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DoE Approach: A Stability Indicating RP-HPLC Method for Simultaneous Estimation of Methylparaben, Mometasone furoate and Eberconazole nitrate in Topical Formulations

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ABSTRACT

A rapid and sensitive stability indicating RP-HPLC method is developed for the simultaneous estimation of Methylparaben, Mometasone Furoate and Eberconazole Nitrate in topical formulations. Chromatographic separation was achieved on Waters Xterra C18 (150 × 4.6 mm, 5µm) using a mobile phase constituted of water and methanol (35:65, v/v) at a flow rate of 1.50 mL/min and column temperature of 30˚C. All three components were measured with uv detection at 235 nm. Force degradation study was conducted to determine Methylparaben, Mometasone Furoate and Eberconazole Nitrate in the presence of degradants and excipients peaks. Developed method was validated for method precision, specificity, linearity, accuracy, robustness and solution stability as per ICH guidelines. Method is showing linearity in the range of 0.25-188, 0.50-75 and 2.0-750 µg/mL for Methylparaben, Mometasone Furoate and Eberconazole Nitrate respectively. The method was proved to be robust by conducting DOE study. The method is suitable for stability studies, routine analysis and quality control of topical formulations containing these components, either alone or in combination.

INTRODUCTION

Mometasone furoate (MTS) is a glucocorticosteroid used topically in the treatment of inflammatory skin disorders (such as eczema and psoriasis), allergic rhinitis and it has vasoconstrictive properties (Bousquet J, 2009; Tan RA, Corren J, 2008). Chemically, Mometasone furoate is 9,21-Dichloro-11β,17-dihydroxy-16 α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate) and having molecular weight of 521.40. Eberconazole nitrate (EBZ) is an imidazole derivative, used topically in the treatment of superficial fungal infections (Barbanoj MJ et al., 2005). Chemically, Eberconazole nitrate is {1-(2,4-dichloro-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yl)-1H- Imidazole nitrate} and having molecular weight of 392.236. Methylparaben (MP) is the methyl ester of p-hydroxybenzoic acid and having molecular weight of 152. It is a preservative used in a topical products and also used as food preservative (Parabens, FDA). Structures of the components MP, MTS and EBZ are presented in Fig. 1. Literature reveals that MTS is official in US Pharmacopoeia and British Pharmacopoeia. Official methods involve determination of MTS by HPLC (United States Pharmacopoeia-36, British Pharmacopoeia, 2013).

EBZ is not official in any of the Pharmacopoeia. Literature reveals that methods such as HPLC (Shabir GA, 2010; Shaikh, Patil, 2013; Shaikh et al., 2009; Katari Srinivasarao et al., 2012; M Vamsi Krishna et al., 2012; N Sharma et al., 2013), HPTLC (Hiral N Dave et al., 2011; Kulkarni Amol A et al., 2010), Super critical fluid chromatography (Wang Zhenyu et al., 2011), TLC densitometry (Wulandari Lestyo et al., 2003) and In vitro permeation study (Ana Cristina Gomes Barros Salgado et al., 2010) reported for the quantification of MP, MTS and EBZ either alone or in combination with other components.

Combine therapy of Eberconazole nitrate and Mometasone furoate is approved for the treatment of mild to moderate inflamed cutaneous mycoses. It is available in cream formulation. There are no such rapid, sensitive, specific and accurate methods available for the simultaneous estimation of MP, MTS and EBZ in topical formulations.

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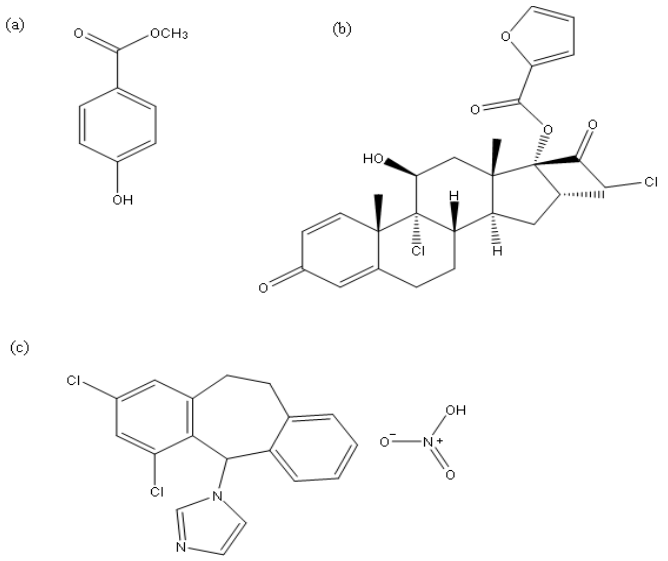

