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Original Article

FAST CHIRAL HPLC PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF DROPROPIZINE ENANTIOMERS AND ITS NONPOLAR IMPURITY IN RAW MATERIAL AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: Levodropropizine is a novel antitussive drug, which occurs as enantiomers. They are levodropropizine (2S) [LDP] and dextrodropropizine (impurity A) (2R) [DDP]. An isocratic chiral high performance liquid chromatographic (Normal phase HPLC) method has been developed and validated for simultaneous determination of dropropizine enantiomers along with non-polar impurity-B, (1-phenyl piperazine) [1-PP] in raw material and in dosage forms.

Methods: The compounds were separated on chiral stationary phase (CSP) Chiralpak AD-H column, with a mixture of n-hexane, anhydrous ethanol, diethyl amine (DEA) in the ratio of 55:45:0.1 v/v as mobile phase at a flow rate of 1.4 ml/min. UV detection was performed at 254 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The developed and validated method was successfully used for quantitative analysis of commercially available Tablets.

Results: Total chromatographic analysis time per sample was ~5 min. with 1-PP, levodrpropizne, dextropropizine eluting with retention times of 2.5 min., 3.05 min., and 3.66 min., respectively. Validation studies revealed the method is specific, rapid, reliable and reproducible for levodropropizne and its impurity A and non chiral impurity B. Calibration plots were linear over the concentration ranges 0.5-5 µg/ml and 0.5-5 µg/ml for levodropropizine and dextrodropropizine respectively.

Conclusion: The high recovery and low relative standard deviation confirm the suitability of the method for determination of dropropizine compounds in commercial tablets.

Keywords: Chiral stationary phase, Chiral separation, Column liquid chromatography, Levodropropizine, Optimization mobile phase.

INTRODUCTION

Levodropropizine [S (-)-3-(4-Phenyl piperizine-1-yl) –propane -1, 2 - diol] (LDP) (Fig.1) is used as an antitussive drug in clinical on the central nervous system, with a reduced sedative effect. The sedative effect is attributable to the chiral impurity A (Fig.2), namely dextro dropropizine enantiomer (DDP) [1, 2]. In the literature, many HPLC methods for the determination of LDP, DDP in formulation and biological matrices are reported. Recently, a chiral method for the determination of enantiomer separation using Chiral OC Column was reported by Huang Yong et al. [3], show RSD ratio area of LDP vs. DDP. The chiral separation of dropropizine enantiomers utilizing a Chiral OJ-H analytical column used normal phase solvents with run time of ~12 min was reported by K. Valliappan et al. [4]. The enantiomeric separation of dropropizine in biological matrices using LC-MS-MS was reported by Yunbiao Tang et al. [5]. A HPLC method for the separation of dropropizine enantiomers using normal phase Chiral OD column, with the run time of > 40 min was reported in BP 2007[6].

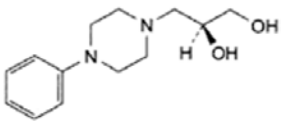

Fig. 1: Levodropropizine (LDP)

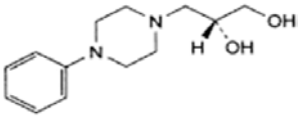

Fig. 2: Impurity A

(2R)-3-(4-phenylpiperazin-1-yl) propane-1, 2-diol (dextro dropropizine, DPP)

All the reported methods present limitations, viz. expensive instrumentation (LC-MS-MS), were having excess run time, making them unsuitable for routine use. The reported methods envisage chiral optimizations as the only goal, without considering the analysis of dropropizine impurities. To overcome these drawbacks, it is necessary to develop a simple chiral HPLC method for the simultaneous determination and optimization of LDP and its impurities A, B, which has not been reported so far. This manuscript describes the development and validation of a rapid, simple, robust enantiospecific HPLC method for simultaneous determination of LDP, and its impurities A, B in pharmaceutical formulations. The availability of an analytical method allows the estimation of active principal LDP, together with its chiral impurity DDP and non-polar achiral impurity B (1-Phenyl Piperazine) (Fig.3) present in a sample, alone or in combination. A commercially available pharmaceutical product (Levotuss) was analysed in order to check the validity of the proposed method.

