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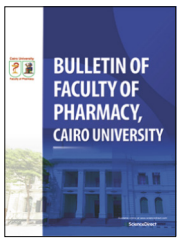

ORIGINAL ARTICLE

Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form

Hanan A. Merey, Sally S. El-Mosallamy *, Nagiba Y. Hassan, Badr A. El-Zeany

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El Aini Post, 11562 Cairo, Egypt

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High performance liquid chromatography;
Isocratic elution

Abstract Two chromatographic methods were developed and validated for the simultaneous determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR). Combination of MO and FOR is used for the treatment of asthma in patients suffering from reversible obstructive airway disease. The first chromatographic method was based on using aluminum TLC plates pre-coated with silica gel GF_{254} as the stationary phase and chloroform:ethyl acetate: methanol:toluene:formic acid (5:2:2:2:0.1, by volume) as the mobile phase followed by densitometric measurement of the separated bands at 233 nm. The second method is a high performance liquid chromatographic method for the separation and determination of MO and FOR using reversed phase C_{18} column with isocratic elution. The mobile phase composed of methanol: 0.5% ammonium acetate pH adjusted with acetic acid (80:20, v/v) at a flow rate of 1.0 mL/min. Quantitation was achieved with UV detection at 220 nm. The specificity of the developed methods was investigated by analyzing the pharmaceutical dosage form. The validity of the proposed methods was assessed using the standard addition technique. The obtained results were statistically compared with those obtained by the reported methods, showing no significant difference with respect to accuracy and precision at $p = 0.05$.

1. Introduction

Asthma is a chronic inflammatory disorder of the airways. During asthma attacks, the smooth muscle cells in the bronchi constrict, the airways become inflamed and swollen, and breathing becomes difficult.[1] Therefore one of the ways of treating it is a combination of inhaled corticosteroids to reduce the inflammation of the airways and prevent the loss of lung functions[2] with long acting β2 agonists (LABA) which acts locally on the lung as a bronchodilator and relaxes muscles

* Corresponding author. Tel.: +20 1223467677.
E-mail address: sally_elmosalamy@hotmail.com (S.S. El-Mosallamy).
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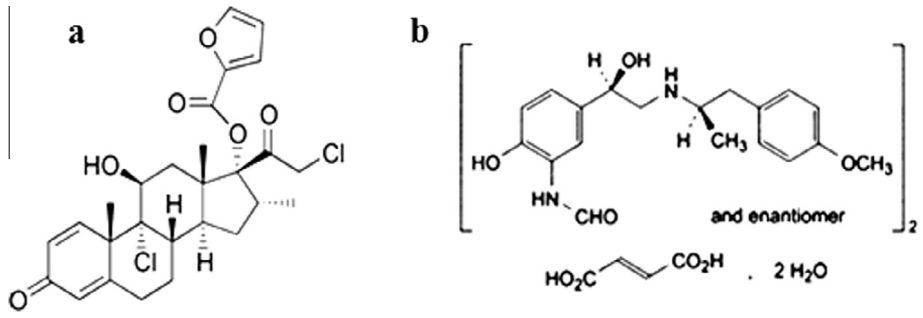

Figure 1 Structural formula of (a) Mometasone furoate MO and (b) Formoterol fumarate dihydrate FOR.

in the airways to improve breathing. An example of this combination is Mometasone furoate (MO), (9α,21-dichloro-1 1β,17-dihydroxy-16α-methylpregna-1,4-diene-17-yl furan-2-carboxylate) (Fig. 1a) which acts as a corticosteroid and Formoterol fumarate dihydrate (FOR), (N-[2-hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino] ethyl]phenyl]formamide(E)-butenedioate dehydrate) (Fig. 1b) which acts as a long acting β2 agonist.[3]

Literature survey reveals that MO and FOR are official drugs in European Pharmacopoeia,[4] also MO is official in United States Pharmacopoeia.[5] Several analytical methods have been reported for the determination of MO alone or in combinations with other drugs including, spectrophotometry,[4,6–9] TLC[10,11] and HPLC.[5,12–20] Besides, several methods have been reported for the determination of FOR alone or in combinations including, non aqueous titration,[4] spectrophotometry,[21–24] voltammetry,[25] capillary electrophoresis,[26] and HPLC.[18–20,27–32] The aim of this work is to develop simple chromatographic methods for the simultaneous determination of MO and FOR in pharmaceutical dosage form.

