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Enantiomeric separation of Midodrine hydrochloride in bulk and pharmaceutical dosage form by chiral HPLC

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ABSTRACT

A simple, sensitive chiral liquid chromatographic method was developed for the separation and quantification of enantiomers Midodrine. A chiral PAK IG3 (150 x 4.6 mm) 3μm column was used for the separation of the enantiomers. The mobile phase consists of 10 mM ammonium bicarbonate in water and acetonitrile in the ratio of 95:5, v/v with a flow rate of 0.7 ml/min. The detection was done at 290 nm with column temperature maintained at 40°C. The method linear ranged between 10 – 110 μg/ml and 5 – 100 μg/ml for (+) and (-) Midodrine enantiomers. The recovery of the method was found to be in the range of 99.1 to 101.2 %. The detection limit for the (+) and (-) enantiomers was found to be 4 μg/ml and 1 μg/ml, respectively. A simple validated chiral HPLC method with reverse elusion is described for the separation and quantification of the enantiomers of Midodrine in bulk and formulation.

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INTRODUCTION

The importance of enantioselective has become increasingly important in the analysis of drugs. Despite the significant differences in the pharmacological, pharmacodynamics and pharmacokinetics of the individual enantiomers racemic mixtures are marketed in the market. It must be taken into account that one of the enantiomers can be more active or toxic. The activity of chiral substances primarily depends upon their stereochemistry due to the chiral environment of the body (Shafaati, 2007).

Enantiomeric drugs generally differ in their bioactivity, toxicity, and metabolic mechanism and have gained attention over the years (Chen *et al.*, 2007). The production of pure enantiomeric compounds has been emphasized by the pharmaceutical industry before studying the pharmacokinetic and toxicological effects in search of drugs with greater therapeutic benefits and low toxicity (Kumar *et al.*, 2015).

Over the past decade, chiral analysis has become more important due to its different potential behavior after its administration. In the chiral drug, one of the enantiomers (the eutomer) will produce the desired therapeutic effect whereas, the other enantiomer (the distomer) has the capability to produce a lower effect of in some cases it may cause side effects (Ates *et al.*, 2013).

Midodrine (2 - amino - *N* - [2 - (2,5 - dimethoxy - phenyl)-2-hydroxyethyl] acetamide) a cardiovascular drug had a chiral carbon in C-2 of the hydroxyethyl portion of the molecule has been studied (Quaglia *et al.*, 1998, 2004). To our knowledge, no chiral HPLC method is reported in the separa-

tion and quantification of enantiomers Midodrine. Hence, the aim of the present work is to separate and quantify Midodrine enantiomers in bulk and its pharmaceutical formulation using a simple, sensitive and validated chiral HPLC method.

