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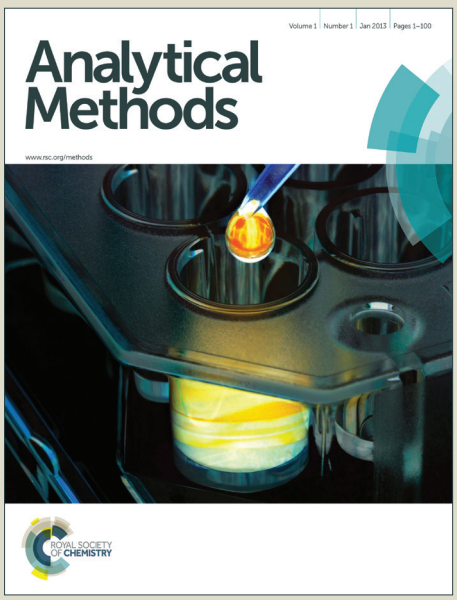

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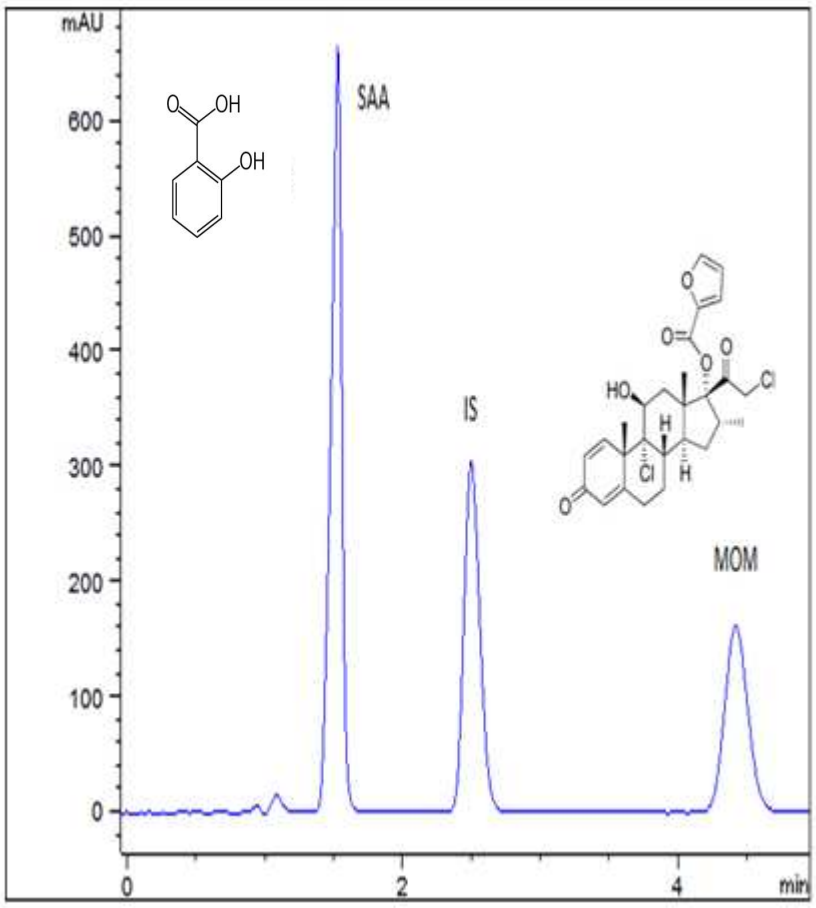

Development of Stability Indicating HPLC-DAD method for Simultaneous Determination of Mometsone Furoate and Salicylic Acid in Ointment Matrix

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Abstract

Validated stability-indicating method has been developed for simultaneous determination of mometsone furoate (MOM) and salicylic acid (SAA) in combined dosage form. This method was based on a reversed- phase high performance liquid chromatographic (HPLC) separation of the cited drugs. The HPLC separation was performed on a RP Zobrax Eclipse XDB-C8 analytical column (150×4.6 mm. 5µm) with isocratic elution system of methanol and 0.02 M aqueous phosphate buffer adjusted to pH 2.3 (70:30) as the mobile phase at the flow rate of 1.5 mL min^{-1}. Quantitation was achieved with the photodiode array detection (DAD) at 260 nm. The calibration graphs for each drug were rectilinear in the range of 0.25-15 and 12.5-750 µg mL^{-1} for MOM and SAA, respectively using dexamethasone acetate (DXM) as internal standard. The proposed HPLC-DAD method was successfully applied for the determination of the investigated drugs in ointment. The method was validated in compliance with ICH guidelines; in terms of linearity, accuracy, precision, robustness, limits of detection and quantitation and specificity.

Keywords: mometsone furoate, salicylic acid, HPLC-DAD, Stability-indicating method

1. Introduction

Mometasone furoate (MOM), 9a,21-Dichloro-11b,17-dihydroxy-16a-methylpregna-1,4-diene- 3,20-dione 17(2-furoate); (11b,16a)-9,21-dichloro-17-[(2-furanylcarbonyl)oxy]- 11-hydroxy-16-methylpregna-1,4-diene-3,20-dione, [Fig. 1] is a synthetic glucocorticoid with anti-inflammatory, anti-allergy effect. Mometasone furoate is effective for various skin diseases, such as neurodermatitis, eczema, atopic dermatitis and psoriasis of the skin caused by skin inflammation and itching. [1-5]

Salicylic acid (SAA), 2-Hydroxybenzoic acid, [Fig. 1] has bacteriostatic, fungicidal and keratolytic actions. It has been extensively used in dermatologic therapy as a keratolytic agent, relieves pain and reduces swelling. Moreover, SAA is effective to treat warts, skin ulcer, psoriasis and other skin conditions. [1, 2]

Nowadays, MOM has been marketed in combination with SAA in semisolid dosage forms, which have lesser side effects and patient specificity. Elicasal® ointment (Mometasone furoate 0.1% and 5% Salicylic acid) is used for glucocorticoid with anti-inflammatory, anti-allergy effect mainly in skin diseases, such as neurodermatitis, eczema, atopic dermatitis and psoriasis of the skin caused by skin inflammation and itching. [4, 6]

Scientific literature reports that there are many methods reported for the determination of MOM individually and in combination with other drugs like fucidic acid, terbinafine hydrochloride, nadifloxacin and formoterol fumarate etc. based on reversed-phase HPLC and HPTLC methods [7-12]. For the determination of SAA either alone or in combination with other drugs several analytical methods were reported includes UV spectroscopic, HPLC, HPTLC and capillary electrophoresis methods [13-16]. MOM and SAA are official in BP and USP individually. [17, 18].

