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COMPUTER-AIDED OPTIMISATION OF DRUG ENANTIOMER SEPARATION IN CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The advent of several new column materials for the resolution of chiral compounds in high-performance liquid chromatography has opened up new possibilities for the analysis of drug enantiomers both in the dosage form and in bioanalytical studies. The utility of simplex optimisation, modified simplex and response surface mapping are considered with reference to the antischistosomal drug, oxamniquine, separated on an α_1-acid glycoprotein column. The resolution of the enantiomers of three closely related benzodiazepines, temazepam, oxazepam and lorazepam, is attempted on three new column systems: cellulose triacetate, β-cyclodextrin and the reversed-phase column porous graphitic carbon with β-cyclodextrin as a mobile phase additive.

INTRODUCTION

The observation that the chirality of a drug or pro-drug can influence one or all of the processes of absorption, distribution, metabolism or excretion has led to the search for suitable methods for separating the individual drug enantiomers, both in the dosage from and in biological fluids. Thus a number of stereoselective separation methods have been developed during the past decade. One group involves the formation of the diastereoisomers by using a suitable, pure chiral reagent. More recently methods have been developed that rely on the chiral selectivity of a new generation of column packing materials or on the addition of a chirally sensitive additive to the mobile phase used with a regular reversed-phase column. Thus any differences in the pharmacokinetic profiles and/or pharmacodynamic effects of a drug can be identified and quantified, thereby permitting the eutomer to be distinguished from the less therapeutically potent distomer and enabling the eudismic ratio of their relative potencies to be calculated [1].

A number of reviews have proposed various classifications of the chiral sta-

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tionary phases (CSP) currently available [2,3] and some have commented on the principal mobile phase additives for chiral recognition [4]. Although a large number of successful chiral separations have been published, little attention has been paid to the use of systematic methods for the optimisation of chiral separations. This is all the more remarkable in view of the delicate nature of the separation processes involved and the very great difficulty in establishing optimum chromatographic conditions. The present work reports some early experience in using the modified simplex approach to optimisation in chiral high-performance liquid chromatography (HPLC) as compared with response surface mapping. The key factors to be considered in optimising the chiral separation of some closely related benzodiazepine enantiomers are also considered.

EXPERIMENTAL

Apparatus and materials

The modular liquid chromatograph assembled in the laboratory comprised a dual reciprocating pump (LKB 2150), a variable-wavelength detector (LKB 2151), a strip chart recorder (Perkin-Elmer Model 56) and a Rheodyne loop-valve injector (Model 7010) equipped with a 20-μl loop.

All eluents were filtered in a Millipore all-glass apparatus equipped with a 0.45-μm membrane filter and degassed by ultrasonication.

The chiral columns used were as follows: α_1-acid glycoprotein (AGP) column, 100 mm$\times$5 mm (Enantiopac; LKB Produkter, Bromma, Sweden); 15–25 μm microcrystalline cellulose triacetate column, 250 mm $\times$5 mm (ConbrioTac; Hewlett-Packard, Waldbronn, F.R.G.); β-cyclodextrin column, 250 mm$\times$4.6 mm (Cyclobond I; Astec, Whippany, NJ, U.S.A.). The reversed-phase column employed in this work was porous graphitic carbon (PGC), 100 mm$\times$4.6 mm (Hypercarb; Shandon-Southern Products, Runcorn, U.K.).

Mobile phase constituents acetonitrile, methanol, ethanol and potassium dihydrogenphosphate were of HPLC grade and used without further purification. β-Cyclodextrin was obtained as a gift from Dr. G. Szepesy (Gedeon Richter, Budapest, Hungary) and used as received.

The drugs oxamniquine (Pfizer, Sandwich, U.K.), oxazepam (Wyeth, Taplow, U.K.), lorazepam and temazepam were kindly supplied by the manufacturers and used as received.

Software

Programs for the modified sequential simplex procedure were written in BASIC after an algorithm by Wright et al. [5] and implemented on a Hewlett-Packard HP 85 microcomputer with a printer–plotter (ThinkJet, Hewlett-Packard).

