J. Pharm. Res. 2020;19(3):1–6

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Resolution and Characterization of Racemic Mixture of Carbinoxamine using Tartaric Acid

B Narendra Boggula[1], M D Karvekar[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

ARTICLE INFO

Article history:
Received 25.06.2020
Accepted 10.09.2020
Published 23.09.2020

* *Corresponding author.*
M D Karvekar
drmdkarvekar@gmail.com

https://doi.org/10.18579/jopcr/v19.3.narendra

ABSTRACT

Racemic H_1-antihistamine carbinoxamine contains enantiomers whose pharmacological characteristics may be different. The enantiomeric resolution of such molecules is important for maximising their efficacy and reducing side effects. The objective of this study was to achieve chiral resolution of racemic carbinoxamine maleate and evaluate the individual enantiomers based on their structural and pharmacological characteristics. Racemic carbinoxamine maleate was resolved by diastereomeric salt formation with O,O'-di-p-toluoyl-D-tartaric acid at R. L. Fine Chemicals, Bangalore. Crystallisation from isopropyl alcohol selectively afforded the (S)-isomer, and the (R)-isomer was obtained from the mother liquor. The free bases were recovered and transformed into maleate and oxalate salts, respectively. The analytical methods involved melting point determination, TLC, IR, optical rotation, and ^{1}H NMR. Antihistaminic activity was tested using the guinea pig ileum at the Krupanidhi College of Pharmacy, Bangalore. The (S)-carbinoxamine base had an optical rotation of -2° and 95.83% inhibition of histamine, whereas the (R)-isomer had +4° rotation and an inhibition of just 9.09–12.5%. Different melting points and NMR singlets were used to verify enantiomeric purity. The IR spectra confirmed the differential interactions with the resolving agent. This study provides an effective resolution approach for carbinoxamine with a high enantiomeric yield and illustrates the higher pharmacological activity of the (S)-isomer. The findings of this study provide support for the development of enantiomerically pure formulations.

Keywords: Carbinoxamine; Chiral Resolution; Tartaric Acid; Antihistamine Activity

INTRODUCTION

Chirality is a key factor for the pharmacological activity and safety of therapeutic compounds. Enantiomers or chiral isomers tend to possess different biological activities; one enantiomer potentially has therapeutic activity, while its mirror image is less active or even toxic.[1] This fact supports the need for enantioselective synthesis and resolution for the development of pharmaceuticals.

Carbinoxamine is an antihistaminic drug often administered as a racemic mixture of equal quantities of its (R)- and (S)-enantiomers. Considering the possibility of differences in pharmacodynamics and pharmacokinetics between enantiomers, individual characterisation and resolution are of important interest. In the past, enantiomers from racemic mixtures have been separated using a variety of methods, such as chiral chromatography and diastereomeric salt crystallisation. The latter involves the creation of diastereomeric salts via a chiral resolving agent, taking advantage of the differences in their physical properties to allow separation. One naturally occurring chiral molecule, tartaric acid, has been widely used in such cases because it can form crystalline diastereomeric salts with many amines. Notably, Louis Pasteur's pioneering work in the 19th century demonstrated the resolution of racemic tartaric acid into its enantiomers by manual separation of their crystals, laying the foundation for stereochemistry.[2]

The resolution of racemic carbinoxamine with tartaric acid derivatives is a promising method for obtaining individual enantiomers. The literature has documented the efficiency of di-p-toluoyl-D-tartaric acid in resolving the racemic mixtures of other amines, which results in the generation of diastereomeric salts separable due to their solubility differences.[3] Subsequent basification resulted in the generation of free enantiomerically pure amines. This process not only permits the separation of single enan-

KRUPANIDHI COLLEGE OF PHARMACY

tiomers but also permits their detailed analysis with methods including melting point analysis, infrared spectroscopy, measurement of optical rotation, and nuclear magnetic resonance spectroscopy.[4]

With carbinoxamine, resolution methods are important to understand the different pharmacological profiles of its enantiomers. Due to the possibility of one enantiomer having better antihistaminic activity, along with a better safety profile, the possibility of isolating and investigating each enantiomer separately is fundamental. Understanding the specific interactions of each enantiomer with biological targets will pave the way for the production of more effective and safer therapeutic compounds.

The present study aims to achieve the resolution of racemic carbinoxamine maleate using di-p-toluoyl-d-tartaric acid and the subsequent exhaustive characterisation of the product enantiomers. Through the application of mixed crystallisation methodologies and analytical techniques, this study revealed the structural and pharmacological differences between the (R)- and (S)-enantiomers of carbinoxamine. These findings are expected to lead to improved antihistaminic therapies and highlight the importance of chiral resolution in medicinal research.