Fig. 1: Chemical structures of molecules a) MP b) MTS & c) EBZ.

The present study was aimed at developing a rapid, sensitive, specific, accurate and precise HPLC method for the simultaneous estimation of MP, MTS and EBZ in topical formulations. Method robustness was proved by conducting DOE study. The method can be suitable for stability studies, routine analysis and quality control of topical formulations containing these components, either alone or in combination.

MATERIAL AND METHODS

Instrumentation

Waters HPLC system containing 2695 separation module, 2996 PDA detector and Empower software for data acquisition were used for the study. XS205 Dual range balance (Make: Mettler Toledo), Orion 3 star pH meter (Make: Thermo electron corporation), Ultrasonic bath (Make: Bandelin Sonorex), Heraeus Biofuge Stratos Centrifuge (Make: Thermo electron corporation), DOE Expert software for the DOE study were used for the research purpose.

Standard, chemicals and reagents

MP (Potency: 99.7%), MTS (Potency: 99.8%) and EBZ (Potency: 99.6%) standards were obtained from Dr. Reddy's Laboratories (Hyderabad, India). Methanol, acetonitrile and tetrahydrofuran of HPLC grade were obtained from RFCL, India. Distilled water was obtained from a Milli-Q system, Millipore (Milford, MA).

PTFE and Nylon syringe filters of 0.45 µm porosity were obtained from MDI, India. Topical products of MTS and EBZ were purchased from market.

Chromatographic condition

The isocratic mobile phase consisted of a combination of water and methanol in the ratio 35:65 (v/v) was run at 1.50 mL/minute throughout analysis. A Waters Xterra C18, 150 × 4.60 mm, 5 µm was used as stationary phase. UV detection at 235nm, column temperature as 30˚C, injection volume as 10 µL and run time of 12 minutes was set as chromatographic conditions.

Extracting solvent (diluent)

Acetonitrile and methanol in the ratio of 80:20, (v/v) was used as diluent.

Standard solution preparation

Accurately weighed and transferred about 50 mg of MP, 20 mg of MTS and 240 mg of EBZ into a 100 mL volumetric flask. Added 50 mL of diluent, sonicated for 5 minutes to dissolve it and made up to volume with diluent. Transferred 5 mL of this solution into another 20 mL volumetric flask, added 2 mL of tetrahydrofuran and made up to volume with diluent to obtain a solution containing 125 µg/mL, 50 µg/mL and 500 µg/mL of MP, MTS and EBZ respectively.

Estimation from formulations

Accurately weighed and transferred about 2.5 g of cream/lotion sample in to a 50 mL volumetric flask. To this, added 5 mL of Tetrahydrofuran and sonicated for 5 minutes with intermittent shaking to disperse the sample. To this, again added 25 mL of diluent and sonicated for 15 minutes to extract drugs from the sample. Allowed the solution to cool at room temperature and made up to volume with diluent. Centrifuged a portion of this solution at 5000 rpm for 10 minutes, collected supernant solution, filtered through 0.45 µm PTFE filter and injected into HPLC.

Quantification

Peak response of all peaks in standard and samples were recorded. Respective peak areas were taken into account to calculate % assay of individual component by using following formula:

% Assay of MP/MTS/EBZ = Au/As ×Ws/100 × 5/20 × 50/Wt × P/100 × 100/L.C.× 100 ×F

Where Au is peak area obtained from MP/MTS/EBZ in the sample solution; As is peak area obtained from MP/MTS/EBZ in the standard solution; Ws is weight of corresponding MP/MTS/EBZ taken in mg to prepare standard solution; Wt is weight of cream/lotion sample taken in mg to prepare sample solution; P is % purity of respective MP/MTS/EBZ standard and L.C. is label claim of respective MP/MTS/EBZ component in %. F is the factor (0.84) for conversion of Eberconazole nitrate to Eberconazole. While for other components, F value considered as 1.

