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Separation and Quantification of Octahydro-1h-Indole-2-Carboxilic Acid and Its Three Isomers by HPLC using Refractive Index Detector

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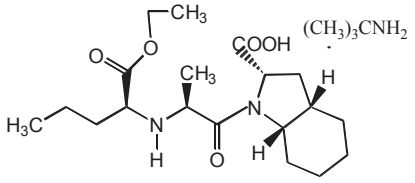
Abstract

Octahydro-1H-indole-2-carboxylic acid is a key starting material for the synthesis of Perindopril and Trandolapril. A rapid, economical, simple, sensitive and reliable stability-indicating reverse phase HPLC method developed and validated for the quantitative determination of all isomers related to Octahydro-1H-indole-2-carboxylic acid and its related substances. The compound is non chromophoric, it has three chiral centers and there is a possibility of four pairs of enantiomers. Refractive index detector was used for the quantification of all its isomers. Optimized mobile phase is 10 mM potassium phosphate buffer with pH-3.0. The C18 column (Inertsil ODS-4, 250 mm×4.6 mm×5 µm) is used as the stationary phase with a mobile phase flow rate of 1.5 mLmin^{-1} and column temperature maintained at 35°C. The developed method was validated as per ICH guidelines for Accuracy, linearity, range, precision, ruggedness, robustness, solution stability, limit of quantification, and limit of detection. A linear range from LOQ to 150% performed for the analyte and its three isomers. The correlation coefficient obtained for all the isomers was more than 0.999. The recoveries obtained for all isomers were found in between 93.9% and 107.9%. The detection limit for all the isomers were about 0.006 mg/mL and the quantification limits were in between 0.022 mg/mL to 0.024 mg/mL respectively. The proposed method can be successfully applied for the quantification of the three isomers in Octahydro-1H-indole-2-carboxylic acid and provides a simple and cost effective quality control tool for routine analysis.

Keywords: Octahydro-1H-indole-2-carboxylic acid; Refractive index detector; Perindopril; Trandolapril; High performance liquid chromatography; Validation and stability indicating method

Introduction

Perindopril erbumine (Figure 1) [1], compound with tert-butylamine (1:1) belongs to a group called angiotensin converting enzyme (ACE) inhibitors. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma renin activity and decreased aldosterone secretion. The overall effect of this is a drop in blood pressure and a decrease in the workload of the heart. ACE inhibitors and particularly perindopril were primarily considered as antihypertensive drugs able to reduce, significantly high blood pressure in hypertensives. They greatly reduced left ventricular (LV) hypertrophy and prevented recurrent stroke in patients with a history of cerebrovascular accident (CVA) or transient ischaemic attack (TIA) (PROGRESS trial) [2]. During this study, there was a significant reduction of major coronary events and this led to the EUROPA trial [3]. EUROPA [3] was the largest study conducted with an ACE inhibitor for secondary prevention of stable coronary artery disease (CAD).



Molecular formula: $C_{23}H_{43}N_3O_5$
Chemicalname:2-Methylpropan-2-amine(2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1H-indole-2-carboxylate.

Figure 1: Perindopril erbumine Chemical Structure.

Blood pressure (BP) is an important determinant of cardiovascular risk in the general population, [4] in which interventions that lower BP have been clearly shown to prevent cardiovascular events [5]. BP levels are commonly elevated in people with chronic kidney disease (CKD); raising the possibility that BP lowering may offer significant benefit in this group. BP-lowering agents acting *via* the renin-angiotensin system have been demonstrated to have renoprotective effects in the proteinuric subgroup of people with CKD [6,7]. The few BP-lowering trials that have been conducted in a broader range of participants with CKD [8-10], have not demonstrated clear benefits for either cardiovascular events or kidney function overall. Subsidiary analyses of one large clinical trial [11] suggested that angiotensin-converting enzyme inhibitors may produce greater benefits in the presence of CKD. However, a number of other studies that used agents acting *via* the RAS and were performed specifically in participants with CKD, all with limited statistical power, failed to demonstrate clear cardiovascular benefits [10,12–14].

