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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

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ABSTRACT

An accurate and reproducible high-performance liquid chromatographic (HPLC) method has been developed and validated for the direct separation of individual enantiomers of lansoprazole, a potent proton pump inhibitor belonging to the family of the substituted benzimidazoles. The enantiomers were resolved on a Chiralpak IA by using a mobile phase consisting of methyl-*tert*-butyl ether (M*t*BE)–ethyl acetate (EA)–ethanol (EtOH)–diethylamine (DEA) in the ratio 60:40:5:0.1 (v/v/v/v). Baseline separation of the enantiomers of lansoprazole was obtained with a resolution factor of 8.14. The standard curves for the two enantiomers were linear ($r^2 > 0.999$) in the concentration range of 10–80 μg/ml with a working concentration of about 60 μg/ml for each enantiomer. Apparent recovery was 100.8% with a relative standard deviation less than 2%. The limit of quantization for each enantiomer of lansoprazole was 0.22 μg/ml. The intra-day precisions were in the range of 0.21–0.36 and 0.59–0.66 while the inter-day precisions were in the range of 0.55–1.24 and 0.66–1.19% in terms of retention times and area response RSD% for (*R*)-(+)- and (*S*)-(−)-lansoprazole, respectively. The method was also able to resolve impurities from the enantiomers of lansoprazole.

1. Introduction

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole, is a potent non-reversible inhibitor of the enzyme gastric proton pump H^+/K^+-ATPase, which is responsible for gastric acid secretion. It is used to treat and prevent two common disorders: gastrointestinal ulcers and gastroesophageal reflux disease (GERD) [1].

Lansoprazole consists of a substituted benzimidazole ring and a pyridine ring connected via a sulfoxide-containing chain (Fig. 1). Due to the presence of the pyridine ring, lansoprazole is a weak base with a pK_a values of 4.15 ± 0.06 and 1.33 ± 0.09, while the N–H proton in the benzimidazole ring is responsible for the acidity of the molecule (pK_a 8.84 ± 0.04). Moreover lansoprazole is soluble in methanol, sparingly soluble in ethyl alcohol, slightly soluble in ethyl acetate and insoluble in water.

Lansoprazole, as other proton pump inhibitors (PPI), such as omeprazole, rabeprazole and pantoprazole, presents an asymmetric sulfoxide centre in its molecule and is used clinically as racemic mixture. Like most compounds of this class, lansoprazole is acid labile and it is reversibly transformed to an achiral cyclic sulfenamide in acidic media [2,3]. In vivo the achiral form reacts with the mercapto groups in the enzyme and inactives it with the formation of a disulphide complex [4].

To date, only omeprazole has been developed and marketed as a single enatiomer under the trade name Nexium™ in the E.U. in 2000, in the U.S. in 2001, and in other jurisdictions. Compared to the racemate, esomeprazole magnesium has unique metabolic properties, with therapeutic advantages that include higher bioavailability in the majority of patients (extensive metabolizers), lower exposure in poor metabolizers, less interindividual variation, and steeper dose–response curve at steady state resulting in a more pronounced inhibition of gastric acid secretion [5,6]. Furthermore, esomeprazole magnesium has been shown to possess superior physicochemical [7] and pharmacological properties as compared with its paired single enantiomer and the racemate [8,9]. The international patent applications W09602535 [10] claim the use of the compounds (*S*)-(−)- and (*R*)-(+)-pantoprazole for treating stomach disorders in humans. Pharmacological studies have shown that the (*S*)-(−)-pantoprazole is more potent than (*R*)-(+)-pantoprazole and (*R*),(*S*)-(±)-pantoprazole in inhibiting the gastric acid secretion and in reducing the gastric and duodenal ulcers [11]. So the (*S*)-(−)-form of pantoprazole has been developed by Emcure Pharmaceuticals Ltd. in 2006 and it is known as Panpureα®.