RESULTS AND DISCUSSION

Method development and optimization

Optimum wavelength of 235 nm was selected based on sufficient response of MP, MTS and EBZ and less base line noise during chromatographic run. During optimization of conditions, different stationary phases along with different mobile phase compositions were tried to achieve desired system suitability

parameters as well as good separation of MP, MTS and EBZ peaks. Different C8 and C18 columns along with mobile phase constituted of different ratio of water/buffer, acetonitrile and methanol were tried to get desired separation of all peaks. Mobile phase constituted of acidic pH buffer and acetonitrile was shown that EBZ was eluted early with broader and splitted peak shape while no impact on retention of MP and MTS peaks was observed. MP peak was co-eluted with placebo peaks in case of Hypersil BDS C8 column. Good separation of MP, MTS and EBZ peaks was achieved by using Xterra C18, 150 × 4.6 mm, 5µm as stationary phase and mobile phase constituted of water and methanol in the ratio 35:65 (v/v).

DoE study was conducted with optimized mobile phase composition, flow rate and column temperature as critical variables and measured responses in the terms of theoretical plate count of all peaks and separation of all peaks. DOE study revealed that mobile phase composition, flow rate and column temperature are significantly impacting on theoretical plate counts and separation of all peaks while combined effect of these variables showing less impact on above responses. Optimized chromatographic conditions as per DoE study are mobile phase constituted of water and methanol in the ratio of 35:65 (v/v), 1.50 mL/minute of flow rate with isocratic mode and column temperature of 30˚C which are showing operating design space with respect to robustness of the method (Fig. 2). Injection volume was scaled up to 10 µL for sufficient response and good peak shape.

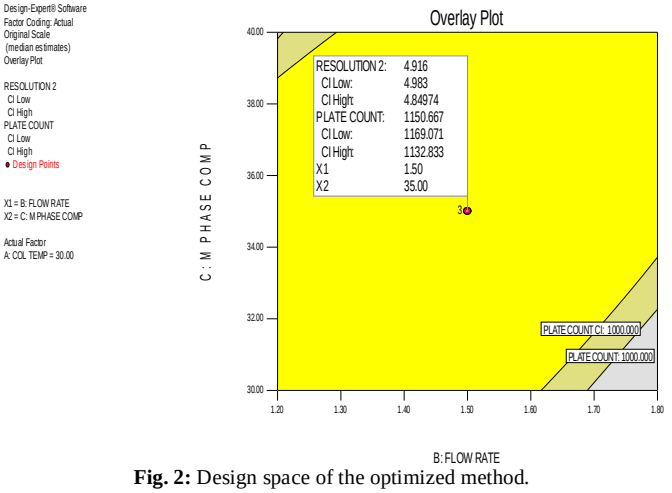

Fig. 2: Design space of the optimized method.

Recovery of the drugs from the topical formulation is challenging compared to other conventional dosage forms due to complex matrix and lower strength of drugs. MP, MTS and EBZ are soluble in acetonitrile, methanol and tetrahydrofuran. To get desired recovery of all components, added tetrahydrofuran in sample preparation to break the cream/lotion sample matrix followed by addition of mixture of acetonitrile and methanol (80:20, v/v) to extract MP, MTS and EBZ from sample matrix. By finalized chromatographic conditions, MP, MTS and EBZ were eluted about 1.8, 5.6 and 9.0 minutes respectively (Fig. 3).