Both MOM and SAA were simultaneously determined using derivative ratio spectrophotometric method in ointment formulation [19]. But literature reviews lack any chromatographic method for simultaneous estimation of mentioned drugs in formulation. Therefore, the objective of this work was to develop validated stability indicating chromatographic method for analysis of MOM and SAA in the bulk powder, and in ointment. This paper describes accurate, specific, repeatable, and stability indicating HPLC-DAD method. This method can also be used for determination of both drugs in the presence of degradation products and for assessment of the purity of bulk powder and the stability of the dosage form of the drugs.

2. Experimental

2.1. Materials and Reagents

Pharmaceutical grade of MOM, SAA and dexamethasone acetate (DXM, IS) were supplied as a gift sample by Borg Pharmaceutical Industries, Alexandria, Egypt. All reagents used were of analytical grade. Orthophosphoric acid, potassium hydrogen phosphate and sodium hydroxide (Sigma-Aldrich®, St. Louis, MO, United States) and HPLC grade methanol and acetonitrile (Tedia, Ohio, USA). The water for LC was reverse osmosis water.

2.2. Pharmaceutical formulations

A commercial product namely Elicasal® ointment produced by Jamjoom Pharmaceuticals (Batch No. MA102) containing 100 mg MOM, 5 g SAA and per 100 g ointment was studied.

2.3. Instrumentation and chromatographic conditions

The HPLC system (Agilent, Germany) consisted of Agilent 1260 Series Quaternary pump G1311C which comprises a solvent cabinet and an integrated Vacuum Degasser and a four-channel gradient pump; Agilent 1260 Series Diode Array and Multiple Wavelength detector

G1315D. The LC system is equipped with Agilent 1260 Series Thermostated Column Compartment G1316A and Agilent 1260 Series Manual Injector which uses a Rheodyne 7725i7-port sample injection valve and fitted with a 100 µL sample loop. LC separations were performed on an Agilent Zobrax Eclipse XDB-C8 analytical column (150 × 4.6 mm, 5 µm). The column was thermostated at 25° C during analysis.

The mobile phase consisted of methanol and 0.02 M aqueous phosphate buffer adjusted to pH 2.3 (70:30). The mobile phase was degassed and filtered by passing through a 0.45 µm pore size membrane filter (Whatman, Hahnestra ße 3 37586 Dassel, Germany) prior to use. The samples were also filtered using 0.45-µm disposable filters. The flow rate was 1.5 mL min^{-1}.

2.4. Construction of calibration graphs

Stock solutions of 50 µg mL^{-1} of MOM, 2500 µg mL^{-1} of SAA and 700 µg mL^{-1} of IS were prepared in methanol. Stock solutions were further diluted with mobile phase to obtain working standard solutions of suitable concentrations (corresponding to the linearity ranges stated in table 1). Triplicate 100 µL injections were made for each concentration and chromatographed under the above mentioned conditions. The peak area ratios of the analytes versus internal standard were plotted against the corresponding concentrations of the analytes to obtain the calibration graph for each compound.

2.5. Analysis of synthetic mixtures

Accurate volumes of each of MOM and SAA stock solutions were transferred into a set of 50-mL volumetric flasks. 1 mL of internal standard stock solution was added. The content of each flask was diluted to volume with mobile phase such that the concentrations of the drugs were within the linearity ranges mentioned in table 1. Portions of each mixture solution (100 µL) were injected in triplicates and chromatographed under the conditions described before.

2.6. Preparation of ointment

Weighed amount of about 1.00 g ointment was accurately transferred into a 100-mL volumetric flask. 40 mL of binary mixture of methanol: acetonitrile, in the ratio of 50:50 v/v, was added. Content of flask was heated in a 65°C water bath to melt the ointment then shaken vigorously. The volume was completed to mark using 0.02M potassium dihydrogen phosphate buffer pH 2.3 and mixed well. Then, it was cooled to about 4ºC (in a freezer). Filteration was performed, on cold, through membrane filter 0.45µ discarding the first 2 portions (wash twice). The procedure was completed on the prepared solutions as under standard solutions and calibration graphs.

2.7. Forced degradation of MOM and SAA

Stress degradation studies were performed in accordance with International Conference on Harmonization (ICH) guidelines [20] in order to demonstrate the stability indicating feature of the assay. Stress testing of the drug substance can help to identify the likely degradation products, the stability, and specificity of the analytical procedures.

To determine whether the analytical method and assay were stability-indicating, MOM and SAA were stressed under different conditions in forced degradation studies. The degradation products were induced in acidic, basic, neutral, oxidative and photolytic conditions.

Accurate volumes of the methanolic stock solutions of each drug (5 mL of each of MOM and SAA) were transferred into 25-mL volumetric flasks. This mixture was subjected to different stress conditions as mentioned below.

2.7.1. Acidic, Alkaline, neutral and oxidative degradation

HCl (0.1 M, 2 mL), NaOH (0.1 M, 2 mL), NaOH (0.01 M, 2 mL),), purified water (2 mL), hydrogen peroxide (33%, v/v, 2 mL) were separately added to the methanolic stock solutions of MOM, SAA and their mixture for acidic, alkaline, neutral and oxidative degradation,

respectively. For all cases, the samples were separately heated in a thermostated water bath at 85^{o}C water bath for 2 hours. The degraded sample solutions were then neutralized with equal volumes and concentrations of either base or acid in case of acidic and alkaline degradation and diluted with mobile phase prior to injection into the HPLC system.

2.7.2. Photostability

Photostability studies were performed by exposing 5 mL in 25-mL volumetric flasks containing the methanolic stock solutions of MOM and SAA as a mixture to simulated daylight for 3 days. Furthermore, the stress degradation study in direct UV radiation was performed by exposing the mixture of the methanolic stock solutions of MOM and SAA to UV radiation at 254 or 365 nm for 2 hours at room temperature. These solutions were then diluted to volume with mobile phase. Samples submitted to identical conditions, but protected of light, were used as a control.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Development of stability indicating HPLC-DAD method for the determination of binary mixture of MOM and SAA was carefully studied. The method was optimized to separate the two intact drugs from degradation products formed under various stress conditions. The main target of the HPLC-DAD method was to get reasonable separation of closely eluting degradation products from MOM and SAA peaks. During method development, different experimental conditions were studied and optimized by using different stationary phases with different mobile phase compositions.

