Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 9, 2015

Original Article

A SIMPLE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ORGANIC IMPURITIES, ENANTIOMER AND ASSAY OF DEXLANSOPRAZOLE

P. BALAMURUGAN[1,3*], K. ANVER BASHA[2], JEENET JAYACHANDRAN[3], MANISH GANGRADE[3], P. PARTHIBAN[4]

[1]Department of Chemistry, Sathyabama University, Jeppiar Nagar, Chennai–119, [2]Department of Chemistry, C. Abdul Hakeem College, Melvisharam, Tamilnadu, [3]Analytical Research and Development Department, Cipla Ltd, Bangalore-49, [4]Centre for Research and Development, PRIST University, Thanjavur, Tamilnadu

Email: communicate2bala@gmail.com

Received: 08 Jun 2015 Revised and Accepted: 22 Jul 2015

ABSTRACT

Objective: To develop a simple and accurate RP-HPLC method for simultaneous estimation of organic impurities, enantiomer and assay of Dexlansoprazole, proposed method was validated according to an International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) guidelines.

Methods: Cellulose *tris*-(3,5-dichlorophenylcarbamate) bonded on silica gel (Chiralpak IC) were used as stationary phase. Acetonitrile (ACN) and 10 mM dibasic potassium phosphate buffer of pH 7.20, adjusted with dilute ortho phosphoric acid, were used as mobile phase in gradient composition at a flow rate of 1.0 ml/min. UV detection was made at 283 nm and run time was 27 min.

Results: Limit of detection (LOD) and Limit of quantification (LOQ) was 42 and 126 ng/ml, respectively. Analytes response was studied from 0.25-1.50 µg/ml and r^2 value of the calibration curve was >0.999. Accuracy was studied in three different concentrations, the mean recovery was observed between 93.8 and 102.5%. To the maximum of 0.01% impurity variation was observed between the results of inter and intra-day precision.

Conclusion: Proposed method is specific, precise, sensitive, linear and accurate. As a significance of this proposed method, the listed known impurities of Lansoprazole monograph [United States, British and European pharmacopoeias (USP/BP/Ph. Eur.)] Were used in method development and separation was demonstrated. Apart from this, enantiomer resolution was achieved and the same chromatogram was used for assay calculation.

Keywords: Chiralpak IC, Cellulose *tris*-(3,5-dichlorophenylcarbamate), Dexlansoprazole, Method development, Simultaneous estimation, Lansoprazole.

INTRODUCTION

Chirality is an important one owing to the stereo dependent nature of biological actions [1]. Research has proved that drug enantiomers are different efficasy, even some drug enantiomers are adverse effect. Hence, enantiomer separation and quantification is the mandatory test along with organic impurities estimation in the field of pharmaceutical analysis. Enantiomers, related compounds estimation with two different methods was well established area in the field of liquid chromatography. In this aspect, combing these two tests and estimation of an assay in a single method was certainly useful for rapid drug analysis. In earlier studies to achieve this goal, combination of chemo (C-18) and enantio selective stationary phases (polysaccharide phenyl carbamate) were used in tandem with SFC technique [2], latter on same kind of study was reported on RP mode [3]. In practice bonded phase was used in achiral components separation, whereas phenyl derivative alkyl bonded stationary phases are recommended for special applications like positional isomer separation, because of additional π-π interaction. Polysaccharide phenyl carbamate derivatives are the best choice for enantiomer separation.

However, there is a scope for chiral stationary phase (CSP) to be used in more efficient way [2]. Polysaccharide phenylcarbamate derivatives are widely used in enantiomer separation in coated form; these columns are limitations in solvent, hence utilisation of these columns to achieve the above goal (combing enantiomer, organic impurities and assay in one test) is challenge. RP mode with the gradient mobile phase composition was the preferred condition to achieve the goal, for these conditions immobilised CSP's are suitable [4]. Dexlansoprazole is chemically known as R (+)-1*H*-Benzimidazole,2-[[[3-methoxyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl] methyl] sulfinyl], is one of important proton pump inhibitor (PPI) that inhibits acid secretion in gastric parietal cells [5]. Numerous literatures were reported in Lansoprazole, for organic impurities estimation in drug substance, dosage form and biological substances [6-14]. Dexlansoprazole is the active drug compared with its enantiomer; this drug will be the therapeutic category of gastric acid secretion inhibitor. Varity of sulphoxide proton pump inhibitors are commercially available, in this aspect all relevant literatures are reviewed thoroughly for simultaneous estimation chemo and enantio selective method. Reports on enantioseparation, retention behaviour of proton pump inhibitors (PPI's) on polysaccharide phenyl carbamate triad of stationary phases is quite abundant in the existing literature [15-24]. Immobilised polysaccharide phenyl carbamate derivative stationary phases are successful in solvent versatility and highest potential of chiral selective in RP-HPLC [4, 24]. Enantio and chemoselective HPLC method were reported for simultaneous estimation of enantiomer and organic impurities in Dexlansoprazole using Chiralpak IA column [19], but isocratic NP mode of separation. Consequently, one reported impurity was not eluted in proposed method (As per EP Lansoprazole Impurity-D, *i.e.*, 2-mercatobenzimidazole).