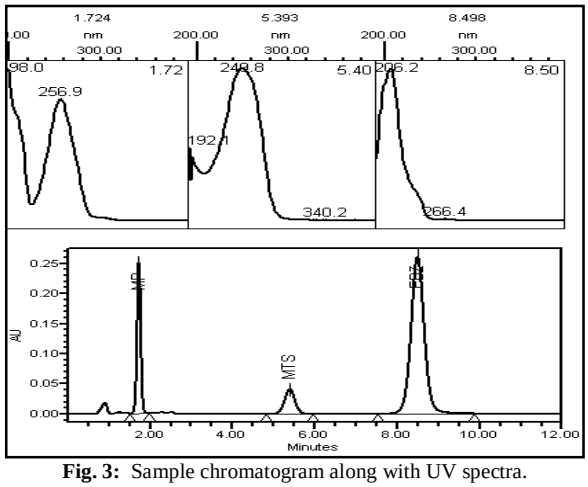

Fig. 3: Sample chromatogram along with UV spectra.

Market samples of topical formulations were analyzed by proposed method and results are presented in Table 1.

Table. 1: Assay results of market formulations.

Sample description	MP	MTS	EBZ
Eberconazole + Mometasone cream (n=3)	99.2 ± 0.88	96.6 ± 1.14	97.4 ± 1.09
Eberconazole cream (n=3)	97.8 ± 0.84	-	99.7 ± 0.82
Mometasone lotion (n=3)	-	105.5 ± 0.90	-

%Assay ± RSD

Method validation

The test method for the simultaneous estimation of MP, MTS and EBZ was validated for parameters like system precision, specificity, method precision, linearity, accuracy, ruggedness, solution stability and robustness as per International Conference on Harmonization guidelines (ICH guideline, Q2).

System precision

Prepared standard solution containing MP, MTS and EBZ and injected five injections into HPLC as per test method. Measured the response of MP, MTS and EBZ in all standard injections. % RSD of peak area of MP, MTS and EBZ from five injections of standard was found to be 0.07%, 0.26% and 0.31% respectively. Results indicated that system is precise.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its degradants and excipients peaks. Cream samples were exposed for acid hydrolysis, base hydrolysis, oxidation reaction by H_2O_2, photo oxidation and thermal degradation. Stressed samples were injected into HPLC with PDA detector as per test method and evaluated for % degradation and peak purity of MP, MTS and EBZ peaks. Peak purity of MP, MTS and EBZ peaks were passed in all stressed samples, this indicate that method is capable to estimate MP, MTS and EBZ in the presence of degradants and excipients peaks (Fig. 4), enabling as stability indicating power of the method. % degradation and peak purity results of all components are presented in Table 2.

Table 2: Results of force degradation study.

Stress conditions	MP			MTS			EBZ		
	% Deg.	PA	PTH	% Deg.	PA	PTH	% Deg.	PA	PTH
Acid Hydrolysis (0.1 N HCl, 1 mL, RT 1 min)	1.6	0.048	1.050	Nil	0.454	1.619	3.1	0.869	1.829
Base Hydrolysis (0.1 N NaOH, 1 mL, RT 1 min)	Nil	0.099	1.083	Nil	1.713	4.350	58.2	2.893	6.417
Oxidation(30% H2O2, 2.50 mL, 60°C 2hr)	5.0	0.077	1.026	2.0	0.187	1.305	4.6	0.116	1.096
Photo degradation(1.2 million lux hours)	Nil	0.116	1.024	4.6	0.178	1.256	1.5	0.099	1.084
Thermal degradation(60°C, 24 hrs)	1.1	0.081	1.028	1.4	0.179	1.309	Nil	0.175	1.115

Peak purity criteria: PA < PTH

Table 3: Results of Repeatability, Inter-day precision, LOD, LOQ and Linearity.

Parameters	MP	MTS	EBZ
Repeatability			
Mean % Assay (n=6)	99.1	96.5	97.1
% RSD: NMT 2.0%	1.0	1.1	1.0
Inter-day precision			
Mean % Assay (n=6)	99.9	95.6	96.4
% RSD: NMT 2.0%	1.3	1.0	0.8
LOD (µg/mL)	0.08	0.15	0.70
LOQ (µg/mL)	0.25	0.50	2.00
Linearity			
Linearity equation	y=13080x – 2032	y=14005x + 1299	y=12588x – 19158
Correlation coefficient (r)	0.99999	0.99995	0.99998

Table 4: Results of recovery study.

