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Note

Determination of isosorbide-5-mononitrate in human serum by gas chromatography–mass spectrometry

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Isosorbide-5-mononitrate (5ISMN) is the major active metabolite of isosorbide dinitrate (ISDN), widely used in the treatment of angina pectoris. 5ISMN shows some advantages over the parent drug: its almost complete oral absorption, the lack of a first-pass effect in the liver, a longer half-life and a more regular kinetics.

Current analytical methodologies for the determination of 5ISMN in blood of serum suffer from various drawbacks. In the case of high-performance liquid chromatography (HPLC), poor sensitivity is observed if a UV detector is used [1,2], and thermal energy analysis detection appears to be extremely expensive and of low applicability [3,4]. Gas chromatography (GC) with electron-capture detection (ECD) is a sensitive technique [5] but the reliability of the analysis is not satisfactory [6–8]. In our experience 5ISMN is too volatile and unstable at high temperature to be assayed by GC.

We decided to derivatize 5ISMN as its bis-silyl ether to create a thermally stable but sufficiently volatile compound. The most suitable silylating mixture

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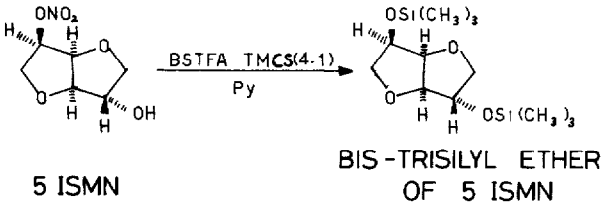

Fig. 1. Derivatization of 5ISMN as bis-TMS ether using BSTFA–TMCS (4.1) in pyridine.

proved to be bis(trimethylsilyl)trifluoroacetamide (BSTFA)–trimethylchlorosilane (TMCS), 4:1 in pyridine. The presence of pyridine and the heating at 80°C for 30 min lead to formation of a trimethylsilyl ether not only at C-2, where a free hydroxyl is present, but also at C-5, where the nitro-group is substituted (Fig. 1).

This bis-silyl derivative (5ISMN bis-TMS) is not detectable by ECD so we have developed a specific and sensitive gas chromatographic–mass spectrometric (GC–MS) assay, using hexa-trimethylsilyl sorbitol (hexa-TMS sorbitol) as internal standard (I.S.).

EXPERIMENTAL

Materials

5ISMN was obtained from Chiesi Farmaceutici (Parma, Italy) and sorbitol from Carlo Erba (Milan, Italy). BSTFA and TMCS were purchased from Fluka (Buchs, Switzerland) and pyridine from Supelchem (Milan, Italy). Methanol, ethyl acetate and dichloromethane were analytical grade.

Gas chromatography–mass spectrometry

GC–MS analyses were performed using an LKB 2091 gas chromatograph–mass spectrometer (Remont, Bromma, Sweden) with an on-line data acquisition system Digital PDP 11 (Digital Equipment, Milan, Italy). Mass spectra were recorded at an ion temperature of 250°C, with an electron energy of 70 eV and a trap current of 25 μA. In the selected-ion monitoring (SIM) analysis an electron energy of 20 eV was used. A capillary column SPB1, 30 m (Supelchem), was used for the separation. The column temperature was initially 100°C for 2 min and was then raised at 16°C/min to 290°C. The glass solventless injection system temperature was set at 180°C, and the helium carrier gas flow-rate was ca. 1 ml/min.

Standards and controls

Aqueous and methanolic stock standard solutions of 5ISMN and sorbitol (1 mg/ml, 10 μg/ml, 1 μg/ml) were prepared and stored at 4°C. Plasmatic standards covering the range 10–500 ng/ml were prepared by adding known amounts

of the aqueous stock standards to lyophilized human serum (Sigma, St. Louis, MO, U.S.A.) reconstituted in 5 ml of water; these standards were used daily to create a calibration curve as a control.

Procedure for sample preparation

Aliquots of 3 ml of fresh serum were pipetted into culture tubes. Dichloromethane–ethyl acetate (1:1) (4 ml) was added, and the tubes were shaken for 1 min on a vortex and centrifuged at 1000 *g* for 5 min. The supernatant was transferred to a clean culture tube, and serum was re-extracted with 4 ml of dichloromethane–ethyl acetate (1:1), followed by another 2 ml. The organic phases with 10 μl of sorbitol (10 μg/ml) added, were collected, evaporated under a stream of nitrogen, then derivatized by heating with 30 μl of BSTFA–TMCS and 60 μl of pyridine at 80°C for 30 min. A 1-μl volume of this sample was injected onto the GC column.