Lansoprazole is extensively metabolized in the liver; the major metabolites present in the plasma are 5-hydroxylansoprazole and

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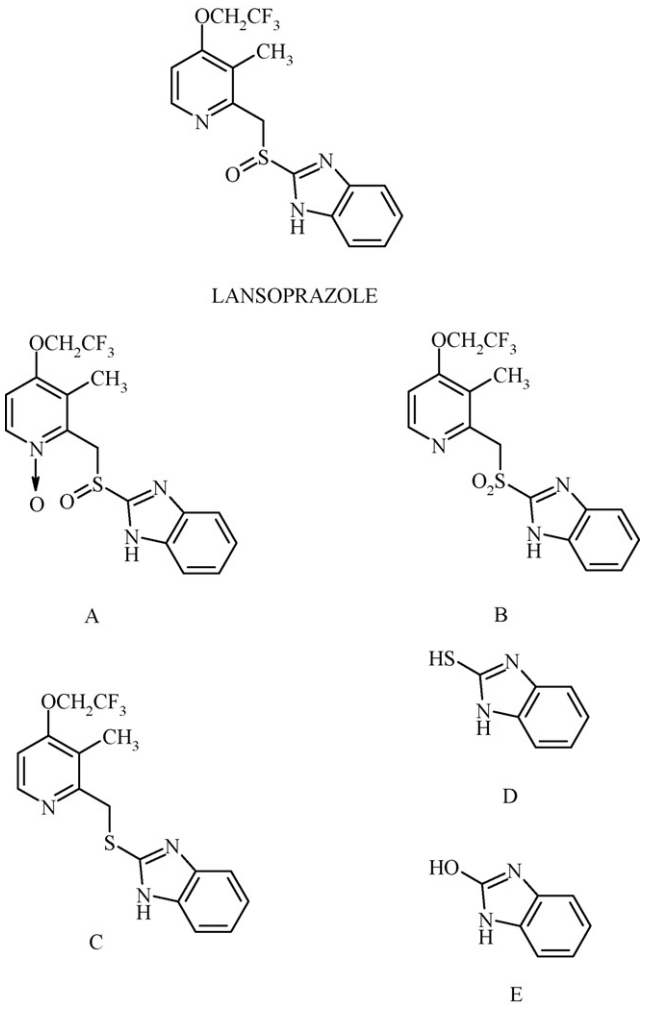

Fig. 1. Lansoprazole and A, B, C, D, E lansoprazole impurities structures.

lansoprazole sulfone with their formation being mediated by cytochrome P450 2C19 (CYP2C19) and CYP3A4, respectively [12]. Despite the fact that identical pharmacological effects of the (*R*)- and (*S*)-enantiomers of lansoprazole, for the inhibition of acidic secretion have been considered until now, there are studies that demonstrate (*S*)-(−)-lansoprazole is more effectively metabolised to pharmacologically inactive 5-hydroxy and sulphone metabolites [13,14]. Studies on the extent of enantioselective binding of lansoprazole to human serum proteins estimated by ultrafiltration techniques show that (*R*)-(+)-lansoprazole is greater bound than (*S*)-(−)-lansoprazole to human serum proteins ($P < 0.05$). Consequently, the (*R*)-(+)-enantiomer may be poorly distributed and slowly eliminated, resulting in a higher serum concentration than those of the (*S*)-(−)-lansoprazole [15]. Therefore, the use of (*R*)-(+)-lansoprazole would be highly desirable for clinical application [16].

