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Enantiomeric drugs — separation by chromatography or by specific immunoassay*

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Introduction

The objective of this paper is to describe an investigation of the usefulness of chiral phase columns in the separation of two enantiomers of a new potential inodilator drug and to compare this chromatographic capability with that of specific antibodies for monitoring plasma concentrations.

The compound, known to date as SK&F 94836, has one chiral centre involving an asymmetric carbon which has two enantiomeric forms by virtue of a methyl group substituent (Fig. 1).

Early preclinical studies of SK&F 94836 relied upon an HPLC/UV technique to quantify the racemate in plasma, but it quickly became apparent that there was a need

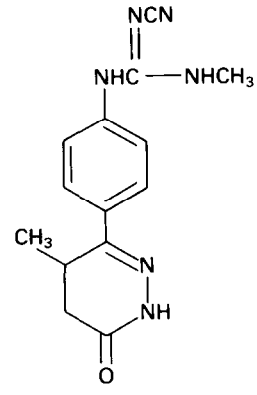

Figure 1
Structure of SK&F 94836.

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for a detector system that was capable of revealing much lower concentrations than did UV absorption, and for a method that would distinguish between the enantiomers. This requirement was established by the discovery that SK&F 94836 was very potent, that most of this potency resided in the R(−) enantiomer and that the plasma clearance of the two enantiomers was different.

Experimental

Columns

The UK suppliers of the chiral phase columns referred to in this communication were as follows: Pirkle Columns, Orbit Laboratories Ltd., Reading RG3 3DT, UK; Cellulose triacetate, BDH Ltd., Poole BH12 4NN, UK; Cyclobond (β-cyclodextrin), Technicol Ltd., Stockport, SK1 3HS, UK; Resolvosil (bovine serum albumen), Macherey-Nagel D 5160 Dueren, BRD, UK; Enantiopac (α-1 acid glycoprotein), LKB Instruments Ltd., Selsdon, CR2 9PX, UK; Apex Chiral, Jones Chromatography, Hengoed, CF8 8AU, UK; Chiralcel Types (Daicel), Anachem, Luton, Beds LU2 0EB, UK.

Results and Discussion

The initial strategy (Fig. 2) was to remove protein from the sample, then apply the resultant "extract" to a chiral phase column and fractionate the eluent for monitoring by means of a radioimmunoassay (RIA). The use of an RIA as the detector system was thought feasible because SK&F 94836 is not metabolised to any observable extent in any species studied.

For this RIA, antibodies have been raised in Soay sheep by the injection of a hapten bound to bovine serum albumin via a carbodiimide bridge (Fig. 3). An iodine-125 labelled antigen was synthesised from an analogue of SK&F 94836 (Fig. 3) to give competitive binding with SK&F 94836 itself. It should be noted however that the hapten used was in the racemic form as was the labelled antigen.

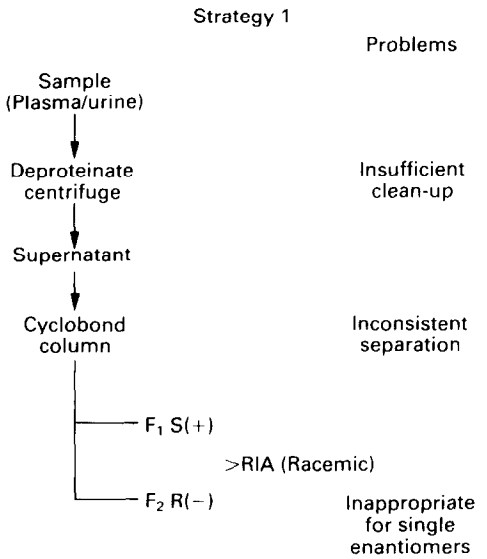


Figure 2
The initial strategy for chiral analysis.

