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Short Communication

Gas chromatographic–mass spectrometric determination of ibuprofen enantiomers in human plasma using *R*(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol as derivatizing reagent

Min-Jie Zhao*, Céline Peter, Marie-Claude Holtz, Nathalie Hugenell, Jean-Claude Koffel, Louis Jung

Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, 74, route du Rhin, 67401 Illkirch, France

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Abstract

A relatively rapid, inexpensive, sensitive and stereospecific gas chromatographic–mass spectrometric method was developed for the quantification of *S*(+) and *R*(−)-ibuprofen in human plasma. This method uses a commercially available internal standard and has no interference from endogenous substances nor metabolites. The method involves derivatization of ibuprofen enantiomers with optically active *R*(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol using oxalyl chloride as the coupling reagent. The subsequently formed diastereoisomers are separated by gas chromatography and analysed by mass spectrometry using selected-ion monitoring. The assay is successfully applied to a pharmacokinetic study. The simplicity, sensitivity and precision of the method make it convenient for the quantification of ibuprofen enantiomers in biological samples.

1. Introduction

Ibuprofen, *R*,*S*-2-(4-isobutylphenyl)propionic acid, is an important non-steroidal antiinflammatory analgesic and antipyretic drug widely used in the treatment of rheumatic disorders, pain and fever [1]. The propionic acid side-chain contains an asymmetric α-carbon, giving two optical isomers. The drug is normally administrated as the racemate, but only the *S*-form is responsible for the pharmacological effect [2]. However, numerous studies have documented the pharmacokinetics of ibuprofen and described the marked unidirectional inversion of the *R*-form to the *S*-form [3–8]. There are a number of gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods available for the separation and quantification of ibuprofen enantiomers from biological fluid samples [4,5,9–14]. Many of these methods require lengthy precolumn derivatization procedures and sample preparation, or lack sufficient sensitivity. Recently a gas chromatographic–mass spectrometric (GC–MS) method via derivatization with dexamphetamine using ethylchloroformate as the coupling reagent was reported for the quantification of ibuprofen enantiomers [10]. That method, however, requires the use of the deuterated internal standards that are not commercially available. This paper reports derivati-

* Corresponding author.

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zation in a single step with $R(-)$-2,2,2-trifluoro-1-(9-anthryl)ethanol using oxalyl chloride as the coupling reagent, followed by GC–MS using selected-ion monitoring (SIM) for the efficient separation and quantification of ibuprofen enantiomers in human plasma samples.

2. Experimental

2.1. Reagents and chemicals

Samples of $S(+)$-, $R(-)$- and R,S-ibuprofen were generous gift of Medice (Iserlohd, Germany). $R(-)$-2,2,2-Trifluoro-1-(9-anthryl)ethanol (R-TFAE), oxalyl chloride and the internal standard 4-methoxyphenylacetic acid were purchased from Aldrich (Saint-Quentin-Fallavier, France). Diethyl ether absolute (Aldrich) was A.C.S. grade with peroxide content ≤ 1 ppm. All other solvents were of analytical grade (Merck, Nogent-sur-Marne, France).

2.2. Standard solutions

The following solutions were prepared and stored at 4°C: 4-methoxyphenylacetic acid, $S(+)$-, $R(-)$- and R,S-ibuprofen (10 mg/100 ml in acetonitrile). From each of these solutions, working solutions (1 mg/100 ml) were made on the day of the experiment.

2.3. Instrumentation

The gas chromatograph–mass spectrometer was a Ribermag R10-10 Delsi (Rueil-Malmaison, France), equipped with a 2.0 m $\times$ 2 mm I.D. glass column packed with 3% phenylmethylsilane on Chromosorb WHP 100–120 mesh. Helium was used as carrier gas at a flow-rate of 20 ml/min. Chromatograph oven, injector block and interface temperatures were set at 320, 310 and 320°C respectively. The ion source temperature was *ca.* 280°C. Mass spectra were recorded at an electron energy of 100 eV in the chemical-ionisation (CI) mode (NH_3). Data aquisation and reduction were done with the Nermag Sidar computing system (Tektronix 4010-1). Quantitative analyses of all samples were performed on the system described above by selected-ion monitoring (SIM) at m/z 482 for derivatized ibuprofen and 442 for the derivatized internal standard in ammonia-CI-MS, including peak area calculations.

