STABILITY-INDICATING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF FORMOTEROL FUMARATE DIHYDRATE AND FLUTICASONE PROPIONATE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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INTRODUCTION

Inhalation is currently the preferred route of drug delivery in asthma in accordance with a global initiative for asthma guideline [1], as it allows the release of drug directly to the site where the action is needed, thus minimizing systemic side effect. Inhalated corticosteroids in combination with a long-acting β2-agonist is the gold standard for the management of persistent asthma, with maximal local targeting and minimal systemic side effects. Formoterol fumarate dihydrate (FFD) is a long-acting selective β-2 agonist used as a bronchodilator in the treatment of asthma. Chemically, FFD is a (E)-but-2-enedioic acid; N-[2-hydroxy-5-[(1S)-1-hydroxy-2-[(2S)-1-(4-methoxyphenyl) propan-2-yl] amino] ethyl] phenylformamide [2,3].

Fluticasone propionate (FP) is chemically 6α, 9-Difluoro-17-[(fluoromethyl) sulphonyl] carbonyl]-11β-hydroxy-16α-methyl-3-oxoandrost-1-4-dien-17α-y1propanoate. FP is a tri-fluorinated glucocorticoid specifically designed to provide enhanced anti-inflammatory effect [2,4]. Both drugs are official in IP, BP, EP, and USP [5-8]. The chemical structures of FFD and FP [8] are shown in Fig. 1a and b, respectively.

Literature survey for FFD and FP revealed that various analytical methods using techniques such as high-performance liquid chromatography [9-20], spectrophotometry [18,21-25], and high-performance thin-layer chromatography (HPTLC) [19,26] were reported for quantitative determination of single or multi-component systems. Gowekar and Wader reported HPTLC method for simultaneous estimation of FFD and FP, but no degradation profile has been stated in the literature [27]. To the best of our knowledge, there is no stability indicating HPTLC method reported for the simultaneous estimation of FFD and FP in bulk drug and pharmaceutical dosage form.

Hence, the objective of the present work was to develop and validate the stability indicating HPTLC method for simultaneous estimation of FFD and FP in bulk drug and pharmaceutical dosage form.

METHODS

Chemicals and reagents

Gift samples of FFD and FP were procured from Yamsi Laboratories Pvt. Ltd. Solapur, Maharashtra, India. The pharmaceutical formulation of capsule Maxiflo-100 Rotacaps containing 6 μg of FFD and 100 μg of FP manufactured by Cipla Ltd. was procured from the market. All analytical grade chemicals and reagents used for the analysis were purchased from Merck, Mumbai, India.

Instrumentation

Pre-coated silica gel aluminum plates 60F-254 (20 cm×10 cm, 250 μm thickness, E. Merck, Darmstadt, Germany) supplied by Anchorn, Mumbai were used. The sampling was done by automated TLC sampler Linomat V applicator (Camag, Muttenz, Switzerland) which was controlled by Win-Cats software (V 3.15, Camag, Muttenz, Switzerland). The standard and sample solutions were spotted in the form of bands of width 6 mm with a Camag 100 μl sample (Hamilton, Bonaduz, Switzerland) syringe. Linear ascending development was carried out in a twin trough glass chamber (20 cm×10 cm, 10 cm×10 cm Camag, Muttenz, Switzerland). The mobile phase consisted of toluene:ethyl acetate:formic acid (98%)

RESULTS

The selected mobile phase resolved peaks of FFD and FP with Rf values 0.27±0.10 and 0.64±0.10, respectively. Determination coefficients of calibration curves were found to be 0.998 and 0.999 in the range of 1-3.5 μg/spot and 10-60 μg/spot for FFD and FP with an accuracy of 99.09% for FFD and 99.20% for FP. The degradation products of FFD and FP were resolved from the pure drug with significant differences in their retention factor values.

Conclusion

The developed method is simple, accurate and can be successfully applied for quantification of FFD and FP in bulk drug and pharmaceutical dosage form, contributing to improve the quality control and assure the therapeutic efficacy.

Keywords: Formoterol fumarate dihydrate, Fluticasone propionate, High-performance thin-layer chromatography, Stability-indicating method, Validation.

