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Enantiomeric resolution of sulindac by chiral-phase chromatography: determination of enantiomeric composition in human urine

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Sulindac is a nonsteroidal anti-inflammatory prodrug which contains a chiral sulphoxide moiety as part of its structure. The metabolism of sulindac involves reversible reduction to the pharmacologically active sulphide, irreversible oxidation to the corresponding sulphone and conjugation with glucuronic acid (Davies and Watson, 1997). The stereochemistry of the metabolism of drugs containing a chiral sulphoxide is complicated as a consequence of the enzymatic interconversion of the sulphide/sulphoxide redox states which results in the loss and generation of chirality. In order to investigate the stereoselectivity of sulindac metabolism we have examined the chromatographic resolution of the drug and developed a sequential achiral-chiral technique suitable for the determination of enantiomeric composition in biological fluids.

The chromatographic resolution of sulindac was achieved using an amylose tris (3,5-dimethyl-phenylcarbamate) chiral stationary phase (Chiralpak AD, 10 μm, 250 x 4.6 mm; mobile phase, hexane:ethanol, 85:15 v/v containing trifluoroacetic acid, 0.05% v/v; flow rate 1.0 mL min^{-1}; detection, UV 340 nm). Under these conditions the retention times of the enantiomers were 18.5 and 26.5 min with separation and resolution values of 1.43 and 2.46 respectively. Semi-preparative resolution was carried out using the same CSP and the enantiomers characterized by optical rotation, circular dichroism and NMR spectroscopy in the presence of the chiral shift reagent (-)-(*R*)-2,2,2-trifluoro-1-(9-anthryl) ethanol. Using these techniques the chromatographic elution order was determined to be (-)-(*S*)- before (+)-(*R*)-sulindac.

Following administration of racemic sulindac (200 mg) to three healthy male volunteers urine samples were collected at two hourly intervals up to 12 h post-dosing followed by a 12-24 h sample. Urine samples were diluted between 2 to 20 fold with water, depending on the drug concentration present, and were analysed directly or following hydrolysis of the acylglucuronide conjugates. Diluted urine (1.0 mL), was acidified (HCl 1.0 M, 200 μL), the internal standard (indomethacin, 100μL, 0.1 mg mL^{-1}) added and the whole extracted with diethylether (8 mL). The mixture was centrifuged, the organic phase removed, evaporated to dryness and the residue reconstituted in mobile phase (100 μL) and analysed by reversed phase HPLC (Spherisorb S5 ODS2, 5 μm, 250 x 4.6 mm; mobile phase, aqueous acetic acid (2% v/v):acetonitrile:THF, 50:48:2 by volume; flow rate 1.0 mL min^{-1}; detection, UV 340 nm). Hydrolysis samples were treated with NaOH (1.0 M; 200 μL) for 2 h at room temperature prior to acidification (HCl, 1.0 M; 400 μL) and treated as described above. Under the above chromatographic conditions the retention times of sulindac, sulindac-sulphone, internal standard and sulindac-sulphide were 4.9, 6.6, 12.7 and 32.6 min respectively. The HPLC eluate (~0.5 mL) containing sulindac was collected, extracted with diethylether (5 mL), the organic phase removed, evaporated to dryness and the residue reconstituted in mobile phase (150 μL) and subjected to chiral phase analysis.

Analysis of 0-24 h urine samples yielded a total drug and metabolite recovery of ~20% of the dose with the enantiomers of sulindac, both free and conjugated, accounting for approximately half the recovered material. The mean enantiomeric ratio (*R*/*S*) over the collection period for both free and conjugated drug was 1.2 and 1.7 respectively. However, the enantiomeric composition varied markedly with collection period, and between volunteers, which is presumably associated with the complex metabolism of the drug.

Davies, N.M. and Watson, M.S. (1997) Clin. Pharmacokin. 32 : 437-459.