Research Article

Development and Validation of UV-Visible Spectrophotometric Method for Simultaneous Estimation of Momentasone Furoate, Hydroquinone and Tretinoin from their Pharmaceutical Dosage Form

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ABSTRACT

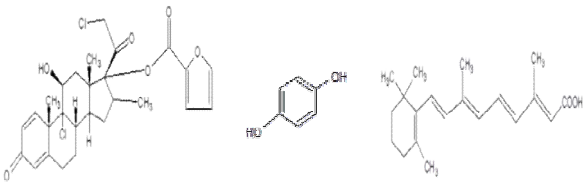
A simple, sensitive, rapid, precise, and accurate UV-Visible Spectrophotometric method has been developed for simultaneous estimation of Momentasone furoate (MOM), Hydroquinone (HYQ) and Tretinoin (TRE) from their pharmaceutical dosage form. The estimation was based on using Simultaneous Equation method. The wavelengths selected for estimation of MOM, HYQ and TRE were 248 nm, 293 nm and 339 nm respectively in methanol. Beer Lamberts law was obeyed in the concentration range of 0.5-50µg/mL for MOM, 5-100 µg/mL for HYQ and 0.125-10 µg/mL for TRE individually and 0.5-4µg/mL for MOM, 10-80 µg/mL for HYQ and 0.125-1 µg/mL in ternary mixture of MOM, HYQ and TRE. The developed method was validated in terms of linearity, precision, accuracy, limit of detection and quantification, robustness as per International Conference on Harmonization Q2 (R1) guidelines. The utility of the developed method has been demonstrated by assay of commercially available cream formulation. The developed UV-Visible spectrophotometric method can be successfully applied for quality control of MOM, HYQ and TRE from their formulations.

Keywords: Momentasone furoate, Hydroquinone, Tretinoin, UV-Visible Spectrophotometric method, Simultaneous equation, validation.

INTRODUCTION

Mometasone furoate (MOM), chemically 9α,21 dichloro 11β-hydroxy-16α-methyl-3,20 dioxopregna 1,4 –diene 17-yl-furan-2-carboxylate (Fig. 1a) is Glucocorticoid used for the relief of inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses. It is official in Indian Pharmacopoeia (2010)[1], United States Pharmacopoeia USP-35 (2004)[2], British Pharmacopoeia (2011)[3], European Pharmacopoeia (2011)[4]. Hydroquinone (HYQ), chemically 1,4-Benzenediol. (Fig. 1b), is a Depigmenting agent and antioxidant, widely used to fade dark skin spots such as freckles and lentigo. It is official in United States Pharmacopoeia USP-35 (2012) and British Pharmacopoeia (2011). Tretinoin (TRE), chemically (2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcylohex-1-enyl)nona-2,4,6,8-tetraacetic acid (Fig. 1c) is antineoplastic agent, keratolytic Agent, cell Stimulant and proliferant with official status in United States Pharmacopoeia USP-35 (2012), British Pharmacopoeia (2011), European Pharmacopoeia (2011). The Combination of MOM (0.1%), HYQ (2%) and TRE (0.025%) has shown beneficial action in treating signs and symptoms of skin allergy like red pigmentation, lentigo, fades dark skin, and so prescribed for treatment of acne and melasma.

Literature review reveals that several Spectroscopic methods have been reported for estimation of MOM, HYQ and TRE individually and in combination with other drugs.[4-15] No analytical method has been reported for simultaneous estimation of MOM, HYQ and TRE from their combined dosage forms. Therefore, it was endeavoured to develop an accurate, precise and sensitive spectroscopic method for simultaneous estimation of MOM, HYQ and TRE from their pharmaceutical dosage forms. The present work aims at development and validation of Spectroscopic method for simultaneous estimation of MOM, HYQ, and TRE from its Pharmaceutical dosage form using Simultaneous equation method.



a b c

Figure 1: Chemical structures of (a) Momentasone Furoate (b) Hydroquinone (c) Tretinoin

MATERIALS AND METHODS

Reagents and Chemicals

Working standards of MOM, HYQ and TRE were received as gratis samples from Glenmark Pharmaceuticals, Nasik; Crystal Quinone ltd, Ahmedabad; and Shalaks Pharmaceuticals, New Delhi respectively. Marketed Formulation containing MOM, HYQ and TRE (0.1: 2: 0.025 %w/w), Melacare, Ajanta Pharma, Mumbai; India was procured from local market. Methanol (AR grade) and tetrahydrofuran (AR grade) were purchased from Loba chemical.

Apparatus

UV Visible spectrophotometer (UV-1800) equipped with UV probe 3.24; Manufactured by Shimadzu Inc., Japan

and Analytical balance (Shimadzu AUX 220), manufactured by SHIMADZU Ltd.; having weighing capacity of 0.01gm to 200gm were used for the study.

