DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF SERTACONAZOLE NITRATE AND MOMETASONE FUROATE IN TOPICAL FORMULATIONS

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ABSTRACT

High-performance liquid chromatography (HPLC) method was successfully developed and validated for simultaneous estimation of Sertaconazole nitrate and Mometasone furoate in their combined dosage form. Chromatographic separation was achieved on a C18 (250 mm × 4.6 mm, 5 µm particle size), using Acetonitrile: 0.01M Sodium Dihydrogen Phosphate (70:30 v/v) (pH 4.69) as mobile phase at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 240 nm. The Sertaconazole nitrate and Mometasone furoate show linearity in the range of 100–500 µg/ml and 5–25 µg/ml respectively. The retention times of Sertaconazole nitrate and Mometasone furoate were found to be 9.929 min and 4.862 min respectively. The developed method was evaluated for precision, accuracy and robustness parameters also. The intraday and inter day precision data found to be less than 2 % RSD showing the method is precise. The accuracy study was performed using standard addition technique and found between 98–102 %. The Limit of Detection was found to be 0.172 and 0.046 for Sertaconazole nitrate and Mometasone furoate respectively. The limit of Quantitation was found to be 0.522 and 0.139 for Sertaconazole nitrate and Mometasone furoate respectively. The % RSD for robustness study was also found below 2%, indicates that the method is robust.

KEY WORDS: Sertaconazole nitrate, Mometasone furoate, RP-HPLC, Simultaneous estimation.

➢ INTRODUCTION

Sertaconazole nitrate is anti-fungal agent. blocks the synthesis of ergosterol by inhibiting the 14α-demethylase enzyme. Mometasone furoate is Glucocorticoids and the mechanism of action of Mometasone furoate is a corticosteroid hormone receptor agonist. Clinically a combination of Sertaconazole nitrate and Mometasone furoate is being used in treatment of fungal skin infections. Sertaconazole nitrate (STZN) is a chemically 1-{2-[(7-chloro-1-benzothiophen-3-yl) methoxy]-2-(2,4-dichlorophenyl) ethyl}-1H-imidazole; nitric acid [1-2]. Mometasone furoate is a chemically 9α,21-Dichloro-11β-hydroxyl-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate [3-4]. Literature survey revealed that there are only RP - HPLC and one HPTLC method was available for this combination. There are several chromatographic [5-15] available to quantify this drug in combination with other drugs in dosage form. So, there is extreme need to develop simple, sensitive, precise and accurate method to quantify these drugs simultaneously.

Fig. 1 Structure of Sertaconazole nitrate

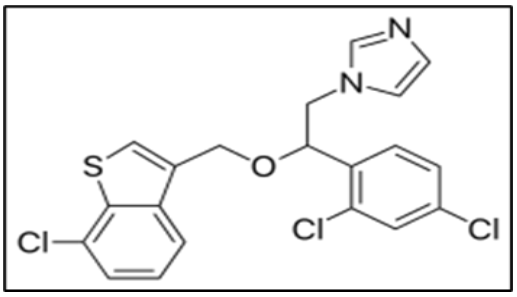
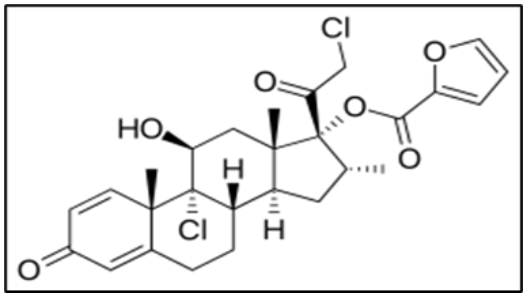


Fig. 2 Structure of Mometasone furoate



➢ MATERIALS AND METHODS:

- **Solvents and Chemicals**

HPLC grade Acetonitrile HPLC grade, Methanol HPLC Grade, Ortho phosphoric acid (HPLC Grade), Potassium dihydrogen Phosphate Buffer, HPLC grade water (milli-pore) was used in the study.

Sertaconazole Nitrate and Mometasone furoate were procured from Triveni Pharmaceutical Ltd, Vapi, India. Acetonitrile, Methanol (Merck, India) and phosphoric acid (Fisher Scientific) were of HPLC grade. Triple distilled water was used. Potassium dihydrogen phosphate was of analytical grade (S. D. fine chemicals Ltd., Mumbai, India). Onabet SD Topical Solution was manufactured by Glenmark Pharmaceutical Ltd. Each tablets claimed to contain 2% w/v STZN and 0.1 % w/v MOF.

