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Enantioseparation of cetirizine by chromatographic methods and discrimination by ^{1}H-NMR

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Cetirizine is an antihistaminic drug used to prevent and treat allergic conditions. It is currently marketed as a racemate. The H1-antagonist activity of cetirizine is primarily due to (*R*)-levocetirizine. This has led to the introduction of (*R*)-levocetirizine into clinical practice, and the chiral switching is expected to be more selective and safer. The present work represents three methods for the analysis and chiral discrimination of cetirizine. The first method was based on the enantioseparation of cetirizine on silica gel TLC plates using different chiral selectors as mobile phase additives. The mobile phase enabling successful resolution was acetonitrile-water 17 : 3, (*v*/*v*) containing 1 mM of chiral selector, namely hydroxypropyl-β-cyclodextrin, chondroitin sulphate or vancomycin hydrochloride. The second method was a validated high performance liquid chromatography (HPLC), based on stereoselective separation of cetirizine and quantitative determination of its eutomer (*R*)-levocetirizine on a monolithic C18 column using hydroxypropyl-β-cyclodextrin as a chiral mobile phase additive. The resolved peaks of (*R*)-levocetirizine and (*S*)-dextrocetirizine were confirmed by further mass spectrometry. The third method used a ^{1}H-NMR technique to characterize cetirizine and (*R*)-levocetirizine. These methods are selective and accurate, and can be easily applied for chiral discrimination and determination of cetirizine in drug substance and drug product in quality control laboratory. Moreover, chiral purity testing of (*R*)-levocetirizine can also be monitored by the chromatographic methods.

Keywords: cetirizine; (*R*)-levocetirizine; enantioseparation; chromatography; ^{1}H-NMR

Introduction

Chiral discrimination has been an important issue in the development of pharmaceutical drugs because drug enantiomers may have different pharmacokinetic and toxicology profiles, and give different clinical responses. The administration of highly pure chiral drugs is a major goal of the pharmaceutical industry, to protect patients from damage caused by variable drug absorption, distribution, metabolism, and toxic side effects.[1]

Cetirizine is a second-generation antihistamine indicated for the treatment of seasonal allergic rhinitis, chronic urticaria, and pollen-induced asthma. Cetirizine is currently marketed as the racemate, which consists of equal amounts of (*R*)-levocetirizine and (*S*)-dextrocetirizine (see Fig. 1). In racemic medicine one of the enantiomers is usually more active or less toxic than the other. A number of studies have shown that cetirizine and its enantiomers follow this rule. The H1-antagonist activity of cetirizine is primarily due to levocetirizine.[2–4] This has led to the introduction of (*R*)-levocetirizine into clinical practice, and the chiral switching is expected to be more selective and safer.[5] Several chiral analytical methods were reported for the determination and separation of racemic compounds, including thin-layer chromatography (TLC), liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy. Thin-layer chromatography and HPLC have been proved to be useful analytical tools for chiral substances.[6–12] Nuclear magnetic resonance spectroscopy is a very selective technique, directly applicable to most samples, with little or no sample preparation being required.[13–15]

In this context, it is necessary to find reliable, sensitive, and simple methods for the analysis of the enantiomers. Few techniques for resolution of racemic cetirizine have been published, including liquid chromatography, which require special and expensive columns[16–18] and capillary electrophoresis.[19,20]

The aim of this work is to apply three methods for chiral discrimination of cetirizine and its eutomer (*R*)-levocetirizine, using TLC, HPLC, NMR, and subsequent determination of (*R*)-levocetirizine by the HPLC method. The chromatographic methods, based on chiral mobile phase additives (CMPAs), tend to be cheap and feasible in contrast with the aforementioned methods. The factors affecting chiral separation, such as mobile phase composition, chiral selectors, pH, temperature, and detective wavelength, were studied. The MS technique was used for confirmation of the resolved peaks of (*R*)- and (*S*)-enantiomers. The NMR technique was applied as a quick and effective method for chiral discrimination of cetirizine and (*R*)-levocetirizine in drug substances without shifting reagent, chiral selector, or chiral solvating agent. The methods developed are robust and selective and can be easily applied for chiral purity analysis of (*R*)-levocetirizine. To the best of the author's knowledge, the procedures described have not been investigated before.