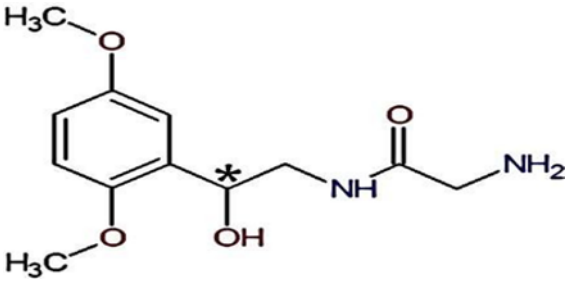

Figure 1: Structure of Midodrine

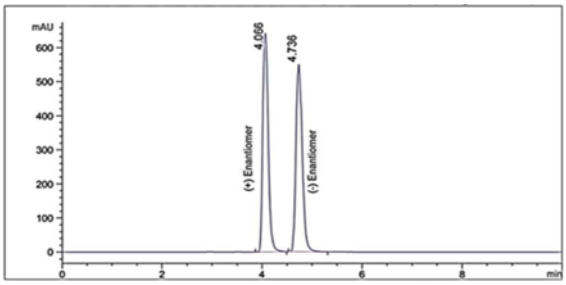

Figure 2: Enantio selective separation of racemic Midodrine in bulk

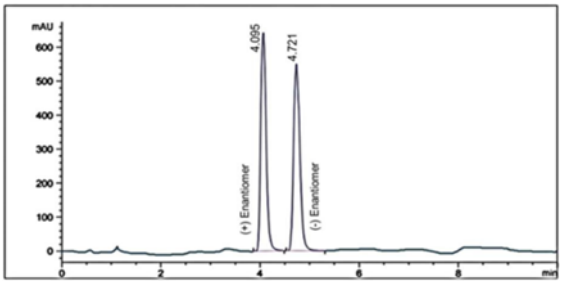

Figure 3: Enantio selective separation of racemic Midodrine in a pharmaceutical formulation

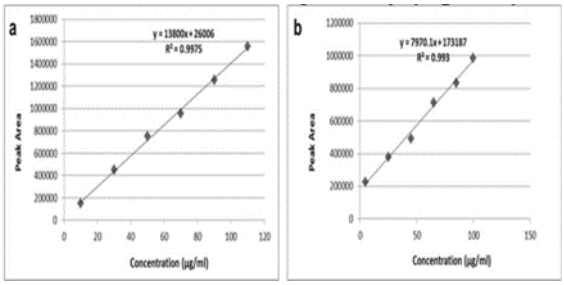

Figure 4: Calibration curve (a)(+) Midodrine enantiomer and (b) (-) Midodrine enantiomer

MATERIALS AND METHODS

Chemicals and reagents

The racemic Midodrine Hydrochloride was procured from PAR Formulations, Chennai, India. The (+) and (-) standard enantiomers were prepared by an in-house method using polarimetry determination. Commercially available Midodrine tablets were procured from Gurmail brother pharmaceutical company, Ludhiana (Punjab), India. Acetonitrile of HPLC grade and AR grade ammonium bicarbonate were procured from Ranbaxy.

Equipment

High-performance liquid chromatography (Shimadzu gradient HPLC system) equipped with a solvent delivery system (Model-LC-10 AT-VP), Rheodyne injector (Model-7725i with 20μl loop), UV detector (Model-SPD M-10A VP). The data were recorded using class-VP data station.

Standard preparation

1mg/ml concentration of Midodrine hydrochloride working standard was prepared by dissolving 10 mg of the drug in a 10 ml volumetric flask with the mobile phase. The volume was made up with the mobile phase. The prepared standard solution was injected, and the chromatogram was recorded.

Selection of wavelength

A solution of 10 μg/ml of Midodrine hydrochloride in water was prepared. The UV spectrum was recorded by scanning the solution in the range of 200 to 400 nm. From the UV spectrum wavelength of 290 nm was selected at which Midodrine hydrochloride showed maximum absorbance.

Method development

The chromatographic separation was achieved using a Chiral PAK IG-3 (150 x 4.6 mm) 3μm column. The mobile phase was 10 mM ammonium bicarbonate in water and acetonitrile in the ratio of 95:5, v/v with the flow rate and column temperature of 0.7 ml/min and 40°C, respectively. The detection was done at a wavelength of 290 nm. The injection volume was set at 10 μL. The retention time of both (+) and (-) enantiomers was about 4.06 and 4.73 min, respectively (Figure 2).

Validation of the Method

The validated of the method was performed for specificity, accuracy, precision, system suitability, linearity, limits of detection (LOD) and quantification (LOQ) and robustness was done in accordance with the guidelines of ICH (International Conference on Harmonization, 1996).

