ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
16 (1997) 425–429

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Gas chromatography-mass spectrometry determination of isosorbide 5-mononitrate and related impurities in raw materials and dosage formulations

Valentina D. Marinković [a], Smiljana S. Milojković [b], Jovan M. Nedeljković [b], Jožef J. Čomor [b,*], Danica Agbaba [c], Dobrila Živanov-Stakić [c]

[a] *Zdravlje Pharmaceutical and Chemical Industry, Quality Control Sector, 16000 Leskovac, Yugoslavia*
[b] *Vinča Institute of Nuclear Sciences, PO Box 522, 11001 Belgrade, Yugoslavia*
[c] *Faculty of Pharmacy, Department of Pharmaceutical Chemistry, PO Box 146, 11000 Belgrade, Yugoslavia*

Received 9 December 1996; received in revised form 5 March 1997

Abstract

A straightforward quantitative method for gas chromatography-mass spectrometry determination of isosorbide 5-mononitrate (IS5MN) and its related impurities such as isosorbide (IS), isosorbide diacetate (ISDA) and isosorbide 2-acetate-5-nitrate (IS2A5N) in raw materials as well as in dosage formulations is developed. The recovery of these materials was found to be 100.4 ± 2.4, 99.3 ± 4.7, 97.8 ± 5.2 and $100.1 \pm 3.1\%$, while the detection limits were 27.2, 1.26, 1.02 and 0.78 μg in dosage formulations for IS5MN, ISDA, IS2A5N, and IS, respectively. The applicability of the method was tested by analysing three different formulations of IS5MN. © 1997 Elsevier Science B.V.

Keywords: Isosorbide; Isosorbide 5-mononitrate; Isosorbide diacetate; Isosorbide-2-acetate-5-nitrate; Gas chromatography-mass spectrometry; Quality control

1. Introduction

Isosorbide 5-mononitrate (IS5MN) is the main metabolite of isosorbide dinitrate (ISDN) and is frequently used for treatment and prevention of angina pectoris. IS5MN has the typical pharmacodynamic action of organic nitrates, but possesses a better pharmacokinetic profile, assuring enhanced biological availability. For this reason, IS5MN is nowadays more frequently used as a coronary vasodilator in various dosage formulations than ISDN.

IS5MN has been the subject of many analytical investigations, concerning its determination as the main component of dosage formulations by colorimetry [1], polarography [2,3], HPLC [4–6], GC [7,8], and TLC [4], and as a degradation product of ISDN [9–11]. Recently its stability has been investigated by capillary gas chromatography-mass spectrometry (GC-MS) [12]. Pharmacokinetic studies of IS5MN, both as the main compound and as an active metabolite of ISDN,

* Corresponding author.

PII S0731-7085(97)00079-4

has been done by HPLC [13,14] and GC [15–18] techniques.

IS5MN can be synthesised by alkaline or acidic hydrolysis of ISDN [19–21] or more frequently, from isosorbide (IS). The synthetic procedure starts with acylation of IS in position 2, followed by nitration in position 5, and finally by the removal of the acyl group from position 2 by hydrolysis [21,22]. Thus, IS can be found in IS5MN as an impurity originating from the synthesis. An additional source of IS in IS5MN is its degradation, especially if storage under dry conditions is not provided [12]. Besides IS, IS5MN can be contaminated with other intermediates or by-products of its synthesis such as isosorbide diacetate (ISDA), isosorbide 2-acetate (IS2A), isosorbide 2-mononitrate (IS2MN), isosorbide 2-acetate-5-nitrate (IS2A5N), isosorbide 5-acetate (IS5A) and isosorbide 5-acetate-2-nitrate [23].

IS and some of the other impurities have been identified in IS5MN and its dosage formulations by GC [23], HPLC [6], and TLC [24], but none of these methods were reported to be applicable for routine quantitative and qualitative determination of all impurities in IS5MN. In this paper our effort to develop a quantitative method for GC-MS determination of IS5MN, as well as IS, ISDA, and IS2A5N (the most common impurities) in bulk IS5MN and its dosage formulations is presented.

