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Determination of the enantiomeric composition of ibuprofen solutions via a rapid and sensitive mass spectrometry method

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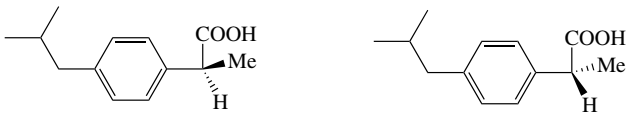
Abstract—A rapid and sensitive method for the determination of the enantiomeric composition of ibuprofen solutions is presented. This approach uses electrospray ionization tandem mass spectrometry and applies the kinetic method to perform the data analysis. Deviations of ca. 1.5% between the actual and experimental enantiomeric composition were observed. The simplicity, sensitivity, and precision of the method make it potentially suitable for the quantification of ibuprofen enantiomers in biological samples.

1. Introduction

Ibuprofen, (*RS*)-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 (Scheme 1).[1] This drug is normally administrated as the racemate, but only the (*S*)-form is responsible for the pharmacological effect.[2] Numerous studies have documented the pharmacokinetics of ibuprofen and described the marked unidirectional inversion of the (*R*)- to the (*S*)-form.[3–6]



(*S*)-ibuprofen (*R*)-ibuprofen

Scheme 1. The enantiomers of ibuprofen.

There is 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.[3,4,7–13] Many of these methods either require lengthy derivatization procedures and sample preparation or lack sufficient sensitivity.

There are few subjects in chemistry that have drawn as much attention as the chiral nature of molecules. It has long been known that the enantiomers of a chiral drug can have very different and even dangerous side effects. The systematic examination of the biological activity of individual enantiomers is a rule for all the new racemic drug candidates, and an increasing number of enantiomerically pure drugs are now approved and marketed.[14,15] However, the development of new chiral products requires not only efficient asymmetric synthetic methods, but also the ability to identify and quantify enantiomeric mixtures.

Noteworthy advances have been made over the past few years on the general methods of chiral identification and quantification based entirely on mass spectrometry (MS) methods. These methods can be conveniently divided into two groups which use: (a) single-stage mass spectrometry and (b) tandem mass spectrometry (MS/MS). Single-stage methods typically rely on peak intensity measurements of intermolecular complexes in the mass spectrum.[16–23] The tandem mass spectrometry approach typically relies on the isolation of a specific diastereomeric ion and its reaction with another reagent, or fragmentation via collision-induced dissociation (CID).[15,24] A number of these experiments have been applied mainly to cyclodextrin–analyte complexes.[25–27] The rate

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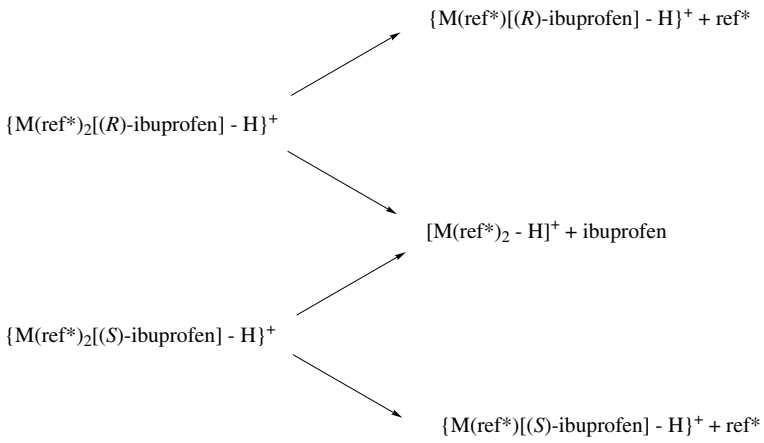


Scheme 2. The dissociation of complexes $[M(ref^*)_2(ibuprofen)–H]^+$.

at which the analyte exchanges to an achiral reagent gas in the cyclodextrin–analyte complex is used to determine the enantiomeric composition of the analyte. In other tandem experiments,[28] higher order complexes are mass-selected and allowed to undergo CID, while the observed relative branching ratios are related to the enantiomeric composition by the kinetic method.[29] This approach[30–36] has been successfully applied to the determination of enantiomeric composition of amino-acids,[33,32,37] α-hydroxyacids,[38] sugars,[39] drugs,[30,40,41] and vitamins.[42]

