Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2013, 4(2):61-67

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Stability indicating RP-HPLC method for simultaneous estimation of salbutamol sulphate and guaifenesin

Sanjay G. Walode*, Shruti D. Deshpande and Avinash V. Deshpande

Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmaceutical Sciences, Lonavala, Pune, Maharashtra, India

---

ABSTRACT

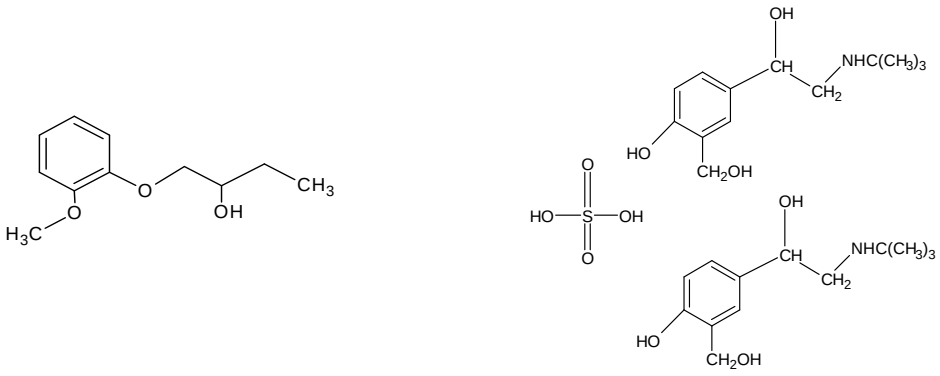
Present work describes a precise, accurate and reproducible RP-HPLC method for simultaneous estimation of salbutamol sulphate and guaifenesin. The drugs were resolved using a mobile phase of acetonitrile: 50 mM disodium hydrogen phosphate buffer containing 0.1% triethylamine (36:64 v/v pH 3.0) on an Inertsil, ODS-3V C18 (250 X 4.6 mm), 5µm column in isocratic mode. Recovery values of 99.82-101.07 %, percentage relative standard deviation of <1.81 and correlation coefficient of 0.998–0.999 shows that the developed methods were accurate and precise. For stability study, the drug was exposed to the stress conditions such as acid, base, oxidation, neutral and sunlight. As per ICH guidelines the results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy and were found to be satisfactory. These methods can be employed for the routine analysis of syrup containing salbutamol sulphate and guaifenesin.

Keywords: Reverse phase liquid chromatography, Guaifenesin, Salbutamol, Method validation,

---

INTRODUCTION

Guaifenesin (GF), (*RS*)-3-(2-methoxyphenoxy) propane-1, 2-diol [**Fig. 1 (a)**], is an expectorant believed to stimulate receptors that initiate a reflex secretion of respiratory tract fluid, thereby increasing the volume while decreasing the viscosity of mucus in the lungs. This action facilitates removal of mucus and reduces irritation of the bronchial tissue [1]. Salbutamol Sulphate (SAL) IS chemically (RS)-1-(4-hydroxy-3-hydroxy methyl phenyl)-2-(tert-butyl amino) ethanol sulphate [**Fig. 1 (b)**]. It is β_2-adrenoceptor agonist widely used as bronchodilator in the treatment of asthma and seasonal allergies [2]. Salbutamol Sulphate is official in IP [3], BP [4] and USP [5].



(a) **(b)**

Figure 1: Chemical structure of (a) Guaifenesin and (b) Salbutamol sulphate

Literature survey revealed spectrophotometry [6], HPLC [7-12], supercritical fluid chromatography [13] and voltammetry [14] methods are reported for the estimation of guaifenesin alone or in combination with other anti-asthmatic agents. Methods such as UV spectrophotometry [15-19], RP- HPLC [20-22] and TLC [23] are reported for estimation of salbutamol sulphate alone or in combination with other agents. Literature survey revealed that no stability indicating RP-HPLC method have been found to be reported for the simultaneous estimation of SAL and GF in combination. The aim of the work was to introduce a simple, accurate and reproducible isocratic stability indicating RP-HPLC method for simultaneous determination of SAL and GF. The proposed methods were optimized and validated as per ICH guidelines [24].