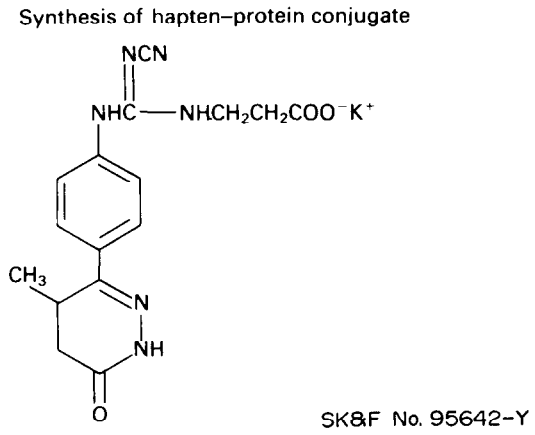
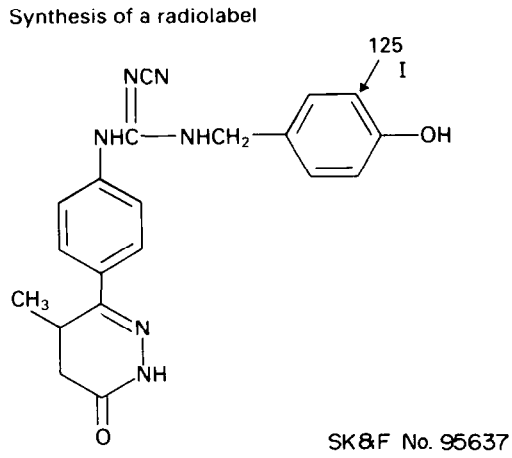


Figure 3
Precursors used to develop the radioimmunoassay.



Thus the antibodies were a mixed population formed as a response to both enantiomeric hapten–BSA complexes. Under these circumstances, it is pertinent to refer to papers by Cook and his co-workers (1982) and Rominger (1985) who point out the errors that can result from the use of racemic antigens in the analysis of enantiomers.

When this strategy was tested using β-cyclodextrin (Cyclobond) columns, several deficiencies emerged: (i) the simple deproteination step of precipitation with acetonitrile and centrifugation, was insufficient clean-up to allow good separation of enantiomers — this could be resolved by running the extract through a C18 column (15 cm), collecting the fraction containing racemic SK&F 94836 and applying this fraction to the β-cyclodextrin column; (ii) changing from one β-cyclodextrin column to another altered the degree of separation of the enantiomers markedly from 80–90% separation for the first column to zero differentiation for subsequent columns — the manufacturers were approached for a possible solution to the problem, but were unable to supply further

columns based on the original packing material, as there had been a change in composition of the silica backbone during the course of the work; (iii) it was found that the RIA became inaccurate when the ratio of (−) to (+) isomer was greater than 3:1 or less than 1:4 (Fig. 4).

In the face of these difficulties, this particular strategy was abondoned and other chiral phase columns were tested for their ability to separate the SK&F 94836 enantiomers. Of the columns tested (Table 1) with normal- and/or reversed-phase eluents, only Apex and Chiralcel OC types showed any promise.

As with the Cyclobond column, the Apex chiral (5 μm) gave 95% resolution initially (100% methanol at 25°C) and a flow rate of 0.2 ml min^{-1}, but this separation could not be sustained in the long term when further columns of the same manufacture were used.

The Chiralcel OC type has been more consistent but requires the complex clean-up of biological samples mentioned previously, i.e. before the enantiomeric separation it is necessary to go through the following steps: (i) precipitate protein with acetonitrile (1 volume); (ii) centrifuge; (iii) apply supernatant fraction to C18 column (mobile phase CH_3CN:buffer); (iv) collect appropriate fraction; (v) evaporate to constant volume to eliminate acetonitrile; (vi) apply residual aqueous fraction to Chiralcel OC and separate enantiomers with a mobile phase of 50% ethanol in hexane at 1.0 ml min^{-1}.

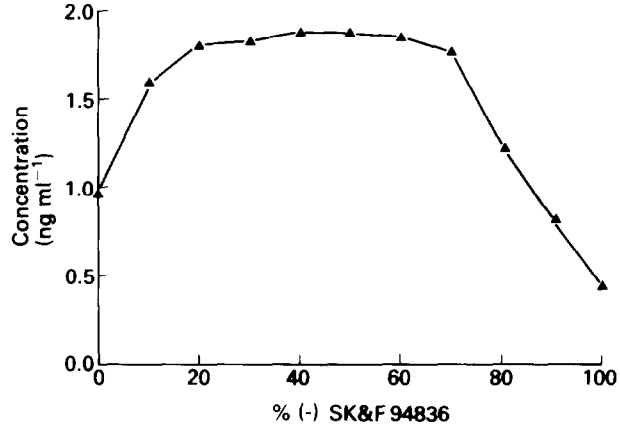


Figure 4
Variation in calculated drug concentration with difference in enantiomeric composition.