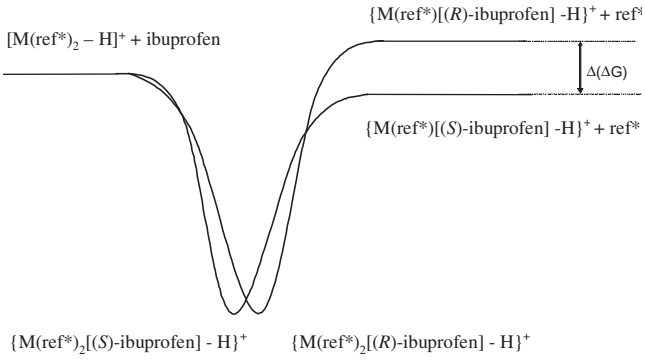
The determination of the enantiomeric composition of ibuprofen solutions based on the kinetic method approach, the subject of this paper, involves, as a first step, the choice of systems that can promote suitable enantiomeric distinction. In order to do that, the singly-charged trimeric complexes, $\{M(ref^*)_2[(R)\text{-ibuprofen}]–H\}^+$ or $\{M(ref^*)_2[(S)\text{-ibuprofen}]–H\}^+$-formed in electrosprayed solutions containing the ibuprofen enantiomers, a chiral reference compound (ref*), and a metal cation (usually M^{2+})- is mass-selected and fragmented upon collision-induced dissociation. Scheme 2 displays the competitive dissociation of both trimeric complexes to form the corresponding dimeric fragments, that is, $[M(ref^*)(ibuprofen)–H]^+$ and $[M(ref^*)_2–H]^+$.

Scheme 3 shows the energy diagram for the competitive dissociations of the trimeric complexes $\{M(ref^*)_2[(R)\text{-ibuprofen}]–H\}^+$ and $\{M(ref^*)_2[(S)\text{-ibuprofen}]–H\}^+$ to form the corresponding dimeric fragments.

Clearly, the difference in energies, Δ(ΔG), of the diastereomeric complexes $\{M(ref^*)[(R)\text{-ibuprofen}]–H\}^+$ and $\{M(ref^*)[(S)\text{-ibuprofen}]–H\}^+$ result in different ratios R_R or R_S, as defined in Eqs. 1 and 2, respectively,

$$R_R = \{M(ref^*)[(R)\text{-ibuprofen}]–H\}^+/[M(ref^*)_2–H]^+ \quad (1)$$

$$R_S = \{M(ref^*)[(S)\text{-ibuprofen}]–H\}^+/[M(ref^*)_2–H]^+ \quad (2)$$



Scheme 3. The energy diagram for the competitive dissociations of the trimeric complexes $\{M(ref^*)_2[(R)\text{-ibuprofen}]–H\}^+$ and $\{M(ref^*)_2[(S)\text{-ibuprofen}]–H\}^+$.

The ratio R_{chiral} (Eq. 3) indicates the level of chiral distinction achievable in a particular experiment.

$$R_{chiral} = R_R/R_S \quad (3)$$

Evidently, when $R_{chiral} = 1$ there is no chiral discrimination, which denotes that this specific array of metal and chiral reference is not able to originate effective enantiomeric distinction. Therefore, the best system is the one that provides R_{chiral} as far as possible from the unity, provided that accurate abundance ratios can still be measured. The enantiomeric composition of a chiral analyte solution can be determined based on a linear relationship between the natural logarithm of the abundance ratio R ($R = [M(ref^*)(ibuprofen)–H]^+/M(ref^*)_2–H]^+$) and the enantiomeric composition, as previously demonstrated.[32,39] Thus, unknown enantiomeric mixtures are analyzed by measuring the ratio of the two fragment ions, that is, $[M(ref^*)(ibuprofen)–H]^+$ and $M(ref^*)_2–H]^+$), in a single tandem mass spectrum. This methodology is already well-established and known as the single ratio method.[43]

Therefore, we herein report the application of the kinetic method strategy to quickly and efficiently determine the enantiomeric composition of ibuprofen solutions with high accuracy and reproducibility.

