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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate and reproducible UV-Spectrophotometric and Stability indicating RP-HPLC methods have been developed for simultaneous estimation of Metoprolol Succinate and Hydrochlorothiazide in tablet dosage form. The first UV method was a determination using Absorption corrected for interference method and the second UV method was a determination using Multi-Component mode method at 276 nm and 316.5 nm over the concentration range 20-120 µg/mL and 10-60 µg/mL for Metoprolol Succinate and Hydrochlorothiazide respectively. The RP-HPLC analysis is carried out using 0.05M potassium dihydrogen orthophosphate buffer (pH adjusted to 3 with orthophosphoric acid) and acetonitrile in the ratio of (80:20 % v/v) as the mobile phase and Thermo C18 column (4.6 mm i.d. × 250 mm), flow rate 1.1 mL/min, with detection wavelength of 222 nm. Linearity was obtained in the concentration range of 20-120 µg/mL and 10-60 µg/mL for Metoprolol Succinate and Hydrochlorothiazide respectively. Both UV-spectrophotometric and stability indicating RP-HPLC methods were developed and statistically validated as per ICH guidelines.

Keywords: Metoprolol Succinate (METO), Hydrochlorothiazide (HCTZ), RP-HPLC.

INTRODUCTION

Metoprolol Succinate is a beta-blocker and Hydrochlorothiazide is a potent thiazide diuretic that enhances natriuresis, leading to reduction in plasma volume and cardiac output. Therefore, it is used widely alone or in combination with other antihypertensive drugs for the treatment of cardiovascular disorders, viz, hypertension, angina and Congestive cardiac failure [1]. Chemically, Metoprolol Succinate is (±) 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate and Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide [2]. Detailed survey of literature for METO alone or in combination with other drugs is reported to be estimated by several methods based on different techniques such as UV spectrophotometry, HPLC [3-5], and HPTLC for its determination from pharmaceuticals. Similarly literature survey for HCTZ alone or in combination with other drugs is reported to be estimated by UV spectrophotometry [6], HPLC [7] and HPTLC method. But no methods have been reported for simultaneous determination of METO and HCTZ. Hence in the present work a successful attempt has been made to estimate both these drugs simultaneously by UV spectrophotometric method (Absorption corrected for interference method and Multi-Component mode method) and RP-HPLC method. To establish stability indicating nature of the RP-HPLC method, forced degradation of drug substances were performed under stress conditions (oxidation, acid and base

hydrolysis) [8, 9]. The proposed methods were optimized and validated as per ICH guidelines [10-12].

MATERIALS AND METHODS

Chemicals and Reagents: Metoprolol Succinate and Hydrochlorothiazide Active Pharmaceutical Ingredient (API) were kindly gifted by EMCURE PHARMACEUTICALS, PUNE. Marketed tablet formulation of Metoprolol Succinate (25 mg) and Hydrochlorothiazide (12.5 mg), brand name METPURE-H, manufactured by EMCURE PHARMACEUTICALS, PUNE. HPLC grade Acetonitrile and Methanol were purchased from Merck specialities Pvt. Ltd. Double distilled water used in all experiments was obtained from Milli-Q System (Millipore). Concentrated Hydrochloric acid AR grade and Sodium hydroxide pellets purified was procured from Merck specialities Pvt. Ltd. and Hydrogen Peroxide 30% AR grade was obtained from Universal laboratories Pvt. Ltd.

Instrumentation and analytical conditions: The UV methods were performed on a Double-beam Shimadzu UV-Visible spectrophotometer, 1700, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1 cm matched quartz cells was used to measure absorbance of solution. The method is based upon determination of Metoprolol Succinate at 276 nm and Hydrochlorothiazide at 316.5 nm. RP-HPLC method was performed on HPLC system (Merck Hitachi) consisting of quaternary gradient pump, column oven, and UV detector (L-7400) was employed for analysis. Chromatographic data was acquired using Winchrome software. Thermo C18 column (4.6 mm i.d × 250 mm) was used as stationary phase. METO and HCTZ were eluted isocratically with a flow rate 1.1 mL/min using a mobile phase consisting of 0.05M phosphate buffer (pH was adjusted to 3.0 using orthophosphoric acid) and acetonitrile in a proportion of 80:20 v/v respectively. The wavelength of UV detector was set to 222 nm. The mobile phase was prepared daily, filtered through 0.45 µm membrane filter (Millipore) and sonicated before use. The HPLC system was operated at 25 ±1°C. The summary of system suitability parameters were shown in (Table 1) [13-18].