In the last few years the interest in generating individual enantiomers has become a priority for the pharmaceutical industry, with many of the top-selling drugs in the world now being sold in the enantiomeric form [17]. The preparation of single enantiomers or enantiomerically forms of PPIs have been performed by enantioselective HPLC methods that use chiral stationary phases based on proteins [18], cyclodextrin [19], and derivatives of cellulose and amylose [20–22]. In the last few years preparative chiral supercritical fluid chromatography (SFC) has emerged as a competitive technique [23]. So far to our knowledge, no chiral HPLC method has been reported for quantitative determination of the chiral impurity and related substance in bulk drug of (*R*)-(+)-lansoprazole. Therefore, in prevision of a racemic switch, it would be useful and imperative to develop a simple and suitable method for the measurement of (*S*)-(−)-lansoprazole and related substance in bulk (*R*)-(+)-lansoprazole to be adapted for routine and in-process quality control analysis or similar studies, as well as the enantiomeric determination of lansoprazole in human plasma by direct sample injection [24]. In the current investigation a simple, rapid, sensitive, selective and accurate method for the quantization of individual enantiomers of lansoprazole is reported. The enantiomers were resolved on a Chiralpak IA CSP with a mobile phase consisting of M*t*BE–EA–EtOH–DEA in the ratio 60:40:5:0.1 (v/v/v/v), a little modification of the previous reported method [25]. The method was also able to separate the impurities named A, B, C, D, E as reported in the European Pharmacopoeia (EP) monograph of this substance (Fig. 1) from the enantiomers of lansoprazole.

2. Experimental

2.1. Compound

Lansoprazole in its racemic form was purchased from Sigma–Aldrich (Gillingham, UK).

Available impurities A, B, C, D, E were obtained by the European Directorate for the Quality of Medicines & Healthcare (EDQM) (France). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). HPLC enantioseparation were performed by using stainless-steel Chiralpak IA columns (250 mm × 4.6 mm i.d. and 250 mm × 10 mm i.d.) (Chiral Technologies, Cedex, France). Analytical HPLC apparatus consisted on a Dionex P580 LPG pump, an ASI-100 T autosampler, a STH 585 column oven, a PDA-100 UV detector; data were acquired and processed by a Chromeleon Datasystem (Dionex Corporation, Sunnyvale, CA). For semipreparative separation a PerkinElmer (Norwalk, CT, USA) 200 lc pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 1 ml sample loop, a PerkinElmer LC oven 101 and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

The mobile phase was filtered through a 0.45-μm PTFE filter (Millipore, USA) and degassed by sonication just before use.

In analytical separation, because of the lability of the active ingredient lansoprazole in acidic means, freshly standard solution of lansoprazole and single impurities were prepared immediately before use by dissolving 1–3 mg of each analyte in the mobile phase. The injection volume was of 20 μl.

The column hold-up time (t_0 = 3.0 min) was determined from the elution of an unretained marker (1,3,5 tri-*tert*-butyl-benzene) (Sigma–Aldrich, Gillingham, UK) and using the mobile phase as eluent.

In semipreparative separations of racemic lansoprazole, a little amount of diethylamine (DEA) was added to the mobile phase to prevent the degradation of the product due to acidic impurities present in the mobile phase and to increase the solubility of the sample. After semipreparative separations, the collected fractions were analysed by chiral analytical column to determine their enantiomeric excess (ee).

2.2. HPLC operating conditions

Analytical chromatographic separations were carried out on a Chiralpak IA column (250 mm × 4.6 mm i.d.) with a mobile phase consisting of M*t*BE–EA–EtOH–DEA in the ratio 60:40:5:0.1 (v/v/v/v) at a flow rate of 1 ml/min and maintaining the column at 25 °C.

The injection volume was 20 or 50 μl, sampler temperature was set at 5 °C, and the detection wavelength was set at 285 nm.

Semipreparative chromatographic separation of racemic lansoprazole was performed on a Chiralpak IA column (250 mm × 10 mm i.d.) with a mobile phase consisting of M*t*BE–EA–EtOH–DEA in the ratio 60:40:5:0.1 (v/v/v/v) at a flow rate of 4 ml/min and maintaining the column at 25 °C.

The injection volume was 1 ml, with a sample loading of 12 mg for run and dissolving it in EtOH–eluent in the ratio 50/50 (v/v). The detection was carried out at 310 nm.