2. Experimental

2.1. Instruments

The thin-layer chromatographic (TLC) system consisted of a Camag Linomat autosampler (Muttenzl, Switzerland), a Camag microsyringe (100 μL) and a Camag 35/N/30319 TLC scanner with winCATS software; an ultraviolet (UV) lamp with a short wavelength at 254 nm (Desaga, Wiesloch, Germany); and TLC plates precoated with silica gel GF_{254} 10 × 20 cm, 0.25 mm thickness (Merck, Darmstadt, Germany).

Shimadzu HPLC system consisted of a pumping system (model LC-10 AD vp), an ultra-violet variable wavelength detector (model SPD-10A vp), Degasser (model DGU-12A) and System controller (model SCL-10A vp) Equipped with a prominence autosampler (model SIL-20A) (Shimadzu, Kyoto, Japan). An Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i. d.) was used as stationary phase (GL Sciences, Tokyo, Japan).

2.2. Materials and reagents

2.2.1. Pure standard

Mometasone furoate was kindly supplied by SIGMA Pharmaceutical Industries, Cairo, Egypt, its purity was found to be 100.12 ± 0.762 according to the official method.[5]

Formoterol fumarate dihydrate was kindly supplied by NOVARTIS pharmaceuticals, Cairo, Egypt, its purity was found to be 100.02 ± 0.592 according to the reported method.[19]

2.2.2. Pharmaceutical dosage form

Dulera® Inhalation aerosol (Batch No. GLG122) labeled to contain 100 μg of MO and 5 μg of FOR per actuation, was manufactured by (MERCK & CO. INC, White House Station, USA) and obtained from the American market.

2.2.3. Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents for HPLC were of HPLC grade. These included methanol (Sigma-Aldrich, Belgium), chloroform (Sigma-Aldrich, Belgium) and double distilled deionized water (Otsuka, Cairo, Egypt). Ethyl acetate, toluene, formic acid and ammonium acetate were purchased from Al-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

2.2.4. Standard solutions

- Standard stock solution of MO: 1.0 mg/mL in methanol.
- Standard stock solution of FOR: 1.0 mg/mL in methanol.

2.2.5. Working Solutions

For TLC-spectrodensitometric method: Working solution of FOR (200 μg/mL) was prepared from its stock solution using methanol as a solvent.

For HPLC method: Working solutions of MO (400 μg/mL) and FOR (100 μg/mL) were prepared from their respective stock solutions using mobile phase as a solvent.

2.3. Procedures

2.3.1. Construction of the calibration curves

2.3.1.1. For TLC-spectrodensitometric method. Accurately measured aliquots of MO stock standard solution (1 mg/mL) and FOR working solution (200 μg/mL) were spotted onto TLC plates using Camag Linomat autosampler with microsyringe (100 μL). The plates were then developed by the ascending technique using chloroform:methanol:ethyl acetate:toluene:formic acid (5:2:2:2:0.1, by volume) as a mobile phase. The plates were then removed and air-dried. The chromatogram was scanned at 233 nm. Calibration curves representing the relationship

between integrated peak area and the corresponding concentrations of each of MO (2–14 μg/band) and FOR (0.1–5 μg/band) were plotted.

2.3.1.2. For HPLC method. Aliquots equivalent to 100–3000 μg of MO and 10–500 μg of FOR were accurately measured and transferred from their working solutions into a set of 10-mL volumetric flasks and the volumes were completed to the mark with the mobile phase (methanol: 0.5% ammonium acetate pH 5.7 (80:20; v/v)). A 20-μL aliquot of each solution was injected into an Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.), using the mobile phase, at flow rate 1.0 mL/min and UV detection at 220 nm. Two calibration curves were constructed by plotting the relative peak area, using 100 μg/mL of MO and 25 μg/mL of FOR as the external standards, against the corresponding concentrations of each drug.

