Analytical method validation on simultaneous estimation of Ozenoxacin and Benzoic acid in pharmaceutical formulation

AMARNATH REDDY RAMIREDDY¹ and DILIP KUMAR BEHARA²

¹ Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu, Andhra Pradesh, 515002, India
² Chemical Engineering, JNTUA College of Engineering (Autonomous), Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu, Andhra Pradesh, 515002, India

ABSTRACT

In this study, an accurate, simple, economical and precise Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of Ozenoxacin and Benzoic Acid in a pharmaceutical cream formulation, according to the International Conference on Harmonisation (ICH) guidelines. Chromatographic separation was achieved by gradient elution, on RP-HPLC Instrument, equipped with column C8 (150 mm × 4.6 mm, 5 μm particle size) using Ultra Violet (UV) detector at 235 nm wavelength, by using Mobile Phase A: triethylamine, trifluoroacetic acid and water (1:1:1000) and Mobile Phase B: methanol and Diluent: water, acetonitrile and triethylamine (500:500:1), at flow rate 0.8 mL min⁻¹; injection volume of 20 μL; column oven temperature 45°C and sample temperature: 25°C; Run time: 15 min. All the validation parameters were within the acceptance criteria, as per ICH requirements, for Ozenoxacin and Benzoic acid. Consequently, this method has found to be validated, simple, rapid and successfully applicable, to the simultaneous estimation of Ozenoxacin and Benzoic acid by RP-HPLC, for routine analytical testing in quality control, with a run time of 15 min and for future research studies. Forced degradation of Ozenoxacin cream 1% w/w formulation was performed and found that validated method has stability indicating potential that needs to be further studied.

KEYWORDS

Ozenoxacin, Benzoic acid, impetigo, RP-HPLC, stability indicating

INTRODUCTION

Ozenoxacin is a novel topical, non-fluorinated quinolone antimicrobial [1]. Ozenoxacin Cream 1% w/w was approved by United States Food and Drug Administration (FDA) in December 2017 to treat Impetigo caused by Staphylococcus aureus or Streptococcus pyogenes in pediatric patients with two months of age and older as well as adults. Twice-daily application of 0.5 g of Ozenoxacin Cream 1% w/w for five days is the recommended dosing regimen [2, 3].

Impetigo is the third most common skin disease in children, after dermatitis and viral warts, with a peak incidence at 2–6 years of age [4, 5]. Lesions are highly contagious and can spread rapidly by direct contact, through a family, nursery, or class. [6].

Ozenoxacin belongs to the quinolone group of antimicrobials and is the first non-fluorinated quinolone, which has a pyridinyl group at C7 [7].

Ozenoxacin appears as a white to pale - yellow crystalline solid material [8]. Ozenoxacin chemical name is “1 - cyclopropyl - 8 - methyl - 7 - (5 - methyl - 6 - methylamino - pyridine - 3 -yl) - 4 - oxo - 1,4 - dihydro - quinolone - 3 - carboxylic acid”, molecular formula is C₂₁ H₂₁ N₃ O₃ and molecular weight is 363.41 g mol⁻¹. Ozenoxacin structure [9] is as shown in Fig. 1 (a).
Innovator formulation XEPITM existing in a cream form and each gram of the cream contains 10 mg of Ozenoxacin and the subsequent inactive excipients: Stearyl alcohol, peglicol 5 oleate (Labrafil M1944CS: Oleoyl macrogol-6 glycerides), pegoxol 7 stearate (Tefose 63: PEG-6 Stearate and Ethylene Glycol Palmito Stearate and PEG-32 Stearate), octyl dodecanol, propylene glycol, Benzoic Acid and purified water [2, 10, 11].

Benzoic Acid is used in Ozenoxacin Cream 1% w/w, as an anti-microbial preservative. Benzoic Acid appears as a white crystalline solid, has a molecular formula of C7H6O2, has a molecular weight of 122.12 g mol⁻¹ and structure [12] is as shown in Fig. 1 (b).

Anti-Microbial Preservatives are the ingredients that are commonly included in pharmaceutical, cosmetic, food and beverages to inhibit initial microbial loads, quash their propagation, or preclude new microbial contamination in a preparation of multiple doses and extend the shelf life of the product [13, 14]. Semisolid dosage forms generally comprise of water or water-based solvents inside them. Chances of microbial growth and contamination increases with the presence of water. Therefore, Anti-Microbial Preservatives are required to inhibit the microbial growth in semisolid dosage forms due to any of these reasons [15].

