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Note

Determination of isosorbide nitrates by high-performance liquid chromatography and their stability in aqueous solution

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Polynitric esters, which are used as vasodilators in the therapy of angina pectoris, are pharmacologically effective in doses of only a few milligrams[1]. These substances are very quickly metabolized in the liver and blood[2,3]. When isosorbide dinitrate (ISDN) is injected intravenously, ISDN and its metabolites, that is, isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-nitrate (5-ISMN), appear in the blood and the metabolites are also pharmacologically effective. However, their stability in aqueous solution has not yet been studied. ISDN, 2-ISMN and 5-ISMN can be measured by gas–liquid chromatography with an electron-capture detector[4,5] but this method, although very sensitive, requires time-consuming extraction. We have established a method for the simultaneous quantitation of ISDN, 2-ISMN and 5-ISMN by high-performance liquid chromatography (HPLC) and used it to study their stability in aqueous solution.

EXPERIMENTAL

Chemicals

ISDN, 2-ISMN and 5-ISMN were gifts from Eisai (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

High-performance liquid chromatography

A Hitachi Model 635-T high-performance liquid chromatograph equipped with a 100-μl loop injector was used for the analyses. The wavelength of the UV detector was set at 220 nm. The sensitivity was kept at 0.08 a.u.f.s. throughout. The mobile phase of methanol–water (25:75) was filtered through a 0.45-μm membrane filter (Toyo Roshi, Tokyo, Japan) and degassed in an ultrasonic bath prior to use. The analyses were carried out at room temperature on a stainless-steel column (15 cm $\times$ 4 mm I.D.) packed with Nucleosil C_{18} (5-μm particles) (Macherey, Nagel & Co., Duren, G.F.R.). The flow-rate was 0.5 ml/min.

Thin-layer chromatography

Thin-layer chromatography (TLC) was carried out on silica gel plates (0.25

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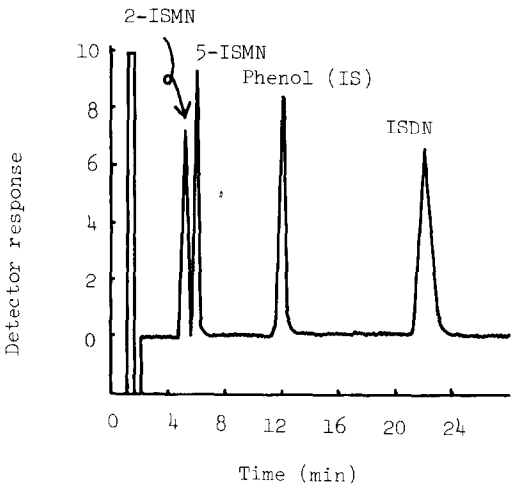

Fig. 1. Chromatogram of isosorbide nitrates. Mobile phase: methanol–water (25:75).

mm Kieselgel F-254 on glass; Merck, Darmstadt, G.F.R.) as described by Rosseel and Bogaert[6]. The solvent used was benzene–ethyl acetate (1:1). Colouring of the nitrate groups was effected by spraying with 1 % diphenylamine in ethanol and irradiation for 10 min with a UV source. The R_F values were ISDN 0.84, 2-ISMN 0.41 and 5-ISMN 0.30.

Calibration

Peak-height ratios were calculated by dividing the heights of the ISDN, 2-ISMN and 5-ISMN peaks by the height of the internal standard (phenol) peak and were plotted against the amounts of ISDN, 2-ISMN and 5-ISMN.

Stability of ISDN, 2-ISMN and 5-ISMN

Two millilitres of compound solution (0.34 m*M*) and 2 ml of 0.2 *N* sodium

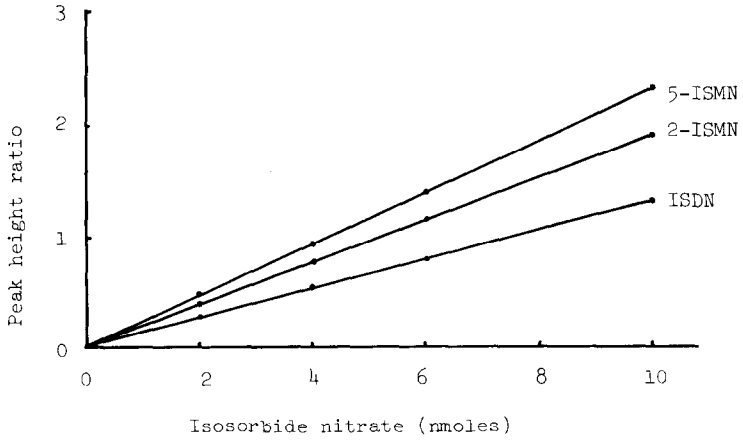

Fig. 2. Calibration graphs for isosorbide nitrates.

