

## SEPARATION OF THE ENANTIOMERS OF IBUPROFEN BY A GAS CHROMATOGRAPHIC– MASS SPECTROMETRIC METHOD

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### SUMMARY

A gas chromatographic–mass spectrometric (GC–MS) method using selected ion ionization (SIS) and tandem mass spectrometry (MS–MS) has been established for separation of the enantiomers of ibuprofen, an analgesic, from tablets. The separation is performed on a chiral capillary column. The chromatographic conditions for enantiomer separation are presented.

### INTRODUCTION

Separation of the enantiomers of chiral drugs has become an important issue in analytical clinical chemistry in recent years, because of differences in the biological activity and pharmacokinetic properties of drug enantiomers [1,2]. Many drugs contain chirality centers; their enantiomers may have considerably different potency, type of pharmacological activity, pharmacokinetics, and metabolism because most enzymatically catalyzed reactions are stereoselective [3]. Thus, in the preparation and analysis of chiral drugs it is of vital importance to separate their enantiomeric forms.

Ibuprofen, *R,S*-2-(4-isobutylphenyl)propionic acid, a derivative of 2-arylpropionic acids (profens), is a chiral non-steroidal anti-inflammatory drug (NSAID) which has pharmacological action similar to other drugs in this class, for example fluribuprofen, fenoprofen, or ketoprofen. It is extensively used because of its excellent pharmaceutical properties and because it is better tolerated than aspirin, indomethacin, and pirazolonic derivatives, with analgesic, antipyretic, and platelet antiaggregant properties. It is used

to relieve moderate pain, acute arthritis, non-rheumatic inflammation, fever, and dysmenorrhea [4]. The propionic acid side-chain of ibuprofen contains an asymmetric  $\alpha$ -carbon, resulting in two optical isomers. Ibuprofen is marketed as a racemate, but its pharmacological activity is due almost exclusively to the *S*-(+) enantiomer [5].

Several assays for measurement of the enantiomers of ibuprofen have been reported in the literature. These include direct and indirect liquid chromatographic methods [6–10], supercritical-fluid chromatography (SFC) [11], combination of micellar electrokinetic chromatographic (MEKC) separation with mass spectrometric (MS) detection [12], capillary electrophoresis [13–15], diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy [16], and gas chromatography (GC) [17]. According to the literature, the enantiomers of ibuprofen have rarely been separated by GC, because of temperature limitations. Most chiral columns have a maximum operating temperature of 230°C, and many drugs with optical activity boil at higher temperatures than this.

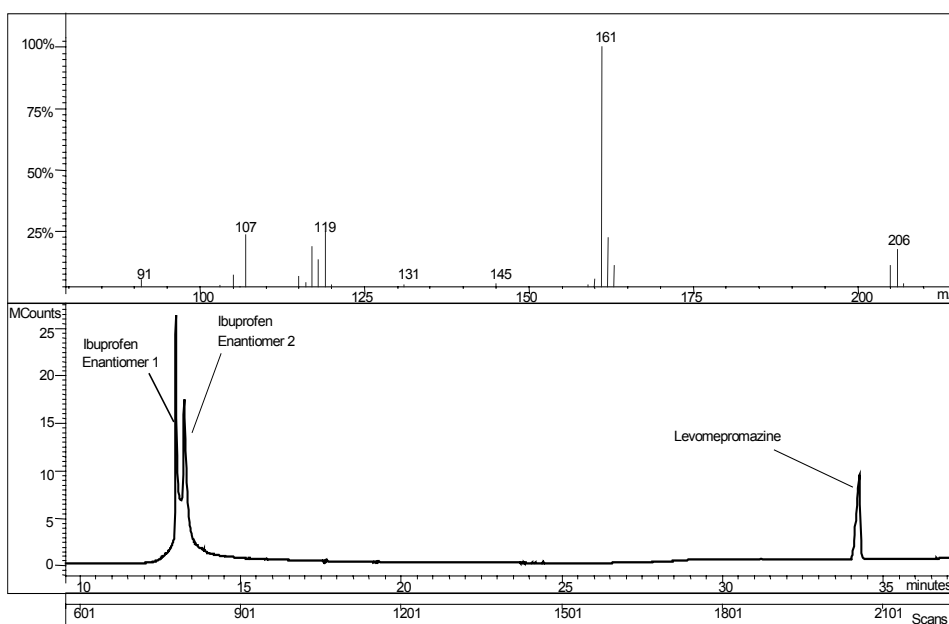
This paper reports the development of a simple gas chromatographic method for separation of the two enantiomers of ibuprofen on an Astec ChiralDEX chiral column with dimethyl- $\beta$ -cyclodextrin as stationary phase. Selected ion ionization (SIS) mode can increase the sensitivity of the method; tandem mass spectrometry (MS–MS) also increases the sensitivity and can also give more information about chemical structure and for identification of the enantiomers. The limitations and advantages of GC–MS or GC–MS–MS for separation of the enantiomers ibuprofen are compared and discussed. The method has a major advantage because separation of the enantiomers was achieved without derivatization.