Sufficient quantity of Benzoic Acid is required to inhibit microbial growth in formulation. For this purpose, in the same run, Ozenoxacin which is an Active Pharmaceutical Ingredients and Benzoic Acid which is an anti-microbial preservative are to be analyzed, for the estimation, in finished product & stability stage of formulation by using same analytical testing procedure.

The literature survey disclosed that there is not one analytical method available for the concurrent measurement of Ozenoxacin and Benzoic Acid. Thus, it was certainly important to develop a single run method of Reversed Phase High Pressure Liquid Chromatography (RP-HPLC), which will decrease cost and save time as compared to the prevailing individual estimation methods. This research paper reports a simple, selective RP-HPLC method for concurrent measurement of Ozenoxacin and Benzoic Acid in cream formulation, which was established, optimized and reported for the first time.

MATERIALS AND METHODS

Materials

Ozenoxacin was collected from Optimus Drugs Private Limited, Hyderabad, India as a gift sample and Benzoic Acid was purchased from Ganesh Benzoplast, Thane, India. The pharmaceutical dosage form of their combination OZANEX cream (Ozenoxacin Cream 1% w/w market sample) was purchased from Cipher Pharmaceuticals Inc., Canada. All solvents used in this work were HPLC grade and purchased from MERCK/RANKEM (India). All other materials used were of analytical reagent grade.

Instrumentation

The chromatographic partition was executed on a RP-HPLC system comprising the following: a Waters Alliance 2695 series system with 2489 Photo Diode Array detector, equipped with quaternary pumps and an autosampler. Data handling system was performed using Waters Empower work station.

Development and optimization of chromatographic conditions

Different aqueous and organic phases were used for best partitioning of Ozenoxacin and Benzoic Acid to develop a robust chromatographic method.
**Analytical method validation**

The analytical method used for simultaneous measurement of Ozenoxacin and Benzoic Acid was validated as per the ICH guideline Q2 (R1) and the method validation parameters [25] were: specificity, precision (repeatability and intermediate precision), linearity, accuracy, range and robustness.

Forced degradation studies were performed to assess stability-indicating potential of the validated method. Filter validation was also performed.

**Chromatographic conditions**

The chromatographic partitioning was accomplished using a Hypersil BDS C8 column (150 mm × 4.6 mm; 5 μm particle size). The total run time was 15 min carried out using gradient elution (Table 1) at the flow rate of 0.8 mL min⁻¹. The column oven temperature was maintained at 45 °C. The Ultraviolet/Visible (UV) detector wavelength was established at 235 nm and the injection volume was 20 μL. All the determinations were performed at room temperature (~25 °C).

**Preparation of Mobile Phase A:** Triethylamine, trifluoroacetic acid and purified water were added at the volume ratio of 1:1:1000 respectively and mixed well.

**Preparation of Mobile Phase B:** Methanol.

**Preparation of Diluent:** Purified water, acetonitrile and triethylamine were added at the volume ratio of 500:500:1 respectively and mixed well.

**Preparation of Needle Wash:** Purified water and methanol were added at the ratio of 20:80 (v/v) respectively and mixed well.

### RESULTS AND DISCUSSION

RP-HPLC method was developed in agreement with the ICH guideline Q2 (R1) for the simultaneous estimation of Ozenoxacin and Benzoic Acid in a pharmaceutical cream formulation.

#### Development and optimization of chromatographic conditions

Different aqueous and organic phases, flow rates and wavelengths were used for best separation of Ozenoxacin and Benzoic Acid to develop a robust chromatographic method for the simultaneous measurement of Ozenoxacin and Benzoic Acid. The improved chromatographic environments were attained, when method development was introduced using an aqueous phase (A) comprising triethylamine: orthophosphoric acid: water at the volume ratio of 1:1:1000 (pH adjustment using orthophosphoric acid to 2.0 ± 0.05) and organic phase (B) comprising Methanol and Acetonitrile at the ratio of 700:300 (v/v) with a flow rate of 1.0 mL min⁻¹, UV detector was set at wavelength of 230 nm; temperature of column oven maintained at 40 °C. Under these situations, elution for Ozenoxacin and Benzoic Acid gave sharp peaks but peak shapes were observed irregular.

