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Short communication

Simultaneous determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by capillary gas chromatography with electron-capture detection

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Abstract

A method for the simultaneous determination of isosorbide dinitrate (ISDN) and its mononitrate metabolites (2- and 5-ISMN) in human plasma by capillary gas chromatography with electron-capture detection was developed. Two internal standards were used: isomannide dinitrate (IMDN) for the determination of ISDN and isomannide mononitrate (IMMN) for the determinations of 2- and 5-ISMN. After addition of the internal standards, the compounds were isolated from plasma by solid–liquid extraction. They were determined by gas chromatography using an electron-capture detector. The reproducibility and accuracy of the method were found suitable in the range of concentrations 2.5–83 ng/ml for ISDN, 2.6–208 ng/ml for 2-ISMN and 2.3–1010 ng/ml for 5-ISMN. The limit of quantitation (LOQ) was about 2.5 ng/ml for each compound. The method was applied to clinical samples.

Keywords: Isosorbide dinitrate; Isosorbide mononitrate

1. Introduction

ISDN is an organic nitrate vasodilator. A glutathione-dependent enzyme system, glutathione S-transferase, catalyses the denitration of ISDN during its passage through the liver to give two isosorbide mononitrates: 2- and 5-ISMN.

Many methods have been already published for the quantitative determination of ISDN or 2- and 5-ISMN in biological fluids. Some of them [1–10] allowed the simultaneous determination of ISDN and its mononitrate metabolites. The most commonly used methods have been based on gas chromatography (GC) with electron-capture detection (ECD), using first packed columns [1–4] and later capillary columns [5–10] without mononitrate derivatisation.

Several procedures used a laborious liquid–liquid extraction [1,5,8] or required a large sample volume [1,2,4,5]. Some of them lacked sensitivity [1,2] or were poorly reproducible in the lower concentration range [4].

In three methods, the laborious liquid–liquid extraction was replaced by a liquid–solid extraction using Extrelut columns [6,9] or ENVI 18 cartridges [10]. The method described by Santoni et al. [6] used two different solvents for elution of ISDN and mononitrates, two chromatographies each with different conditions, and the limits of quantitation were 0.5, 2 and 10 ng/ml for ISDN, 2- and 5-ISMN, respectively. In the method reported by Edlund et al.

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[9], where the extracts were purified by gel permeation chromatography (GPC) prior to GC determination, reproducibility and accuracy were obtained for 4.5 ng/ml of each compound. The LOQ values obtained by Gremeau et al. [10] were 10 ng/ml for ISDN and 2-ISMN, and 20 ng/ml for 5-ISMN.

The present paper describes a rapid and sensitive method for the simultaneous determination of ISDN and its mononitrate metabolites in human plasma, by capillary gas chromatography using an electron-capture detection.

2. Experimental

2.1. Chemicals and reagents

The chemical structures of ISDN, mononitrate metabolites and internal standards are shown in Fig. 1. 5-ISMN was supplied by Ciba (Basle, Switzerland), 2-ISMN and ISDN were provided by Nobel Chemicals (Karlskoga, Sweden). The internal standards were provided by Nipa Laboratories (Sandycroft, UK). IMDN was used for the determination of ISDN and IMMN for the determinations of 2-and 5-ISMN.

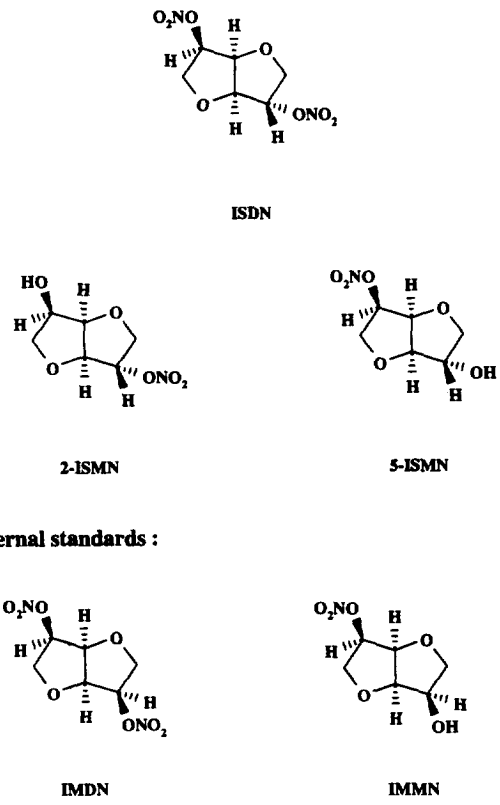

Fig. 1. Chemical structures of ISDN, mononitrate metabolites and internal standards.