## EXPERIMENTAL

A Saturn 2000 ion-trap mass spectrometer from Varian Instruments (Mecro Systems, Romania) was used. The integrated gas chromatograph was a GC Star model 3800 fitted with an autosampler (Combi-Pal), a split–splitless programmed temperature injector (1079), and an Astec ChiralDEX chiral column with dimethyl- $\beta$ -cyclodextrin as stationary phase. The ion-trap mass spectrometer was operated in electron impact (EI) mode (without ion preparation–normal EI, and with ion preparation–SIS) with the MS–MS option for ibuprofen. PMW, NIST98 and Wiley6 were used as libraries for EI. The carrier gas used was helium (purity 99.99%).

Dilute samples (1  $\mu\text{L}$ ) were injected with a 1:10 split ratio. The injector temperature was programmed to 230°C. The oven temperature was held at 100°C for 1 min after injection then programmed at 10°  $\text{min}^{-1}$  to 180°C which was held for 2 min, then at 4°  $\text{min}^{-1}$  to 200°C, which was held for 10 min, then finally at 10°  $\text{min}^{-1}$  to 230°C which was held for 21 min; the total programming time was 50 min. The MS conditions were: solvent-delay time 5 min; electron energy 70 eV; scan rate 1 scan  $\text{s}^{-1}$  and scanned-mass range ( $m/z$ ) 91–205.

Chromatographic-quality solvent (methanol) and phenobarbital (used as internal standard) were purchased from Merck. All other chemicals and solvents were of analytical-reagent grade and used without further purification. Pharmaceutical tablets were used as the source of ibuprofen enantiomer separation. A 50-mg tablet was extracted with methanol, with ultrasound, and the extract was filtered and diluted 1000-fold, so the final ibuprofen concentration was 50  $\mu\text{g mL}^{-1}$ . The same method was investigated with *n*-hexane as solvent, but with poor results.



**Fig. 1**

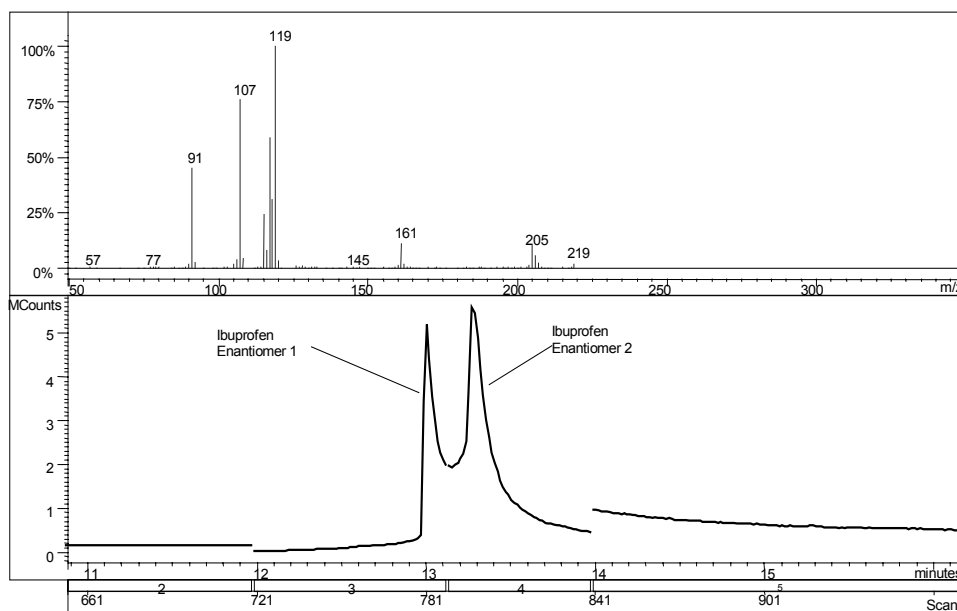
Chromatogram and mass spectrum of ibuprofen enantiomers obtained in EI normal mode

## RESULTS AND DISCUSSION

The chromatograms and mass spectra obtained from the enantiomers of ibuprofen in normal EI mode and in SIS mode are presented Figs. 1 and 2, respectively. The ions of  $m/z$  91, 107, 119, 161, and 205 were stored. There are small differences between the two spectra and sensitivity is higher in SIS mode because noise was reduced. Figure 3 shows the chromatogram and mass spectrum obtained from the enantiomers of ibuprofen in MS–MS mode. The parent ion was situated at  $m/z$  205. Because all the noise was ejected from the ion trap, the sensitivity was increased.