2.3. Specificity

The selectivity of the analytical method was valuated by the analysis of a solution containing lansoprazole enantiomers and its related substances. The ability to separate all the compounds was assessed by the resolution between the peaks corresponding to the various substances. The tailing factor for lansoprazole enantiomers and related compounds was also assessed.

2.4. Preparation of stock and standard solutions

Fresh standard solutions of (*S*)-(−)- and (*R*)-(+)-lansoprazole obtained by semipreparative separations, accurately protected from light, were prepared and used daily for calibration purpose. Standard solutions were examined in the range from about 20% to about 140% relative to the working concentration of about 60 μg/ml (100%) of each enantiomer of lansoprazole. Two set of stock solutions of (*S*)-(−)- and (*R*)-(+)-lansoprazole were prepared by dissolving about 11 and 16 mg for each enantiomer in 10 ml of volumetric flasks separately with the mobile phase and kept at −20 °C. Aliquots of 0.5 and 1 ml of these solutions were transferred into 10, 20, 50 ml volumetric flasks and diluted with the mobile phase. The final concentration of standard solutions were 11, 20, 27.5, 40, 55 and 80 μg/ml. The vials containing the solutions for the injections were put in the autosampler set at 5 °C before the analyse.

2.5. Linearity

The linearity evaluation was performed with the standard solutions of (*R*)-(+)- and (S)-(−)-lansoprazole at the concentrations described ranging from 11 to 80 μg/ml. Three injections of each solution were made under the chromatographic conditions described above, using an injection volume of 50 μl. The peak areas response at t_R 7.59 ± 0.016 min and 11.31 ± 0.041 min were plotted against the corresponding concentration and the linear regression equations were computed.

2.6. LOD and LOQ

The limit of detection (LOD) and the limit of quantization (LOQ) represent the concentration of the analyte that would yield a S/N of 3 and 10, respectively, following the EP [26]. The LOD and LOQ of (*R*)-(+)- and (*S*)-(−)-lansoprazole were determined by injecting a series of dilute solutions.

2.7. Precision and repeatability

Method precision was determined by measuring the repeatability (intra-day precision) and intermediate precision (inter-day precision) of retention times and peak areas for lansoprazole enantiomers. The intra-day variability was performed by the same analyst over 1 day, while intra-day precision was carried out by another independent analyst over 3 days. In order to determine the repeatability of the method, replicate injections (n = 6) of 55 μg/ml of lansoprazole enantiomers were carried out. The intermediate precision was evaluated over 3 days by performing six consecutive injections each day. Precision was reported as % of relative standard deviation (%RSD).

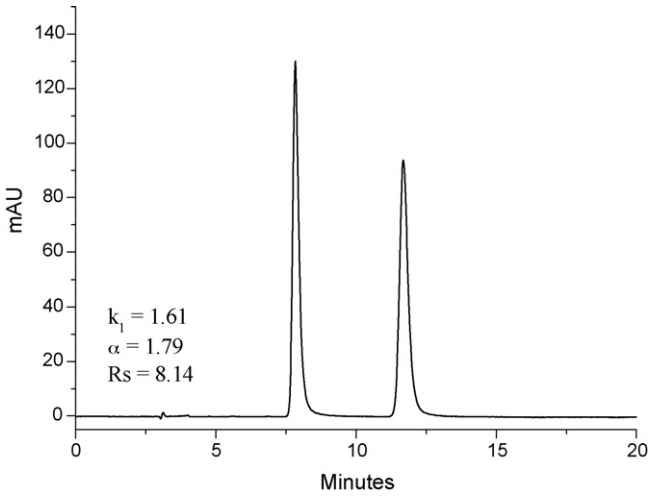

Fig. 2. Representative chromatogram of racemic lansoprazole on Chiralpak IA CSP (250 mm × 4.6 mm i.d.), eluent: M*t*BE–EA–EtOH–DEA (60:40:5:0.1, v/v/v/v); flow rate: 1 ml/min; temperature: 25 °C; UV detection: 285 nm.