Trandolapril (Figure 2) is a colorless, crystalline substance that is soluble in chloroform, dichloromethane and methanol. It is practically insoluble in water and sparingly soluble in hydrochloric acid. It is available in the brand names Zetpril (Hetero HC (Genx)), Mavik

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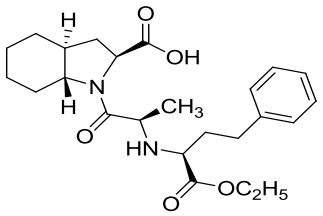
(Abbott), Tarka (Abbott) Trandolaprilum (Halo). It is an angiotensin converting enzyme (ACE) inhibitor widely used as an efficient orally active antihypertensive drug. It is especially recommended for the treatment of arterial hypertension in patients after myocardial infarction with dysfunction of the left heart ventricle [15]. The synthesis of this generic drug has been patented in 1981 by Hoechst [16] and no more synthetic details were later described. It is believed to exert its antihypertensive effect through the renin-angiotensin-aldosterone system. The effect of trandolapril in hypertension appears to result primarily from the inhibition of circulating and tissue ACE activity there by reducing angiotensin II formation, decreasing vasoconstriction, decreasing aldosterone secretion, and increasing plasma renin. Decreased aldosterone secretion leads to diuresis, natriuresis, and a small increase of serum potassium. Literature survey reveals that several researchers developed different chromatographic methods to determine the amount of trandolapril present in human plasma, in bulk, dosage forms, and in combination with verapamil. Trandolapril is a newer angiotensin-converting enzyme (ACE) inhibitor that is approved by the US Food and Drug Administration for the treatment of hypertension and for use in stable patients who have evidence of left ventricular (LV) systolic dysfunction or symptoms of chronic heart failure within the first 2 days after an acute myocardial infarction (AMI). The fixed-dose combination of trandolapril and verapamil extended release (ER) is approved for the treatment of hypertension only. Bulk drug and pharmaceutical dosage forms of trandolapril are qualitatively and quantitatively analyzed [17-19] by reverse phase HPLC. Stability-indicating high-performance thin-layer chromatographic (HPTLC) method for analysis of trandolapril in pharmaceutical dosage forms has been reported in the literature [20].

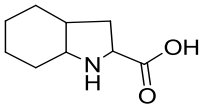
Octahydro-1H-indole-2-carboxylic acid (Figure 3) is a key starting material for the synthesis of Perindopril and Trandolapril. Octahydro-1H-indole-2-carboxylic acid has three chiral centers and it exists in four pairs of enantiomers. Using reverse phase HPLC with refractive index detector, raw material and its three isomers were separated, in which (2S,3aS,7aS)-octahydro-1H-indole-2-carboxylic acid is a key starting material for the synthesis of Perindopril.

The key raw material in trandolapril synthesis is (2S,3aR,7aS)-octahydro-1H-indole-2-carboxylic acid. This compound exists in racemic form and is prepared by catalytic hydrogenation of the corresponding imine-acid salt (I) (Figure 4). According to the patent literature [16] the main product of this hydrogenation in presence of Pt ([16], general description) or on a Pd catalyst ([16], experimental), is a racemic stereoisomer (IA). The reaction is not dependent on catalyst, resultant into mixture of two major diastereoisomers (IA) and (IB). When the conditions of reduction reactions are appropriately



Molecular formula: C24H34N2O5
Chemicalname:(2s,3aR,7aS)-1-[(2s)-2[[(1s)-1-ethoxycarbonyl-3-phenylpropyl]-amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid.

Figure 2: Trandolapil Chemical Structure.



Molecular formula: C9H15NO2
Chemicalname: Octahydro-1H-indole-2-carboxylic acid

Figure 3: Octahydro-1H-indole-2-carboxylic acid Chemical Structure.

