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Note

Separation of nitrate esters and nitrate–acetate esters of isosorbide

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It was recently found[1] that isosorbide-5-mononitrate (5ISMN) is more active and less toxic than other coronary vasodilators. As a consequence, pathways for the stereospecific synthesis of 5ISMN have been sought. For effective control of the reaction mixture a quick and reliable analytical method is necessary.

Various methods are available for polynitrate purity determination, such as polarography[2], thin-layer chromatography (TLC)[3] and gas chromatography (GC)[4]. However, for our purposes only a separation methods would be satisfactory. GC suffers from the disadvantage that thermal decomposition can occur. In addition, salts and inorganic acids may be present in the sample mixture. The published high-performance liquid chromatographic (HPLC) methods do not consider all the substances which could be present in such a synthetic mixture. Recently, an HPLC method was proposed[5] for the separation and determination of isosorbide dinitrate (ISDN), isosorbide-5-mononitrate (5ISMN) and isosorbide-2-mononitrate (2ISMN), but other compounds could be present in the reaction mixture.

In this paper a TLC method for separation of all possible compounds (mononitrates, monoacetates, mononitrate–monoacetate, dinitrate and diacetate esters) as well as an HPLC method for determination of all nitrate esters are given.

EXPERIMENTAL AND RESULTS

Reagents

5ISMN, 2ISMN, isosorbide-2-monoacetate (2ISMA), isosorbide-5-monoacetate (5ISMA), isosorbide-2-nitrate-5-acetate (IS2N5A), isosorbide-2-acetate-5-nitrate (IS2A5N), isosorbide-2,5-diacetate (ISDA), isosorbide and isosorbide-2,5-dinitrate were synthesized in our laboratory.

TLC was carried out on silica gel plates (0.2-mm Kieselgel 60F 254 Alufolien; E. Merck, Darmstadt, F.R.G.). Two mobile phases were chosen: I, benzene–ethyl acetate–isopropanol (7:3:1.5); II, dichloromethane–dipropyl ether–dioxane–isopropanol (20:4:2:1).

The R_F values obtained are given in Table I. The spots from the nitrates could be observed under UV light at 254 nm. All the substances were visualized after spraying with 50% H_2SO_4 in ethanol and heating for 10 min at 160°C. While the isosorbide spot is readily formed, the nitrates require more extensive heating. The mixed esters IS2A5N and IS2N5A could not be separated.

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TABLE I

R_F VALUES OF NITRATE AND ACETATE ESTERS OF ISOSORBIDE

Compound	R_F	
	System I	*System II*
Isosorbide-2,5-dinitrate (ISDN)	0.93	0.82
Isosorbide-2-nitrate-5-acetate (IS2N5A)	0.86	0.77
Isosorbide-2-acetate-5-nitrate (IS2A5N)	0.86	0.77
Isosorbide-2,5-diacetate (ISDA)	0.82	0.72
Isosorbide-2-mononitrate (2ISMN)	0.67	0.50
Isosorbide-5-mononitrate (5ISMN)	0.57	0.35
Isosorbide-2-monoacetate (2ISMA)	0.52	0.43
Isosorbide-5-monoacetate (5ISMA)	0.38	0.27
Isosorbide (IS)	0.16	0.10

The qualitative separation of the nitrate esters from the acetate esters required two spray reagents. The nitrate esters were sprayed with an ethanolic solution of H_2SO_4 (50%) followed by 1% diphenylamine in 95% ethanol yielding yellow-green spots on a white background after heating. For the acetate esters three solutions were necessary: A, 1 g $NH_2OH \cdot HCl$ in 9 ml water; B, 2 g NaOH in 8 ml water; C, 4 g $Fe(NO_3)_2 \cdot 9H_2O$ in 60 ml water and diluted in acetic acid to 100 ml. The plate was first sprayed with a 1:1 mixture of solutions A and B, dried, heated at 110°C for 10 min and then sprayed with a mixture of 45 ml of solution C and 6 ml concentrated HCl, yielding violet spots.

The TLC method provided information on the presence or absence of all possible compounds. A semiquantitative estimation is also possible.

HPLC method

A Perkin-Elmer Series 3B Model HPL chromatograph equipped with a LC-75 UV detector was used. Two wavelengths were used for detection: 220 nm for quantities < 1% in the reaction mixture; 250 nm in all other cases. A gradient elution was performed as follows: the methanol content in water was increased from 30 to 60% in 8 min, and then to 95% in 10 min. The analyses were carried out at room temperature on a LiChrosorb RP-18 column (250 × 4 mm I.D., particle size 5 μm; E. Merck). The flow-rate was 1 ml/min.

A typical chromatogram is shown in Fig. 1. Not only is there a good separation of 2ISMN and 5ISMN, but also the pair IS2A5N–IS2N5A unresolved by TLC is also separated. The response of the UV detector to 5ISMN is twice that to 2ISMN at 250 nm. Even IS2A5N and IS2N5A have different molar absorbances at the ana-

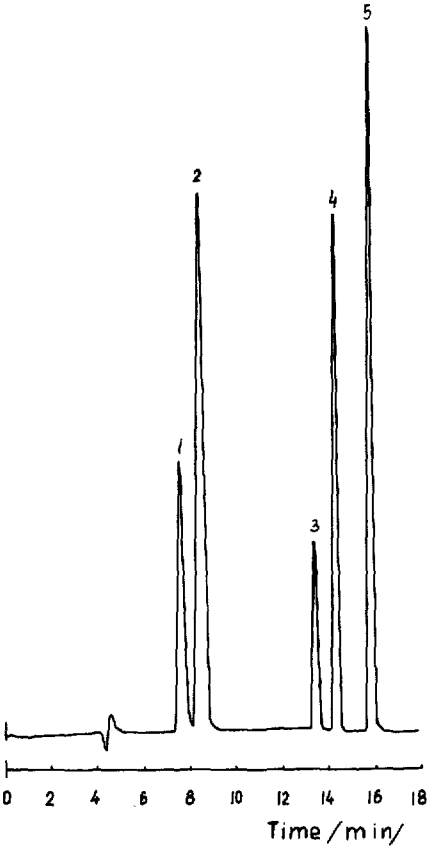

Fig. 1. HPLC analysis of 2ISMN (1), 5ISMN (2), IS2N5A (3), IS2A5N (4) AND ISDN (5). Column: LiChrosorb RP-18, 5 μm. Two-stage gradient from 30 to 95% methanol in water. UV detection at 250 nm (0.32 a.u.f.s.).

lytical wavelengths. Thus, for quantitations an internal normalization is not possible and the use of an internal standard (for example phenol) is preferable.

REFERENCES

1 A. Dengler, *Med. Welt*, 14A (1981) 32; *Scrip*, 9 (1982) 717.
2 B. Persson and L. Rosen, *Anal. Chem.*, 123 (1981) 115.
3 M. T. Rosseel and M. G. Bogaert, *Biochem. Pharmacol.*, 22 (1973) 67.
4 E. Doyle, L. F. Chasseand and T. Taylor, *Biopharm. Drug Dispos.*, 1 (1980) 141.
5 N. Mizuno, Ch. Shimizu, E. Morita, D. Shinkuma and Y. Yamanaka, *J. Chromatogr.*, 264 (1983) 159.