

# Comparative Chromatographic Study on the Chiral Separation of the 1-Naphthylamine Derivative of Ketoprofen on Cellulose-Based Columns of Different Sizes

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## INTRODUCTION

The enantiomeric separation of several 2-arylpropionic acids on a cellulose-based chiral stationary phase was studied (van Overbeke *et al.*, 1994). Chiral discrimination could not be obtained until after precolumn derivatization of the carboxylic acid moiety. The separation of ketoprofen enantiomers as their 1-naphthylamine derivative on cellulose columns of different sizes (equal lengths of 15 cm, different i.d. of 4.6 and 2.1 mm) is presently reported.

## CHROMATOGRAPHIC CONDITIONS

For chromatographic conditions see Table 1.

## RESULTS

### Reproducibility of derivatization

1-Ethyl-3-dimethylaminopropyl-carbodiimide (EDC) acts as a coupling agent for the formation of an amide binding between the carboxylic acid moiety and the

primary amine, in the presence of hydroxybenzotriazole (HOBT) as catalyst.

To 1 mL ketoprofen solution containing 61.7 mg in 50 mL methylene chloride, 300  $\mu$ L HOBT solution (1 mg/mL in methylene chloride with 1% pyridine), 300  $\mu$ L/EDC solution (550 mg/50 mL) and 300  $\mu$ L 1-naphthylamine (173.7 mg/50 mL), both dissolved in methylene chloride, were added. The mixture was vortexed and left for 1 h before it was evaporated under a nitrogen stream. The residue was dissolved in 5.0 mL methanol.

This procedure was repeated ten times and each sample was analysed once.

### Linearity

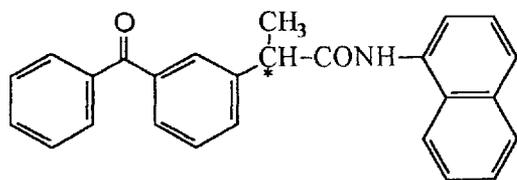
For a limited study on the linearity, derivatization of one tenth to a double amount of ketoprofen (compared to the experiment above) was carried out. Chromatography was monitored at 220 and 256 nm. Since problems on the narrow-bore scale arose at 220 nm owing to detector overload, the size of the loop was reduced from 3 to 1  $\mu$ L. For the latter, the correlation coefficient was not better than 0.998, whereas correlation exceeded 0.9996 in the other cases.

Table 1. Chromatographic conditions

Column	Conventional See <sup>a</sup> 150 $\times$ 4.6 mm i.d.	Narrow-bore See <sup>a</sup> 150 $\times$ 2.1 mm i.d.
Eluent	See <sup>b</sup>	See <sup>b</sup>
Flow	1.0 mL/min	0.21 mL/min
Pump	Varian 9010 SDS	Shimadzu LC 9A
Injection system	Rheodyne 7125, 20 $\mu$ L/loop	Rheodyne 7725i, 3 $\mu$ L external loop, peek tubing or Valco, 1 $\mu$ L internal loop
Detection	HP series 1050 DAD flow cell 8 $\mu$ L	Shimadzu SPD-10 A UV-VIS flow cell 0.2 $\mu$ L

<sup>a</sup> Tollycellulose EXP B101 consists of a tris(4-methylbenzoate)cellulose derivative covalently bonded onto a 10  $\mu$ m aminopropylsilica layer with a mean pore size of 300  $\text{Å}$ .

<sup>b</sup> To a NaClO<sub>4</sub> solution (14.05 g/L, 0.1 M), perchloric acid was added to pH 2.0 and mixed with methanol up to a ratio of 15:85.



**Figure 1.** Structure of 1-naphthylamine derivative of ketoprofen.

**Table 2.** Integration results for the reproducibility test

	Conventional		Narrow-bore	
	Average	RSD	Average	RSD
AUC 1st peak, 256 nm	6475	0.94	19,006,058	1.25
AUC 2nd peak, 256 nm	6452	0.69	19,079,772	1.66

### Detection limit

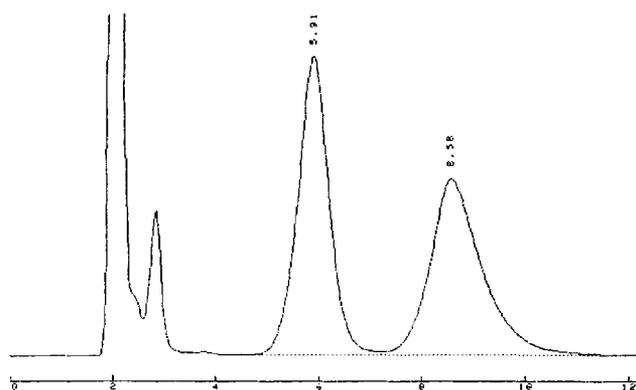
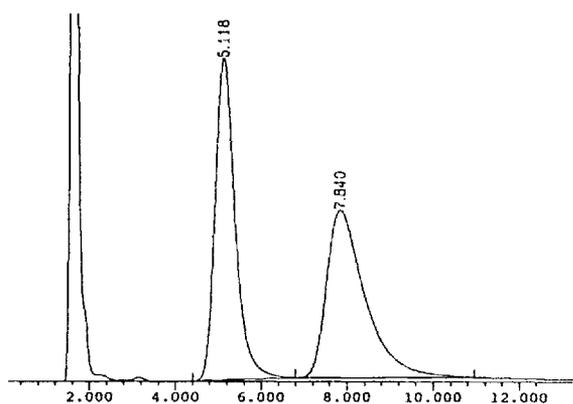
A detection limit ( $S/N > 3$ ) of 2.5 ng ketoprofen, submitted to derivatization, was found for the conventional application, 74 ng on the narrow-bore scale, both at 256 nm. At 220 nm 100 pg was recorded as the detection limit for the narrow-bore chromatography.

### CONCLUSION

Chromatography performed on columns of classical sizes can be transferred to narrow-bore scale without serious problems. In spite of lower plate numbers and smaller resolutions for the narrow-bore application, the enantiomers of the 1-naphthylamine derivative of ketoprofen were discriminated adequately, consuming less eluent and improving the detection limit.

### Acknowledgement

The authors wish to thank the Shimadzu Company for providing the set of narrow-bore instruments described.



**Figure 2.** Chromatograms of the 1-naphthylamine derivative of ketoprofen. (a) Conventional:  $k'1$ , 2.73;  $k'2$ , 4.71;  $\alpha$ , 1.73;  $R_s$ , 2.27;  $N1$ , 594;  $N2$ , 398. (b) Narrow-bore:  $k'1$ , 2.53;  $k'2$ , 4.12;  $\alpha$ , 1.63;  $R_s$ , 1.67;  $N1$ , 389;  $N2$ , 291.

### REFERENCES

- Van Overbeke, A., Baeyens, W., Van den Bossche, W. and Dewaele, C. (1994). *J. Pharm. Biom. Anal.* **12**(7) 901.