2.3.2. Application to pharmaceutical formulations

The actuator after shaking was inverted and placed in a beaker containing 4 mL methanol, and then the beaker was covered tightly. Ten actuations were delivered in the beaker, then the actuator was washed with methanol. The solution was transferred accurately into 10-mL volumetric flask and the volume was completed with methanol to prepare dosage form solution containing 100 μg/mL MO and 5 μg/mL FOR. For TLC-spectrodensitometric determination of both drugs, 40 μL was applied onto TLC plates.

For HPLC analysis, dosage form solution was prepared as mentioned above but completing the volume with the mobile phase instead of completing with methanol and then injected to the column. The procedure was completed as mentioned under construction of calibration curves for each method. The concentration of MO and FOR was calculated from the corresponding regression equations.

3. Results

Several trials were conducted to develop the optimum chromatographic conditions for the sufficient separation of both drugs including chloroform:methanol (2:8, v/v), methanol:toluene (8:2, v/v) and chloroform:ethyl acetate:toluene (5:4:3, by volume) but bad resolution was obtained. The results of the TLC system were satisfactory when using chloroform:ethyl a cetate:methanol:toluene:formic acid (5:2:2:2:0.1, by volume) as the mobile phase. R_f values were found to be 0.81 ± 0.02 and 0.17 ± 0.02 for MO and FOR, respectively as shown in (Fig. 2). This separation allows the determination of MO and FOR at 233 nm without any interference from each other.

HPLC method was also tried to separate MO and FOR, therefore several trials have been undertaken to reach the optimum stationary/mobile phases matching. Good chromatographic separation of the two drugs in their binary mixtures could be achieved using an Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.), with a mobile phase consisting of (methanol: 0.5% ammonium acetate (80:20, v/v) pH 5.7 adjusted by glacial acetic acid) at flow rate 1.0 mL/min, followed by UV detection at 220 nm, (Fig. 3). Calibration curves were plotted for both TLC and HPLC for the determination of the cited drugs.

An overall system suitability testing was calculated (Table 1) to determine whether the operating system performed properly.

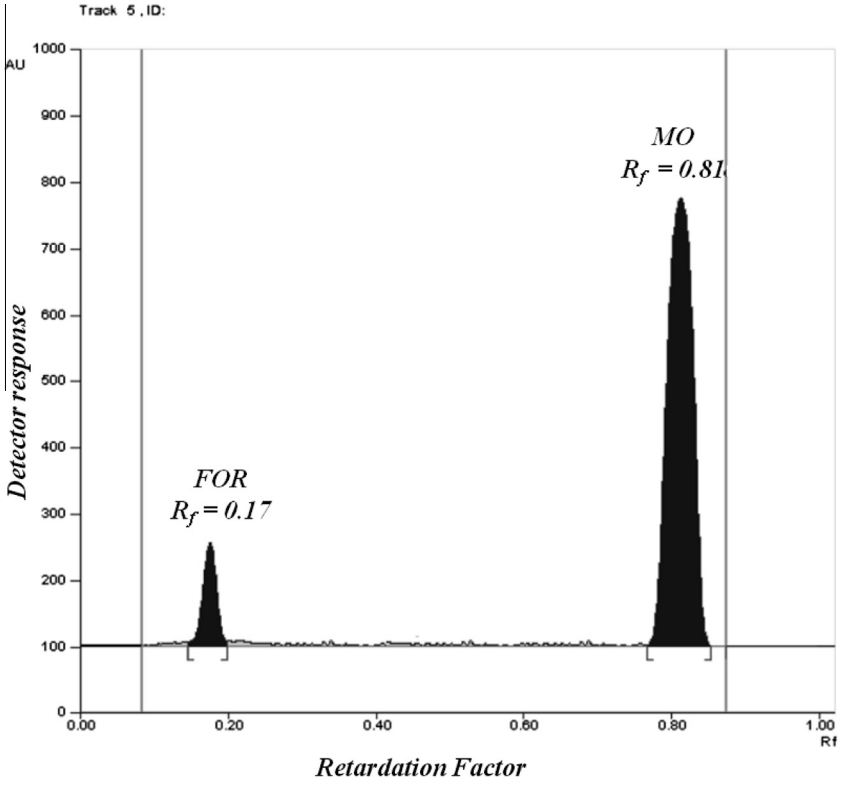

Figure 2 TLC chromatogram of a resolved mixture containing 0.5 μg/band of FOR and 10 μg/band of MO.