The stock solutions were prepared by dissolving the compounds in methanol. The working solutions were obtained by dilution of the corresponding stock solutions with water.

The concentrations of the internal standard solutions were 84 ng/50 μl for IMMN and 56.6 ng/50 μl for IMDN.

All the chemicals were of analytical grade: dichloromethane and ethyl acetate (Pestipur SDS) were obtained from Solvants Documentation Synthèse (Pépin, France).

Pre-packed columns for extraction were Extrelut 1 obtained from Merck (Darmstadt, Germany).

2.2. Equipment

All the glassware was pretreated to prevent adsorption. It was immersed in toluene containing hexamethyldisilazane, trimethylchlorosilane and pyridine [1% (v/v) each] for 15 min and rinsed with methanol. This treatment was repeated every month. Between these treatments, the glassware was cleaned as usual and rinsed with methanol.

A Hewlett-Packard 5890 gas chromatograph equipped with a capillary inlet system and an HP 7673 automatic sampler was used (Hewlett-Packard, Palo Alto, CA, USA). The column was a 12 m×0.2 mm fused-silica capillary column coated with cross-linked methyl silicone with a film thickness of 0.33 μm (HP 19091A-101). The carrier gas was helium with an inlet pressure of 100 kPa (15 p.s.i.) with a split flow of 45 ml/min and a septum purge of 3.0 ml/min. Splitless injection was used with a deactivated injection liner in quartz (internal diameter 2 mm) which was replaced daily after 40–50 injections. The injector temperature was 150°C with a 0.5-min splitless-period and the electron-capture detector was set at 220°C with argon–methane (90:10) at a flow-rate of 66 ml/min. The initial column temperature was 100°C for 0.5 min and the temperature was raised at a rate of 12°C/min up to 180°C and then increased at a rate of 30°C/min up to 250°C.

A HP 3365 Series II ChemStation was used to

control GC and injector instruments and for data acquisition and processing.

2.3. Calibration, validation and clinical samples

For calibration and validation, aliquots of working solutions were added to 1 ml of drug-free human plasma to produce reference samples in the concentrations ranges of 2.5–83 ng/ml for ISDN, 2.6–208 ng/ml for 2-ISMN and 2.3–1010 ng/ml for 5-ISMN. A constant amount of internal standards (84 ng IMMN and 56.6 ng IMDN) was added to each reference sample.

For clinical samples, a constant amount of internal standards (84 ng IMMN and 56.6 ng IMDN) was added to 1 ml of plasma (obtained from blood collected on solid heparin–lithium and centrifuged).

2.4. Extraction from plasma

The mixture was transferred to the top of an Extrelut 1 column and allowed to soak for 10 min. The column was eluted twice with 3 ml of dichloromethane. The eluate was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μl of ethyl acetate, transferred into a 100-μl glass insert contained in a vial and 1 μl was injected into the gas chromatograph.

3. Results and discussion

3.1. Plasma interferences

Typical chromatograms obtained from extracts of drug-free plasma and plasma spiked with ISDN, 2- and 5-ISMN metabolites are shown in Fig. 2. ISDN, 2- and 5-ISMN metabolites and internal standards are well separated from the components of the plasma extract.

3.2. Calibration curves

Calibration curves were obtained by plotting the peak-height ratio of either compound (ISDN, 2- and 5-ISMN metabolites) and of the respective internal standard versus the concentration of either compound. Their equations were calculated by using weighted linear least-squares regression with a weighting factor of $1/(\text{conc.})^2$. The linear calibration ranges were 2.5–83 ng/ml for ISDN, 2.6–208 ng/ml for 2-ISMN and 2.3–1010 ng/ml for 5-ISMN. Calibration curves were established on each day of analysis.

3.3. Accuracy and precision

The accuracy and precision were studied from replicate sets of analyte samples of known concentrations at levels corresponding to the lowest, near the lowest, near the middle and the highest concentration values of the calibration curve range. Accuracy was determined by calculating the mean recovery for the found concentrations as a percent of the nominal concentrations in standard samples. Precision was assessed from the coefficient of variation (C.V.) in % of the mean recoveries. The following validation criteria for accuracy and precision were used to assess the method suitability: mean recoveries should be within 85–115%, except at the LOQ where it should be within 80–120%; C.V. should not exceed 15%, except at the LOQ where it should not exceed 20% [11].