- **Apparatus and Instrument**

Model: Chrome Leon Console (Thermo Scientific), Column: C18 (250 mm × 4.6 mm, 5.0 µm), Injector: Auto injector, Detector: UV Detector, Software: LC solution, Electronic analytical balance (GR 202), Digital pH meter (Seven compact pH system), Ultrasonic cleaner (DTL 08 HEXATEC), Filter paper: Vacuum filter: Membrane filter 0.45-micron, Syringe filter: Membrane filter 0.27-micron, Volumetric flask and pipettes

- **Reagent and Materials**

Mometasone Furoate (Gift sample, Sumit Laboratories, VAPI), Sertaconazole Nitrate, Acetonitrile (HPLC grade, Merck), Methanol (HPLC grade, Merck), Water (Milli-Q-Water), Orthophosphoric acid (HPLC grade Fisher Scientific), Sodium dihydrogen Phosphate (Finar Extra pure)

- **Selection of Wavelength**

Aliquots of 2.5 ml from working solution of MOF (100 µg/ml) and 2.5 ml from standard stock solution of STZN (1000 µg/ml) were pipette out into two separate 10 ml of volumetric flask and volume was made up to the mark with methanol to get 60 µg/ml of MOF and 5 µg/ml of STZN. Each Solutions of MOF and STZN were scanned between 200-400 nm using UV-Visible Spectrophotometer. Wavelength was selected from the overlay spectra of above solutions

➢ PREPARATION OF STANDARD AND WORKING SOLUTION:

1. Preparation of MOF standard stock solution (1000 µg/ml)

10 mg of MOF was weighed and transferred to 10 ml volumetric flask. It was dissolved in acetonitrile and volume was made up to the mark with acetonitrile to give a solution containing 1000 µg/ml.

2. Preparation of MOF working stock solution (100 µg/ml)

Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with acetonitrile.

3. Preparation of STZN standard stock solution (1000 µg/ml)

10mg of STZN was weighed and transferred to 10 ml volumetric flask. It was dissolved in acetonitrile and volume was made up to the mark with acetonitrile to give a solution containing 1000 µg/ml.

4. Preparation of STZN working stock solution (100 µg/ml)

Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with acetonitrile.

5. Preparation of Binary Mixture of STZN and MOF

Aliquots of 1ml from standard solution of STZN 1000 µg/ml and 1ml from standard solution of MOF 1000 µg/ml were taken into common volumetric flask and diluted up to 10 ml with mobile phase to make final concentration STZN and MOF.

- **Selection of Mobile Phase:**

The mobile phase selection was based on the observation like best peak separation, theoretical plate, resolution, peak symmetry etc. So, number of trials was taken for the selection of mobile phase.

- **VALIDATION OF PROPOSED HPLC METHOD:**

1. System suitability

Evaluation of system suitability was done by analyzing five replicates of STZN and MOF in a mixture of concentration of STZN and MOF. The column efficiency, peak asymmetry and resolution were calculated for each replicate.

2. Specificity

Specificity involves quantitative detection of analytes in the presence of those components that may be expected to be part of sample matrix. Specificity of developed method was established by spiking of STZN and MOF in placebo and expressing that analytical peak was not interfered from excipients.

3. Linearity

The linearity response was determined by analyzing five independent levels of concentration in the range of 5-60 µg/ml for STZN and MOF respectively.

Preparation of calibration curves

Calibration curve for MOF:

Calibration curve for MOF consisted of five different concentrations solution ranging from 5-60 µg/ml. Calibration curve of peak area vs concentration was plotted and regression equation determined.

Calibration curve for STZN:

Calibration curve for STZN consisted of five different concentrations solution ranging from 5-60 µg/ml. Calibration curve of peak area vs concentration was plotted and regression equation determined.

4. Precision

a) Repeatability

Repeatability of the developed method was assessed by analyzing samples from the same batch 5 times with standard solution containing concentration for STZN and MOF and %RSD was calculated.

b) Intraday

It was assessed by analyzing samples from the same batch with 3 sample solutions containing concentrations for STZN and MOF. Solutions were analyzed thrice on the same day within short interval of time and %RSD was calculated.

c) Interday

It was assessed by analyzing samples from the same batch with 3 sample solutions containing concentrations for STZN and MOF. Solutions were analyzed thrice on the three different day and %RSD was calculated.