To resolve this, finally, aqueous phase (A) containing triethylamine: trifluoroacetic acid: water at the volume ratio of 1:1:1000 and organic phase (B) containing methanol, measured at wavelength of 235 nm using UV detector; column oven temperature 45 °C; flow rate of 0.8 mL min⁻¹ were used. These changes allowed very good separation of Ozenoxacin and Benzoic Acid and resolution was found to be greater than 4.0, sharp regular peak shapes were observed. The effectiveness of a separation can be quantified by measuring the resolution. Resolution is a measure of the degree of separation between two adjacent signals, detector responses, or, in the case of optical instruments, objects [26]. All of the system suitability parameters (theoretical plates, tailing factor, resolution and relative SD) were contented the FDA requirements [27]. The Typical chromatogram of Ozenoxacin and Benzoic Acid Standard solution is shown in Fig. 2.

![Fig. 2. Typical chromatogram of ozenoxacin and benzoic acid standard solution](image-url)
Analytical method validation

System suitability. To establish the system suitability, standard solution of Ozenoxacin and Benzoic Acid was prepared and injected five times. Results were given in Supplemental file (Table S1 & Table S2).

Theoretical plates of Ozenoxacin and Benzoic Acid peaks in standard solution were found to be 3,308 and 7,921 respectively (Acceptance Criteria: more than 2,000). The tailing factor was found to be 1.22 and 1.22 respectively (Acceptance Criteria: not more than 2.0) for Ozenoxacin and Benzoic acid peaks in standard solution. The % Relative SD (RSD) of the Ozenoxacin and Benzoic acid in five replicate injections from standard solution was found to be 0.1% and 0.1% respectively (Acceptance Criteria: less than 2.0%). Resolution between Ozenoxacin and Benzoic acid peaks was found to be 4.23 (Acceptance Criteria: more than 2.0). All of the system suitability parameters were contented the FDA requirements [27].

System precision. The standard solution was prepared and injected six times as per the method of analysis to establish the system precision. Results were given in Supplemental file (Table S3).

%RSD of the Ozenoxacin and Benzoic Acid peak areas of six replicate injections from standard preparation was found to be 0.1% and 0.1% respectively (Acceptance Criteria: less than 2.0%).

Specificity. Solutions of individual impurities, Ozenoxacin sample, Ozenoxacin Placebo and Spiked Solution were prepared and analyzed. Results were summarized in Table 2.

Peak purity complies as per the Ozenoxacin and Benzoic Acid peak requirement obtained from the chromatogram of spiked sample solution. Benzoic Acid was well separated from Ozenoxacin obtained from the chromatogram of spiked sample solution and all process related impurities were well separated from Ozenoxacin and Benzoic Acid. A typical chromatogram of the spiked sample is shown in Fig. 3.

Absolute difference between % Assay during specificity was given in Supplemental file (Table S4). Specificity Chromatograms were given in Supplemental file (Figure S1 to Figure S11).

Repeatability (Method precision). A sample solution of Ozenoxacin Cream 1% w/w at working concentration for six times (six individual sample preparations) was analyzed to determine the method precision. Results were given in Supplemental file (Table S8).

Ruggedness (Intermediate precision). A sample solution of Ozenoxacin Cream 1% w/w at working concentration for six times (six individual sample preparations) was analyzed to determine the ruggedness of the method using different analysts, different instruments, different reagents and different columns on different days. Details of variations, System Suitability Parameters & Areas for ruggedness and results of Method Precision & Intermediate Precision were given in Supplemental file (Table S5 to Table S8).

The % assay values obtained from six sample preparations (method precision/intermediate precision) for Ozenoxacin and Benzoic Acid were within specification (95%–105%). The %RSD for % assay values obtained from six sample preparations (method precision/intermediate precision) for Ozenoxacin and Benzoic Acid was within specification (less than 2%). The overall %RSD for % assay values obtained from replicate sample preparations (12 results) of method precision and intermediate precision was within specification (less than 3.0%).