MATERIALS AND METHODS

SAL and GF were obtained as gift samples from Gens Pharma International Pvt. Ltd., Pune and Elder Pharmaceuticals Ltd., Mumbai, respectively. Disodium hydrogen phosphate, HPLC grade Water and Acetonitrile were procured from Merck Ltd, Mumbai, India. Orthophosphoric acid was purchased from Research Lab., Fine Industries, Mumbai. The commercial formulation of SAL and GF {Asthlin expectorant} procured from local market.

Instrumentation:
The HPLC system, Jasco PU-2080 Plus, with manual Rheodyne injector facility operates at 20 µL capacity per injection was used. The column used was Inertsil, C18 (250 X 4.6 mm), 5µm and the detector consisted of UV/VIS (Jasco UV 2075-Plus) operated at 225 nm. The data were acquired and processed using Borwin software version 1.5

Chromatographic Conditions:
The mobile phase containing acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH 3.0 adjusted by using orthophosphoric acid) (36:64 v/v) was found to resolve SAL and GF. The mobile phase was filtered through 0.45 micron nylon filter and then sonicated for 5 min. The flow rate was set to 0.8 ml/min. Both the drugs shows good absorbance at 225 nm, this wavelength was selected for further analysis. All determinations were performed at constant temperature (18^0C). A typical chromatogram shown in **Fig.2**

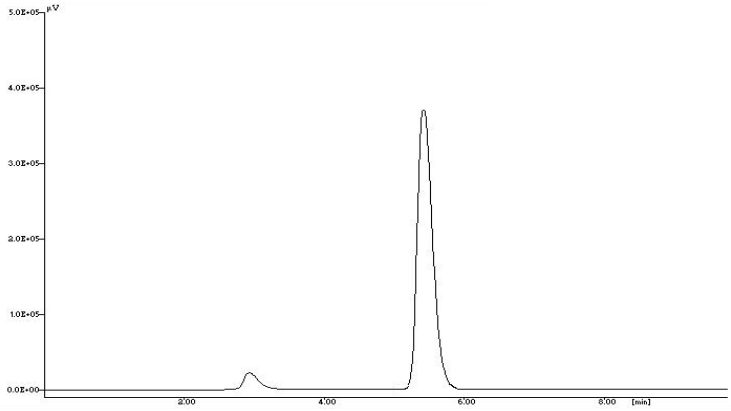


Figure 2: Chromatogram of Standard Salbutamol Sulphate and Guaifenesin

Preparation of standard stock solution:
Weighed accurately 1 mg of salbutamol sulphate and 50 mg of guaifenesin, transferred to a 10 ml volumetric flask, add 5 ml of mobile phase, sonicate for 10 min and volume was made up to the mark with mobile phase.

Preparation of working standard solution: From the standard stock solution, 0.2 ml sample was pipette out and diluted to 10 ml with mobile phase.

Analysis of syrup formulation:
An accurately weighed quantity of syrup equivalent to 1 mg salbutamol sulphate and 50 mg guaifenesin was transferred to 10 ml volumetric flask, add 5 ml mobile phase, sonicated for 10 min. The resulting solution was filtered through 0.45µ Whatmann filter and volume was adjusted to mark with same solvent. From this solution, 0.2 ml was pipette out and diluted to 10 ml with mobile phase and injected to HPLC system (**Table 1**).

Table 1.Analysis data of Salbutamol sulphate and Guaifenesin

Sample	Labelled claim	% estimated	S.D.	% RSD
SAL	1 mg	101.53	0.4103	0.4042
GF	50 mg	99.69	0.8483	0.8509

S.D.-standard deviation, RSD- relative standard deviation

VALIDATION:

Limit of detection (LOD) and limit of quantification (LOQ):

The signal-to-noise ratio (*S/N*) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the *S/N* ratio and the limit of quantification was estimated as ten times the *S/N* ratio.

Specificity:

Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of SAL and GF and was demonstrated by assaying samples of SAL and GF syrup.

Linearity:

Different standard solutions were prepared by diluting standard stock solution with mobile phase in concentration 0.5, 1, 2, 3, 4, 5 µg/ml for SAL and 25, 50, 100, 150, 200, 250 µg/ml for GF, injected to HPLC system and chromatograms were taken under standard chromatographic conditions.

