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Note

High-performance liquid chromatographic analysis of glucose, fructose, sorbitol and low levels of the aldose reductase inhibitor sorbinil in human lens and plasma

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Much interest has been generated in the use of aldose reductase inhibitors to delay or prevent diabetic complications, particularly neuropathy and cataract [1, 2]. Methods presently available [3] are unable to resolve the aldose reductase inhibitor sorbinil [*d*-6-fluoro-spiro(chroman-4,4′-imidazolidine)-2′,5′-dione] from interfering peaks at the low levels which may be of clinical benefit. In addition, to determine the effect of aldose reductase inhibitors on the polyol pathway in lens [1], it is necessary to have an assay which is capable of resolving glucose, sorbitol and fructose quantitatively from this tissue. We describe here methods for the quantitative determination of sorbinil in human plasma and lens, at the level of sensitivity required for possible therapeutic dosage; and for the derivatisation and quantitative determination of glucose, sorbitol and fructose in human lens.

EXPERIMENTAL

Chromatographic system

The high-performance liquid chromatographic (HPLC) equipment was as described previously [4]. Wavelength setting was either 284 nm for sorbinil or 260 nm for carbohydrate analysis. For sorbinil determination a stainless-steel column (150 mm × 4.6 mm I.D.) packed with Spherisorb ODS 2, particle size 5 μm (Phase Sep., Clwyd, U.K.), was preceded by a stainless-steel pre-column (50 mm × 2.1 mm I.D.) packed with pellicular ODS (Whatman, Maidstone, U.K.).

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Chemicals

Solvents for extractions, derivatisation and mobile phases were all HPLC grade (Fisons, Loughborough, U.K.). Pyridine and *p*-nitrobenzoyl chloride were as described previously [4]. Other reagents were from Sigma (Poole, U.K.), and of the purest grade available. Sorbinil and *d*-6-chloro-spiro(chroman-4,4′-imidazolidine)-2′,5′-dione, the internal standard (I.S.), were from Pfizer U.K. (Sandwich, U.K.).

Sample preparation and chromatography

Cataractous human lenses (obtained from the Oxford Eye Hospital) were homogenised in 4.0 ml of 47.6 m*M* sodium fluoride, and the homogenate was filtered using the Centrifree system (Amicon, Stonehouse, U.K.) in an angle-head rotor at 2400 *g* for 30 min. Plasma was obtained from sodium heparin-treated blood by centrifugation at 500 *g* for 10 min.

Sorbinil determination

Volumes of 1.0 ml of either plasma or lens ultrafiltrate were pipetted into separate tubes. Sorbinil calibration standards were constructed as follows: for plasma 0—250 ng sorbinil and 500 ng I.S.; and for lens 0—100 ng sorbinil and 200 ng I.S. were added to each tube. Extraction was as follows. Samples were adjusted to pH 1.5 with 70 μl of 4 *M* hydrochloric acid and mixed on a vortex mixer. Solvent extraction was performed with 5.0 ml diethyl ether by vortex-mixing for 1.0 min, and the layers were separated by centrifugation (500 *g* for 30 sec). The diethyl ether layer was washed with 5.0 ml of 0.25 *M* phosphate buffer pH 7.0, and the layers were again separated by centrifugation. The diethyl ether layer was removed and evaporated to dryness under nitrogen. The residue was redissolved in 500 μl (plasma) or 200 μl (lens) acetonitrile—water (25:75), and 100-μl samples taken for analysis by HPLC in the same solvent.

Glucose, sorbitol and fructose determination

Separation of glucose, fructose and sorbitol was performed essentially by the previous method [4] with the following exceptions. Derivatisation was at 37°C for 30 min, the water content of the HPLC solvent was increased to 0.2% and the column was equilibrated with this solvent overnight at a flow-rate of 0.5 ml/min. A 100-μl sample of the chloroform layer was injected into the chromatograph.