RESULTS AND DISCUSSION

AGP separation of oxamniquine

Although for optically active drugs with only one chiral centre there are only two enantiomers, with four or more optical isomers being more rarely observed

(e.g. labetolol with two chiral centres), it would be inappropriate to assume that optimisation of such separations is necessarily trivial, since the selectivity obtained is often very sensitive to experimental conditions. The modified sequential simplex method directs the optimisation process through the response surface defined by the key parameters that determine the separation. In the case of the AGP column, the X and Z coordinates of the response surface are propan-2-ol and pH, respectively, while the Y coordinate represents the chromatographic response function (CRF) defined in a manner suitable for the separation desired. For the separation of oxamniquine enantiomers, for example, this was derived from that proposed by Wright et al. [5]:

$$\mathrm{CRF} = (R_s/1.5)^2 - 0.25(T_L - 16) - 0.05(7 - T_F)$$

where T_F = time to appearance of first peak, T_L = time to last peak and R_s = resolution between enantiomer peaks; the constants $1/1.5^2$, 0.25 and 0.05 were determined empirically, to give a function that sharply discriminated manifestly unsatisfactory separations from better separations.

In the simplex procedure, the CRF is calculated for n sets of starting conditions, where n is given by one more than the number of factors to be optimised; in this case, therefore, n is 3. The point corresponding to the lowest value of CRF is then reflected about the axis defined by the two other points to give a fourth set of starting conditions. Once again, the point with the lowest CRF is reflected and the process repeated sequentially until an apparent optimum has been obtained. In the modified version of simplex, the search space is extended or contracted, in order to more rapidly focus on the optimum point in the response surface. In principle this procedure can be applied to any number of variables, represented in hyperspace. In this example, however, only two variables are involved, and these can be readily visualised, as indicated in Fig. 1, where the sequential movements of the simplex are represented. The 'optimum' was defined as that region where less than 2% change was observed in the CRF for successive points in the simplex. This corresponded to an eluent composition of (10 m*M* phosphate buffer + 0.1 *M* sodium chloride)–propan-2-ol (99.5:0.5, v/v) at pH 5.85. At this composition the α value was 2.00, the resolution R_s was 2.0 and the k' values for each enantiomer were 1.22 (laevorotatory) and 1.44 (dextrorotatory), respectively (Fig. 2). This optimum in the response surface was obtained after only thirteen experiments and it corresponded (within experimental error) exactly with the global optimum established by mapping the response surface by varying pH and propan-2-ol in a systematic manner. It was interesting to observe in the response surface contour plot of CRF versus pH and propan-2-ol, that a very sharp ridge in the pH dimension defines the optimum, illustrating the difficulty of locating the optimum response when employing the traditional 'single parameter at a time' approach.

However, despite the excellent resolution in this case, the performance of the column was ca. 900 theoretical plates per metre, a very low figure compared with reversed-phase achiral separations. Although the on-column detection limit (signal-to-noise ratio = 2) was ca. 0.5 ng oxamniquine and the detector response at

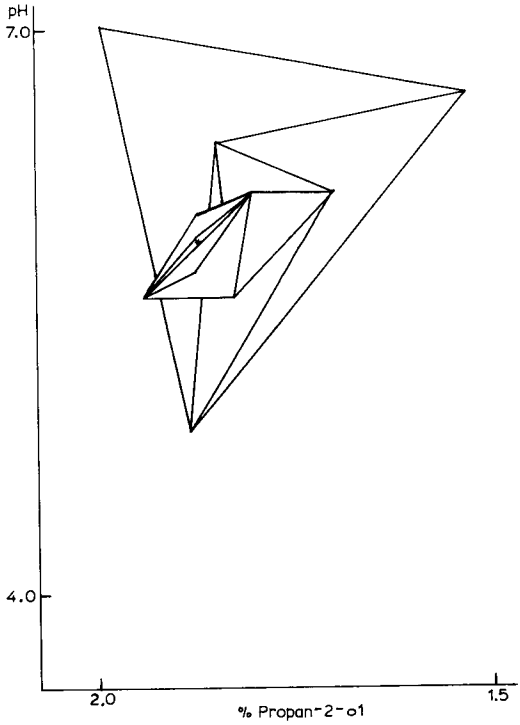

Fig. 1. Simplex optimisation of enantiomers of oxamniquine separated on an AGP column at room temperature (for chromatographic conditions: see text).