Preparation of Standard Stock Solution of MOM (100µg/mL), HYQ (100µg/mL) and TRE (100µg/mL)

10 mg of MOM, HYQ and TRE were weighed separately and transferred to three different 100 mL volumetric flasks. Each of them was dissolved in few mL of methanol. The flasks were shaken and volume was made up to the mark with methanol to give a final solutions containing 100 µg/mL of MOM, 100 µg/mL of HYQ and 100 µg/mL of TRE respectively.

Preparation of ternary mixture containing MOM, HYQ and TRE (10, 200 and 2.5 µg/mL)

To a 100 ml volumetric flask, accurately weighed 20 mg of HYQ was added. To the same flask 10mL of MOM standard stock solution (100 µg/mL) and 2.5 mL of TRE standard stock solution (100µg/mL) were added. The volume was made up to the mark with methanol to obtain a ternary mixture containing 10 µg/mL of MOM (200 µg/mL of HYQ and 2.5 µg/mL of TRE).

Method validation

The simultaneous equation method was validated in terms of linearity, accuracy, precision, limit of detection(LOD), limit of quantification (LOQ) , robustness, specificity as per ICH guideline Q2(R1).[16]

Linearity

Appropriate aliquots (0.5, 1, 1.5, 2, 3 and 4 ml) from ternary mixture solution were transferred to different volumetric flasks of 10 mL capacity. The volume was made up to the mark with methanol to obtain concentration of 10, 20, 40, 50, 60, 80 µg/ml of HYQ (0.5, 1, 1.5, 2, 3, 4 µg/ml of MOM and 0.125, 0.25, 0.375, 0.5, 0.75, 1µg/ml of TRE). Calibration curve was constructed by plotting average absorbance Vs. conc. and regression equation was computed.

Precision

For *intraday* precision, lower, middle and higher level concentrations of linearity range were selected and solutions were analyzed under the optimized conditions for three times in a same day and absorbance was recorded. For *inter day* precision, lower, middle and higher level concentrations of linearity range were selected and solutions were analyzed under the optimized conditions for three consecutive days and absorbance was recorded. *Repeatability* was established by performing the experiment for six times consecutively and measuring the absorbance of the solution having concentration nearer to assay concentration.

Accuracy

To check the accuracy of proposed method, recovery studies were carried out from pre analyzed samples at three different levels of standard addition 80%, 100% and 120% of label claim. The validity and reliability of proposed method was assessed by recovery studies by standard addition method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions.Limit of Quantification (LOQ) is lowest amount of analyte in a sample which can be quantitatively determined. LOD & LOQ were calculated by using standard deviation and slope value obtained from calibration curve.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the developed method was tested by varying detection wavelength (±2 nm) of optimized conditions.

Specificity

The specificity of the developed method was established by comparing the spectra of blank, standard (prepared by pipetting 2 mL from ternary mixture solution and diluting to 10mL with methanol) and sample (prepared by pipetting 2 mL from *solution A* and diluting to 10mL with methanol)

Analysis of Marketed Formulation by Vierodt's Method:

A quantity of Cream equivalent to 20 mg of HYQ (1mg of MOM and 0.25mg of TRE) was taken and dissolved in 20 mL of tetra hydro furan (THF). The cream was triturated for 10-15 min and filtered through whatman filter paper no 41. The volume was made up to 100 mL with methanol. The final solution obtained has concentration of 10 µg/mL of MOM (200 µg/mL of HYQ and 2.5 µg/mL of TRE). From this 2 mL was pipetted in 10mL volumetric flask and volume was made up to mark with methanol to obtain concentration of 2 µg/mL of MOM (40 µg/mL of HYQ and 0.5 µg/mL of TRE). This solution was analysed using the developed method.

RESULTS AND DISCUSSION

Simultaneous Equation Method (Vierodt's Method)

The solutions were scanned within the wavelength range of 200-400 nm. The wavelength selection was based on choosing the wavelength at which one drug shows maximum absorbance and other two drugs shows less absorbance. The overlay spectra of MOM, HYQ and TRE revealed that at 248nm, HYQ and TRE showed less absorbance whereas MOM has maximum absorbance. Similarly, at 293 nm HYQ has maximum absorbance and MOM and TRE showed less absorbance and at 339nm TRE has maximum absorbance whereas HYQ and MOM showed less absorbance.

So the wavelength selected for estimation of MOM, HYQ and TRE were 248, 293 and 339nm respectively using Simultaneous Equation method. The mean absorptive values were obtained by measuring the absorbance of MOM (0.5-50µg/mL), HYQ (5-100 µg/mL) and TRE (0.125-10) µg/mL.The equation framed from the mean absorptive values was as below.

