Simultaneous analysis of mometasone furoate, miconazole nitrate, and nadifloxacin in cream formulation by HPTLC

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ABSTRACT
A simple and precise High performance thin layer chromatography method was developed for the simultaneous investigation of mometasone furoate (MOM), miconazole nitrate (MIC), and nadifloxacin (NAD). This method was used to analyze three drugs in a cream formulation without the interference of excipients. HPTLC separation of the drugs was achieved using the mobile phase system containing toluene, ethyl acetate, ethanol, and formic acid (10:3:2:0.5 v/v/v/v) on a precoated aluminum plate of silica gel 60 F
254
 at 235 nm. Linearity was achieved over the range of 60–220, 1,200–4,400, and 600–2,200 ng/band, with mean accuracy of 99.004 ± 1.008, 99.182 ± 1.324, and 99.169 ± 1.421 for MOM, MIC, and NAD, respectively. The limits of detection (ng/band) were found to be 14.075, 326.945, and 191.611, and the limits of quantification (ng/band) were 42.653, 990.741, 580.639 for MOM, MIC, and NAD, respectively, which show the sensitivity of the method. After successful development and validation, the established method was used for the assessment of mometasone furoate, miconazole nitrate, and nadifloxacin in 3 Mix cream.

INTRODUCTION
Mometasone furoate (MOM), [(8S,9R,10S,11S,13S,14S,16R,17R)-9-chloro-17-(2-chloroacetyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthrene-17-yl]furan-2-carboxylate (Fig. 1a) is a white or almost white powder which shows high solubility in acetone and methylene chloride, and insolubility in water, whereas in alcohol it shows slight solubility (Martindale, 2012). It is a topical corticosteroid (corticosteroid which can be directly applied on skin) useful in relieving symptoms of itching and inflammation, and is known to have vasoconstrictive properties. Therefore, it can be used to treat skin atopic dermatitis (Prakash and Benfeld, 1988; Zanwar et al., 2014).

Miconazole nitrate (MIC), 1-[2-(2,4-dichlorophenyl)-2-[2,4-dichlorophenyl]methoxy] ethyl] imidazole; nitric acid (Fig. 1b) is an antifungal imidazole derivative, which is widely used as a nitrate salt to treat fungal infections. It is slightly soluble in water and methyl alcohol. Nadifloxacin (NAD), 9-fluoro-6,7-dihydro-8(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo-(ij)-quinolizine-2-carboxylic acid (Fig. 1c) is a fluoroquinolone showing antibacterial activity which is a second-generation broad spectrum category, and is used to cure bacterial skin infection and acne (The Merck Index, 2001; Nenof, 2006). It is soluble in Dimethyl sulfoxide (DMSO) and is slightly soluble in ethyl alcohol and water. MOM and MIC are official in British Pharmacopoeia (BP), USP, and Indian Pharmacopoeia (IP), whereas NAD is official in BP and IP (British Pharmacopoeia, 2008; Indian Pharmacopoeia, 2007; USP 31 NF, 2008). These three drugs are marketed in a combined dosage form as a cream/ointment in the ratio of 0.1:1:2% w/w/w (MOM:NAD:MIC) for the treatment of various skin infections. After analyzing the literature, High Performance Liquid Chromatography (HPLC) method for investigation of MOM in bulk, pharmaceutical formulation and biological fluid (Teng et al., 2001) was reported. Moreover, the assessment of MOM along with drugs like terbinafine, eberconazole nitrate, oxymetazoline, ketoconazole, miconazole nitrate, formoterol fumarate, and fusidic acid, using HPLC (El-Bagarya et al., 2012; Katari et al., 2012; Patel, 2014; Roy and Chakrabarty, 2013; Shaikh and Patil, 2009; Shaikh et al., 2009; Sharma et al., 2013), UV spectroscopy (Ramzia et al., 2013), and in combination with nadifloxacin by applying...
High performance thin layer chromatography (HPTLC) (Kulkarni et al., 2010), was published. There are many reported stability indicating methods using HPLC and HPTLC for the investigation of MIC alone (Birsan et al., 2014; De Zan et al., 2009; Pagare et al., 2012; Tyler and Genzale, 1989). Moreover, MIC in combination with other drugs, such as hydrocortisone, mometasone furoate, lidocaine, econazole, metronidazole, etc., using HPLC (Aboul-Enein and Ali, 2002; Akay et al., 2002; Belal et al., 2012; Cavrini et al., 1989; El Bagary et al., 2012; Erk and Altun, 2001), UV spectroscopy (Cavrini et al., 1989; Ekiert and Krzek, 2009; Erk and Altun, 2001), and with fluocinolone acetonide by applying HPTLC (Patel and Patel, 2014) has been published. The assessment of nadifloxacin in bulk and dosage forms has been reported by HPLC and HPTLC methods in stability studies (Kumar et al., 2010; Devhadrao et al., 2014; Murthy et al., 2014; Sharma and Singh, 2012) and also in combination with ibuprofen by UV spectroscopy (Kalantre and Pishwikar, 2012).