3.3.1. Intra-day measurements

Samples (6 replicates of 6 different concentrations) were analysed on the same day. Mean concentrations, mean recoveries and corresponding C.V. are presented in Table 1.

3.3.2. Inter-day measurements

Samples (5 different concentrations) were analysed on 5 different days over a period of one week using a daily calibration curve. Mean concentrations, mean relative recoveries and corresponding C.V. are presented in Table 2.

3.4. Limits of quantitation

The LOQ is defined as the lowest concentration on the calibration curve that can be measured with acceptable accuracy, precision and variability. As indicated previously, the mean recovery should be within 80–120% of the expected value with a C.V. not exceeding 20%. The lowest concentration value

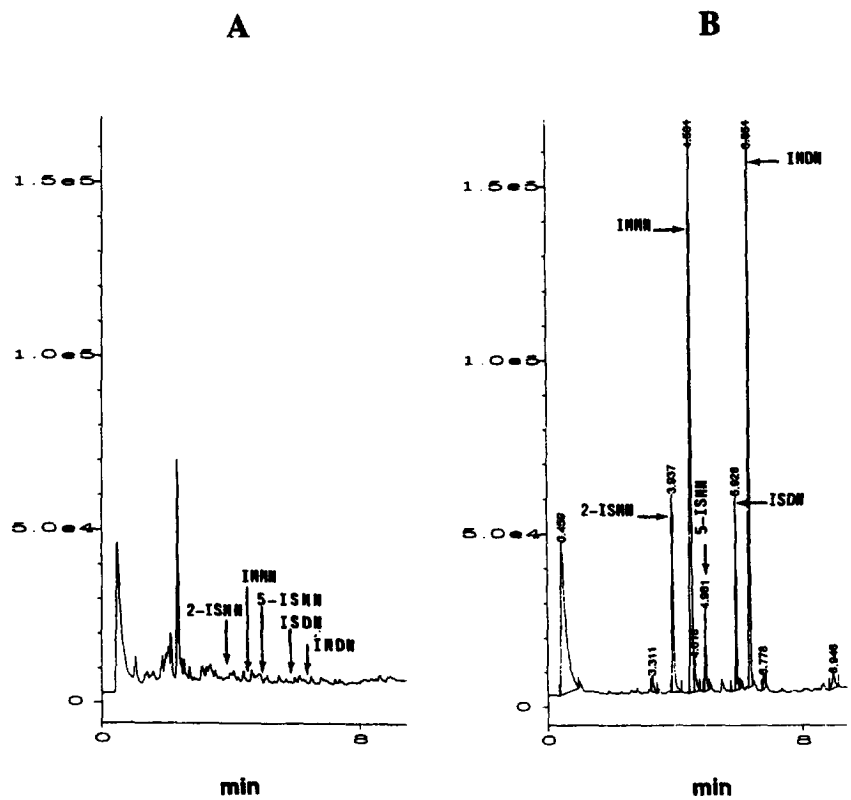

Fig. 2. Examples of chromatograms: (A) extract of 1 ml drug-free plasma; (B) plasma samples spiked with 14.6 ng of ISDN, 18.5 ng of 2-ISMN, 12.2 ng 5-ISMN, 56.6 ng IMDN and 84 ng IMMN.

of 2.5 ng/ml for ISDN, 2- and 5-ISMN whose accuracy and precision (Table 1 and Table 2) were within the proposed criteria is quoted as the LOQ.

3.5. Specificity

Previous studies in our laboratories [12] showed that the major urinary metabolite of ISDN is isosorbide. The determination of isosorbide by chromatography with electron-capture detection needs a derivatization procedure and does not interfere with the chromatography of ISDN, 2- and 5-ISMN. This paper describes a method in plasma applied to bioavailability studies not including urinary determination of isosorbide.

3.6. Storage stability

The storage stability results were already reported in previous studies [13,14]. No decrease in the 2- and 5-ISMN content was observed in plasma when stored frozen for 1 year at −20°C [13]. A decrease of around 15% in the ISDN content was observed in plasma samples when stored frozen for 3 months at −20°C; this decrease reaches 30% after 9 months [14].

3.7. Comparison with previous methods

Liquid–solid extraction on a Extrelut column with elution by dichloromethane was a suitable and rapid method for the isolation of ISDN and its mononitrate metabolites from plasma.