Precision:

Precision of analytical methods were expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

Recovery:

To check the accuracy of the proposed method, recovery studies were carried out by applying standard addition method. A known amount of standard SAL and AMB corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

FORCED DEGRADATION:

Acid and base induced degradation product:

To 10 ml of standard stock solution, 10 ml of 0.1 N HCl and 10 ml of 0.1N NaOH were added separately. These mixtures were reflux separately for 1 hr at 50ºC. The forced degradation study in acidic and basic media was performed in the dark in order to leave out the possible degradative effect of light. 0.4 ml of each resultant solution was diluted to 10 ml with the mobile phase and resultant solution injected into the system.

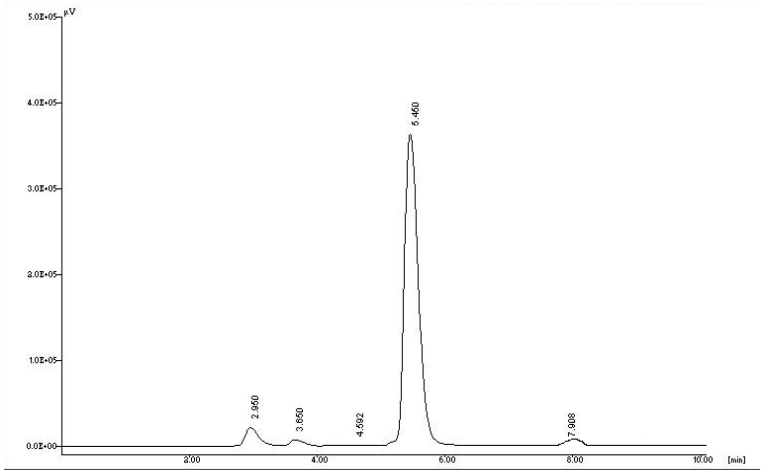


Figure 3: Chromatogram of acid [0.1N HCl (reflux for 1 hr at 50°C)] treated sample
Degradants [Rt = 3.650, 4.592, 7.908]
Salbutamol and guaifenesin [Rt = 2.950 and 5.450]

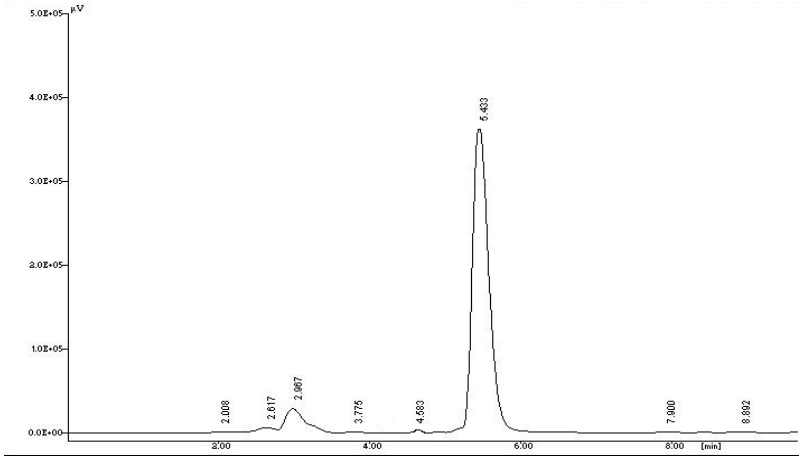


Figure 4: Chromatogram of base [0.1N NaOH (reflux for 1 hr at 50°C)] treated sample
Degradants [Rt =2.008, 2.617, 3.775, 4.583, 7.900, 8.892]
Salbutamol and guaifenesin [Rt = 2.967 and 5.433]

Hydrogen peroxide induced degradation product:

To 10 ml of standard stock solution, 10 ml of hydrogen peroxide (3 % v/v H_2O_2) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and reflux for 30 min at 50°C. 0.4 ml of resultant solution was diluted to 10 ml with the mobile phase and injected into the system.

