Simultaneous spectrophotometric estimation and validation of three component tablet formulation containing paracetamol, nimesulide and tizanidine

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In the present study, two spectrophotometric methods that do not require prior separation for simultaneous estimation of three drugs: paracetamol, nimesulide, and tizanidine in tablet formulation have been reported. Shimadzu UV-1700, capable of multi-component analysis was used for quantitation. Method I was based on derivative spectrophotometry and the absorbances were measured at 229.5, 271, and 323.0 nm, being the zero crossing points for paracetamol, nimesulide and tizanidine, respectively. Method II is based on multi-wavelength spectroscopic method, absorbances of standard solutions were measured at 229.0 nm, 272.0 nm, 262.0 nm and 323.0 nm based on statistical calculations and results of the sample solutions. All the three drugs obey Beer’s law in the concentration range employed for the methods. The results of the analysis for both methods were tested and validated for various parameters according to International Conference on Harmonization Q 2B guidelines. The utility of the developed methods has been demonstrated by analysis of commercially available tablet dosage form.

Keywords: Derivative spectrophotometry, Validation, Paracetamol, Nimuslide, Tizanidine

Paracetamol (PCM) is \(N\)-(4-hydroxyphenyl) acetamide\(^1\). It is a popular analgesic and antipyretic drug, used for the relief of fever, headache, and other minor aches and pains. Nimesulide (NIMS) is an anti-inflammatory drug. Chemically, NIMS is \(N\)-(4-nitro-2-phenoxy phenyl) -ethane sulphonamide\(^2\). It is approved for the treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis and dental pain and inflammation. Tizanidine (TZN), 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiadiazole is \(\alpha_2\)-adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants\(^3\).

A tablet dosage form containing all the three, (PCM 325 mg, NIMS 100 mg and TZN 2 mg), is commercially available and used in pain associated with musculoskeletal disorders. PCM is official in I.P\(^4\), B.P\(^5\). NIM is mentioned in British pharmacopoeia\(^3\) while TZN is reported in Merck index\(^6\). A literature survey revealed that PCM has been analyzed separately and in combination with other drugs by HPLC\(^7\), HPTLC\(^8\) and UV spectrophotometry\(^9\). Some HPLC\(^10,11\) and spectrophotometric\(^12,13\) methods have been reported for the estimation of NIMS alone. HPLC\(^14\) and spectrophotometric\(^15\) methods have been reported for simultaneous estimation of NIMS and PCM. RP-HPLC analysis of NIMS and TZN have also been reported\(^16-19\). However, no spectrophotometric and chromatographic method for simultaneous analysis of PCM, NIMS, and TZN in a combined dosage form has been reported yet; hence it is essential to develop a spectrophotometric method for simultaneous estimation of all the three drugs in a tablet formulation.

**Experimental Procedure**

UV/Visible double beam spectrophotometer (Shimadzu, model-1700) was employed for analysis work. Pure PCM, NIMS and TZN were provided as gift samples by Pharma French Pharmaceuticals Pvt Ltd, Baddi, India. The tablet dosage form, Niciflex T was manufactured by Cipla Pharmaceutical Ltd, India (Label claim: 325 mg PCM, 100 mg NIMS and 2 mg TZN). Methanol was used as solvent in which all the three drugs are very much stable.

**UV-Spectrophotometry**

A standard stock solution of PCM having a concentration of 100 \(\mu\)g mL\(^{-1}\) was prepared by dissolving 10 mg of PCM in 100 mL methanol. From
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this stock solution, working standard solution having a concentration of 20 µg mL⁻¹, was prepared by appropriate dilution. Standard stock solutions (100 mg mL⁻¹) of NIMS and TZN were prepared from respective standard drugs in a similar manner. Working standard solutions (20 µg mL⁻¹) of each of the drugs were scanned in the range 400-200 nm to obtain the first order derivative overlain spectra and multicomponent spectra for all the three drugs.