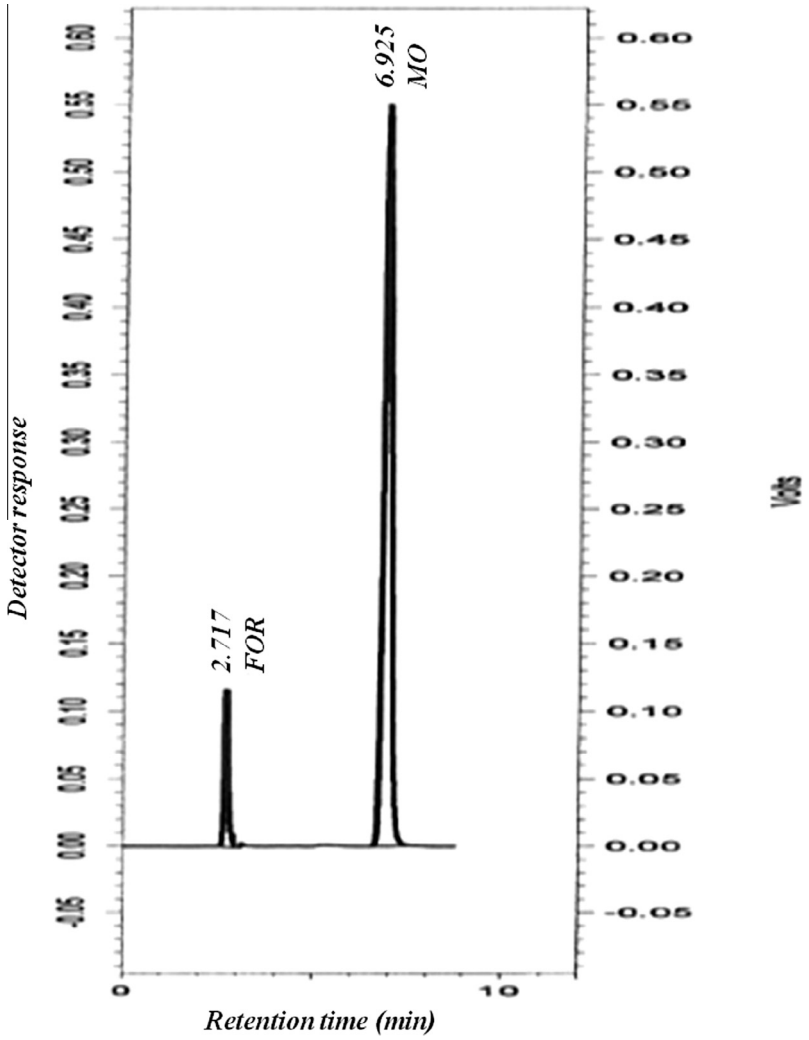

Figure 3 HPLC chromatogram of resolved mixture containing 15 μg/mL of FOR and 300 μg/mL of MO.

Table 1 Parameters required for system suitability test of TLC-densitometric and HPLC methods.

Parameter	TLC		HPLC		Reference values (5)
	MO	FOR	MO	FOR	
Retention time (t_R) [min]			6.925	2.717	
Retardation factor (R_f)	0.81	0.17			
Tailing factor (T)	0.89	1.2	1	1	$T = 1$, for a typical symmetrical peak
Capacity factor (K')			7.343	2.273	$1 < K' < 10$
Selectivity factor (α)	4.76		3.23		$\alpha > 1$
Resolution factor (Rs)	12.8		12.27		$Rs > 2$
Column efficiency (N)			3503	2485	$N > 2000$
HETP[a] [mm]			0.071	0.100	

HETP[a] = height equivalent to theoretical plates (length of column in mm/N).

The proposed methods were validated according to International Conference on Harmonization (ICH) guidelines (Table 2). The table also shows the assay parameters of the regression equations and the ranges of concentration.

The proposed methods were successfully applied for the determination of MO and FOR in *Dulera*® inhaler. The results shown in Table 3 were satisfactory. The validity of the proposed methods was assessed by applying the standard addition technique, no interference due to excipients was observed as shown from the results in Table 3.

The results obtained by applying the proposed methods for the analysis of pure MO and FOR compared to those obtained by applying the official[5] and reported methods,[19] respectively, they showed no significant difference regarding accuracy and precision Table 4.