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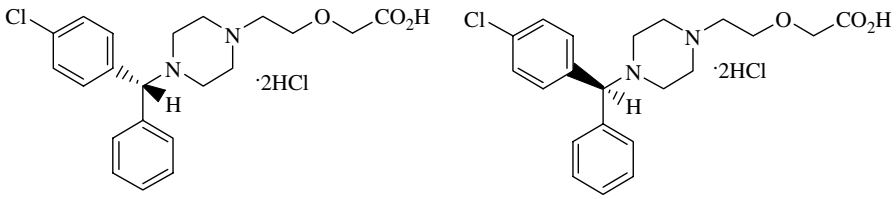

Figure 1. The chemical structures of *(R)* – levocetirizine and (*S*) – dextrocetirizine.

Experimental

Instruments

- Pre-coated TLC plates (10 × 10 cm, aluminium plates coated with 0.25 mm silica gel F254) were purchased from Merck, UK. UV-radiation (Cole-Parmer Instrument, France) detective wavelength was 254 nm.
- A Waters-2525 LC system was used, equipped with a dual wavelength absorbance detector 2487, an auto-sampler injector and Mass Lynx v 4.1. The HPLC column was a C18 reverse-phase column (4.6 mm diameter × 100 mm length, 5 µm particles), monolithic (Phenomenex). Ultra-pure water (ELGA, UK) was used for the preparation of a buffer. Nylon membrane filters 0.45 µm, 47 mm (Whatman, Schleicher & Schuell, England).
- Mass spectrometric analyses were conducted using negative electrospray ionization of Micromass LCT (Micromass UK Ltd), which is a high-performance orthogonal acceleration reflecting TOF mass spectrometer and coupled to the Waters 2795 HPLC. It is fully integrated with the MassLynx software v 4.1. The mass spectrometer uses a Z-flow atmospheric pressure ionization source and samples were introduced by ESI, allowing constant infusion of a 'lock mass' calibrant independent of the sample, giving 5 ppm (RMS) mass accuracy with no sample suppression. The system settings were as follows: electrospray voltage, 3 kV; capillary temperature, 150 °C; sample solution flow rate, 0.1 mL/min. All samples were dissolved in a 50% acetonitrile aqueous solution before being analysed.
- ^{1}H -NMR experiments were performed on a Bruker Avance 400 MHz spectrometer operating at 400.13 MHz. Analysis and acquisition were carried out using Brukers Topspin 2.0 software. The pulse repetition period was 5 s. Sixteen scans were recorded into 64 k data points with a tip angle of 30 degrees. An exponential window function with a line-broadening factor of 0.3 Hz was applied before Fourier transformation. Automatic phase correction and base line correction were then applied. For estimation of cetirizine, symmetrical well separated signal was automatically integrated using the belt-in integration protocol supported with software package used for running the ^{1}H-NMR spectrometer.

Materials and reagents

All chemicals were of analytical grade if not otherwise stated. Cetirizine dihydrochloride was kindly supplied by Glaxo Co., Egypt. Its purity was found to be 99.55% according to the official method.[21] (*R*)- Levocetirizine dihydrochloride was kindly supplied by Western Pharmaceutical Industries, India. Its purity was 99.80%. Cetirizine tablets (Zyrtec®) were purchased from GlaxoSmithKline Co., Egypt, and were labelled as containing 10 mg cetirizine dihydrochloride per tablet. Chondroitin sulphate (Eva Co., Egypt), Vancomycin HCl and Hydroxypropyl-β-cyclodextrin were purchased from Sigma-Aldrich, UK. Potassium dihyrogen phosphate (BDH, UK), sodium hydroxide (Analar, UK), orthophosphoric acid (85%) and triethylamine (TEA) (Fluka, UK), trifluoroacetic acid (TFAA) (Aldrich, UK) were used without further purification. Methanol, acetonitrile (Fisher Scientific, UK) and tetrahydrofuran (THF), (Sigma, UK) were HPLC grade; 99.9% D methanol-d_4 (Cambridge Isotope Laboratory, CIL) was used as a solvent for NMR.