Method I: Derivative spectrophotometry

The first derivative (D1) overlain spectra (Fig. 1) of each pure drug was found to show zero crossing point (ZCP) and assisted in their simultaneous estimation. The first derivative wavelength considered for TZN was 323 nm at which PCM and NIMS show zero absorbance. The estimation of PCM and NIMS was carried out at 229.5 nm and 271.0 nm by employing simultaneous equation method at which TZN shows zero absorbance.

Calibration curves were plotted between absorbances observed at D1, for three drugs at all the three wavelengths against the concentrations, in the range of 5–80 µg mL⁻¹, 5–50 µg mL⁻¹, and 0.2–30 µg mL⁻¹ for PCM, NIMS and TZN respectively. Estimation of TZN was done by solving the following regression equation,

\[ y = 0.0039x + 0.0004 \]  

...(1), for TZN

Estimation of PCM and NIMS was done by framing and solving simultaneous equations, by measuring absorbance of PCM at 229.5 nm and NIMS at 271 nm in derivative mode at which TZN shows zero absorbance. The concentration of both the drugs in mixture was calculated by using the following equations.

\[ C_{PCM} = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \]  

...(2), for PCM

\[ C_{NIMS} = \frac{A_2 ax_1 - A_1 ax_2}{ax_2 ay_1 - ax_1 ay_2} \]  

...(3), for NIMS

where \( A_1 \) and \( A_2 \) are the absorbances of mixed standards at 229 nm and 271 nm respectively; \( ax_1 \) and \( ax_2 \) are the absorptivities E (1%, 1 cm) of PCM at 229 and 271 nm respectively; \( ay_1 \) and \( ay_2 \) are the absorptivities of NIMS at 229 and 271 nm respectively.

Method II: Multi-wavelength spectroscopy

The overlain spectra of PCM, NIMS and TZN are shown in Fig. 2. The use of five mixed standards and four sampling wavelengths: 229, 272, 262 and 323.0 nm were found to serve the purpose of this experiment.

Five mixed standard solutions of each containing PCM, NIMS and TZN in the concentration ratio of 32.5:10:0.2, 48.75:15:0.3, 65:20:0.4, 81.25:25:0.5 and 97.50:30:0.6 (µg mL⁻¹) as in NICIFLEX T was prepared in methanol. All the mixed standard solutions were scanned over the range of 400 -200 nm in the multi-component mode using the previously mentioned four sampling wavelengths. Recording of the absorbances of the mixed standard solutions was processed by the instrument by means of matrix equations and then corrected to determine the concentrations of all the drugs in the tablet sample solutions.

Fig. 1—First derivative overlain spectra of PCM, NIMS and TZN

Fig. 2—Overlain spectra of mixed standards of PCM, NIMS and TZN
Sample preparation and analysis

Twenty NICIFLEX T tablets were weighed and powdered and powder equivalent to 50 mg of PCM (corresponding amount of NIMS 15 mg and TZN 0.33 mg) was dissolved in methanol by thorough mixing and diluted to a volume of 100 mL. The extracts were filtered separately through Whatman # 41 filter paper. The sample solutions of 10 mL of each PCM were prepared in methanol by transferring appropriate amount of each filtrate to obtain an equimolar solution containing approximately 50 µg mL\(^{-1}\) of PCM and corresponding amounts of NIMS (15 µg mL\(^{-1}\)) and TZN (0.33 µg mL\(^{-1}\)). Then these sample solutions were scanned using proposed two methods and the results were recorded (Table 1).

Validation of the developed methods

The methods were validated according to International Conference on Harmonization (ICH) Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Both precision and accuracy were determined with standard samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range.