A literature search concluded that several methods have been published for the assessment of MOM, MIC, and NAD as single component or along with other components, although there is an HPTLC analytical method that has been described for the assessment of MOM, MIC, and NAD drugs in their combined formulations (Patel et al., 2016). This study proposes an alternative, validated HPTLC method for the simultaneous investigation of MOM, MIC, and NAD in creams using suitable mobile phase consisting of a lesser number of solvent components. Moreover, linear and accurate studies were carried out as per the ratio of drugs present in the 3 Mix cream. Routine investigation of these drugs in their semisolid formulation was carried out by applying this simple method based on the outcome in terms of accuracy and precision.

**MATERIALS AND METHODS**

The MOM reference standard was gifted for research purpose by Sun Pharmaceutical Industries Ltd., Vadodara, India, NAD was gifted by Wockhardt Ltd., Aurangabad, India, and MIC was purchased from Hem-Deep Organics Pvt. Ltd., Ankleshwar, India. The 3 MIX cream 7.5 g, manufactured by Abbott Healthcare Pvt. Ltd, containing MOM (0.1% w/w), NAD (1% w/w), and MIC (2% w/w) was purchased from a local pharmacy store. AR grade solvents, such as toluene, ethanol, acetonitrile, methanol, ethanol triethylamine, orthophosphoric acid, and glacial acetic acid, were supplied by Loba Chemie Pvt. Ltd. Mumbai, India.

**Chromatographic conditions**

Prior to development, accurately prepared standard solutions were applied on a precoated silica gel 60 F\textsubscript{254} on aluminum sheets with a dimension of 10 × 20 cm (E. Merck, Darmstadt, Germany) in the form of a band. The applicator named Linomat V (Camag, Muttenz, Switzerland) along with 100 µl Hamilton (Reno, Nevada) microsyringe was used to apply the desired solution as 6 mm bands, 11.6 mm apart, and 10 mm from the bottom.

Before application of the sample, the plates were washed with methyl alcohol and kept in a desiccator. A twin trough Thin layer chromatography (TLC) chamber, by Camag, was utilized for the development (ascending) of the TLC plate, which was saturated earlier with the solvent system for 15 minutes. Toluene, ethyl acetate, ethanol, and formic acid (10:3:2:0.5 v/v/v/v) as solvents were used for the development of the plate at 25 ± 2°C. The distance traveled by the solvent front was 80 mm in approximately 15 minutes. The developed TLC plate was oven-dried (80°C) for 5 minutes, followed by densitometric scanning at 235 nm using Camag’s TLC scanner three with the support of the Wincats software (1.3.0). The silt dimension was set as 6.00 × 0.45 mm and the scanning speed was 40 mm/s. After development, the analyte concentration was estimated based on the intensity of the diffused reflected light and the respective peak areas, and R\textsubscript{f} values were measured.

