Validated liquid chromatographic method for simultaneous estimation of niacinamide and salicylic acid in semi-solid dosage form

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Received 30 May 2011; accepted 21 February 2013

A simple, precise and accurate RP-HPLC method has been described for estimation of niacinamide and salicylic acid in semi-solid dosage form. The separation is done by using column Oyster BDS C₁₈ (250 mm × 4.6 mm) at 25°C and 74:26 (v/v) water: methanol as mobile phase at flow rate of 1 mL/min and pH adjusted to 3 with triethylamine and glacial acetic acid. Detection is carried out at 280 nm. The method has been validated according to ICH guideline in terms of linearity, precision, accuracy, specificity, and solution stability. The linearity of proposed method is investigated in the range of 250-750 µg/mL ($r^2=0.999$) for niacinamide and 100-300 µg/mL ($r^2=0.999$) for salicylic acid. The percentage recoveries of niacinamide and salicylic acid are found to be 99.07-100.08% and 99.57-100.25% respectively. The proposed method provides an accurate and precise quality control tool for analysis of niacinamide and salicylic acid in semisolid dosage forms.

Keywords: Chromatographic method, Niacinamide, RP-HPLC, Salicylic acid

Niacinamide is chemically pyridine-3-carboxamide¹. The role of topical niacinamide in acne treatment is the reduction of inflammation and redness that occurs in most acne lesions. Niacinamide is an effective topical anti-inflammatory agent that has mild exfoliating action, enabling the skin to shed old skin cells and prevent pore blockage. The mild exfoliating action of niacinamide is attributed in its ability to speed up the differentiation or cell division of keratinocytes. Topical niacinamide can also mildly reduce the amount of sebum on the oil gland. Salicylic acid is chemically 2-hydrxy-benzoic acid¹. It is used traditionally as an anti-fungal agent for the treatment of warts and corns. For the treatment of acne, salicylic acid doesn’t help much in killing those acne-causing bacteria. Salicylic acid only helps in exfoliating the skin. Exfoliation makes the skin peel at a faster rate which unblocks the pores of the skin. Open pores reduce the growth of acne-causing bacteria by starving them with trapped sebum. Since salicylic acid is oil soluble, it can penetrate deeper into the skin. This makes salicylic acid more effective in treating oily skin with lots of whiteheads, blackheads and acne breakouts. Literature survey reveals that analytical methods including HPLC²-⁴ and LC/MS/MS⁵ for estimation of niacinamide alone or simultaneously with other drugs have been reported. Analytical methods including UV⁶, HPLC⁷, HPTLC⁸, LC/MS/MS⁹, fluorimetry¹⁰ and GC¹¹ have been reported for the estimation of salicylic acid alone or simultaneously with other drugs. However, no method has yet been reported for the simultaneous estimation of niacinamide and salicylic acid in semi-solid dosage form. The present work describes RP-HPLC method for the simultaneous estimation of niacinamide and salicylic acid in semi-solid dosage form.

Experimental Procedure

Instruments, apparatus and equipments

The HPLC system ( Dionex, Ultimate 3000) consisting of a system controller, on-line degasser, low-pressure gradient flow control valve, solvent delivery module, auto injector, column oven, UV–VIS detector and Chromeleon software version 6.8), analytical balance (Sartorius CP224S, Germany), double beam UV-visible spectrophotometer (UV-2450, Shimadzu, Japan) having two matched cells with 1 cm light path, pH meter (Labindia, India),

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sonicator (5510, Branson Ultrasonics Corporation, USA), corning volumetric flasks and pipettes of borosilicate glass were used for the study.

**Materials**

Niacinamide and salicylic acid standard both having purity of 99.1% were obtained from Lincoln Pharmaceutical Limited (Gujarat, India). The commercial fixed dose product containing 5% and 2% w/w concentration of niacinamide and salicylic acid respectively and placebo cream were used from Lincoln Pharmaceutical Limited (Gujarat, India). HPLC grade glacial acetic acid (Rankem, India), methanol, 1-hexane sulphonic acid anhydrous and triethylamine (Merck, India) were also used.