5. Accuracy

6. LOD and LOQ

The LOD was assessed from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula:

LOD = 3.3 * S.D./SLOPE

Where, S.D. = Standard deviation of the Y-intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

The LOQ was assessed from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula:

LOQ = 10 * S.D./SLOPE

Where, S.D. = Standard deviation of the Y-intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

- **SIMULTANEOUS ESTIMATION OF MOMETASONE FUROATE AND SERTACONAZOLE NITRATE IN TOPICAL FORMULATION**

Topical formulation equivalent to 500 mg of MOF was taken into 25 ml of volumetric flask and added acetonitrile, the solution was ultrasonicated for 15 mins and was make up with acetonitrile. From the above solution 0.5 ml was pipette out and transferred to 10ml volumetric flask and volume was made up mark with acetonitrile to give a solution STZN and MOF.

RESULT AND DISCUSSION:

- **SELECTION OF MOBILE PHASE:**

The mobile phase should be sufficiently transparent at the detection of wavelength. Acetonitrile and sodium dihydrogen phosphate are the solvents used for mobile phase provided optimum polarity for proper separation and resolution for Mometasone Furoate and Sertaconazole Nitrate. The ratio selected is Acetonitrile and Sodium dihydrogen phosphate 70:30 v/v.

Fig. 3 Chromatograph of Sertaconazole Nitrate

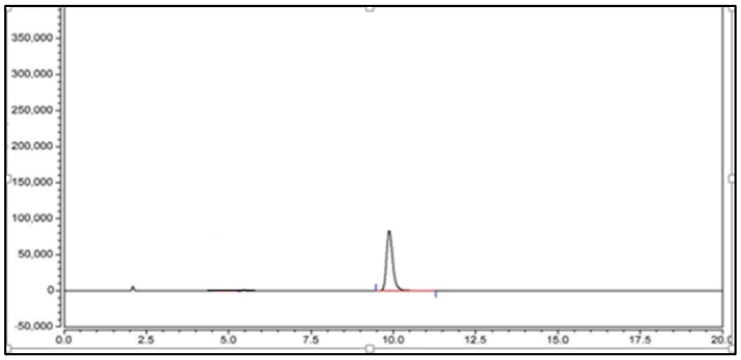


Fig. 4 Chromatograph of Mometasone Furoate

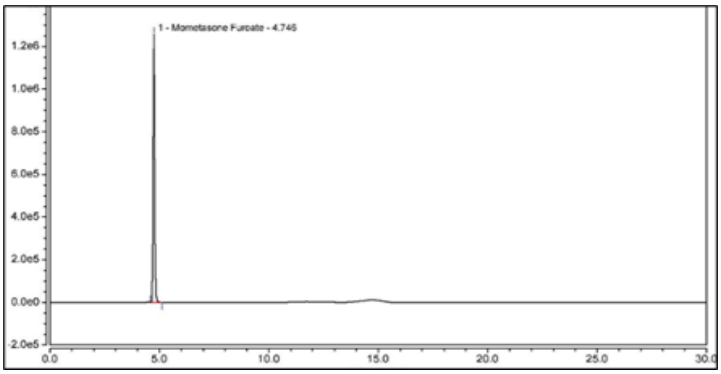
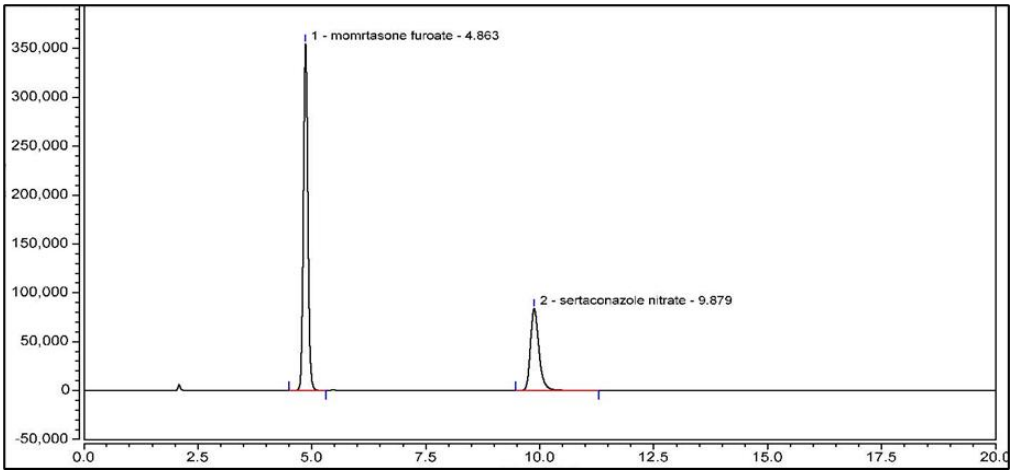


Fig. 5 Chromatograph of Mometasone Furoate and Sertaconazole Nitrate in Acetonitrile: 0.01M Sodium Dihydrogen Phosphate Buffer (pH 4.69); (70:30 v/v)



DEVELOPMENT OF METHOD

After optimization of mobile phase, final optimized chromatographic conduction is as given below in the table.