MATERIALS AND METHODS

Resolution and synthesis were conducted at the R&D Laboratory of R. L. Fine Chemicals, Yelahanka, Bangalore. Pharmacological screening was performed in the Department of Pharmacology, Krupanidhi College of Pharmacy, Bangalore.

Chemicals Used

All chemicals used in this study were of Laboratory Reagent (LR) and Analytical Reagent (AR) grade. These were obtained from recognised sources, such as Lancaster, Sigma Aldrich, NR Chem., Rolex, S. D. Fine Chem. Ltd., and Merck.

Analytical and Characterization Techniques

- **Melting Point Determination:**

The melting points of the synthesised and separated isomers were determined using the open capillary tube method. The measurements were performed using a digital melting point apparatus (MP Digital) and were not corrected.

- **Thin Layer Chromatography (TLC):**

To determine compound purity, TLC analysis was performed using silica gel G as the stationary phase. The mobile phase was comprised of a mixture of acetone and chloroform. The chromatograms were detected using an iodine chamber, resulting in brown spots.

- **Infrared Spectroscopy (IR):**

IR spectra of the resolved and synthesised compounds were obtained using the KBr pellet technique in the range of 4000–400 cm^{-1}. The analyses were carried out at R.L. Fine Chemicals, Bangalore, using a Shimadzu 8700 FT-IR spectrophotometer. The peaks are represented in terms of wavenumbers (cm^{-1}).

- **Proton Nuclear Magnetic Resonance (^{1}H-NMR):**

^{1}H-NMR spectra were recorded at the Sophisticated Analytical Instrumentation Facility (SAIF), IISc Bangalore, on a 200 MHz Amx-200 NMR spectrometer. Samples were dissolved in deuterated chloroform ($CDCl_3$) and chemical shifts were reported in parts per million (ppm) against tetramethylsilane (TMS).

- **Optical Rotation:**

The optical rotations were determined using a Jasco P-2000 Polarimeter. Measurements were performed in a 20% w/v methanol solution, and rotation was recorded in degrees.

Synthetic and Resolution Procedure

- **Basification of (±)-Carbinoxamine Maleate:**

Racemic carbinoxamine maleate (250 g) was dissolved in 500 ml of distilled water. The solution was basified with dilute NaOH to pH 10–11. Thereafter, 1000 ml of toluene was added, and the mixture was shaken thoroughly. The organic layer was separated, and the aqueous phase was extracted again with 500 ml toluene.

The combined toluene extracts were washed twice with 600 ml water, dried with 4–5 g of anhydrous sodium sulfate, and allowed to stand for 15–20 min. The organic phase was vacuum-distilled, and the residual solvent was removed by passing nitrogen gas through it to produce 174.6 g of (±)-carbinoxamine base with a yield of 97.7%.

- **Synthesis of (±)-Carbinoxamine Di-p-Toluoyl-D-Tartaric Acid Salt:**

In a 1000 ml round-bottomed flask, 140 g of (±)-carbinoxamine base was dissolved in 700 ml ethyl acetate and stirred under nitrogen pressure at 40–50°C. 182.6 g of di-p-toluoyl-D-tartaric acid was added slowly. The mixture was maintained for 1.5 hours and then cooled overnight at room temperature. The resulting salt was vacuum filtered and dried at 40°C for 2 h, and again at 65-70°C for 7 h, yielding 300 g (92%) of crystalline salt.

- **Resolution into (R)- and (S)-Diastereomers:**

One hundred grams of the salt was dissolved in 300 ml isopropyl alcohol and warmed to 77–80°C until a clear solution was formed. The solution was cooled and left to stand at room temperature for 48 h, resulting in

the crystallisation of the (S)-diastereomer. The crystals were then vacuum-filtered and dried. The mother liquor, which contained the (R)-diastereomer, was evaporated, re-dissolved in ethyl acetate, and recrystallised in the same way.

- **Basification of Diastereomeric Salts:**

Both (S)- and (R)-carbinoxamine diastereomeric salts were separately dissolved in water, basified with NaOH (pH 10–11), and extracted with toluene. The toluene layers were dried over sodium sulfate, filtered, and distilled under a vacuum. The (S)-base had an optical rotation of -20°, whereas the (R)-base had an optical rotation of +40°, validating the successful resolution.