2. Experimental

2.1. Materials

IS5MN, IS, ISDA, and IS2A5N as well as bulk IS5MN in lactose (1:1) were obtained from Kali Chemie Pharma (Hanover, Germany) and were used as received. Ancorbid 60 capsules, with prolonged effect, were obtained from Zdravlje (Leskovac, Yugoslavia), while the Monosan 40 tablets were obtained from Slaviamed (Belgrade, Yugoslavia). *N*,*O*-bis-trimethylsilyl-trifluoroacetamide (BSTFA) was a Sigma product, while diethylether was purchased from Merck.

2.2. Preparation of test and reference solutions

The standard stock solutions of IS, ISDA and IS2A5N were prepared in diethylether at a concentration of 1 mg ml^{-1} while the stock solution of IS5MN was prepared at a concentration of 0.5 mg ml^{-1} in the same solvent. Standard calibration solutions in the concentration range from 0.001 to 0.2 mg ml^{-1} were prepared from these stock solutions by dilution.

For recovery determinations, 10 g powdered raw IS5MN was spiked with 2% (by weight) IS, ISDA and IS2A5N and mixed thoroughly by repeated grinding.

The raw or spiked IS5MN, and the grounded tablets or pellets were precisely weighed on a

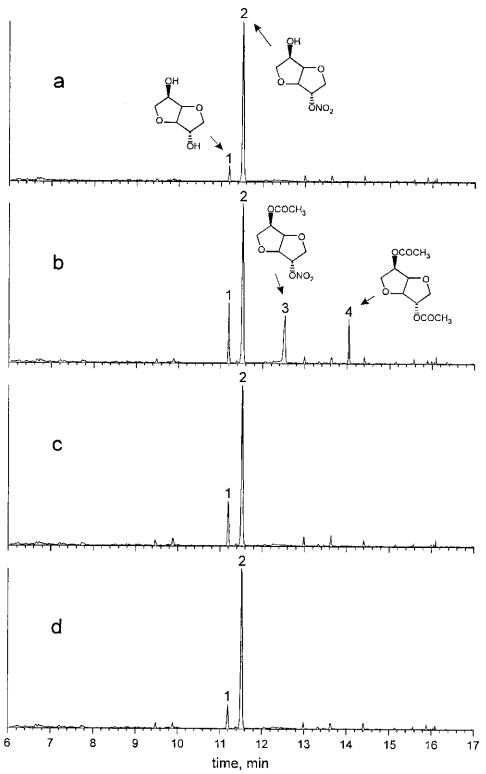

Fig. 1. Chromatograms of raw (a) and spiked (b) IS5MN, as well as samples of Ancorbid 60 (c) and Monosan 40 (d) formulations. The actual compounds detected by GC-MS are the trimethylsilyl derivatives of (1) IS, (2) IS5MN, (3) IS2A5N and (4) ISDA.

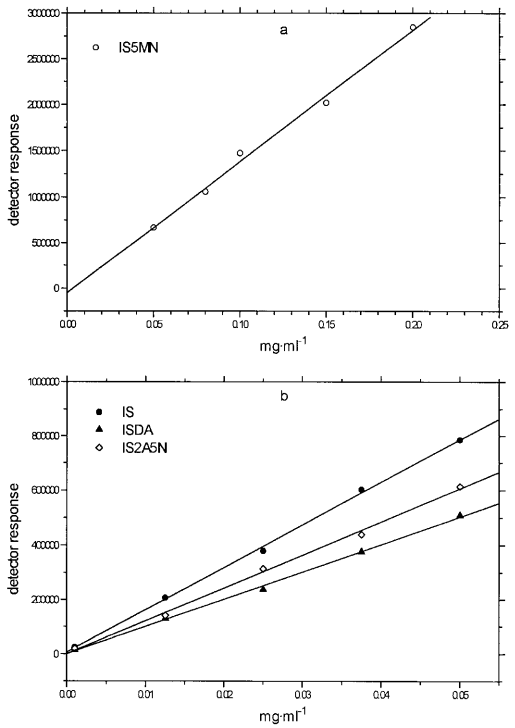

Fig. 2. Calibration diagrams of (a) IS5MN and (b) IS, IS5MN and IS2A5N.

balance and an amount equivalent to 50 mg of IS5MN (according to the declaration of the supplier) was extracted with 50 ml diethylether by vigorous shaking for 5 min. This solution was used for the determination of IS, ISDA and IS2A5N, while for the determination of IS5MN the solution was diluted 10-fold with diethylether.