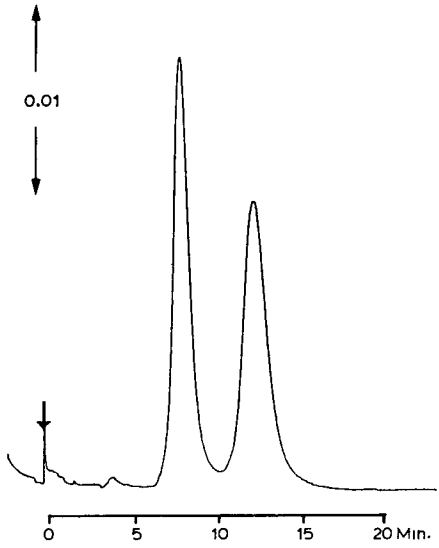

Fig. 2. Resolution of laevo- and dextrorotatory enantiomers of oxamniquine on an AGP column at room temperature.

260 nm was linear over the range 20 ng to 2 μg injected racemate, the low efficiency of this system limited its practical utility for bioanalytical separations.

Separation of enantiomers of benzodiazepines

In order to explore more fully the parameters that determined the resolution of a group of closely related enantiomeric benzodiazepines, a number of experiments were carried out utilising a new cellulose triacetate column, the well established β-cyclodextrin column and its transpose, namely the addition of β-cyclodextrin as a mobile phase additive in the eluent with the new PGC column developed recently by Gilbert et al. [6]. Temazepam, oxazepam and lorazepam feature a chiral centre in common, namely the 3-hydroxy-substituted benzodiazepine ring carbon. Temazepam is methylated at the C-1 ring position, whereas oxazepam and lorazepam are desmethyl. The only other distinguishing feature in this series is the 2-chloro substituent in the 5-phenyl group of lorazepam, the other two drugs bearing a phenyl substituent at C-5.

An initial analysis of this group of drugs would lead to the tentative prediction that their chiral separatory requirements should be closely similar. In a first analysis of the data from the cellulose triacetate column, this does not turn out to be the case. A mobile phase of ethanol–water (97.5:2.5, v/v) at neutral pH was found to yield partial resolution of temazepam (R_s about 0.8; Fig. 3), but no useful resolution of the enantiomers of the other two drugs (detection wavelength 340 nm). By contrast, the β-cyclodextrin column with a mobile phase of acetonitrile–methanol–0.05 *M* phosphate buffer (pH 5.0) (15:20:65, v/v/v) partially resolved oxazepam (Fig. 4) but not the other two benzodiazepines. Interestingly, the addition of β-cyclodextrin at 3 m*M* to the mobile phase of acetonitrile–0.005 *M* phosphate buffer (pH 12) yielded a successful separation on PGC both of the oxazepam enantiomers and of lorazepam, albeit with poor efficiency (Figs. 5 and 6).

It is apparent that even in the case of very closely related compounds, such as

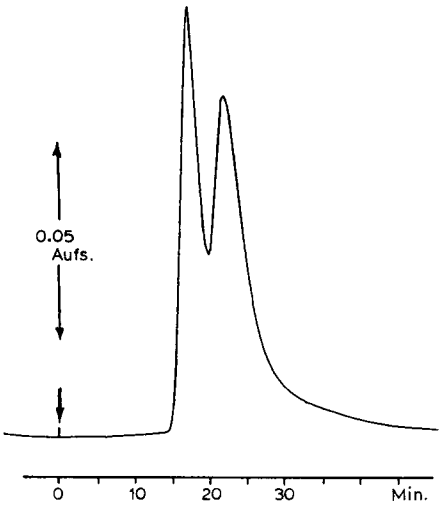

Fig. 3. Separation of enantiomers of temazepam on a cellulose triacetate column (for chromatographic conditions: see text).