Standard stock solutions

A solution of 1 mg/mL cetirizine or (*R*)-levocetirizine in methanol was freshly prepared for TLC assay. The solution was subsequently used to prepare a working standard solution in the range of 2.5–200 µg/mL for HPLC analysis by dilution with mobile phase containing 1 mM HP-β-CD.

Laboratory-prepared mixtures

Mixtures of (*R*)-levocetirizine and cetirizine dihydrochloride in different proportions ranging from 10–90% were laboratory prepared and analysed. The recovery percentage of (*R*)-levocetirizine was calculated.

Chiral discrimination and method development

TLC-based method

The TLC plates were developed in acetonitrile water 17 : 3, (*v*/*v*) containing 1 mM of different chiral selectors. The chromatographic tank was saturated with the mobile phase for 10 minutes before development of the plates. For separation and detection, 20 µL of racemic centirizine and 10 µL of standard solution of levocetirizine were applied as separate compact spots 20 mm apart and 10 mm from the bottom of the TLC plates using a 25 µL Hamilton micro syringe. The chromatograms were developed up to 8 cm in the usual ascending way, air dried and visualized under UV wavelength of 254 nm or by exposure to I_2 vapours.

HPLC-based method

The mobile phase was prepared by mixing acetonitrile-methanol-tetrahydrofuran-0.05 M potassium dihydrogen orthophosphate containing 1 mM HP-β-CD 50 : 25 : 5 : 120, (v/v) pH 5.2 ± 2. The pH was adjusted with phosphoric acid and/or triethanolamine. All solutions and samples were filtered through a 0.45 µm membrane filter prior to use. The HPLC flow rate was set at 1 mL/min and a UV wavelength of 230 nm. The column was equilibrated for 45 minutes prior to assay. All analysis was performed at 25 ± 2 °C with a sample injection volume of 20 µL. The resolved fractions corresponding to (*R*)-levocetirizine and (*S*)-dextrocetirizine were collected and freeze dried. The samples were dissolved in a 50%

solution of acetonitrile in water; 10 µL of each sample was injected to the mass spectrometer and the spectra were recorded.

For quantitative determination of (*R*)-levocetirizine aliquots of standard solution (1 mg/mL) equivalent to 0.025–2.0 mg were transferred into 10 mL volumetric flasks and made up to volume with the mobile phase. Triplicate 20 µL injections were made of each concentration. The average peak areas were calculated and plotted *versus* concentrations, a linear relationship was obtained and the regression equation was recorded.

^{1}H-NMR method

Cetirizine dihydrochloride and (*R*)-levocetirizine dihydrochloride, 10 mg of each, were dissolved in 600 µL methanol-d_4 in epamdrof tubes. The solutions were transferred into NMR tubes, and the standard spectra were recorded. The chemical shifts were referenced to the centre of the peaks of the solvents residual signal of methanol at 3.31 ppm (^{1}H-NMR). Each sample was measured in triplicates.

Application to pharmaceutical dosage form

Ten accurately weighed tablets were finely powdered. A portion equivalent to 50 mg cetirizine was transferred into 50 mL volumetric flask followed by the addition of 30 mL methanol. The solution was stirred with a magnetic stir bar for 10 minutes, filtered and made up to volume with the same solvent. The TLC and HPLC analyses were performed as described above. The concentration of (*R*)-levocertirizine was calculated from the regression equation.