2.3. Silylation procedure

1 ml Of the solution (standard solutions or diethylether extracts of samples) was put into a 2 ml vial, the solvent was evaporated under vacuum at 40°C, and the vial was closed by a PTFE septum. Finally, 0.1 ml of BSTFA was injected into the vial through the septum and the vial was heated to 100°C for 10 min in order to complete the silylation of the sample [25]. 1 μl Of this derivatized sample was injected into the GC. The whole process (evaporation, derivatization and GC determination) was repeated 5 times for every sample.

2.4. GC-MS analyses

A HP 5890 series II + gas chromatograph (Hewlett Packard) equipped with an HP 5891 mass spectrometer (Hewlett Packard) and an HP-5 (50 m, 0.32 mm, 0.17 μm) capillary column (Hewlett Packard) was used for the measurements. The temperature programme started at 100°C for 2 min, than increased at 8°C min^{-1} to 300°C. The injector was operated in splitless mode at 170°C, in order to avoid thermal degradation of the samples [12]. Helium was used as a carrier gas at a flow rate of 2 ml min^{-1}.

The MS transfer line was operated at 200°C, the ion source at 250°C, the detector was operated at 3000 V and the MS scanning rate was 1.1 s for the m/e range from 50 to 600. The identification of compounds was based on their retention times and corresponding mass spectra.

3. Results and discussions

Fig. 1 presents the chromatograms of the extracts of bulk IS5MN (a), spiked IS5MN (b), as well as Ancorbid 60 (c) and Monosan (d) formulations. Though all of the compounds are baseline resolved, ISDA and IS2A5N were identified only in the spiked samples, revealing the high quality of the investigated materials.

For quantitative measurements, the linearity of the detector was investigated in the concentration range 0.05–0.2 mg ml^{-1} for IS5MN, and 0.001–0.05 mg ml^{-1} for IS, ISDA and IS2A5N by measuring the peak areas for 5 different concentrations (see Fig. 2). The average R.S.D. of integrated peak areas after 5 successive determinations of the same sample was 2.4%. The regression coefficients obtained are summarized in Table 1. The detection limits for the above mentioned compounds were determined as a mean value of S.D. of 5 successive determinations, when the actual concentrations of the compounds were 10 times higher than the detection limit. The values obtained are also shown in Table 1.

The recoveries of the compounds investigated were determined by repeated analysis of raw IS5MN spiked with IS, ISDA and IS2A5N. The

Table 1
Regression coefficients of the detector's linearity test, recovery and detection limit values obtained from the analysis of spiked samples

Compound	Regression coefficient	Recovery (%)	Detection limit in dosage formulations (μg)
IS5MN	0.9970	100.4 ± 2.4	27.2
IS	0.9994	100.1 ± 3.1	0.78
ISDA	0.9991	99.3 ± 4.7	1.26
IS2A5N	0.9987	97.8 ± 5.2	1.02

Table 2
Results of the GC-MS analysis of raw IS5MN, Ancorbid 60 and Monosan 40 formulations

Sample	Declared content of IS5MN (mg)	Determined values (mg)			
		IS5MN	IS	ISDA	IS2A5N
Raw IS5MN	50	49.8 ± 1.0	0.46 ± 0.01	N.D.[a]	N.D.
ANCORBID 60	60	55.3 ± 1.3	4.6 ± 0.1	N.D.	N.D.
MONOSAN 40	40	39.1 ± 0.9	0.80 ± 0.02	N.D.	N.D.

[a] Not detected, concentrations below the detection limit of the method.

results are presented in Table 1, as the mean values of 5 determination. It is evident, that the developed method is quite efficient, since the recovery values are at least 98% for all compounds of interest.