2.8. Accuracy

The accuracy of the method was tested by analyzing different samples of lansoprazole enantiomers at various concentration levels.

3. Results and discussion

3.1. Specificity and chromatography

In recent years, the direct separation of enantiomers by chiral chromatography has been the target of intense research. Enantioselectivity can be achieved by the appropriate choice of the chiral stationary phase and the mobile phase composition. Chiralpak IA, a new generation of chiral packing materials for resolution of enantiomers by chromatography, has been produced by improved immobilisation technologies. The chiral selector is 3,5-dimethylphenylcarbamate of amylose immobilised on a silica matrix. This chiral stationary phase (CSP) has proved to be able to separate a wide series of chiral analytes both at analytical and semipreparative scale [27–33].

As previously reported [25] by using non-conventional solvents, lansoprazole enantiomers were resolved with an enantioselectivity and resolution factors of 1.75 and 7.00 with a mobile phase consisting of M*t*BE–EA–EtOH in the ratio 60:40:5 (v/v/v). In the present work the method has been only slightly modified in terms of mobile phase composition, and a little amount (0.1%) of the basic additive diethylamine was added. This resulted in better values of enantioselectivity (1.79) and resolution (8.14). In Fig. 2 the chromatogram obtained is shown.

In order to obtain single enantiomers of lansoprazole at mg-scale to be employed for validation purpose, racemic sample was subjected to semipreparative separations. The immobilisation of the polysaccharide derivatives on the chromatographic matrix offers the possibility of developing separation methods with no constraint on the choice of the mobile phase and sample solvent [27]. This last point has practical relevance for enantioseparations at preparative levels, where sample solubility in the mobile phase is a prerequisite for successful separation. In fact in order to render a preparative separation practically feasible and/or economically attractive, the racemic compound must be soluble enough in the solvent eluent.

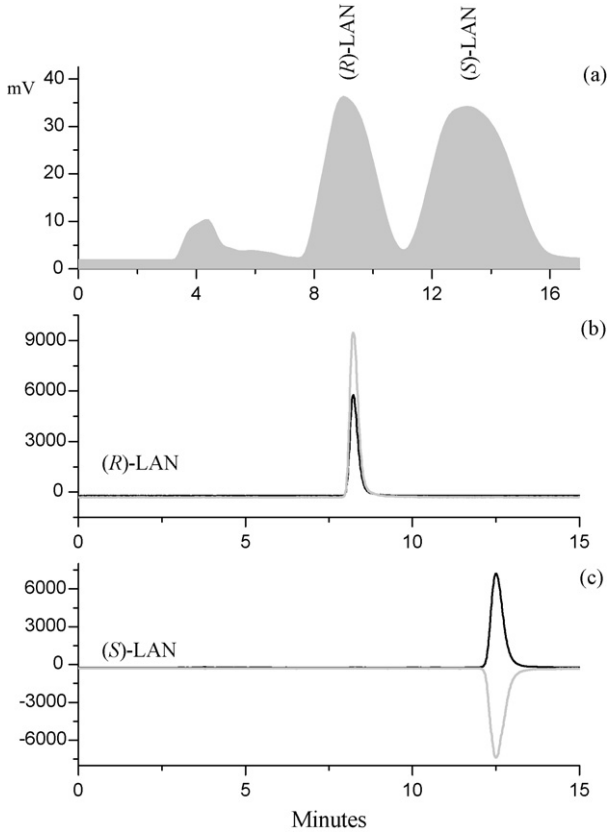

Fig. 3. (a) Semipreparative resolution of 12 mg of racemic lansoprazole; (b and c) UV (black) and CD (gray) traces of the isolated enantiomers. Column: (a) Chiralpak IA 250 mm × 10 mm i.d.; (b and c) Chiralpak IA 250 mm × 4.6 mm i.d. Eluent: (a–c) M*t*BE–EA–EtOH–DEA (60:40:5:0.1, v/v/v/v); flow rate: (a) 4 ml/min, (b and c) 1 ml/min. Detector: (a) UV at 310 nm, (b and c) UV and CD: 285 nm. Temperature: (a–c): 25 °C.