However, the goal was not achieved. Proposed method was used as a chemoselective method for enantiomer estimation, hence method validation demonstrated with enantiomer and Dexlansoprazole. Continuation of the previous work Zanitti *et al.* proposed a method for direct enantio separation of omeprazole and its chiral impurities [20]. There is no other literature is available to describe about the simultaneous estimation of enantiomer, organic impurities and assay in Dexlansoprazole. Considering all the above literatures, and achieve the goal (combing enantiomer, organic impurities and assay in one test), this work was initiated. Lansoprazole USP listed impurities, enantiomer were used for method development and validation. Latter on additional two impurities which is listed in EP was purchased and resolution was demonstrated. Dexlansoprazole and related impurities structure is shown below.

MATERIALS AND METHODS

Materials

HPLC grade acetonitrile, methanol, di potassium hydrogen orthophosphate anhydrous, ammonium acetate, phosphoric acid and

potassium hydroxide were obtained from Merck, Mumbai, India-400 018. Dexlansoprazole, racemic Lansoprazole, related compounds-A, B, C and racemic N-oxide are prepared in Cipla Rand D, Bangalore, India-500 049.

Related compound-D was purchased from Ph. Eur. Millipore Milli-Q plus water purifying system was used to purify the water (Millipore, USA). Cellulose *tris*-(3,5-dichlorophenylcarbamate) bonded on silica gel, Chiralpak IC column (250 mm x 4.6 mm, 5 µm) was procured from Daicel chiral technologies (India) Pvt. Ltd., Hyderabad, India-500 078.

HPLC and LCMS

HPLC used for this study is Waters 2695 module series apparatus. It is equipped with the quaternary pump, degasser, auto injector and column oven compartment with 2996 module multi wave length PDA detector. Data was collected and processed using Empower 2 chromatographic software. Agilent 1290 series UPLC equipped with binary gradient pump and detector was coupled with AB Sciex 5500 series mass spectrometer was used for MS study. It is equipped with turbo spray ion source and Qtrap mass analyser, MS and MS/MS data were acquired and processed in Analyst software.