- **Synthesis of (S)- and (R)-Carbinoxamine Maleates:**

Each base (10 g) was treated with equimolar amounts of maleic acid in ethyl acetate, heated to 40–50°C, and stirred. Upon cooling, the resulting precipitate was vacuum filtered and dried at 40°C and then at 60–65°C to obtain pure (S)- and (R)-carbinoxamine maleates.

- **Synthesis of (S)- and (R)-Carbinoxamine Oxalates:**

Each purified base was also reacted with oxalic acid in ethyl acetate under the same conditions. Upon precipitation, filtering, and drying, (S)- and (R)-carbinoxamine oxalates were obtained with good purities.

RESULTS

- **(±)-Carbinoxamine Maleate Resolution and Characterization**

The resolution of (±)-carbinoxamine maleate was successfully achieved through diastereomeric salt formation with O,O'-di-p-toluoyl-D-tartaric acid as the resolving agent. The crude racemic salt was crystallised with isopropyl alcohol (1:3 ratio). Crystals of the (S)(−)-isomer were isolated after two days of vacuum filtration, with a yield of 92%. After ethyl acetate treatment, the mother liquor gave the (R)(+)-isomer. The two isomers were basified with NaOH to provide free bases.

- **Melting Point Determination**

The melting points of the resolved compounds verified their successful resolution and revealed their purity. The racemic salt (R)(S)-carbinoxamine di-p-toluoyl-D-tartaric acid had melting points of 124–127°C, whereas the resolved salts of (S)- and (R)-carbinoxamine di-p-toluoyl-D-tartaric acid had melting points of 101–103°C and 95–98°C, respectively. The corresponding maleate and oxalate salts also have different melting points (Table 1).

- **Infrared Spectroscopy**

The IR spectral information further validated the structural resolution and integrity. The racemic compound showed specific peaks like 3427.51 cm^{-1} (O–H stretch) and 1718.58 cm^{-1} (C=O stretch). On resolution, the (S)-isomer showed peaks at slightly shifted positions of 3423.65 cm^{-1} and 1720.50 cm^{-1}, and the (R)-isomer showed peaks at 3398.57 cm^{-1} and 1716.65 cm^{-1}, which indicate enantiomer-specific interactions with the resolving agent (Table 2).

Table 1: Melting point of carbinoxamine derivatives

Compound	Melting point
(R)(S)-Carbinoxamine di-p-toluoyl-D-tartaric acid	124-127 °C
(S)-Carbinoxamine di-p-toluoyl-D-tartaric acid	101-103 °C
(R)-Carbinoxamine di-p-toluoyl-D-tartaric acid	95-98 °C
(S)-Carbinoxamine maleate	114-117 °C
(S)-Carbinoxamine oxalate	83-86 °C
(R)-Carbinoxamine maleate	125-127 °C
(R)-Carbinoxamine oxalate	92-94 °C

Table 2: IR spectral data of the racemic mixture, (S)-isomer and (R)-isomer of carbinoxamine di-p- toluoyl-D-tartaric acid

Compound	IR KBr λ cm^{-1}
(R)(S)-Carbinoxamine di-p-toluoyl-D-tartaric acid	3427.51 cm^{-1} (OH str.), 3059.10 cm^{-1} (Ar. C-H str.), 2956.87 cm^{-1} (Alip. C-H str.), 2350 cm^{-1} (quaternary nitrogen atom (asymmetric str.)), 1718.58 cm^{-1} (C=O str.), 1612.49 cm^{-1} (Ar. C=C str.), 1267.23 cm^{-1} (C-N str.), 1018.41 cm^{-1} (C-Cl Ar.).
(S)-Carbinoxamine di-p-toluoyl-D-tartaric acid	3423.65 cm^{-1} (OH str.), 3037.89 cm^{-1} (Ar. C-H str.), 2960.73 cm^{-1} (Alip. C-H str.), 2370.51 cm^{-1} (quaternary nitrogen atom (asymmetric str.)), 1720.50 cm^{-1} (C=O str.), 1616.35 cm^{-1} (Ar. C=C str.), 1267.23 cm^{-1} (C-N str.), 1020.34 cm^{-1} (C-Cl Ar.).
(R)-Carbinoxamine di-p-toluoyl-D-tartaric acid	3398.57 cm^{-1} (OH str.), 3059.10 cm^{-1} (Ar. C-H str.), 2970.38 cm^{-1} (Alip. C-H str.), 2372.44 cm^{-1} (quaternary nitrogen atom (asymmetric str.)), 1716.65 cm^{-1} (C=O str.), 1616.35 cm^{-1} (Ar. C=C str.), 1265.30 cm^{-1} (C-N str.), 1020.34 cm^{-1} (C-Cl Ar.).