Specificity

Specificity is the ability to assess the presence of components which may be expected to be

Table 1: Recovery study for (+) and (-) enantiomer of Midodrine

Label claim (mg)	Amountpresent(mg/tablet) ± % RSD (n = 3)			% Recovery ± % RSD (n = 3)		
	(+) & (-)	(+)	(-)	(+) & (-)	(+)	(-)
2.5	2.48 ± 0.40	1.49 ± 0.51	0.99 ± 0.21	99.1 ± 0.40	60.0 ± 0.12	40.0 ± 0.23
	2.47 ± 0.61	1.52 ± 0.37	0.90 ± 0.35	99.6 ± 0.61	63.2 ± 0.42	36.8 ± 0.33
	2.49 ± 0.23	1.51 ± 0.47	0.98 ± 0.65	101.2 ± 0.23	65.4 ± 0.62	34.6 ± 0.53

Table 2: System suitability study for (+) and (-) enantiomer of Midodrine

S.No	Parameters	(+) Midodrine	(-) Midodrine
1	Linearity range	10 - 110 μg/ml	5 - 100 μg/ml
2	Regression equation	y = 13800x + 26006	y = 7970.1x + 173187
3	Correlation coefficient	0.997	0.993
4	Theoretical plate/meter	6724	6578
5	Resolution factor	3.10	
6	Asymmetric factor	0.81	0.79
7	LOD(μg/ml)	4	1
8	LOQ(μg/ml)	10	5

present along with the analyze such as impurities, degradant, etc.

Linearity

The linearity was evaluated by analyzing the (+) and (-) Midodrine enantiomers of Midodrine in the range 10–110μg/ml and 5– 100μg/ml, respectively. The correlation coefficient, slope and intercept were calculated from the graph.

The Midodrine (Gutron, 2.5mg) tablets were ground to a fine powder. The amount equivalent to 0.13 g was weighed and transferred into 100 ml volumetric flask which was into the mobile phase by ultrasonication (45 kHz) in a water bath for 30 min. The use of ultra-sonication had not influenced much on the quality and stability of the extracted compounds. The extractant was filtered through a 0.45 μm membrane and used to prepare the test solution. Chromatograms were recorded and then measured for peak areas (Figure 3).

Detection (LOD) and quantification limit (LOQ)

The detection and quantitation limit was determined by the signal-to-noise ratio of 3:1 and 10:1, respectively.

Accuracy

The accuracy of the method was calculated by recovery studies at three levels low, middle and high concentration from the linearity range. The mean, standard deviation and % RSD were calculated.

System suitability study

System suitability study is an essential part of the method development process. In which a number of theoretical plates (N), tailing factor (T) are evaluated for three replicates injections of the sample solution.

RESULTS AND DISCUSSION

Specificity

The specificity test demonstrated that the use of excipients did not interfere with the main peak and no peaks were eluted with the retention time of the (+) and (-) enantiomers (Figure 3).

Linearity

The linearity was evaluated at six determinations for both (+) and (-) enantiomers of Midodrine with the range 10–110 μg/ml and 5–100 μg/ml, respectively. The coefficient of regression was found to be 0.997 and 0.993 for (+) and (-) Midodrine enantiomer. The regression line equation was y = 13800x + 26006 R^2= 0.997 and y=7970.1x + 173187 R^2 = 0.993 for (+) and (-) Midodrine enantiomer, respectively (Figure 4).

Accuracy

The accuracy of the method was evaluated by recovery studies, and the percentage recovery was calculated. The accuracy of the optimized methods was determined by absolute recovery experiments. The percentage recovery value for (+) and (-) enantiomers was found to be 99.1 to 101.2% (Table 1).

Detection and quantification Limit

The limit of detection for this method was found to be 4μg/ml and 1μg/ml for both (+) and (-) enantiomers, respectively. The limit of quantification was found to be 10μg/ml and 5μg/ml for (+) and (-) Midodrine enantiomers

System suitability study

System suitability study was performed to check parameters such as column efficiency, resolution factor and an asymmetric factor of Midodrine hydrochloride peak. Results obtained from six replicate injections of the standard solution are found to be, and the results are summarized in (Table 2).

CONCLUSION

A simple normal phase chiral HPLC method with reverse elusion described for the separation and quantitative determination of enantiomers of Midodrine in bulk and formulation. The developed direct chiral reverse phase HPLC method was found to be simple, rapid, accurate, precise and linear.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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