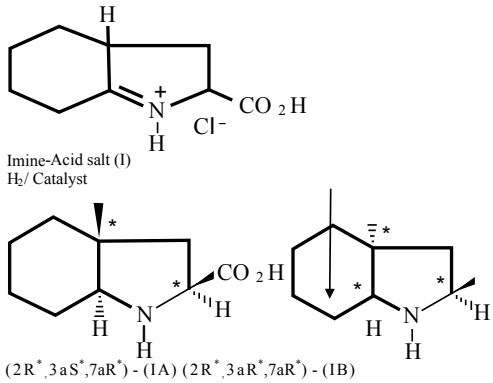


Figure 4: Scheme of octahydro—IH—indole-2—carboxylic acid synthesis.

modified viz, type of catalyst, solvent, temperature, acid addition etc. followed by recrystallization, then both diastereoisomers were synthesized in an almost pure form. Out of these two diastereoisomers, only one stereoisomer is required to synthesize the drug. Quantitative determination of the desired stereoisomer in the mixture is the essential requirement to way forward. HPLC technique was successfully used for the separation of the diastereoisomers.

HPLC methodology for determination of content of diastereoisomers using a C-18 column as well as indirectly via preparation of (N-quino1-6-y1)-carbamoil-derivatives of octahydro-1H-indole-2-carboxylic acids is referred in AccQ-Tag Amino Acid Analysis Method [21]. This methodology was developed and recommended for the determination of amino acid mixtures by Milipore Corporation Waters [22], but was never used for octahydro-IH-indole-2-carboxylic acids. However this literature explains only about two diastereomers of Trandolapril Raw material but not discussed remaining two diastereomers. No validation or quantification data was published about four diastereomers of this Raw material in any journal or literature. Harlikar J N and Amlani A M quantified this raw material in API and formulations of Perindopril but not discussed its other isomers.

The objective of the current study was to develop a stability-indicating reverse phase HPLC method for the quantitative determination of all isomers related to Octahydro-1H-indole-2-carboxylic acid and its related substances. Major challenge of the methodology was non-chromophoric compounds. Refractive index detector is a universal detector which is most preferable for non-UV compounds. After some trials, separated analyte and its three isomers with good resolution and validated as per ICH guidelines. This method can be applicable for quantification of its isomers as well as to check the chromatographic purity of the analyte. In the current study (2S,3aS,7aS)-octahydro-1H-indole-2-carboxylic acid was used as a raw material for Perindopril and quantified the purity of raw material as well as quantified the percentages of remaining isomers present in the raw material. This method can be useful to quantify all the isomers

related to raw material present in Perindopril and Trandolapril Active pharmaceutical ingredients (Figure 1).

Experimental

Chemicals and reagents

Potassium dihydrogen phosphate anhydrous (KH_2PO_4) and Ortho Phosphoric acid (88%) were purchased from Merck. Above 93% purity of (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid and remaining three diastereomers are taken from Pharma industries of Hyderabad, India.

Instrumentation

An Agilent HPLC 1100 series equipped with an auto sampler with refractive index detector was used in the experiment; a binary gradient pump system with temperature control oven used. Data acquisition and processing were conducted using the Agilent Chemistation software. Metrohm pH meter is used for adjusting pH of the mobile phase.

Preparation of stock, standard and test solutions

The stock solutions of (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid and the remaining three diastereomers were prepared by dissolving 25 mg of each isomer in 10 mL of mobile phase separately (50%). A series of dilutions prepared using 50% solutions for raw material, isomer-1, isomer-2 and isomer-3. The sample solution was prepared by weighing about 50 mg of the (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid substance into a 10 mL volumetric flask and dissolved and diluted to 10 mL with mobile phase.

Preparation of system suitability

50 mg of (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid dissolved in 10 mL of mobile phase and further 1 ml of solution taken and diluted to 10 ml with mobile phase. Sample itself contains about 5% of 1st Isomer. As a part of system suitability, two criteria were defined (i) resolution between (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid and 1st Isomer and (ii) Theoritical plates of (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid.

Chromatographic conditions

The chromatographic column used was Inertsil ODS-4 (250 mm, 4.6 mm-ID; 5 µm), make of GL Sciences, USA. pH 3.0 Buffer solution of 10 mM anhydrous Potassium dihydrogen phosphate (adjusted the ph with dilute ortho phosphoric acid) was optimized as mobile phase as well as diluent. The flow rate of the mobile phase was 1.5 $mLmin^{-1}$ and the column temperature set at 35°C. The injection volume was 10 µL. The concentration of the sample was 5 $mLmin^{-1}$. HPLC column separated isomers were quantified by Refractive index detector (RID).

