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Short Communication

Simultaneous determination of nitroglycerin and its dinitrate metabolites by capillary gas chromatography with electron-capture detection

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ABSTRACT

A major problem in the analysis of nitroglycerin (propanetriol trinitrate, GTN) and its metabolites [propanetriol 1,2-dinitrate (1,2-GDN) and propanetriol 1,3-dinitrate (1,3-GDN)] has been the adsorption of the nitro compounds to glass apparatus during sample preparation or injection. In this paper we present a method determining GTN, 1,2-GDN and 1,3-GDN in plasma at low nanomolar level using capillary gas chromatography with electron-capture detection. The detection limits are about 0.2 n*M* in plasma. Adsorption of the nitro compounds to glassware is prevented by using triethylamine, giving a simple sample preparation and accurate results. Thus, the laborious and time-consuming silanization of the glassware is avoided.

INTRODUCTION

Nitroglycerin (propanetriol trinitrate, glyceryl trinitrate, GTN) is used in the treatment of angina pectoris [1]. GTN is metabolized in the liver and blood to its two therapeutically active metabolites, propanetriol 1,2-dinitrate (1,2-GDN) and propanetriol 1,3-dinitrate (1,3-GDN) [2].

The development of an analytical method for the simultaneous determination of GTN and its dinitro metabolites in human plasma at the picomolar level has previously been described [3–7]. However, the adsorption of the nitro compounds to glass surfaces is a major difficulty in developing an analytical method that provides reliable analytical results. In several of the previously published articles [3–6] glass equipment was silanized in order to avoid the adsorption problems. However, silanization of glass equipment is a tedious and time-consuming procedure.

We have found that triethylamine (TEA) is very efficient in deactivating active glass surfaces, and we therefore present a method in which the adsorption of 1,2-GDN and 1,3-GDN is effectively eliminated, making possible the quantitative determination of GTN as well as the two dinitrate metabolites in the picomolar range using a simple sample preparation.

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EXPERIMENTAL

Apparatus

A Packard (Packard Instruments, Downersgrove, IL, USA) Model 438 gas chromatograph equipped with a Packard on-column injector, a Packard Model 902 nickel-63 electron-capture detector and a Spectra-Physics (San Jose, CA, USA) Model SP 4270 integrator was used. The column was an HP-1 (cross-linked methyl-silicone gum), 30 m × 0.53 mm I.D. with a 0.88-μm film thickness (Hewlett Packard, Avondale, PA, USA). An IEC (Needham Heights, MA, USA) Centra-7R centrifuge and a Savant (Farmingdale, NY, USA) SVC 200 vacuum centrifuge were used.

Chemicals

Helium of 99.9995% purity and argon–methane (95:5) were obtained from AGA (Copenhagen, Denmark). Helium as well as argon–methane were further cleaned by passing through oxygen and moisture filters (GAS-clean, Chrompack, Middelburg, Netherlands). GTN, 1,2-GDN and 1,3-GDN were from Nobel Industries (Karlskoga, Sweden). *n*-Hexane (ACS, nanograde) was from Mallinckrodt (Paris, KY, USA). TEA was obtained from Fluka (Buchs, Switzerland).

All other chemicals were of analytical grade from Merck (Darmstadt, Germany).

Chromatography

Helium was used as carrier gas at a flow-rate of 3.8 ml/min (43 cm/s) and argon–methane was used as make-up gas at a flow-rate of 68 ml/min. The following temperatures were set in the gas chromatograph: injector, 250°C; oven, 120°C; detector, 250°C.

When each sample had been chromatographed, the temperature was increased by 30°C/min to 280°C. After 5 min at 280°C the temperature was returned to the original 120°C. After injection of approximately 50 samples, 20 cm of the top of the column were cut off and the glass liner was cleaned.

Sample preparation

A 1-ml aliquot of plasma previously cooled to 4°C was extracted with 4 ml of pentane–dichloromethane (1:1, v/v) in a rotating apparatus for 10 min followed by centrifugation for 5 min at 1000 *g* at 10°C. A 3-ml aliquot of the organic phase was evaporated in the vacuum centrifuge, which was stopped at the precise moment when all the organic phase had been evaporated. The residue was dissolved in 75 μl of *n*-hexane containing 2% TEA, and 2 μl were injected into the gas chromatograph.

