ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 8, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

METHOD DEVELOPMENT AND FORCE DEGRADATION STUDIES FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULFATE, ETOFYLLINE AND BROMHEXINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

NUTAN RAO[1]*, KAJAL D GAWDE[2]

[1]Department of Pharmaceutical Chemistry and Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi Mumbai – 400 705, Maharashtra, India. [2]Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi Mumbai – 400 705, Maharashtra, India. Email: nutan.rao@ocp.edu.in

Received: 21 March 2018, Revised and Accepted: 08 May 2018

ABSTRACT

Objective: The present study describes the stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous estimation of salbutamol sulfate (SAL), etofylline (ETO), and bromhexine hydrochloride (BROM) in pharmaceutical dosage forms.

Methods: The proposed RP-HPLC method was developed using Shimadzu prominence-i LC-2030 HPLC system equipped with ultraviolet (UV) detector and chromatographic separation was achieved isocratically using Shim-pack C18 (250 mm×4.6mm, 5 µ) column at a flow rate of 1 ml/min and the run time was 13 min. The mobile phase consisted of acetonitrile: 0.1M potassium dihydrogen phosphate buffer (35:65) with pH adjusted to 3.0 and eluents were scanned using UV detector at 225 nm.

Result: The retention time of SAL, ETO, and BROM was found to be 2.319 min, 2.698 min, and 10.329 min, respectively. The calibration curve was linear over the concentration ranges of 1.6–3.2 µg/ml, 160–320 µg/ml, and 6.4–12.8 µg/ml for SAL, ETO, and BROM, respectively.

Conclusion: The stability indicating method was developed by subjecting the drugs to stress conditions such as acid and base hydrolysis, oxidation, humidity, and photo- and thermal degradation and the degraded products formed were resolved successfully from the samples. Therefore, the proposed method can be used as a more convenient and efficient option for the simultaneous estimation of all the three drugs in bulk and combined dosage form.

Keywords: Salbutamol sulfate, Etofylline, Bromhexine hydrochloride, RP-HPLC, Validation, Forced degradation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i8.26119

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease that interferes with normal breathing and is not reversible. The chronic limitation of airflow is an important characteristic of COPD, which can be caused by a mixture of obstructive bronchiolitis (small airway disease) and emphysema (parenchymal destruction). COPD has extra-pulmonary effects, as it is a multicomponent disease. The British Medical Research Council defined chronic bronchitis as "daily productive cough for at least 3 consecutive months for more than 2 successive years" [1]. As of 2015, COPD affected about 174.5 million of the global population. Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation.

Salbutamol sulfate (SAL) is a beta-adrenoceptor agonist. Activation of β_2-adrenergic receptors present in airway smooth muscle; results in the activation of adenyl cyclase and lead to an increase in the intracellular concentration of cyclic-3′,5′-adenosine monophosphate (cyclic AMP). There is an increase in cyclic AMP which results in the activation of protein kinase-A, which inhibits the phosphorylation of myosin and lowers intracellular ionic calcium concentrations, resulting in the relaxation. It is used for the relief of bronchospasm in a condition such as asthma and chronic obstructive pulmonary disease. Chemical name of SAL is 4-[2-(tert-butylamine) -1-hydroxyethyl] -2-(hydroxymethyl) phenol; sulfuric acid. It has a molecular formula of $C_{26}H_{44}N_2O_{10}S$ and molecular weight of 576.702 g/mol.

Etofylline (ETO) is a xanthine bronchodilator which inhibits phosphodiesterase enzyme and intracellularly degrades cyclic nucleotides resulting in the intracellular accumulation of the cyclic AMP and causing bronchodilation. Chemical name of ETO is 7-(2-hydroxyethyl)-1,3-dimethylpurine-2,6-dione. It has a molecular formula of $C_9H_{12}N_4O_3$ and molecular weight of 224.22 g/mol.

Bromhexine hydrochloride (BROM) acts as an oral mucolytic agent. It disrupts the structure of mucopolysaccharide fibers in mucoid sputum and produces less viscous mucus, which is easier to expectorate [2]. Chemical name of BROM is 2,4-dibromo-6[[cyclohexyl(methyl) amino]methyl]aniline, hydrochloride. It has a molecular formula of $C_{14}H_{21}Br_2ClN_2$ and molecular weight of 412.594 g/mol.

