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Short communication

Determination of isosorbide-5-mononitrate in human plasma by high-resolution gas chromatography

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Abstract

A method for the determination of isosorbide-5-mononitrate (5-ISMN) in human plasma by capillary gas chromatography with electron-capture detection was developed and applied to clinical samples. 9-Fluorenone was used as an internal standard, ethyl acetate was employed for liquid–liquid extraction. The advantage of the extraction procedure is the possibility of a direct injection of the plasma extract, without solvent removal/reconstitution of the sample. The precision and accuracy of the method were satisfactory in the concentration range 10–1600 ng/ml. The lower limit of quantification was 10 ng/ml.

Keywords: Isosorbide mononitrate

1. Introduction

Isosorbide-5-mononitrate (5-ISMN) is an organic nitrate vasodilator used in the treatment of angina pectoris [1]. This substance can also be formed from isosorbide dinitrate [2]. A number of methods have been described for the determination of 5-ISMN in biological fluids. Gas–liquid chromatography with electron-capture detection (GC-ECD) has most often been employed. The GC-ECD technique offers high selectivity and sensitivity, advantageous in pharmacokinetic studies [3–25], and is more easily accessible than GC-MS methods [26–28].

9-Fluorenone was chosen as an internal standard [5]. For the liquid-liquid extraction step, ethyl acetate was employed [4]. Solvent evaporation was omitted due to the volatility of the nitrates [22], and the ethyl acetate extract was directly injected. The gas chromatographic method according to Pommier et al. [21] was used, with a modification using a capillary column coated with polydimethylsiloxane (SPB-1).

2. Experimental

2.1. Chemicals and reagents

The chemical structures of 5-ISMN and the internal standard (9-FN) are shown in Fig. 1. All chemicals were analytical grade or better. 5-ISMN was supplied by PRO.MED.CS Praha a.s. (Prague, Czech Rep.). 9-Fluorenone (internal standard) was obtained from Sigma–Aldrich St. Louis, MO, (USA). Methanol SupraSolv®, Ethyl acetate SupraSolv® and anhydrous Sodium sulfate Suprapur® were from Merck Darmstadt (Germany).

2.2. Equipment

An Agilent Technologies 6890 Series GC System gas chromatograph with a ^{63}Ni-ECD detector, equipped with an Agilent Technologies 7683 Series Injector and 7683 Series Autosampler was used (Agilent Technologies, Palo Alto, CA) together with a 30 m × 0.32 mm fused silica column coated with polydimethylsiloxane (SPB-1) with a film thickness of 1 μm (Supelco, Belle tante, PA). Helium at the flow rate of 3 ml/min was used as the carrier gas. The inlet temperature was set at 150 °C. Splitless injection (1 μl) was used with a deactivated injection liner, which was replaced after every

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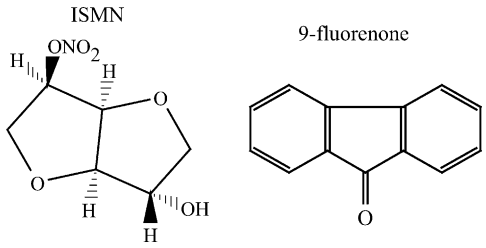

Fig. 1. Structures of isosorbide-5-mononitrate (5-ISMN) and 9-fluorenone.

60 injections. The electron-capture detector was set at 250 °C with a nitrogen makeup flow of 60 ml/min. The initial column temperature was 110 °C. After 0.1 min at 110 °C, the temperature was gradually raised to 140 °C at the rate of 15 °C/min, and this temperature was maintained for 13.5 min. Following this period, the temperature was elevated to 220 °C, and this temperature maintained for 5 min. A Hewlett-Packard ChemStation (Agilent Technologies, CA, USA) was used to control the GC apparatus and to acquire and process the data.

2.3. Extraction procedure

9-Fluorenone (200 ng) was added as the internal standard to 0.5 ml or 0.2 ml of the plasma, and the solution was shaken for 10 s. Ethyl acetate (5.0 ml) was added to the sample, and the tube was shaken for 6 min and centrifuged. One milliliter of the organic layer was transferred into another tube, containing 0.2 g of anhydrous sodium sulfate. The ethyl acetate extract was allowed to stand over the drying agent for 1 h, and the supernatant solution was then decanted. One microlitre of the solution was injected into the gas chromatograph.

2.4. Preparation of standards, calibration and clinical samples

The stock solutions were prepared by dissolving the compounds in methanol. The calibration solutions were obtained by the dilution of the stock solutions with water. The concentration of the internal standard (9-fluorenone) was 50 μg/ml.