Both acetonitrile and methanol have been tried as organic modifiers during the optimization of the HPLC method. Methanol was considered optimum and was chosen for the separation of

the two drugs (and the internal standard) within reasonable retention times. Different ratios of methanol were tried in the mobile phase consisting of methanol and phosphate buffer pH 2.3. At lower concentrations of methanol, separation occurred but with increased retention times for MOM resulting in tailing and distortion of the peak shape. Increasing methanol concentration led to less resolution of SAA from solvent front (lower retention factor, K'). Optimum resolution within reasonable total running time was achieved using 70% methanol (Fig. 2).

Phosphate buffer of different pH values from 2.1-3.0 together with methanol in a ratio of 30:70 v/v were used to study the effect of pH of the aqueous phase. The pH showed great effect on the shape and retention time of SAA peak while showed no effect in case of MOM and internal standard. As pH decreases, the retention time of SAA increases and better resolution from solvent front is met. Optimum pH for separation of SAA was 2.3 (Fig. 3).

A wavelength of 260 nm was selected for a compromised display of the three peaks (Fig.4). In spite of the presence of broad SAA spectrum that shows minimum absorption values at 260 nm, being a major component in the binary mixture not affect the sensitivity of the method and avoid loss of linearity. In addition, selection of this wavelength near the maximum peak of the UV spectrum of MOM at 250 nm allows simultaneous determination of MOM and SAA with acceptable sensitivity.

An isocratic system consisting of methanol: 0.02M phosphate buffer, pH 2.3 (70:30, v/v) was able to separate the two drugs and the internal standard in both standard solution and stressed samples.

3.2. Optimization of extraction procedure

For obtaining successful extraction of both drugs from ointment matrix, many organic solvents were tried. Acetonitrile offers efficient extraction of MOM but gives distorted SAA

peak (Peak fronting was overlapped with solvent front). The latter problem was overcome by the addition of methanol to extracting solvent in varying proportions. The most efficient extraction and best peak shape of both drugs were obtained upon using Acetonitrile: Methanol in a ratio (50:50%V/V).

Filtration is done on cold to decrease the solubility of the fatty matter from the ointment base. Washing twice is important to remove any fatty matter residue from previous sample. Ignoring washing steps will result in distorted peak shape with a seriously reduced number of theoretical plates.

3.3. Internal standard selection

Many internal standards were tried. Beclomethasone dipropionate was mentioned in the USP assay method of MOM in an ointment matrix. It showed relative retention time of 1.6, i.e. more retained than MOM, not in the middle of both MOM and SAA and also there was a suspect of possible interference with the alkaline hydrolysis degradate of MOM. Betamethasone dipropionate was also tried and has shown the same problems of beclomethasone dipropionate. Dexamethasone acetate was selected as the optimum internal standard because it shows a peak in the middle between peaks of both drugs (Fig. 2). Its concentration was optimized to give reasonable peak area (between those of the two drugs) at the detection wavelength.

3.4. System Suitability

The system suitability parameters of the chromatographic system were checked as they are integrated parts of the analytical method and it ascertains the suitability and effectiveness of the operating system. Method performance data are listed in table 2. All were satisfactory and indicative of the good specificity of the method for assessment of the stability of MOM and SAA.

3.5. Stress degradation

Stress testing provides evidence on how the quality of a drug may be affected under the influence of different stress conditions. Drug decomposition may result in loss of potency and advent of possible adverse effects due to the formation of degradation products [21]. Under different stress conditions, MOM concentrations decreased over time with appearance of different degradation product peaks. Analyses revealed the behavior of the two drugs, as summarized in table 3.

3.5.1. Acidic and alkaline degradation

Only MOM showed degradation in both acidic and alkaline conditions. SAA was found to be stable in both acidic and alkaline conditions (Fig. 5). Meanwhile, MOM showed extensive degradation in alkaline conditions more than other conditions, this degradation product eluted after 5.599 min in the HPLC chromatogram (Fig.6). Also, degradation of MOM was assessed using 0.01 M NaOH and showed 83.15% recovery of the parent drug.

3.5.2. Neutral degradation

Regarding the neutral hydrolytic degradation, both drugs demonstrated to be thermotostable. (Fig. 5)

3.5.3. Oxidative degradation

Reaction of the two drugs with 33% hydrogen peroxide was studied. It was found that MOM was degraded but at a relatively slower rate than that in acidic and alkaline conditions. (Fig. 5)

3.5.4. Photolytic degradation

Under different photolytic degradation conditions including exposure to simulated daylight for 72 h, UV radiation (254 and 365 nm) for 2 h, both drugs demonstrated to be photostable. (Fig.5)

3.6. Validation

Using the optimized chromatographic conditions, the developed HPLC-DAD method was validated in terms of linearity, accuracy, precision, robustness and specificity.

3.6.1. Linearity

Calibration graphs for MOM and SAA using the proposed HPLC-DAD were found to be linear, that is adherence of the system to Beer's law was found over the concentration ranges stated in table 1. Peak ratio and concentration (μg mL^{-1}) was subjected to least squares linear regression analysis to calculate the calibration equations and other statistical parameters [22]. Linearity results are depicted in table 1. The 99.0% confidence interval of the intercept of both drugs is more symmetric about the zero in case of peak ratios (using IS) than in case of peak areas.

3.6.2. Limits of detection (LOD) and limits of quantitation (LOQ)

The concentrations of the analyte showing signal-to-noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively. LOD and LOQ of SAA and MOM using the proposed HPLC method are presented in table 1.

3.6.3. Accuracy and Precision

In order to test for accuracy and precision of the proposed methods, three replicate determinations of laboratory prepared mixtures of the three drugs were carried out. The concentrations of the three drugs in the prepared synthetic mixtures are within the linearity range of each drug. The assay was repeated three times in the same day (for studying the intra-day precision) or in other day (for studying the inter-day precision) for each mixture. The percentage relative error Er (%) values and calculated RSD% for intra-and inter-day precision were found to be less than 2% indicating good accuracy and precision of the proposed method (Table 4).