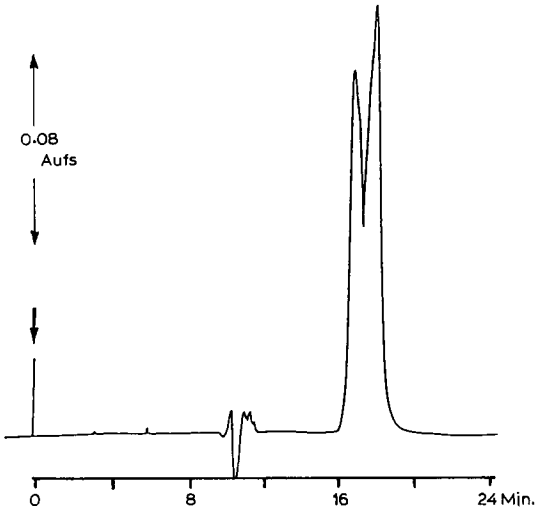

Fig. 4. Separation of enantiomers of oxazepam on a β-cyclodextrin-bonded column (for chromatographic conditions: see text).

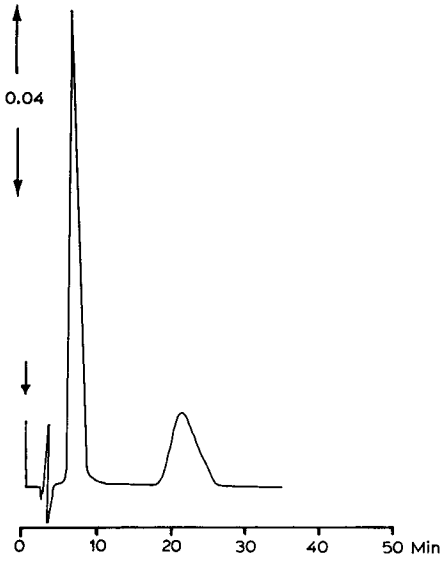

Fig. 5. Separation of enantiomers of oxazepam using β-cyclodextrin as a mobile phase additive with PGC (for chromatographic conditions: see text).

these benzodiazepines, subtle interactions come into play in determining the chiral resolution achievable. In such cases the application of systematic optimisation methods, including the iterative lattice method [5], would be expected to contribute in fine tuning these difficult separations. Nevertheless, it would seem that for bioanalytical purposes, at least, hybrid or multicolumn techniques should be considered, so that an achiral column is used to isolate the chiral solute before valve-switching onto a second chiral column, as advocated by Wainer [7]. However, the methods developed by Edholm [8] would seem to hold more promise for

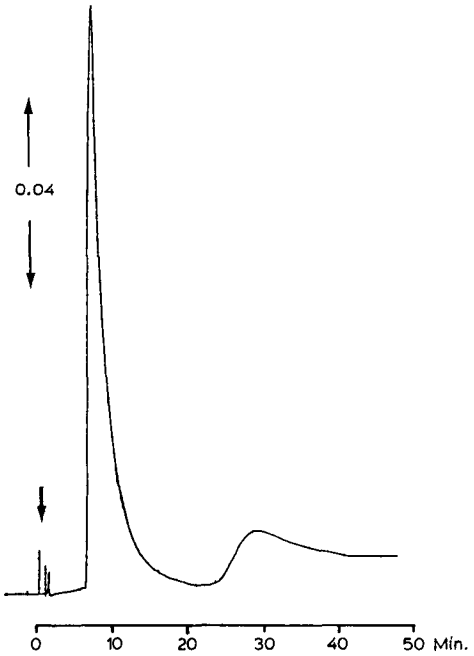

Fig. 6. Separation of enantiomers of lorazepam using β-cyclodextrin as a mobile phase additive with PGC (for chromatographic conditions: see text).

biofluid analysis, in that the chiral column separation is followed by a second column where peak compression occurs, thus improving the apparent efficiency and therefore the detection limits. A substantial body of work remains to be carried out in order to establish effective optimisation strategies for such coupled-column separations, where it is clear that simplex optimisation, iterative lattice and their extensions will have a vital role to play in future.

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