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The developing solvent was run up to 80 mm and development was performed at room temperature (25°C±2°C) at a relative humidity of 60%±5%. The development time was 20 min. Plates were scanned at 233 nm with CAMAG TLC scanner 3. Deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm was used as a source of radiation.

HPTLC method and chromatographic conditions

Preparation of standard stock solutions

Accurately weighed 10 mg of FFD was transferred to 10 ml volumetric flask, dissolved and diluted up to the mark with methanol (1 mg/ml). Accurately weighed 10 mg of FP was transferred to 10 ml volumetric flask, dissolved and diluted up to the mark with chloroform (1 mg/ml).

Preparation of sample solution

Powder from 20 capsules (Maxiflo-100 Rotacaps containing 6 μg of FFD and 100 μg of FP per capsule) were weighed, their average weight determined (3.038 mg) and crushed to fine powder. The quantity of powder equivalent to 10 mg of FP and 0.6 mg of FFD was transferred into a 10 ml volumetric flask containing 5 ml of methanol and mixed well. The solution was ultrasonicated for 20 min, and then diluted to 10 ml with methanol. The solution was filtered through Whatman filter paper (0.45 µm). The amount of each drug present in the sample was determined by comparing mean peak areas with that of the standard.

Pre-washing of plates

Densitometric estimation was carried out on 20 cm×10 cm pre-coated silica gel 60F–254 plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 15 min at 110°C before chromatography.

Selection of the solvent

Methanol and chloroform were selected as solvents for preparing sample solutions.

Selection of stationary phase

Identification and separation of FFD and FP were carried out on 20 cm×10 cm, 10 cm×10 cm, pre-coated silica gel aluminium plates 60 F-254 (250 μm thickness E. Merck, Darmstadt, Germany).

Sample application

The standard and formulation solution of FFD and FP was spotted on pre-coated TLC plates in the form of narrow bands of length 6 mm, at 8 mm from the bottom, and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nl/s.

Selection of wavelength

An evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 233 nm using methanol as a blank solution. The selection of detection wavelength is shown in Fig. 2. The FFD and FP were satisfactorily resolved with Rf value 0.27±0.10 and 0.64±0.10, respectively. Pre-saturation of TLC chamber for 20 min assured better reproducibility in the migration of FFD and FP with better resolution which is shown in Fig. 3.

Method validation

The developed HPTLC method was validated as per the ICH guidelines Q1A (R2), Q1B, Q2 (R1) for linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and specificity [28-33].

Optimization of the mobile phase

Various solvent systems such as mixtures of (1) n-hexane:ethyl acetate:methanol:acetic acid (2.0:2.5:2.0:0.2; v/v/v/v), (2) n-hexane:ethyl acetate:methanol:formic acid (2.0:2.5:2.0:0.2; v/v/v/v), (3) n-hexane:ethyl acetate:acetic acid (5:10:0.2; v/v/v), and (4) toluene:ethyl acetate:formic acid (7:3:0.1; v/v/v) were tried to separate and resolve spots of FFD and FP from each other and other excipients of formulation. The mixture of n-hexane:ethyl acetate:methanol:acetic acid (2.0:2.5:2.0:0.2; v/v/v/v) and n-hexane:ethyl acetate:methanol:formic acid (2.0:2.5:2.0:0.2; v/v/v/v) provided well-resolved peaks but tailing was observed. Good peak shape was observed with a mixture of toluene:ethyl acetate:formic acid (7:3:0.1; v/v/v), but the FFD did not resolve from FP. Finally, the mixture of toluene:ethyl acetate:formic acid (6:4:0.1; v/v/v) showed well-resolved peaks with better peak shape. FFD and FP were satisfactorily resolved with R value 0.27±0.10 and 0.64±0.10, respectively. Pre-saturation of TLC chamber with the mobile phase for 20 min assured better reproducibility in the migration of FFD and FP with better resolution which is shown in Fig. 3.
Slope, intercept, and coefficient of determination (r^2) of the calibration curves were calculated to ascertain linearity of the method.