**Preparation of standard stock solution of niacinamide and salicylic acid**

Accurately weighed niacinamide standard (100 mg) and salicylic acid standard (40 mg) solutions were transferred in 100 mL volumetric flask, dissolved in water with the aid of ultrasonic bath and final volume was adjusted up to mark with water.

**Preparation of working standard solution of niacinamide and salicylic acid**

Stock solutions (5 mL) of niacinamide and salicylic acid were transferred to a 10 mL volumetric flask and the volume was diluted up to mark with water to get final concentration for niacinamide (500 µg/mL) and salicylic acid (200 µg/mL).

**Chromatographic condition**

Chromatographic separation was achieved at 25°C on Oyster BDS C\textsuperscript{18} (250 mm × 4.6 mm) column using mobile phase consisting of water and methanol [74:26 (v/v)], 0.1% triethylamine and 0.15 g hexane sulfonic acid. pH was adjusted at 3.0 with glacial acetic acid. The flow rate was kept at 1 mL/min and detection was carried out at 280 nm. The injection volume was 20 µL of standard preparation containing niacinamide (500 µg/mL) and salicylic acid (200 µg/mL).

**Calibration curve**

The linearity of the response for niacinamide and salicylic acid assay method was determined by preparing and injecting mixture of standard stock solutions suitably diluted to achieve concentrations of about 250, 375, 500, 625 & 750 µg/mL and 100, 150, 200, 250 & 300 µg/mL of niacinamide and salicylic acid respectively. The values of coefficient of correlation (r\textsuperscript{2}), slope and intercept were 0.999 (r\textsuperscript{2}), 3225 (slope) & -65562 (intercept) and 0.999 (r\textsuperscript{2}), 12399 (slope) & -25597 intercept for niacinamide and salicylic acid respectively. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied for both the drugs.

**Analysis of marketed formulation**

Assay of cream having combination of niacinamide (50 mg) and salicylic acid (20 mg) was performed. Accurately weigh and transfer about 1.0 g sample cream (Equiv. to 50 mg of niacinamide WS and 20 mg salicylic acid WS) to a 100 mL volumetric flask, slightly heat on water bath to melt, add about 50 mL of diluent and further heat on water bath for 10-15 min. Cool and make up the volume with diluent and mix. Filter the solution through Whatman filter paper and inject the solution.

**Results and Discussion**

**Method development**

For method development several trials were made to resolve the peak of niacinamide and salicylic acid. Niacinamide 10 mcg/mL and salicylic acid 20 mcg/mL in water was scanned on a UV-visible spectrophotometer in the wavelength range 200-400 nm. The spectra show peaks for niacinamide at 262 nm and for salicylic acid at 304 nm. The intersection point is found to be at 280 nm. Hence, the wavelength 280 nm is selected for the determination of niacinamide and salicylic acid. The mobile phase compositions used were 50:50, 60:40, 90:10, 80:20 and 74:26. The different pH for mobile phase were 2, 3, 4 and 6. Different flow rates for mobile phase were 1.5, 0.8, 2 and 1 mL/min. Hexane sulphonic acid sodium salt ion-pair reagent was used to increase resolution of niacinamide for better separation of both drugs. Acidic ion-pair reagent was reacting with basic niacinamide for better retention and separation. Triethylamine was used to decrease tailing. The optimum mobile phase containing 74:26 water: methanol at pH 3 adjusted with glacial acetic acid and 1 mL/min flow rate is selected, because it gives better results than other trials. Water was selected as diluents. As per USP XXIII28, system suitability tests were carried out on freshly prepared standard solution of the drugs and parameters obtained with 20 µL.
injection volume are summarized in Table 1. Retention time of niacinamide and salicylic acid are found to be 3.953 and 11.673 min respectively.

**Analytical method validation\(^\text{12}\)**

**Specificity**
 Specificity study was performed by analyzing standard solution in the presence (\(C_p\)) and absence (\(C_a\)) of excipients/placebo. The two drug concentrations were expressed and compared as [\(\%\) interference = \((C_p - C_a)/C_a \times 100\)]; acceptance criteria being \(\%\) interference <0.5%. Percentage interference was found to be 0.01% and 0.02% for niacinamide and salicylic acid respectively which is within the acceptance limits. Hence, the excipients do not interfere with the estimation of drugs.