The applicability of the method was tested by analysing three different samples, namely the raw IS5MN mixed with lactose (1:1), five capsules of Ancorbid 60 (expired validation date) and five tablets of Monosan 40. Table 2 shows the average results obtained from these measurements. Except for the isosorbide content of Ancorbid 60, all values are within the limits declared by the supplier. The increased isosorbide concentration in Ancorbid 60 is probably a result of degradation of IS5MN during the long storage time [12].

4. Conclusion

The results presented clearly indicate that the proposed method can be applied for the determination of IS5MN, IS, ISDA and IS2A5N in the quality control of pharmaceuticals based on IS5MN. The method is simple, straightforward and sensitive and gives reliable results.

Acknowledgements

This work was partially supported by the Ministry of Science and Technology of the Republic of Serbia.

References

[1] P.N. Ivakhnenko, L.S. Chigarenko, G.M. Kilyakova, E.S. Ventsel, L.Y. Vasilchenko, Farmatsiya 25 (1976) 67.
[2] S. Pintanti, E. La Porta, P. Gratteri, G. Dapeshi, V. Giannellini, Pharm. Acta Helv. 64 (1989) 125.
[3] P. Kazandzhieva, N. Firkova, M. Yakovich, Farmatsiya 37 (1987) 1.
[4] T. Azcona, A. Martin-Gonzales, P. Zemoreno, C. Pascual, C. Grant, M. Garcia De Mirasirra, J. Pharm. Biomed. Anal. 9 (1991) 725.
[5] D. Agbaba, V. Janjić, D. Živanov-Stakić, S. Vladimirov, J. Liq. Chromatogr. 17 (1994) 3983.
[6] C.S. Olsen, H.S. Ceroggins, J. Pharm. Sci. 73 (1984) 1303.
[7] A. Marzo, E. Treffner, J. Chromatogr. 345 (1985) 390.
[8] L. Nobile, M.A. Raggi, V. Cavrini, A.M. DiPetro, J. Pharm. Belg. 44 (1989) 210.
[9] W.Y. Zin, Yuaxue Muebe 24 (1989) 797.
[10] N. Mizuno, Ch. Shimizu, E. Morita, D. Shinkuma, Y. Yamanaka, J. Chromatogr. 264 (1983) 159.

[11] K. Niemienen, R. Aaltonen, D. Kahela, Acta Pharm. Fenn 97 (1988) 21.
[12] V.D. Marinković, B. Gudžić, S.S. Milojković, J.M. Nedeljković, J.J. Čomor, J. Chromatogr. A 746 (1996) 286.
[13] J. Maddok, P.A. Lewis, A. Woodward, P.K. Massey, R. Kenny, J. Chromatogr. 272 (1983) 129.
[14] C.Y. Wing, E. Goff, Anal. Chem. 55 (1983) 29.
[15] P. Zucearo, S.M. Zucearo, R. Pacifici, S. Pichini, Z. Bonoforti, J. Chromatogr. 525 (1990) 447.
[16] G. Michael, L. Fay, M. Prest, J. Chromatogr. 493 (1989) 188.
[17] J. Halkiewich, W. Sawichi, Pharmazie 43 (1988) 865.
[18] H. Jaeger, D. Lutz, K. Michaelis, Z.B. Salama, Drugs 33 (1987) 9.
[19] D.L. Haxward, Can. J. Chem. 45 (1967) 2191.
[20] O. DeLucchi, A. Angius, F. Filiuzzi, G. Modena, E. Camera, Gazz. Chim. Ital. 117 (1987) 173.
[21] USA Patent No. 4065448.
[22] Eur. Patent No. 0045076.
[23] N. Russeva, N. Dimova, G. Spyrov, M. Jurovska, J. Chromatogr. 295 (1984) 255.
[24] N. Dimov, N. Agapova, St. Levy, Iv. Yanchov, J. Chromatogr. 285 (1984) 515.
[25] Alan E. Pierce, Silylation of Organic Compounds, Pierce Chemical Company, Rockford, 1968.