Conc. Level	Component	Added conc. (µg/mL)	Recovered conc. (µg/mL)	% Recovery	% RSD
At 50% (n=3)	MP	63.0	61.8	98.0	0.47
	MTS	25.1	24.7	98.4	0.81
	EBZ	250.5	247.4	98.8	0.68
At 100% (n=3)	MP	126.0	125.6	99.7	0.32
	MTS	50.2	49.9	99.5	0.12
	EBZ	501.0	498.2	99.4	0.58
At 150% (n=3)	MP	189.0	187.0	99.0	0.16
	MTS	75.3	74.7	99.2	0.20
	EBZ	751.5	746.0	99.3	0.16

Table 5: Results of Robustness study.

	System suitability parameters								
	EBZ			MTZ			MP		
Parameters	**PA**	**TP**	**%RSD**	**PA**	**TP**	**%RSD**	**PA**	**TP**	**%RSD**
Flow rate									
1.30 mL/min	1.1	3303	0.62	1.0	2162	0.43	0.9	1574	0.33
1.50 mL/min	1.0	3405	0.31	1.0	2105	0.26	1.0	1600	0.07
1.70 mL/min	1.1	3000	0.30	1.0	1949	0.38	0.9	1413	0.18
Col. Temperature									
25˚C	1.1	3054	0.24	1.0	1986	0.48	0.9	1446	0.32
30˚C	1.0	3405	0.31	1.0	2105	0.26	1.0	1600	0.07
35˚C	1.1	3216	0.82	1.0	2126	0.95	0.9	1491	0.83
Mobile phase comp. (Water: Methanol)									
30 : 70, % v/v	1.1	3007	0.31	1.0	1914	1.79	0.9	1361	1.02
35 : 65, % v/v	1.0	3405	0.31	1.0	2105	0.26	1.0	1600	0.07
40 : 60, % v/v	1.0	2753	0.40	1.0	2245	0.46	1.0	1766	0.41

PA(Peak Assymetry): NMT 2.0, TP(Theoretical plates): NLT 1000, %RSD: NMT 2.0

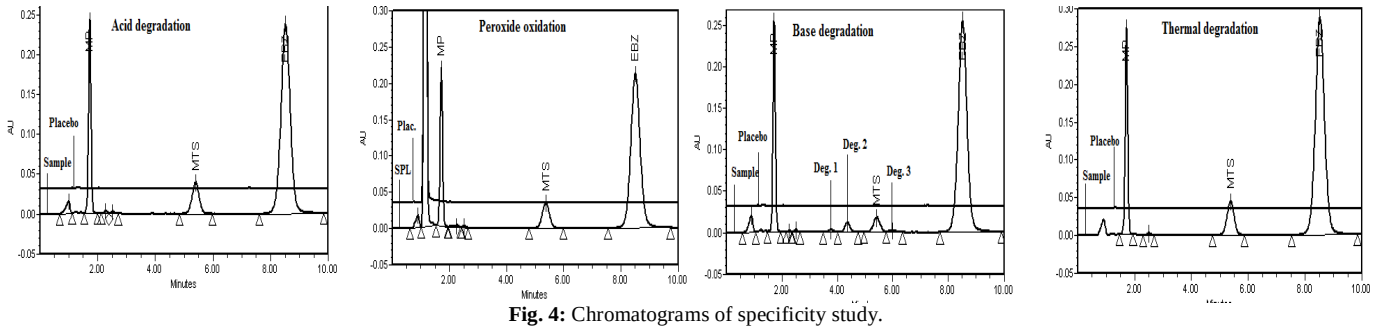

Fig. 4: Chromatograms of specificity study.

Precision

Method precision or intra-day precision was performed by preparation of six sample preparations and injected into HPLC as per test method. Calculated % assay of MP, MTS and EBZ in each of the sample preparation and % RSD for the assay of six sample preparations. Intermediate precision was performed by preparation of six sample preparations by different analyst using different HPLC system and HPLC column as per test method. Calculated % assay of MP, MTS and EBZ in each of the sample preparation and % RSD for the assay of six sample preparations. Intraday and intermediate precision results are presented in Table3

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

LOD is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as exact value while LOQ is the lowest amount of analyte in sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ for MP, MTS and EBZ were determined as per ICH guideline (Fig. 5). LOD and LOQ values for MP, MTS and EBZ are presented in Table 3.