2.4. Drug extraction and derivatization

A 100-μl volume of internal standard solution was introduced into a 10-ml tube fitted with a PTFE-lined screw cap; after evaporation of the organic solvent, aliquots (0.1–0.2 ml) of plasma were added, followed by 0.5 ml of 1 *M* hydrochloric acid and 5 ml diethyl ether. The tube was vortex-mixed for 15 s and then centrifuged for 15 min at 3000 *g*. The organic phase was transferred to a 5-ml tube (fitted with a PTFE-lined screw cap). The extraction of the acid aqueous solution was repeated with 5 ml of diethyl ether. Organic phases were combined and evaporated to dryness under a gentle stream of nitrogen gas. The residue was dissolved in 50 μl of R-TFAE (10 mg/ml) in acetonitrile and 150 μl of oxalyl chloride were added. The reaction mixture was allowed to stand for 2 h at 60°C and then evaporated to dryness under a gentle stream of nitrogen gas. The residue was reconstituted with toluene (20 μl) and a 2-μl aliquot of this solution was analysed by GC.

2.5. Calibration curves

For the purposes of calibration, 0.1 ml samples of blank plasma were spiked with various aliquots of the standard $R(-)$- and $S(+)$-ibuprofen solutions to give final quantities of 0.01, 0.1, 0.5, 1, 1.5, 2, 2.5, 3.5, and 5.5 μg ibuprofen. Following the addition of 100 μl (1 μg) 4-methoxyphenylacetic acid solution, the extraction and derivatization procedure was carried out as described above. Quantification was based on peak-area ratio [m/z 482 (derivatized ibuprofen) to m/z 442 (derivatized internal standard)].

3. Results and discussion

The esters of ibuprofen and internal standard were synthetized by converting the corresponding acids into their acid chlorides with oxalyl chloride followed by the addition of *R*-TFAE (Fig. 1). We observed that the transformation of ibuprofen to its ester derivative via two separate steps resulted in a variable amount of end-product. In fact, the acid chloride of ibuprofen, formed in the first step, was unstable and relatively volatile. In order to solve this problem, *R*-TFAE was added at the beginning of the reaction. Consequently, the acid chloride of ibuprofen reacted with *R*-TFAE immediately after its formation. The reaction could be monitored by ^{1}H nuclear magnetic resonance since the chemical displacement of the proton on the asymmetric carbon in ibuprofen and its ester derivative is different (−0.4 ppm). At the end of the reaction, the signal of the proton in ibuprofen should completely disappear and the reäction was estimated to be completed. Different reaction temperatures for derivatization were compared: 30, 40, 50 and 60°C. It was observed that the time needed to complete the reaction was much longer when the temperature was low. At 60°C, the reaction was complete after 2 h. In contrast, at 30°C only *ca.* 20% of ibuprofen had reacted over the same period of time.

Under the assay conditions described, the derivatized ibuprofen isomers were clearly separated from each other with retention times of 2.15 min for *S*(+)-ibuprofen and 2.55 min for *R*(−)-ibuprofen. The derivatized internal standard had a retention time of 2.52 min. Typical SIM chromatograms of the ibuprofen diastereoisomers and derivatized internal standard are shown in Fig. 2. For ibuprofen, SIM of m/z 482 gave the cleanest trace without interfering peaks, whilst m/z 442 was best for the internal standard. Hence, quantification of ibuprofen was carried out by selecting the ions m/z 482 and m/z 442.