SAL, ETO, and BROM are available in the market as a combined tablet dosage form, which is widely used in the treatment of asthma and COPD.

The literature survey reveals that high-performance liquid chromatography (HPLC) and ultraviolet (UV) methods were reported for the estimation of SAL, ETO, and BROM alone or in combination with other drugs in bulk and dosage forms.[4-14] However, so far there is no stability indicating method reported for the same. This initiated an interest to develop a new, simple, and rapid HPLC method of these drugs in combination with the marketed formulation used for the treatment of COPD and asthma. The proposed method was used successfully to separate the degraded products from the samples and it is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [3] (Figs. 1-3).

EXPERIMENTAL CONDITIONS

Materials and reagents

SAL, ETO, and BROM were obtained as gift samples from Centaur Pharmaceuticals, Mumbai. A commercial preparation (ALBUTAMOL PLUS TABLET) used for analysis was procured from Pharmaceuticals market. Each tablet contains 2 mg of SAL, 200 mg of ETO, and 8 mg of BROM. HPLC grade acetonitrile (Thomas Baker) and water, potassium dihydrogen phosphate (LOBA CHEM), and orthophosphoric acid were used.

Instrumentation

The RP-HPLC chromatographic separation was carried out by Shimadzu prominence-i LC-2030 HPLC system containing software of LAB solution with pump p-5000, UV/VIS detector and a fixed injector equipped with 20 µL loop. The Lab Solution software was used for signal monitoring and processing.

Chromatographic conditions

- Column: Shim-pack C18 (250 x 4.6 mm, 5 µm)
- Mobile phase:acetonitrile: 0.1 M potassium dihydrogen phosphate buffer (35:65), adjusted to pH 3.0 with orthophosphoric acid
- Flow rate: 1.0 ml/min
- Wavelength: 225 nm
- Injection volume: 20 µL
- Runtime: 13 min
- Elution: Isocratic.

Preparation of 0.1 M potassium dihydrogen orthophosphate (pH 3.0)

About 13.609 g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 1000 ml of water and adjusted pH with o-phosphoric acid to 3.0±0.05. The solution was then filtered using 0.45 µ membrane filter.

Preparation of mobile phase

The pH of (0.1 M) potassium dihydrogen orthophosphate was adjusted to 3.0 with orthophosphoric acid and mixed with acetonitrile in the proportion 65:35 and was sonicated.

Preparation of standard solution

100 mg of SAL, 100 mg of ETO, and 100 mg of BROM standard were accurately weighed and transferred into individual 100 ml volumetric flasks. About 70 ml of the mobile phase was added, sonicated to dissolve and diluted to 100 ml using mobile phase. Suitable dilutions were made to obtain a final concentration of 2,200 and 8 µg/ml SAL, ETO, and BROM, respectively.

Preparation of sample solution

10 tablets were weighed and powdered. The quantity of powder equivalent to 2 mg of SAL, 200 mg of ETO, and 8 mg of Bromhexine were transferred into a 100ml volumetric flask. About 70 ml mobile phase was added, and the solution was sonicated for 30 min with intermittent shaking. The volume was made up using the mobile phase, mixed and filtered through 0.45 µ PVDF filter. Suitable dilutions were made to obtain a final concentration of 2200 and 8 µg/ml SAL, ETO, and bromhexine hydrochloride, respectively.

Statistical analysis

To evaluate the contribution of each factor with different levels on responses, two-way analysis of variance was performed using GraphPad Prism 7.04 software.

RESULTS AND DISCUSSION

The proposed RP-HPLC method was validated as per the ICH guidelines.

Selectivity and specificity

To assess the selectivity of the developed method solutions of all three drugs were injected into the system, three sharp peaks of SAL, ETO, and BROM were obtained at a retention time of 2.319, 2.698, and 10.329 min, respectively, in reference to the standard solution. Specificity was determined by comparison of the chromatogram of mixed standards and sample solutions Fig 4,5. As the retention time of standard drugs and the retention time of the drugs in sample solutions were same, so the method was specific. The parameters such as resolution (Rs) and asymmetric factor were calculated. A good correlation was found between the results of mixed standards and sample solutions. Results are shown in Table 1.