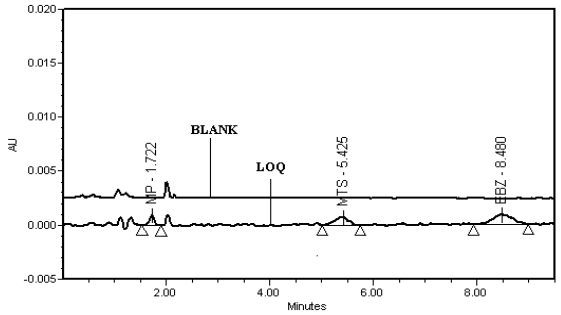

Fig. 5: Overlaid chromatograms of blank and LOQ solution.

Linearity

Peak area versus concentration in µg/mL were plotted for MP, MTS and EBZ at the concentration range between LOQ and 150% of the target level (Fig. 6). Correlation co-efficients of linearity plots were found more than 0.999 for all three components, this indicate linear behavior of the method in established concentration range. MP, MTS and EBZ showed linearity in the range 0.25-188, 0.50-75 and 2.0-750 µg/mL respectively. Linear regression equations and correlation coefficient are presented in Table 3.

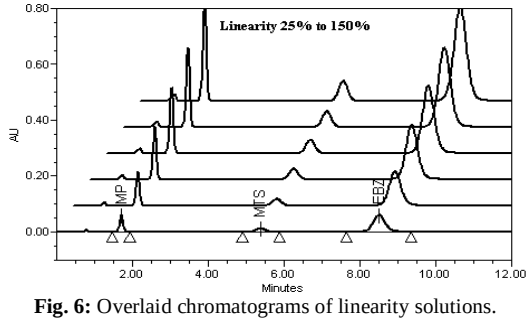

Fig. 6: Overlaid chromatograms of linearity solutions.

Accuracy

Accuracy of the proposed method was evaluated by spiking standard stock solution containing MP, MTS and EBZ into placebo equivalent to amount present in sample preparation to achieve 50%, 100% and 150% of the target concentration. Measured recovered concentration versus added concentration for MP, MTS and EBZ and calculated % recovery. % recovery for all components were found more than 97.0%, this indicate accuracy of the method. Recovery results are tabulated in Table 4.

Robustness

Robustness of the propose method was performed by keeping chromatographic conditions constant with the following differences: (a) Changing mobile phase flow rate from 1.30 mL/min to 1.70 mL/min (b) Changing column temperature from 25°C to 35°C (c) Changing mobile phase composition from water: methanol (30:70, %v/v) to water:methanol (40:60, %v/v). Standard solution was injected five times for each minor change and evaluated for system suitability parameters like peak asymmetry, theoretical plates and % RSD. System suitability parameters were found to be within acceptance criteria for MP, MTS and EBZ peaks, this indicate that method is robust with respect above mentioned conditions. The results are presented in Table 5.

Solution stability

Prepared standard and sample solution and injected into HPLC as per test method. Kept standard and sample solution on bench top at room temperature and again reanalyzed after 24 hrs against freshly prepared standard. Calculated similarity factor for standard and % difference in assay of MP, MTS and EBZ for the sample against initial results. Similarity factor of standard for all three components were found between 0.98 and 1.02, while % difference in assay of all three components in samples were found less than 2.0%, this indicate that standard and sample solutions are stable at room temperature for 24 hrs.

CONCLUSION

The proposed HPLC method is rapid, specific, sensitive, accurate and precise for the simultaneous determination of MP, MTS and EBZ from topical formulations. The method is capable to determine MP, MTS and EBZ in the presence of degradants and excipients peaks of formulations in all stressed conditions, enabling as stability indicating power of the method. The proposed method was proved to be robust by conducting DOE study. The proposed method is suitable for stability studies, routine analysis and quality control of topical formulations containing these components, either alone or in combination.

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