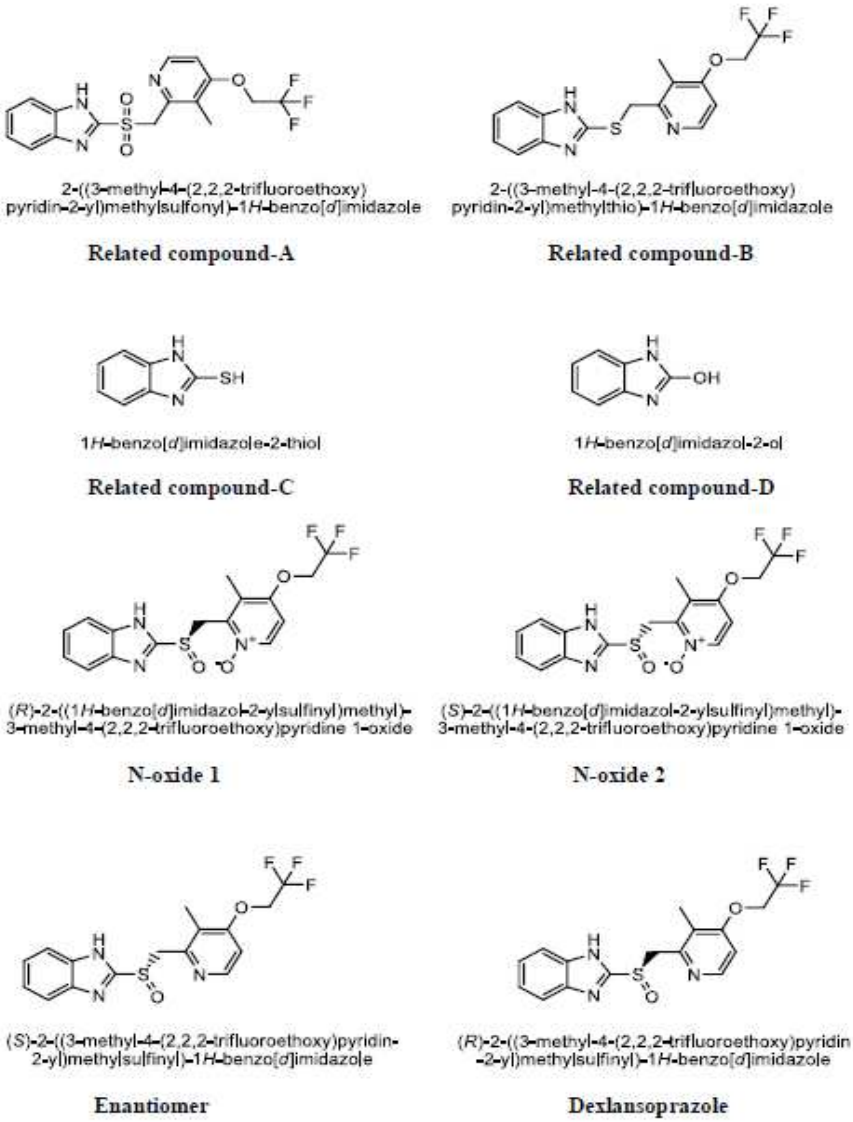

Fig. 1: Chemical structure of Dexlansoprazole and its impurities

Method scouting

Immobilised phenyl carbamate polysaccharide derivative CSP's are considered for combined approach, because of proven selectivity and solvent versatility [25]. Amylose *tris*-(3, 5-dimethyl phenyl carbamate), Cellulose *tris*-(3, 5-dichloro phenyl carbamate) stationary phases were used for method scouting; these columns are compatible in reverse phase mode. USP related compounds (N-oxide, Sulfone and Sulphide) along with related compound-C and enantiomer impurity was used in initial method development. Mobile phase gradient were applied to retain the polar and elute the non-polar compound in short analysis time.

Methods (HPLC and LCMS)

Preparation of phosphate buffer: 10 mM dibasic potassium phosphate buffer was prepared and pH of the solution was adjusted to 7.20 with dilute orthophosphoric acid. This buffer was used as mobile phase-A and ACN was used as a mobile phase-B at the flow rate of 1.0 ml/min in gradient composition. Gradient programme: Time (min)/A (v/v): B (v/v); $T_{0.0}$/70:30;$T_{20.0}$/ 30:70;$T_{22.0}$/ 70:30;$T_{27.0}$/70:30). Chiralpak IC 250 mm, 4.6 mm, 5 µm column was used for separation. UV detection was at 283 nm and run time was 27 min. Column oven temperature was maintained at 30 ºC. Sample concentration was 500 µg/ml and injection volume 10 µl. Instead of

phosphate buffer, 10 mM ammonium acetate pH 7.20 buffer was used for LCMS analysis other chromatographic conditions are remain same.

Method validation

Proposed method was validated as per ICHQ2 guidelines. Specificity, inter and intra-day precision, linearity, sensitivity and accuracy were performed.

Specificity

Gradient/diluent interference was checked at the retention time of analyte and its impurities, 1 µg/ml of individual impurities and Dexlansoprazole were injected in chromatographic system. 1 µg/ml of impurities are spiked in Dexlansoprazole and injected for system suitability.

Repeatability and reproducibility

Precision of the method was verified by repeatability and reproducibility studies. Six test solutions were prepared and injected in chromatographic system in a day and results are compared. Reproducibility was carried out, same as per repeatability study but different day in different instrument and results are compared.

Sensitivity

Series of dilute solutions was prepared and 10 µl injected in the chromatographic system. Sensitivity was reported as LOD and LOQ, signal to noise(S/n) ratio was the criterion set for sensitivity. S/n ratio ≥3 set for LOD, S/n ratio ≥ 10 with precision was set for LOQ. Triplicate was injected for LOD and six replicate injections were carried out for LOQ. Relative standard deviation of peak area response was calculated for LOQ replicates and 5% RSD set for limit.