RESULTS AND DISCUSSION

Determination of low levels of sorbinil

For determination of sorbinil in the range 0—250 ng/ml of plasma the method of Foulds et al. [3] produces interfering peaks, and the recovery of sorbinil and internal standard is lower than that obtained using larger amounts of sorbinil (Fig. 1). The extraction procedure described here has been found to provide a method which does not produce interfering or spurious peaks in the range 0—250 ng/ml plasma, 0—400 ng/ml lens sorbinil (Fig. 1). Furthermore, at this level of sensitivity recoveries of sorbinil and I.S. are better with this method

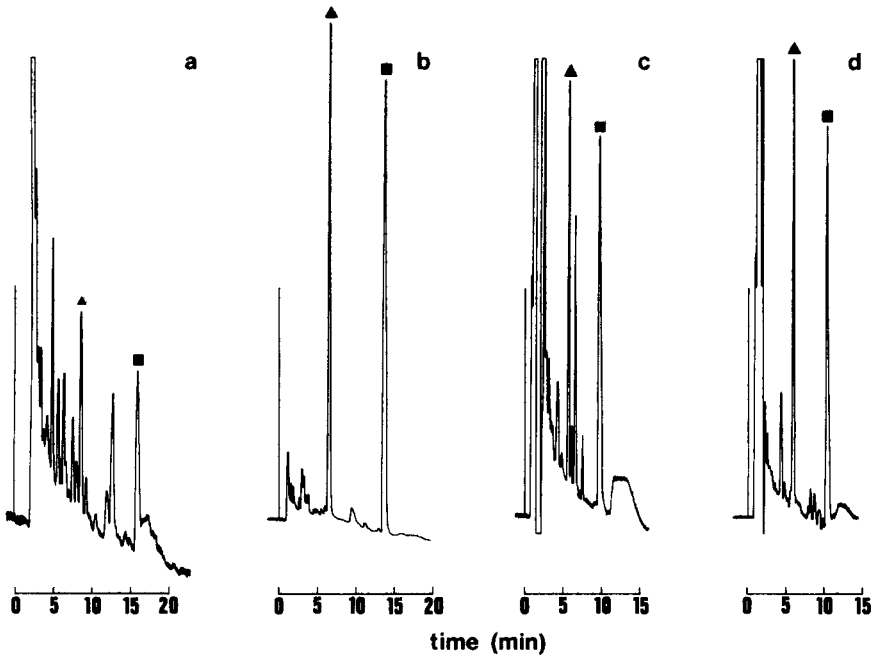

Fig. 1. Chromatograms of sorbinil (▲) and I.S. (■) extracted from plasma (a, b and c) and lens (d). Detection of 250 ng/ml sorbinil and 500 ng/ml I.S. by the method of Foulds et al. [3] produces interfering peaks (a), a similar profile is obtained after extraction from lens. The method of Foulds et al. [3] using 1500 ng/ml sorbinil and 3000 ng/ml I.S. is free from interference (b). The present method gives better recoveries of sorbinil and I.S. at the lower detection level, and is free from interference: 250 ng/ml sorbinil and 500 ng/ml I.S. for plasma (c); 400 ng/ml sorbinil and 800 ng/ml I.S. for lens (d). 0.005 a.u.f.s. except for b: 0.02 a.u.f.s.

(72% and 78%, respectively, Fig. 1c; 83% and 92%, respectively, Fig. 1d) than with the previously published method [3] (45% and 53%, respectively, Fig. 1a). At higher concentrations, the recoveries of sorbinil and I.S. by the method of Foulds et al. [3] were 83% and 80%, respectively (Fig. 1b). The addition of charcoal as a purification step in the method of Foulds et al. [3] cannot be used to improve the extraction procedure described as charcoal itself binds sorbinil in the presence of diethyl ether.