**Precision**

To evaluate intraday precision, three samples at three different concentrations were analyzed on the same day. The interday precision was studied by comparing assays performed on three different days.

The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions.

The intraday and interday variation for determination of FFD and FP were carried out at three different concentration levels 1.5, 2, and 2.5 µg/spot for FFD and 20, 30, and 40 µg/spot for FP.

**Repeatability**

Repeatability of sample application was assessed by spotting 2 µg/spot for FFD and 30 µg/spot FP of standard drug solution 6 times on a TLC plate at different times on the same day by sample applicator, followed by the development of plate and recording of the peak areas for six spots.

**Accuracy**

Accuracy studies were carried out at 80–120% levels, by mixing a known quantity of standard drug (0.5, 0.6, and 0.7 µg for FFD and 8, 10, and 12 µg for FP) with the sample formulation and the contents were analyzed by the proposed method.

**Specificity**

The specificity of the method was ascertained by analysis of drug standards and samples. The identities of the peaks for FFD and FP were confirmed by comparing the Rf with those of standards. The peak purity of FFD and FP was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot.

**Robustness**

The proposed HPTLC method was tested for robustness. The parameters selected for the robustness study were, change in the amount of toluene in mobile phase composition, change in time from spotting to chromatography and time from chromatography to scanning, and change in saturation time. By introducing small changes in these parameters, the effect on the results was examined.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

LOD and LOQ values represent the sensitivity of the proposed analytical method. To estimate the LOD and LOQ, blank methanol was spotted 6 times. Different concentrations 0.1, 0.3, 0.5, 0.8, and 1 µg/spot for FFD were analyzed.
and 0.1, 0.2, 0.4, 0.6, and 0.8 µg/spot for FP were spotted. The peak was detected at 0.3 µg/spot for FFD and 0.2 µg/spot for FP with a signal-to-noise ratio of 3:1. The peak was detected with the quantifiable area at 1 µg/spot for FFD and 0.6 µg/spot for FP with a signal-to-noise ratio of 10:1.

**Forced degradation studies**

**Acid- and base-induced degradation**

To 6 mg of FFD and 100 mg of FP, 10 ml of 0.1 N HCl and 10 ml of 0.1 N NaOH were added separately and refluxed at 50°C for 1 h. Samples were withdrawn (0.5 ml) at different time intervals for 1 h.

Further acidic and alkaline degradation were carried out for a combination of FFD and FP by refluxing them together with 10 ml 0.1 N HCl and 10 ml 0.1 N NaOH at 50°C for 1 h. Samples were withdrawn (0.5 ml) at different time intervals for 1 h. A 3 µL solution was applied on TLC plate in such a way that final concentration achieved was 1.8 µg/spot for FFD and 30 µg/spot for FP and densitograms were developed.

**Oxidative degradation**

To 6 mg of FFD and 100 mg of FP, 10 ml of 3% H₂O₂ was added separately and refluxed at 50°C for 2 h. Samples were withdrawn (0.5 ml) at different time intervals for 2 h.

Further oxidative degradation was carried out for a combination of FFD and FP by refluxing them together with 10 ml of 3% H₂O₂ at 50°C for 2 h. Samples were withdrawn (0.5 ml) at different time intervals for 2 h. A 3 µL solution was applied on TLC plate in such a way that final concentration achieved was 1.8 µg/spot for FFD and 30 µg/spot for FP and densitograms were developed.

**Photolytic degradation**

Solid forms of FFD and FP were exposed directly to sunlight during the daytime for 2 days to study their photolytic stability. Samples were weighed, dissolved and 3 µL solution was applied on TLC plate in such a way that final concentration achieved was 1.8 µg/spot for FFD and 30 µg/spot for FP and densitograms were developed.

**Neutral hydrolysis**

To 6 mg of FFD and 100 mg of FP, 10 ml of water was added separately and refluxed at 50°C for 2 h. Samples were withdrawn (0.5 ml) at different time intervals for 2 h.

The neutral degradation of FFD and FP in combination was induced by refluxing them together with 10 ml of water at 50°C for 2 h. Samples were withdrawn (0.5 ml) at different time intervals for 2 h. A 3 µL solution was applied on TLC plate in such a way that final concentration achieved was 1.6 µg/spot for FFD and 30 µg/spot for FP and densitograms were developed.