Study in humans

Five hospitalized patients, suffering from angina pectoris, who gave consent to the study, received orally 20 mg of 5ISMN as one tablet. Blood samples (6 ml) were collected before and 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h after administration. They were immediately centrifuged at 1000 *g* for 5 min and serum was collected.

RESULTS AND DISCUSSION

Extraction procedure

After other compounds proved unsuitable, sorbitol was chosen as I.S. It permitted an excellent separation and a good reliability, but its introduction before the extraction step interfered with these results because the extraction conditions for 5ISMN were different from those required for sorbitol.

GC–MS conditions

The mass spectra of silylated 5ISMN and silylated sorbitol (Figs. 2 and 3) were recorded initially in order to choose fragment ions for the SIM analysis of serum samples. The molecular ion peak at m/z 290, and the ions at m/z 275, 129 and 101 were observed for the bis-silyl derivative of 5ISMN, and ions m/z 319, 205 and 147 were observed for hexa-TMS sorbitol. Only m/z 101 was used for the quantitation of 5ISMN, but m/z 129 and 275 were also monitored because these values were present only for the bis-silyl derivative and showed the complete derivatization of 5ISMN; m/z 319 was chosen for the quantitation of I.S. A typical SIM chromatogram obtained from the serum of a patient is shown in Fig. 4. No detectable interferences were observed. The retention times of silylated 5ISMN and silylated sorbitol were 7.6 and 11 min, respectively. The limit of detection observed was 10 ng/ml 5ISMN (R.S.D. 13%).

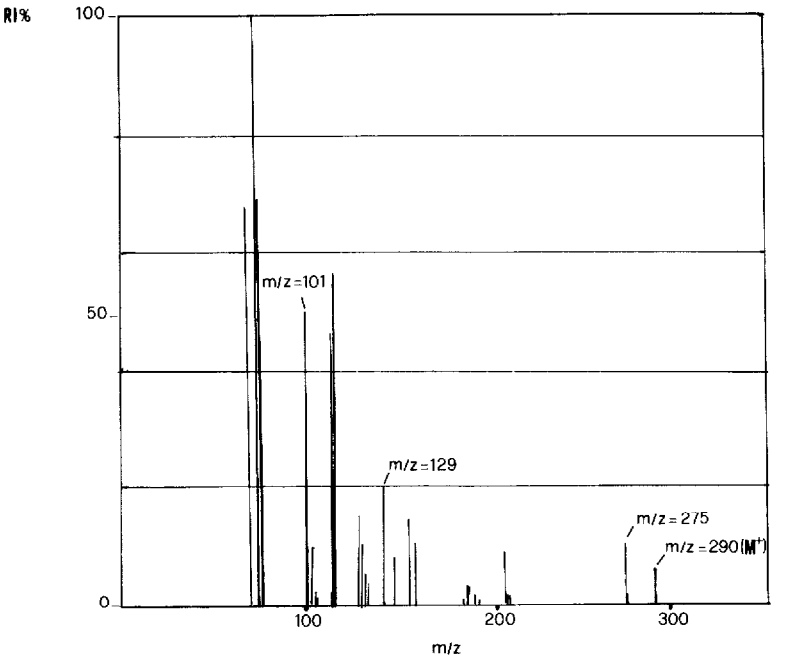

Fig. 2. Mass spectrum of bis-TMS 5ISMN.