Calibration curve

Analytes responses were studied in the range of 0.25-1.50 µg/ml (50 to 300% of limit level concentration). Five point calibrations were carried out, in each level triplicate injections were made and average peak area responses are calculated and calibration curve was drawn against their respective concentrations. Linearity was evaluated by linear regression analysis, which was evaluated by least square regression method. Acceptance criteria set for linearity study were r^2>0.999.

Recovery

Accuracy is one of the important parameter in method validation to evaluate the sample matrix effect on impurities response. Accuracy was carried out by recovery experiments. Related compounds-A, B, racemic Lansoprazole and N-oxide were spiked into the sample at 0.25, 0.50, 1.0 µg/ml concentrations. Three preparations were prepared and injected in the chromatographic system. %Recovery was calculated and 90 to 110% of the recovery was the limit set for accuracy study.

RESULTS AND DISCUSSION

Column selection challenge

Late 1980's enantiomer separation is novel area, now very much familiar. Hence, last three decades variety of coated CSP's was introduced in the market. Quite abundant literatures were available for enantio separation on coated CSP's in NP mode. Separation of polar and non polar impurities in short run time on coated CSP in NP isocratic mode is challenge, such instance is, Cirilli *et-al* unable to elute one reported impurity, in his proposed method [19]. RP gradient mobile phase condition is the preferred choice for short and sensitive method, though immobilised CSP's are chosen for combined method development, however very few immobilised chiral stationary phase columns are commercially available.

MS study

Racemic N-oxide was used in method development and validation study, though two peaks were eluting at the relative retention (RRT) of 0.68 and 0.76 with respect to Dexlansoprazole. Configuration of these two N-oxide peaks was unknown, thus denoted as N-oxide 1 and 2. One impurity was observed in trace level at 0.57 RRT of Dexlansoprazole by MS and identified as *m/z* [$(M+H)^+$] 402. Exact mass of this specified impurity was 401 Da (i.e., 32 Da more than Dexlansoprazole and 16 Da more than N-oxide), hence N-oxide and specified impurity was further fragmented in MS/MS to predict the probable structure. Common daughter ions (*m/z* 238, 204,119 and 106) was observed in MS/MS though specified impurity is the related structure of N-oxide. 386, 268 mass was found in N-oxide fragments and corresponding 16 Da more mass 402 and 284 was observed in specified impurity. Hence specified impurity could be Suphone N-oxide. MS, MS/MS spectrum of specified impurity and N-oxide were presented in fig. 2 for better understanding. From the evident of MS/MS fragments probable schemas were drawn and shown in fig. 3.

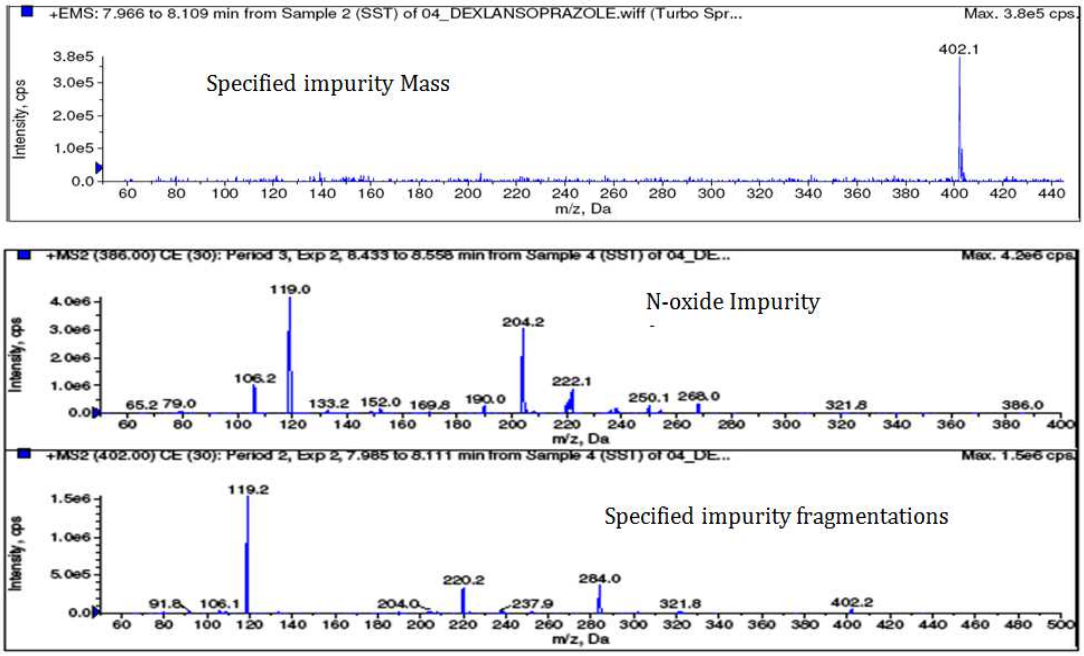

Fig. 2: MS spectrum of specified impurity and MS/MS spectrum of N-oxide and specified impurity