**RESULTS**

**Optimization of chromatographic conditions**

Toluene:ethyl acetate:formic acid (98%) (6:4:0.1 v/v/v) mixture provided best resolution with better peak shape. The R<sub>f</sub> values were found to be 0.27 and 0.64 for FFD and FP, respectively.

**Table 1: Linearity and range for FFD and FP**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/spot)</th>
<th>Regression coefficient (r²)</th>
<th>Linearity equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFD</td>
<td>1–3.5</td>
<td>0.998</td>
<td>y=677.6x+472.73</td>
</tr>
<tr>
<td>FP</td>
<td>10–60</td>
<td>0.999</td>
<td>y=1013.3x+10059</td>
</tr>
</tbody>
</table>

FFD: Formoterol fumarate dihydrate, FP: Fluticasone propionate

**Table 2: Precision studies for FFP and FP**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Precision</th>
<th>Concentration (µg/spot)</th>
<th>Area</th>
<th>Average area</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFD</td>
<td>Intraday</td>
<td>1.5</td>
<td>1476</td>
<td>1452</td>
<td>1448</td>
<td>1460</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1791</td>
<td>1798</td>
<td>1757</td>
<td>1782</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>2234</td>
<td>2204</td>
<td>2252</td>
<td>2230</td>
</tr>
<tr>
<td></td>
<td>Interday</td>
<td>1.5</td>
<td>1481</td>
<td>1462</td>
<td>1498</td>
<td>1480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1771</td>
<td>1742</td>
<td>1789</td>
<td>1767</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>2267</td>
<td>2244</td>
<td>2212</td>
<td>2241</td>
</tr>
<tr>
<td>FP</td>
<td>Intraday</td>
<td>20</td>
<td>38152</td>
<td>38869</td>
<td>37878</td>
<td>38299</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>49962</td>
<td>49758</td>
<td>48935</td>
<td>49551</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>57998</td>
<td>57993</td>
<td>56901</td>
<td>57630</td>
</tr>
<tr>
<td></td>
<td>Interday</td>
<td>20</td>
<td>38282</td>
<td>37095</td>
<td>37248</td>
<td>37808</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>47989</td>
<td>48102</td>
<td>48966</td>
<td>48322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>57767</td>
<td>56894</td>
<td>56587</td>
<td>57082</td>
</tr>
</tbody>
</table>

FFD: Formoterol fumarate dihydrate, FP: Fluticasone propionate, RSD: Relative standard deviation

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Fig. 9: Densitogram of degradation products of formoterol fumarate dihydrate (FFD) and fluticasone propionate (FP) in 0.1N HCl at 50°C after 30 min at R<sub>f</sub> value 0.15 (peak 1; FFD 1), 0.47 (peak 3; FFD 2), and 0.72 (peak 5; FP 1), respectively

Fig. 10: Densitogram of degradation products of formoterol fumarate dihydrate (FFD) and fluticasone propionate (FP) in 0.1N NaOH at 50°C after 15 min at R<sub>f</sub> value 0.42 (peak 2; FFD 3) and 0.78 (peak 4; FP 2), respectively
Validation of the method

**Linearity (calibration curve)**

Linearity was demonstrated with six different concentration levels for both FFD and FP, which were found to be linear in the range of 1–3.5 µg/spot for FFD and 10–60 µg/spot for FP. The values are given in Table 1. Regression coefficient and concentration of the drugs correlated well. The calibration curves are shown in Figs. 4 and 5. The residual plots are shown in Figs. 6 and 7.

**Precision**

The values of intraday and interday precision are given against sample application and scanning of peak area and results are expressed in terms of percentage relative standard deviation (RSD). The measurement of peak areas at three different concentration levels showed a low value of percentage RSD (<2) for intra- and inter-day variation, which suggested that the method was precise (Table 2).

**Repeatability**

The percentage RSD for repeatability of the drugs was found to be <2 (i.e., 1.05 for FFD and 1.17 for FP). Hence, it was concluded that the proposed method for estimation of FFD and FP was repeatable in nature; the data for the same are shown in Table 3.

