Blood was collected in ice-cooled EDTA- or heparin-containing vacuum tubes (Venoject, Terumo, Japan). It was kept on ice for 5 min and then centrifuged at 400 *g* for 5 min at room temperature. Plasma was transferred with disposable glass pipettes to other vacuum tubes, capped and stored at −20°C until assayed, maximum 1 month, or at −70°C, maximum 3 months.

RESULTS AND DISCUSSION

Nitroglycerine was successfully analysed on SP-2550/OV-225 as the stationary phase with our work-up procedure described previously[1]. Its most characteristic fea-

0021-9673/85/$03.30

ture was the single, rapid extraction step, where nitroglycerine was extracted from plasma into hexane on a rotary mixer for 5 min. This was followed by a concentration step, where it became crucial to avoid evaporation of the solvent to dryness. Loss of nitroglycerine was as high as 23% after 1 min in the dry state. Evaporation to about 100 μl was found to be optimal.

This extraction procedure was adopted by Sioufi and Pommier[2], but the samples were extracted on a mechanical shaker for 20 min. The recovery was not mentioned. We have demonstrated that the recovery does not increase after 5 min of extraction with hexane in either shaking or rotating extraction procedures. An increase in extraction of unwanted endogenous plasma components was observed with mechanical shaking.

Fig. 1 shows a typical chromatogram of a sample containing 3.0 nmol/l nitroglycerine, with 25 nmol/l 1,3-dinitrobenzene as internal standard. The retention times for nitroglycerine and the internal standard were 1.8 and 2.5 min, respectively. Blank plasma assayed by this procedure gave no peaks that interfered with either the nitroglycerine or the internal standard.

Using the new stationary phase, the operating conditions were modified as follows: the carrier gas flow-rate was increased from 25 to 60 ml/min, and the column and detector temperatures were decreased from 145 to 135°C and 250 to 200°C, respectively. The detector response, tested with nitroglycerine in hexane solution, increased with increasing gas flow-rate by a factor of about 3 (Fig. 2) and with decreasing detector temperature by a factor of about 2 (Table I), whereas the response to the internal standard decreased by a factor of about 2 in both cases. A high gas flow-rate and low column and detector temperatures minimize the thermal decomposition of nitroglycerine.

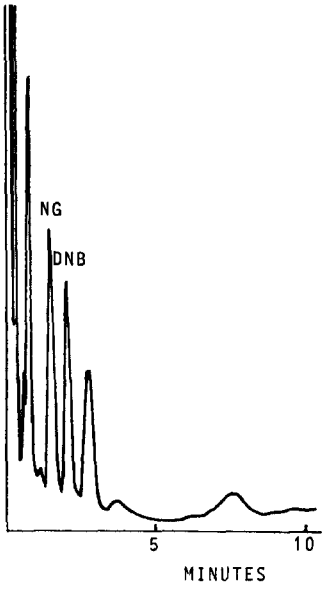

Fig. 1. Chromatogram of human plasma containing 3.0 nmol/l nitroglycerine (NG) with 25 nmol/l 1,3-dinitrobenzene (DNB) as internal standard.

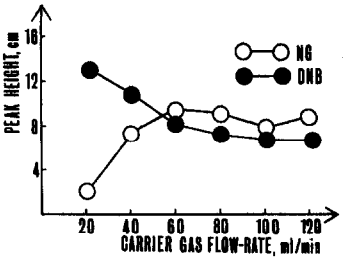

Fig. 2. Detector response, as the peak height (cm) for nitroglycerine (NG) and 1,3-dinitrobenzene (DNB), as a function of the carrier gas flow-rate (ml/min).

The sensitivity of 0.05 nmol/l achieved was due to the modifications described. This is an improvement by a factor of 4 over the previous method. The sensitivity was calculated from the minimum detectable amount at twice the noise level. Table II shows the day-to-day precision of the calibration curve (12 days). The results demonstrate good precision in the concentration range tested.

The blood sampling is critical, mainly because of the short half-life of nitroglycerine (6.2 min)[3] in blood at 37°C, and because of interfering substances originating from, *e.g.*, rubber stoppers and plastic syringes. A well designed sampling technique with immediate cooling of the blood samples will avoid any loss of nitroglycerine.

TABLE I

DETECTOR RESPONSE, AS THE PEAK HEIGHT, FOR NITROGLYCERINE (NG) AND 1,3-DINITROBENZENE (DNB), AS A FUNCTION OF THE DETECTOR TEMPERATURE

Detector temperature (°C)	*Peak height (cm)*	
	NG	*DNB*
150	12.60	2.70
200	13.45	5.10
250	6.70	8.25
300	1.25	10.45
350	0.30	10.80

TABLE II

DAY-TO-DAY PRECISION (12 DAYS) OF THE CALIBRATION CURVE FOR NITROGLYCERINE IN PLASMA

Concentration added to plasma (nmol/l)	*Mean peak height ratio*	*Coefficient of variation (%)*
0.25	0.06	2.2
0.5	0.12	2.5
1.0	0.25	2.2
5.0	1.37	1.9

A pharmacological study of transdermal nitroglycerine[4] was successfully carried out using the method described. The plasma concentrations found ranged from 0.2 to 7.2 nmol/l.

CONCLUSION

The method described includes a single, rapid, yet efficient extraction step. It is sensitive, reproducible and convenient for pharmacokinetic as well as occupational studies.

REFERENCES

1 K. Langseth-Manrique, J. E. Bredesen and T. Greibrokk, in A. Frigerio (Editor), *Chromatography and Mass Spectrometry in Biomedical Sciences, 2*, Elsevier, Amsterdam, Oxford, New York, 1983, p. 97.
2 A. Sioufi and F. Pommier, *J. Chromatogr.*, 339 (1985) 117.
3 J. A. Armstrong, S. E. Slaughter, G. S. Marks and P. W. Armstrong, *Can. J. Pharmacol.*, 58 (1980) 459.
4 T. F. Barkve, K. Langseth-Manrique, J. E. Bredesen and K. Gjesdal, *Am. Heart J.*, submitted for publication.