3.6.4. Robustness

Robustness of the proposed methods was evaluated by analyzing MOM and SAA under various parameters using three synthetic mixtures containing MOM, SAA and IS. Various parameters in the proposed HPLC method were slightly changed including pH of the aqueous phase, organic phase percentage, flow rate of the mobile phase, temperature of the thermostated column compartment and wavelength of detection. This study demonstrated that slight intended variations in the previously mentioned parameters have no significant effect on the determination of MOM and SAA using the proposed method. Good robustness of the proposed methods was indicated by nearly unchanged retention factor (k') values of MOM, SAA and IS (Table 5).

3.6.5. Specificity

The peak purity of the MOM, SAA and IS in stressed samples was checked by using a G1315D photo diode array detector (DDA) for the HPLC method. The purity angle was within the purity threshold limit in all of the stressed samples, indicating that no additional peaks were co-eluting with each of the analytes and evidencing the ability of the method to assess the analytes of interest in the presence of potential interferences. Baseline resolution was achieved for all investigated compounds. The FDA guidance indicates that well-separated peaks, with resolution, *R*s> 2 between the peak of interest and the closest eluted peak, are essential for reliable quantification [23]. All the peaks met this specification where the resolution factors for MOM, SAA and IS peaks were more than 2 (8.73, 4.28 and 6.82, respectively, from the nearest resolving peak) in all cases of degradation.

No interferences from the results of stress testing studies, diluents, impurities, and excipients were observed, indicating a high degree of specificity of this method for the determination of MOM and SAA in pharmaceutical formulations.

3.5. Application to Pharmaceutical Formulation

The proposed method was successfully used to determine MOM and SAA in Elicasal® ointment. Three replicate determinations were performed. Satisfactory results were obtained for each compound and they were in a good agreement with label claims (Table 6). The results obtained were compared statistically with those from reported method by use of Student's t-test (for accuracy) and the variance ratio F-test (for precision). The results in table 6 show that the t and F values were smaller than the critical values, indicating there were no significant differences between the results obtained from the proposed method and from the reported methods [19, 24]

4. Conclusion

The proposed HPLC-DAD method provide simple, accurate, reproducible and stability indicating assay for the quantitative determination of MOM and SAA in ointment, without interference from the excipients and in the presence of their acidic, alkaline, neutral, oxidative and photolytic degradation products. The developed HPLC-DAD method solved the two drugs in the presence of all forms of degradates. All of the degradation products were well separated from the drug substances demonstrating the stability-indicating power of the method. The behavior of the two drugs under various stress conditions was studied. Moreover, The new developed extraction method is better than the extraction method of the USP assay method of MOM (24) as it requires less heat and less effort in addition to the advantageous cooling step with washing twice that prevents passage of soluble (melt) fatty matter from the ointment base which results in distorted peak shape with a seriously reduced number of theoretical plates.

In addition, there are not any reported HPLC methods for simultaneous determination of MOM and SAA in ointment making the proposed HPLC-DAD method of great interest for quality

control of both drugs in their binary mixture. Also, the proposed stability-indicating method uses simple reagents, with minimal preparation procedures and short run time thus encouraging application in routine analysis.

Table 1: Regression and statistical parameters for the determination of SAA and MOM mixture using the proposed HPLC-DAD method

Parameter	SAA	MOM
Linearity range (µg mL^{-1})	12.50-750.00	0.25-15.00
LOQ (µg mL^{-1})	12.00	0.20
LOD (µg mL^{-1})	4.00	0.07
Intercept	6.80×10^{-3}	9.00×10^{-4}
Slope	3.10×10^{-3}	7.27×10^{-2}
Correlation coefficient	0.99999	0.99999
S_a	3.50×10^{-3}	1.70×10^{-3}
S_b	9.45×10^{-6}	2.00×10^{-4}
$S_{y/x}$	5.40×10^{-3}	2.50×10^{-3}
a/S_a*	1.94	0.53
S_b^2	8.93×10^{-11}	4.00×10^{-8}
S_b%	3.05×10^{-1}	2.75×10^{-1}
F	106221.34	107176.22
Significance F	6.37×10^{-8}	6.29×10^{-8}

S_a is standard deviation of intercept, S_b is standard deviation of slope, and $S_{y/x}$ is standard deviation of residuals
*Theoretical value of t (a/S_a) = 2.31 at the 95% confidence level

Table 2: HPLC system suitability parameters

Analyte	Retention time (min) R_t	Capacity factor k	Selectivity α	Resolution (R_s)	Tailing Factor (T_f)	Efficiency (plates m^{-1})
SAA	1.662	0.75	---	---	1.08	29150
IS	2.515	1.64	2.28	6.83	1.17	30280
MOM	4.304	3.52	2.18	8.72	1.15	29136

System suitability recommendations: k (1-10), $\alpha > 1$, $R_s > 2$, A_s (0.9-1.2) and plates m^{-1} (>2000)[23]

Table 3: Effect of stress conditions on the degradation of SAA and MOM using proposed HPLC-DAD Method

Stress condition	Temperature(°C)	Time (h)	% degradation		Purity factor	
			SAA	MOM	SAA	MOM
Standard solution	----	----	----	----	998.501	999.588
Acidic (0.1M HCl)	85	2	Stable (96.34%)	39.22%	998.224	999.804
Alkaline (0.1M NaOH)	85	2	Stable (95.41%)	100.00%	997.790	----- (Degradant: 999.742)
Alkaline (0.01M NaOH)	85	2	Stable (96.85%)	16.85%	999.762	998.790
Oxidation (33% H2O2)	85	2	Stable (94.94%)	28.35%	997.945	999.524
Neutral hydrolysis	85	2	Stable (100.44%)	Stable (101.14%)	998.098	999.641
Day light	Room temperature	72	Stable (100.70%)	Stable (95.54%)	998.920	998.596
UV radiation (254 nm)	Room temperature	2	Stable (100.37%)	Stable (100.10%)	999.280	998.681
UV radiation (365 nm)	Room temperature	2	Stable (101.49%)	Stable (101.33%)	999.321	998.565

Table 4. Intraday and Interday precision and accuracy for the determination of SAA and MOM using the proposed HPLC-DAD method