The sensitivity of the method described here, allowing the simultaneous determination of ISDN and its mononitrate metabolites, was comparable to that obtained with previous methods. In comparison to methods for ISDN and metabolites using a comparable solid–liquid extraction procedure [6,9], the preparation of extracts is faster and the duration of chromatography is reduced (only one run of 9 min instead of 2 runs of 10 min [6] or 20 min [9] each).

Table 1
Intra-day accuracy and precision for ISDN, 2- and 5-ISMN in spiked human plasma samples

Compound	Given (ng/ml)	Mean found (n=6, ng/ml)	Intra-day accuracy (mean recovery[a], %)	Precision (C.V.[b], %)
ISDN	2.50	2.57	103	6.2
	6.25	5.89	94.3	9.2
	18.8	19.2	102	3.7
	31.3	30.4	97.0	1.3
	62.5	63.1	101	2.1
	83.3	97.1	117	1.7
2-ISMN	2.56	2.67	104	7.6
	6.40	6.38	99.7	10.0
	19.2	20.6	107	6.5
	32.0	32.7	103	2.7
	64.0	66.8	104	2.7
	208	205	98.5	1.6
5-ISMN	2.34	2.60	111	7.8
	5.85	5.55	95.0	2.9
	17.6	19.4	111	6.4
	29.3	30.6	104	2.8
	58.5	59.7	102	3.0
	1010	1010	100	2.0

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]C.V.: Coefficient of variation on recovery.

3.8. Application

The present method was used to determine the plasma concentrations of ISDN and its two mononitrate metabolites after oral administration of 120 mg ISDN to 12 healthy male subjects. Fig. 3 shows mean plasma concentration–time profiles of ISDN, 2-ISMN and 5-ISMN.

Table 2
Inter-day accuracy and precision for ISDN, 2- and 5-ISMN in spiked human plasma samples

Compound	Given (ng/ml)	Mean found (n=5, ng/ml)	Inter-day accuracy (mean recovery[a], %)	Precision (C.V.[b], %)
ISDN	2.60	2.66	102	5.7
	8.32	7.63	91.7	8.4
	18.7	19.4	104	8.3
	33.3	37.1	111	6.9
	83.3	94.6	113	6.5
2-ISMN	2.60	2.83	109	4.8
	8.32	7.79	93.6	7.8
	18.7	18.4	98.4	8.2
	83.2	85.7	103	3.2
	208	212	102	4.6
5-ISMN	2.51	2.52	100	11.2
	40.4	38.6	95.5	6.0
	90.9	92.2	101	3.6
	404	446	110	5.6
	1010	1041	103	6.7

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]C.V.: Coefficient of variation on recovery.

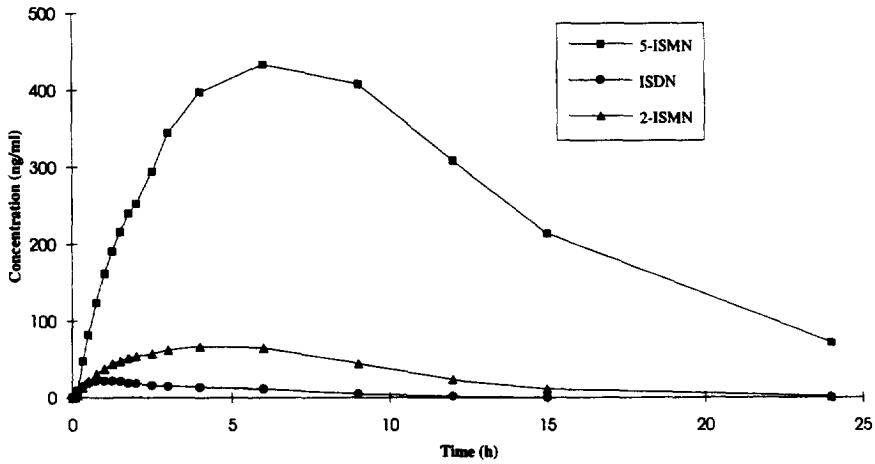

Fig. 3. ISDN, 2-ISMN and 5-ISMN mean plasma concentration–time profiles obtained from 12 healthy male volunteers after oral administration of 120 mg ISDN.

4. Conclusions

The proposed GC technique allows the simultaneous determination of ISDN and its 2- and 5-ISMN metabolites in plasma, with suitable reproducibility and accuracy as demonstrated for plasma obtained from healthy volunteers.

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