Results and Discussion

Chiral separation

HP-β-CD, chondroitin and vancomycin have shown to be effective chiral selectors for the enantiomeric separation of different pharmaceutical compounds using TLC, HPLC and ^{1}H-NMR.[1,13,22] The chiral recognition between analyte and HP-β-CD depends primarily on the size and geometry of the analyte relative to the cyclodextrin cavity and to the hydrophobic and hydrogen bonding interactions. Similarly, the steric hindrance derived from the sulphate position of chondroitin sulphate may contribute to the interaction with enantiomers.[22] The enantioselectivity of vancomycin is related to its semi-rigid, basket-shaped aglycan structure. The chiral environment formed by 18 asymmetrical centres with various functional groups provides the essential interactions for chiral recognition. A previous study has shown that vancomycin may form stable non-covalent dimmers or micelles in aqueous media.[1]

^{1}H-NMR spectroscopy is one of the powerful experimental techniques for the investigation of chiral recognition.[14] In the NMR method described no chiral shifting reagent, chiral selector or chiral solvating agent was used.[13]

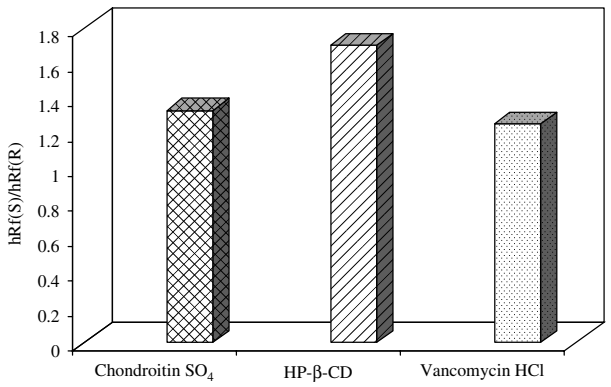

Figure 2. Effect of different chiral selectors (1 mM) on enantiomeric resolution of racemic cetirizine dihydrochloride, 20 µg/spot.

TLC–based method

The TLC method was based on chiral separation of racemic cetirizine depending on the difference in R_F values between – (*R*) and – (*S*) forms. The experimental conditions such as mobile phase composition, chiral selector, pH and temperature were optimized to provide precise results. Different CMPAs (1 mM) were tried including HP-β-CD, chondroitin sulphate and vancomycin.

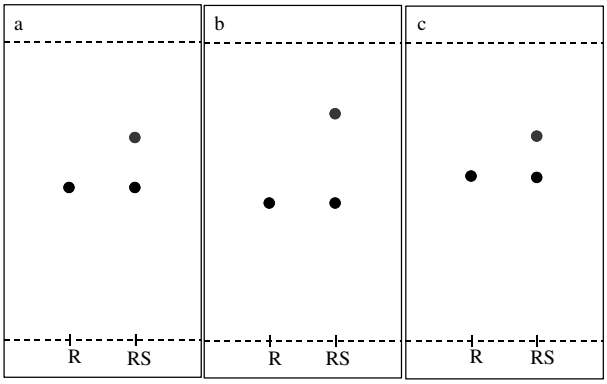

Figure 3. Thin layer chromatogram showing resolution of racemic cetirizine dihydrochloride, 20 µg/spot, using 1 mM of different chiral selectors, a) chondroitin SO_4, b) HP-β-CD, c) vancomycin HCl, solvent front 8 cm, temp. 25 ± 2 °C, solvent system, MeCN:H_2O 17 : 3, (*v*/*v*).