Table 2. Specificity Results

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Retention Time (Minutes)</th>
<th>Purity Angle</th>
<th>Purity Threshold</th>
<th>Peak Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozenoxacin</td>
<td>5.57</td>
<td>0.130</td>
<td>0.268</td>
<td>Pass</td>
</tr>
<tr>
<td>Benzoic Acid</td>
<td>6.98</td>
<td>0.357</td>
<td>1.114</td>
<td>Pass</td>
</tr>
<tr>
<td>Quinoline Ester Impurity Peak-1</td>
<td>11.16</td>
<td>Not Applicable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoline Ester Impurity Peak-2</td>
<td>11.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Impurity</td>
<td>12.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OZE Stage 1 Impurity</td>
<td>12.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>No interference was observed at Ozenoxacin &amp; Placebo Solution</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Typical Chromatogram of Spiked sample
Linearity. Ozenoxacin and Benzoic Acid composite solutions, with five concentrations of 80%, 90%, 100%, 110%, 120%, were prepared from the standard stock solution and the experiments were performed in triplicate for each concentration level to determine the linearity. 80%, 90%, 100%, 110%, 120% are concentration levels relative to the label claim/declared content. The linearity curves were acquired between concentrations and peaks areas (mAU: milli Ampere Units) of five different solutions of Ozenoxacin and Benzoic Acid at concentration $32 - 48 \mu \text{g mL}^{-1}$ and $3.2 - 4.8 \mu \text{g mL}^{-1}$ respectively. Ozenoxacin and Benzoic Acid linearity results were shown in Table 3.

Calibration curves of Ozenoxacin & Benzoic acid were given in Supplemental file (Figure S12 & Figure S13).

Correlation coefficient values were not less than 0.999 and 0.999 for Ozenoxacin and Benzoic Acid respectively. Y-Bias was found to be within ±2.0%. The relationships between peak areas and concentrations were satisfactory considering the calibration curve regression data.

Accuracy. Ozenoxacin and Benzoic Acid composite solutions containing 80%, 100% and 120% of were analyzed to determine the method’s accuracy. Each solution was prepared individually in triplicate and analyzed to determine recovery values by component recovery method, which involves calculating the amount added (spiked) and the amount measured (recovered). Results are given in Table 4.

The individual % of recovery was between 97.0% and 103.0%. The mean % of recovery was between 98.0% and 102.0%. The %RSD was less than 2% at each level.

Range. This method validation test was performed from the lower concentration (80%) to upper concentration (120%) of both Ozenoxacin and Benzoic Acid, which were used in precision, accuracy and linearity. As per ICH requirements, the results demonstrated that all system suitability and method validation parameters were within the acceptance criteria [25].

Robustness. Flow rate and column oven temperature were varied to establish robustness of the method. Analysis of

### Table 3. Linearity data of Ozenoxacin and Benzoic Acid

<table>
<thead>
<tr>
<th>Linearity Level</th>
<th>Linearity of Ozenoxacin</th>
<th>Linearity of Benzoic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (μg/mL)</td>
<td>Average Area (mAU)</td>
</tr>
<tr>
<td>80%</td>
<td>32.1792</td>
<td>3,452,159</td>
</tr>
<tr>
<td>90%</td>
<td>36.2016</td>
<td>3,886,263</td>
</tr>
<tr>
<td>100%</td>
<td>40.2240</td>
<td>4,320,794</td>
</tr>
<tr>
<td>110%</td>
<td>44.2464</td>
<td>4,795,229</td>
</tr>
<tr>
<td>120%</td>
<td>48.2688</td>
<td>5,170,025</td>
</tr>
</tbody>
</table>

- Correlation coefficient 0.9995
- Coefficient of determination 0.9996
- Intercept 108,013
- Slope 19,804
- Regression equation ($y = mx + c$) $y = 108,013 \times 19,804$
- Y-Bias (%)
- Residual Sum of Squares 1901692651.6