The MS fragmentation patterns for each diastereoisomer prepared from authentic standards

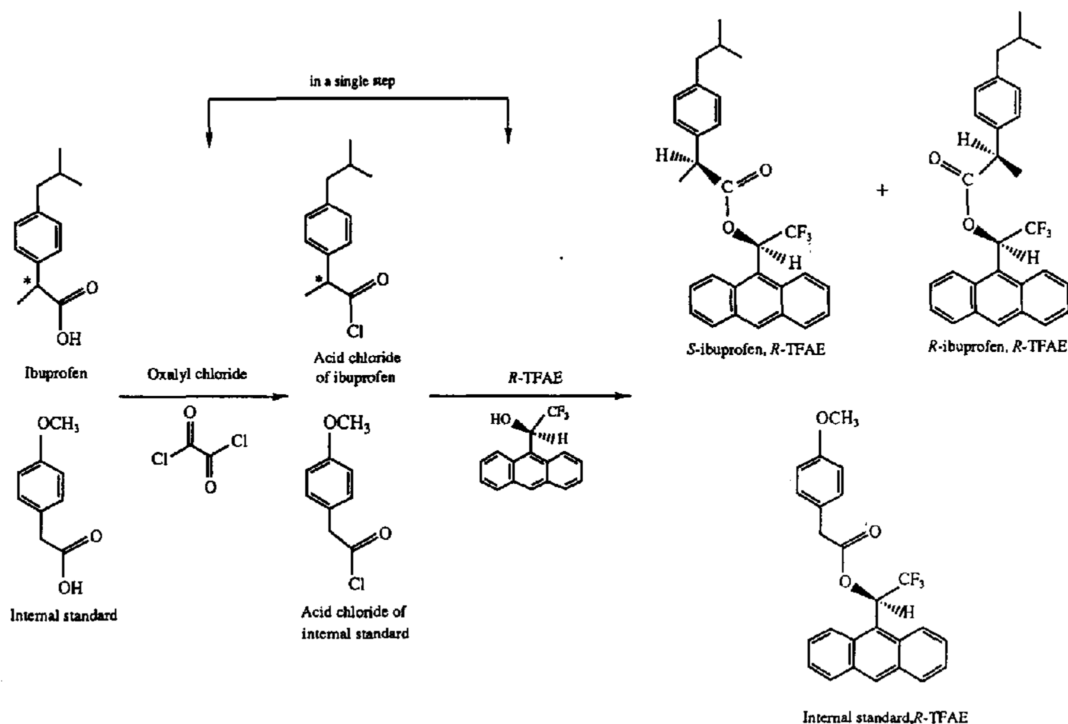

Fig. 1. Reaction scheme of the derivatization of *R*,*S*-ibuprofen enantiomers and the internal standard.