Concentration ($\mu g\ mL^{-1}$)		Mean % recovery ± SD		RSD (%)		Er (%)	
SAA	**MOM**	**SAA**	**MOM**	**SAA**	**MOM**	**SAA**	**MOM**
(a) Intra-day precision and accuracy							
375.00	10.00	$99.43 \pm 1.50 \times 10^{-3}$	$101.22 \pm 1.58 \times 10^{-4}$	0.15	0.16	-0.57	1.22
625.00	10.00	$99.34 \pm 1.13 \times 10^{-3}$	$100.75 \pm 5.77 \times 10^{-4}$	0.11	0.06	-0.66	0.75
500.00	10.00	$101.23 \pm 1.62 \times 10^{-3}$	$101.54 \pm 6.22 \times 10^{-4}$	0.16	0.06	1.23	1.54
500.00	0.00	$99.06 \pm 2.38 \times 10^{-3}$	____	0.15	____	-0.94	____
500.00	7.50	$100.23 \pm 1.13 \times 10^{-3}$	$101.02 \pm 1.49 \times 10^{-4}$	0.07	0.01	0.23	1.02
500.00	12.50	$101.87 \pm 1.32 \times 10^{-3}$	$100.93 \pm 8.76 \times 10^{-4}$	0.08	0.09	1.87	0.93
0.00	10.00	____	$101.21 \pm 3.14 \times 10^{-4}$	____	0.04	____	1.21
(b) Inter-day precision and accuracy							
375.00	10.00	$99.82 \pm 3.03 \times 10^{-3}$	$102.72 \pm 3.68 \times 10^{-4}$	0.25	0.04	-0.18	2.72
625.00	10.00	$99.00 \pm 4.60 \times 10^{-4}$	$102.48 \pm 9.77 \times 10^{-4}$	0.02	0.10	-1	2.48
500.00	10.00	$101.07 \pm 2.45 \times 10^{-3}$	$101.24 \pm 5.84 \times 10^{-4}$	0.15	0.06	1.07	1.24
500.00	0.00	$99.11 \pm 8.04 \times 10^{-4}$	____	0.05	____	-0.89	____
500.00	7.50	$99.97 \pm 4.11 \times 10^{-4}$	$98.63 \pm 1.03 \times 10^{-3}$	0.03	0.10	-0.03	-1.37
500.00	12.50	$101.07 \pm 3.35 \times 10^{-4}$	$100.14 \pm 4.78 \times 10^{-4}$	0.02	0.05	1.07	0.14
0.00	10.00	____	$101.62 \pm 3.68 \times 10^{-4}$	____	0.07	____	1.62

Mean ± standard deviation of three determinations.

Table 5: Robustness of the proposed HPLC-DAD method.

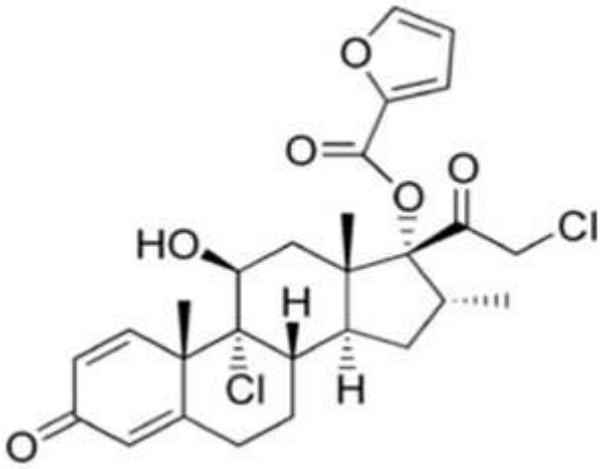
Parameter	SAA		IS	MOM	
	k±SD	RSD% of peak area	*k*±SD	*k*±SD	RSD% of peak area
1) pH of the aqueous phase (2.7, 2.6, 2.5, 2.4, 2.3 and 2.1)	$0.70\pm4.6\times10^{-3}$	$1.56\pm7.0\times10^{-3}$	$1.65\pm5.1\times10^{-3}$	$3.54\pm9.0\times10^{-3}$	$0.68\pm1.4\times10^{-2}$
2) Flow rate of the mobile phase (1.4, 1.5 and 1.6 mL min^{-1})	$0.62\pm3.5\times10^{-3}$	$1.58\pm1.1\times10^{-3}$	$1.54\pm5.6\times10^{-3}$	$3.31\pm7.7\times10^{-3}$	$0.75\pm5.3\times10^{-3}$
3) Organic phase percentage (65, 70 and 75%)	$0.72\pm5.6\times10^{-3}$	$1.57\pm5.3\times10^{-3}$	$1.83\pm7.2\times10^{-3}$	$4.15\pm1.02\times10^{-2}$	$0.76\pm7.1\times10^{-3}$
4) Temperature of the column (23, 25, 27 °C)	$0.69\pm4.8\times10^{-3}$	$1.58\pm5.5\times10^{-3}$	$1.64\pm5.3\times10^{-3}$	$3.52\pm8.9\times10^{-3}$	$0.76\pm6.5\times10^{-3}$
5) Wavelength of detection (257, 258, 259, 260, 261, 262 and 263)	$0.62\pm3.2\times10^{-3}$	$1.66\pm1.9\times10^{-3}$	$1.55\pm5.2\times10^{-3}$	$3.33\pm5.8\times10^{-3}$	$0.70\pm6.0\times10^{-3}$
6) Strength of the aqueous phase (0.019, 0.020 and 0.021 M)	$0.70\pm2.7\times10^{-3}$	$1.60\pm1.9\times10^{-3}$	$1.60\pm3.9\times10^{-3}$	$3.40\pm3.3\times10^{-3}$	$0.74\pm6.9\times10^{-3}$

Table 6: Assay of SAA and MOM in ointment using the proposed HPLC-DAD method

Pharmaceutical preparations	Mean % recovery[a] ± RSD%			
	SAA		MOM	
	HPLC	Reference method [19]	HPLC	Reference method [24]
Elicasal® ointment[b]	99.18 ±0.74	99.57±0.47	99.15±0.70	99.07±0.36
t[c]	0.78		0.17	
F[c]	2.46		3.76	

[a] Results are average of three experiments.
[b] (Batch No. MA102) each gram of Elicasal ointment contains containing 1mg MOM and 50m g SAA.
[c] Theoretical values of t and F are 2.78 and 19, respectively, at 95% confidence limit

(a)



(b)

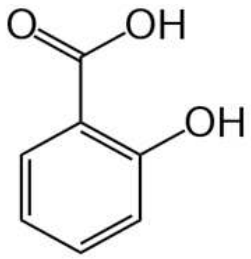


Fig. 1: Chemical structure of MOM (a) and SAA (b).