Table 2 Assay validation sheet of the proposed methods for the simultaneous determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR).

Parameter	TLC		HPLC	
	MO	FOR	MO	FOR
Range	2–14 μg/band	0.1–5 μg/band	10–300 μg/mL	1–50 μg/mL
Linearity				
Slope	Slope 1 = −0.1286 Slope 2 = 3.6519	Slope 1 = −0.3501 Slope 2 = 4.5039	0.0083	0.04
Intercept	3.0026	0.2202	0.1688	−0.008
SE of intercept	1.7826	0.3482	0.0048	0.0005
Correlation coefficient (*r*)	0.9999	1	0.9999	1
Accuracy (mean ± SD)	100.10 ± 1.039	99.96 ± 0.970	100.24 ± 0.494	100.24 ± 0.819
Precision				
Intraday[a]	0.970	0.870	0.664	0.735
Interday[b]	1.269	0.879	0.753	0.916
LOD	0.26 μg/band	0.01 μg/band	2.52 μg/mL	0.06 μg/mL
LOQ	0.78 μg/band	0.04 μg/band	7.63 μg/mL	0.18 μg/mL
Robustness[c]	0.881	0.933	0.845	0.966

[a] The intraday RSD% for *TLC* RSD% of three concentrations (3, 7 and 11 μg/band) of MO and (0.26, 0.46 and 0.66 μg/band) of FOR repeated three times within the day. For *HPLC* RSD% of three concentrations (20, 70 and 140 μg/mL) of MO and (2.5, 10 and 20 μg/mL) of FOR repeated three times within the day.

[b] The inter-day RSD% for *TLC* RSD% of three concentrations (3, 7 and 11 μg/band) of MO and (0.26, 0.46 and 0.66 μg/band) of FOR repeated three successive days. For *HPLC* RSD% of three concentrations (20, 70 and 140 μg/mL) of MO and (2.5, 10 and 20 μg/mL) of FOR repeated three successive days.

[c] For *TLC*, RSD% of three concentrations (3, 7 and 11 μg/band) of MO and (0.26, 0.46 and 0.66 μg/band) of FOR) using 0.2 mL of formic acid instead of 0.1 mL. For *HPLC*, RSD% of three concentrations (20, 70 and 140 μg/mL) of MO and (2.5, 10 and 20 μg/mL) of FOR at flow rate 1.2 mL/min instead of 1 mL/min.

Table 3 Determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR) in their Dosage form and application of standard addition technique using the proposed methods.

Product	Method	Drug	Recovery%* ± RSD	Standard addition			
				Claimed amount taken	Added**	Found**	Recovery%*
Dulera® inhalation aresol (Batch No. GLG122)	TLC	MO	99.60 ± 0.351	4 μg/band	2	2.01	100.50
					4	3.99	99.75
					6	5.97	99.50
					Mean ± RSD		99.91 ± 0.521
		FOR	101.00 ± 0.910	0.2 μg/band	0.1	0.100	100.00
					0.2	0.201	100.50
					0.3	0.299	99.67
					Mean ± RSD		100.06 ± 0.418
	HPLC	MO	100.83 ± 0.630	100 μg/mL	60	61.16	101.93
					100	102.98	102.98
					120	122.13	101.78
					Mean ± RSD		102.23 ± 0.639
		FOR	101.16 ± 0.436	5 μg/mL	3	3.06	102.00
					5	5.00	100.00
					7	7.11	101.57
					Mean ± RSD		100.17 ± 1.050

* Average of three determinations of dosage form.

** Added and found concentrations were μg/band in case of TLC method and μg/mL in case of HPLC method.

4. Discussion

Planar chromatography with precise application of the samples and computer controlled evaluation and quantification of the developed chromatograms has been considered to be a reliable technique for purity control and for quantitative drug testing.[33] Therefore the aim of this work is to develop simple, accurate, rapid, specific and valid spectro-densitometric method. This separation allows the determination of MO and FOR without any interference from each other. A polynomial relationship was found to exist between the integrated peak area of the separated spots at the selected wavelength (233 nm) and the corresponding concentrations of MO and FOR in the range of 2–14 μg/band and 0.1–5 μg/band for MO and FOR, respectively.