Table. 1 Optimized chromatographic condition

Sr. No.	Parameter	Condition
1	Mobile Phase	Acetonitrile: 0.01 Sodium Dihydrogen Phosphate pH adjusted with 4.69 (60:40 v/v)
2	Flow Rate	1 ml/min
3	Run Time	20 min
4	Volume Of Injection	10 µL
5	Detection Wavelength	240 nm

1. System Suitability Data:

Table. 2 System suitability data for MOF and STZN

Drugs	Parameters	Mean ± S.D	%RSD
MOF	Retention Time	4.8692 ±0.00164	0.0337
	Theoretical Plates	11663.6±46.65	0.3999
STZN	Retention Time	9.9442±0.003492	0.0351
	Theoretical Plates	53211.2±72.967	0.430
	Resolution	19.69±0.1354	0.687

2. Specificity

The specificity of the method was determined by analyzing standard drugs and sample of MOF and STZN. The results suggested that proposed method is specific, the excipients present in the synthetic mixture does not affect the result. The chromatogram taken by running only with the mobile phase and after injection of the sample are given in Fig 6 and 7.

Fig. 6 Calibration curve of MOF

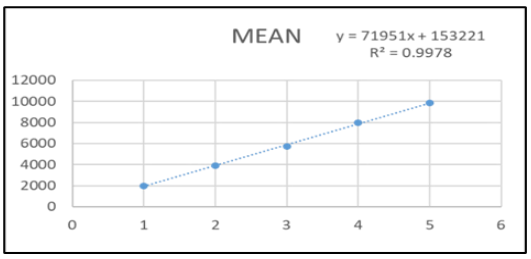


Fig. 7 Calibration curve of STZN

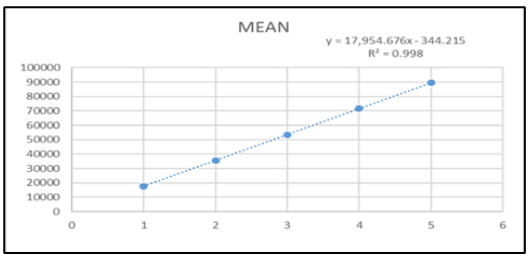


Fig. 8 Chromatogram of Mobile Phase

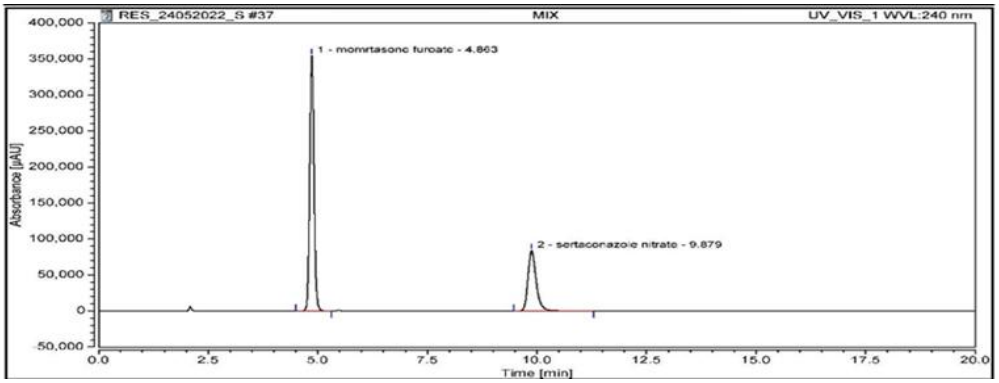
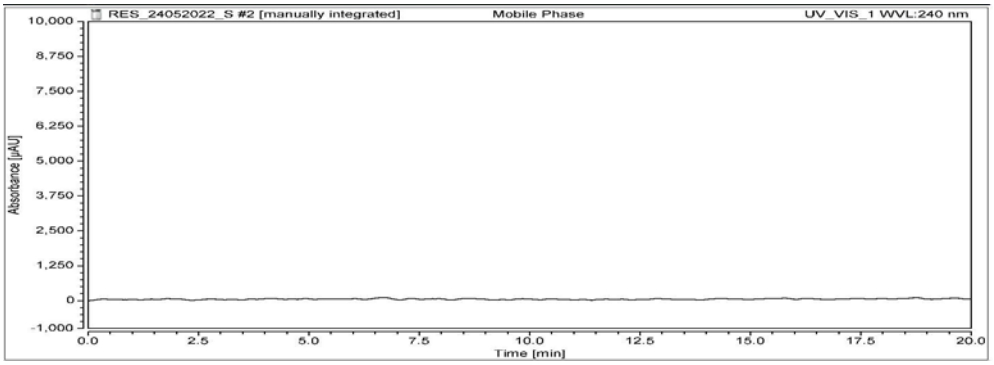