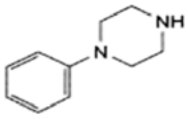

Fig. 3: Impurity B: 1-phenyl piperazine, (1-PP)

MATERIALS AND METHODS

Apparatus

Chromatographic measurements were made on an Isocratic Shimadzu (Tokyo, Japan) model which consisted of one LC 20AD solvent delivery

module, a SPD-M 20A PDA detector, and a Rheodyne injector (model 7725i, USA) valve fitted with a 20 µl loop. The system was controlled through a system controller (CBM10A) and a personal computer using a Shimadzu chromatographic software (LC solution. Release 1.11 SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonics, USA). Absorbance spectra were recorded using an UV-Visible spectrophotometer (Model UV-1601PC; Japan) employing quartz cell of 1.00 cm of path length.

Software

Capacity factor k_1, Selective Retention α, Resolution $Rs_{1,2}$ $Rs_{2,3,}$ Tailing factor, Theoretical plates were calculated by Shimadzu LC solution software. The rest of the calculations were performed by use of Microsoft Excel 2007 software (Microsoft, USA)

Chemicals and reagents

Working standards of LDP and certified reference sample mixture (CRS) of LDP and DPP were provided by M/S FDC, Mumbai, India. Non polar impurity B (1-PP) (>99%) make Aldrich used as internal standard was procured from M/S Chandanmal, Chennai. HPLC grade n-hexane and diethyl amine AR grade supplied by M/S SD Fine Chemicals (Mumbai, India). Ethanol absolute for analysis Ph Eur supplied by M/S Merck chemicals (Mumbai India). The pharmaceuticals Levotuss tablet was free gift from Hyundai Pharm Co. Kr.

Standard solutions

Stock standard solutions of LDP, Chiral Impurity A (DDP) CRS mixture and relative non polar impurity B (1-PP) were prepared in solvent mixture of anhydrous ethanol, n-hexane (40:60 v/v). The prepared stock solution was stored at 4 ^{o}C protected from light. Working standard solutions were freshly obtained by diluting the standard solutions with the solvent mixture during the analysis day. Calibration curves reporting peak area ratios of LDP and DDP were established in the range of 0.5 – 25 µg/ml for all analytes in the presence of impurity B (1-PP).

Sample preparation

Twenty tablets were weighed and finely powdered. An amount of Pharmaceutical products equivalent to 100 mg of LDP was accurately weighed and transferred in a 50 ml volumetric flask. A suitable quantity of IS (1-PP) 5 mg was added followed by 25 ml of solvent mixture. The mixture was subjected to sonication for 10 min for complete extraction of drugs and the solution was made up to the mark with solvent mixture to obtain a concentration of LDP and 1-PP as 10.0 and 0.5 µg/ml respectively. The solution was centrifuged at 4000 rpm for 10 min. The clear supernatant was collected and filtered through 0.2 µm membrane filter (Gelman Science, India) and 20 µl of this was injected for HPLC analysis.

Chromatographic procedure

Chromatographic separations were carried out on a Daicel CSP namely, Chiralpak AD-H (150 mm x 4.6 mm i. d., 5 µm) connected with a daicel security guard cartridge (4 mm x 10 mm id. 5 µm). Based on the PDA data, a wavelength of 254 nm was selected for detection. The mobile phase of hexane: anhydrous alcohol: DEA (55:45:0.1% v/v) was used as the mobile phase. An injection volume of the sample was 20 µl. The HPLC system was used in an air conditioned laboratory atmosphere (25± 2^{o}C).

Validation

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in ICH guidelines Q2(R1) - ICH [7]. Key analytical parameters including specificity, accuracy, precision, linearity, detection limit, and quantitation limit were evaluated. Calibration curves constructed in a low region of 0.05- 1.0% of the target analytes concentration for the limit of detection and quantification.