- **Optical Rotation**

Optical rotation analysis was used to establish enantiomeric purity. The racemic base demonstrated 0° rotation, whereas the resolved (S)-carbinoxamine had a specific rotation of − 2°, indicating that it was levorotatory in nature. (R)-Carbinoxamine had a rotation of +4° and was thus

determined to be dextrorotatory. These findings clearly establish successful chiral resolution (Table 3).

- **Pharmacological Screening**

The antihistaminic activity of maleate and oxalate salts of the resolved isomers was evaluated in the guinea pig ileum. (S)(–)-Carbinoxamine maleate and oxalate showed 95.83% inhibition of histamine, but the (R)(+)-isomers showed only 9.09% and 12.5% inhibition, respectively. This clearly showed that the (S)-isomer was pharmacologically more active than the (R)-isomer (Figure 1).

Table 3: Optical rotation of carbinoxamine bases and Antihistamine activity of the synthesized compounds

Optical rotation of carbinoxamine bases	
Compound	**Optical rotation**
(R)(S)-Carbinoxamine base	0°
(S)-Carbinoxamine base	-2°
(R)-Carbinoxamine base	+4°
Antihistamine activity of the synthesized compounds	
Drug	**% Histamine inhibition**
(S)(-)-Carbinoxamine maleate	95.83%
(R)(+)-Carbinoxamine maleate	9.09%
(S)(-)-Carbinoxamine oxalate	95.83%
(R)(+)-Carbinoxamine oxalate	12.5%

- **^{1}H NMR Spectroscopy**

The ^{1}H NMR spectra provided detailed structural information. In the racemic mixture (Figure 1), N,N-dimethyl groups existed as two singlets at 5.450–5.600 ppm, indicating the coexistence of both enantiomers. The resolved (S)-carbinoxamine spectrum (Figure 2) showed a single singlet at 5.4517 ppm for the 6H of the dimethyl groups, proving the enantiomeric purity. (R)-Carbinoxamine (Figure 3) displays a singlet at 5.5690 ppm, reflecting its purity. These spectral variations further confirmed the effective separation and identification of enantiomers.

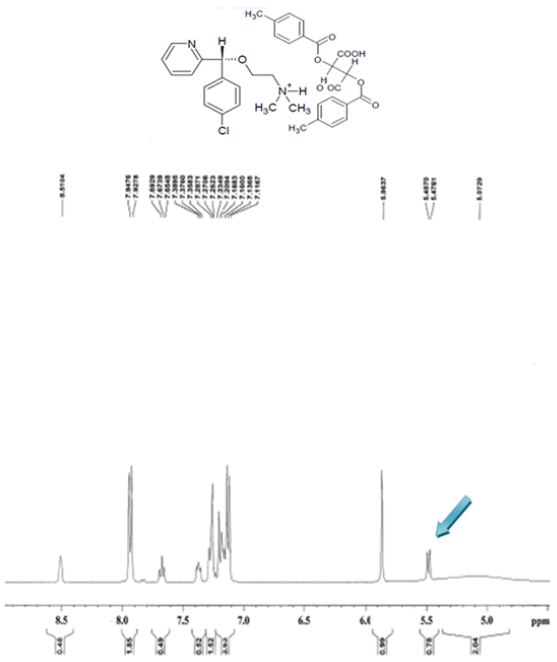

Fig. 1: Structure and ^{1} H NMR spectra of (R)(S)-carbinoxamine di-p-toluoyl-D-tartaric acid

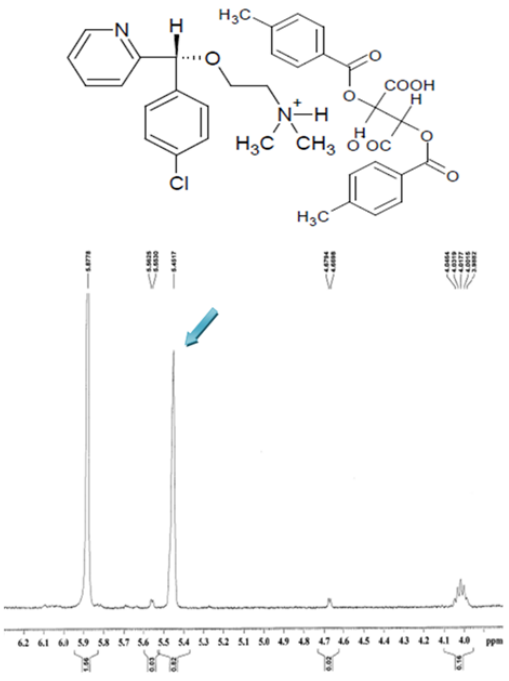

Fig. 2: Structure and ^{1} H NMR spectra of (S)(-)-carbinoxamine di-p-toluoyl-D-tartaric acid