2. Results and discussion

Chiral reference compounds (see Experimental) were chosen due to the following reasons: (a) they can be easily obtained in enantiomerically pure forms; and (b) in previous studies, some of these compounds proved to be useful reference compounds in chiral analysis.[38,39] Five divalent metal cations ($M = Cu^{2+}$, Zn^{2+}, Co^{2+}, Fe^{2+}, and Ni^{2+}) were selected as the central metal ions owing to their well-known ability to form complexes with oxygen- and nitrogen-containing compounds, as well as to their recent use in the chiral analysis of several compounds by the kinetic method.[32,30,38,40,37,41,42] Figure 1 shows the ESI-MS spectrum of a 1:1 water/methanol solution containing a racemic mixture of ibuprofen, D-glucose, and Zn^{2+}. The formation of the trimeric complex $[Zn(D\text{-glucose})_2(ibuprofen)–H]^+$ (*m/z* 629) was observed and indicated in this spectrum. Other intense ionic adducts, such as $[Zn(D\text{-glucose})(ibuprofen)–H]^+$ (*m/z* 449), $[Zn(ibuprofen)_2–H]^+$ (*m/z* 475), $[Zn(D\text{-glucose})(ibuprofen)_2–H]^+$ (*m/z* 655), and $[Zn(ibuprofen)_3–H]^+$ (*m/z* 681), are also detected.

Figure 2a and b show the collision-induced dissociation spectrum of the mass-selected trimeric complexes $\{Zn(D\text{-glucose})_2[(S)\text{-ibuprofen}]–H\}^+$ [formed in a solution containing pure (*S*)-ibuprofen] and $\{Zn(D\text{-glucose})_2[(RS)\text{-ibuprofen}]–H\}^+$ (from a solution with racemic ibuprofen), respectively. The R_{chiral} for this system, calculated by using Eq. 3, was found to be 0.75 and reflects the difference in stability of the diastereomeric ions $\{Zn(D\text{-glucose})[(R)\text{-ibuprofen}]–H\}^+$ and $\{Zn(D\text{-glucose})[(S)\text{-ibuprofen}]–H\}^+$ (Scheme 3).

The ratio R_R ($R_R = \{Zn(D\text{-glucose})[(R)\text{-ibuprofen}]–H\}^+/[Zn(D\text{-glucose})_2–H]^+$), related to the dissociation of the trimeric complex $\{Zn(D\text{-glucose})_2[(R)\text{-ibuprofen})]–H\}^+$ hypothetically formed in a solution containing pure *R*-ibuprofen, was obtained by extrapolation to zero in a plot of ln(*R*) versus the molar fraction of (*S*)-ibuprofen, as shown in Figure 3.

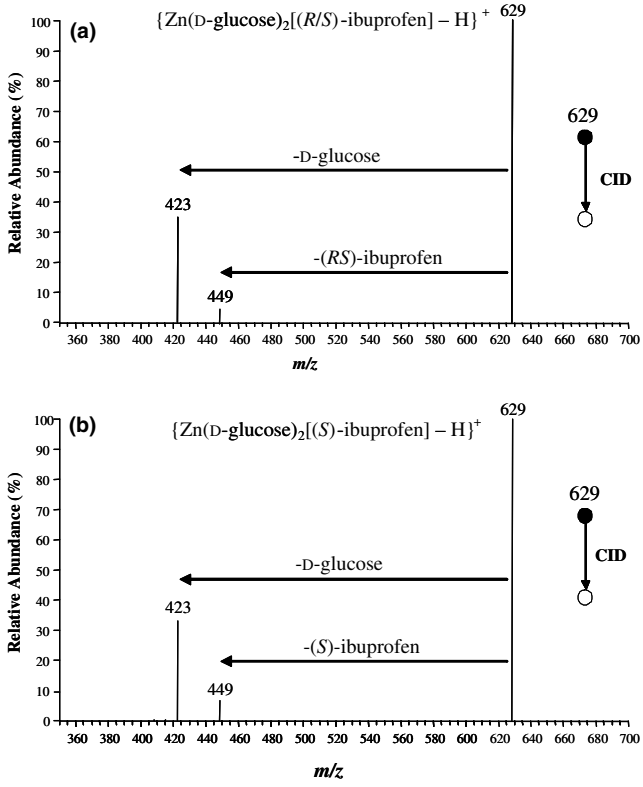


Figure 2. MS/MS product ion spectra of (a) $\{Zn(D\text{-glucose})_2[(RS)\text{-ibuprofen}]–H\}^+$ (*m/z* 629) and (b) $\{Zn(D\text{-glucose})_2[(S)\text{-ibuprofen}]–H\}^+$ (*m/z* 629). The CID activation level of 10.6% was chosen, which corresponds to approximately 265 mV AC.