**Accuracy**

To check the accuracy of the method, recovery studies were carried out by standard addition of drug solution to pre-analyzed sample solution at

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**Table 3: Repeatability study for FFD and FP (n=6)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/spot)</th>
<th>Peak area</th>
<th>Average area</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formoterol fumarate dihydrate</td>
<td>2</td>
<td>1763</td>
<td>1773.33</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1748</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1768</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1774</td>
<td></td>
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<td></td>
<td>2</td>
<td>1802</td>
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<tr>
<td></td>
<td>2</td>
<td>1785</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>30</td>
<td>48369</td>
<td>48707.16</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>49435</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48885</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48598</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48687</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>30</td>
<td>49269</td>
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<td></td>
</tr>
</tbody>
</table>

FFD: Formoterol fumarate dihydrate, FP: Fluticasone propionate, RSD: Relative standard deviation

**Table 4: Recovery studies for FFD and FP by HPTLC method (n=3)**

<table>
<thead>
<tr>
<th>Label claim (µg/capsule)</th>
<th>% Level of spiked standard drug</th>
<th>Conc. added</th>
<th>Formulation</th>
<th>Pure drug</th>
<th>Total amount (µg)</th>
<th>Average area n=3</th>
<th>Amount recovered (µg)</th>
<th>% Recovery</th>
<th>Mean (%) recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFD 6 µg</td>
<td>80</td>
<td>0.6</td>
<td>0.5</td>
<td>1.1</td>
<td>1212</td>
<td>1.09</td>
<td>99.09</td>
<td>99.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.6</td>
<td>0.6</td>
<td>1.2</td>
<td>1279</td>
<td>1.18</td>
<td>99.08</td>
<td>99.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.6</td>
<td>0.7</td>
<td>1.3</td>
<td>1348</td>
<td>1.29</td>
<td>99.23</td>
<td>99.23</td>
<td></td>
</tr>
<tr>
<td>FP 100 µg</td>
<td>80</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>28136</td>
<td>17.83</td>
<td>99.05</td>
<td>99.22</td>
<td></td>
</tr>
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<td></td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>30195</td>
<td>19.87</td>
<td>99.35</td>
<td>99.27</td>
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<tr>
<td></td>
<td>120</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>32196</td>
<td>21.84</td>
<td>99.27</td>
<td>99.27</td>
<td></td>
</tr>
</tbody>
</table>

FFD: Formoterol fumarate dihydrate, FP: Fluticasone propionate, RSD: Relative standard deviation

**Table 5: Results of robustness evaluation of FFD and FP (n=3)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>FFD Average area</th>
<th>% RSD</th>
<th>FP Average area</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Change in amount of toluene in mobile phase composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene: ethyl acetate: formic acid (5.9:4:0.1% v/v/v)</td>
<td>1413</td>
<td>1.13</td>
<td>57047</td>
<td>1.14</td>
</tr>
<tr>
<td>Toluene: ethyl acetate: formic acid (6.1:4:0.1% v/v/v)</td>
<td>1471</td>
<td>1.12</td>
<td>57006</td>
<td>1.12</td>
</tr>
<tr>
<td>B: Change in saturation time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>1433</td>
<td>1.10</td>
<td>56953</td>
<td>1.13</td>
</tr>
<tr>
<td>25 min</td>
<td>1464</td>
<td>1.14</td>
<td>57118</td>
<td>1.07</td>
</tr>
<tr>
<td>C: Time from spotting to chromatography (+10 min)</td>
<td>1433</td>
<td>1.10</td>
<td>56833</td>
<td>1.10</td>
</tr>
<tr>
<td>D: Time from chromatography to scanning (+10 min)</td>
<td>1407</td>
<td>1.13</td>
<td>57426</td>
<td>1.11</td>
</tr>
</tbody>
</table>

FFD: Formoterol fumarate dihydrate, FP: Fluticasone propionate, RSD: Relative standard deviation
three different levels 80, 100, and 120%. The percent mean recoveries were found to be 99.13% for FFD and 99.22% for FP (Table 4).