Preparation of standard solutions:

For UV method: Standard stock solution of METO and HCTZ were prepared by transferring accurately weighed METO (10 mg) and HCTZ (10 mg) to a 100 mL volumetric flask separately, dissolved and diluted to a mark with methanol to obtain a standard solution of METO (100 µg/mL) and HCTZ (100 µg/mL). From these solutions 4 mL of METO and 2 mL of HCTZ standard stock solutions were mixed in a 10 mL volumetric flask and made up the volume with methanol, to get the concentration of 40 µg/mL of METO and 20 µg/mL of HCTZ.

For HPLC method: Standard stock solution of METO and HCTZ were prepared by transferring accurately weighed METO (10 mg) and HCTZ (10 mg) to a 100 mL volumetric flask separately, dissolved and diluted to a mark with mobile phase to obtain a standard solution of METO (100 µg/mL) and HCTZ (100 µg/mL). From these solutions, 4 mL of METO and 2 mL of HCTZ standard stock solutions were mixed in a 10 mL volumetric flask and made up the volume with mobile phase, to get the concentration of 40 µg/mL of METO and 20 µg/mL of HCTZ.

Preparation of the sample solutions:

For UV method: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 25 mg of METO and 12.5 mg of HCTZ was transferred to 100 mL volumetric flask; diluted to a mark with methanol and sonicated for 10 min. The resulting solution was filtered through Whatmann filter paper and filtrate was appropriately diluted with methanol to get concentration of 40 µg/mL of METO and 20 µg/mL of HCTZ.

For HPLC method: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 25 mg of METO and 12.5 mg of HCTZ was transferred to 100 mL volumetric flask; diluted to a mark with mobile phase and sonicated for 10 min. The resulting solution was filtered through Whatmann filter paper and filtrate was appropriately diluted with mobile phase to get concentration of 40 µg/mL of METO and 20 µg/mL of HCTZ.

Procedure for forced degradation study: Degradation studies were performed in tablet solutions containing 40 µg/mL of METO and 20 µg/mL of HCTZ.

Stress degradation by hydrolysis under acidic conditions: For acid degradation, 1mL of 1M HCl was added to final drug solution, and it was refluxed for 1 h. at 80° C. After 1 h. this solution was injected in stabilized chromatographic condition.

Stress degradation by hydrolysis under alkaline conditions: For alkali degradation, 1mL of 1M NaOH was added to final drug solution, and it was refluxed for 1 h. at 80° C. After 1 h. this solution was injected in stabilized chromatographic condition.

Oxidative degradation: For oxidation, 1mL of 3% v/v H_2O_2 was added to final drug solution, and it was refluxed for 1 h. at 80° C. After 1 h. this solution was injected in stabilized chromatographic condition.

Method Validation

The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity: The calibration curve for UV and RP-HPLC method were obtained with concentrations of the standard solutions 20-120 µg/mL and 10-60 µg/mL of Metoprolol Succinate and Hydrochlorothiazide respectively. The solutions were prepared in triplicate. Linearity was evaluated by regression analysis, which was calculated by the least square regression method.

Precision: Precision of UV and RP-HPLC method were checked by analyzing the samples at three different time intervals of the same day (intraday precision) as well as on different days (interday precision).

Accuracy: To check the degree of accuracy of UV and RP-HPLC method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%.

Robustness: Robustness for RP-HPLC method was determined by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 1.1 mL/min to 1 mL/min and 1.2 mL/min. The ratio of the mobile phase was changed by ± 2%. The effect on retention time and peak parameter were studied.