**Working standard solution**

A standard stock solution containing 200, 4,000, and 2,000 µg/ml of MOM, MIC, and NAD, respectively, was prepared by weighing 10 mg of MOM, 200 mg of MIC, and 100 mg of NAD standard drug accurately, and then transferred into a 50-ml volumetric flask. An additional 10 ml of methyl alcohol was added, sonicated, and the volume was adjusted. A working standard solution containing 20, 400, and 200 µg/ml of MOM, MIC and NAD was prepared by diluting with methyl alcohol in a 10-ml volumetric flask.
Application of study for formulation assay

A 1.5 g of cream formulation equivalent to 1.5 mg of MOM, 30 mg of MIC, and 15 mg of NAD was weighed accurately and transferred into a 50-ml screw centrifuge tube containing 15 ml mixture of methanol: acetonitrile (1:1). Furthermore, it was warmed with occasional swirling for the melting of the semisolid cream. The solution was further vortexed for 10 minutes on the vortex shaker. It was further diluted up to the desired level with methyl alcohol. Furthermore, centrifugation was carried out at 2,500 rpm for 10 minutes and the membrane filter (0.45 μm) was used to filter the obtained solution. The extracted marketed sample solution was diluted again with methyl alcohol to obtain the required concentration of 12 μg/ml MOM, 240 μg/ml MIC, and 120 μg/ml NAD. From the resulting solution, optimum volume (5 μl) was applied on the TLC plate. The developed plate was a scanned chromatogram and the data were recorded and stored for interpretation. Based on the recorded peak area of the component, the concentration and % assay were calculated.

Specificity

Method specificity was checked by assessing the chromatographic peak of the drugs for peak purity. The peak purity of the drugs MOM, MIC, and NAD was judged by matching their corresponding spectra at different positions of the spots, like peak start, peak apex, and peak end.

Linearity and range

Linearity of the method was checked by applying various volumes, i.e., 3, 5, 7, 9, and 11 μl, of the working standard solution (20 μg/ml MOM, 400 μg/ml MIC, and 200 μg/ml NAD) on the plate. The developed precoated plate was analyzed and the response was documented. The calibration curve was established between the amount of analyte (ng/band) and the peak area. The least squares method was applied to obtain a regression equation for evaluating the slope, intercept, and coefficient of correlation.

Sensitivity

The limits of detection and the limits of quantification of the above-mentioned method were evaluated by employing 3 a/S and 10 a/S equations, where a denotes the root-mean square deviation of the y intercepts and S is calibration curve’s mean slope.

Precision

The precision of the method was checked by carrying out repeatability, intraday, and interday precision. To check the repeatability parameter, 100, 2,000, and 1,000 ng/band of MOM, MIC, and NAD, respectively, of the standard stock solution were applied on the precoated plate six times and analyzed; % Relative Standard Deviation (RSD) was computed.

Intraday and interday precision were executed by spotting a band of three different aliquots of the standard solution of MOM (60, 140, 220 ng/band), MIC (1,200, 2,800, 4,400 ng/band), and NAD (600, 1,400, 2,200 ng/band) in triplicate in a day and on three different days, respectively, and the analysis value which was obtained was stated in %RSD.

Accuracy

Recovery analysis was executed by the deliberate incorporation of the standard analyte with the initially analyzed sample solution of cream dosage form (MOM: 50 ng/band; MIC: 1,000 ng/band; and NAD: 500 ng/band) at three different levels, 50%, 100%, and 150%, to investigate the accuracy of the method. The resulting solutions were reanalyzed and %recovery was computed. The obtained value of the accuracy study was predicted based on the percentage of MOM, MIC, and NAD recovered from the 3 Mix formulations.