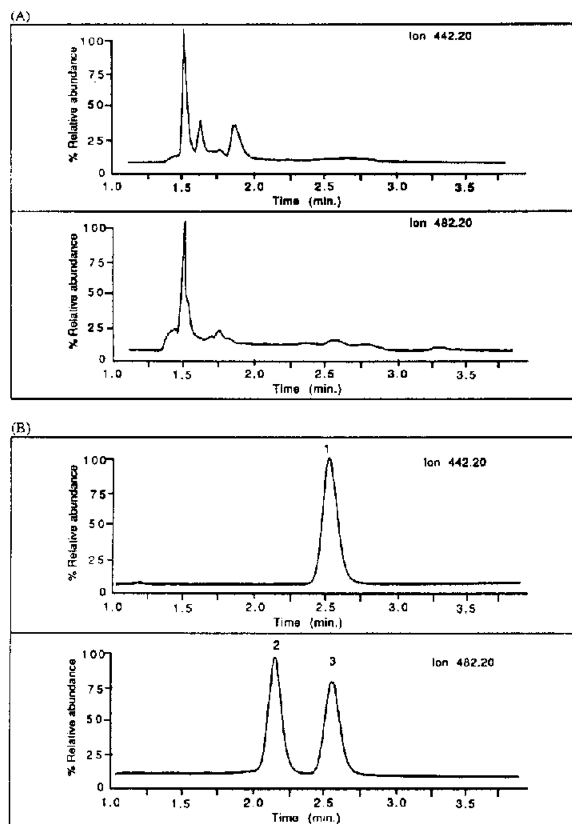

Fig. 2. SIM chromatograms of (A) control plasma before administration of ibuprofen, and (B) plasma, 1.25 h after a single oral dose of 800 mg of *R*,*S*-ibuprofen. Drug levels were: 40.9 μg/ml *S*(+) ibuprofen (peak 2) and 31.0 μg/ml *R*(−) ibuprofen (peak 3). Peaks: 1 = internal standard-*R*-TFAE (100% = 9 837 421); 2 = *S*(+)-ibuprofen-*R*-TFAE; 3 = *R*(−)-ibuprofen-*R*-TFAE (100% = 4 230 412).

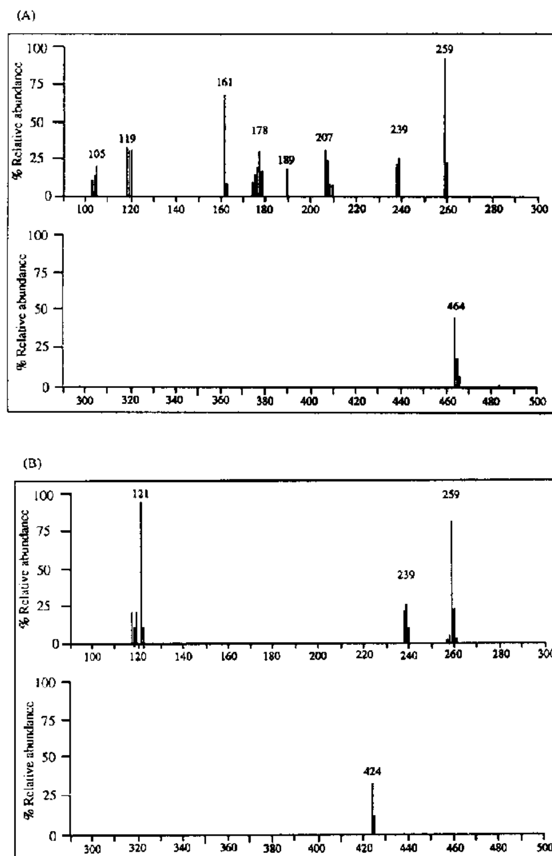

Fig. 3. Mass spectra of *R*,*S*-ibuprofen-*R*-TFAE (A), internal standard-*R*-TFAE (B).

were identical to those of the respective isomers observed in the samples (Fig. 3A). Characteristic fragment ions of significant abundance (relative abundance >35%) were present in both spectra and readily identified: *m*/*z* [identity]: 464 $[M^+]$; 259 $[M - OCOCH(CH_3)PhC_4H_9]^+$; 161 $[M - O{=}C{-}O{-}CH(CF_3)C_{14}H_9]^+$. The MS fragmentation pattern for the derivatized internal standard is shown in Fig. 3B. Characteristic fragment ions of significant abundance (relative abundance >35%) were identified: *m*/*z* [identity]: 424 $[M]^+$, 259 $[M - OCOCH_2PhOCH_3]^+$; 121 $[M - O{=}C{-}O{-}CH(CF_3)C_{14}H_9]^+$.

The recovery of *S*(+)- and *R*(−)-ibuprofen was determined by spiking 0.1 ml of plasma with 1 μg of each enantiomer. After extraction, 0.1 ml internal standard solution was added, and the samples were derivatized and analyzed. From the difference between the measured quantities and those theoretically expected extraction efficiency was calculated. A variety of extraction solvents were compared: dichloromethane, *n*-hexane, *n*-hexane–diethyl ether (50:50, v,v), and diethyl ether. The latter gave the cleanest and most efficient extraction. The recoveries (mean ± S.D.) found for *S*(+)- and *R*(−)-enantiomers were 58.9 ± 3.7% ($n = 5$) and 59.5 ± 5.7% ($n = 5$), respectively.