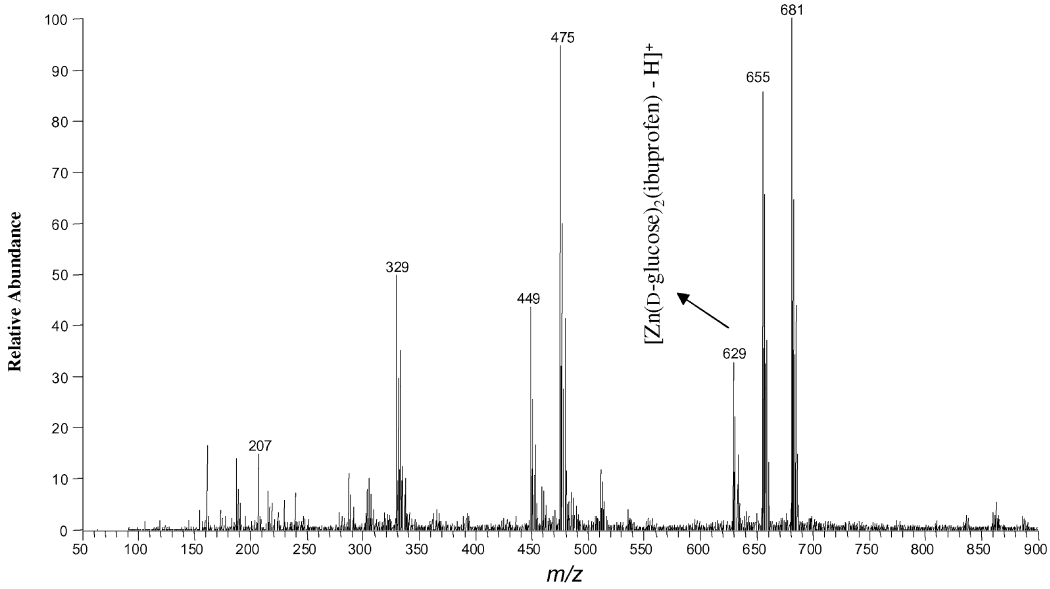


Figure 1. ESI-MS spectrum of a 1:1 water/methanol solution containing (*RS*)-ibuprofen, D-glucose, and Zn^{2+}. The presence of the trimeric complex $\{Zn(D\text{-glucose})_2[(RS)\text{-ibuprofen})]–H\}+$ (*m/z* 629) is indicated.

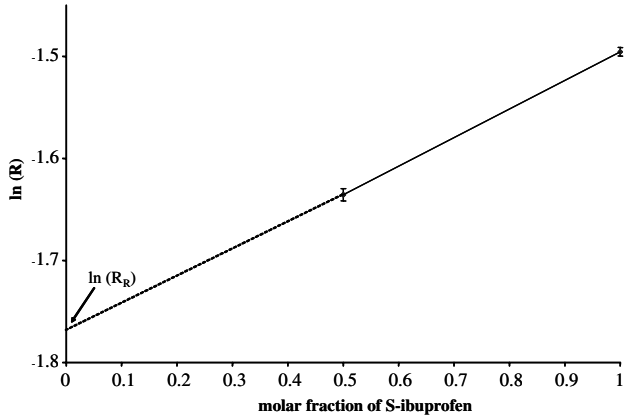


Figure 3. Two-point calibration curve for enantiomeric quantification of ibuprofen solutions using Zn^{2+} as the metal cation and D-glucose as the chiral reference compound. The chiral selectivity factor (R_{chiral}) is 0.75. Each point represents an average of five measurements and the error bars are given with 95% confidence interval.

Other systems, that is, different combinations of chiral reference compounds and metal cations (see Experimental), furnished worse R_{chiral} values (Table 1). However, for most of the systems tested, the R_{chiral} values could not be determined owing to at least one of the following reasons: (a) no formation of the required trimeric complexes $[M(ref^*)_2(ibuprofen)–H]^+$; (b) no production of either dimeric ions during the dissociation of the $[M(ref^*)_2(ibuprofen)–H]^+$ trimeric complexes.

Table 1. Chiral selectivity factor (R_{chiral}) of several systems used for the enantiomeric quantification of ibuprofen solutions

Reference	Metal ion	R_{chiral}[a]
D-Glucose	Cu^{2+}	0.84
D-Mannose	Cu^{2+}	0.79
D-Tartaric acid	Co^{2+}	0.86
D-Galactose	Cu^{2+}	0.93
D-Ribose	Cu^{2+}	1.07

[a] Only the combinations of metal cations and chiral reference compounds that furnished measurable R_{chiral} values are listed above.