Table 1. Robustness results for TLC- and HPLC-based methods

Parameters	TLC method		HPLC method	
	Conditions	R_F (*S*)/R_F (*R*)	*Conditions*	*RS*
PH	7.8	1.63	5.0	3.56
	8.0	1.65	5.2	3.91
	8.2	1.70	5.5	3.60
* Mobile phase composition	17.5 : 2.5	1.50	50 : 23 : 7 : 120	3.77
	17.0 : 3.0	1.65	50 : 25 : 5 : 120	3.91
	16.5 : 3.5	1.55	50 : 27 : 3 : 120	3.82
Temperature (°C)	23	1.52		
	25	1.65	–	–
	27	1.57		
Flow rate (mL/min)			0.8	3.86
	–	–	1.0	3.91
			1.2	3.76
Wavelength (nm)			228	3.90
	–	–	230	3.91
			232	3.85

* TLC: MeCN:H_2O; HPLC: MeCN : MeOH : THF : 0.05M KH_2PO_4;1 mM HP-β-CD

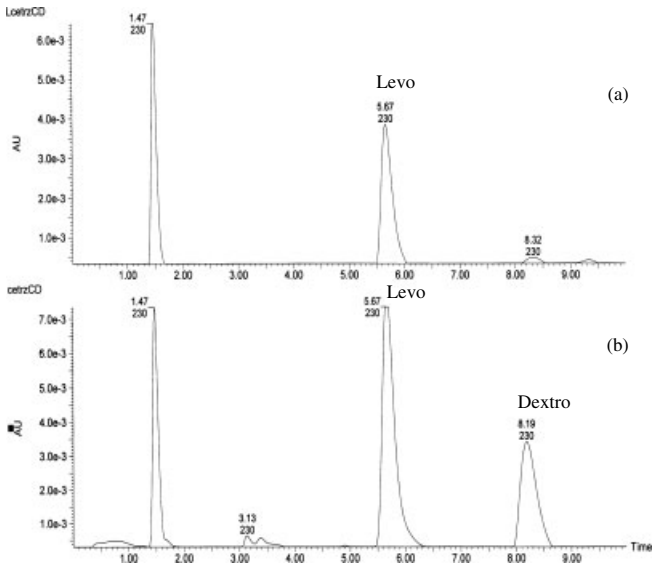

Figure 4. HPLC separation: a levocetirizine dihydrochloride, 10 µg/mL; b cetirizine dihydrochloride, 20µg/mL. HPLC conditions: MeCN : MeOH : THF : 0.05M KH_2PO_4 50 : 25 : 5 : 120, (*v*/*v*), pH 5.2 containing 1 mM HP-β-CD, 25 ± 2 °C, 1 mL/min flow rate.

Complete separation was achieved upon using acetonitrile-water 17 : 3, (*v*/*v*) as a mobile phase containing 1 mM of each selected chiral additive. The order of enantioselectivity was found to be HP-β-CD > chondroitin > vancomycin (see Fig. 2). This may be attributed to the size and geometry of (±) cetirizine relative to chiral selectors. The R_F values were 0.46, 0.51, 0.55 for levocetirizine and 0.76, 0.68, 0.69 for dextrocetirizine for the three selected chiral additives respectively (see Fig. 3). The study of the effect of HP-β-CD concentrations (0.25–2.00 mM) showed that the best resolution was achieved using 1 mM with respect to selectivity. As chiral interactions between the chiral selector and analyte are affected by pH and temperature,[23] the effect of pH and temperature on the resolution of cetirizine was investigated. The best discrimination was observed at pH 8.0 and 25 ± 2 °C. A control experiment was performed by eluting the plate without HP-β-CD resulted in no resolution of the racemic mixture. The robustness of the method was studied by slightly changing mobile phase ratios, pH and temperature. The results indicate that the method remained unaffected by small changes in these parameters, as presented in Table 1.[24] The limit of detection (LOD) was estimated by preparing different concentrations of cetirizine ranging from 0.05–40 µg/spot and it was found to be 0.025 µg/spot after detection with iodine vapours.

