Research Article

Stability Indicating Validated RP-HPLC Method for Simultaneous Determination of Perindopril Erbumine and Amlodipine Besylate in Bulk and Pharmaceutical Dosage Form

SK. Mastannamma*, I.S.Tejaswini, Reehana.SK, P. Saidulu,
Department of Pharmaceutical Analysis, University College of Pharmaceutical Science, Acharya Nagarjuna University, Nagarjunanagar, Guntur, Andhra Pradesh, India.
***Corresponding author's E-mail:** masthanamma.sk@gmail.com

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ABSTRACT

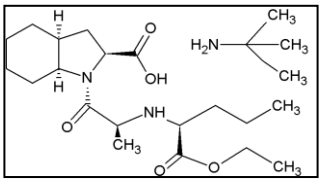
The aim of this work was focused on to develop and validate an accurate, simple, precise, rapid, and stability-indicating reversed phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Perindopril Erbumine (PDE) and Amlodipine Besylate (AMD) in bulk drugs and their combined commercial tablets**.** The chromatographic separation was performed on a YMC C18 (250mm×4.6mm I.D; 5 µm) column, with a mobile phase comprising of a mixer of Orthophosphoric acid (0.1%) pH 2.1 and Methanol (40:60), at a flow rate of 1 ml/min, with detection at 262nm.Retention times of Perindopril Erbumine and Amlodipine Besylate were found to be 2.2 min and 3.1min respectively. The developed method was validated according to ICH guidelines. Linearity of Perindopril Erbumine was found to be in the range of 4-12µg/ml and that for Amlodipine Besylate was found to be in the range of 5-15µg/ml. The percentage recoveries for both drugs were found in the range of 97-102%. The limits of detection values were found to be 2.96µg/ml and 2.72µg/ml for Perindopril Erbumine and Amlodipine Besylate respectively. The proposed RP-HPLC method is simple, rapid, isocratic, specific, accurate and precise. Hence, this method can be recommended for the estimation of Perindopril Erbumine (PDE) and Amlodipine Besylate (AMD) in pharmaceutical dosage form.

Keywords: RP-HPLC, Perindopril Erbumine, Amlodipine Besylate, YMC C18, Forced degradation studies.

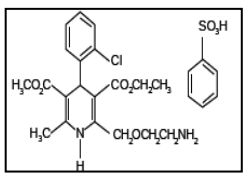
INTRODUCTION

Perindopril Erbumine[4] is chemically 2- Methyl Propane-2-amine (2S, 3As, 7As)-1-[(2S)-2- 2[[(1S)-1-(ethoxy carbonyl) butyl [amine] propanoyl] octahydro-1H-indol-2-carboxylate, with molecular formula-$C_{19}H_{32}N_2O_5.C_4H_{11}N$ and 441.60 mg molecular weight. It is freely soluble in water and sparingly soluble in methaline chloride. Perindopril Erbumine is Angiotensin Converting Enzyme Inhibitor. It is used for the treatment of hypertension. It may be used alone or in combination with other antihypertensive.

Amlodipine Besylate[4] (AB) is 3-ethyl 5-methyl 4RS-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5- dicarboxylate benzene sulphonate. Its molecular weight is 567.1 mg. It is slightly soluble in water and proponol,sparingly soluble in ethanol and freely soluble in methanol. It is a calcium channel blocker, used as an anti-hypertensive and in the treatment of angina; it lowers the blood pressure, relaxes heart muscles and dilates the heart blood vessels to prevent spasm.



(a)



(b)

Figure1a: chemical Structure of (a) Perindopril Erbumine (b) Structure of Amlodipine Besylate

Literature include few spectophotometric and chromatographic methods were established for simultaneous estimation as well as individual drugs However, the developed analytical methods lack stability indicating nature incase of Amlodipine Besylate[8] and Perindopril Erbumine[6] combination using water as solvent. In the present investigation, an attempt was made to develop a simple, rapid, precise and accurate stability indicating RP-HPLC assay method for simultaneous estimation of PDE and AMD in presence of their degradation products[7]. The major advantage of the proposed method was that Perindopril Erbumine and Amlodipine can be determined on a single chromatographic system with the same detection wavelength. This proposed method can be successfully employed for quality control during manufacture and for assessment of the stability of both drugs in bulk samples and their combined tablet dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

Perindopril Erbumine and Amlodipine Besylate were obtained as gift sample from Lara drugs, Hyderabad, India. Pharmaceutical tablet formulation of COVERSYL-AM4/5 was purchased from local pharmacy. Methanol (HPLC Grade; MERCK), Orthophosphoric acid (HPLC grade, MERCK), Hydrochloric acid (AR), sodium hydroxide (AR), hydrogen peroxide (AR) and HPLC grade water were used for the entire study.

Preparation of standard solutions for HPLC

Preparation of Buffer

0.1%OPAwas prepared by taking 1ml of OPA in 1000ml HPLC grade water.

Preparation of Mobile phase

Mobile phase was prepared by mixing OPA (pH-2.1, 0.1%) and Methanol (40/60). It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram and it was sonicated for 15min to remove the undisolve gases.

Preparation of Perindopril Erbumine and Amlodipine Besylate Stock solution

Accurately weighed and transferred 4mg and 5mg of Perindopril Erbumine and Amlodipine Besylate into 100 ml volumetric flasks separately. Add required amount of diluents and sonicated for 5min and make up the final volume with HPLC grade water, then the solutions consists of 8µg/ml and 10µg/ ml of PDE and AMD respectively. From the above stock solution take 5ml from each flask and transfer into two separate 25ml volumetric flasks and make up the final volumes with diluents. The final standard solution consisting of 8µg/ml of PDE and 10µg/ ml of AMD respectively.

HPLC Instrumentation and chromatographic condition

Analysis was performed on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in isocratic mode using Auto sampler. Data collection and processing was done using EMPOWER PDA 2 software. The analytical column used for the separation was YMC C18, 250× 4.6 mm I.D., 5µm particle size, Flow rate was kept at 1ml/min. the column temperature was maintained at 30°C. The mobile phase was made up of Orthophosphoric acid (0.1%) pH 2.1 and Methanol in 40:60 ratio. The method was optimized at 262nm. Run time was taken as 6min. The injection volume of samples was 10 µl. Other equipment's used were ultra-sonicator (model 3210, Branson Ultrasonic Corporation, Connecticut, USA), Analytical balance (contech balance).The retention times for PDE and AMD were found to be 2.22 and 3.15 mins respectively. Typical chromatogram of PDE and AMD show in fig. 1b and optimized chromatographic conditions as shown in below

Column: YMC C18, 250× 4.6 mm I.D., 5µm

Mobile phase: 0.1%OPA (pH 2.1), Methanol (40/60 v/v)

Flow rate: 1ml/min

Column temperature: 30°C

Injection volume: 10 µl

Wavelength: 262nm

Run time: 10 min

Retention times: 2.221(PDE), 3.156(AMD) min

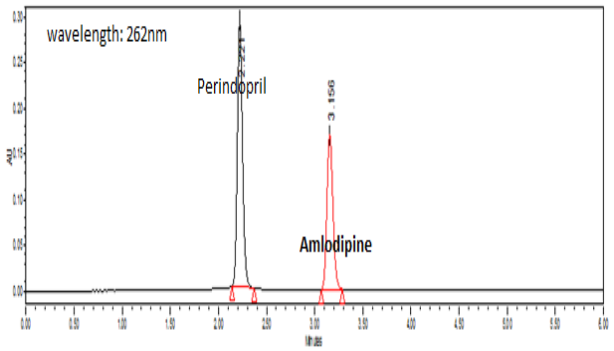


Figure 1b: Typical chromatogram of PDE and AMD

METHOD DEVELOPMENT

To saturate the column, the mobile phase was pumped for about 30 minutes thereby base line correction can be achieved. The separate standard calibration curves were constructed for each drug. A series of aliquots were prepared from the above stock solutions using HPLC grade water to get the concentrations 4-12µg/ml PDE and 5-15µg/ml AMD. Each concentration was injected 6times into chromatographic system. Each time peak area and retention time were recorded separately for both the drugs. Calibration curves were constructed by taking average peak area on Y-axis and concentration on X-axis separately for both the drugs. From the calibration curves regression equations were calculated, these regression equations were used to calculate drug content in formulation.

Estimation of PDE & AMD in tablet dosage forms

20 tablets were weighed and triturated in a glass mortar and quantity of powder equivalent to 4mg of Perindopril was transferred to 100ml volumetric flask and dissolved in sufficient quantity of HPLC grade water. It was sonicated for 5mins and volume was made up to 100ml HPLC grade water. It was filtered through 0.45µ membrane filter. From the filtered solution, 5ml was pipette out into 25ml volumetric flask and the final volume was made up to the mark with HPLC grade water to get the concentrations of 8µg/ml of PDE and 10µg/ ml of AMD respectively. The test concentration is injected 6 times into chromatographic system. Each time peak area and retention time was recorded and the results obtained are shown in the table 1

Table 1: Results of marketed formulation analysis

Drug name	Labeled claim(mg)	Test Conc. (µg/mL)	Mean Amount found (µg/mL)	%Estimated Amount	%RSD
Perindopril	4	8	7.98	99.75	0.30
Amlodipine	5	10	9.93	99.33	0.25

Method validations

The described method has been validated for linearity, accuracy, limit of detection, precision, and robustness, as per the ICH guidelines.

Linearity

The linearity of the method was determined by preparing six different concentrations of both PDE and AMD in the concentration range of 4-12 µg/ml and 5-15 µg/ml. Each solution was prepared in triplicate. The calibration curves were obtained by plotting peak area versus concentration. Linearity was checked over the same concentration range on three consecutive days and the results obtained from as shown in table no.2.

Table 2: Results of Linearity studies

Parameters	PDE	AMD
Linearity range (µg/ml)	4-12	5-10
Regression line equation	y = 13433x +86.6	y = 74874x + 47.4
Correlation coefficient (r^2)	0.999	0.999
Accuracy (% Recovery)	99.33	99.73
LOD (µg/ml)	2.963	2.7273
LOQ (µg/ml)	9.877	9.0909
Method precision (RSD)	0.25	0.11
Intermediate precision(RSD)	0.25	0.11

Specificity and Selectivity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by examining blank matrix samples for any interfering peaks. The specificity of the method was evaluated with regard to interference caused by presence of any other placebo. Two different samples were injected and studied with respective placebo. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and placebo) showed almost no interfering peaks with in retention time ranges. The obtained figures shows that the selected drugs were cleanly separated. Thus, the HPLC method proposed in this study was selective.

Accuracy, as Recovery

The accuracy of the method was determined at three different concentration levels 50%, 100%, and150% by spiking known quantities of the drug analyte and % of recovery were calculated. And the results obtained from as shown in table no.2.

Precision

Method precision (repeatability)

The method precision was determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD),%assay were calculated.

Intermediate precision

The intermediate precision was determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD), %assay was calculated and results obtained from as shown in table no 2.

LOD and LOQ

LOD: It is lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value under the stated experimental conclusions. The detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope and the results obtained.

LOD = 3.3*standard deviation (б)/s

LOQ: The quantification limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope and the results obtained. The results of LOD &LOQ are shown in the table 2

LOQ= 10* standard deviation (б)/s

Robustness

The robustness can be determined by varying the following parameters

1. Flow rate: It was determined by varying the flow rate from 0.8ml/min to 1.2ml/min. The standard solution of PDE and AMD were prepared and injected by varying the flow rate from 0.8ml/min to 1.2ml/min along with the optimized method
2. Column temperature: a study was conducted to determine the effect of variation in column temperature. Standard solution of PDE and AMD were prepared and injected by keeping the column temperature at 28°C and 32°C along with the optimized method. The results were tabulated in table 3.

Table 3: Robustness Studies for PDE & AMD

Method Parameters	Conditions	Retention Time(R_t)		Area		%Recovery	
		PDE	AMD	PDE	AMD	PDE	AMD
Temp1	28°C	3.352	4.486	4686059	3236283	100.01	100.33
Temp3	32°C	2.677	3.593	6073408	4162866	100	100.2
Flow2	1 µg/ml	2.228	3.599	4691349	3278731	102.56	102.56
Flow3	1.2 µg/ml	2.678	3.593	3883234	3245647	98.1	100.2

System suitability parameters

For assessing system suitability, six replicates of working standards samples of PDE and AMD were injected and studied the parameters like plate number(N), tailing factor(K), resolution, relative retention time and peak asymmetry of samples. The results were tabulated in table 4

Table 4: System suitability parameters of PDE and AMD

Parameters	Values obtained (n=6)	
	PDE	AMD
Plate count	8375	12044
Tailing Factor	1.17	1.14
R_t (min)	2.221	3.156
Resolution	0	8.64
Asymmetry	1.1	1.2

FORCED DEGRADATION STUDIES

Forced degradation of each drug substance and product was carried out under different stress studies like oxidation, acid, alkaline, thermal, photolytic degradation studies. The results from forced degradation studies were summarized in Table no.5.

Degradation sample Preparation

Weigh accurately 20tablets and crush into fine powder from this weigh equivalent to 25mg of powdered sample into a 25ml volumetric flask dissolve and dilute to volume with HPLC grade water to get concentration of 40ug/ml of PDE , 50ug/ml of AMD and filter the solution using 0.45µ Nylon filter.

Peroxide (oxidation) degradation studies (3%v/v of H_2O_2)

To 2ml of a stock solution, 1ml of 3%v/v of H_2O_2 was added and refluxed for 30min at 60°C. For HPLC study, the resultant solution was diluted to obtain 8µg/ml and 10µg/ml of PDE and AMD respectively.10µl solution were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation studies (0.1N HCL)

To 2ml of a stock solution, 1ml of 0.1N HCL was added and refluxed for 30min at 60°C. Cool to room temperature and neutralize with 1ml of 0.1N NaOH. For HPLC study, the resultant solution was diluted to obtain 8µg/ml and 10µg/ml of PDE and AMD respectively.10µl solution were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies (0.1N NaOH)

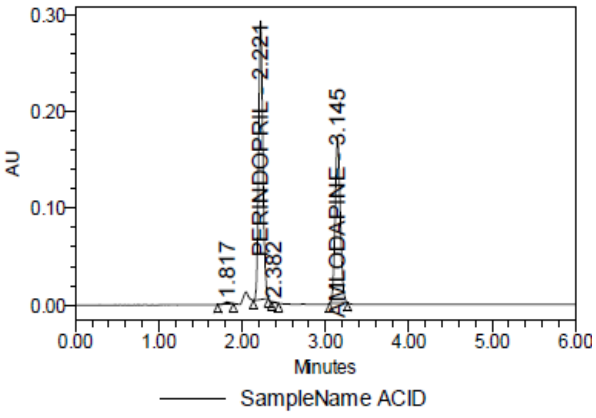
To 2ml of a stock solution, 1ml of 0.1N NaOH was added and refluxed for 30min at 60°C. Cool to room temperature and neutralize with 1ml of 0.1N HCl. For HPLC study, the resultant solution was diluted to obtain 8µg/ml and 10µg/ml of PDE and AMD respectively.10µl solution were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Thermal Degradation studies (6hrs at 105°C)

Weigh accurately 20tablets and crush into fine powder and transfer powder into petridish. Heat the sample in oven for about 6hrs at 105°C. From this weigh equivalent to 25mg of powdered sample into a 25 ml volumetric flask dissolve and dilute to volume with HPLC grade water. For HPLC study the resultant solution was diluted to 8µg/ml and 10µg/ml of PDE and AMD respectively. 10µl solution was injected in to the system and the chromatograms were recorded to assess the stability of sample.

Photolytic Degradation (2600 lux for 24 hr)

Photolytic degradation study was carried out by exposing the accurately weighed 200mg (8mg PDE and 10mg AMD) of tablet powder to UV light in a photolytic chamber at 2600 lux for 24 hr, after 24hrs weigh equivalent to 25 mg of powdered sample into a 25 ml volumetric flask dissolve and dilute to volume with HPLC grade water. For HPLC study the resultant solution was diluted to 8µg/ml and 10µg/ml of PDE and AMD respectively. 10µl solution was injected in to the system and the chromatograms were recorded to assess the stability of sample.



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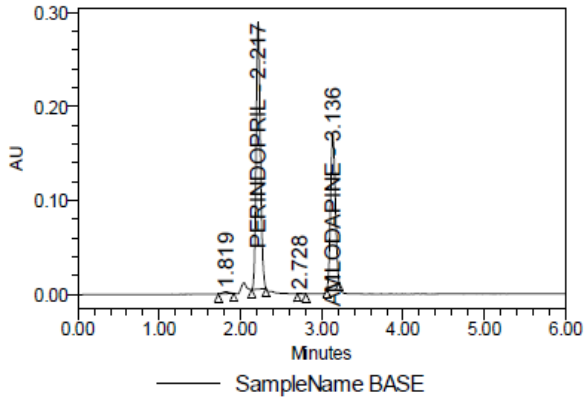

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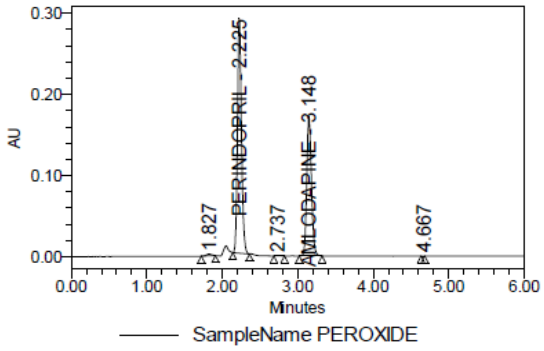

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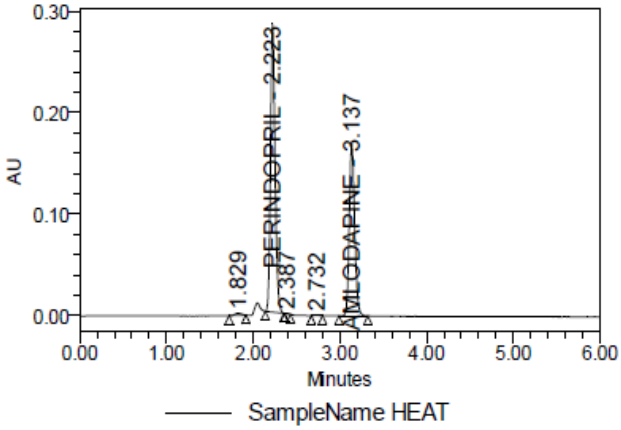

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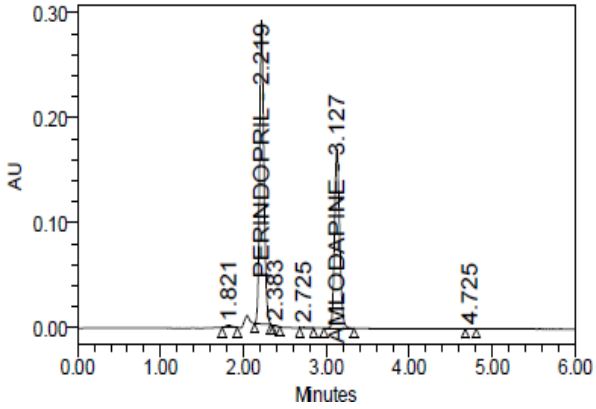

(e)

Figure 2: (a-e): chromatograms of degradation-(a) acid degradation (b) Alkali degradation (c) peroxide degradation (d) thermal degradation (e) photo degradation sample.

DISCUSSIONS

Optimized chromatographic conditions

Attempts made towards development of a Simple and better method on commonly used C18 column with good resolution were successful. Different logical modifications were tried to get good separation among the drugs and the degraded products. These changes include change in mobile phase composition in isocratic elution as well as gradient modes on different C18 columns.

The optimized chromatographic conditions (figure1b). The best peak shape and maximum separation was achieved with mobile phase composition of 0.1% OPA (pH 2.1) and methanol (a: b, 40/60).the best separation, peak symmetry and reproducibility were obtained on YMC C18 250mm×4.6mm I.D; 5 µm) .The optimum wavelength for detecting the analyte was found to be 262nm, a flow rate of 1ml/min yielded optimum separation and peak symmetry as shown in table 1.

Degradation studies

Results are tabulated in table no.5.

Table 5: Forced Degradation Studies Data

Stress conditions	Perindopril Erbumine	Amlodipine Besylate
	% degradation	% degradation
Acidic/0.1 M HCl/60°C reflux/48 h	21.37	19.67
Basic/0.1 M NaOH/60°C reflux/48 h	10.54	9.12
Oxidizing/3% H2O2/cool at RT/30min	11.02	27.54
Thermal/105°C/6hr	0.5	0.74
Photolysis/UV light	3.5	3.2

A. Acid hydrolysis (figure 2a)

Upon performance of acid degradation studies 21.37% of Perindopril and 19.67% of Amlodipine Besylate was degraded.

B. Base hydrolysis (fig.2b)

Upon performance of base degradation studies 10.54% of Perindopril and 9.12% of Amlodipine Besylate was degraded.

C. Peroxide hydrolysis (fig.2c)

Upon performance of peroxide degradation studies 11.02% of Perindopril and 27.54% of Amlodipine Besylate was degraded.

D. Thermal degradation (fig.2d)

Upon performance of Thermal degradation studies 0.05% of Perindopril and 0.74% of Amlodipine Besylate was degraded.

E. Photolytic degradation (fig.2e)

Upon performance of Photolytic degradation studies 3.5% of Perindopril and 3.2% of Amlodipine Besylate was degraded.

Linearity, LOD and LOQ

The calibration plot was linear over the concentration range investigated (4-12µg/ml; *n* = 3) and (5-15µg/ml; *n* = 3) for PDE and AMD respectively. Average correlation coefficient r=0.9999 for both drug candidates with %RSD values ≤2.0 across the concentration ranges studied, were obtained from regression analysis. The LOQ that produced the requisite precision and accuracy was found to be 9.877µg/ml for PDE and 9.0909µg/ml for AMD. The resultant % RSD values were ≤1.00 %(table no.2).The LOD for PDE and AMD were found to be 2.963µg/ml and 2.727µg/ml respectively. The regression results indicate that method was linear in the concentration range studied (table no.2) and can be used for detection and quantification of PDE and AMD in a very wide concentration range.

Accuracy and Precision

Accuracy as recovery was evaluated by spiking previously analyzed test solution with additional Standard drug at three different concentration levels (table no.3). Recovery of standard drugs added was found to be 99.32±0.47% for Perindopril Erbumine and 99.93±0.46% for Amlodipine Besylate with the value of RSD less than 1% indicating that the proposed method is accurate for the simultaneous estimation of both drugs from their combination drug products in presence of their degradation products. The low RSD values indicate the repeatability and reproducibility of the Method (table-2)

Robustness

Results of the robustness study are depicted in Table no.3. The elution order and resolution for both components were not significantly affected. RSD of peak areas were found to be well within the limit of 2.0%.

CONCLUSION

A simple, rapid, accurate and precise stability-indicating HPLC[3] analytical method has been developed and validated for the routine quantitative analysis of Perindopril Erbumine and Amlodipine Besylate in API and combined dosage forms. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability-indicating. The proposed method has the ability to separate these drugs from their degradation products in tablet dosage forms and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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