RESULTS AND DISCUSSION

From the literature it is obvious that the first attempts at the analysis of GTN and its dinitro metabolites in low concentrations in plasma were accompanied by problems caused by adsorption to glass surfaces. When setting up a method for this analysis we observed similar difficulties. These difficulties were found to be connected to the glass equipment. Significant losses of the two dinitro metabolites, but no loss of GTN, were observed (Fig. 1). Thus, the problems are as-

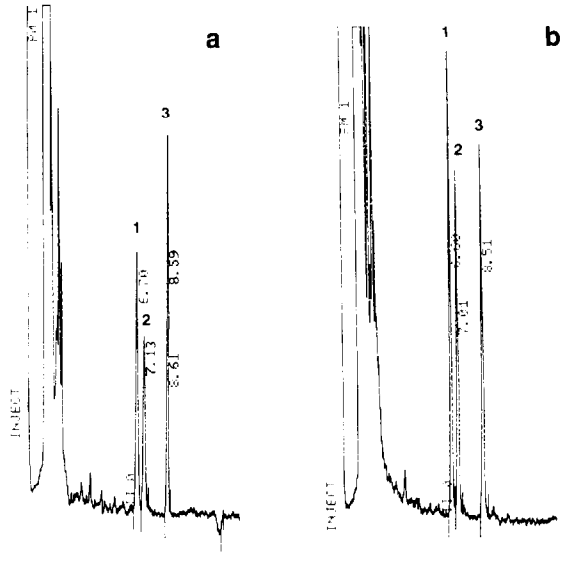

Fig. 1. Chromatogram showing the separation of nitroglycerin and its two metabolites (a) without using triethylamine (TEA) in the sample preparation and (b) using TEA in the sample preparation. A 100 n*M* solution of each of the three substances was chromatographed. For chromatographic details see text. Peaks: 1 = 1,3-GDN; 2 = 1,2-GDN; and 3 = GTN.

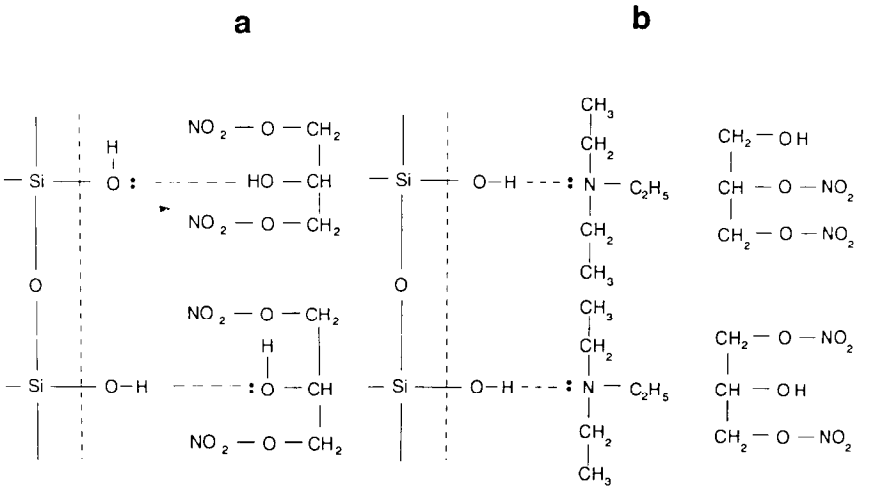

Fig. 2. Schematic representation of the interaction of the dinitro metabolites and triethylamine (TEA) with the silanol groups on the glass surface (a) without addition of TEA and (b) after the addition of TEA.

cribed to interactions between the free alcohol group in the two dinitro metabolites and silanol groups on the glass surfaces (Fig. 2a). Therefore, the glass equipment, including the glass liner of the gas chromatograph, was silanized, but this treatment was found to be a very tedious and time-consuming procedure and, in addition, not always sufficiently efficient.

It is well known (*e.g.* ref. 8) that TEA is useful for masking silanol groups through strong electrostatic interaction. TEA as an inhibitor of the adsorption of the two dinitro metabolites of GTN was therefore investigated. As the loss of metabolites was found to take place during evaporation of the pentane–dichloromethane extract or later in the sample procedure, TEA was added to the apolar *n*-hexane used for redissolving the residue. This solved the problem of loss of the dinitro metabolites (Fig. 2b).

Before the validation of the new method was performed, the stability of GTN, 1,2-GDN and 1,3-GDN dissolved in *n*-hexane–TEA (98:2, v/v) was investigated. Fig. 3 shows that while 1,2-GDN and 1,3-GDN were stable in this solution GTN was hydrolysed, although only very little water was present. The hydrolysis takes place according to a pseudo first-order reaction with an apparent half-life of approximately six days. This means that, if samples are analysed within 4 h after dissolving the residue in *n*-hexane containing 2% TEA, less than 2% GTN is lost. This was found to be acceptable for routine analysis.

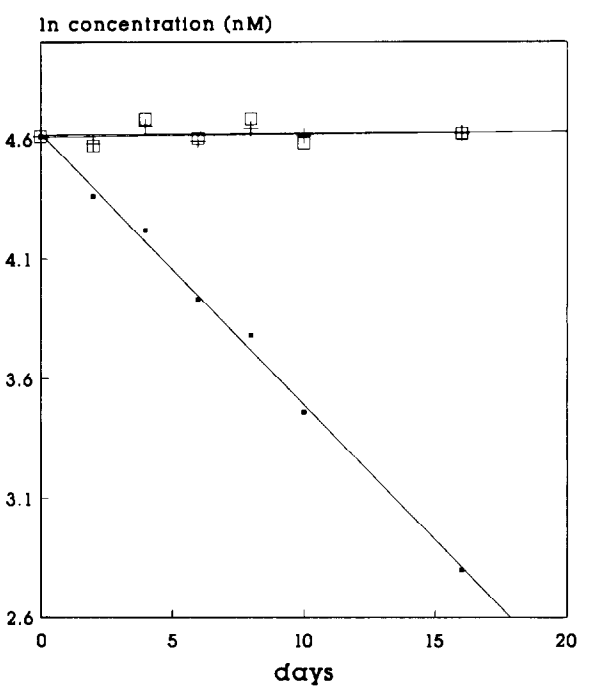

Fig. 3. Pseudo first-order plot for hydrolysis of GTN in *n*-hexane with 2% TEA added. The half-life of GTN is approximately six days. No change in the concentrations of 1,2-GDN and 1,3-GDN are observed in the same period of time. Symbols: ■ = GTN; + = 1,2-GDN; and □ = 1,3-GDN.

TABLE I

ACCURACY, REPEATABILITY AND BETWEEN-DAY VARIATION FOR GTN, 1,2-GDN AND 1,3-GDN IN PLASMA

C.V. = coefficient of variation (n = 4).

Compound	Concentration added (nM)	Day 1		Days 1–4	
		Found (nM)	C.V. (%)	Found (nM)	C.V. (%)
GTN	4.0	4.0	4.3	4.0	1.2
	20.0	19.7	5.6	19.7	1.3
1,2-GDN	4.0	4.2	9.2	4.0	4.6
	20.0	20.3	6.2	19.9	1.4
1,3-GDN	4.0	4.1	4.5	4.1	3.2
	20.0	19.9	4.4	20.1	1.9

Validation

The recoveries of GTN, 1,2-GDN and 1,3-GDN from human plasma using pentane–dichloromethane (1:1, v/v) as the extraction solvent were 76, 58 and 55%, respectively. The minimum detectable quantity, defined as the quantity that gives a signal twice the noise (signal-to-noise ratio = 2), was found to be 1 pg, corresponding to a concentration of 0.2 nM (the detection limit) in human plasma.

The linearity was tested in the range 0.4–20.0 nM for GTN, 1,2-GDN and 1,3-GDN. All standard curves were linear in the range investigated (r >0.998) and passed through the origin. The accuracy, repeatability and between-day variation of the method were investigated at the concentration levels 4.0 and 20.0 nM for all three solutes. The results summarized in Table I show that a method of obtaining reliable results has been developed.

CONCLUSION

A method for the simultaneous determination of nitroglycerin (GTN) and it two dinitro metabolites (1,2-GDN and 1,3-GDN) in human plasma using capillary gas chromatography with electron-capture detection is presented. The method differs from previously published methods in not using silanized glass equipment. Instead of deactivating the active silanol groups on the glass surfaces by chemical derivatization, the silanol groups are masked with TEA as a component of the sample solvent.

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