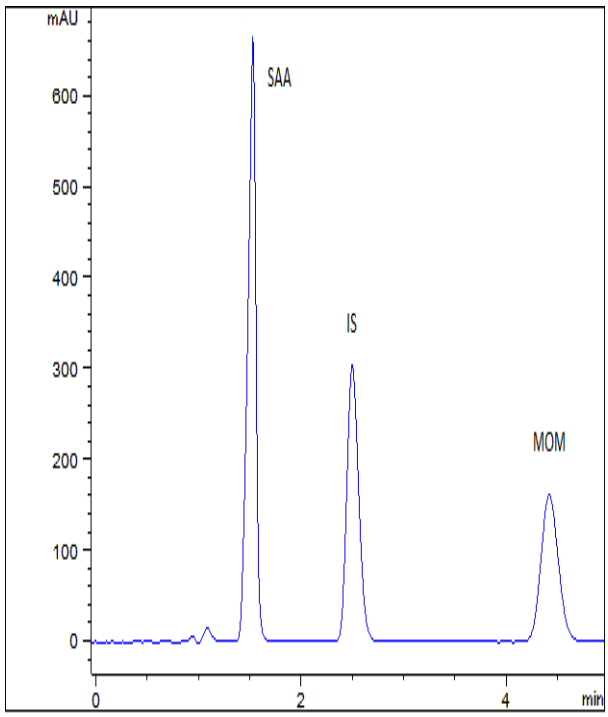
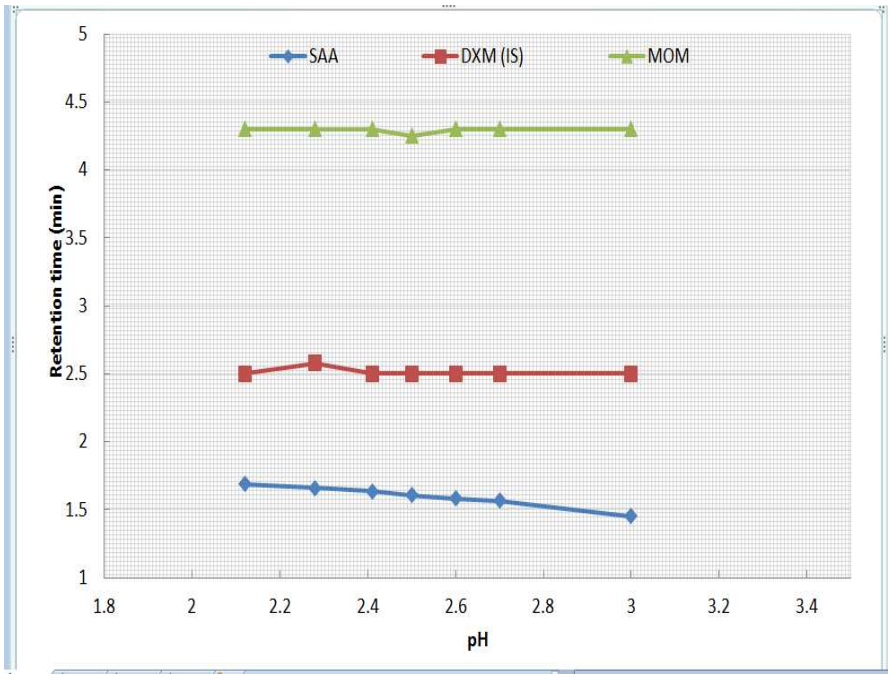


Fig. 2: Typical HPLC chromatogram of 500, 28 and 10 µg mL^{-1} of SAA, IS and MOM, respectively

(a)



(b)

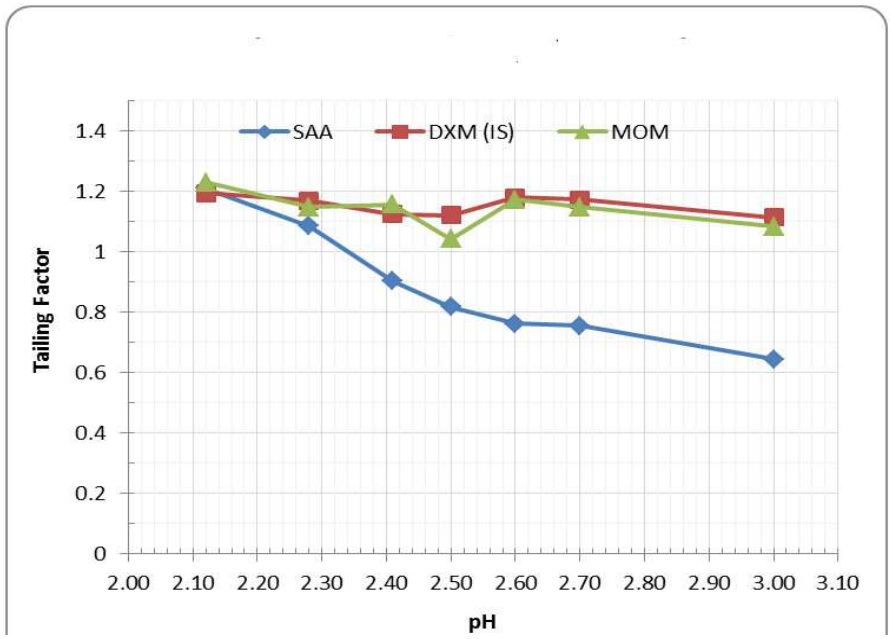


Fig. 3: Effect of pH on (a) retention time and (b) tailing factor of SAA, DXM and MOM peaks using proposed chromatographic method