Results and Discussion

Method development and optimization

The HPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Octahydro-1H-indole-2-carboxylic acid and its diastereomers and also complied the general requirements of system suitability criterias. The main challenge of this methodology was the determination of non-chromophoric compound. Refractive index detector is a universal detector which is most preferable for non-UV compounds. So many trials were done on RID with different pH, different flow and different column oven temperature. Different types of mobile phases prepared with different combinations of buffers like Acetonitrile and Methanol, but combination of buffer with small quantity of organic solvent are giving very poor peak shapes. i.e the peaks were not in Gaussian shape. Finally decided 100% buffer with pH-3.0, 1.5 mL·min–1 flow and column oven temperature 35°C. The run time was fixed for 35 minutes. In this method (2S,3aS,7aS)-Octahydro-1H-indole-2-carboxylic acid and remaining three isomers are well separated. In the present study 1.0% of specification is kept for the three isomers with respect to 5 $mgmL^{-1}$. The RID detector is having its own limitations so stabilized the system for hours prior to analysis (Figure 5-8).

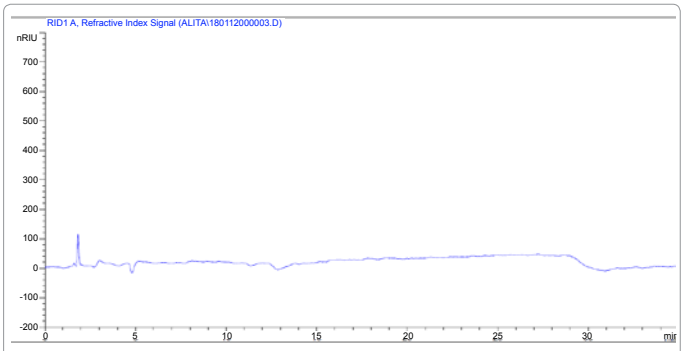


Figure 5: A typical chromatogram of blank mobile phase (Inertsil ODS-4 column with dimensions 25cm×4.6mm×5µm, flow: 1.5$mLmin^{-1}$, column oven temperature 35°C).

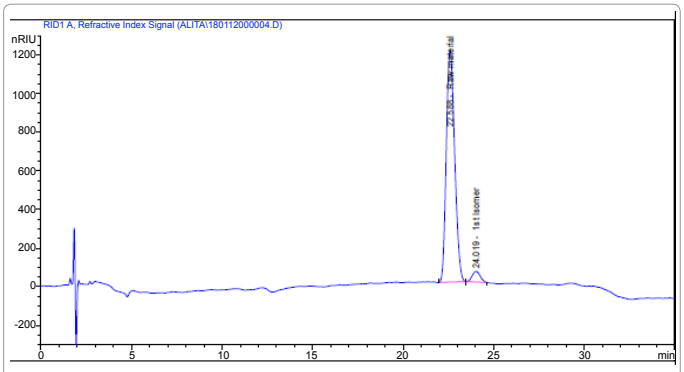


Figure 6: A typical chromatogram of system suitability (Inertsil ODS-4 column with dimensions 25cm×4.6mm×5µm, flow: 1.5$mLmin^{-1}$, column oven temperature 35°C).

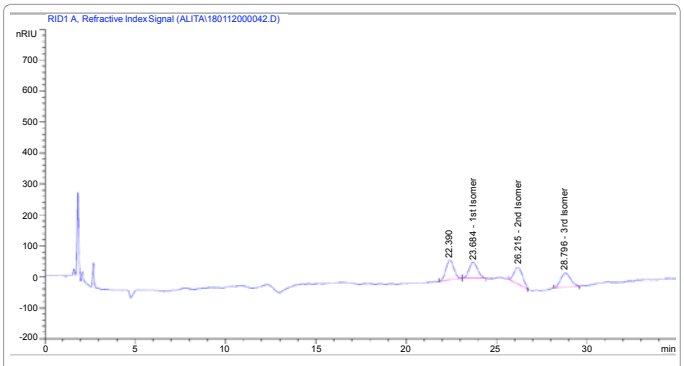


Figure 7: A typical chromatogram of Isomers blend from LOQ Solution (Inertsil ODS-4 column with dimensions 25cm×4.6mm×5µm, flow: 1.5$mLmin^{-1}$, column oven temperature 35°C).