Limit of detection and limit of quantitation: LOD, LOQ of UV and RP-HPLC method were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

RESULT AND DISCUSSION

UV method: The proposed UV methods, allows a rapid and accurate quantitation of METO and HCTZ in tablet preparation without any time consuming sample preparation (Table 4). Moreover, the spectrophotometric methods involve simple instrumentation compared with other instrumental techniques. The absorption spectra of METO and HCTZ in methanol are shown in Figure 1. Wavelengths selected for analysis are 276 nm (λ_{max} of METO) and 316.5 nm (λ_{max} of HCTZ). Calibration curves were constructed in the concentration range of 20-120 µg/mL and 10-60 µg/mL of METO and HCTZ respectively. Beer's law was obeyed over this concentration range, and the coefficient of regression for both the drugs was found to be nearer to 1 (Table 2). The accuracy of proposed method were determined (Table 5), indicating an agreement between the true value and found value.

Precision was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for estimation of METO and HCTZ under intraday and interday variations were found to be less than 2 (Table 3).

HPLC method*:* Different proportions of acetonitrile and 0.05M phosphate buffer was tried for selection of mobile phase. Ultimately, 0.05M phosphate buffer (pH was adjusted to 3.0 using orthophosphoric acid) and acetonitrile in a proportion of 80:20 v/v respectively was finalized as the mobile phase. Figure 2 shows typical chromatogram obtained from the analysis of standard solution of METO and HCTZ using the proposed method. The elution order was HCTZ (Rt = 6.84 min) and METO (Rt = 10.55), at a flow rate of 1.1 mL/min. The chromatogram was recorded at 222 nm.

The calibration curves for METO and HCTZ were constructed in the concentration range of 20-120 µg/mL and 10-60 µg/mL of METO and HCTZ respectively, and the coefficient of regression for both the drugs was found to be nearer to 1 (Table 2). The accuracy of proposed method was determined (Table 5), indicating an agreement between the true value and found value. Precision was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for estimation of METO and HCTZ under intraday and interday variations were found to be less than 2 (Table 3) and for robustness studies in all deliberately varied conditions percent relative standard deviations were found to be less than 2 % (Table 6). The experimental values obtained for the determination of METO and HCTZ in tablet formulation are showed in (Table 4).
METO and HCTZ were subjected to,

Acid hydrolysis: Both the drugs were degraded in acidic condition shown in (Fig.3).

Alkaline hydrolysis: Both the drugs were degraded in alkaline condition shown in (Fig.4).

Oxidative degradation: Both the drugs were degraded in hydrogen peroxide (3%) shown in (Fig.5).

The percent amount of drug recovered after degradation studies and the Rt of degradation products are given in (Table 7).

CONCLUSION

The two proposed methods based on the spectrophotometry and RP-HPLC were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. The RP-HPLC method could selectively quantitate METO and HCTZ in presence of its degradation products it can be employed as a stability indicating method. Hence, it can be concluded that the developed spectrophotometric and chromatographic methods are accurate, precise and selective and can be employed successfully for the estimation of METO and HCTZ in tablet dosage form.

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Table 1: System suitability parameters

Parameter	Observation*	
	METO	HCTZ
Retention Time (min.)	10.55	6.84
No. of theoretical Plates	3609.60	2064.79
Tailing Factor	1.06	1.05
Asymmetry factor	1.12	1.05

**Denotes average of three estimations.*

Table 2: Validation data of Metoprolol Succinate and Hydrochlorothiazide

Parameters	Metoprolol Succinate			Hydrochlorothiazide		
	Method I	Method II	Method III	Method I	Method II	Method III
Working wavelengths	276	276	222	316.5	316.5	222
Beer-Lamberts range (µg/mL)	20-120	20-120	20-120	10-60	10-60	10-60
LOD (µg/mL)*	1.65	1.65	0.2441	0.33	0.33	0.0697
LOQ (µg/mL)*	5.00	5.00	0.7399	1.00	1.00	0.2113
Régression coefficient (r^2)*	0.9991	0.9991	0.9983	0.9980	0.9980	0.9963

**Denotes average of three estimations, Where, Method-I – Absorption corrected for interference method, Method-II- Multi-Component mode method, Method-III – RP-HPLC.*

Table 3: Intra-day and Inter-day Precision

Precision*	Metoprolol Succinate			Hydrochlorothiazide		
	Method I	Method II	Method III	Method I	Method II	Method III
Interday (% RSD)	0.6833	0.5791	0.2709	0.9449	0.6482	0.2428
Intraday (% RSD)	0.6836	0.4472	0.2860	0.8453	0.5240	0.2448

**Denotes average of six estimations.*

Table 4: Results of simultaneous estimation of tablet formulation

Method	Tablet content	Label claim (mg/tab)	Amount found (mg/tab)	Label claim* (%)	% RSD	SE
I	METO	25.0	25.12	100.48	1.0170	0.4174
	HCTZ	12.5	12.50	100.05	0.8509	0.3476
II	METO	25.0	25.05	100.20	0.6508	0.2663
	HCTZ	12.5	12.52	100.20	0.7898	0.3231
III	METO	25.0	25.02	100.09	0.3143	0.1284

	HCTZ	12.5	12.40	99.26	0.2290	0.0928

**Denotes average of six estimations, SE- Standard error of mean.*

Table 5: Result for recovery studies

Level of % recovery	Method	*% Recovery		% RSD		SE	
		METO	HCTZ	METO	HCTZ	METO	HCTZ
80	I	100.04	100.58	0.9943	0.3642	0.5744	0.2115
	II	100.12	100.75	1.1146	1.1304	0.6441	0.6576
	III	100.19	100.77	0.6581	0.3816	0.3807	0.3816
100	I	99.82	100.11	0.6422	0.2359	0.3701	0.1364
	II	100.25	100.15	0.4175	0.5019	0.2417	0.2902
	III	100.03	99.79	0.5352	0.4644	0.3091	0.4644
120	I	100.37	100.03	0.5267	0.4999	0.3052	0.2888
	II	100.44	99.75	0.2138	0.4471	0.1240	0.2576
	III	100.39	99.31	0.7036	0.9107	0.4034	0.5279

**Denotes average of three estimations.*

Table 6: Result for robustness studies

Parameter	Level	Retention time*		Tailing factor*	
		METO	HCTZ	METO	HCTZ
Flow rate (±0.1 mL/min)					
1.0	-0.1	10.58	6.86	1.05	1.04
1.1	0	10.55	6.84	1.06	1.05
1.2	+0.1	10.49	6.79	1.06	1.06
	(±) SD	0.0458	0.0360	0.0057	0.0100
	% RSD	0.4345	0.5270	0.5428	0.9523
Mobile phase Change					
78	-2	10.61	6.86	1.05	1.06
80	0	10.55	6.84	1.06	1.05
82	+2	10.51	6.80	1.07	1.05
	(±) SD	0.0503	0.0305	0.0100	0.0033
	% RSD	0.4767	0.4465	0.9433	0.3142

**Denotes average of three estimations.*

Table 7: Result for forced degradation Studies.

Stress conditions	Time (h.)	% Assay of active substance*		Rt of degraded product*	
		METO	HCTZ	METO	HCTZ
1M HCl	1	86.52	90.14	6.04	2.74
1M NaOH	1	92.04	89.34	4.93	3.08
3% H_2O_2	1	80.02	90.90	5.99	3.08

**Denotes average of three estimations.*

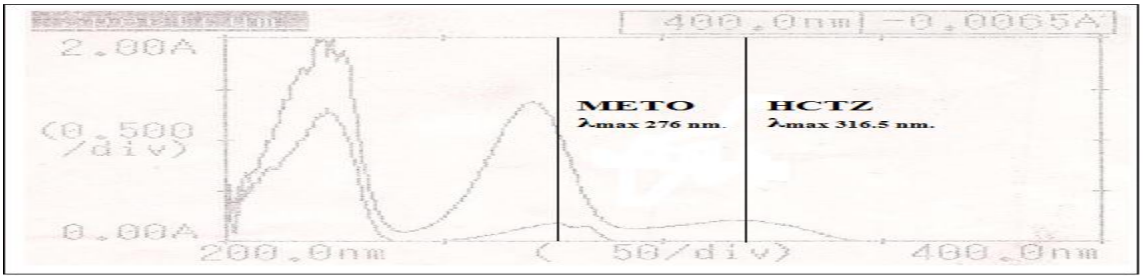

Figure 1: Overlay spectra of METO and HCTZ

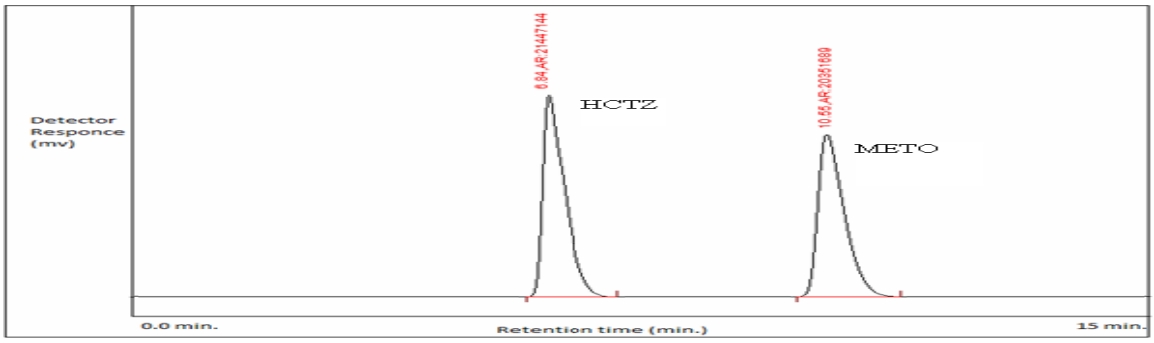

Figure 2: RP-HPLC chromatogram of HCTZ and METO at 222 nm

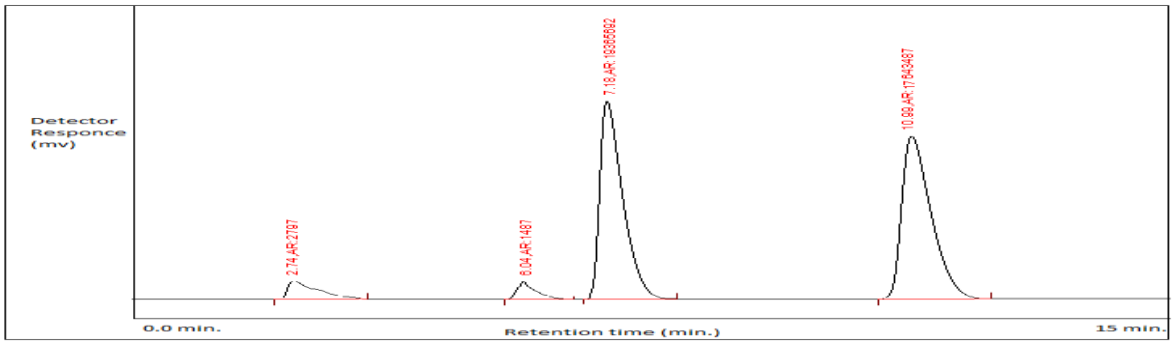

Figure 3: Chromatogram of acid hydrolysis 1M HCl 1 h. reflux at 80^{0}C

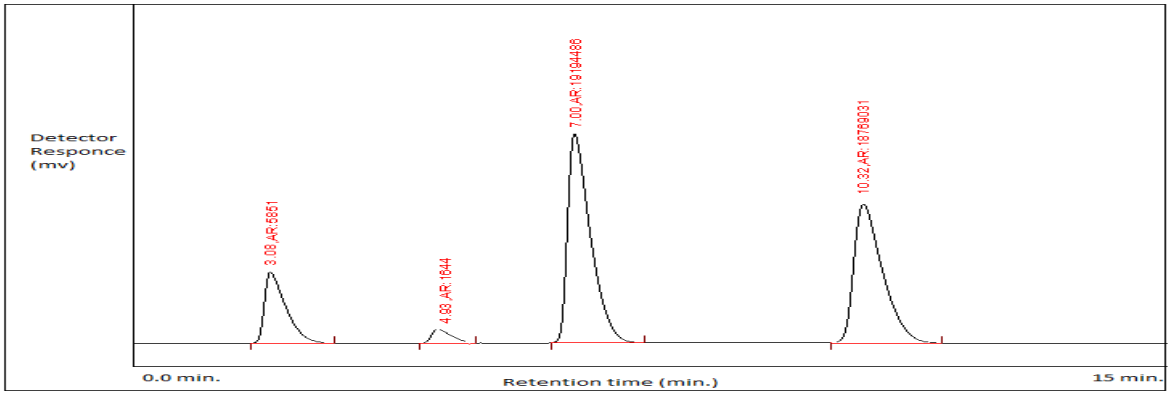

Figure 4: Chromatogram of alkaline hydrolysis 1M NaOH 1 h. reflux at 80^{0}C

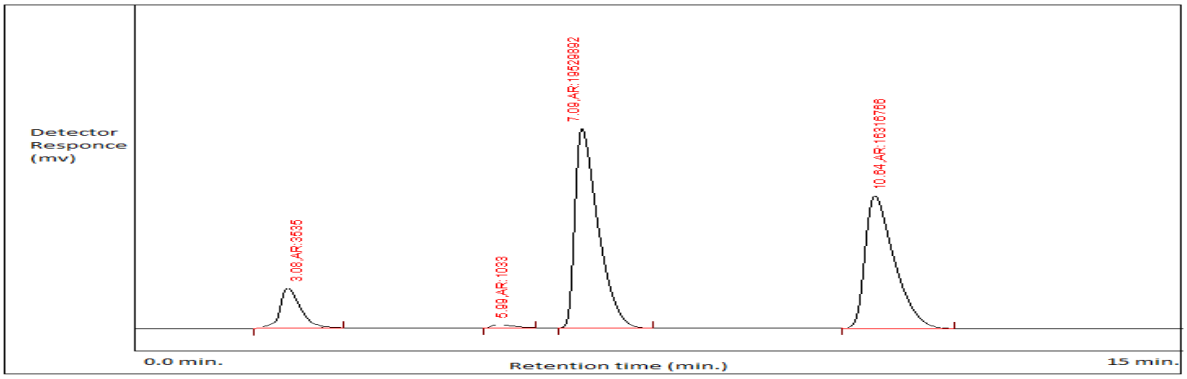

Figure 5: Chromatogram of oxidative degradation 3 % v/v H_2O_2 1 h. reflux at 80^0C

REFERENCES

1. Rang HP, Dale MM, Ritter JM, More PK. Pharmacology. 5th ed., Elsevier Science Publisher: 2003, pp. 313, 315, 377, 378.
2. Indian Pharmacopoeia, volume II, New Delhi, Indian pharmacopoeia commission, Ghaziabad: 2007, pp. 370,763.
3. Bhargavi DK, Naga MI, Shajhan SK, Srinivasarao N. Int J Sci Innovations Discov, 2011; 1(2): 151-57.
4. Hussain S, Munjewar RR, Farooqui M. Curr Pharm Res, 2011; 2(1): 443-49.
5. Joshi P and Kumar M. Der Pharm Sinica, 2011; 2(3): 211-19.
6. Qutab SS, Razzaq SN. ACTA Chromatographia, 2007; 2(19): 119-29.
7. Godse VP, Bhosale AV. Eurasian J Anal Chem, 2010; 5(2): 137-44.
8. Reynolds DW, Facchine KL, Motto MG. Available Guidance and Best Practices for Conducting Forced Degradation Studies, Pharmaceutical Technology: 2002, pp. 48-56.
9. FDA, International Conference on Harmonization: Draft Revised Guidance on Q1A(R) Stability Testing of New Drug Substances and Products, Federal Register 65 (78), 21446–21453 (21 April 2000) [ICH Q1A(R)].
10. Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), November, 2005.
11. ICH, Q2 (R1). Validation of analytical procedures: Text and Methodology International Conference on Harmonization, Geneva: 2005, pp. 1- 13.
12. Nash RA, Wachter AH. Validation of analytical methods and pharmaceutical process, 3rd ed., Marcel Dekker Inc., New York: 2003, pp. 507- 524.
13. Sethi PD. High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publishers and Distributors, New Delhi: 2001, pp. 3-72, 116-120.
14. Christen GD. Analytical Chemistry. 6th ed., John Wiley and Sons: 2003, pp. 35-42, 131-132.
15. Skoog DA, Holler FJ, Timothy A, Nieman NW. Principle of Instrumental Analysis. Saunders College Publications, London: 1998, pp. 1-12.
16. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. CBS Publishers and Distributors, New Delhi, Part-2: 2002, pp. 275-288.
17. Snyder R. Practical HPLC method development. 2nd ed., Wiley Interscience Publication: 1997, pp.120, 234-240, 266-278, 688.
18. Cazes J. EWING'S Analytical Instrumentation Handbook. 3rd ed., Marcel Dekker, New York, pp. 995-996, 1016.