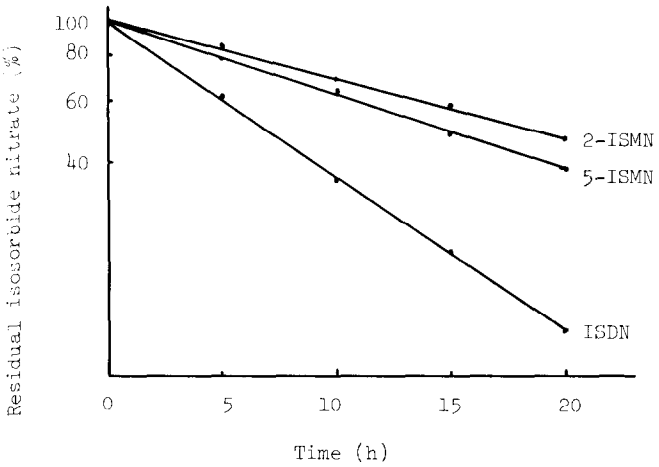

Fig. 3. Denitration of isosorbide 2-mononitrate, isosorbide 5-mononitrate and isosorbide dinitrate in 0.1 *N* hydrochloric acid at 100°C.

hydroxide solution were placed in a 10-ml erlenmeyer flask to which a reflux condenser was attached. The mixture was heated for various periods at 100°C, then immediately cooled to 5°C to stop the reaction. To 1 ml of reaction solution, 1 ml of internal standard solution (2 μg/ml) in 0.15 *N* hydrochloric acid was added and the compounds in the reaction solution were determined by HPLC.

RESULTS AND DISCUSSION

A typical chromatogram is shown in Fig. 1. 2-ISMN, 5-ISMN, ISDN and the internal standard are well separated. The retention times are 2-ISMN 5.4, 5-ISMN 6.2, internal standard 12.2 and ISDN 22.2 min.

The calibration graphs were linear over the range of 1.0–10 nmole of ISDN, 2-

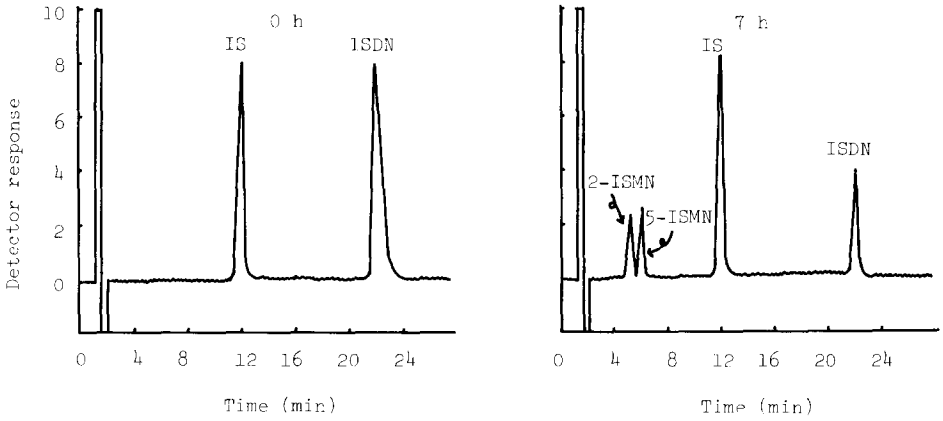

Fig. 4. High-performance liquid chromatograms of isosorbide dinitrate after dinitration in 0.1 *N* hydrochloric acid at 100°C for 0 and 7 h.

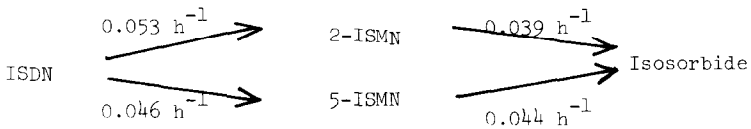

Fig. 5. Denitration rate constants of isosorbide dinitrate, isosorbide 2-mononitrate and isosorbide 5-mononitrate in 0.1 *N* hydrochloric acid at 100°C. Numerical values are denitration rate constants.