HPLC-based method

The HPLC method depended on separation of cetirizine and determination of its eutomer levocetirizine using HP-β-CD as CMPAs. Different factors affecting the enantioseparation were thoroughly studied and optimized, including mobile phase composition, chiral selector concentration, pH, detection wavelength, and flow rate. Different mobile phase systems containing potassium dihydrogen orthophosphate (0.05 M) and different organic modifiers were tried, such as acetonitrile, methanol, isopropyl alcohol, and tetrahydrofuran. Using 0.05 M potassium dihydrogen orthophosphate-acetonitrile 60 : 40 (*v*/*v*), 0.1% trifloroacetic acid the enantiomers were separated giving 88% (*R*)-levocetirizine and 12% (*S*)-dextrocetirizine. The mobile phase system consisting of acetonitrile-methanol-tetrahydrofuran-0.05 M potassium dihydrogen orthophosphate sulphate, containing 1 mM HP-β-CD 50 : 25 : 5 : 120 (*v*/*v*), pH5.2 ± 0.2, was accepted as an optimum with respect to separation time and resolution. The concentration of HP-β-CD was an important optimization parameter. Different molarities (0.25–4 mM) were tried with a significant improvement in resolution being achieved with 1 mM HP-β-CD. Variations in the pH were also investigated from pH 3.5–7.0. The optimum separation was achieved at pH 5.2 ± 0.2. The UV detection at 230 nm was found to be sensitive, giving high absorbance. The method was proved to be robust as no significant change on resolution was obtained by small change of flow rate, wavelength and mobile phase composition as stated in Table 1. The comparative HPLC chromatograms of (*R*)–levocetirizine and cetirizine resolution are shown in Fig. 4. The HPLC fractions were collected and analysed by MS. The similarity of molecular mass ions corresponding to each of (*R*)–levocetirizine-HP-β-CD and (*S*)–dextrocetirizine- HP-β-CD were confirmed by mass spectrometry as shown in (Fig. 5).

Table 2. Determination of levocetirizine in presence of (±)cetirizine in laboratory prepared mixtures by HPLC method

Sample	Levocetirizine:(±) Cetirizine (µg : µg)	* Recovery (%)	RSD (%)
1	10 : 90	100.60	1.60
2	20 : 80	99.70	2.00
3	50 : 50	98.00	1.03
4	60 : 40	99.85	1.28
5	90 : 10	98.67	0.60

* All measurements were made in triplicates, 25 ± 2 °C.

Table 3. Validation report of HPLC method for the determination of levocetirizine in drug substance

Parameters	HPLC-method
Linearity range	2.50–200 µg/mL
Regression equation	
slope	48.83
SE of slope	00.48
Intercept	176.73
SE of Intercept	42.15
Correlation coefficient (r)	0.9996
SE of estimation	99.51
Accuracy	
Mean[a] ± RSD%	100.17 ± 1.10
Precision	
Intraday[b]	
Mean ± RSD%	100.88 ± 0.40
Interday[b]	
Mean ± RSD%	99.92 ± 1.06
LOD(µg/mL)	0.03
LOQ(µg/mL)	0.83

[a] n = 6; [b] n = 9

^{1}H-NMR-based method

The NMR technique was used for chiral discrimination of cetirizine and its enantiomer (*R*)-levocetirizine. The aromatic region of the spectrum shows three distinct multiplets: one multiplet with an integral of one and two multiplets with an integral of four. The three multiplets are shifted downfield in the spectrum of the racemate relative to the spectrum of the pure (*R*)-levocetirizine. The chemical shifts of the centre of the three multiplets are 7.34, 7.40 and 7.62 ppm in the racemate and 7.36, 7.42 and 7.67 ppm in the (*R*)-levocetirizine (see Fig. 6). Further studies are being conducted by the authors.