DISCUSSION

The present study effectively showed the resolution of (±)-carbinoxamine maleate to its enantiomers by O,O'-di-p-toluoyl-D-tartaric acid through diastereomeric salt formation, a tried and tested chiral resolution method.[5] Crystallization from isopropyl alcohol provided the (S)(–)-isomer with high purity and yield (92%), and the (R)(+)-isomer was then recovered from the mother liquor. This method is in line with earlier studies that employed tartaric acid derivatives to resolve simple pharmaceutical compounds.[6,7] Melting point analysis confirmed successful resolution, with the resolved enantiomers having different melting ranges from one another and from the racemic mixture.

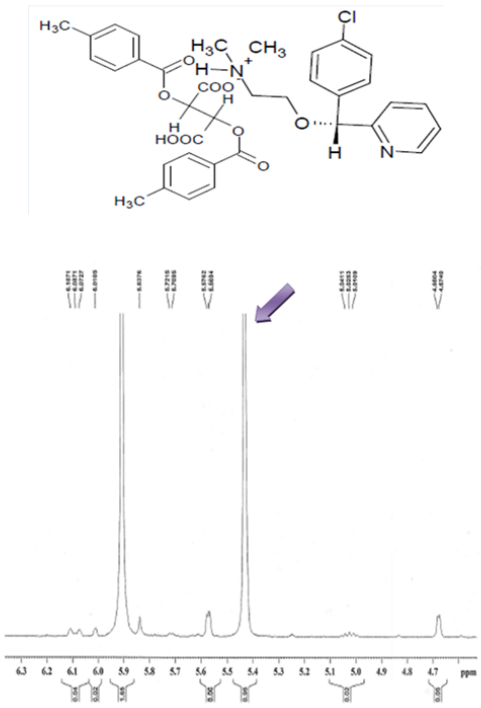

Fig. 3: Structure and ^{1} H NMR spectra of (R)(+)-carbinoxamine di-p-toluoyl-D-tartaric acid

This heating pattern heating was in accordance with expectation of the formation of pure diastereomeric salts, consistent with literature accounts on enantiomer separation with tartaric acid where melting point discrimination has provided a principal basis for purity determination.[8]

Infrared spectral data of the present study also confirmed the structural integrity of the resolved enantiomers. Minor differences in O–H and C=O stretching frequencies reflected minor variations in hydrogen bonding geometries and molecular interactions with the resolving agent, a process widely observed in earlier spectroscopic work on resolved drug isomers.[9] Optical rotation measurements clearly demonstrated successful chiral resolution. As predicted, the racemic mixture was not optically active; however, the resolved (S)- and (R)-enantiomers exhibited specific rotations of -2° and +4°, respectively. These observations were consistent with previous characterizations of resolved carbinoxamine and other H1-antihistamines, which have similar optical properties upon resolution.[10]

Pharmacological screening also supported enantiomeric discrimination. The (S)(–)-carbinoxamine maleate and oxalate salts exhibited significantly higher antihistaminic activities than the (R)(+)-enantiomers, suggesting that the biological activity was largely confined to the (S)-enantiomer. These findings are consistent with earlier reports, where one enantiomer of chiral antihistamines was found to be much more potent through selective binding to H1-receptors.[11,12]^{1}H NMR spectroscopy provided unequivocal proof of the enantiomeric purity. The separated isomers exhibited single sharp singlets for the N,N-dimethyl protons, whereas the racemic mixture exhibited overlapping peaks. This was consistent with previous study, using NMR as a confirmatory technique for resolution of chiral amines, specifically in detecting differences in chemical shifts between enantiomers.[13]

Overall, the resolution of carbinoxamine by O,O'-di-p-toluoyl-D-tartaric acid was not only effective but also produced enantiomers whose physical, spectral, and pharmacological characteristics are well known. (S)-Enantiomer exhibited superior antihistaminic activity, affirming its potential use as a single-enantiomer drug, a pattern increasingly followed in contemporary drug development for enhanced efficacy and fewer side effects.[14]

