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Quantitative determination of the enantiomeric composition of thalidomide solutions by electrospray ionization tandem mass spectrometry

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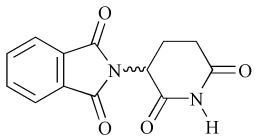
Received (in Cambridge, UK) 9th May 2002, Accepted 22nd August 2002
First published as an Advance Article on the web 9th September 2002

Rapid and simple chiral analysis of thalidomide solutions is demonstrated by using electrospray ionization tandem mass spectrometry and analysis of cluster ion dissociation by the kinetic method. Average deviations of 1% between the actual and experimental enantiomeric compositions are observed.

There are few topics in biological and pharmaceutical science that have drawn as much interest as the chiral nature of drug molecules. Today, systematic investigation of the biological activity (including pharmacology and toxicology) of individual enantiomers is the rule for all new racemic drug candidates, and an increasing number of optically pure drugs has been approved and marketed.[1] Thalidomide, originally developed as a sedative drug, was responsible for severe birth defects across Europe in the early 1960s. It was reported later that its teratogenic effect is caused by the *S*-enantiomer. However, it has also been suggested that thalidomide enantiomers can interconvert in the body, indicating that the exclusive use of the *R*-enantiomer is not enough to prevent these undesirable side effects.[2] After a long period of relative obscurity during which thalidomide was mainly employed in the treatment of leprosy, interest in this drug is rising again. Promising results have been reported in patients with multiple myeloma or myelodysplasias, a group of proliferative disorders of the bone marrow.[3]



Thalidomide

Significant progress has been made during the past few years on methods of chiral identification and quantification based exclusively on mass spectrometry. It is possible to classify these methods into four broad types: (a) generation of host–guest diastereomeric adducts using a chiral guest;[4,5] (b) determination of rates of ion/molecule reactions between an enantiomeric guest and a host molecule;[6,7] (c) collision-induced dissociation (CID) of diastereomeric adducts in a MS/MS experiment;[8] (d) utilization of the kinetic method to quantify the MS/MS chiral effect.[9] Successful applications to quantitative analysis of amino acids,[9,10] α-hydroxyacids,[11] sugars[12] and some drugs[13,14] have been reported. In comparison with chromatographic methods,[15] which are usually employed in quantitative chiral analysis, the kinetic method approach is faster and also requires smaller amounts of sample. The present study describes the application of the kinetic method to enantiomeric quantitation of thalidomide, a special drug of wide interest.

All experiments were performed using a commercial LCQ ion trap mass spectrometer (Thermo Finnigan, 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 follow: 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 examined contained a mixture of thalidomide (*R* or *S*, 2×10^{-5} mol L^{-1}), a chiral reference compound (ref* = D-glucose, D-mannose, D-galactose, D-ribose, L-tartaric acid, L-3-phenyllactic acid, L-citramalic acid, L-malic acid, L-Phe, L-Tyr, L-Glu, L-Leu, L-Cys, N-*t*-Boc-L-Phe or N-Fmoc-L-Pro, 2×10^{-4} mol L^{-1}) and a transition metal ion (M = Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} or Fe^{2+}, 1×10^{-4} mol L^{-1}).

The singly-charged trimeric ions $[M(ref^*)_2(thalidomide) - H]^+$, formed in the mass spectra of electrosprayed solutions containing enantiomerically pure thalidomide (*R* or *S*), were mass-selected and fragmented by collision-induced dissociation to form a pair of dimeric product ions [M(*R*-thalidomide)(ref*) − H]$^+$ or [M(*S*-thalidomide)(ref*) − H]$^+$ and $[M(ref^*)_2 - H]^+$. The difference in energy between the diasteromeric ions [M(ref*)(*R*-thalidomide) − H]$^+$ and [M(ref*)(*S*-thalidomide) − H]$^+$ results in differences in the relative abundance ratios (R_R or R_S), defined in eqns. (1) and (2), respectively:

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

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

The ratio of R_R to R_S, defined as R_{chiral} [eqn. (3)], indicates the level of chiral discrimination achievable in a particular experiment.

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

The best systems are those that provide values for R_{chiral} as far as possible from unity, provided accurate abundance ratios can still be measured. The results of chiral recognition experiments on thalidomide using the chiral reference compounds and the metal cations listed above are summarized in Table 1. The best of these system is that composed of the sugar D-galactose and Zn^{2+}. In Fig. 1, chiral distinction between *R*- and *S*-thalidomide is demonstrated under particular conditions using this selected system. The difference between R_R and R_S, which reflects the difference in stability of the diastereomeric ions [Zn(*R*-thalidomide)(D-galactose) − H]$^+$ and [Zn(*S*-thalidomide)(D-galactose) − H]$^+$, is large and R_{chiral} is 0.17. Linear relationships of ln (*R*) *versus* the molar fraction of *R*-thalidomide (Fig. 2) were observed with an excellent correlation coefficient (R^2 = 0.9987). Such linear correlations are intrinsic to the kinetic method, as shown elsewhere.[9] Using such calibration curves, the enantiomeric compositions of prepared thalidomide solutions were determined and excellent results were obtained, as illustrated by the data of Table 2. Significantly, it was verified that the relative concentrations of thalidomide *vs.* D-galactose did not affect the chiral discrimination in these systems.[12,16] This is an indispensable result for successful quantitative analysis of unknown samples. It was also verified that the accuracy of the measurements was not affected by the possible presence of products formed from the hydrolysis of thalido-