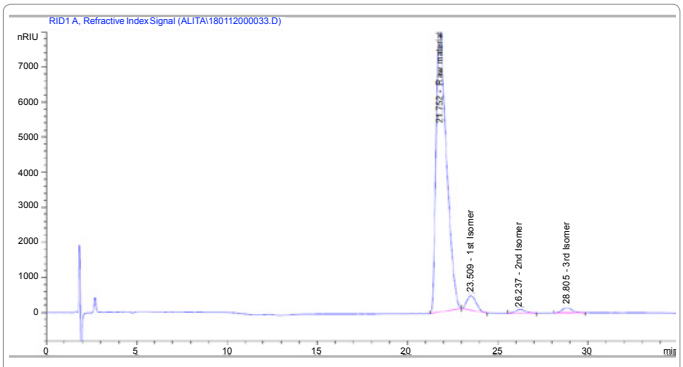


Figure 8: A typical chromatogram of 1.0% isomers spiked with raw material solution (Inertsil ODS-4 column with dimentions 25cm×4.6mm×5µm, flow: 1.5mL·min–1, column oven temperature 35°C). Concentration of raw material 5mg/mL.

Method validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines [23,24] the validated method parameters include LOD, LOQ, accuracy, precision, linearity, ruggedness and robustness.

Sensitivity: Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for raw material, isomer-1, isomer-2 and isomer-3 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The detection limit (LOD) of all the isomers were found about 0.006 mg/mL and the quantification limit (LOQ) in between 0.022 mg/mL to 0.024 mg/mL. The precision study was also carried out at LOQ level by injecting six individual preparations of isomer-1, isomer-2 and isomer-3 and calculated the %RSD for the areas of each impurity. The precision values at LOQ concentration for isomer-1, isomer-2 and isomer-3 were below 2%. The recovery at LOQ concentration level for isomer-1, isomer-2 and isomer-3 were in between 95 to 105%. The sample containing 1st Isomer about 5% during LOQ recovery study was diluted because the sample lowers the LOQ level as it already contains the 1st isomer of about 5% and spiked with all isomers at LOQ level.

Precision: The precision of the related substance method was performed by injecting six individual preparations of raw material (5 $mgmL^{-1}$) spiked with 1.0% (100%) of each isomer-1, isomer-2 and isomer-3. The %RSD for area% of each isomer-1, isomer-2 and isomer-3 were calculated. Precision study was also determined by performing the same procedures at higher level (at 150%). The %RSD values for precision study at 100% concentration and 150% concentration for isomer-1, isomer-2 and isomer-3 were below 2%. Intermediate precision performed for Raw material and its three isomers at 100% level and the %RSD values for all the isomers found to be below 1.0%.

Linearity and range: A linearity test solution for related substance method was prepared by diluting the raw material and its three isomers stock solutions to the required concentrations. The solutions were prepared at five concentration levels. From LOQ to 150% of the 1% level of the impurity (i.e. LOQ, 75%, 100%, 125% and 150%) was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The correlation coefficients for all isomers were more than 0.999 (Table 2). The coefficient of determination (R^2) obtained for isomer-1, isomer-2 and isomer-3 are within the acceptance criteria.

Accuracy: Accuracy of the method was determined by analyzing raw marerial (n=3) spiked with (50% to 150%) 0.5%, 1.0% and 1.5% of the isomer-1, isomer-2 and isomer-3. The recoveries obtained for all isomers in between 93.9% and 107.9% respectively. The percentage of recoveries for isomer-1, isomer-2 and isomer-3 were calculated and tabulated (Table 1).