Robustness

Flexibility of the method was checked in terms of robustness based on slight alterations in the ethanol content of the mobile phase, i.e., ethanol (2 ± 0.1 ml), mobile phase volume (15.5 ± 5%, ml), saturation time (20 ± 5 minutes), development distance (80 ± 5 mm), time difference from spotting to development (25 ± 5 minutes), and time gap from development to scanning (25 ± 5 minutes). It was carried out to check its effects on the retardation factor and peak area of MOM, MIC, and NAD.

Analysis of the marketed formulation

The marketed formulation was extracted to obtain the desired concentrations of MOM 12 μg/ml, MIC 240 μg/ml, and NAD 120 μg/ml, as described earlier in the sample preparation, and 5 μl was spotted on the precoated TLC plate. The applied spots were developed and scanned under optimized conditions. From peak area of the component, the concentration and %assay were calculated using the calibration graph. The mean percentage assay was calculated by repeating the analysis six times.

RESULTS AND DISCUSSION

The chromatographic specifications were optimized to obtain the best possible assay method for the assessment of the MOM, MIC, and NAD by simultaneously using HPTLC. Initially, the migration pattern of all the three components was studied using single solvents, such as methanol, ethyl acetate, toluene, acetone, ethanol, etc. It was observed that ethyl acetate can migrate all the three components at different %RSD values, but in diffused band of MIC and NAD. Therefore, methanol, instead of ethanol, was also tried but showed satisfactory %RSD value of NAD. Therefore, it was difficult to obtain optimum resolution between MOM, MIC, and NAD. However, to improve the peak shape of NAD, some organic modifiers, such as formic acid, glacial acetic acid, ammonia, etc., were tried (Kumar et al., 2010; Kulkarni et al., 2010). After trying with the above-mentioned organic modifiers in the solvent system containing toluene, ethyl acetate, and...
ethanol (10:3:2 v/v/v), only formic acid improved the NAD peak shape. But further addition of an amount of formic acid was found to be detrimental to the MIC peak shape. Therefore, toluene, ethyl acetate, ethanol, and formic acid (10:3:2:0.5 v/v/v/v), as solvent system, were used for the development of the plate. To obtain an acceptable resolution and peak symmetry, as well as reproducible Rf value of all the three analytes, chromatographic conditions, like chamber saturation time, development distance, volume of mobile phase, detection wavelength, activation time, etc., were assessed. All the components were scanned at 235 nm and reproducible Rf values were observed as 0.75, 0.61, and 0.39 for MOM, MIC, and NAD, respectively. Additionally, the peak of nitrate salt of miconazole was observed at Rf 0.05 (Popovic et al., 2004). The chromatograph is shown in Figure 2.

Validation of the developed chromatographic method

The parameters of validation were carried out in agreement with the International Conference on Harmonization guideline for the proposed method (ICH, 2005).

Specificity

The specificity parameter was verified by investigating standard drugs and formulation solution. The mobile phase was capable enough to resolve all the three analytes successfully, as shown in Figure 2. The sample bands of MOM, MIC, and NAD were assured by matching the retention factor (Rf) and UV spectra with that of the standard. The peak purity of all the three drugs, MOM, MIC, and NAD, were judged by matching their corresponding UV spectra at peak start, apex, and at the end of the band. A good correlation, i.e., in range of 0.9999–0.9996, was attained for all the spectra at start (s), apex (m), and end (e) of the peaks, serving as a sign of peak purity for all the three drugs, which indicates that no other component, such as degradation products or impurities, traveled with the peaks (Shinde et al., 2015).

Linearity

The correlation coefficient ranging between 0.998 and 0.999 was achieved after regression analysis (linear) over the concentration gap of 60–220, 1,200–4,400, and 600–2,200 ng/band for the drugs MOM, MIC, and NAD, respectively, which is indicative of an excellent linear relationship. The summary of the results is shown in Table 1, and the overlain 3D chromatogram of the standard solution of MOM, MIC, and NAD is shown in Figure 3.

Sensitivity

The limits of detection (ng/band) were found to be 14.075, 326.945, and 191.611, and the limits of quantification (ng/band) were 42.653, 990.741, and 580.639 of MOM, MIC, and NAD, respectively, which showed that the method is reasonably sensitive (Table 1).