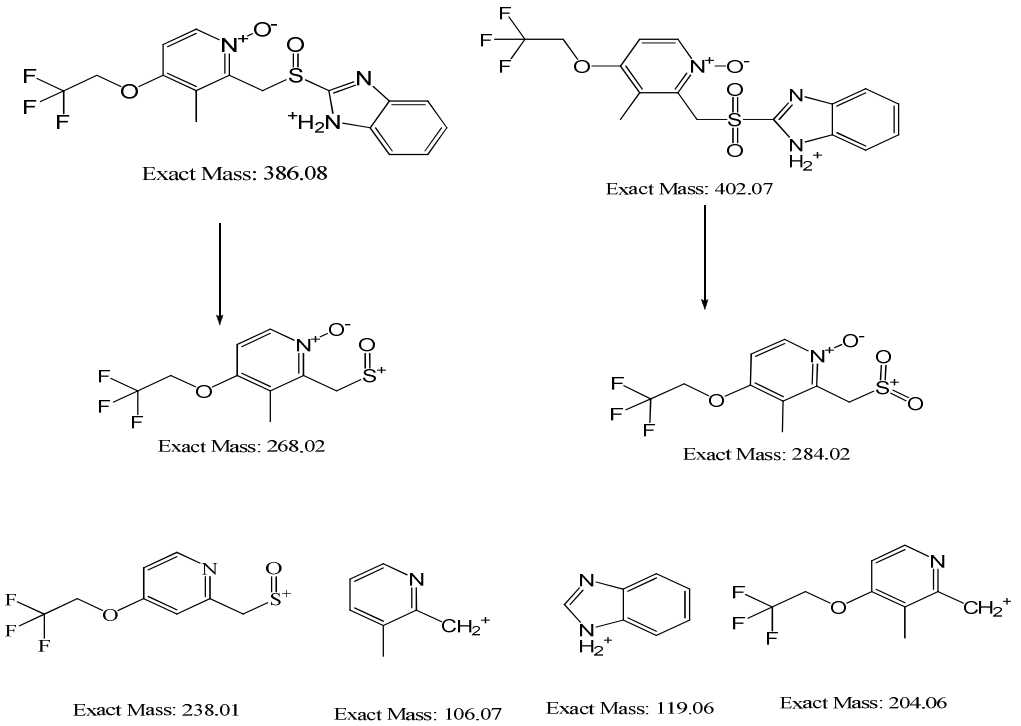

Fig. 3: Probable fragmentation pattern of N-oxide and specified impurity

Specificity, Repeatability and reproducibility

USP listed impurities are well separated with base line separation. Obtained peak purity values are evident for method specific. Related compound-D (i. e EP impurity-E) is not available during the method development, in order to make fully applicable for the developed method, this impurity was purchased from European directorate for the quality of medicines and healthcare (EDQM) and specificity was demonstrated along with the adjacent peak of related compound-C.

Typical system suitability chromatogram was shown in fig. 4. The chromatogram of dexlansoprazole related compounds C and D is shown in fig. 5 and specificity values are presented in below for better understanding.

Table 1: Specificity results

Name of the analyte	Retention time	Capacity factor (k)	Resolution (Rs)	Tailing factor (T)
Related compound-D	3.97	0.32	-	0.93
Related compound-C	4.44	0.48	-	1.10
N-oxide 1	8.80	2.62	15.23	1.04
Related compound-A	9.41	2.86	1.95	1.01
N-oxide 2	9.94	3.08	1.64	1.06
Enantiomer	11.76	3.84	5.49	1.09
Dexlansoprazole	13.02	4.37	3.94	1.03
Related compound-B	16.79	5.91	11.43	1.04

500 µg/ml of Dexlansoprazole spiked with 1 µg/ml of impurities and injected for specificity and system suitability purpose