Method validation

The specificity of the method was assessed by analysing laboratory-prepared mixtures of (*R*)-levocetirizine and cetirizine in different proportions as shown in Table 2. The linearity was investigated for ten concentrations in the range of 2.5–200 µg/mL revealed good linearity. The accuracy based on the mean percentage recovery of measured concentrations (n = 6) to the actual concentration is shown in Table 3. The precision of the method was assessed by determining RSD% values of intra- and inter-day analysis (n = 9) of (*R*)-levocetirizine over three days. Two different analyses were performed the intermediate precision experiments with separate buffer solution according to the assay of pharmaceutical preparations. Each sample solution was assayed in triplicate. The RSD% values of the intermediate precision are less than 2% for (*R*)-levocetirizine in drug substance and tablets. The LOD and quantification (LOQ) for (*R*)-levocetirizine were found to be 0.03 and 0.83 µg/mL respectively as shown in Table 3. The LOD of less than 1.0% (*S*)-dextrocetirizine in (*R*)-levocetirizine was estimated by visual (S/N) evaluation. The system suitability was tested according to USP guidelines and the results are given in Table 4. The standard addition recoveries were carried out by adding a known amount of (*R*)-levocetirizine to Zyrtec tablet at three different levels (10 µg, 50 µg, 150 µg) and each level in triplicates (n = 3). The percentage of recovery was evaluated by the ratio of the amount found and the amount added. The average recovery was calculated and is presented in Table 5.

Table 4. Results of system suitability tests of HPLC method

Parameters	Values	Comments
Retention time of levocetirizine	5.65 ± 0.45	±Standard deviation
Retention time of dextrocetirizine	8.19 ± 0.13	±Standard deviation
Injection repeatability	1.08%	RSD for five injections
K′1 – Levocetirizine	2.82	Capacity factor
K′2 – Dextrocetirizine	4.57	Capacity factor
Selectivity factor(α)	1.60	Separation factor calculated as K2/K1
Tailing factor	1.0 ± 0.01	Calculated at 5% of peak height
Theoretical plates (N)	3076	Column efficiency plate/column
HETP	0.003	Height equivalent theoretical plate
Resolution	3.91	Calculated by $2(t_2 - t_1)/w_2 + w_1$

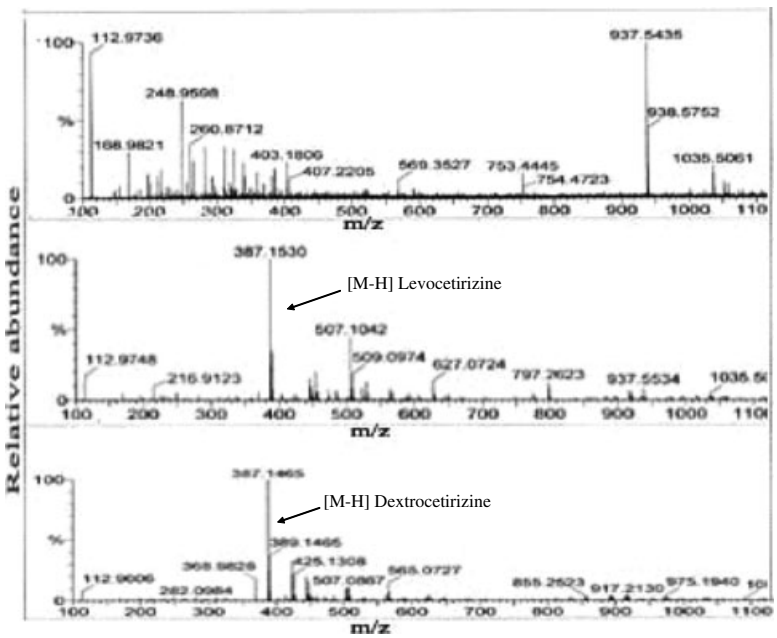

Figure 5. Mass spectra of HPLC fraction collection peaks: mobile phase containing HP-β-CD (top), (*R*)-levocetirizine–HP-β-CD (middle), and (*S*)-dextrocetirizine–HP-β-CD (bottom).