RESULTS AND DISCUSSION

Optimizing parameters and analysis

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvents in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so the peak from the first eluting compound did not interfere with those from the solvent, excipients and other components. Other criteria, viz. time required for analysis, appropriate k range (1< k <10) for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase, the column was re-equilibrated by passage of at least ten column volumes of the new mobile phase. [8]

BP 2007 monographs specified Chiralcel OD column for separation of LDP and DPP. Normal phase Chiralcel column contains cellulose backbone in the helical structure, which is loosely packed can readily accommodate enantiomer interaction with greater binding to site leading to very lengthy separation run time of around 40 min under the conditions for these compounds. The experiment used Daicel Chiralpak AD-H column having amylose core as backbone, which being more tightly coiled leading to early separation run time of around 12 min under the same conditions. Moreover Chiralpak AD-H having 5 µm particle sizes withstood high pressure (operate up to 150 kgf), leading to reasonable theoretical plates ~ 4996, meaning longer lifetime for the column.

To investigate the appropriate wavelength for simultaneous determination of LDP, DDP, solutions of these compounds in mobile phase were scanned by UV–visible spectrometry (Shimadzu, Japan; model UV-1601 PC) in the range of 200-300 nm. The compounds were showing wavelength choices considered monitoring the drugs were around 254 nm. The CRS mixture of LDP, DDP were injected in to HPLC with PDA detector, recording the peak purity from 229 nm to 279 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 254 nm with peaks LDP, DDP having tR of ~3.05 min. and ~3.66 min. Respectively. It was, therefore, concluded that 254 nm, as the most appropriate wavelength for the compounds (Fig.4) with suitable sensitivity.

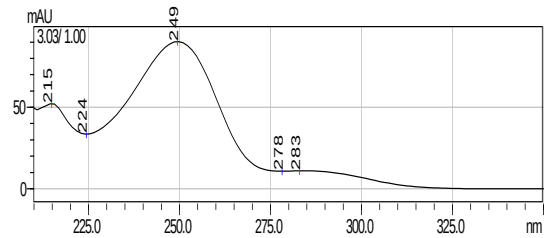

Fig. 4: LDP UV Spectrum from 210 to 350 nm. Optimum wavelength of 254 nm selected

The compounds of interest namely 1-PP, LDP, DDP were relatively non polar and of low molecular mass, a polar polysaccharide normal phase column Chiralpak AD-H (150 mm x 4.6 mm i. d., 5 µm) was tried. Several binary mobile phases of hexane (70-50%v/v) and anhydrous ethanol (30-50%v/v) were evaluated with the Chiralpak AD-H column. The retention times of the solutes decreased with increasing concentration of polar anhydrous ethanol.

It was noticed that k value for IS 1-PP was low (k< 1) at the highest concentration of anhydrous ethanol. In contrast, hexane concentrations which were high resulted in k values for DDP that were relatively high, resulting in excessively long run times of ~ 12 min. It was well known that multiple- component mobile phases result in better separation efficiency than binary mobile phases, because with these solvent strength and selectivity was varied simultaneously to obtain the retention times desired [9,10].

However resolution between LDP and DDP was lacking. Hence a third component DEA (as solubilising agent for LDP, DDP resolution), was therefore included in the mobile phase and ternary mixtures of hexane, anhydrous ethanol in proportions 55:45, 60:40, 65:35%v/v and DEA solution (0.1±0.05%v/v) were tried. Use of the

first of these with 0.1%v/v DEA resulted in a quality separation in terms of peak symmetry, optimum resolution, reasonable run time, and acceptable k values, particularly for LDP (Table 1). No further improvement in peak symmetry was observed with higher DEA concentration. (0.1%v/v) was accepted for use. Increasing the flow rate from 1.0 to 1.4 ml/min reduced the runtime to less than 5 min. The final mobile phase system of 55%v/v hexane, made up to 100%v/v with anhydrous ethanol, 0.1% v/v DEA and 1.4 ml flow rate (60 kgf), was suitable in all respects to detect impurities to limits and quantify LDP in a single run of 5 min. Elution order of separation was, for non polar impurity B (1-PP) ~ 2.5 min. away from system peak, followed by LDP peak ~ 3.05 min. and DPP peak ~3.66 min. A result of 12 runs carried in random order replicates, as shown in (Table 1) for the various parameters and responses with their Average, Standard deviation calculated. Also the Area Ratio of LDP, DDP was found to be R^2 = 0.965 and R^2 = 0.966 respectively.