Using this method, addition of I.S. gave a linear correlation of peak height ratio with sorbinil over the concentration range 25—250 ng/ml in plasma (r = 0.994) and 40—400 ng/ml in lens (r = 0.997), the amounts detected by HPLC being 5—50 ng. The method is suitable for the extraction of sorbinil from either plasma or lens ultrafiltrate at levels which are expected to be found in current clinical trials [5]. Recoveries of I.S. were 78.4 ± 1.4% (n = 19) and 86.5 ± 2.0% (n = 13) from plasma and lens, respectively. For analysis of unknown amounts of sorbinil in lens, 800 ng I.S. were added at the homogenisation step.

Determination of glucose, sorbitol and fructose in lens tissue

Modifications to the method of Petchey and Crabbe [4] were necessary for the determination of fructose. The derivative formed from fructose and *p*-

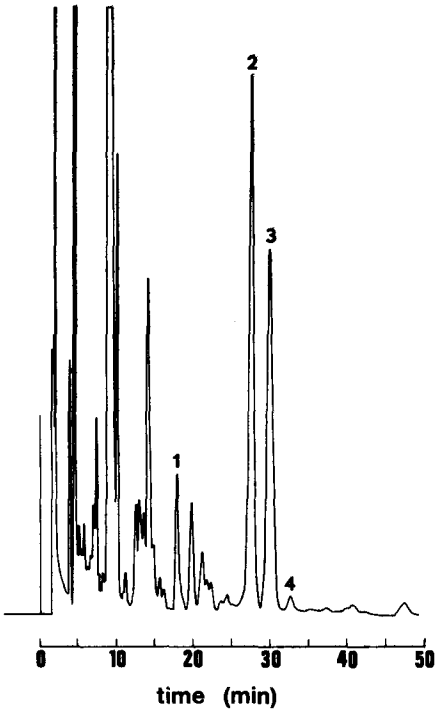

Fig. 2. Chromatogram of derivatised sugars from lens ultrafiltrate. Peaks: 1 = D-glucose; 2 = myoinositol; 3 = sorbitol; 4 = fructose. 0.32 a.u.f.s.

nitrobenzoyl chloride is rapidly destroyed at 60°C, but shows greater stability at 37°C [6]. In addition, glucose and sorbitol are fully derivatised after 30 min at 37°C in this system. Inclusion of an internal standard (perseitol) was omitted, as this would require a longer derivatisation time at higher temperature (1 h; 60°C), at the expense of fructose determination. The separation of fructose and sorbitol as nitrobenzoate derivatives is particularly sensitive to the water content of the solvent, and it is essential to fully equilibrate with solvent before loading samples.

This present assay allows the determination of glucose, sorbitol and fructose in lens tissue against an external standard, and provides a simple assay procedure for the efficacy of aldose reductase inhibitors such as sorbinil in reducing sorbitol accumulation. Linear calibration graphs were obtained for peak height against initial concentrations of glucose (r = 0.999), sorbitol (r = 0.999) and fructose (r = 0.999) in the range 5–50 μg/ml, the amount detected by HPLC being 50—500 ng. A typical chromatogram is shown in Fig. 2. The assay is currently being used to evaluate the effect of sorbinil on carbohydrate levels in cataractous lenses extracted from diabetic patients.

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REFERENCES

1 J.H. Kinoshita, Ann. Intern. Med., 101 (1984) 82.
2 J.J. Harding and M.J.C. Crabbe, in Davson (Editor), The Eye, Vol. 1B, Academic Press, New York, 3rd ed., 1984, p. 207.
3 G. Foulds, M.M. O'Brien, J.R. Bianchine and K.H. Gabbay, Clin. Pharmacol. Ther., 30 (1981) 693.
4 M. Petchey and M.J.C. Crabbe, J. Chromatogr., 307 (1984) 180.
5 M.J.C. Crabbe, M. Petchey, P. Burgess and H. Cheng, Exp. Eye. Res., 40 (1985) 95.
6 F. Nachtmann and K.W. Budna, J. Chromatogr., 136 (1977) 279.