A_1 = 556.3616Cx + 5.56375Cy + 1.876528Cz

A_2= 16.45687Cx + 308.4184Cy +1.3732 Cz

A_3 = 214.6328Cx + 567.8011Cy + 1938.3481Cz

Where, A1, A2 and A3 are the absorbance of mixture at 248 nm, 293 nm and 339 nm respectively, ax1 ,ax2 and ax3 are absorptivities of MOM at λ1, λ2 and λ3 respectively. ay1, ay2 and ay3 are absorptivties of HYQ at λ1, λ2 and λ3 respectively. az1, az2, and az3 are absorptivties of TRE at λ1, λ2 and λ3 respectively. Cx, Cy, and Cz are concentrations of MOM, HYQ and TRE respectively.

Method validation

The developed simultaneous equation method showed linear response with the correlation value of 0.999 (Table 1 and Fig 2). The %RSD values for intra day, (table 2) inter day (table 2) and repeatability (table 4) were found to be less than 2%, indicating the method was precise. The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The recovery values between 98-102% indicated that the method is free from interference of excipients and the drugs can be recovered accurately. (Table 3). The %RSD values of less than 2% indicated that the method is robust for changes in ±2 nm wavelength of estimation. (Table 4). The specificity of developed method is indicated in figure 3. The summary of validation parameters are mentioned in table 5. Concentration of the individual drug present in the marketed formulation was determined by measuring the absorbance at 248, 293 and 339 nm and placing these values in framed simultaneous equations. The results of assay were found to be 99.41% for MOM, 99.28% for HYQ and 99.45% for TRE. (Table 5)

Table 1: Calibration curve data for MOM (0.5-4µg/mL), HYQ (10-80 µg/mL) and TRE (0.125-1µg/mL) at 248nm, 293nm and 339nm in mixture

MOM			HYQ			TRE		
Conc. (µg/mL)	Mean Abs ± SD*	% RSD	Conc. (µg/mL)	Mean Abs ± SD*	% RSD	Conc. (µg/mL)	Mean Abs ± SD*	% RSD
0.5	0.0385±0.0007	1.818	10	0.2730±0.0018	0.692	0.125	0.0169±0.0001	1.159
1	0.0982±0.0010	1.101	20	0.6555±0.0035	0.541	0.25	0.0477±0.0007	1.657
1.5	0.1332±0.0016	1.201	30	0.9250±0.0027	0.297	0.375	0.063±0.0008	1.420
2	0.1865±0.0012	0.693	40	1.1488±0.0077	0.674	0.5	0.08051±0.0007	0.947
3	0.2960±0.0037	1.261	60	1.7894±0.0288	1.611	0.75	0.1248±0.0018	1.459
4	0.4113±0.0062	1.525	80	2.4765±0.0218	0.881	1	0.1673±0.0005	0.309
Linearity Equation: Y = 0.1054X - 0.0169			**Linearity Equation: Y = 0.0307X - 0.0156**			**Linearity Equation: Y= 0.167X – 0.00008**		
r^2=0.997			**r^2=0.996**			**r^2=0.995**		

Table 2: Intraday Precision and Inter day Precision Data for MOM, HYQ and TRE in Mixture at 248nm, 293nm and 339nm by developed method (*n = 3)

MOM (248nm)			HYQ (293nm)			TRE (339nm)		
Intraday Precision								
Conc. (µg/mL)	Mean Conc. ± SD*	% RSD	Conc. (µg/mL)	Mean Conc. ± SD*	% RSD	Conc. (µg/mL)	Mean Conc. ± SD*	% RSD
1	0.99 ±0.025	1.032	20	19.71 ± 0.290	1.515	0.25	0.247 ± 0.0036	1.339
2	2.02 ± 0.022	0.985	40	39.69 ± 0.501	1.228	0.5	0.499 ± 0.0092	0.958
4	3.96 ± 1.706	0.665	80	78.74 ± 0.683	0.808	1	0.993± 0.0100	0.891
Inter day Precision								
Conc. (µg/mL) (MOM)	**Mean Conc. ± SD***	**% RSD**	**Conc.(µg/mL) (HYQ)**	**Mean Conc. ± SD***	**% RSD**	**Conc.(µg/mL) (TRE)**	**Mean Conc. ± SD***	**% RSD**
1	1.01± 0.030	1.386	**20**	19.92±0.331	1.601	**0.25**	0.26± 0.009	1.517
2	1.95± 0.031	1.222	**40**	39.95±0.603	1.438	**0.5**	0.51± 0.007	1.231
4	3.93± 0.060	0.852	**80**	78.82±0.745	0.945	**1**	1.01± 0.010	0.999