CONCLUSION

The present study achieved the resolution of racemic carbinoxamine maleate with O,O'-di-p-toluoyl-D-tartaric acid, yielding enantiomerically pure (S)- and (R)-isomers. Characterisation by melting point, IR, NMR, and optical rotation analyses validated the purity and identity of the resolved products. The pharmacological activity revealed that the (S)-enantiomer had a much greater antihistaminic activity, indicating its therapeutic value. These findings support the development of enantiomerically pure (S)-carbinoxamine as a potent and targeted antihistamine drug.

REFERENCES

1. Nguyen LA, He H, Pham-Huy C. Chiral Drugs. An Overview. *International Journal of Biomedical Science*. 2006;2(2):85–100. Available from: https://dx.doi.org/10.59566/ijbs.2006.2085.
2. Drayer DE. The early history of stereochemistry: From the discovery of molecular asymmetry and the first resolution of a racemate by Pasteur to the asymmetrical chiral carbon of van't Hoff and Le Bel. *Clinical Research and Regulatory Affairs*. 2001;18(3):181–203. Available from: https://dx.doi.org/10.1081/crp-100108172.
3. Helmchen G. Asymmetric Synthesis with Chemical and Biological Methods. John Wiley & Sons. 2007. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9783527610648.
4. Maier NM, Franco P, Lindner W. Separation of enantiomers: needs, challenges, perspectives. *Journal of Chromatography A*. 2001;906(1-2):3–33. Available from: https://dx.doi.org/10.1016/s0021-9673(00)00532-x.
5. Eliel EL, Wilen SH. Stereochemistry of Organic Compounds. John Wiley & Sons. 1994. Available from: https://books.google.co.in/books?id=OAUPEAAAQBAJ.
6. Lindner W, Hirschböck I. Tartaric acid derivatives as chiral sources for enantioseparation in liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*. 1984;2(2):183–189. Available from: https://dx.doi.org/10.1016/0731-7085(84)80069-2.
7. McConathy J, Owens MJ. Stereochemistry in Drug Action. *The Primary Care Companion For CNS Disorders*. 2003;5(2):70–73. Available from: https://dx.doi.org/10.4088/pcc.v05n0202.
8. Hutt A, Valentova J. The chiral switch: the development of single enantiomer drugs from racemates. *Universitas Comeniana Acta Facultatis Pharmaceuticae*. 2003;50:7–23. Available from: https://www.researchgate.net/publication/284658722_The_chiral_switch_The_development_of_single_enantiomer_drugs_from_racemates.

9. Tardioli S, Gooijer C, van der Zwan G. Structure elucidation of fluorescent H₁ antihistamines by ultraviolet resonance Raman spectroscopy: solvent structures of tripelennamine and mepyramine. *Journal of Raman Spectroscopy*. 2011;42(5):1016–1024. Available from: https://dx.doi.org/10.1002/jrs.2810.
10. Tang YF, Wu HL, Wu SM, Chen SH, Kou HS. Quantitative chiral analysis of carbinoxamine, doxylamine, and orphenadrine by capillary zone electrophoresis. *Journal of Microcolumn Separations*. 2000;12(6):366–370. Available from: https://dx.doi.org/10.1002/1520-667x(2000)12:6<366::aid-mcs5>3.0.co;2-c.
11. Nicholson AN, Pascoe PA, Turner C, Ganellin CR, Greengrass PM, Casy AF, et al. Sedation and histamine H1-receptor antagonism: studies in man with the enantiomers of chlorpheniramine and dimethindene. *British Journal of Pharmacology*. 1991;104(1):270–276. Available from: https://dx.doi.org/10.1111/j.1476-5381.1991.tb12418.x.
12. Testa B, Mayer JM. Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry, and Enzymology. Wiley. 2003. Available from: http://dx.doi.org/10.1002/9783906390444.
13. Ward TJ, Ward KD. Chiral Separations: A Review of Current Topics and Trends. *Analytical Chemistry*. 2012;84(2):626–635. Available from: https://dx.doi.org/10.1021/ac202892w.
14. Srinivas NR, Barbhaiya RH, Midha KK. Enantiomeric drug development: Issues, considerations, and regulatory requirements. *Journal of Pharmaceutical Sciences*. 2001;90(9):1205–1215. Available from: https://dx.doi.org/10.1002/jps.1074.