The ratio R for any solution containing Zn^{2+}, D-glucose, and ibuprofen can be easily determined by measuring the intensities of the dimeric complexes [Zn(D-glucose)(ibuprofen)–H]$^+$ (m/z 423) and [Zn(D-glucose)$_2$–H]$^+$ (m/z 449) formed upon the dissociation of the mass-selected trimeric complex [Zn(D-glucose)$_2$(ibuprofen)–H]$^+$ (m/z 629), as shown in Figure 2a and b. The reliability of the two-point calibration curve (Fig. 3) was evaluated by the analysis of several solutions of ibuprofen with 'unknown' enantiomeric composition. Good agreement between the real and experimental values was observed with an average absolute difference of 1.5%, as shown in Table 2.

It was also verified that the relative concentrations of ibuprofen versus D-glucose did not affect the performance of this system.[39,37,41] For instance, a systematic study of the influence of the change in the [(*RS*)-ibuprofen]/[D-glucose] concentration ratios on the ion abundance ratio (R = {Zn(D-glucose)[(*RS*)-ibuprofen]–H}$^+$/[Zn(D-glucose)$_2$–H]$^+$) was carried out, and the results are summarized in Table 3. The study shows that, at least for the case examined, the ion abundance ratio R is virtually independent of the relative concentration of the analyte (ibuprofen) and the chiral reference (D-glucose) in solution. Thus, it is clear that the addition of different concentrations of the chiral reference to unknown samples has little effect on the accuracy of the measurements, a practical advantage for this approach. This is an indispensable result for the successful quantitative analysis of real samples.

Table 2. Actual and experimental values of enantiomeric composition of ibuprofen solutions

Fraction of (*S*)-ibuprofen (%)		
Actual	Experimental[a,b]	Difference
60	61 ± 2	1
70	72 ± 1	2
80	82 ± 1	2
90	89 ± 1	1

[a] Values obtained from the two-point calibration curve (Fig. 3).
[b] Average of five measurements (95% confidence interval).

Table 3. Ion abundance ratio R = {Zn(D-glucose)[(*RS*)-ibuprofen]–H}$^+$/[Zn(D-glucose)$_2$–H]$^+$ for various ratios of [(*RS*)-ibuprofen]/[D-glucose]

[*R*/*S*-Ibuprofen]/[D-glucose]	R[b]
5:1	0.1967
4:1	0.1958
3:1	0.1971
2:1	0.1962
1:1[a]	0.1959
1:2	0.1965
1:3	0.1955
1:4	0.1973
1:5	0.1970

[a] [*R*/*S*-ibuprofen]=2×10^{-5} mol L^{-1}; [D-glucose] = 2×10^{-5} mol L^{-1}.
[b] Average of five measurements.

3. Conclusion

The analytical method reported herein is of wide relevance both for the general demonstration of a simple method of enantiomeric quantification and because ibuprofen represents a valuable substance, particularly in the pharmaceutical industry. The two-point calibration curve allowed the fast and reliable determination of the enantiomeric composition of ibuprofen solutions. Finally, it can be envisaged that this methodology could be advantageously applied to monitoring of the interconversion ibuprofen enantiomers in complex samples such as human plasma.

4. Experimental

All the reagents were purchased from Sigma–Aldrich and used without purification. The experiments were conducted in a commercial LCQ advantage ion trap

mass spectrometer (ThermoElectron, San Jose, CA) equipped with an ESI source and operated in the positive ion mode. The mass spectra reported are the average of about 40 scans, each requiring 0.2 s. Samples were infused into the ESI source via a syringe pump at a flow rate of 2.00 μL min^{-1}. Typical ESI conditions were as follows: heated capillary temperature, 150 °C; sheath gas (N_2) flow rate, 0.75 L min^{-1}; spray voltage 5 kV; capillary voltage 3 V; tube lens off set voltage, 40 V. Aqueous methanol 1:1 solutions were prepared by the mixture of the following reagents: (a) 2×10^{-5} mol L^{-1} of ibuprofen (as either the *S*-enantiomer or the racemic mixture); (b) 2×10^{-5} mol L^{-1} of a chiral reference compound (ref*), namely: α-hydroxy-acids (L-tartaric acid, L-3-phenyl lactic acid, L-citramalic acid, L-mandelic acid), sugars (D-glucose, D-mannose, D-galactose, D-ribose, and D-maltose), natural aminoacids (L-Tyr, L-Glu, L-Phe, L-Cys, and L-Leu), modified aminoacids (*N*-*t*-Boc-L-Phe, *N*-Fmoc-L-Pro), and other compounds [(*R*)-talidomide]; and (c) 1×10^{-5} mol L^{-1} of a metal cation (nitrate salt of Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, and Ni^{2+}).

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