Table 3: Accuracy (%Recovery) Data for MOM, HYQ and TRE in Mixture at 248nm, 293nm and 339nm by developed method (*n = 3)

MOM (248nm)		HYQ (293nm)		TRE (339nm)	
Level of recovery (%)	Mean % Recovery	Level of recovery (%)	Mean % Recovery	Level of recovery (%)	Mean % Recovery
80	99.20	**80**	101.42	**80**	98.69
100	100.04	**100**	99.30	**100**	99.45
120	99.47	**120**	101.61	**120**	99.10

Table 4: Summary of validation parameters for MOM, HYQ and TRE by developed method

Parameters	MOM	HYQ	TRE
λmax	248nm	293nm	339nm
Range	0.5 to 50 µg/ml	5 to 100 µg/ml	0.125 to 10µg/ml
Linearity	0.5 to 4 µg/ml	10 to 80µg/ml	0.125 to 1µg/ml
R^2	0.997	0.996	0.995
Regression Equation	y = 0.1054x - 0.0169	y = 0.0307x - 0.0156	y = 0.167x – 0.00005
LOD (µg/mL)	0.060	0.774	0.003
LOQ (µg/mL)	0.182	2.347	0.011
Intraday Precision (% RSD) (n=3)	0.665 to 1.032	0.868 to 1.513	0.891 to 1.339
Inter day Precision (% RSD) (n=3)	0.8525 to 1.3869	0.945 to 1.601	0.999 to 1.517
Repeatability (% RSD) (n=6)	0.6452	0.5187	0.9529
%Recovery	99.20 to 100.04	99.30 to 101.61	98.69 to 99.45
Robustness (% RSD)	(+2nm) 0.882	(+2nm) 1.307	(+2nm) 1.370
	(-2nm) 1.152	(-2nm) 0.935	(-2nm) 1.433

Table 5: Analysis of marketed formulations (*n = 6)

Brand	Drug	Label Claim (%w/w)	Mean Abs	% Assay ± SD*	%RSD
MELACARE	HYQ	2	0.1851	99.41±0.001802	0.967
	MOM	0.1	1.2584	99.28±0.011041	0.877
	TRE	0.025	0.1029	99.45±0.000975	0.947

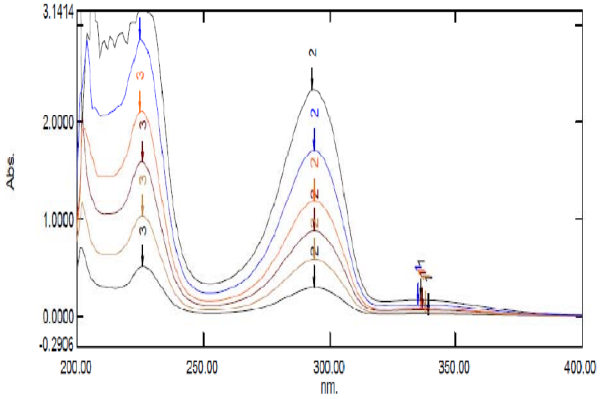


Figure 2: Overlay spectra of Mixture (MOM 0.5 to 4 µg/ml, HYQ 10- 80 µg/mL and TRE 0.125 to 1 µg/mL)

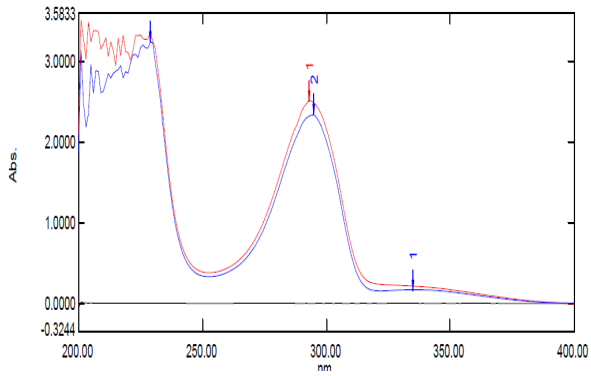


Figure 3: Spectra of blank, standard (2, 40, 0.5 µg/ml) and sample (2, 40, 0.5 µg/ml) of MOM, HYQ and TRE to check specificity of developed method.

CONCLUSION

A simple, accurate, precise, robust and rapid UV visible spectrophotometric method has been developed for simultaneous estimation of MOM, HYQ and TRE from their pharmaceutical formulations. The results reveal that the proposed method could be successfully applied for the routine analysis and quality control of pharmaceutical dosage forms containing MOM, HYQ and TRE.

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