DOI: 10.1039/b204421h

Table 1 Chiral selectivity factor (R_{chiral}) recorded using different metal cations and various chiral reference compounds[a]

Reference	Metal	R_{chiral}[b]
L-tartaric acid	Co^{2+}	1.34
L-citramalic acid	Cu^{2+}	1.05
	Zn^{2+}	1.17
L-malic acid	Co^{2+}	1.17
	Zn^{2+}	0.94
	Fe^{2+}	1.29
L-glutaric acid	Ni^{2+}	0.99
	Fe^{2+}	0.98
D-mannose	Cu^{2+}	0.64
	Zn^{2+}	1.19
D-galactose	Cu^{2+}	0.68
	Zn^{2+}	0.17
D-ribose	Cu^{2+}	1.95
	Zn^{2+}	0.82
D-maltose	Ni^{2+}	0.61

[a] Of the combinations of metal cations and chiral reference compounds listed in the text, only those that furnished measurable values of R_{chiral} are shown. [b] Calculated using eqn. (3).

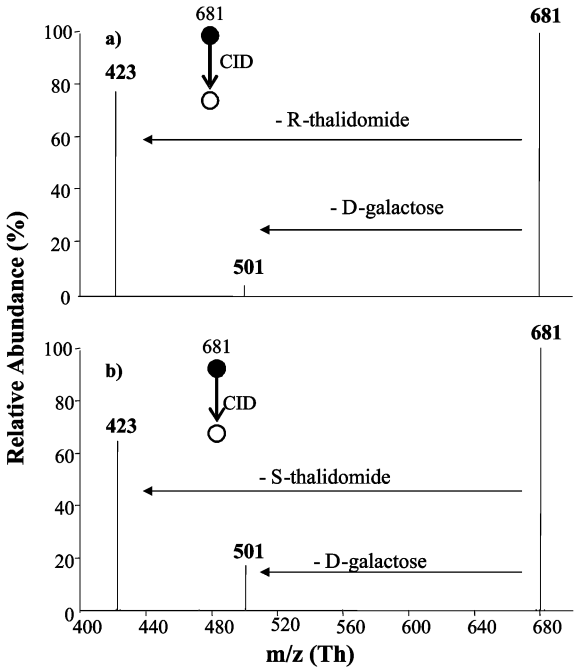


Fig. 1 MS/MS product ion spectra of (a) [Zn(*R*-thalidomide)(D-galactose)$_2$ − H]$^+$ (*m*/*z* 681) and (b) [Zn (*S*-thalidomide)(D-galactose)$_2$ − H]$^+$ (*m*/*z* 681). The CID activation level is chosen as 10.6%, corresponding to approximately 265 mV AC.

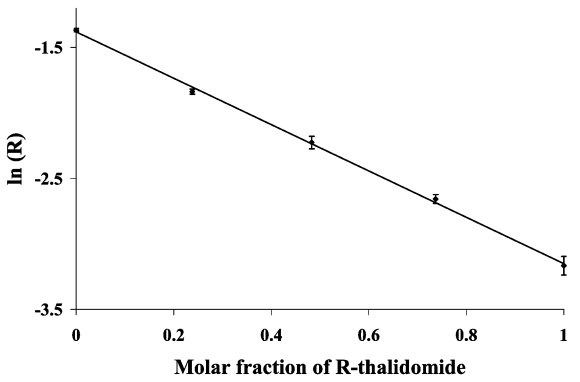


Fig. 2 Calibration curve for chiral analysis of thalidomide using Zn^{2+} as the metal cation and D-galactose as the chiral reference compound. The chiral selectivity factor (R_{chiral}) is 0.17, R^2 is 0.9987 and error bars represent the 95% confidence level.

Table 2 Actual and experimental values for the enantiomeric composition of thalidomide solutions

Fraction of R-thalidomide (%)		
Actual (%)	Experimental (%)[a,b]	Difference (%)
77	77 ± 3	0
73	72 ± 1	1
48	48 ± 1	0
30	29 ± 1	1
25	26 ± 1	1
19	17.9 ± 0.8	1.1

[a] Values obtained from the calibration curve (Fig. 2). [b] Average of five measurements (95% confidence).

mide[17] or by the several isomeric forms which are in equilibrium in an aqueous solution of D-galactose.[18] Note that the other sugars tested, including such diastereomers of D-galactose as D-glucose and D-mannose, furnished smaller values for R_{chiral}.

Once the linearity of the semi-log plot has been established for a chiral analysis, a two-point calibration curve allows a faster but still reliable method of quantitative chiral analysis. By the use of this two-point calibration curve, which was built using the two enantiomerically pure solutions, the enantiomeric compositions of some prepared solutions of thalidomide were determined. Good agreement between the actual and the measured values was observed, with an average deviation of 1%.

The subject reported in this paper is of wide relevance, particularly to the pharmaceutical industry, both for the general demonstration of a simple method of drug enantiomer quantitation and in particular because thalidomide continues to represent a controversial although still valuable drug with renewed possibilities of application. Furthermore, for the first time, a sugar (D-galactose) is used as a chiral reference compound for chiral recognition using the kinetic method.

This work was supported by the National Science Foundation, grant no. CHE 97-32670. D. V. Augusti and R. Augusti thank the Brazilian National Research Council (CNPq) for financial support.

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