ISMN and 5-ISMN (Fig. 2). The correlation coefficient for each compound was greater than 0.998 ($n = 8$). To examine the stability of ISDN in aqueous solution, we studied the denitration of ISDN at pH 1.2, 7.4, 10 and above 12 and 37°C for 48 h. ISDN was stable at pH 1.2–10 but unstable at pH above 12. Thus, the stability of ISDN, 2-ISMN and 5-ISMN in 0.1 *N* hydrochloric acid and 0.1 *N* sodium hydroxide solution were studied at 100°C. Fig. 3 shows the stability of ISDN, 2-ISMN and 5-ISMN in 0.1 *N* hydrochloric acid at 100°C. The denitration of these compounds obeyed first-order kinetics in both solutions. The HPLC traces of the reaction solution of ISDN are shown in Fig. 4. The solution was heated at 100°C and portions of the solution taken at 0 and 7 h were analysed by HPLC. The denitration rate constants of ISDN, 2-ISMN and 5-ISMN in 0.1 N hydrochloric acid at 100°C are shown in Fig. 5. Denitration rate constants of ISDN were calculated according to literature[7]. As the rate constants are approximately equal, denitration of isosorbide nitrates occurs with about the same velocity. The formation of 2-ISMN and 5-ISMN as intermediates has been confirmed by HPLC and TLC.

Fig. 6 shows the stability of ISDN, 2-ISMN and 5-ISMN in 0.1 *N* sodium hydroxide solution at 100°C and their denitration rate constants. The value for 2-ISMN is 10 times that of 5-ISMN. ISDN has an elimination rate constant of 1.444 h^{-1} from blood in man and 2-ISMN and 5-ISMN have shorter rate constants of 0.396 and 0.091 h^{-1}, respectively[8]. This order is the same as that of the denitration rate constants in 0.1 *N* sodium hydroxide solution. 2-ISMN is metabolized at a faster rate than 5-ISMN. Thus, Rosseel and Bogaert[6] and Sisenwine and Ruelius[9] explained that the dinitrates of isosorbide have one nitrate group in the exo-position and one

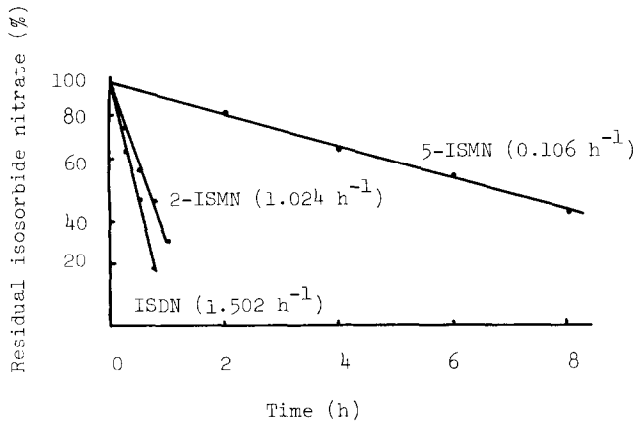

Fig. 6. Denitration of isosorbide 2-mononitrate, isosorbide 5-mononitrate and isosorbide dinitrate in 0.1 *N* sodium hydroxide solution at 100°C. Numerical values in parentheses are denitration rate constants.

nitrate group in the endo-position. 2-ISMN and 5-ISMN are exo-mononitrate and endo-mononitrate compounds, respectively. The nitrate group in the exo-position is more readily available than the endo-group, the latter being sterically shielded by the sugar derivative. The fact that the denitration rate constant of 5-ISMN is much larger than that of 2-ISMN in 0.1 *N* sodium hydroxide solution may be related to the fact the 5-position lends itself less easily to OH^- attack than 2-position in the same manner as enzymatic attack. However, the denitration rate constants of 2-ISMN and 5-ISMN in 0.1 *N* hydrochloric acid are approximately equal. Therefore, the attack by H_3O^+ on nitrate at the 2- and 5-positions is not influenced by steric hindrance.

The present method has been applied successfully to the investigation of the degradation kinetics of isosorbide nitrates. The advantages of our chromatographic technique are that 2-ISMN, 5-ISMN and ISDN in aqueous solution can be assayed without pre-treatment and extraction and the accuracy and reproducibility are excellent. This method will allow the assay of the purity and contents of drugs in dosage forms.

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