The optimized chromatographic conditions were, therefore, use of the Chiralpack AD-H column with hexane: anhydrous ethanol: DEA solution, 55:45:0.1(v/v) as mobile phase at 1.4 ml/min. This method was therefore validated in accordance with ICH guidelines.

Table 1: Results replicates of 12 runs, CRS mixture of LDP, DDP

Independent Parameters			Responses							
Hexane	**DEA**	**Flow Rate**	**$Rs_{1,2*}$**	**K_1**	**$Rs_{2,3*}$**	**tF_1**	**tF_2**	**α**	**tR_2**	**Area ratio**
55	0.05	1.0	2.64	1.78	3.85	1.35	1.19	1.38	5.59	0.82
60	0.10	1.2	2.69	1.75	3.89	1.27	1.22	1.40	5.07	0.831
65	0.15	1.0	3.35	1.70	4.53	1.26	1.33	1.42	6.88	0.82
65	0.05	1.0	3.42	1.67	4.57	1.31	1.44	1.43	6.86	0.82
55	0.15	1.4	2.15	1.71	3.40	1.30	1.18	1.37	3.96	0.81
60	0.10	1.2	2.70	1.71	3.87	1.28	1.22	1.39	5.01	0.81
65	0.15	1.4	2.15	1.70	3.93	1.21	1.33	1.46	4.84	0.82
60	0.10	1.2	2.67	1.70	3.87	1.28	1.23	1.39	5.01	0.83
65	0.05	1.4	2.77	1.71	3.98	1.21	1.31	1.42	4.82	0.82
55	0.15	1.0	2.71	1.73	3.89	1.29	1.17	1.37	5.53	0.82
60	0.10	1.2	3.05	1.80	4.02	1.22	1.15	1.41	5.23	0.83
55	0.05	1.4	2.78	2.40	3.32	1.27	1.12	1.37	3.99	0.82
Avg			**2.76**	**1.69**	**3.74**	**1.28**	**1.25**	**1.39**		**0.819**
STDEV				**0.15**	**0.36**	**0.039**	**0.085**	**0.028**		**0.006**

$Rs_{1,2*}$ - Average resolution between Non chiral Impurity B vs LDP, $Rs_{2,3*}$ - Average resolution between LDP vs chiral Impurity A

Assay method validation

The next step of the present study was to check method's validation for specificity, linearity, accuracy, intra/inter-day precision, and robustness [11]. The optimized HPLC method was specific in relation to the placebo blank used after every run in this study. An excellent linearity was established at five levels in the range of 0.5- 5 µg/ml LDP, DDP, 1-PP with R^2 of more than 0.965 for all analytes. The LOD and LOQ were estimated as 0.95 and 2.05 ng/ml for LDP and 1.15 and 2.55 ng/ml for DDP and 0.3 and 0.95 ng/ml for 1-PP respectively. Accuracy (n=9), assessed by spike recovery were found to be 99.69, 99.66, and 99.65% for LDP, DDP and 1-PP respectively, which were within acceptable ranges of 100 ± 2%. The intra and inter-assay precision (n=6) was confirmed since the % CV were well within the target criterion of <2 respectively.

Robustness study revealed that small change did not alter retention time, retention factor, and resolutions more than 2% and therefore it would be concluded that the method conditions are robust. The assay method validation of individual parameter and results are shown in (Table 2).

Application of the method

The proposed isocratic CSP method was applied to the quantitative analysis of LDP in real samples (Levotuss) containing DDP and 1-PP in detectable limits. Representative chromatograms are presented in Fig.5 and Fig.6. The results achieved when analyzing Levotuss tablets were 60.5 (0.3) mg of LDP and 0.5 (0.38) mg of DDP, with values within parenthesis being the % CV of the six replicates. Good agreement was found between assay results and the label claim of the product.