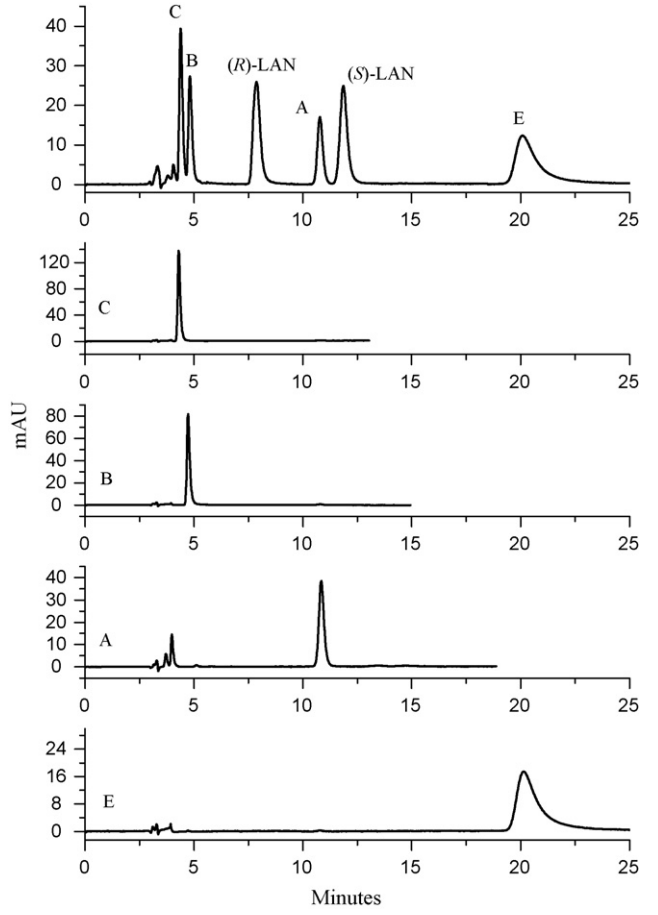

Fig. 4. HPLC separation of the enantiomers of lansoprazole and its impurities A, B, C and E (top) and single impurities (bottom) on Chiralpak IA CSP (250 mm × 4.6 mm i.d.). Detector: UV 285 nm.

Thus, to achive mg-scale separation of the enantiomers of lansoprazole on the semipreparative IA CSP, the EA-based eluent in combination with ethanol in the 1:1 ratio was selected as solvent. Repetitive injections of 12 mg of the racemic sample dissolved in one ml of the eluent were performed. The temperature of the column was 25 °C and the flow rate was 4 ml/min. The first fraction was recovered with a yield of 90% with ee% >99%; the second fraction was obtained with a yield of 80% and ee% >99%.

Fig. 3 shows the chromatogram obtained by injecting 12 mg of the racemic sample and typical traces of the two enantiomers using UV and CD detectors.

The CSP/eluent combination was applied not only for the separation of the enantiomers of lansoprazole, but also for the separation of impurities named A, B, C, D, E from the EP monograph of this substance. Under the above mentioned chromatographic conditions, all the impurities but impurity D, that was not eluted in this chromatographic condition, were well separated from the principal compound (Fig. 4), with resolution factors greater than 2.00, as reported in Table 1. A resolution value minor of 2.00 was only obtained between impurity C and B (1.49). Impurity C was chosen for the system suitability parameters of the method. Six consecutive runs were performed and the results of the suitability test are reported in Table 2.