Linearity

The linearity of an analytical method has ability to obtain results, which are directly proportional to the concentration of an analyte in the sample. It was done by preparing the sample solutions containing 2 µg/ml, 200 µg/ml, and 8 µg/ml of SAL, ETO, and BROM, respectively. A calibration curve was drawn by plotting concentration on an X-axis versus area on Y-axis and regression equation, correlation coefficient, y-intercept, and slope of the equation were calculated. The result is shown in Table 2 and Figs. 6-8.

Accuracy

The accuracy of the proposed methods was estimated by recovery studies at three different levels, i.e. 80%, 100%, and 120%. The recovery studies were carried out by adding known amounts of standard SAL, ETO, and BROM and were added to the pre-analyzed samples, and they were subjected to a proposed HPLC method. The recoveries results of standards in pharmaceutical preparation are shown in Table 3.

Precision

The precision study was carried out to find out intraday and interday variations. The intraday and interday precision study of SAL, ETO, and BROM was carried out by estimating the correspondence response

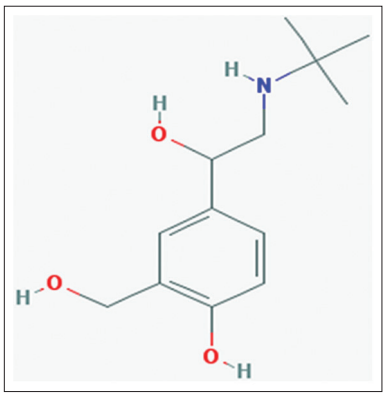

Fig. 1: Structure of salbutamol

Table 1: System suitability parameters

System suitability parameters	SAL	ETO	BROM
Retention time (min)	2.319	2.698	10.329
USP plate count	3539	14621	4683
USP tailing	1.602	1.219	1.109

SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride

Table 2: Linearity studies

Parameters	SAL	ETO	BROM
Linearity range (µg/ml)	1.6–3.2	160–320	6.4–12.8
Slope	54263	35048	33400
Intercept	28846	22877	2309
Correlation coefficient	0.998	0.998	0.999

SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride

3 times on the same day and on 3 different days for 3 different concentrations, and the results were reported in terms of percentage relative standard deviation (% RSD), however, all results fall within an acceptance limit (RSD <2), as shown in Table 4.