Table 4 Statistical comparison of the results obtained by the proposed methods and the reported methods for the analysis of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR).

Parameter	TLC		HPLC		Reported methods	
	MO	FOR	MO	FOR	MO (5)[b]	FOR (19)[c]
Mean	100.10	99.96	100.24	100.24	100.12	100.02
SD	1.039	0.969	0.494	0.819	0.762	0.592
Variance	1.060	0.939	0.244	0.670	0.581	0.350
n	7	7	7	7	5	5
Student's *t*-test	0.038 (2.228)[a]	0.132 (2.228)[a]	0.531 (2.228)[a]	0.542 (2.228)[a]		
F-test	1.86 (6.16)[a]	2.68 (6.16)[a]	2.38 (4.53)[a]	1.91 (6.16)[a]		

[a] The figures in parenthesis are the corresponding theoretical values at $p = 0.05$.
[b] Official method is HPLC for MO determination using C_8 column (5 μm, 4.6 × 250 mm), methanol:water (65:35, v/v) as a mobile phase at a flow rate of 1.7 mL/min and UV detection at 254 nm.
[c] HPLC method for FOR determination using C_{18} column (5 μm, 4.6 × 150 mm), sodium dihydrogen phosphate buffer:acetonitrile (50:50, v:v) as a mobile phase. pH = 3 adjusted by diluted ortho-phosphoric acid at a flow rate of 1 mL/min and UV detection at 220 nm.

The regression equations were computed for MO and found to be:

$$A = -0.1286X^2 + 3.6519X + 3.0026 \qquad r = 0.9999 \quad \text{for MO}$$

$$A = -0.3051X^2 + 4.5039X + 0.2202 \qquad r = 1 \qquad \text{for FOR}$$

where A is the integrated peak area multiplied by (10^{-3}), X is the corresponding concentration in μg/band and r is the correlation coefficient.

The suggested chromatographic system for the HPLC method allows complete base line separation at reasonable time. The linearity of the detector's response of the studied drugs was determined by plotting a relative peak area (calculated following the external standard technique using 100 μg/mL of MO and 25 μg/mL of FOR as the external standards for MO and FOR, respectively) versus concentrations and linear correlation was obtained. The regression equations were computed for MO and FOR and found to be:

$$A = 0.0083C + 0.1688 \qquad r = 0.9999 \qquad \text{for MO}$$

$$A = 0.04C + 0.0011 \qquad r = 1 \qquad \text{for FOR}$$

where A is the relative peak area, C is the corresponding concentration in μg/mL and r is the correlation coefficient.

By comparing the developed HPLC method with the reported methods[18–19] we found that, the developed method is more sensitive and liner over a wider range of concentration 1–50 μg/mL for FOR and 10–300 μg/mL for MO than that of the reported method[18] 3–9 μg/mL for FOR and 100–300 μg/mL for MO. Also the developed HPLC method shows a shorter retention time, where FOR and MO eluted after 2.7 min. and 6.9 min., while eluted after 3.4 min. and 9.2 min., respectively in the reported method.[19]

4.1. Method validation

The proposed methods were validated according to the ICH Q2 (R1) recommendations.[34] The method was validated for parameters such as system suitability, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and selectivity.

4.1.1. System suitability

The system suitability test is an integral part of chromatographic method development and it is used to verify that the system is adequate for the analysis to be performed; the system suitability parameters for MO and FOR were evaluated. The suitability of the chromatographic system was determined according to USP guidelines and with acceptance of the obtained parameter values.[5]

4.1.2. Linearity and ranges

Under the above mentioned experimental conditions, linear relationships were obtained by plotting the drug concentrations either against relative peak areas or integrated peak areas for each drug, for HPLC and TLC methods, respectively.

4.1.3. Accuracy

The accuracy of the proposed methods was validated by analyzing pure samples of each MO, FOR in triplicate. The concentrations of the active drugs were calculated from the corresponding regression equations.

4.1.4. Precision

It was evaluated by calculating intra and inter-day precisions. By repeating the assay of three different concentrations of each of the cited drugs three times in the same day and assaying the same samples in triplicate on three successive days using the developed chromatographic methods and calculating the recovery% and RSD%.