### Table 4. Accuracy or Recovery data of Ozenoxacin and Benzoic Acid

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spike Level</th>
<th>Amount added in μg mL$^{-1}$</th>
<th>Amount found in μg mL$^{-1}$</th>
<th>% Recovery</th>
<th>Mean</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OZENOXACIN</td>
<td>80% Level</td>
<td>32.11</td>
<td>32.44</td>
<td>101.0</td>
<td>101.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>90% Level</td>
<td>40.13</td>
<td>40.63</td>
<td>101.2</td>
<td>101.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>100% Level</td>
<td>48.16</td>
<td>48.50</td>
<td>100.7</td>
<td>100.7</td>
<td>0.0</td>
</tr>
<tr>
<td>BENZOIC ACID</td>
<td>80% Level</td>
<td>3.22</td>
<td>3.24</td>
<td>100.6</td>
<td>100.6</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>90% Level</td>
<td>4.03</td>
<td>4.07</td>
<td>101.0</td>
<td>101.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>100% Level</td>
<td>4.83</td>
<td>4.90</td>
<td>101.4</td>
<td>101.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>
standard solution and sample solution was performed in each varied condition. One parameter was changed while keeping the other one unchanged from the actual parameter. Retention time & Assay results are given in Table 5.

Parameters varied, System Suitability Parameters, Areas observed during Robustness are given in Supplemental file (Table S9 to Table S15).

The assay values of Ozenoxacin and Benzoic Acid were within specification (95%–105%).

**Bench top solution stability of standard solution and sample solutions.** Standard solution and sample solution containing Ozenoxacin and Benzoic Acid were kept at room temperature. The solution stability was monitored by analyzing standard solution and sample solution in the initial and 48 h intervals.

It was observed that the standard solution and sample solution were stable for 48 h. The difference in % assay between initial and solution stability samples was found to be not more than ±2.0%. The similarity factor for standard was found to be in the range of 0.98–1.02.

Summary results were given in Supplemental file (Table S16).

**Bench top stability of mobile phase.** Mobile phase was kept at room temperature and monitored by analyzing the standard solution in the initial and 48 h intervals.

The Resolution between Ozenoxacin and benzoic acid peaks was 4.41. No Haziness and particles were observed in Mobile Phase up to 2 days.

Summary results were given in Supplemental file (Table S17).

**Filter validation.** Two different filters namely, 0.45 μm Nylon filter and 0.45 μm Polyvinylidene fluoride filter were used for filter validation of the method. Sample solution was prepared using the homogenous solution. Different portions of sample solution were centrifuged, filtered and injected into the HPLC system.

The difference between filtered samples observed to be not more than 2%. The assay values of Ozenoxacin and Benzoic Acid were within specification (95%–105%). Any significant interference was not found to be between nylon filter and Polyvinylidene fluoride filter.

Filter Details & Assay Results are given in Supplemental file (Table S18 & Table S19).

**Forced degradation study.** To assess the stability-indicating nature of the Assay by HPLC method in Ozenoxacin Cream 1% w/w, the sample has been stressed by acid, base, hydrogen peroxide, aqueous hydrolysis, heat, humidity, white fluorescent light, and UV light. The degraded samples were injected into the chromatographic system and analyzed. Results were summarized in Table 6 & Table 7.

Ozenoxacin in the cream formulation was found to be stable during exposure to humidity, white fluorescent light, UV light conditions, water hydrolysis, thermal condition, acid hydrolysis and base hydrolysis. Drastic degradation was observed in oxidation conditions. The Ozenoxacin peak was pure and spectrally homogenous in all the above degradation conditions, proving that degrading peaks were not co-eluting with Ozenoxacin peak.

Benzoic Acid in the cream formulation was found to be stable during exposure to humidity, white fluorescent light, UV light conditions, water hydrolysis, thermal condition, acid hydrolysis and base hydrolysis.

### Table 5. Robustness data for Ozenoxacin and Benzoic Acid

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Condition</th>
<th>Retention Time (Minutes)</th>
<th>Assay (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ozenoxacin</td>
<td>Benzoic Acid</td>
</tr>
<tr>
<td>1</td>
<td>Actual</td>
<td>5.72</td>
<td>7.28</td>
</tr>
<tr>
<td>2</td>
<td>Low Column Temp.</td>
<td>5.91</td>
<td>7.44</td>
</tr>
<tr>
<td>3</td>
<td>High Column Temp.</td>
<td>5.54</td>
<td>7.12</td>
</tr>
<tr>
<td>4</td>
<td>Low Flow Rate</td>
<td>6.42</td>
<td>8.07</td>
</tr>
<tr>
<td>5</td>
<td>High Flow Rate</td>
<td>5.16</td>
<td>6.62</td>
</tr>
</tbody>
</table>