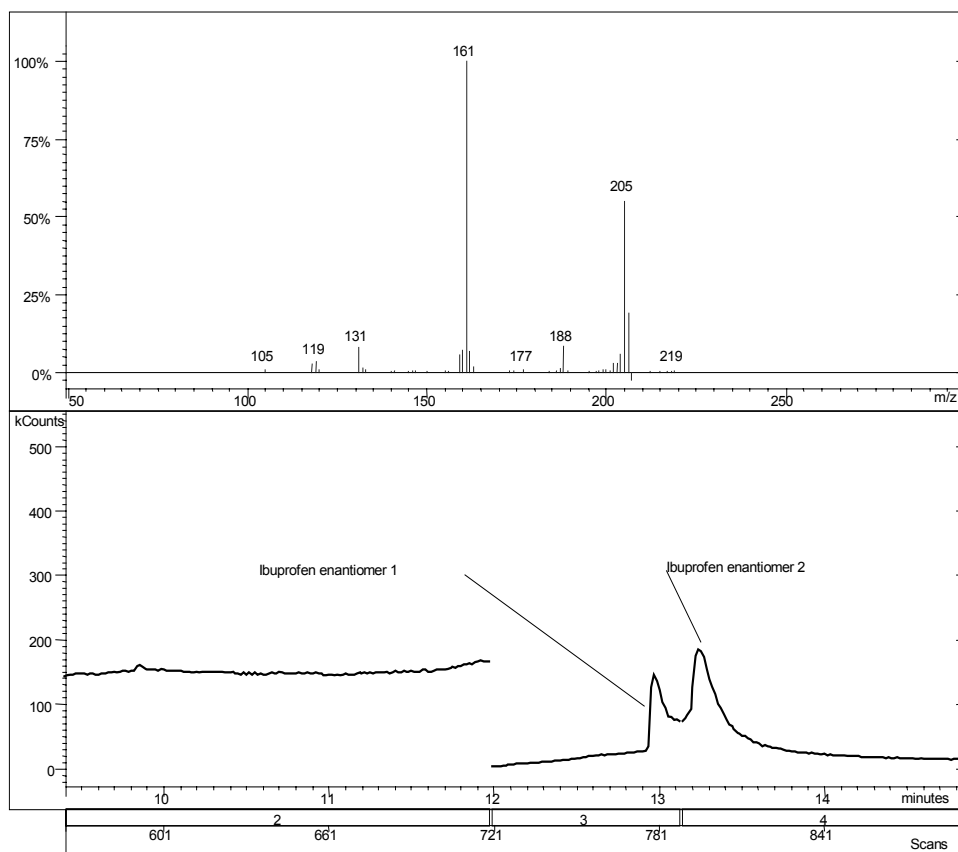
It is apparent that in EI mode without ion preparation the peak of enantiomer 1 is higher than that of enantiomer 2 whereas in the ion preparation mode and in MS–MS the opposite is true.

MS–MS–MS mode was used by storing and fragmenting the ions at  $m/z$  205 and 161 as ion parent and first daughter ion, but the results did not show any advantages over MS–MS mode with regard to sensitivity or structural information.



**Fig. 2**

Chromatogram and mass spectrum of ibuprofen enantiomers obtained in SIS mode



**Fig. 3**

Chromatogram and mass spectrum of ibuprofen enantiomers obtained in MS–MS mode

**Table 1**

Ratio of the areas and heights of the peaks of the ibuprofen enantiomers to area and height of the phenobarbital (IS) peak

	Chromatogram 1	Chromatogram 2	Chromatogram 3
Area ratio enantiomer 1/IS	3.2881	5.0226	4.8106
Height ratio enantiomer 1/IS	3.8714	6.1095	5.8231
Area ratio enantiomer 2/IS	4.1807	6.0542	6.7258
Height ratio enantiomer 2/IS	2.4581	3.3778	3.4637

To check the reproducibility of the methods, three analyses of the same concentration of ibuprofen were performed, using phenobarbital as internal standard (IS). Table I shows the ratios of the areas and heights of the peaks of the ibuprofen enantiomers to those of phenobarbital.

## CONCLUSIONS

GC–MS can be used for separation and analysis of the enantiomers of ibuprofen in biological samples obtained after treatment with this analgesic and anti-inflammatory drug. It is also useful for analysis of pharmaceutical products. The method is very sensitive and very specific in comparison with other methods, e.g. HPLC with classic detection; the latter is not useful for analysis of unknown biological samples because of their complex matrices. Although the sensitivity of the method was not evaluated it was apparent that it was possible to measure concentrations at ppm levels. When SIS and MS–MS are used quantification at sub-ppm levels is possible, because use of SIS and MS–MS result in improved sensitivity in trace analysis of drugs in biological samples.

The main advantage of the method is that separation of the enantiomers was achieved without derivatization. MS–MS–MS had no important advantages over SIS and MS–MS.

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