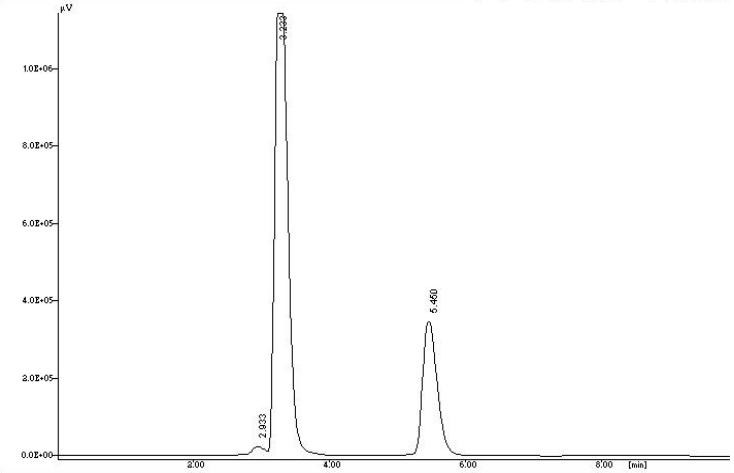


Figure 5: Chromatogram of Hydrogen peroxide [3% H_2O_2 (reflux for 30 min at 50°C)] treated sample
Degradants [Rt =3.333]
Salbutamol and guaifenesin [Rt = 2.933 and 5.450]

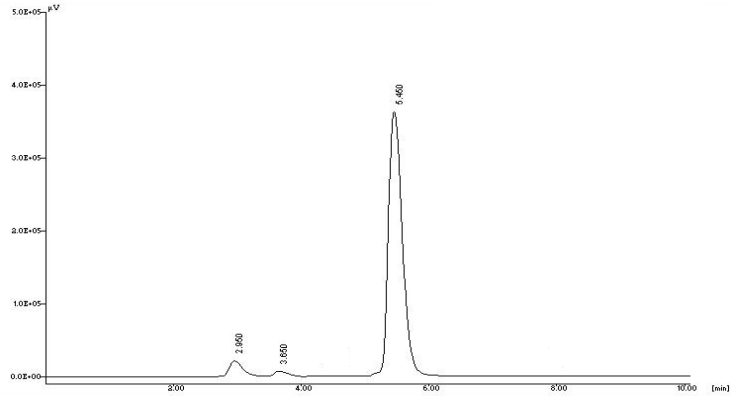


Figure 6: Chromatogram of neutral hydrolysis (reflux for 60 min at 60°C)]
Degradant [Rt =2.950]
Salbutamol and guaifenesin [Rt = 2.950 and 5.450]

Neutral hydrolysis:
Ten millilitres of standard stock solution was mixed with 10 ml water and reflux for 60 min at 60ºC. 0.4 ml this solution was diluted to 10 ml with the mobile and resultant solution injected into the system.

Photolytic induced degradation product:
Ten millilitres of standard stock solution was exposed to direct sunlight for 4 hr on a wooden plank and kept on terrace. 0.2 ml of resultant exposed solution was transferred to 10 ml volumetric flask, diluted with the mobile phase and solution was injected into the system.

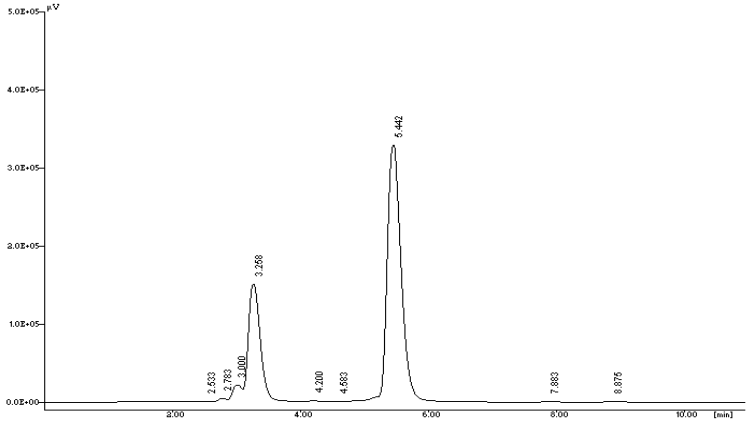


Figure 7: Chromatogram of sunlight exposed (for 4 hr) sample
Degradants [Rt =2533, 2.783, 3.258, 4.200, 4.583, 7.883, 8.876]
Salbutamol and guaifenesin [Rt = 3.00 and 5.442]

RESULTS AND DISCUSSION

The parameters were focused for improvisation of retention time, separation of degradation products and column life. The Inertsil C18 column provided good peak shapes and no peak splitting was observed. The retention time for SAL and GF was found to be 2.9 and 6.5 respectively. SAL and GF showed good linear responses in concentrations level ranging from 0.5-5µg/ml (0.998) for SAL and 25-250 (0.999) µg/ml for GF [**Fig.8 (Table 2**)].

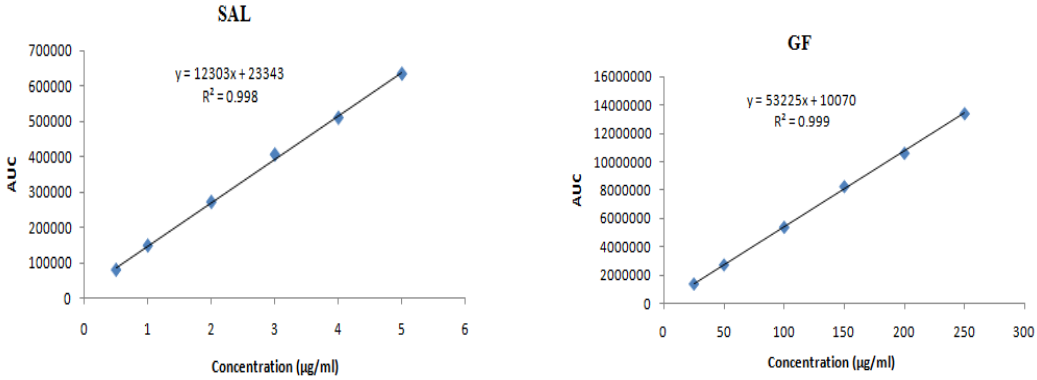


Figure 8: Linearity plots of Salbutamol sulphate and Guaifenesin

Table 2: Result Linear Regression data of Salbutamol sulphate and Guaifenesin

Parameter	RP-HPLC	
	SAL	**GF**
Limit of detection	0.15	0.30
Limit of quantitation	0.46	1.0
Retention time (min) and Rf	2.9	6.5
Correlation coefficient (r^2)	0.998	0.999
Calibration range	0.5-5 µg/ml	25-250 µg/ml
Regression equation	Y=12303x+23343	Y=53225x+10070

The measurement at three different concentration levels showed low value of % R.S.D. (<2) and low value of S. E. (<2) for intra-day and inter-day variation, which suggested an excellent precision of the method (**Table 3**).

Table 3: Precision data of Salbutamol Sulphate and Guaifenesin

Parameter	(% estimated ± % RSD)	
Precision (n=3)	**SAL**	**GF**
Intra-day	101.18 ± 1.66	99.46 ± 1.13
	101.95 ± 1.17	100.53 ± 1.52
	100.37 ± 1.53	102 ± 0.70
Inter-day	99.14 ± 1.17	101.33 ± 1.63
	100.60 ± 1.75	99.84 ± 0.96
	101.18 ± 0.88	101.82 ± 0.38

The recovery of drug was determined by spiking drug at three different levels and was found to be between 99.82-101.07 % (**Table 4**).

Table 4: Recovery data of Salbutamol sulphate and Guaifenesin

Level of Standard Addition (%)	% Recovery	S.D.	% RSD
SAL			
80	99.82	1.3111	1.3134
100	100.02	1.1231	1.1228
120	100.73	1.6852	1.6729
GF			
80	100.90	1.8343	1.8178
100	101.07	1.5428	1.5264
120	100.02	1.6822	1.6818

Forced degradation of drug was carried out as per the ICH guidelines (ICH Q2B) by subjecting SAL and GF to various stress conditions. The percent area decreased at the level of 4.22-29.80 % and it indicates that SAL and GF undergoes degradation in acidic, basic, oxidative, neutral and photolytic conditions. Summary of force degradation data are given in Table 5.

Table 5: Summary of force degradation data

Sample stress condition	Stress condition	SAL & GF (R.T.)	Degradants (R.T.)	% Area decreased		Fig. No.
				SAL	GF	
Acid degradation	0.1 N HCl reflux for 1 hr.	2.950 & 5.450	3.650, 4.342, 4.592, 7.908	7.75	4.22	**3**
Alkaline degradation	0.1 N NaOH reflux for 1 hr.	2.967 & 5.433	2.008, 2.617, 3.775, 4.583, 7.900, 8.892	15.09	12.53	**4**
Oxidative degradation	3 % H_2O_2 reflux for 30 min.	2.933 & 5.450	3.333	8.68	6.05	**5**
Neutral hydrolysis	Purified water reflux for 1 hr.	2.950 & 5.450	2.950	8.12	5.23	**6**
Photolytic degradation	Kept in sunlight for 4 hr.	3.00 & 5.442	2.533, 2.783, 3.258, 4.30, 4.58, 7.88, 8.87	29.80	12.11	**7**

REFERENCES

[1] Hajian R, Shams N, Davarpanah Z; *E-Journal of Chemistry*, **2011**, 8, 966-976.
[2] Murthy N, Hiremath S; *Int J Pharm*, **2004**, 287, 47-53.
[3] *Indian pharmacopoeia*.Vol.3.Ghaziabad: Indian pharmacopeia commission, Ghaziabad on behalf of Ministry of health and family welfare, Government of India; **2007**, 1687-92.
[4] *British pharmacopoeia*. Vol. 2. London, United Kingdom: Stationary office on behalf of Medicine and health care products regulatory agency; **2005**, 1756-60.
[5] *United State Pharmacopoeia*. Rockville, USA: United State Pharmacopeia Convention; **2005**, 57-58.
[6] Pappano N, De-Micalizzi Y, Debattista N, Ferretti F; *Talanta*, **1997**, 44, 633-639.
[7] Donmez O, Asci B, Bozdogan A, Sungur S; *Talanta*: **2011**, 83, 1601-1605.
[8] Korany M, Maher H, Galal S, Fahmy O, Ragab M; *Talanta*, **2010,** 83, 93-109.
[9] Elkady E; *Talanta*,**2010,** 82, 1604-1607.
[10] Louhaichi M, Jebali S, Loueslati M, Adhoum N, Monser L; *Talanta*, **2009**,78, 991-997.
[11] Wen J, Zhang H, Xia C, Hu X, Xu W; *Y. Biomed Chromatogr*, **2010**, 24, 351-357.
[12] Schieffer G, Hughes D; *J Pharm Sci*, **1983**; 72: 55-59.
[13] Phinney K, Sander L, Wise S; *Anal Chem*, **1998**, 70, 2331-2335.
[14] Tapsoba I, Belgaied J, Boujlel K; *J Pharm Biomed Anal*, **2005**, 38, 162-165.
[15] Dave H, Mashru R, Thakkar A; *Anal Chim Acta*, **2007**, 597,113-120.
[16] Mukherji G, Aggarwal N; *Int J Pharmaceutics*, **1991**, 71,187-191.
[17] Mukherji G. Aggarwal N; *Int J Pharmaceutics*, **1992,** 86, 153-158.
[18] Mishra A, Kumar M, Mishra A, Verma A, Chattopadhyay P; *Arch Apl Sci Res*, **2010,** 2, 207-211.
[19] Parimoo P, Umapathi P, Ilango K; *Int J Pharmaceutics*, **1993**, 100, 227-231.
[20] AbuRuz S, Millership J, Heaney L, McElnay J; *J Chromatogr B*, **2003**, 798, 193-201.

[21] Ossama T, Hoda M, Hadir M; *J Adv Res*, **2011**, 2, 121-130.
[22] El-Gindy A, Emara S, Shaaban H; *J Pharm Biomed Anal*, **2007,** 43, 973-82.
[23] Dave H, Mashru R, Patel A; *Int J Pharm Sci*, **2010**, 2, 390-394.
[24] ICH Q2B, Validation of Analytical Procedures: Methodology, *International Conference on Harmonization*, ICH Harmonized Tripartite Guideline, Geneva, Fed. Regist. **1997,** 62, 27463.