4.1.5. Specificity

The specificity of the developed methods was investigated by analyzing the pharmaceutical dosage form. The spots of the active drugs in the dosage form were confirmed by comparing their R_f values and densito-spectra of the spot with that of a standard drugs solutions (in TLC method).

4.1.6. Robustness

It was evaluated by calculating the RSD% of three different concentrations of each of the cited drugs after making a deliberate change in the assay conditions of both TLC and HPLC.

5. Conclusion

The suggested chromatographic methods provide simple, sensitive, accurate and reproducible methods for quantitative analysis of MO and FOR in their binary mixtures and pharmaceutical dosage form. The developed TLC method is highly sensitive. It has the advantages of short run time, large sample capacity and use of minimal volume of solvents. HPLC method gives a good resolution between the proposed components within suitable analysis time; it is highly specific but more expensive. The proposed methods have advantage than other published methods of being more sensitive, simple, lower time consuming and easy in application on inhaler dosage form.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Murphy W. *Asthma*. New York: Twenty-First Century Books; 2011.
2. Schleimer RP. *Inhaled steroids in asthma: optimizing effects in the airways*. New York: Marcel Dekker Inc.; 2001.
3. O'Neil MJ. The Merck index: an encyclopedia of chemicals, drugs and biologicals. 15th ed. Whitehouse Station: RSC Publishing; 2013.
4. European Pharmacopoeia. 7th ed. European Directorate for the Quality of Medicine and Healthcare. London; 2011.
5. United States Pharmacopoeia and National Formulary (USP 37-NF 32). United States Pharmacopoeial Convention Rockville; 2014.
6. Vanani DR, Desai SD, Patel KG, Shah PA. Application of ratio derivative spectrophotometry for simultaneous determination of mometasone furoate and salicylic acid in semisolid dosage form. *Int J Anal Bioanal Chem* 2013;**3**:67–71.
7. El-bagary RI, Fouad MA, El-shaal MA, Tolba EH. Derivative, derivative of the ratio spectrophotometric and stability-indicating RP–HPLC methods for the determination of mometasone furoate and miconazole nitrate. *J Chem Pharm Res* 2013;**5**:368–78.
8. Patel MM, Patel HD. Development and validation of UV spectrophotometric method for simultaneous estimation of terbinafine hydrochloride and mometasone furoate in combined dosage form. *Asian J Res Chem* 2013;**6**:29–34.
9. Bhangale PR, Jain HK. Spectrophotometric method for simultaneous estimation of formoterol fumarate and mometasone furoate in respicaps. *Int Res J Pharm* 2013;**4**:220–3.
10. Kulkarni AA, Nanda RK, Ranjane MN, Ranjane PN. Simultaneous estimation of nadifloxacin and mometasone furoate in topical cream by HPTLC method. *Der Pharma Chem* 2010;**2**:25–30.
11. Wulandari L, Kiauw Sia T, Indrayanto G. TLC densitometric determination of mometasone furoate in topical preparations: validation. *J Liq Chromatogr Relat Technol* 2003;**26**:109–17.
12. Ourique AF, Contri RV, Guterres SS, et al. Set-up of a method using LC–UV to assay mometasone furoate in pharmaceutical dosage forms. *Quím Nova* 2012;**35**:818–21.
13. Shaikh KA, Patil AT. Stability-indicating HPLC method for the determination of mometasone furoate oxymetazoline phenyl ethanol and benzalkonium chloride in nasal spray solution. *J Trace Anal Food Drugs* 2013;**1**:14–21.
14. Shaikh S, Muneera MS, Thusleem OA, Tahir M, Kondaguli AV. A simple RP–HPLC method for the simultaneous quantitation of chlorocresol, mometasone furoate, and fusidic acid in creams. *J Chromatogr Sci* 2009;**47**:178–83.
15. Teng XW, Foe K, Brown KF, Cutler DJ, Davies NM. High-performance liquid chromatographic analysis of mometasone furoate and its degradation products. *J Pharm Biomed Anal* 2001;**26**:313–9.