Precision

In repeatability studies, the %RSD was in the range of 0.734–1.008, also the %RSD of precision studies (intraday and interday) were in the range of 1.112–1.332 and 1.359–1.616, respectively (Table 1). All the obtained results were within the ICH guideline’s acceptable limits, i.e., <2, confirming the preciseness of the proposed method (Table 1).

Accuracy

Results of the recovery studies (98.314%–99.986%) for all three drugs revealing the accuracy of the method are shown in Table 2. It indicates that the inactive components of formulation were not interfering with the active constituents, i.e., MOM, MIC, and NAD.

Robustness

This study is confirmed to be robust, since the Rf value and peak area were not affected much by introducing slight changes to the experimental conditions that are shown in Table 3. The standard deviations of the chromatographic responses for every parameter were calculated and %RSD <2 was obtained, authenticating the robustness of the projected method.

Figure 2. HPTLC chromatogram of MOM (100 ng/band), MIC (2,000 ng/band), and NAD (1,000 ng/band).
Assay of the marketed formulation

Quantitative assessment of MOM, MIC, and NAD in the 3 Mix cream (10, 200, and 100 mg in 10 g of cream) were effectively carried out by the proposed HPTLC method. Average assay values for MOM, MIC, and NAD were found to be 99.088 ± 1.793, 99.368 ± 0.814, and 99.613 ± 1.520% w/w, respectively, after assessing the 3 Mix cream six times (Table 4). One-way analysis of variance (ANOVA) was used to measure the difference between the assay results of published and developed methods which are shown in Table 5. The

![Overlain 3D chromatogram of MOM, MIC, and NAD.](image)

**Table 1.** Summary of linear regression and method validation data for the method.

| Parameters                        | MOM          | MIC          | NAD         |
|-----------------------------------|--------------|--------------|-------------|
| Linearity range (ng/band)         | 60–220       | 1,200–4,400  | 600–2,200   |
| Correlation coefficient           | 0.999        | 0.999        | 0.998       |
| Regression equation               | $y = 11.57x + 726.4$ | $y = 0.835x + 2,184$ | $y = 1.759x + 2,541$ |
| Precision (%RSD)                  | 1.116        | 1.112        | 1.332       |
| Intraday ($n = 3$)                | 1.359        | 1.505        | 1.616       |
| Interday ($n = 3$)                | 0.734        | 0.972        | 1.008       |
| Repeatability of injection ($n = 6$) | No interference |             |             |
| LOD (ng/band)                     | 14.075       | 326.945      | 191.611     |
| LOQ (ng/band)                     | 42.653       | 990.741      | 580.639     |
obtained value justifies that the developed method does not have considerable variation from the reported method.

CONCLUSION

MOM, MIC, and NAD in the 3 Mix cream were investigated simultaneously by the developed HPTLC method which serves as a quality control tool. It has the potential to determine these drugs in the cream formulation precisely, without any interference. The statistical outcome reveals that there is no considerable variation between the developed and reported methods, but the developed method can be utilized as a substitute method which has advantages over the reported method in terms of composition of mobile phase. Hence, different quality control laboratories can use this projected method to investigate these drugs individually or in combination in the present pharmaceutical formulation.

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| Table 2. Results of recovery studies. |
|--------------------------------------|
| Recovery level (%) | 50 | 100 | 150 |
|---------------------|----|-----|-----|
| MOM*               | 99.986 ± 0.937 | 98.712 ± 1.095 | 98.314 ± 1.035 |
| MIC*               | 99.233 ± 1.633 | 99.226 ± 1.131 | 99.086 ± 1.207 |
| NAD*               | 98.737 ± 1.603 | 99.105 ± 1.357 | 99.666 ± 1.303 |

% Recovered mean ± SD* (n=3).
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CONFLICT OF INTEREST

Authors declared that they do not have any conflict of interest.

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