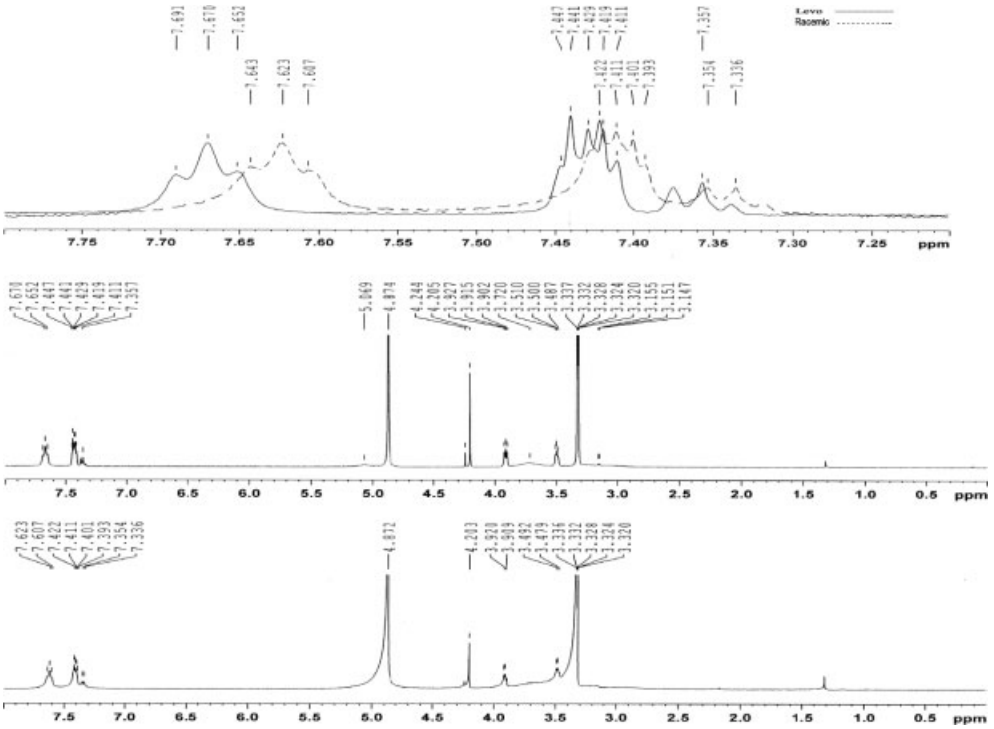

Figure 6. ^{1}H-NMR spectra of cetirizine and (*R*)-levocetirizine (400 MHz, CD_3OD).

Table 5. Analysis results for determination of levocetirizine in tablet and application of standard addition technique by HPLC

		Standard addition	
Sample	* *Recovery* ±*RSD%*	*Authentic added* µg/mL *R-Levocetirizine*	* *Found recovery* ± *RSD%*
1	97.50 ± 1.55	10	98.00 ± 1.65
2	98.00 ± 1.43	50	99.38 ± 1.24
3	99.00 ± 1.57	150	100.10 ± 1.80

* n = 3

Application

The developed TLC- and HPLC-based methods were applied for the enantioseparation of cetirizine and the determination of its enantiomer (*R*)-levocetirizine in the drug substance and the drug product. ^{1}H-NMR was used to discriminate cetirizine and (*R*)-levocetirizine in the drug substance.

Conclusion

Three selective and robust TLC, HPLC and ^{1}H-NMR methods were applied for the chiral discrimination of cetirizine and its enantiomer (*R*)-levocetirizine in the drug substance and the drug product. The stereospecificity was achieved by incorporating chiral selectors directly into TLC and HPLC mobile phases. The TLC method successfully detected as low as 0.025 µg/spot of each enantiomer. Using a conventional HPLC column, the two enantiomers were separated and (*R*)-levocetirizine was quantified. The resolved peaks of (*R*)- and (*S*)-forms of racemic cetirizine were confirmed by mass spectrometry. ^{1}H-NMR used for the chiral discrimination of cetirizine and its (*R*)-levocetirizine has an advantage over TLC and HPLC methods which the time of analysis is two minutes and there is no need for method development. This approach greatly simplified the overall procedure, resulting in rapid and efficient sample analysis while maintaining precision and accuracy.

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