**Precision**
 Precision was measured in terms of repeatability of measurement, performed by injecting the standard solution six times and measuring the peak areas. The RSD is found to be 0.464 and 0.387 for niacinamide and salicylic acid respectively. This shows that precision of the method is satisfactory as relative standard deviation is not more than 2.0%.

**Intermediate precision**
 Intermediate precision (intermediate precision and reproducibility are the terms currently accepted/advocated in ICH guidelines which were earlier collectively referred to as ruggedness) of the method was determined by analyzing standard solutions with two different analysts, using different instruments, in two different labs and on different days (set-I and set-II conditions). The values of RSD obtained under set-I conditions are found to be 0.62 and 0.70 and those under set-II conditions are 0.710 and 0.561 for niacinamide and salicylic acid respectively. As the values of RSD for the two sets of conditions are below 2% for both the drugs, intermediate precision of the method is established.

**Accuracy**
 The accuracy of the method was determined by recovery study carried out using standard addition method at three different concentration levels. The resulting spiked sample solutions were assayed in triplicate and the results obtained were compared with the expected results and expressed in percentage. The mean \% recoveries of niacinamide and salicylic acid are found to be in the range 99.07-100.08% and 99.57-100.25% respectively, which are within the acceptance limit.

**Robustness**
 Robustness of the method was determined by analyzing standard solutions at normal operating conditions and also by changing some operating analytical conditions such as flow rate, column oven temperature and mobile phase ratio. The RSD values for assay are found to be <2%. Hence, the robustness of the method is established to the extent of variations applied to the analytical conditions (Table 2).

Summary of the validation parameters is presented in Table 3.

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**Table 1** — System suitability parameters and chromatographic condition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Niacinamide ± RSD ((n = 6))</th>
<th>Salicylic acid ± RSD ((n = 6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time, min</td>
<td>3.947 ± 0.149</td>
<td>11.544 ± 0.273</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.02 ± 0.439</td>
<td>1.56 ± 0.300</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>5240 ± 0.708</td>
<td>5668 ± 0.840</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
<td>19.36 ± 1.04</td>
</tr>
</tbody>
</table>

**Table 2** — Robustness study for niacinamide and salicylic acid

<table>
<thead>
<tr>
<th>Condition</th>
<th>Assay</th>
<th>Resolution factor</th>
<th>Tailing factor</th>
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<tr>
<td></td>
<td>Niacinamide</td>
<td>Salicylic acid</td>
<td>Niacinamide</td>
</tr>
<tr>
<td>Flow rate, mL/min</td>
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<tr>
<td>0.9</td>
<td>98.19</td>
<td>97.91</td>
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<td>1.0</td>
<td>98.23</td>
<td>98.16</td>
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<td>1.1</td>
<td>98.18</td>
<td>98.47</td>
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<td>Column oven temperature, °C</td>
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<tr>
<td>20</td>
<td>98.20</td>
<td>98.67</td>
<td>19.20</td>
</tr>
<tr>
<td>25</td>
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<tr>
<td>30</td>
<td>98.23</td>
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<td>19.34</td>
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<td>Detection wavelength, nm</td>
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<td>279</td>
<td>97.81</td>
<td>98.11</td>
<td>19.08</td>
</tr>
<tr>
<td>280</td>
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<td>281</td>
<td>98.12</td>
<td>97.89</td>
<td>19.13</td>
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</table>
Analysis of marketed formulations

The developed method was applied for the simultaneous analysis of the drugs in semi-solid dosage form. The results of analysis are given in Table 4. The contents of niacinamide and salicylic acid are found to be in the range of 100±2% with less than 2% RSD, which indicates the suitability of the method for routine analysis of both the drugs in pharmaceutical dosage forms.

Conclusion

The developed liquid chromatographic method is specific, precise, accurate and robust. It can be used as an alternative method for rapid and routine simultaneous determination of niacinamide and salicylic acid in semi-solid dosage form.

Acknowledgement

The authors are thankful to Lincoln Pharma Ltd., Gujarat, for providing samples of niacinamide and salicylic acid.

References