### Specificity

The peak purity of FFD and FP was assessed by comparing their respective densitograms at peak start, peak apex, and peak end positions of the spot. The positions for FFD are: start (0.25–0.27), middle (0.57–0.65), and end (0.65–0.68); for FP, they are: start (0.27–0.29), middle (0.57–0.65), and end (0.65–0.68). The densitogram of the capsule sample showed peaks at Rf values of 0.27 and 0.64 for FFD and FP, respectively (Fig. 8), indicating that there is no interference of the excipients present in the capsule formulation indicating the specificity of the method.

### Robustness

The percentage RSD of the peak areas was calculated for change in scanning, change in saturation time and change in solvent run distance (Table 5).

### Limits of detection and quantitation

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3:1). The LOD was found to be 0.3 µg/spot for FFD and 0.2 µg/spot for FP. LOD is the smallest concentration of the analyte, which gives the response that can be accurately quantified (signal to noise ratio of 10:1). The LOQ was 1 µg/spot for FFD and 0.6 µg/spot for FP, which indicates that the proposed method was sensitive enough to detect the drugs at very low concentration level (Table 6).

### Forced degradation studies

Selectivity of the method was demonstrated by enhancing degradation of FFD and FP under various stressed conditions (acid, base hydrolysis, oxidation, neutral, and photochemical), to show that FFD and FP were separated from their possible degradation products. The number of degradation products with their Rf, was calculated and listed in Table 7.

### Acid-induced degradation

The densitograms for acid degraded FFD and FP showed additional peaks at Rf 0.15, 0.47 and 0.72, respectively. The rate of degradation for FFD (18.00%) was more as compared to FP (13.22%) in acid-induced degradation (Fig. 9).

### Base-induced degradation

The densitogram of hydrogen peroxide-induced degradation showed the additional peaks at Rf 0.50 for FFD and 0.72 and 0.84 for FP, respectively. The percent of degradation was found to be 13.06% for FFD and 11.29% for FP (Fig. 10).

### Oxidative degradation

The drugs were found to be susceptible to oxidative degradation. The densitogram of hydrogen peroxide-induced degradation showed the additional peaks at Rf value 0.47 for FFD and 0.84 for FP, respectively. The percent of degradation was found to be 13.06% for FFD and 11.29% for FP (Fig. 11).

### Photolytic degradation

FP and FFD were found to undergo photolytic degradation after exposure of solid drugs direct to sunlight during the daytime for 2 days. Degradation of FFD was observed (5.74%) with degradation product r (start, middle) = (0.25–0.27) and r (middle, end) = (0.65–0.68) for FP. The chromatogram of capsule sample showed peaks at Rf values of 0.27 and 0.64 for FFD and FP, respectively (Fig. 8), indicating that there is no interference of the excipients present in the capsule formulation indicating the specificity of the method.

### Neutral degradation

The FFD and FP showed two additional peaks when treated in water at 50°C for 30 min. Peaks of degraded products were found at Rf value 0.47 (peak 2; FFD 2) and 0.74 (peak 4; FP 5).

### Discussion

The proposed stability indicating HPTLC method provides precise, accurate, and reproducible quantitative analysis for simultaneous estimation of FFD and FP in bulk drug and pharmaceutical dosage form. The method was validated as per the ICH guidelines. The linearity was found to be in the range of 1–3.5 µg/spot and 10–60 µg/spot for FFD and FP, respectively. Percentage RSD of intraday and interday precision was found to be <2% making the method more precise. Degradation study revealed that FFD was more prone to degradation under acid (18%, 30 min) stress followed by the stress conditions such as neutral
(14.3%, 30 min), base (9.11%, 15 min), oxidative (13.06%, 1 h), and photolytic (5.74%, 2 days). FP showed more degradation in basic (18.12, 15 min) and photolytic (18.3%), 24 h) conditions.

CONCLUSION
The developed method was able to separate the drugs from its degradants and impurities. It can be successfully applied as stability indicating method for combination of FFD and FP. Thus, the reported method is of considerable importance and has sound industrial applicability for quality control and stability analysis of FFD and FP from bulk drug and pharmaceutical dosage form.

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CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES