EJAC
Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 7(1): 28-42, 2012

Development and Validation of Reversed-Phase LC Method for Simultaneous Determination Telmisartan, Amlodipine and Their Degradation Products in Fixed Dose Combination Tablets

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Received: *09/03/2010****; Accepted:*** *19/05/2010*

Abstract

A simple, rapid and reproducible stability indicating RP-HPLC method was developed and validated for the simultaneous determination of telmisartan and amlodipine in binary mixture and fixed dose combination tablets. The chromatography was carried out on a 4.6 mm I.D x 250 mm, 5 µm particle size SGE make Wakosil C18-AR column with 0.025 mol L^{-1} potassium di-hydrogen orthophosphate (KH2PO4) buffer (pH adjusted to 3.5 with ortho-phosphoric acid) and acetonitrile (65:35 v/v) at a flow rate of 1.0 mL min^{-1} and UV detector was set at 238 nm. The developed method was validated in terms of specificity, linearity, range, accuracy, ruggedness, robustness, limit of detection and limit of quantification. The developed method shows excellent linearity over a range of 4-80 µg mL^{-1} (r^2=0.9999) for telmisartan and 0.5-10 µg mL^{-1} (r^2=0.9997) for amlodipine. The recoveries of telmisartan and amlodipine were 100.15% and 99.98%. The relative standard deviation (% RSD) values of intermediate precision were 0.03 and 0.39, and reproducibility were 0.32 and 0.11 respectively for telmisartan and amlodipine. The limits of detection were 0.01 and 0.05, and the limits of quantification were 0.05 and 0.20 for telmisartan amd amlodipine respectively. The developed method was applied successfully for quantification of telmisartan and amlodipine in bulk drug and its fixed dose combination tablets formulations.

Keywords:

Telmisartan; Amlodipine besylate; reverse phase chromatography; fixed dose combination; stability indicating

1. Introduction

Monotherapy for control of blood pressure has been shown to be inefficient, only approximately 40% to 50% of hypertensive patients will achieve goal blood pressures of <140/90 mm Hg with monotherapy, regardless of the medication used. On the contrary fixed-dose combination therapy with two different classes of antihypertensive agents will achieve goal pressures in more than 70%. [1-2]. Fixed-dose combination decreases the risk of medication non-compliance and should be considered in patients with chronic conditions like hypertension for improving medication compliance which can translate into better clinical outcomes [3]. Many different combinations of diuretics and β-blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (AIIRAs), as well as ACE inhibitors and calcium antagonists are available. The following fixed dose combination of two AIIRAs with amlodipine are newly developed fixed dose combination

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ISSN: 1306-3057,

formulation for treatment of patients suffering for hypertensions. The pharmaceutical products of telmisartan and amlodipine combinations are commercially available. However, at this moment, there are no methods published for the simultaneous quantitative analysis of telmisartan and amlodipine as active pharmaceutical ingredient or finished product.

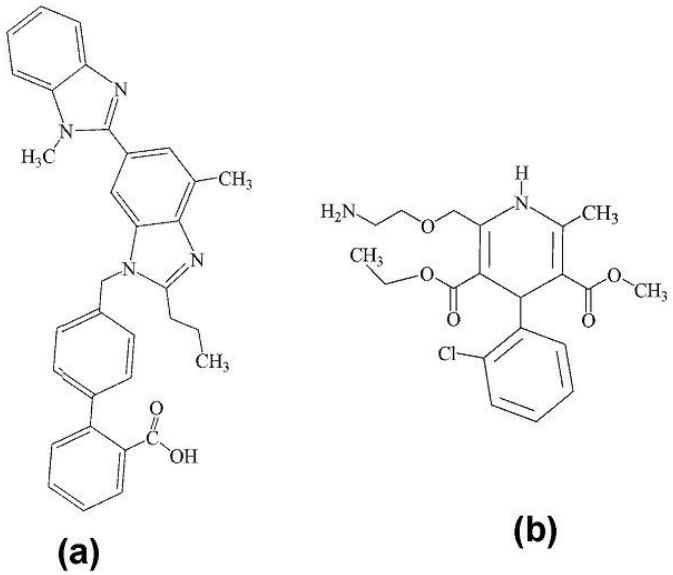

Fig. 1. Chemical structure of (a) Telmisartan and (b) Amlodipine

Telmisartan(4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl) methyl) -biphenyl-2-carboxylic acid) is an Angiotensin II Type I blocker [4-5]. It is widely used in the treatment of mild to moderate hypertension and heart failure [6].

A detailed survey of literature of telmisartan revealed several methods reported for the determination of telmisartan alone from pharmaceutical preparations based on different techniques, viz., HPLC [7-9], UV-spectrophotometry [10], sweep polarography [11] and parallel catalytic hydrogen wave method [12]. Methods for the determination of telmisartan in biological samples based on HPLC [13-15], LC coupled with mass spectrometry [16-17], as well as simultaneous determination of telmisartan in a combined dosage form with other drugs by TLC [18] and HPLC [19-21] are also reported.

Amlodipine is chemically a 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5- pyridine dicarboxylic acid- 3-ethyl 5-methyl ester and is a di-hydro pyridine calcium antagonist [22] which belongs to the class of calcium channel blocker.[23] amlodipine is useful in the management of angina pectoris and hypertension [24]. Many different combinations of diuretics and β-blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists, as well as ACE inhibitors and calcium antagonists are available. The combination of telmisartan with amlodipine is useful to treatment of hypertensive diabetic patients with microalbuminuria [25-26].

A survey of literature of amlodipine revealed that several methods based on spectroscopy [27], thin-layer chromatography [28] and RP-HPLC [29-30] have been reported for the estimation of amlodipine alone and in combination with other drugs. Different methods for the determination of amlodipine from biological samples such as, LC-MS/MS [31-32], LC-MS [33] and liquid chromatography (LC) with amperometric detection [34-35] have been reported. Few methods are reported for angiotensin II receptor antagonists and calcium antagonist amlodipine [36-37]. This method described only separation of active

substance and not enough specific methods to detect impurity or degraded products and hence cannot be applied for the stability testing of the drugs.

Telmisartan and amlodipine combination is an effective treatment for patients with moderate or severe hypertension [38-39] and shows superior antihypertensive efficacy versus respective mono-therapies. [40]. The fixed dose combination tablet containing telmisartan 40 mg and amlodipine 5 mg is available in the market. The combination is useful in the treatment of moderate to severe hypertension.

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires stress testing to be performed to elucidate the inherent stability characteristics of the active substance, hydrolytic, oxidative, thermal, and photolytic stability should be determined [41-42]. It is noteworthy that assay of both telmisartan and amlodipine have been performed by HPLC as part of a stability testing study of fixed dose combination involving concurrent method, the two assays were carried out independently.

Therefore, it is felt necessary to develop stability indicating methods for simultaneous determination of above drugs. Here we describe the development and validation of RP–LC with UV detection for the simultaneous quantification of above drugs, and separation of their all degradation products from a combined dosage form.

2. Experimental

2.1. Reagents and Chemical

Telmisartan (purity 99.96%) and amlodipine (purity 99.78%) besylate reference standards were gift samples from Zydus Cadila Healthcare Ltd. (Gujarat, India). HPLC grade acetonitrile was purchased from Merck fine chemicals (Mumbai, India). Purified water was obtained from Millipore (synergy) system. Potassium di-hydrogen orthophosphate and ortho-phosphoric acid were obtained from Lancaster. The 0.45 µm membrane filter was obtained from Millipore (Bedford, USA). Commercially available tablets containing telmisartan (40 mg) and amlodipine (5 mg) (TELSARTAN -AM) manufactured by Dr. Reddy's laboratories Ltd. (India) were purchased and used within their shelf life. All other chemicals and reagents used were of HPLC grade and supplied by Merck (India).

2.2. Equipment

The development and validation of the method was performed on a HPLC system consisting of a CROMPACK ISOS isocratic pump and a CROMPACK variable wavelength UV-visible detector (UV var), a manual injector with a 20 µl loop (RHEODYNE 7125, USA) and detector output was processed and recorded by SPINCHROM software (version 2.4.1.93) on a Pentium computer. The peak purity test was performed on a Shimadzu LC system (Kyoto, Japan) which consist a SPDM 10ADVP photodiode array (PDA) detector. A JASCO V-570 spectrophotometer was used for scanning and selecting detection wavelength.

2.3. Chromatographic Conditions

The chromatographic separation was achieved on a stainless steel (250 mm X 4.6 mm I.D., 5µm particle size) SGE make Wakosil C18 AR analytical column. Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase consisting of (0.025 M) phosphate buffer with the pH adjusted to 3.5 using phosphoric acid and acetonitrile (65:35, % v/v). Before use the mobile phase was

filtered by passing through a 0.22 µm membrane filter. The flow rate was 1.0 mL min-1. The effluent was monitored by UV detector at a wavelength of 238 nm.

2.4. Standard and Stock Solutions Preparation

Preparation of standard stock solution A: 40 mg of telmisartan working standard was put in to 100 mL volumetric flask, and added about 70 mL of acetonitrile and sonicated to dissolve. It was dilute to volume with acetonitrile and mixed.

Preparation of standard stock solution B: 25 mg of amlodipine besylate working standard was put in to 100 mL volumetric flask, an added about 70 mL of acetonitrile and sonicated to dissolve. It was diluted to volume with acetonitrile and mixed.

Preparation of working standard solution: 5 mL of standard stock solution A and 1 mL of standard stock solution B were transferred into 50 mL volumetric flask and diluted to volume with mobile phase and mixed well. In this solution, there was 40 µg mL^{-1} of telmisartan, and 5 µg mL^{-1} of amlodipine.

2.5. Sample preparation

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to 40 mg of telmisartan and 5 mg of amlodipine was transferred into a 100 mL volumetric flask. 50 mL of acetonitrile was added into this flask, and the solution was sonicated for 15 min. The solution was cooled to ambient temperature. Then the volume was made up with acetonitrile and centrifuged at 10,000 rpm for 10 min. The centrifuged solution was filtered through a 0.45µm filter. From the filtered solution, 5 mL was transferred into a 50 mL volumetric flask and diluted to volume with mobile phase.

2.6. Procedure for Stress Study of Drug Substances and Tablet Samples

Stress testing of the drug substance can help to identify the likely degradation products which can in turn help to establish the degradation pathways and the stability of the molecule. The stability indicating capability of the method was determined by subjecting sample under stress degradation conditions to evaluate the interference in the separation of drugs. Stress degradation of each drug substance and the drug product (tablets) were carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions.[41-42] Singh and Bakshi [42] suggested a target degradation of 20–80% for establishing stability indicating nature of the assay method. All the samples were stored at room temperature (23+1°C) to compare the results of stress degradation studies.

2.6.1. Acidic and Alkaline Degradation

Taken 40 mg of telmisartan, 5 mg amlodipine and tablet sample containing telmisartan: amlodipine (40:5 v/v) in to three separate 100 mL volumetric flasks in duplicate and dissolved in 10 mL of acetonitrile, then added 10 mL 1 N HCl for acid degradation and 10 mL 1 N NaOH for alkali degradation in to different set of flasks and the mixtures were kept at 60 °C for 4 h in a water bath. The solutions were allowed to attain ambient temperature, and then acid degraded samples were neutralized by 1 N NaOH and alkali degraded samples were neutralized by 1 N HCl and finally the volume was made up to 100 mL with mobile phase.

2.6.2. Oxidative Degradation

Taken 40 mg of telmisartan, 5 mg amlodipine and tablet sample containing telmisartan : amlodipine (40:5 v/v) in to three separate 100 mL volumetric flasks and dissolved in 10 mL

of acetonitrile, then added 10 mL 30% H_2O_2 in to each flasks and the mixtures were kept at 60 °C for 4 h in a water bath. The solutions were allowed to attain ambient temperature and the volume was made up to 100 mL with mobile phase.

2.6.3. Thermal Degradation

About 40 mg of drug substances and tablet samples were kept at 80 °C for 24 h. Then the solutions were prepared to achieve 40 µg mL^{-1} of telmisartan, and 5 µg mL^{-1} of amlodipine.

2.6.4. UV Degradation

About 40 mg of drug substances and tablet samples were exposed to UV short (254 nm) light for 24 h and UV long light (366 nm) for 48 h. Then the solutions were prepared to achieve 40 µg mL^{-1} of telmisartan, and 5 µg mL^{-1} of amlodipine.

2.6.5. Placebo degradation

During stress analysis of the drug product, placebo formulation and stressed placebos must yield a blank chromatographic baseline [46]. There for during the specificity studies of method the placebo solution was injected along with the solution of placebo under stress conditions. Placebos were treated under the above stress condition and these solutions were further injected on to the chromatographic system.

2.7. System Suitability

The system suitability test was performed to ensure that the complete testing system is suitable for the intended application. A standard solution containing mixture of 40 µg mL^{-1} of telmisartan and 5 µg mL^{-1} of amlodipine was injected six times. The parameter measured were peak area, retention time, retention factor, theoretical plates and asymmetry factor. The test sample chromatogram shall correspond to the reference chromatogram of working standard solution with respect to retention time and resolution.

2.8. Method Validation

The method was validated using tablet samples containing 40 mg telmisartan in combination with 5 mg amlodipine by the determination of specificity, linearity and range, precision, accuracy, limit of detection, limit of quantification and robustness following ICH guidelines [43] and USP [44].

3. Results and Discussions

In this work an analytical LC method with UV detection was developed and validated for the simultaneous determination of telmisartan and amlodipine. To prove the method is stability indicating, the drugs were assayed in presence of its degradation products obtained under stress conditions like acidic, basic, oxidative condition, photolytic and thermal degradation

3.1. Optimization of Chromatographic Conditions

During the method development process different stationary phase like C-8, C-18, different mobile phase buffer like acetate buffer sodium hydrogen phosphate and potassium hydrogen phosphate buffer at different pH and different solvent like methanol and acetonitrile were tried. From the method optimization it was observed that the ideal condition for separation was 0.025M potassium di-hydrogen orthophosphate (KH2PO4) (pH adjusted to 3.5

with ortho-phosphoric acid) and acetonitrile in the ratio of 65:35 (%v/v) with a mobile phase flow rate of 1.0 mL min^{-1} and C-18 column at ambient temperature. The common detection wavelength which was optimized for analyzing both analyte in a single run was 238 nm (Fig. 2). The analytes of combination have adequate retentions, peak shape, less asymmetry, more resolution and less run time. Thus, the method is specific and sensitive. Typical retention times of amlodipine and telmisartan were about 3.30 min and 7.61 min.

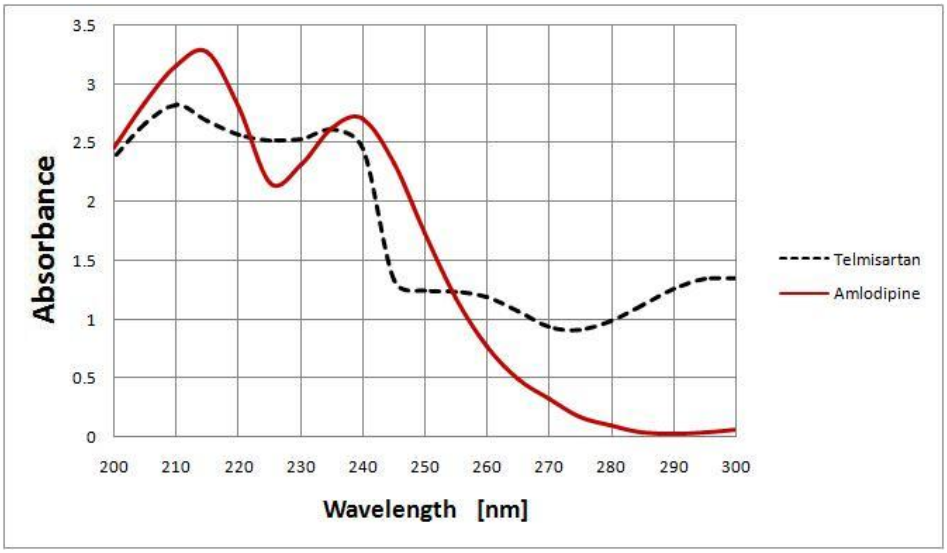

Fig. 2. UV spectra of Telmisartan and Amlodipine.

3.2. System suitability

The experimental results (Table 1) show that the parameters tested were within the acceptable range (RSD < 2.0 %) indicating that the system is suitable for the analysis intended.

Table 1. Result of system suitability study of method

Parameters	Telmisartan	Amlodipine
Resolution from nearest DP*	2.01	2.94
% RSD of Peak area†	0.78	0.63
% RSD of retention time	0.11	0.13
Peak purity	999.896	999.783
Asymmetry factor	1.11	1.13
Retention factor	2.0	5.91
Theoretical plates	18317	4522

*DP= degraded product †RSD relative standard deviation

3.3. Method Validation

3.3.1. Specificity/ Stress Degradation Studies.

Standard solutions containing telmisartan, amlodipine and placebo (Micro crystalline cellulose, L-HPC, Hydroxy propyle methyl cellulose, Lactose, Klucel-LF, PEG 6000, Talc, Magnesium stearate, Purified water) were determined separately under the proposed chromatographic conditions. The chromatograms obtained were shown in Fig. 3. The

chromatograms were indicating the satisfactory resolution between the telmisartan and amlodipine peaks, and found no interferences of tablet excipients (placebo) at any level.

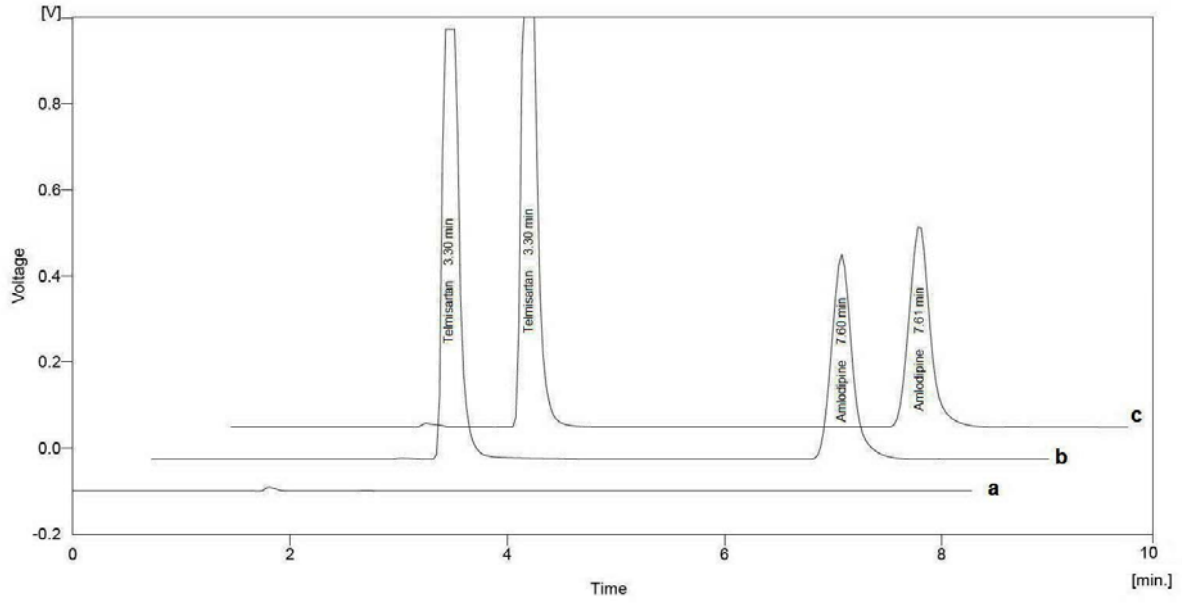

Fig. 3. A typical overlay chromatogram of the (a) placebo, (b) standard solution containing telmisartan (3.30 min) and amlodipine (7.61min) and (c) tablet sample.

Table 2. Result of Stress Degradation study of telmisartan and amlodipine

	Telmisartan		Amlodipine	
Stress condition	% Degradation	Peak purity*	% Degradation	Peak purity*
Acidic (1N HCl 60°C for 4 h)	21.63	996.310	78.12	998.531
Alkali (1N NaOH 60°C for 4 h)	24.99	998.256	29.79	998.590
Oxidative (30% H_2O_2 60°C for 4 h)	31.89	999.568	24.83	997.360
Thermal (80 °C for 24 h)	No degradation	999.816	No degradation	999.409
UV short (254 nm 24 h)	No degradation	999.967	No degradation	999.849
UV long (366nm 48 h)	No degradation	998.854	No degradation	996.732

*Peak purity values > 990 indicating homogeneous peak.

Both drugs were found to be stable under photolytic and thermal conditions in solid form. The obtained LC chromatograms for the separation of both drugs from its degraded products were shown in Fig. 4 (acidic hydrolysis), Fig. 5 (alkali hydrolysis), and Fig. 6 (oxidative degradation). Fig. 4 to 6 and Table 2 indicate the extent of degradation of amlodipine and telmisartan under various stress conditions. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogenous peak thus establishing the specificity of the method. The separation of telmisartan and amlodipine from all the degraded products of the tablets sample confirms the stability indicating power of the method.

Telmisartan was degraded approximately 21.63% during acid degradation (Fig. 4b), 24.99%under basic (alkaline) condition (Fig. 5b) and 31.89% under oxidizing condition (Fig. 6b). Acidic degradation of amlodipine was superfluous than other stress. It degrad approximately 78.12% during acid degradation (Fig. 4c), 29.79%under basic (alkaline) condition (Fig. 5c) and 24.83% under oxidizing condition (Fig. 6c). Telmisartan and

amlodipine exhibits a symmetric peak shape with a retention time at about 3.30 min and 7.61 min respectively, which can be well resolved from all the degradation products of both drugs. The run time for one analysis is less than 10 min, which are favorable to the routine quality control of the product.

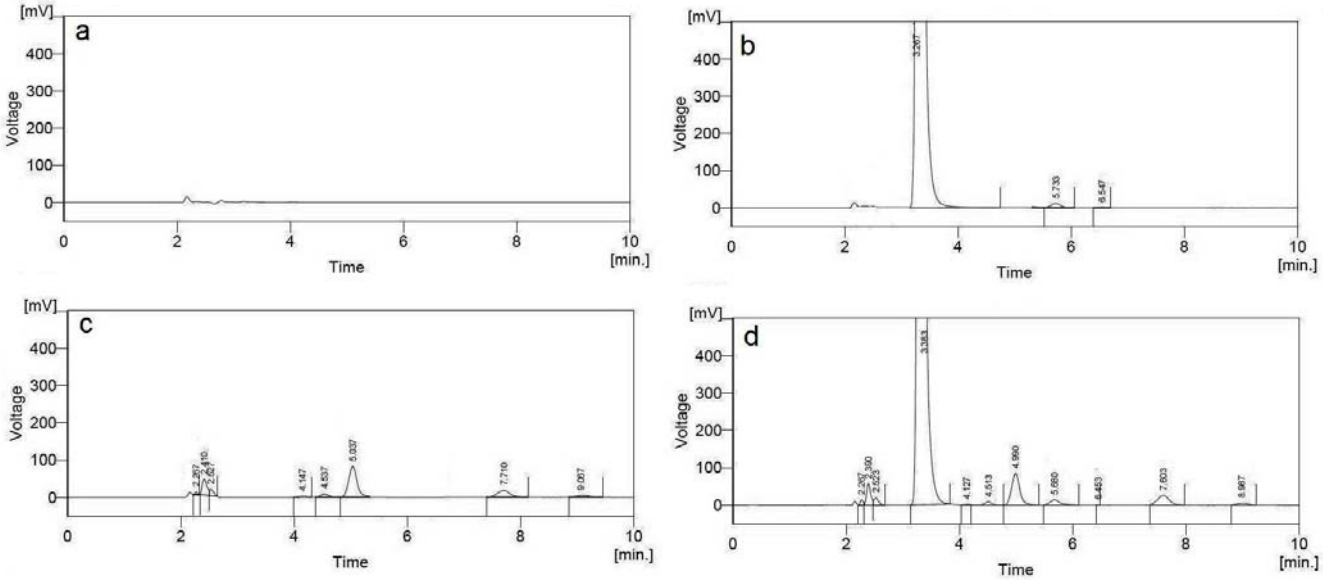

Fig. 4. Chromatograms of acid hydrolysis degraded samples (a) acidic blank, (b) degraded amlodipine, (c) degraded telmisartan and (d) degraded tablet solution.

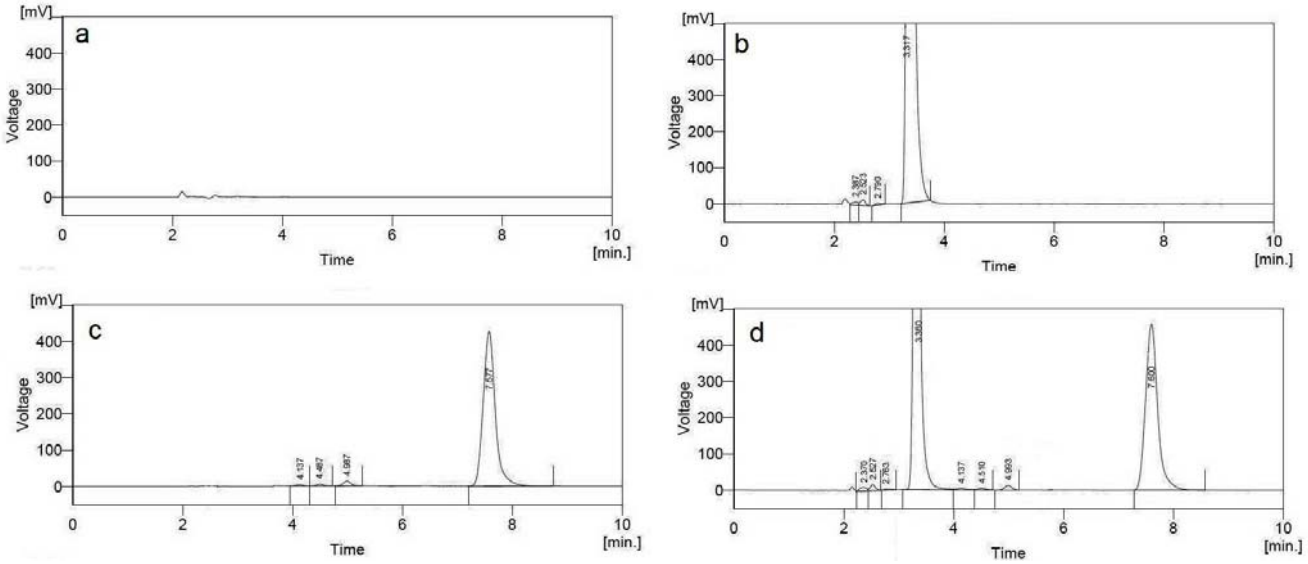

Fig. 5. Chromatograms of alkali hydrolysis degraded samples (a) alkali blank, (b) degraded amlodipine, (c) degraded telmisartan and (d) degraded tablet solution.

3.3.2. Linearity and Range

The linearity of the developed method was determined at 11 concentration levels ranging from 10-200 % of the targeted level of the assay concentration. The calibration curve solutions contained 4-80 µg mL^{-1} of telmisartan and 0.5-10 µg mL^{-1} of amlodipine. The equations of the linear calibration curves for telmisartan and amlodipine were y = 99.64x + 46.06 and y = 100.52x + 0.86, respectively. In the simultaneous determination the calibration graphs were found to be linear in the aforementioned concentrations with calibration coefficients (r2) 0.9999 and 0.9997 for telmisartan and amlodipine respectively. The results

show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated above.

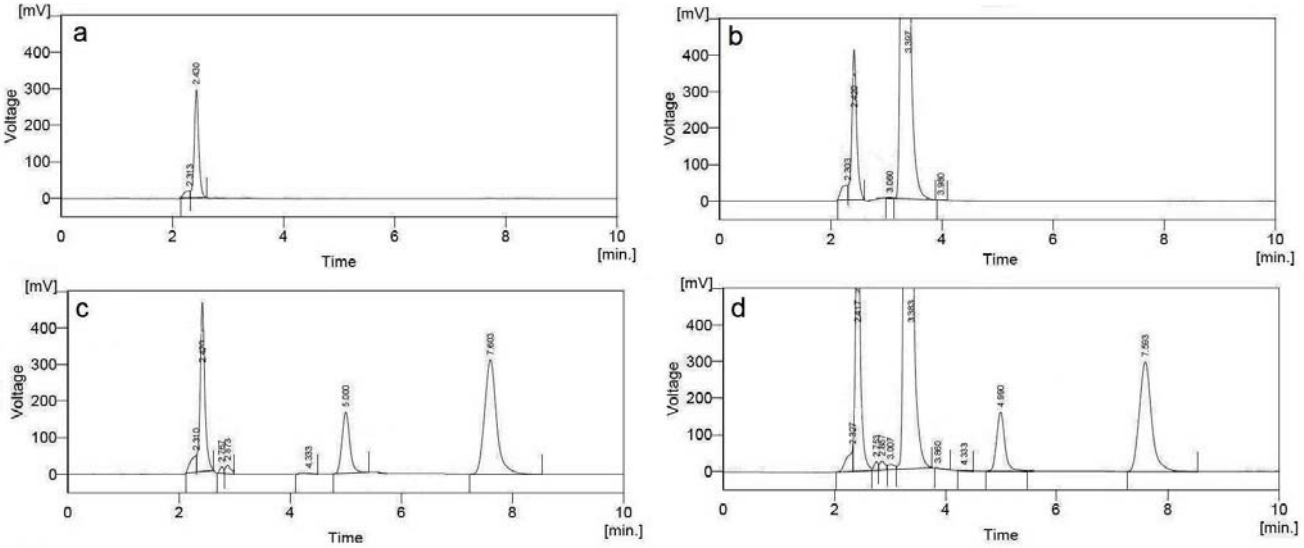

Fig. 6. Chromatograms of oxidative degraded samples (a) oxidative blank, (b) degraded amlodipine, (c) degraded telmisartan and (d) degraded tablet solution.

3.3.3. Limit of Detection and Limit of Quantification

The limit of detection (LOD) of telmisartan and amlodipine were found to be 0.01 µg mL^{-1} and 0.05 µg mL^{-1} respectively. The LOQ of telmisartan and amlodipine were found to be 0.05 µg mL^{-1} and 0.20 µg mL^{-1}, respectively. The recovery of spiked telmisartan is 101.30 % and spiked amlodipine is 99.87% in sample at the LOQ levels.

3.3.4. Precision

The system precision (repeatability) was determined by performing six replicate analyses of the same mix standard solution and the obtained R.S.D. value for peak area of telmisartan and amlodipine were 0.48% and 0.45%. The Intermediate precision was determined by analyzing samples from same lot three times on the same days. Reproducibility (between analyst precision) of the method was determined by analyzing same sample by three different analysts on three different occasions, and calculating the mean assay values and RSD. The results for the repeatability, intermediate precision (Table 3) and reproducibility (Table 3) indicate the good precision of the developed method. The results of precision are shown in Table 3.

Table 3. Result of Precision study of telmisartan and amlodipine

Sample	Intermediate Precision (Inter-Day)				Reproducibility (Between Analysts) Precision			
	analysis	Assay (%)*	Mean assay %	% †RSD	Analyst	Assay (%)*	Mean assay %	% †RSD
Telmisartan	1.00	100.11	100.10	0.03	A	100.13	100.47	0.32
	2.00	100.07			B	100.50		
	3.00	100.13			C	100.77		
Amlodipine	1.00	99.51	99.72	0.39	A	100.17	100.04	0.11
	2.00	99.47			B	99.99		
	3.00	100.17			C	99.96		

*Mean of Three Replicate
†RSD relative standard deviation

3.3.5. Accuracy

The accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of excipients to which known amount of telmisartan and amlodipine corresponding to 80-120 % of label claim had been added. The recovery results are shown in Table 4. Mean recovery of five levels for telmisartan and amlodipine from the formulation were 100.15% (RSD 0.18) and 99.98% (RSD 0.70), indicating that the developed method is very accurate for the determination of telmisartan and amlodipine from tablet formulation.

Table 4. Evaluation of the accuracy of the method.

Level,%	Telmisartan			Amlodipine		
	Amount added (mg)	Amount found (mg)	Recovery (%)*	Amount added (mg)	Amount found (mg)	Recovery (%)*
80	32	32.02	100.06	4	3.99	99.75
90	36	35.99	99.97	4.5	4.45	98.89
100	40	40.01	100.03	5	5.02	100.40
110	44	44.13	100.30	5.5	5.51	100.18
120	48	48.18	100.38	6	6.04	100.67

*Mean of Three Replicate

3.3.6. Robustness

During the deliberate changes in chromatographic conditions i.e. flow rate, detection wavelength, and mobile phase composition, the resolution of telmisartan and amlodipine from the nearest eluting peak was found to be greater than 2. The detection wavelength was varied + 1 nm, the mobile phase ratio of buffer: acetonitrile was varied 2%, whereas the flow rate was varied + 0.1 mL min^{-1}. The RSD value of assay determined for the same sample under developed method and under the varied conditions are 0.70% and 0.51% for telmisartan and amlodipine respectively indicating that the developed method is robust over. Results from evaluation of the robustness of the method are shown in Table 5.

Table 5. Result from evaluation of the robustness of the method

Conditions	System suitability Data					
	Telmisartan			Amlodipine System suitability Data		
	Assay* (%)	Theoretical Plates*	Asymmetry*	Assay* (%)	Theoretical Plates*	Asymmetry*
Optimized condition	101.11	18317	1.11	100.13	4522	1.13
Mobile phase flow 0.9 mL min^{-1}	100.83	18221	1.13	99.98	4512	1.31
Mobile phase flow 1.1 mL min^{-1}	100.92	18582	1.01	99.88	4618	1.12
Buffer-acetonitrile 67:33(%v/v)	101.13	18229	1.20	99.67	4478	1.23
Buffer-acetonitrile 53:37(%v/v)	99.38	18634	1.01	99.24	4695	1.11
Detection at 237 nm	100.21	18392	1.11	100.53	4596	1.13
Detection at 239 nm	101.49	18413	1.11	100.76	45.81	1.13

*Mean of Three Replicate

3.4. Solution stability

To confirm the stability of working standard and tablet sample solution during the analytical process, both solutions were analyzed over a period of 24 h at room temperature

(23+1°C). The results (Table 6) show that both the retention time and the peak area of telmisartan and amlodipine were unchanged. %RSD of area was 1.27 % and 2.35% for telmisartan and amlodipine respectively, and no significant degradation was observed within the indicated period which was sufficient for performing analytical process.

Table 6. Result from Evaluation of stability of sample

Time in hr.	Telmisartan Peak area*	Amlodipine Peak area*
0	4100.113	363.471
2	4164.040	357.076
4	4091.234	375.454
8	4124.552	351.820
16	4048.224	369.556
24	4019.949	360.539
Average peak area	4091.352	362.986
% RSD of peak area†	1.27	2.35

*Mean of Three Replicate
†RSD relative standard deviation

3.5. Method application

The validated method was then successfully applied for the stability testing of telmisartan and amlodipine in fixed dose combination tablet. Assay results for three batches of telmisartan amlodipine tablets are shown in Table 7. Results show that the content of telmisartan and amlodipine in the tablet formulation was to the counter requirements (90-110 %).

Table 7. Method application results

Sample No	Telmisartan			Amlodipine		
	Label claim (mg)	Amount found (mg)	Mean Recovery (%)*	Label claim (mg)	Amount found (mg)	Mean Recovery (%)*
1	40.00	39.78	99.45	5.00	5.03	100.60
2	40.00	40.13	100.33	5.00	5.07	101.40
3	40.00	40.36	100.90	5.00	5.02	100.40
4	40.00	40.46	101.15	5.00	5.05	101.00
5	40.00	40.37	100.93	5.00	5.11	102.20
6	40.00	40.43	101.08	5.00	5.13	102.60
Average		100.64			101.37	
% RSD†		0.65			0.87	

*Mean of Three Replicate
†RSD relative standard deviation

4. Conclusion

The RP-HPLC method was developed for the simultaneous quantitative analysis of telmisartan and amlodipine in combined tablet formulations. The method was fully validated showing satisfactory data for all method validation parameters tested. The results of the various validation studies show that the LC method is fast, simple, specific, and possesses

significant linearity in the range of 10-200 % of target concentration. Moreover, the method was found to be accurate and precise, as indicated by recovery studies and %RSD not more than 2.0. The summary of validation parameters of proposed LC method is given in Table 8. A stability indicating method was developed and validated which separates all degradation products formed under varieties of stress conditions. The method can be conveniently applied for the testing of telmisartan and amlodipine in combined tablet formulations and for determination of stability or drug compatibility studies of drug with excipients commonly used in solid dosage form by industry.

Table 8. Summary of Method Validation Parameters.

Parameters	Results	
	Telmisartan	Amlodipine
Linearity range (µg mL^{-1})	4-80	0.5-10
Correlation coefficient (r^2)	0.9999	0.9997
Limit of detection	0.01	0.05
Limit of quantification	0.05	0.2
Recovery at the LOQ levels	101.30	99.87
Accuracy(% recovery)(n=5)	100.15	99.98
Precision (%RSD)*		
• Repeatability (n = 6)	0.48	0.45
• Intermediate Precision (n=3)	0.03	0.39
• Reproducibility (n=3)	0.32	0.11
Robustness(%RSD)	0.70	0.51
Solution stability for 24 h at (23±1°C) (%RSD)	0.39	0.41

†RSD= Relative Standard Deviation.
n= Number of determination.

Acknowledgement

Financial assistance from UGC, New Delhi is gratefully acknowledged.

References

1. Mosera M and Blac H R (1998) The Role of Combination Therapy in the Treatment of Hypertension. Am J Hypertens 11 (6): 73S.
2. Marin R, Sierra A, Roca-Cusachs A, Olivan J, Redon J, Luque M, Figuera M, Pontes C and Delgadillo J (2003) Comparison of a fixed-dose combination vs dose tiration in second line therapy of hypertension. Am J Hypertens 16 (5): A195.
3. Bangalore S, Kamalakkannan G, Parkar S and Messerli F H (2007) Fixed-Dose Combinations Improve Medication Compliance: A Meta-Analysis. Am J Med 120 (8): 713.
4. Budavari S (2001) The Merck Index, 13th ed., Merck & Co. Inc. White House Station NJ p 1628.
5. McClellan K J and Markham A, (1998) Telmisartan. Drugs. 56(6) : 1039.
6. Pitt B and Konstam M A (1998) Overview of angiotensin II-receptor antagonists. Am J Cardiol 82: 47.

7. Rao R N, Sen S, Nagaraju P, Reddy V S, Krishnamurthy P R and Bhaskar S U (2006) HPLC determination of Telmisartan in bulk and pharmaceutical formulation. Asian. J Chem 18: 775.

8. Palled M S , Rajesh P M N, Chatter M and Bhat A R (2005) RP-HPLC determination of Telmisartan in tablet dosage forms. Ind. J. Pharm. Sci. 67 : 108.

9. Kumar M V and Muley P R (2005) Stability indicating RP-HPLC method for determination of telmisartan in solid dosage forms. Ind Pharmacist 4: 69.

10. Palled M S, Rajesh P M N, Chatter M and Bhat A R (2006) Difference spectrophotometric determination of telmisartan in tablet dosage forms. Ind J Pharma Sci 68: 685–686.

11. Xu M T , Song J F and Liang Y D (2004) Rapid determination of Telmisartan in pharmaceutical preparation and serum by linear sweep polarography. J Pharm Biomed Anal 4 : 681–687.

12. Xu M T, Song J F and Li N (2003) Rapid determination of Telmisartan in pharmaceutical and serum by parallel catalytic hydrogen wave method. Anal Bioanal Chem 377 : 1184.

13. Shen J, Jiao Z, Li Z D, Shi X J and Zhong M K (2005) HPLC determination of Telmisartan in humans plasma and its application to pharmacokinetic study. Pharmazie 60: 418.

14. Torrealday N, Gonzalez L, Alonso R M, Jimenez R M and Lastra E O (2003) Experimental design approach for optimization of HPLC fluorimetric method for quantitation of angiotensin II receptor antagonist Telmisartan in urine. J Pharm Biomed Anal 32: 847.

15. Jing N, Zhang M, Fan Y, Wen Y, Xing B and Feng Y Q (2005) Biocompatible in-tube solid-phase microextraction coupled to HPLC for the determination of angiotensin II receptor antagonists in human plasma and urine. J Chromatogr B 828: 62.

16. Chen B M, Liang Y Z, Wang Y L, Deng F L, Zhou P, Guo F Q and Huang L F (2005) Development and validation of LC-MS method for determination of Telmisartan in human plasma. Anal Chim Acta 540: 367.

17. Li P, Wang Y, Tang Y, Fawcett J P and Cui Y (2005) Determination of telmisartan in human plasma by liquid chromatography–tandem mass spectrometry. J Chromatogr B 828: 126.

18. Lories I B, Samah S A, Laila A F and Heba H R (2005) Application of first derivative ratio derivative spectrophotometry TLC densitometry and spectrofluorimetry for the simultaneous determination of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage forms and plasma. Farmaco 60: 859.

19. Bhat L R, Godge R K, Vora A T and Damle, M C (2007) Validated RP-HPLC Method for Simultaneous Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Formulation. J Liq Chromatogr Related Technol 30(20): 3059-3067.

20. Patil K R, Rane V P, Sangshetti J N and Shinde D B (2008) A Stability-Indicating LC Method for the Simultaneous Determination of Telmisartan and Ramipril in Dosage Form. Chromatographia 67: 575.

21. Wankhede S B, Tajne M K, Gupta K R and Wadodkar S G (2007) RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Ind J Pharma Sci 69: 298.

22. Kungys G, Naujoks H and Wanner C (2003) Pharmacokinetics of amlodipine in hypertensive patients undergoing haemodialysis. Eur J Clin Pharmacol 59: 291.

23. Abernethy D R (1989) The pharmacokinetic profile of amlodipine. Am Heat J 118 : 1100.

24. Reid J L, Meredith P A, Donnelly R and Elliot H L (1988) Pharmacokinetics of calcium antagonists. J Cardiovasc Pharmacol Suppl 7: 22.

25. Fogari R, Derosa G, Zoppi A, Preti P, Lazzari P, Destro M, Fogari E, Rinaldi A and Mugellini A (2007) Effect of telmisartan-amlodipine combination at different doses on urinary albumin excretion in hypertensive diabetic patients with microalbuminuria. Am J Hypertens 20: 417.

26. Negro R and Hassan H (2006) The effects of telmisartan and amlodipine on metabolic parameters and blood pressure in type 2 diabetic hypertensive patients. J. Renin Angiotensin Aldosterone Syst. 7 : 243.

27. Gohil K, Trivedi P and Molvi K I (2005) Spectrophotometric analysis of amlodipine besylate in bulk and in tablet dosage forms. Ind J Pharma Sci 67: 376.

28. Meyyanathan S N and Suresh B (2005) HPTLC method for simultaneous determination of amlodipine and benazepril in their formulations. J Chromatogr Sci 43 : 73.

29. Ravi S S, Nanjan M J, Vasudevan M, Shaat N, Suresh B and Sankar S R (1997) Simultaneous estimation of atenolol and amlodipine in formulation by RP-HPLC. Ind J Pharma Sci 59: 171.

30. Naidu K R, Kale U N and Shingare M S (2005) Stability indicating RP-HPLC method for simulatneus determination of amlodipine and benzapril hydrochloride from their combination drug product. J Pharm Biomed Anal 39: 147.

31. Bhatt J, Singh S, Subbaiah G, Shah B, Kambli S and Ameta S (2007) A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the estimation of amlodipine in human plasma. J Biomed Chromatogr Sci Appl 21: 169.

32. Streel B, Laine C, Zimmer C, Sibenaler R and Ceccato A (2002) Enantiomeric determination of amlodipine in human plasma by liquid chromatography coupled to tandem mass spectrometry. J Biochem Biophys Methods 54: 357.

33. Feng Y, Zhang L, Shen Z, Pan F and Zhang Z (2002) Analysis of amlodipine in human plasma by liquid chromatography- mass spectrometry. J Chromatogr Sci Appl 40:49.

34. Shimooka K, Sawada Y and Tatematsu H (1989) Analysis of amlodipine in serum by a sensitive high-performance liquid chromatographic method with amperometric detection. J Pharm Biomed Anal 7: 1267.

35. Josefsson M, Zackrisson A L and Norlander B (1995) Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation. J Chromatogr B 672: 310.

36. Chitlange S S, Bagri K and Sakarkar D M (2008) Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. Asian J Research Chem 1: 15.

37. Pedroso C F, de Oliveira J G, Campos F R, Goncalves A G, Trindade A C L B and Pontarolo R (2009) A Validated RP–LC Method for Simultaneous Determination of Losartan Potassium and Amlodipine Besilate in Pharmaceutical Preparations. Chromatographia 69: S201.

38. Littlejohn T (2008) Telmisartan and amlodipine combination therapy is powerful at lowering 24 hour blood pressure: findings of an ABPM sub study in hypertensive patients presented at the 22nd Scientific Meeting of the International Society of Hypertension. Berlin, Germany.

39. Littlejohn T (2008) Telmisartan and amlodipine combination provides an effective treatment option for patients with moderate or severe hypertension: sub analysis from a factorial design study presented at 18th the 22nd Scientific Meeting of the International Society of Hypertension. Berlin, Germany.

40. Littlejohn T (2008) Superior antihypertensive efficacy of the combination of telmisartan and amlodipine versus respective mono-therapies in patients with hypertension presented at the 22nd Scientific Meeting of the International Society of Hypertension Berlin Germany.

41. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use (2003) " Stability testing of new drugs substance and products". Q1A(R2).

42. Singh S and Bakshi M (2000) Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Technol 26: 24.

43. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use (2005) "Validation of analytical procedures: text and methodology". Q2(R1).

44. USP United States pharmacopoeia (2007) 30th edn. United States Pharmacopeial Convention Rockville.

45. Paul Worsfold Alan Townshend Colin Poole (2005) Encyclopedia of Analytical Science (2nd Edition) Academic Press.