Ruggedness: Ruggedness of the method was performed by doing precision study by injecting six individual preparations of (5 mg·mL–1) raw material spiked with 100% level of each isomer-1, isomer-2 and isomer-3 using different column, different analyst and different system from the same laboratory. The % RSD for area% of each isomer-1, isomer-2 and isomer-3 were calculated. The %RSD for all the three isomers were in between 1% to 3.0%.

Robustness: To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (Rs) between raw material, isomer-1, isomer-2 and isomer-3 were evaluated. The flow rate of the mobile phase was 1.5 mL·min–1. To study the effect of flow rate on the developed method, 0.2 units of flow was changed (i.e. 1.3 and 1.7 mL·min–1). The effect of column temperature on the developed method was studied at 30°C and 40°C instead of 35°C. The effect of pH on resolution of isomers was studied by varying ± 0.2 pH units (i.e. buffer pH altered from 3.0 to 2.8 and 3.2). In the all above varied conditions, the components of the mobile phase were held constant. System suitability and 100% spiked sample injected in the above changed conditions to check the resolution and RRT of three isomers. No peak is merged with each other and the resolution between 1st isomer and raw material was more than 1.5. The Robustness data was tabulated (Table 3, Table 4).

Solution stability: Solution study was performed for sample, system suitability solutions up to 48 hours at room temperature and the solution was found to be highly stable at room temperature during 48 hours study and no impurities were observed during this study.

Conclusion

A simple and sensitive reverse phase HPLC method with refractive index detector has been developed and validated for the quantitative

Level of Accuracy	Isomers	Isomers added (mg/mL)	Isomers Recovered (mg/mL)	% Isomers Recovery (n=3)
at 50%	1	0.025	0.0270	107.9
	2	0.025	0.0255	102.0
	3	0.025	0.0256	102.5
at 100%	1	0.050	0.0490	97.9
	2	0.050	0.0477	95.3
	3	0.050	0.0492	98.3
at 150%	1	0.075	0.0717	95.7
	2	0.075	0.0732	97.6
	3	0.075	0.0704	93.9

Table 1: Accuracy Results.

S.NO.	NAME	Range	Correlation coefficient(R)	Coefficient of determination(R^2)
01.	Raw material	LOQ to 150%	0.9993	0.9987
02.	Isomer-1	LOQ to 150%	0.9992	0.9984
03.	Isomer-2	LOQ to 150%	0.9999	0.9998
04.	Isomer-3	LOQ to 150%	0.9999	0.9998

Table 2: Linearity results.

S. No.	Parameter	1.3 mL/min	1.7 mL/min	30°C	40°C	pH-2.8	pH-3.2
01	The resolution between 1st Isomer and Raw material	1.84	1.85	1.73	1.88	1.52	1.78
02	The theoretical plates of the Raw material peak	12517	11337	11762	11845	10722	11122

System suitability Results

Table 3: Robustness Data.

S. No.	Isomer name	Relative retention time	1.3 mL/min	1.7 mL/min	30°C	40°C	pH-2.8	pH-3.2
01	Isomer-1	---	1.1	1.08	1.08	1.09	1.06	1.07
02	Isomer-2	---	1.21	1.21	1.21	1.21	1.18	1.18
03	Isomer-3	---	1.33	1.33	1.33	1.32	1.28	1.30

Relative retention time of Isomers during Roboustness

Table 4: Robustness Data.

determination of all isomers related to Octahydro-1H-indole-2-carboxylic acid and its related substances. Compared with the previously reported methodologies there is no specific method reported for Octahydro-1H-indole-2-carboxylic acid related substances with RID detector. This method utilizes a RID detector, which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect all isomers 0.006 mg/mL and can quantify up to 0.024 mg/mL. This raw material is manufacturing and supplying by so many industries, if they want to check the purity and isomers content this method is useful. If any unwanted isomers are present excess in the raw material because of its activity there is a huge impact on the final yield and quality of the API drug. Quantification of each isomer was of importance because only the correct stereoisomer could be used for the synthesis of Perindopril and Trandolapril.

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