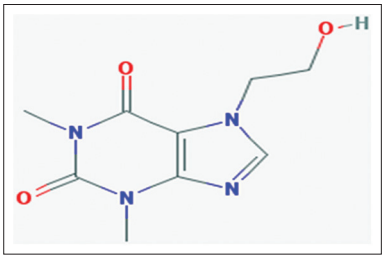

Fig 2: Structure of etofylline

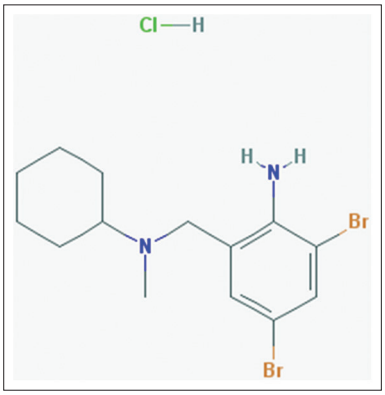

Fig. 3: Structure of bromhexine hydrochloride

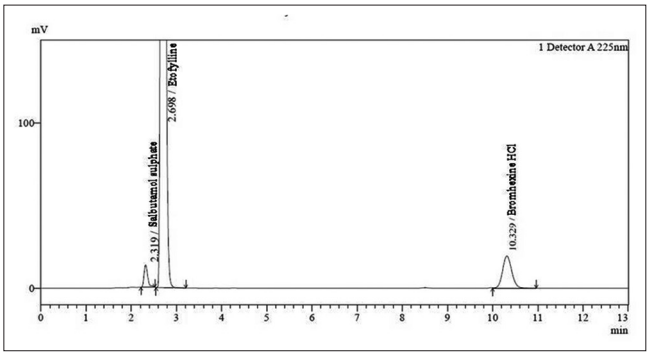

Fig. 4: Chromatogram of standard solution

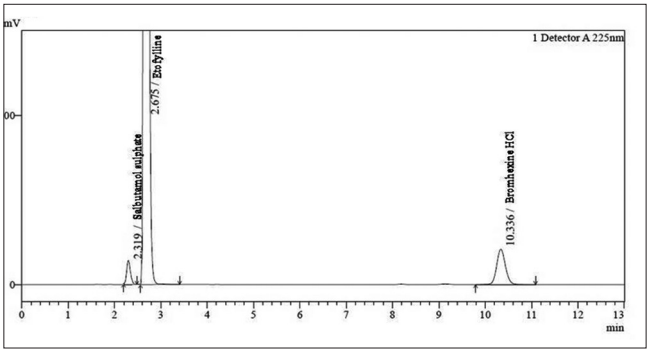

Fig. 5: Chromatogram of the sample solution

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

LOD is the ability of analytical method able to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte which can be quantitatively analyzed with acceptable precision and accuracy. It was calculated based on the slope and blank response