### Table 6. Ozenoxacin Parameters observed during Forced degradation Study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Condition</th>
<th>Assay (% w/w)</th>
<th>Purity Angle</th>
<th>Purity Threshold</th>
<th>Peak Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Sample</td>
<td>99.50</td>
<td>0.148</td>
<td>0.253</td>
<td>Pass</td>
</tr>
<tr>
<td>2</td>
<td>Acid Sample (1N HCl, 24 h at 60 °C)</td>
<td>98.88</td>
<td>0.142</td>
<td>0.252</td>
<td>Pass</td>
</tr>
<tr>
<td>3</td>
<td>Base Sample (1N NaOH, 24 h at 60 °C)</td>
<td>96.78</td>
<td>0.162</td>
<td>0.249</td>
<td>Pass</td>
</tr>
<tr>
<td>4</td>
<td>Water Sample (24 h at 60 °C)</td>
<td>99.76</td>
<td>0.143</td>
<td>0.257</td>
<td>Pass</td>
</tr>
<tr>
<td>5</td>
<td>Peroxide Sample (1% H2O2, 15 min at 60 °C)</td>
<td>74.18</td>
<td>0.413</td>
<td>0.752</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>Thermal Degradation Sample (60 °C for 48 h)</td>
<td>98.71</td>
<td>0.149</td>
<td>0.259</td>
<td>Pass</td>
</tr>
<tr>
<td>7</td>
<td>White Fluorescent Exposure Sample</td>
<td>99.84</td>
<td>0.161</td>
<td>0.260</td>
<td>Pass</td>
</tr>
<tr>
<td>8</td>
<td>UV light Exposure Sample (200-W hours/square meter)</td>
<td>98.13</td>
<td>0.156</td>
<td>0.257</td>
<td>Pass</td>
</tr>
<tr>
<td>9</td>
<td>90% RH Humidity Exposure Sample</td>
<td>100.00</td>
<td>0.159</td>
<td>0.260</td>
<td>Pass</td>
</tr>
</tbody>
</table>
acid hydrolysis, base hydrolysis and oxidation conditions. The Benzoic Acid peak was pure and spectrally homogenous in all the above degradation conditions, proving that degrading peaks were not co-eluting with Benzoic Acid peak.

CONCLUSION

The results obtained in this study demonstrated that the concurrent measurement of Ozenoxacin and Benzoic Acid, in pharmaceutical cream formulation using RP-HPLC by gradient elution was specific, accurate, precise, linear and rugged. The method was found robust at column oven temperature variation and flow rate variation. Solution stability established and found standard solution and sample solutions are stable up to 48 h at room temperature. Mobile phase stability established and found stable up to 48 h at room temperature. Filter validation established and found standard solution and sample solutions are suitable in 0.45 μm Nylon filter and Polyvinylidene fluoride filter. Forced degradation of Ozenoxacin cream 1% w/w formulation was performed and found that validated method has stability indicating potential that needs to be further studied.

The method was validated according to the ICH guideline and presented equivalent outcomes that demonstrate that this method will be valuable for concurrent measurement of Ozenoxacin and Benzoic Acid by RP-HPLC, which was established, optimized and reported first time. This method can be used for routine analysis of Ozenoxacin and Benzoic Acid in any form of pharmaceutical and throughout the stability study with a run time of just 15 min. This unique method can play a dynamic role for the development of other studies (e.g., In Vitro Release Testing and In Vitro Permeation Testing of Ozenoxacin) for the quantitative analysis for future use.

DECLARATIONS

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Conflict of interest section: The authors declare that no potential conflicts of interest for this article’s research, authorship, and/or publication.

Author contributions: Amarnath Reddy Ramireddy: The acquisition, analysis, interpretation of data for the work and drafting of the work. Dilip Kumar Behara: The conception, design of the work and final approval of the version to be published.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

ACKNOWLEDGEMENTS

Authors are thankful to Optimus Pharma Private Limited, Hyderabad for providing facilities to perform this research. One of the authors Amarnath Reddy Ramireddy is thankful to the Jawaharlal Nehru Technological University, Anantapur, for enrolling as a research scholar.

SUPPLEMENTARY MATERIALS

Supplementary data to this article can be found online at https://doi.org/10.1556/1326.2020.01064.

REFERENCES