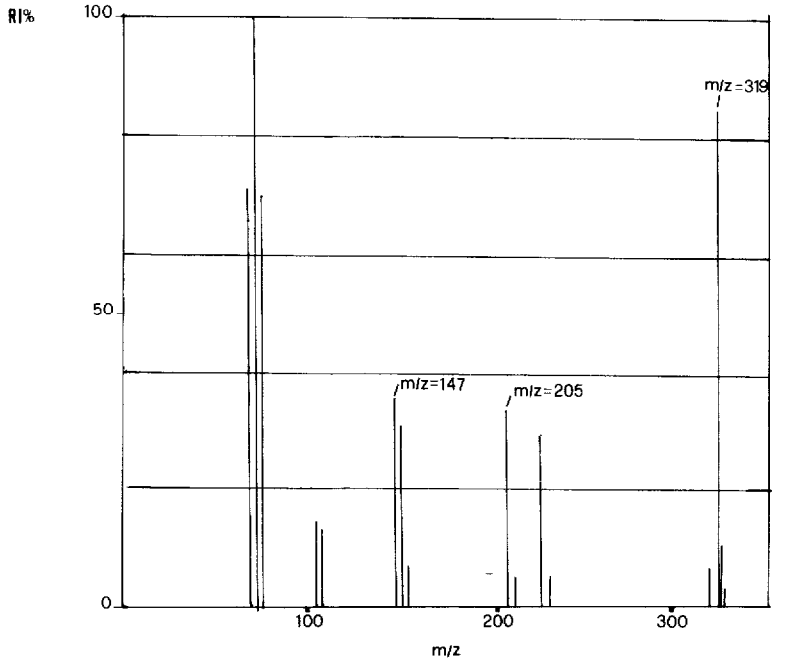

Fig. 3 Mass spectrum of hexa-TMS sorbitol.

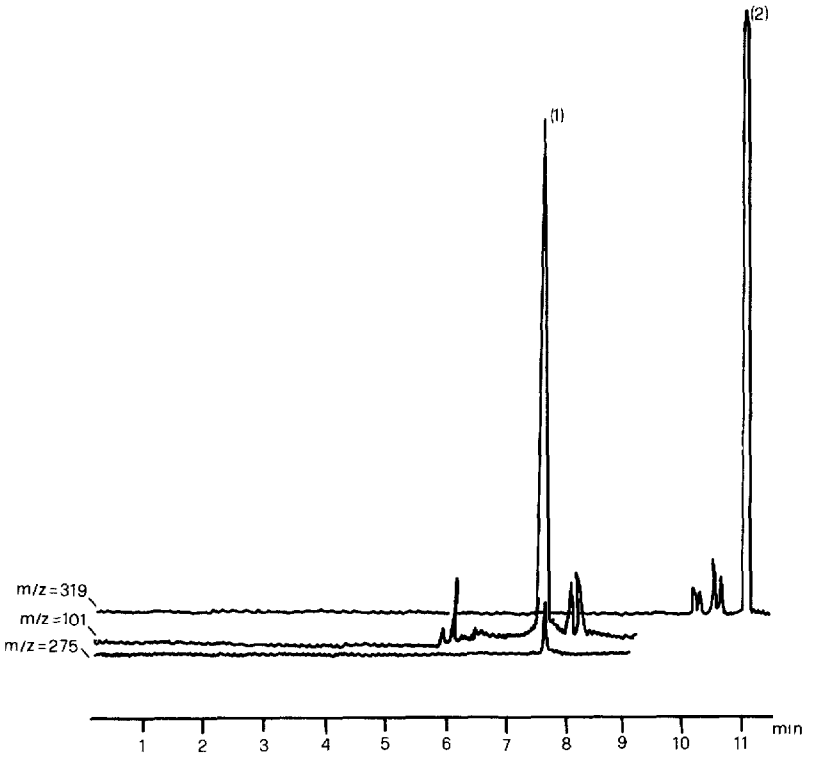

Fig. 4. SIM chromatogram of serum obtained from one patient, with I.S. Peaks: 1 = bis-TMS 5ISMN; 2 = hexa-TMS sorbitol.

TABLE I

RECOVERY OF 5ISMN FROM SPIKED SERUM SAMPLES

Concentration added (ng/ml)	*n*	Recovery (mean ± S.D.) (%)
50	10	94 ± 1.7
500	10	92 ± 1.5

TABLE II

WITHIN-DAY PRECISION

Concentration added (ng/ml)	Mean concentration found (ng/ml)	R.S.D. ($n=4$) (%)	Relative error (%)
50	46.47	5.2	−7.1
500	463.43	3.9	−7.3

TABLE III

BETWEEN-DAY PRECISION

Concentration added (ng/ml)	Mean concentration found (ng/ml)	R.S.D. ($n=6$) (%)	Relative error (%)
50	47.31	5.4	−5.4
500	468.91	3.7	−6.2