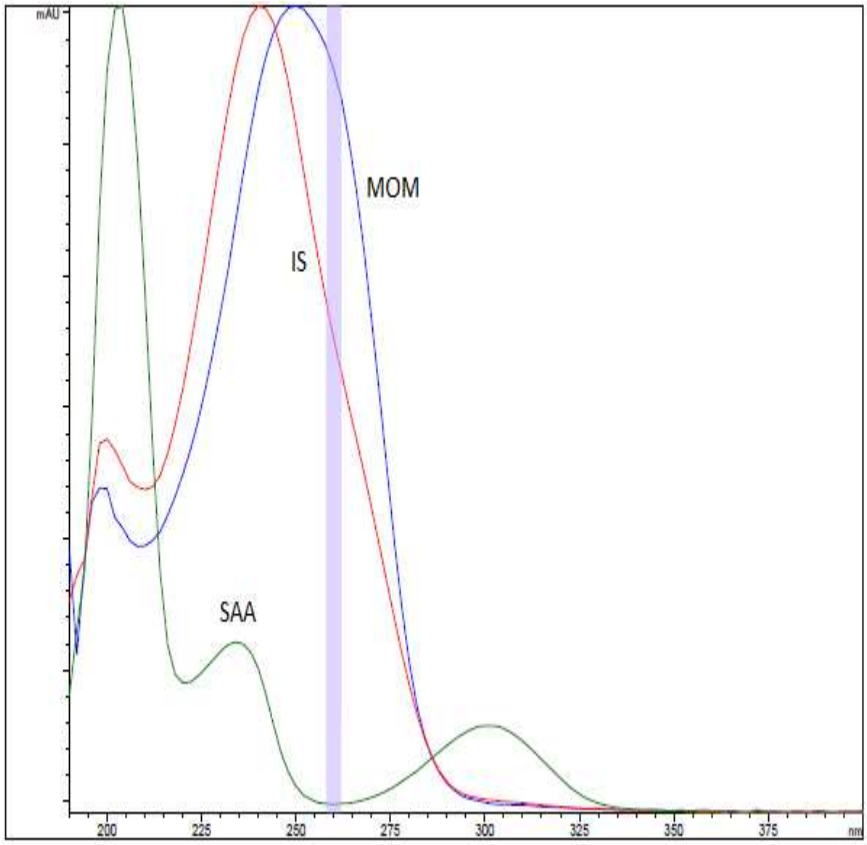


Fig.4: UV absorption spectra of MOM, IS and SAA

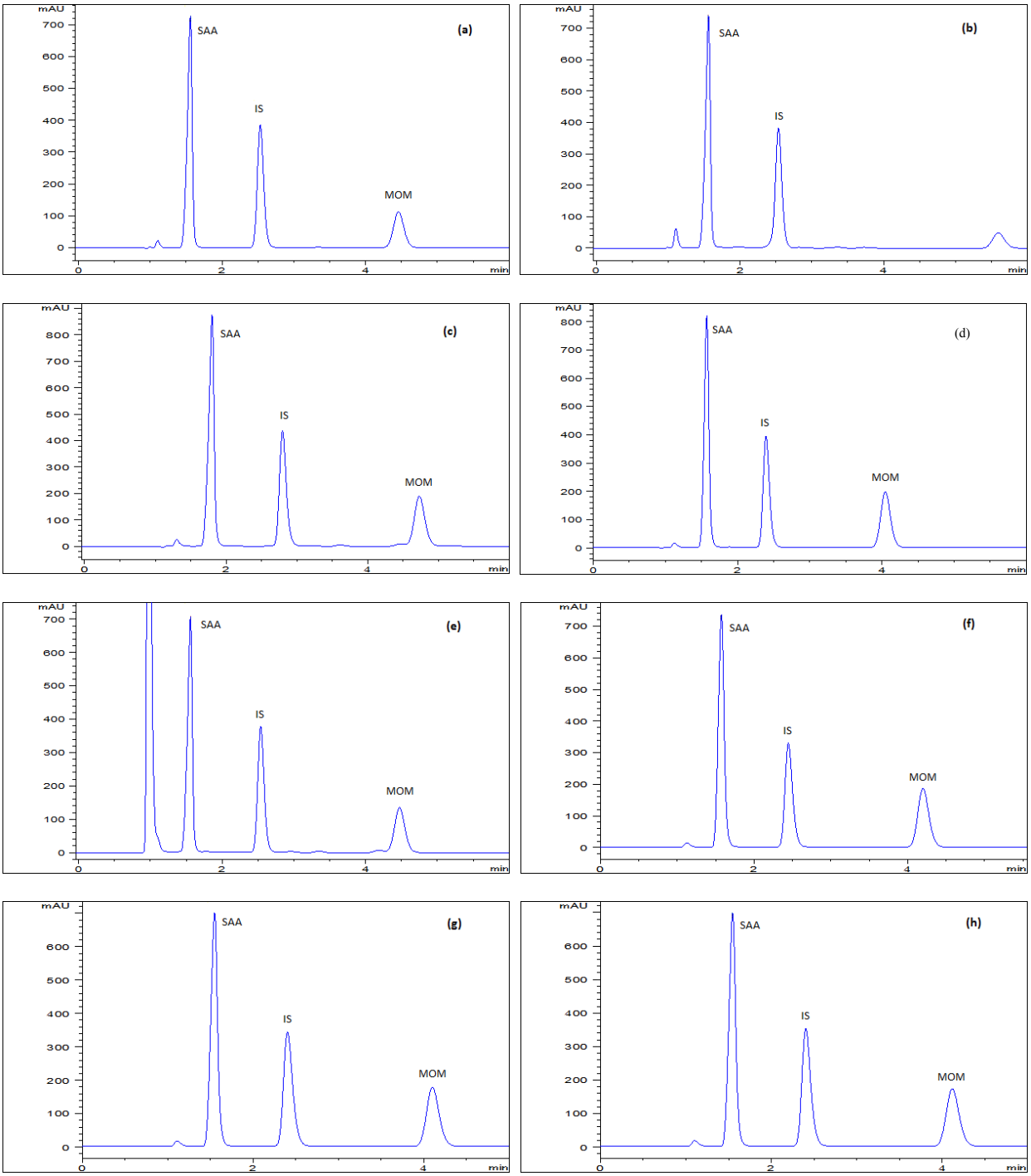


Fig. 5: HPLC chromatogram of 500, 28 and 10 µg mL^{-1} of SAA, IS and MOM, respectively, after acid-induced degradation (a), alkaline-induced degradation by 0.1 M sodium hydroxide (b) , alkaline-induced degradation by 0.01 M sodium hydroxide (c), neutral hydrolytic degradation (d), oxidative-induced degradation (e), photolytic-induced degradation including [day light, (f), UV radiation at 254 nm, (g) and UV radiation at 365 nm (h)]

