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Quantitative determination of nitroglycerin by capillary gas chromatography–electron capture detection*

J. J. JANSSENS, M. I. SELALA, F. F. DAELEMANS, S. W. ANDRIES and P. J. C. SCHEPENS†

Laboratory of Toxicology, Department of Pharmaceutical Sciences, University of Antwerp (UIA), B2610 — Antwerp-Wilrijk, Belgium

Abstract: A rapid and sensitive capillary gas chromatographic method based on the one described by Noonan *et al.* [1] was used to evaluate the nitroglycerin content in serum samples of healthy volunteers, who had orally received a special preparation of the drug (Nisconitrine 6.5®, Bio-Therabel). Concentrations were monitored up to 12 h after administration. In accordance with other literature data [2], no detectable amounts of the mother compound were found (limit of detection: 50 pg ml^{-1}). Yet, significant amounts of the active metabolites, 1,2- and 1,3-dinitroglycerin could be demonstrated. Due to the low mass spectrometric response (electron impact ionization) of the different nitroglycerins, positive confirmation of the results with GC–MS was not possible. However, the concentrations reported here do agree with literature data [2], i.e. the ng ml^{-1} level.

Keywords: *Nitroglycerin; nitroglycerin metabolites; gas chromatography with electron capture detection.*

Introduction

Trinitroglycerin (TNG) is a potent vasodilator used in the treatment of angina pectoris, congestive heart failure, and acute myocardial infarction. Even though nitroglycerin was first synthesized over a century ago, very little is known regarding the pharmacokinetics of this drug, primarily due to the lack of a sensitive and specific assay for the drug. Several analytical methods (e.g. HPLC) have been developed, but none of them were capable of detecting nitroglycerin concentrations in the subnanogram range. This report describes the capillary gas chromatography (GC) determination using an electron capture detector. This method, based on the one of Noonan *et al.* [1], possesses the precision, sensitivity and selectivity required to analyse subnanogram concentrations of nitroglycerin in human plasma.

The method is used to study the bioavailability of Nisconitrine 6.5® (Bio-Therabel), consisting of capsules containing 6.5 mg nitroglycerin each. Noonan *et al* [2] already reported that after an oral intake of nitroglycerin, no traces of the mother compound are

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†To whom correspondence should be addressed.

found in plasma. On the other hand, the mono- and dinitro-derivatives were detected. Since the monoderivative is pharmacologically inactive, only the mother compound TNG and the dinitroglycerin (DNG) derivatives were studied.

Experimental

Volunteers

Thirteen healthy volunteers were chosen by Prof. Dr L. Vanhaelst, pharmacologist at the Academic hospital of the VUB (Laarbeeklaan 103, 1090 Brussels, Belgium). At 8 o'clock in the morning, every volunteer took one capsule of Nisconitrine 6.5®, which contained 6.5 mg TNG.

Blood sampling

Blood samples (10 ml each) of all volunteers were collected prior to intake and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after the intake. The blood was collected in chilled heparinized evacuated tubes (Vacutainers; Becton Dickenson) and immediately centrifuged for 3 min at 2°C and 3000*g*. The plasma was then immediately frozen in a dry ice bath.

Chemicals and reagents

All standards, TNG, 10.063 mg ml^{-1} ethanol (Dynamit Nobel); DNG, 10.190 mg ml^{-1} ethanol, 82% 1,3-DNG and 18% 1,2-DNG (Dynamit Nobel); 2,4-dinitrotoluene, 98% pure (Merck) which was used as internal standard (i.s.), were commercially obtained and their identity was confirmed mass spectrometrically. All solvents used were of analytical grade (PA) quality. All glassware containing the standards and samples was wrapped in aluminium foil to prevent light degradation.

Glassware silanization procedure

Special precautions must be taken in order to avoid decomposition or adsorption of the highly labile nitroglycerins. Therefore, all glassware was silanized before usage. It was cleaned with alcoholic KOH, chromic acid, then soaked in a 5% (v/v) dimethyldichlorosilane solution in toluene, rinsed with toluene and methanol and finally, dried.

Instruments

The gas chromatograph (Hewlett–Packard 5890) was equipped with a ^{63}Ni-electron capture detector (ECD) and with a splitter equipped with a silanized direct injection insert. The split flow rate was 19 ml min^{-1}. Helium was used as carrier gas at a flow rate of 1.3 ml min^{-1} and argon–methane (95:5) was used as reagent and make-up gas at a flow of 60 ml min^{-1}. The column was a 25 m × 0.32 mm (film thickness 0.52 μm) HP5 fused silica capillary column coated with a 5% diphenyl 95% dimethylpolysiloxane stationary phase. The injector- and detector temperatures were maintained at 150 and 300°C, respectively. The oven temperature was kept at 125°C during the first 30 s and then programmed to 225°C at 8°C min^{-1}. Chromatograms were recorded and integrated with a Trivector Computer integrator.

Sample preparation

Sample pretreatment must be minimal in order to reduce adsorption losses. To 1 ml of plasma, a solution of 20 ng 2,4-dinitrotoluene (20 ng ml^{-1} methanol, Merck, chromato-

graphic purity) was added as internal standard. The sample was vortexed twice with 2 ml *n*-hexane (Merck) and twice with t-butylmethylether (Janssen Chimica, glass distilled). The organic layers were combined, centrifuged for 10 min and deep frozen in order to eliminate any traces of water. The organic phase was then transferred to a clean silanized point-bottom test tube in which it was brought to dryness under reduced pressure at room temperature. The extracts were redissolved in 25 μl absolute ethanol (Merck). The extraction efficiency for the spiked plasma was 90 ± 5% for DNG and 98 ± 4% for TNG ($n = 4$). When only *n*-hexane was used as extraction solvent, the extraction efficiency for DNG dropped to 60%. This finding is in agreement with Yap and Fung [3]. The concentrations of the nitroglycerins in the plasma samples were derived from a calibration curve that was constructed using spiked plasma treated in the same way as the plasma samples. In Fig. 1(a), a typical gas chromatogram of a positive serum extract is shown (patient 9 and 3 h after intake); Fig. 1(b) shows a negative serum extract (patient 9, 0 h).

Results and Discussion

Method evaluation

Reproducibility. The response factors for the standards (mentioned above) were subjected to a day-to-day comparison. The standard deviation, SD (σ_{n-1}) of the average response factor for both DNG and TNG was approximately 5%.

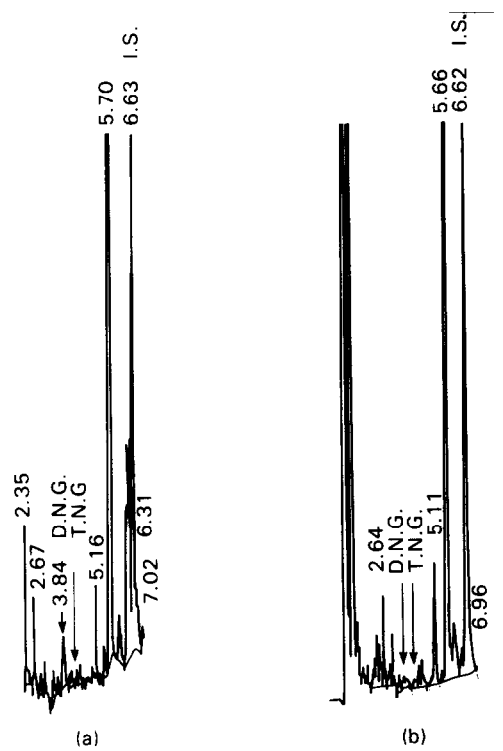

Figure 1
(a) A typical gas chromatogram of a positive serum extract (patient 9, 3 h after intake). (b) A gas chromatogram of a negative serum extract (patient 9, 0 h).

Linearity. Six different solutions, with a concentration ratio DNG or TNG:i.s. varying from 0.02 to 25 (4 decades), were analysed. The linear correlation coefficient for both DNG and TNG was 0.999. Extremely low concentrations can be calculated using average response factors.

Detection limit. The practical detection limit is dependent on the injected amount of sample and ranges between 0.02–0.08 ng ml^{-1} for DNG and between 0.02–0.05 ng ml^{-1} for TNG.

Conclusions

As expected, all nitroglycerin plasma concentrations in each of the subjects were below the minimum measurable concentration (0.02 ng ml^{-1}). An accurate dosage of DNG was possible and for reasons of analytical precision, we preferred to quantitate the sum of 1,2- and 1,3-DNG. Table 1 shows the DNG (both isomers) concentrations for each volunteer, the mean concentration and the SD as a function of time. Ten hours after the intake, the concentration of the active metabolites no longer reached the detection limit. The presence of these active metabolites of the active ingredient of Nisconitrine 6.5® indicated the resorption of the latter. The 13 volunteers tolerated the product well, only a few experienced a mild headache as a side effect.

It can be concluded that Nisconitrine 6.5® has a progressive and regularly prolonged release during at least 8 h.

Table 1
DNG concentrations (both isomers) as a function of time (concentrations in ng ml^{-1})

Patient	0′	30′	60′	90′	2 h	3 h	4 h	5 h	6 h	8 h	10 h	12 h
1	<dl	<dl	0.2	1.6	2.3	1.5	1.9	0.9	0.1	<dl	<dl	<dl
2	<dl	1.3	1.7	2.1	1.9	2.8	1.9	0.1	0.05	0.1	<dl	<dl
3	<dl	0.2	0.4	0.3	0.6	1.2	0.4	0.2	0.2	0.1	<dl	<dl
4	<dl	1.0	0.6	3.7	1.2	1.7	0.3	0.05	0.05	<dl	<dl	<dl
5	<dl	0.7	0.8	3.1	1.7	3.5	5.4	1.3	1.0	0.1	<dl	<dl
6	<dl	0.6	0.2	2.3	2.9	3.1	3.1	1.0	0.2	0.1	<dl	<dl
7	<dl	0.3	1.0	0.8	1.0	1.1	2.8	2.0	1.1	0.4	<dl	<dl
8	<dl	0.2	1.4	1.6	2.0	1.7	1.8	0.9	0.8	0.07	<dl	<dl
9	<dl	1.1	0.5	0.6	1.2	3.3	3.7	1.5	1.5	0.3	<dl	<dl
10	<dl	0.4	1.4	4.3	2.5	2.1	1.9	0.9	0.2	0.2	<dl	<dl
11	<dl	1.0	1.9	2.5	1.1	0.8	1.2	0.5	0.3	<dl	<dl	<dl
12	<dl	0.5	0.8	1.6	1.6	0.6	0.7	0.3	0.3	0.1	<dl	<dl
13	<dl	0.4	2.5	1.9	2.7	3.3	2.4	0.2	0.3	<dl	<dl	<dl
Mean	<dl	0.59	1.03	2.03	1.75	2.05	2.12	0.82	0.50	0.12	<dl	<dl
σ_{n-1}		0.40	0.71	1.17	0.71	1.03	1.42	0.57	0.45	0.11		

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