For calibration and validation, aliquots of 5-ISMN solutions were added to 0.5 and 0.2 ml of a drug-free human plasma to obtain reference samples in the concentration range 10–800 ng/ml for 0.5 ml of the plasma volume, and 600–1600 ng/ml for 0.2 ml of the plasma volume. The same amount of the internal standard (200 ng of 9-fluorenone) was added both to 0.5 and 0.2 ml of the plasma. The recovery of 5-ISMN was determined upon the comparison of the peak area measured after the extraction of the spiked plasma samples with that measured on external standards, prepared in ethyl acetate. Intra- and inter-day precision was determined with the spiked plasma standards. The statistical evaluation of the inter-day assay method performance includes the data from an intra-day validation and two inter-day validations, determined over three consecutive days.

The blood samples were withdrawn from a forearm vein, collected into lithium-heparin tubes, and centrifuged.

3. Results and discussion

Typical chromatograms of a blank plasma sample and a spiked plasma sample are shown in Fig. 2. The analytes (5-ISMN and 9-fluorenone) are well separated from the other components of the plasma extract. Using the described procedure, the extraction recovery of 5-ISMN was 87.1 ± 8%. A stable detector response was achieved by 10 consecutive injections of the standard prior to sample analysis, similar

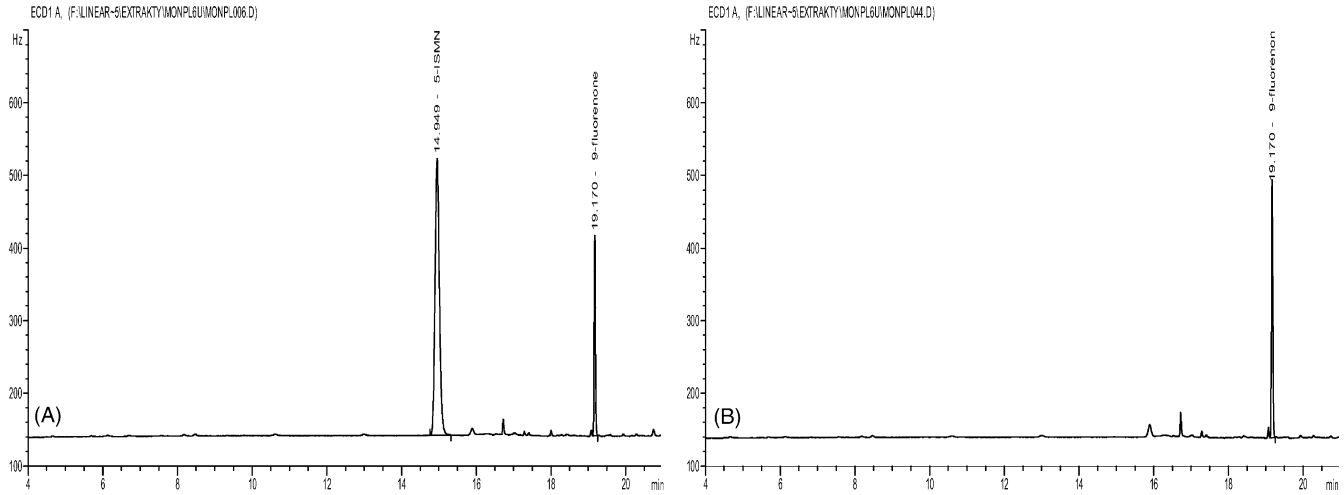

Fig. 2. (A) Chromatogram of 0.5 ml of human blank plasma extract spiked with 800 ng/ml of 5-ISMN (R_t = 14.9 min) and 200 ng of 9-fluorenone (R_t = 19.17 min). (B) Chromatogram of 0.5 ml of human blank plasma extract spiked with 200 ng of 9-fluorenone (internal standard).

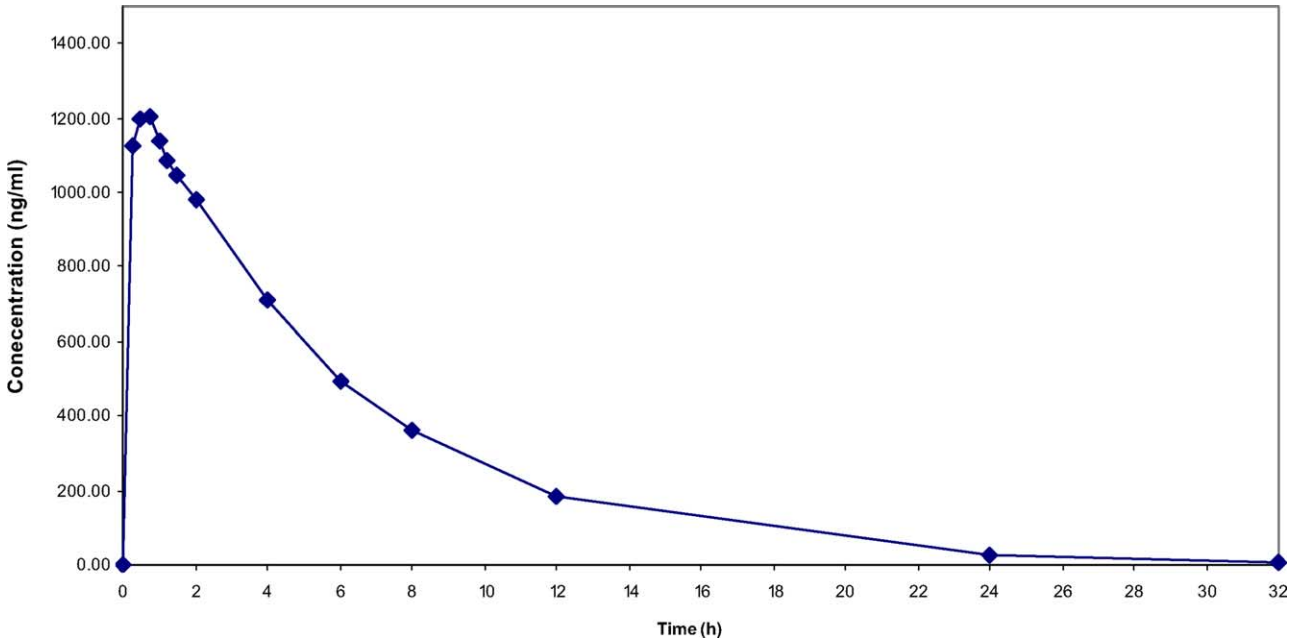

Fig. 3. Representative 5-ISMN plasma concentration–time profile obtained from one healthy female volunteer after an oral administration of 40 mg of 5-ISMN (Monosan 40 mg tbl., PRO.MED.CS Praha a.s., Prague, Czech Rep.).

Table 1
Intra-day quality control results of ISMN

Nominal concentration (ng/ml)	Sample amount (μl)	Mean concentration found (ng/ml)	R.S.D. (%)	Nominal (%)
10	500	11.91	13.4	119
50	500	45.45	5.19	91
100	500	94.03	9.38	94
250	500	226.24	9.62	91
500	500	517.12	6.21	103
800	500	821.17	6.66	103
800	200	779.04	3.4	97
1000	200	1013.41	5.1	101
1200	200	1238.90	4.0	103
1600	200	1584.29	1.7	99

to the procedure by Pennings and de Haas [22]. The reproducibility of the detector response was also found to be dependent on the purity of the injector glass liner. Thus, it was necessary to replace the liner after some 50–60 injections. The linearity of the detector response for the 0.5 ml plasma samples was confirmed only in the concentration range 10–800 ng/ml. Consequently, we decreased the extraction volume of the plasma (0.2 ml) in case of the clinical samples, where we expected plasma concentration levels higher then 800 ng/ml (interval 0.5–2 h after an oral administration of the drug, see Fig. 3).

The statistical evaluation of the intra- and inter-day assay method performance is shown in Tables 1 and 2. The method was found to be precise with R.S.D. values lower than 10%, and hence suitable for application to clinical samples. The lower limit of quantification was 10 ng/ml (R.S.D. < 15%). The lower limit of detection, defined as the peak equal to three times the signal-to-noise ratio of the detector in height, was 2.8 ng/ml 5-ISMN, using 0.5 ml plasma sample.

Table 2
Inter-day quality control results of ISMN

	Nominal concentration (ng/ml)		
	50	250	500
Mean	48.04	239.56	510.76
R.S.D. (%)	8.10	5.90	5.33
Nominal (%)	96.09	95.82	102.15
n	18	18	18

4. Conclusion

A simple method for the determination of 5-ISMN in the human plasma was developed. The advantage of the extraction procedure is the direct injection of the plasma extract, avoiding the steps of solvent evaporation and reconstitution of the samples. The method was used for a comparative clinical study of the bioavailability of two 5-ISMN drug formulations.

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