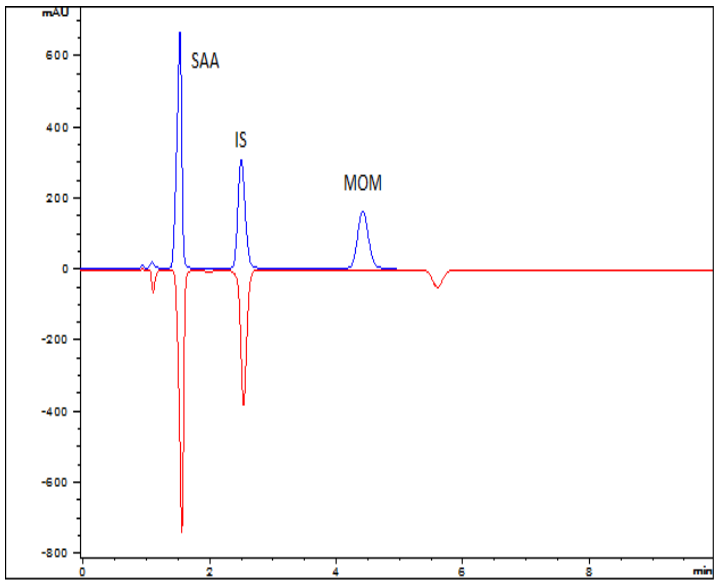


Fig. 6: Overlay signals of standards mixture (above) and standards after alkaline-induced degradation by 0.1 M sodium hydroxide (below).

References

1. Moffat, A.C.; *Clarke's Analysis of Drugs and Poisons* **2011**, Pharmaceutical Press.
2. Sweetman, S.C.; *Martindale: The Complete Drug Reference*. 2011: Pharmaceutical Press.
3. Rang, H.P.; Dale, M.M.; Ritter, A.M.; Flower, R.J.; Lenderson, G. *Rang & Dale's Pharmacology: with STUDENT CONSULT Online Access* **2011**, Elsevier Health Sciences UK.
4. Tripathi, K.D.; *Essentials Of Medical Pharmacology* **2008**, Jaypee Brothers Medical Publishers.
5. Brunton, L.; Blumenthal, D.;Buxton, L.; Parker, K. *Goodman and Gilman's Manual of Pharmacology and Therapeutics* **2007**, Mcgraw-hill.
6. http://www.freepatentsonline.com/EP0735885.pdf, *Formulation approval*.
7. Prakash, A; Benfield,P. Topical mometasone. A review of its pharmacological properties and therapeutic use in the treatment of dermatological disorders. *Drugs* **1998**, *55(1)*: p. 145-63.
8. Rekha, U.; Manjula, B.P.; Formulation and evaluation of microsponges for topical drug delivery of mometasone furoate. *Int J Pharm Pharm Sci.* **2011**, *3(4)*: p. 5.
9. Shaikh, S.; Muneera, M.S.; Thusleem, O.A.; Tahir, M.; Kondaguli, A.V. A simple RP-HPLC method for the simultaneous quantitation of chlorocresol, mometasone furoate, and fusidic acid in creams. *J Chromatogr Sci.* **2009**, *47(2)*: p. 178-83.

10. Srinivasarao, K.; Gorule, V.; Venkata, R.C.; Venkata, K.A. Validated Method Development for Estimation of Formoterol Fumarate and Mometasone Furoate in Metered Dose Inhalation Form by High Performance Liquid Chromatography. *J Anal Bioanal Tech.* **2012**, *3(7).*
11. Kulkarni, A.A.; Nanda, R.K.; Ranjane, M.N.; Ranjane, P.N. Simultaneous estimation of nadifloxacin and mometasone furoate in topical cream by HPTLC method. Der Pharma Chemica. **2010,** *2(3)*: p. 25-30.
12. Shaikh, K.A.;Patil, A.T. Stability-Indicating HPLC Method for the Determination of Mometasone Furoate, Oxymetazoline, Phenyl Ethanol and Benzalkonium Chloride in Nasal Spray Solution. *Journal of Trace Analysis in Food and Drugs* **2013**, *1*: p. 14-21.
13. Ahmad, I.; Vaid, F.H. Determination of benzoic acid and salicylic acid in commercial benzoic and salicylic acids ointments by spectrophotometric method. *Pak J Pharm Sci*. **2009**, *22(1)*: p. 18-22.
14. El-Yazbi, F.A.; Hammud, H.H.; Assi, S.A. Derivative-ratio spectrophotometric method for the determination of ternary mixture of aspirin, paracetamol and salicylic acid. *Spectrochim Acta A Mol Biomol Spectrosc*. **2007**, *68(2)*: p. 275-8.
15. Salinas, F.; Nevado, J.J.B.; Mansilla A.E. A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of salicylic and salicyluric acids. *Talanta.* **1990**, *37(3)*: p. 347-351.
16. Jhariya, A.N.; Joshi, A.; Parashar, A.K.; Nema, R.K. Application of Sodium Citrate As Hydrotropic Agent In Spectrophotometric Analysis of Salicylic Acid. *Int J Recent Adv Pharm Res.* **2013,** *3(1)*: p. 36-38.
17. Commission, B.P., *British Pharmacopoeia: 2012 Edition*. **2011**: Stationery Office.
18. Convention, U.S.P., *USP36 NF31, **2013**: U. S. Pharmacopoeia National Formulary*. **2012**: United States Pharmacopeial Convention.
19. Vanani, D.R.; Desai, S.D.; Patel, K.G.; Shah, P.A. Application of Ratio Derivative Spectrophotometry for Simultaneous, Determination of Mometasone furoate and Salicylic acid in Semisolid dosage form. *International Journal of Analytical and Bioanalytical Chemistry* **2013,** *3(3)*: p. 67-71.
20. ICH, *Validation of Analytical Procedures: Text and Methodology*, International Conference on Harmonisation, Geneva **2005**, (http://www.ich.org/LOB/media/ MEDIA417.pdf).
21. Tonnensen, H.H. *Photostability of Drugs and Drug Formulations*. **1996**: Taylor & Francis, London, UK. pp. 1–7.
22. Armitage, P.; Berry, G. *Statistical Methods in Medical Research, 3 rd ed.* **1994**: Blackwell Scientific Publications, Oxford, England. pp 283-285.
23. *FDA, Center for Drug Evaluation Research (CDER), Reviewer Guidance: Validation of Chromatographic Methods,Washington, USA, (www.fda.gov/cder/guidance/cmc3.pdf).* **1994**.
24. Convention, U.S.P., *The United States Pharmacopeia: USP 30 ; The National Formulary : NF 25*. **2006**: United States Pharmacopeial Convention.