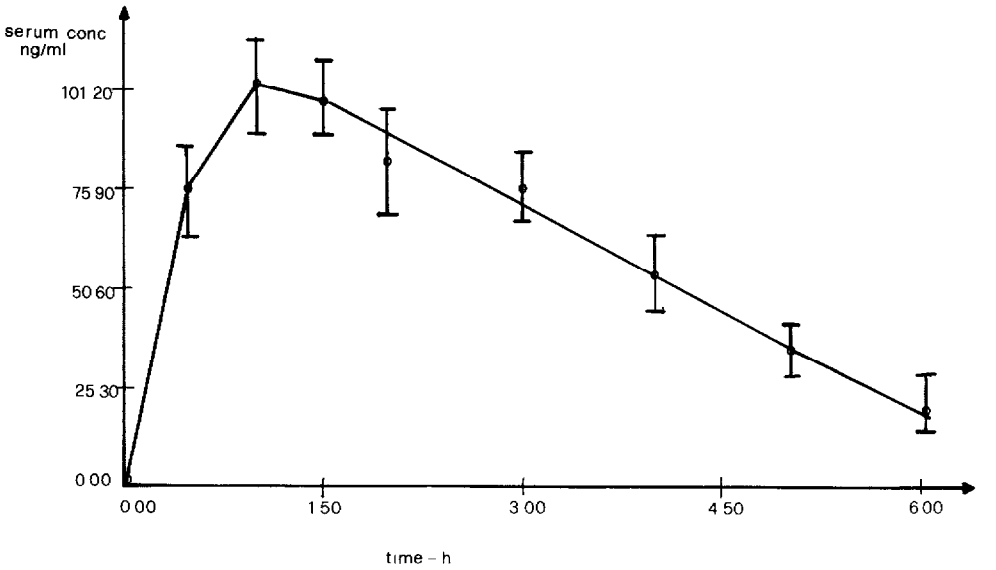

Fig. 5. Mean serum concentrations of 5ISMN in five hospitalized patients after oral administration of 20 mg of drug.

Calibration curve

The calibration curve was prepared and checked daily from spiked serum carried through the entire procedure; it was linear over the range 10–500 ng/ml, which encompassed all the serum levels that we investigated (regression equation: $y=108.1x+6.31$; $r=0.997$). Analytical recoveries from serum containing 50 and 500 ng of added 5ISMN per ml, were assessed in ten replicate samples (Table I). The ratio of m/z 101 to m/z 319 was compared in the samples and in the methanolic standards prepared daily.

Within-day precision

The within-day precision was checked by determining four serum samples spiked with two different concentrations of 5ISMN (Table II).

Between-day precision

Two concentrations were determined in duplicate every day for six days (Table III).

CONCLUSIONS

The method described was applied to the quantitation of the serum concentrations of 5ISMN in five patients after the oral administration of 20 mg of the drug. The mean serum levels are indicated in Fig. 5. The mean maximum concentration of 101.2 ng/ml was observed 1 h after administration; thereafter the concentration decreased to the mean value of 22 ng/ml after 6 h.

The specificity, reproducibility and reliability of the described method are therefore useful for pharmacokinetic control of 5ISMN in hospitalized patients.

REFERENCES

1 C. Cavazzutti, L. Gagliardi, A. Amato, E. Gattavecchia and D. Tonelli, J. Chromatogr., 244 (1982) 391
2 N. Mizuno, C. Shimizu, E. Morita, D. Shinkuma and Y. Yamanaka, J. Chromatogr., 264 (1983) 159.
3 J. Maddock, P.A. Lewis, A. Woodward, P.R. Massey and S. Kennedy, J. Chromatogr., 272 (1983) 129.
4 W.C. Yu and E.U. Goff, Biopharm. Drug Dispos., 4 (1983) 311.
5 A. Sioufi and F. Pommier, J. Chromatogr., 305 (1984) 95.
6 M.G. Bogaert and M.T. Rosseel, Methodol. Anal. Toxicol., 2 (1985) 183.
7 M.T. Rosseel and M.G. Bogaert, J. Pharm. Sci., 68 (1979) 659.
8 P. Straehl and R.L. Galeazzi, J. Pharm. Sci., 73 (1984) 1317.