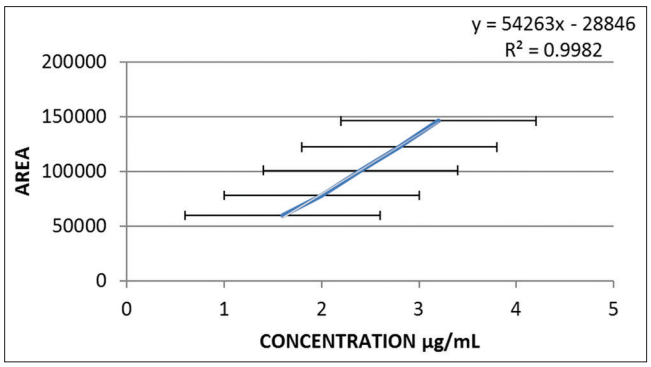

Fig. 6: Calibration curve of salbutamol sulfate

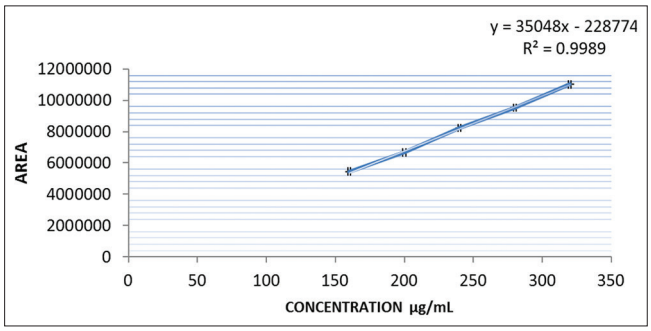

Fig. 7: Calibration curve of etofylline

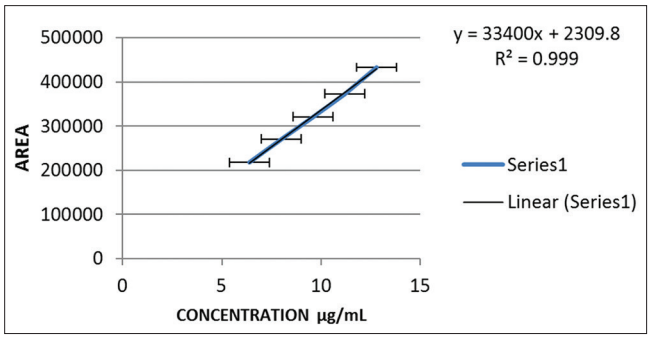

Fig.8: Calibration curve of bromhexine hydrochloride

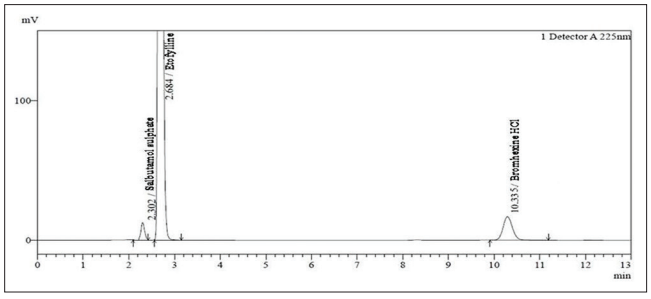

Fig. 9: Chromatograph of acid degradation

Table 3: Results of accuracy studies

Pre-analyzed sample solution (µg/ml)	Sample concentration (µg/ ml)	Excess drug added (µg/ml)	Amount recovered (µg/ ml)	% recovery
SAL	1	0.8	1.8	100.39
	1	1	2	99.29
	1	1.2	2.2	100.32
ETO	100	80	180	98.17
	100	100	200	98.25
	100	120	220	98.58
BROM	4	3.2	7.2	99.90
	4	4	8	100.16
	4	4.8	8.8	99.92

SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride

Table 4: Results of precision and LOD and LOQ

Parameters	Precision (% RSD)		
	SAL	ETO	BROM
Intraday (n=3)	0.85	0.41	0.50
Interday (n=3)	0.63	0.82	0.54
LOD	0.10	8.10	0.30
LOQ	0.31	24.56	0.91

LOD: Limit of detection, LOQ: Limit of quantification, SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride, % RSD: % Relative standard deviation

Table 5: Assay determination of SAL, ETO, and BROM

Brand		% amount found
Albutamol plus (2 mg SAL+200 mg ETO+8 mg BROM)	SAL	99.64
	ETO	99.57
	BROM	99.79

SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride

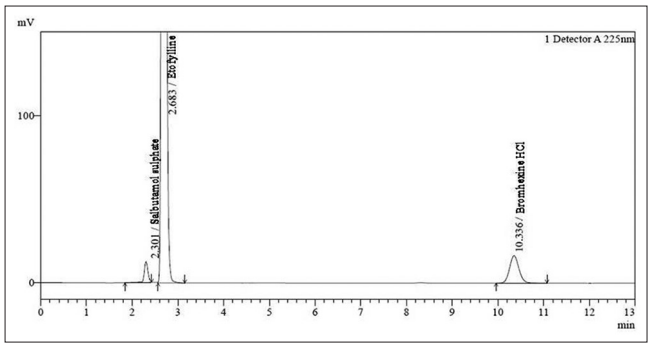

Fig. 10: Chromatograph of base degradation

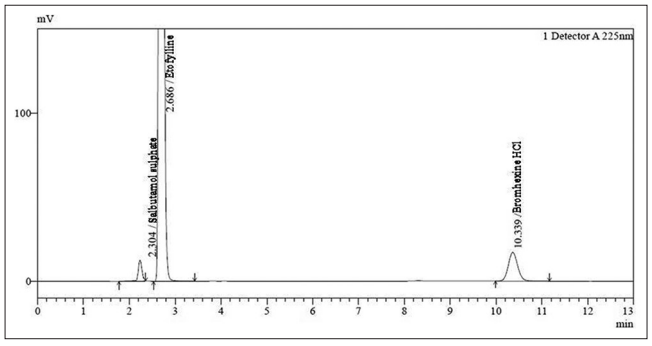

Fig. 11: Chromatograph of oxidative degradation

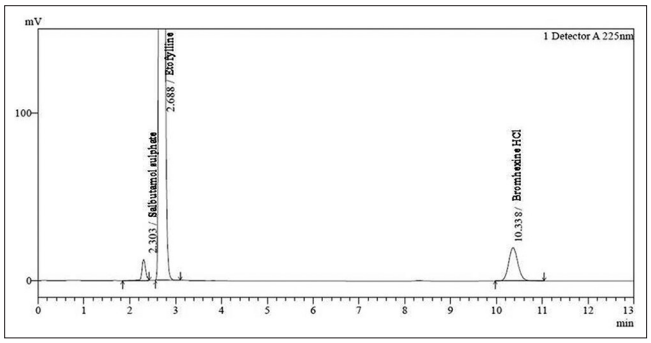

Fig. 12: Chromatograph of photolytic degradation

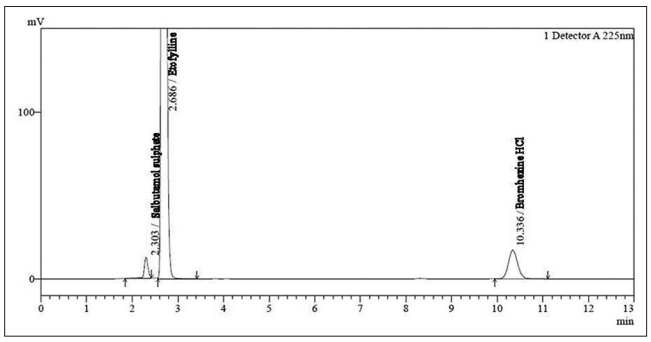

Fig. 13: Chromatograph of thermal degradation

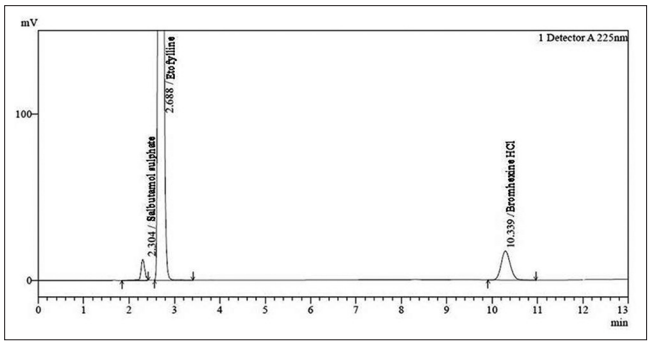

Fig. 14: Chromatograph of humidity degradation

from the calibration curve as per the ICH guidelines. LOD and LOQ were calculated based on the standard deviation of the response and slope. The result is shown in Table 4.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ±0.2 ml change in flow rate, ±2°C

Table 6: Force degradation of SAL, ETO, and BROM

S.NO	Stress condition	SAL		ETO		Bromhexine hydrochloride	
		% assay	% difference w.r.t control	% assay	% difference w.r.t control	% assay	% difference w.r.t control
1	Control	99.64	NA	99.57	NA	99.79	NA
2	Acid degradation	94.85	4.78	91.43	8.13	87.11	12.67
3	Base degradation	98.51	1.12	88.77	10.79	84.23	15.55
4	Oxidative degradation	96.72	2.91	91.67	7.89	87.96	11.82
5	Photolytic degradation	93.53	6.10	98.51	1.05	95.13	4.65
6	Thermal degradation	95.32	4.31	92.63	87.98	6.93	11.80
7	Humidity degradation	96.86	2.77	94.40	5.16	89.18	10.60

SAL: Salbutamol sulfate, ETO: Etofylline, BROM: Bromhexine hydrochloride

change in temperature, and ±2 nm change in wavelength. There was no significant impact on the retention time and tailing factor.

Assay

The amount of SAL, ETO, and BROM per tablet was calculated by comparing the peak area of the standard solution and sample. The result is shown in Table 5.

Forced degradation studies

Forced degradation study was carried out by treating the sample under the following conditions: Sample was subjected to acid degradation using ^{1}N HCl, base degradation using ^{1}N NaOH, oxidative degradation using 3.0% v/v of H_2O_2, photolytic degradation by exposing the sample in UV light for 1 day, thermal degradation by heating at 105°C for 1 h, humidity degradation using 25°C, and 80% RH in a humidity chamber. The results of stress studies were shown in Table 6 and Figs. 9-14.

CONCLUSION

In the present study, stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of SAL, ETO, and BROM in the pharmaceutical dosage form. The developed method was validated as per the ICH guidelines, and the results were within limits. The stress testing studies revealed that the method was successfully employed to resolve the degraded products from the sample. This method can be utilized in routine quantitative and qualitative analysis of SAL, ETO, and BROM in the pharmaceutical dosage form. The proposed HPLC method is one of the simple, rapid, reproducible, accurate and economical methods for estimation of SAL, ETO, and BROM simultaneously and method can reduce the time for routine quality control analysis in their dosage form.

ACKNOWLEDGMENT

The authors are thankful to Centaur Pharmaceuticals for kindly providing gift samples of SAL, ETO, and BROM.

AUTHOR'S CONTRIBUTION

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Kajal D. Gawdecollected and analyzed the data, performed laboratory work and wrote introduction, discussion, material, and method part.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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