16. Youssef RM, Korany MA, Afify MA. Development of a stability indicating HPLC–DAD method for the simultaneous determination of mometasone furoate and salicylic acid in an ointment matrix. *Anal Methods* 2014;**6**:3410–9.
17. El-Bagary RI, Elkady EF, Tammam MH, Abo Elmaaty A. Simultaneous determination of miconazole and hydrocortisone or mometasone using reversed phase liquid chromatography. *Eur J Chem* 2012;**3**:421–5.
18. Gujarati PZ, Thula KC, Maheshwari DG. Stability indicating HPLC method for simultaneous estimation of mometasone furoate and formoterol fumarate in combined dosage form. *Pharmacophore* 2014;**5**:219–30.
19. Srinivasaro K, Gorule V, Reddiah CV, Krishna AV. Validated method development for estimation of formoterol fumarate and mometasone furoate in metered dose inhalation form by high performance liquid chromatography. *Pharmacophore* 2012;**3**:301–6.
20. El-Bagary RI, Fouad MA, Manal A, Tolba EH. Forced degradation of mometasone furoate and development of two RP–HPLC methods for its determination with formoterol fumarate or salicylic acid. *Arabian J Chem* 2015. http://dx.doi.org/10.1016/j.arabjc.2015.05.005.
21. Gousuddin M, Raju SA, Sultanuddin, Manjunath S. Development and validation of spectrophotometric methods for estimation of formoterol bulk drug and its pharmaceutical dosage forms. *Int J Pharm Pharm Sci* 2011;**3**:3–6.
22. Prakash KV, Rao JV, Raju NA, Himabindu V. Spectrophotometric estimation of formoterol fumarate in pharmaceutical formulations. *Asian J Chem* 2007;**19**:5129–33.
23. Prasad AVSS. Simultaneous spectrophotometric determination of formoterol fumarate and budesonide in their combined dosage form. *Indian J Chem Technol* 2006;**13**:81–3.
24. Shah PD, Koradia S. Simultaneous determination of beclomethasone dipropionte and formoterol fumarate in rotacap dosage form using two different spectrophotometric methods. *World J Pharm Pharmacol Sci* 2014;**3**:611–23.
25. Demircigil BT, Özkan SA, Coruh Ö, Yılmaz S. Electrochemical behavior of formoterol fumarate and its determination in capsules for inhalation and human serum using differential-pulse and square-wave voltammetry. *Electroanalysis* 2002;**14**:122–7.
26. Song JZ, Chen J, Tian SJ, Sun ZP. Assay for the determination of low dosage form of formoterol dry syrup by capillary electrophoresis with head-column field-amplified sample stacking. *J Pharm Biomed Anal* 1999;**21**:569–76.
27. Ahmed S, JayaKar B, Aleem MA. Development of reverse phase high performance liquid chromatography method and its validation for estimation of formoterol fumarate rota caps. *Int J Pharm Sci Res* 2011;**2**:319–24.
28. Campestrini J, Lecaillon JB, Godbillon J. Automated and sensitive method for the determination of formoterol in human plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Sci Appl* 1997;**704**:221–9.
29. Patil AT, Patil SD, Shaikh KA. Sensitive LC method for simultaneous determination of ciclesonide and formoterol fumarate in dry powder inhaler. *J Liq Chromatogr Relat Technol* 2011;**34**:1568–77.
30. Shah BD, Kumar S, Seth AK, Ghelani TK, Deshmukh GJ. Analytical method development and method validation of tiotropium bromide and formoterol fumarate metered dose inhaler (MDI) by using RP–HPLC method. *Asian J Biochem Pharm Res* 2011;**1**:145–58.

31. Trivedi RK, Chendake DS, Patel MC. A rapid, stability-indicating RP–HPLC method for the simultaneous determination of formoterol fumarate, tiotropium bromide, and ciclesonide in a pulmonary drug product. *Sci Pharm* 2012;**80**: 591–603.
32. Parmar VK, Patel HN, Patel BK. Sensitive and robust methods for simultaneous determination of beclomethasone dipropionate and formoterol fumarate dihydrate in rotacaps. *J Chromatogr Sci* 2014;**1–12**.
33. Renger B*Proceedings of the sixth international symposium on Instrumental planner chromatography*. Bad Duerkheim: Institute for chromatography; 1991. p. 291.
34. ICH, Q2 (R1) Validation of analytical procedures. In: Proceeding of the International Conference on Harmonization, Geneva; 2005.