Fig. 9 Chromatogram of Sample



3. Linearity

The linearity study was carried out for both drugs at five different concentration levels. The linearity of MOF was in the range 5-25 µg/ml and STZN was in the range of 100-500 µg/ml.

Table. 3 Linearity data for MOF and STZN

Sr. No.	MOF			STZN		
	Conc (µg/mL)	Mean Peak Area ± S.D. (n=5)	% R.S.D.	Conc (µg/mL)	Mean Peak Area ± S.D. (n=5)	% R.S.D.
1	5	1981.5360±11.450	0.577	100	17728.8795±139.125	0.784
2	10	3930.4327±31.55	0.802	200	35525.1141±205.852	0.579
3	15	5750.05809±28.52	0.496	300	53299.3857±241.903	0.453
4	20	7955.2316±13.51	0.169	400	71561.7299±339.258	0.474
5	25	9848.4575±49.66	0.504	500	89483.9503±439.143	0.490

4. Precision

a) Repeatability

The data of repeatability for MOF and STZN are below in

Table. 4 Repeatability data for MOF and STZN

Drugs	Concentration (µg/ml)	Mean peak area ± S.D.	%RSD
MOF	20	7643.685433±44.69	0.791
STZN	400	73139.85093±107.29	0.201

b) Intraday and Interday precision

The data for intraday and interday precision for MOF and STZN are below in

Table. 5 Intraday & Interday precision for MOF and STZN

Drug	Concentration (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± S.D (n=5)	% RSD	Mean ± S.D (n=5)	% RSD
MOF	10	3859.7953 ±28.089	0.727	3970.16±5.0395	0.126
	15	5672.8709±48.318	0.851	5795.99±34.1002	0.588
	20	7993.2474±34.407	0.430	8113.04±65.714	0.809
STZN	200	34246.14547±321.385	0.938	34246.14547±272.553	0.772
	300	53188.71563±192.379	0.361	53188.71563±521.881	0.976
	400	71554.7501±574.145	0.802	71721.97747±577.502	0.805

5. Accuracy

Table. 6 Accuracy data for MOF and STZN

Drug	Level	Amount of sample (µg/ml)	Amount of std spiked (µg/ml)	Total amount	Mean Peak Area ± S.D. (n=3)	Amount of sample found (µg/ml)	Mean % Recovery
MOF	0%	20	-	20	15914.8931 ± 248.55	20.80	98.24
	80%	20	16	36	13676.8538 ± 24.77	35.98	99.33
	100%	20	20	40	15265.7743 ± 25.00	39.11	99.63
	120%	20	24	44	16977.1433 ± 76.77	43.55	100.27
STZN	0%	400	-	400	7158.2917 ± 50.04	389.42	98.02
	80%	400	320	720	7523.0064 ± 17.658	718.92	99.44
	100%	400	400	800	8372.9770 ± 139.355	783.94	99.50
	120%	400	480	880	8922.5877 ± 181.619	872.63	100.22

6. LOD and LOQ

The LOD for MOF was found to be 0.046 µg/ml. and STZN was found to be 0.172 µg/ml.

The LOQ for MOF was found to be 0.139 µg/ml and STZN was found to be 0.522 µg/ml.

APPLICABILITY OF THE PROPOSED METHOD

Table. 7 Determination of Assay of MOF and STZN

Marketed formulation	Amount taken (µg/ml)		Amount obtained (µg/ml)		MOF % Purity ± S.D. (n=3)	STZN % Purity ± S.D. (n=3)
	MOF	STZN	MOF	STZN	MOF	STZN
	20	400	19.48	399.27	99.71% ± 48.07	99.57% ± 108.51

CONCLUSION:

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of MOF and STZN in bulk and in pharmaceutical formulations without interference and with good sensitivity.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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