Table 1

"Chiral" columns examined

Normal phase	Aqueous phase
Pirkle	B.S.A.
Triacetyl cellulose	α-Acid glycoprotein
Cyclodextrins	Cyclodextrins
Acetylated cyclodextrin	Acetylated cyclodextrin
Apex	Chiralpak
Chiralcel	Chiralcel

This system produces 80–90% resolution in a run-time of 25 min, which is only useful for samples that contain enough of each enantiomer for UV detection and quantification using deconvolution techniques. Such a system is used to determine whether test animal species are eliminating the two enantiomers from the plasma at different rates, but cannot be applied to the monitoring of clinical samples because the concentrations in Phase I studies are below the limit of sensitivity for this method.

During the time of testing the various chiral phase chromatographic columns, further work on the RIA was undertaken. Enantiomeric haptens were used to generate antigens and to give the resultant antibodies from sheep. It was found that the antibodies raised in the response to the R(−) enantiomer form did not cross-react with S(+) SK&F 94836 to any significant extent. However the antibodies raised to the S(+) form in another animal did bind R(−) SK&F 94836 to the extent of about 10% of its affinity for the S(+) form.

When the enantiomers are present in a 1:1 ratio, this cross-reaction is unimportant and assay results from the (−), (+) and racemic assays are in agreement. However, when the ratio favours the (−) enantiomer by 3:1 or more, both the (+) and racemic assays become progressively less accurate. Results of studies to date in test animals indicate that the (−) enantiomer is eliminated more slowly from plasma with time and so the assay of both enantiomers must be applied frequently during the phase of elimination when enantiomer ratio does not exceed 3:1. Indeed by the time the ratio is inappropriate the (+) enantiomer assay has virtually reached its limit of sensitivity (about 20 pg ml^{-1}).

The accuracy of the RIA for (−) enantiomers of SK&F 94836 has been checked by comparing the concentrations recorded by the RIA with those calculated from ^{14}C counts in the plasma of animals which received radiolabelled (−) SK&F 94836, together with (+) enantiomer in the C12 form (Fig. 5). Again, it is important to note that both techniques are only applicable where metabolism of the compound in question is negligible, so that there is no cross-reactivity to interfere in the RIA and all of the ^{14}C can be attributed to unchanged SK&F 94836.

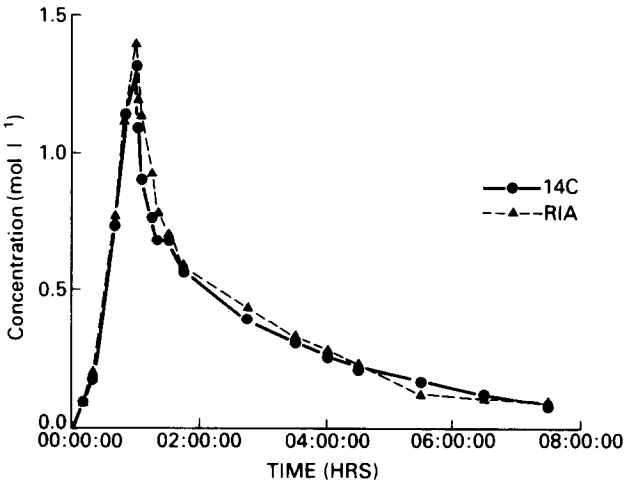


Figure 5
Comparison of R(−) SK&F 94836 RIA results with 14C − R(−) SK&F 94836.

The conversion of (+) and (−) SK&F 94836 to diastereoisomers has been considered but not attempted — the structure of the molecule does not offer any obvious site for derivatisation.

In future, chiral phases that separate the two enantiomers sufficiently may be found for accurate fraction cutting and subsequent assay on- or off-line by a technique with the detecting sensitivity of an immunoassay. Alternatively it may be possible to improve the selectivity of the antibodies generated in response to different haptens or the same haptens in different animals — thereby eliminating the cross-reactivity and permitting accurate assay of one enantiomer even in the presence of, say, a 10-fold excess of its partner.

Presently the pharmacological distinctiveness of methyl-based enantiomers continues to pose a problem for the analyst attempting to produce good data for the kinetic assessment of the distribution of such compounds in man and experimental animals.

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References

[1] C. E. Cook, T. P. Seltzman, C. R. Tallent and J. D. Wooten *J. Pharmacol. Exp. Ther.* **220**, 568–573 (1982).
[2] K. L. Rominger and H. J. Albert *Arzneim. Forsch.* **35**, 415–420 (1985).