Table 2: Validation of method for the determination of LDP, DDP and 1-PP

Validation Parameters	Concentration	LDP Results	DDP Results	1-PP Results
Linearity (n=6)	0.5-5.0 µg/ml	y=0.723x -0.062 R^2 = 0.965	y=0.723x -0.063 R^2 = 0.966	y=0.723x -0.098 R^2 = 0.989
LOD		0.95 ng/ml	1.05 ng/ml	0.3 ng/ml
LOQ		2.05 ng/ml	2.55 ng/ml	0.95 ng/ml
Specificity	The method is specific with respect to tablet excipients (starch, lactose, aerosil, hpmc, titanium dioxide and magnesium stearate)			
Accuracy (mean % recovery) (n=3)				
	80% w/w	99.53	99.69	99.64
	100%w/w	99.86	99.41	99.92
	120%w/w	99.96	99.82	99.35
(mean % recovery, %CV) (n=9)		99.69, 0.33	99.66, 0.33	99.65, 0.68
Precision (%CV) (n=6)				
(a) Intraday precision	0.5	1.2	1.48	1.57
	2.5	0.65	1.06	1.351.08
	5.0	0.83	0.75	
(b) Interday precision	0.5	1.89	1.82	1.681.93
	2.5	0.82	1.46	
	5.0	0.96	0.65	0.68
Robustness (%assay, % CV)				
n-hexane conc.	(55.0 ± 0.5%)	99.79, 0.33	99.68, 0.65	99.05, 0.59

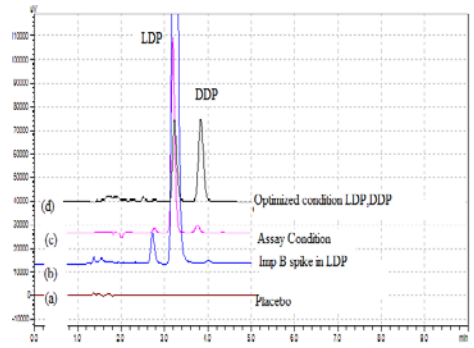

Fig. 5: Chromatograms corresponding to (a) a placebo solution;

(b) Impurity B spike in LDP

(c) Assay condition of Levotuss along with Impurity A (DDP) and Impurity B (1-PP);

(d) Optimized CRS mixture of LDP and DDP

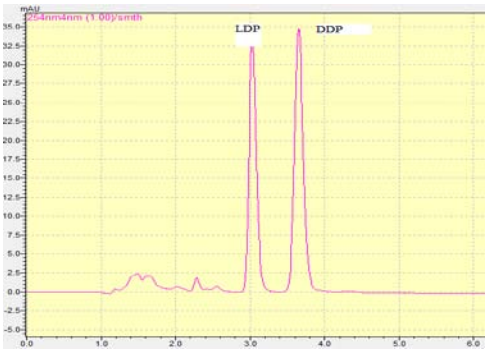

Fig. 6: Chromatogram obtained at 254 nm following the injection of 20 µl of a solution containing 10µg of CRS mixture (LDP and DDP)

To establish the practical limit of detection of non chiral impurity B in LDP containing pharmaceuticals as well as in bulk drugs, standard solutions of LDP were first chromate graphed at high instrumental sensitivity to verify the absence of any peaks at retention times corresponding to the Imp B, and then deliberately spiked IS namely 1-PP, with LDP. The level of impurity added to the active principle was in 0.1% (w/w), less than BP limit of 0.5%. (Fig.5), shows the chromatogram obtained with the solution. From the results of these experiments it can be established that when injecting amounts of 100µg of LDP, a 0.01% level of impurities be quantified, much less than the prescribed limit of 0.5% as per BP2007.

The content of the active principle LDP together with the levels of the impurities A and B in pharmaceutical formulations, commercially available was determined in triplicate by using the proposed method. The quantities found in LDP were in conformity with the target values.

CONCLUSION

An efficient Isocratic CSP HPLC method was developed, optimized and validated for the simultaneous estimation LDP and chiral impurity in pharmaceutical formulation and bulk drugs. This method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic factors and their interaction effects on the attributes of separation. The analytical results obtained lead to the conclusion that the developed method performs well with regard to both precision and accuracy and also the detection of chiral impurity A. Therefore it could be successfully adopted for the routine analysis of LDP and their chiral impurity A in bulk drug and pharmaceutical formulation.

CONFLICT OF INTERESTS

Declared None.

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