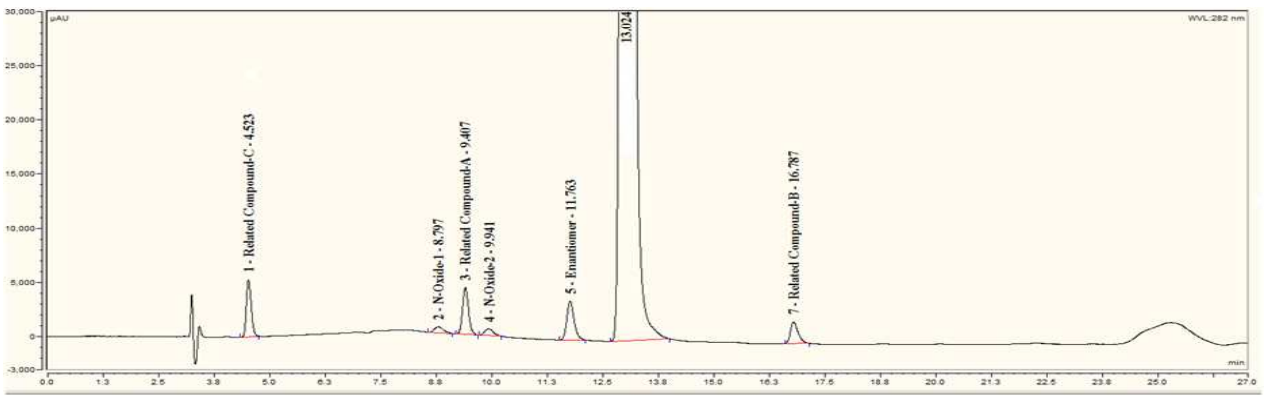

Fig. 4: Typical system suitability chromatogram of dexlansoprazole spiked with impurities

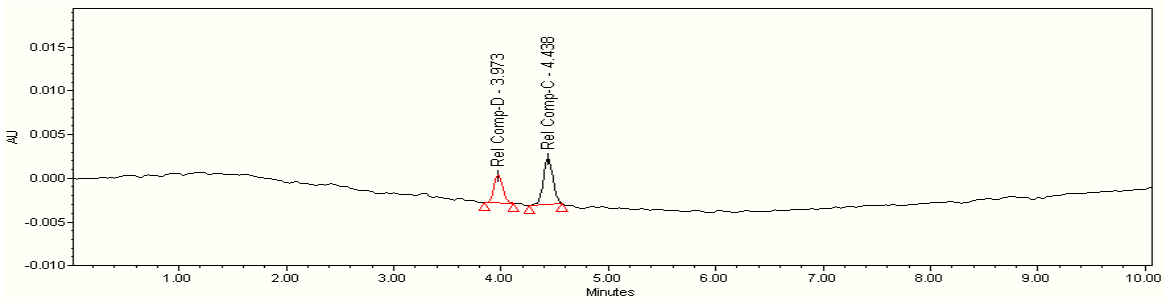

Fig. 5: Zoomed chromatogram of dexlansoprazole related compounds C and D (EP impurities D and E)

Results obtained in repeatability and reproducibility results are comparable and maximum 0.01% of impurity variation observed between the replicates. Hence the proposed method is repeatable and reproducible.

Sensitivity (LOQ and LOD)

Racemic N-oxide, related compound-A, B, and racemic Lansoprazole were diluted and injected in the chromatographic system. S/n ratio>3 were observed for 0.008% of analytes concentration with respect to sample, hence this concentration was considered as LOD. S/n ratio>10 were observed for 0.025% of analytes concentration with<4% RSD of peak area responses of LOQ replicates. Quantification limit of impurities in the proposed method is 0.025%, thus<0.025% of impurities can be disregarded in purity test. Sensitivity results are presented in table 2.

In method sensitivity aspect, minimum 50% of the limit level concentration to be achieved as a LOQ. Proposed method LOQ has been observed apparently 17% of limit level concentrations with good precision. 500 µg/ml sample concentration were used for impurity and assay estimation as well. Considering this test concentration LOD, LOQ was established. Above results clearly insist that developed method is good sensitive.