The calibration curves for both ibuprofen enantiomers were linear within the examined

concentration range. Typical curves for $S(+)$- and $R(-)$-ibuprofen could be described by $y = 0.06640\ x - 0.00434$ ($r = 0.993$, $n = 9$) and $y = 0.06017\ x - 0.00427$ ($r = 0.992$, $n = 9$), respectively [where y is the peak-area ratio and x is the enantiomer concentration (μg/ml)]. The minimum quantifiable concentration of each derivatized enantiomer was *ca.* 5 ng/ml in plasma with a precision better than 8% (signal-to-noise ratio = 3). Contamination from endogenous substances and metabolites did not interfere with the analysis.

Reproducibility and accuracy of the procedure are described in Table 1. The differences between the concentrations assayed and the theoretical values were <5% and reproducibility was satisfactory for all the 3 concentrations studied (S.D. < 5%).

Although all samples in the current study were generally analysed within 24 h after derivatization, the results of sample stability studies demonstrated that the samples could be stored at 4°C for at least 10 days without significant degradation. These assays were then used to determine the plasma concentration of $S(+)$- and $R(-)$-ibuprofen in serial plasma samples (Fig. 4).

In conclusion, the method described here is facile, sensitive, precise, reproducible and readily applied to the separation and quantification of ibuprofen enantiomers in human biological samples. The resolution of the derivatized enantiomers is good and no interference was found. Since the internal standard is commercially available and the retention times for the derivatized ibuprofen enantiomers and the internal standard are short, the developed method is relatively rapid, inexpensive and considered suitable for the analysis of large numbers of patient plasma samples.

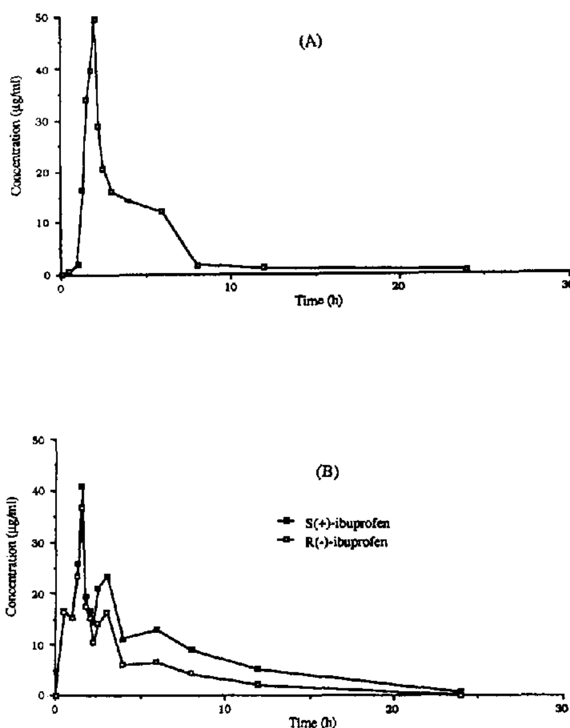

Fig. 4. Plasma concentration–time profiles of *R,S*-ibuprofen enantiomers of an arthritic patient following a single oral dose of 400 mg of $S(+)$-ibuprofen (A), or 800 mg of *R,S*-ibuprofen (B).

Table 1
Reproducibility and accuracy of determination of ibuprofen enantiomers in plasma

Concentration added (μg/ml)	Concentration found (mean ± S.D.) (μg/ml)	Difference from theoretical values (%)
R(−)-Ibuprofen		
0.1	0.104 ± 0.005	+4.0
1	1.018 ± 0.033	+1.8
16	16.22 ± 0.650	−1.4
S(+)-Ibuprofen		
0.1	0.103 ± 0.004	+3.0
1	0.971 ± 0.022	−2.9
18	18.220 ± 0.689	+1.2

4. Acknowledgements

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5. References

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