3.2. Linearity

The linearity of the HPLC method was evaluated by injecting standard concentrations of samples of single enantiomers ranging from 11 to 80 μg/ml (20–140%). The peak area response of each enantiomer was plotted versus nominal concentration of the enantiomers. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method [34]. The calibration curve obtained showed correlation coefficient greater than 0.999. For *R*-(+)-lansoprazole the sequent regression equation was obtained $y = 0.0204x + 0.2893$ ($r^2 = 0.9993$) and for *S*-(−)-lansoprazole $y = 0.0204x + 0.2982$ ($r^2 = 0.9993$) where y is the peak concentration of *R*- or *S*-lansoprazole and x is the area of *R*- or *S*-lansoprazole.

3.3. LOD and LOQ

The LOD and LOQ concentrations were estimated to be 0.2 and 0.6 μg/ml for both the enantiomers, *R*-(+)- and *S*-(−)-lansoprazole, when the S/N of 3 and 10 were used as the criteria.

Table 1
Chromatographic parameters for lansoprazole enantiomers and related substances.

Compound	k'	Rs	Asymmetry factor
Imp C	0.46		1.51
Imp B	0.61	1.49	1.44
R-(+)-Lanso	1.62	6.60	1.24
Imp A	2.59	5.40	1.16
S-(−)-Lanso	2.96	2.00	1.21
Imp E	5.69	7.30	1.89

Column: Chiralpak IA (250 mm × 4.6 mm i.d.); eluent: M*t*BE–EA–EtOH–DEA (60:40:5:0.1, v/v/v/v); flow rate: 1 ml/min; temperature: 25 °C; UV detection: 285 nm.

Table 2
System suitability parameters of the chromatographic method for impurity C and lansoprazole enantiomers.

Compound	Tr[a]	RDS%	Rs[a]	RSD%	Theoretical plates[a]	RSD%	Asymmetry factor[a]	RSD%
Imp C	4.32	0.15			6618	0.85	1.56	5.49
R-(+)-Lanso	7.86	0.13	11.44	0.41	5927	0.48	1.58	2.95
S-(−)-Lanso	11.74	0.2	7.89	0.50	6693	0.78	1.40	3.65

[a] Mean value of six replicates. Chromatographic condition as reported in the test.

Table 3
Accuracy of the HPLC method for the determination of lansoprazole enantiomers in standard solutions.

Compound	Standard concentration (μg/ml)	Standard solutions observed concentration (μg/ml)	Apparent recovery (%)
R-(+)-Lanso	10.46	10.54	100.76
	26.15	26.54	101.50
	38.75	39.40	101.7
	77.5	77.20	99.61
Overall recovery			100.89
%RSD			0.93
S-(−)-Lanso	10.46	10.54	100.76
	26.15	26.50	101.33
	38.75	39.33	101.50
	77.5	77.20	99.61
Overall recovery			100.80
%RSD			0.85

3.4. Precision and repeatability

The precision of the HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day). Method precision has a relative standard deviation (RSD%) below 1.0% for repeatability (0.62%) and for intermediate of precision (0.92%), which comply with the acceptance criteria proposed (RSD%: not more than 2.0%) [26].

3.5. Accuracy

The accuracy for the determination of enantiopure lansoprazole was determined by preparing drug substance sample at 20, 60, 100 and 140% of the target (10–80 μg/ml). The apparent recovery [35] ranged from 99.4 to 103.86%. The data are presented in Table 3. The overall percent recovery was 100.89 (RSD% 0.93) and 100.80 (RSD% 0.85) for the first and second of the enantiomers.

4. Conclusion

Since racemic switch of PPIs provides therapeutic advantages, such as reducing metabolic load on the body, simplifying pharmacokinetics, providing benefit to the non-responders to prior standard dose of racemate, in the present study a simple chiral HPLC method has been reported, that was validated for the analysis of lansoprazole enantiomers and its related substances. The method showed good resolution between lansoprazole enantiomers and the available impurities. The method was also linear and precise. The present method could be applicable to routine analysis when racemic lansoprazole will be replaced by a single enantiomer of lansoprazole for the quantitative determination of chiral impurity in bulk drug samples of *R*-(+)-lansoprazole.

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