Table 2: Sensitivity data

Analytes	Limit of detection		Limit of quantification			
	Conc. (ng/ml)	S/n ratio	Conc. (ng/ml)	S/n ratio	%RSD RT (n=6)	%RSD Area(n=6)
N-Oxide 1	42	3	126	14	0.1	3.6
Related compound-A	42	5	126	17	0.1	3.9
N-Oxide 2	42	3	126	13	0.1	1.4
Enantiomer	42	6	126	14	0.1	2.5
Dexlansoprazole	42	6	126	13	0.1	1.4
Related compound-B	42	5	125	15	0.1	1.5

n=6, six determinations. 0.025% and 0.008% is the LOQ and LOD concentrations with respect to test concentration (500 µl/ml test concentration was used for assay and impurities estimation)

Table 3: Linearity data

Analyte	Conc. µg/ml (50%)	Conc. µg/ml (100%)	Conc. µg/ml (150%)	Conc. µg/ml (200%)	Conc. µg/ml (300%)	r^2	Linearity Equation y =
N-Oxide 1	0.2501	0.5002	0.7503	1.0004	1.5005	0.9999	2535x-16
Related compound-A	0.2529	0.5058	0.7586	1.0115	1.5173	0.9999	1994x-17
N-Oxide 2	0.2501	0.5002	0.7503	1.0004	1.5005	0.9999	2524x-44
Enantiomer	0.2510	0.5020	0.7530	1.0040	1.5060	0.9999	2227x-37
Dexlansoprazole	0.2510	0.5020	0.7530	1.0040	1.5060	0.9993	2231x-43
Related compound-B	0.2498	0.4997	0.7495	0.9993	1.4990	0.9999	2301x-55

0.05, 0.10, 0.15, 0.20and0.30% with respect to sample concentrations were injected in triplicate for linearity study

Table 4: Average % recovery

Spiked conc.	N-Oxide-1	Related compound-A	N-Oxide-1	Enantiomer	Related compound-B
50%	98.79	96.12	102.11	98.40	99.20
100%	95.22	93.78	102.52	96.36	101.99
200%	97.82	97.42	102.29	98.79	99.82

0.05, 0.10and0.20% with respect to sample concentrations of known impurities were spiked and injected in triplicate for accuracy study and average values are reported

Calibration curve

Linear response was observed over the range of 50-300% against the respective concentrations. Analytes calibration curve was evaluated thoroughly, regression coefficient r^2 was observed>0.999. Concentration details, r^2, and linearity equation were presented in table 3.

Recovery

Results obtained in accuracy study were well within the limit set for acceptance criteria. There is no impact on the results with sample matrix. Lowest average recovery was 93.8% and highest was 102.5%. Accuracy data were presented in table 4. Hence, proposed method was accurate.

CONCLUSION

Proposed method is chemo and enantio specific with short run time (27 min), and the same method is applicable to assay estimation. Interestingly, immobilised phenyl carbamate chiral stationary phase was used in this study in efficient way, and as a benefit of this, the analysis time of Dexlansoprazole drug was reduced. Overall, the proposed and validated short and simple method of the immobilised polysaccharide phenylcarbamate derivative with gradient reverse phase mode is the best approach for a successful "combined method development".

ACKNOWLEDGEMENT

We are grateful to the management of Cipla Ltd for supporting this work. Useful discussion and co-operation from colleagues of Analytical RandD department is highly appreciated. We are thankful to Mr. P. L. Srinivas, RandD, Cipla Ltd, Bangalore, India-560 049 for helpful advice and guidance to carry out this work successfully.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest

REFERENCES

1. Andersson T. Single-Isomer drugs true therapeutic advances. Clin Pharmacokinet 2004;43:279-85.
2. Khorassani MA, Taylor LT, Williams DG, Roston DA, Catalan TT. Demonstrative validation study employing a packed column pressurized fluid chromatography method that provides assay, achiral impurities, chiral impurity, and IR identity testing for a drug substance. J Pharm Biomed Anal 2001;26:725-38.
3. Perara RWH, Lough WJ. Assessment of chiral stationary phases for suitability for combined enantiomeric impurity/related substances assays. J Chromatogr A 2011;1218:8655-63.
4. Zhang T, Nguyen D, Franco P. RP-screening strategies for liquid chromatography on polysaccharide-derived chiral stationary phases. J Chromatogr A 2010;1217:1048-55.
5. Miura M, Tada H, Suzuki T. Simultaneous determination of lansoprazole enantiomers and their metabolites in plasma by liquid chromatography with solid-phase extraction. J Chromatogr B 2004;804:389-95.
6. Karol MD, Granneman GR, Alexander K. Determination of lansoprazole and five metabolites in plasma by HPLC. J Chromatogr B 1995;668:82-186.
7. Al-zehouri J, El-subbagh HI, Al-badr AA. Analytical profiles of drug substances and excipients; 2001;28:117-51.
8. Uno T, Yasui-Furukori N, Takahata T, Sugawara K, Tateishi T. Determination of lansoprazole and two of its metabolites by liquid–liquid extraction and automated column-switching HPLC: application to measuring CYP2C19 activity. J Chromatogr B 2005;816:309-14.
9. Song M, Gao X, Hang T, Wen A. Simultaneous determination of lansoprazole and its metabolites 5-hydroxy lansoprazole and lansoprazole sulphone in human plasma by LC–MS/MS: Application to a pharmacokinetic study in healthy volunteers. J Pharm Biomed Anal 2008;48:1181-6.
10. Hishinuma T, Suzuki K, Yamaguchi H, Yamagishi H, Koike T, Ohara S, *et al.* Simple quantification of lansoprazole and rabeprazole concentrations in human serum by liquid chromatography/tandem mass spectrometry. J Chromatogr B 2008;870:38-45.
11. Luo Y, Xu L, Xu M, Feng J, Tang X. A validated, Specific, Stability-indicating HPLC method for determination of Lansoprazole enteric capsules and related impurities. Asian J Pharm Sci 2012;7:149-54.
12. USP monograph 37 and NF 32.
13. Ramulu K, Rao BM, Rao NS. Identification, Isolation and characterization of potential degradation products in Lansoprazole drug substance. Rasayan J Chem 2013;6:274-83.
14. Pai NR, Patil SS. Development and validation of a liquid chromatography method for the analysis of Lansoprazole and its related production impurities. J Chem Pharm Res 2013;5:121-8.
15. Katsuki H, Hamad A, Nakamur C, Arimori K, Nakano M. HPLC assay for the simultaneous determination of lansoprazole enantiomers and metabolites in human liver microsomes. J Chromatogr B 2001;757:127–33.
16. Miura M, Tada H, Satoh S, Habuchi T, Suzuki T. Determination of rabeprazole enantiomers and their metabolites by high-performance liquid chromatography with solid-phase extraction. J Pharm Biomed Anal 2006;4:565-70.
17. Rao RN, Raju AN, Nagaraju D. Enantiospecific resolution of rabeprazole by liquid chromatography on amylose-derived chiral stationary phase using PDA and polarimetric detectors in series. Talanta 2006;70:805-10.
18. Belaz KRA, Coimbra M, Barreir JC, Montanari CA, Cass QB. Multimilligram enantioresolution of sulfoxide proton pump inhibitors by liquid chromatography on polysaccharide-based chiral stationary phase. J Pharm Biomed Anal 2008;47:81-7.
19. Cirilli R, Ferretti R, Gallinella B, Turchetto L, Zanitti L, Torre FL. Development and validation of an enantioselective and chemoselective HPLC method using a Chiralpak IA column to simultaneously quantify (*R*)-(+)-and (*S*)-(−)-lansoprazole enantiomers and related impurities. J Pharm Biomed Anal 2009;50:9-14.
20. Zanitti L, Ferretti R, Gallinella B, Torre FL, Sanna ML, Mosca A, *et al.* Direct HPLC enantioseparation of omeprazole and its chiral impurities: Application to the determination of enantiomeric purity of esomeprazole magnesium trihydrate. J Pharm Biomed Anal 2010;52:665-71.
21. Cirilli R, Ferretti R, Gallinella B, Zanitti L. Retention behaviour of proton pump inhibitors using immobilized polysaccharide-derived chiral stationary phases with organic-aqueous mobile phases. J Chromatogr A 2013;1304:147-53.
22. Chennuru LN, Choppari T, Duvvuri S, Dubey PK. Enantiomeric separation of proton pump inhibitors on new generation chiral columns using LC and supercritical fluid chromatography. J Sep Sci 2013;36:3004-10.
23. Dixit S, Dubey R, Bhushan R. Normal and polar-organic-phase high-performance liquid chromatographic enantioresolution of omeprazole, rabeprazole, lansoprazole and pantoprazole using monochloro-methylated cellulose-based chiral stationary phase and determination of Dexrabeprazole. Biomed Chromatogr 2014;28:112-9.
24. Peng L, Jayapalan S, Chankvetadze B, Farkas T. Reversed-phase chiral HPLC and LC/MS analysis with *tris*-(chloromethylphenylcarbamate) derivatives of cellulose and amylose as chiral stationary phases. J Chromatogr A 2010;1217:6942-55.
25. Zhang T, Nguyen D, Franco P. Enantiomer resolution screening strategy using multiple immobilised polysaccharide-based chiral stationary phases. J Chromatogr A 2008;1191:214-22.