Results and Discussion

Linearity

The linearity range was optimized with 5–80, 5–50, and 0.2–30 µg mL\(^{-1}\) for PCM, NIMS, and TZN respectively for both methods. Linear regression analysis of the responses (y) on the theoretical concentrations (x) gave the following equations: at 229 nm, \(y = 0.0002x + 0.0002\) (for PCM, \(r^2 = 0.9992\)); at 271 nm, \(y = 0.0004x + 0.0007\) (for NIMS, \(r^2 = 0.9994\)); and at 323 nm, \(y = 0.0039x + 0.0004\) (for TZN, \(r^2 = 0.9992\)) for method I; at 229.0 nm, \(y = 0.2485x + 0.0125\) (for PCM, \(r^2 = 0.9999\)); at 271.0 nm, \(y = 0.006 x + 0.001\) (for NIMS, \(r^2 = 0.9993\)) and at 323.0 nm, \(y = 0.0321x + 0.0025\) (for TZN, \(r^2 = 0.9996\)) for method II.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies by standard addition method. The results are shown in Table 2. The value for the mean of recovery (%) were found to be <1.0 for both the methods, which indicate excellent recoveries ranging from 98.60 to 101.40%. These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods.

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The results are presented in Table 3. The intermediate precision study is expressed within the laboratory variation on different days. The % COV in

### Table 1 — Analysis data of tablet formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCM</td>
<td></td>
<td>99.44</td>
</tr>
<tr>
<td>NIMS</td>
<td></td>
<td>99.35</td>
</tr>
<tr>
<td>TZN</td>
<td></td>
<td>99.36</td>
</tr>
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</table>

### Table 2 — Results of recovery study (Niciflex-T Tablet)*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (µg mL(^{-1}))</th>
<th>Amount added (µg mL(^{-1}))</th>
<th>% Recovery Method I</th>
<th>% Recovery Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCM</td>
<td>50</td>
<td>40</td>
<td>99.04±0.63</td>
<td>100.67±0.92</td>
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<tr>
<td>NIMS</td>
<td>15</td>
<td>12</td>
<td>99.30±0.91</td>
<td>98.90±0.23</td>
</tr>
<tr>
<td>TZN</td>
<td>0.33</td>
<td>0.04</td>
<td>99.20±0.81</td>
<td>99.60±0.42</td>
</tr>
<tr>
<td>PCM</td>
<td>50</td>
<td>50</td>
<td>99.39±0.61</td>
<td>99.63±0.37</td>
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<tr>
<td>NIMS</td>
<td>15</td>
<td>15</td>
<td>98.92±0.89</td>
<td>101.40±0.50</td>
</tr>
<tr>
<td>TZN</td>
<td>0.33</td>
<td>0.33</td>
<td>98.60±0.49</td>
<td>98.80±0.31</td>
</tr>
<tr>
<td>PCM</td>
<td>50</td>
<td></td>
<td>98.70±0.32</td>
<td>99.10±0.42</td>
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<tr>
<td>NIMS</td>
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<td>120</td>
<td>98.80±0.42</td>
<td>98.71±0.32</td>
</tr>
<tr>
<td>TZN</td>
<td>0.33</td>
<td>0.39</td>
<td>99.30±0.43</td>
<td>98.67±0.23</td>
</tr>
</tbody>
</table>

*Value for drug content (%) is the mean of 5 estimations, S.D.: Standard deviation, R.S.D.: Relative standard deviation, S.E.: Standard error, Method I: Simultaneous equation method, Method II: Multiwavelength spectroscopy.

*Mean of five determinations Method I: Derivative spectrophotometry; Method II: Multi wavelength spectroscopy. *Manufactured by Cipla Pharmaceuticals Ltd, Baddi, India.
intra and inter-day precision studies for both the methods was not more than 1.0%, which indicates excellent repeatability and intermediate precision.

**LOD and LOQ**

The values of the LOD and LOQ were 0.13 and 0.42 µg mL\(^{-1}\) for PCM, 0.60 and 1.46 µg mL\(^{-1}\) for NIMS, 0.1 and 0.3 µg mL\(^{-1}\) for TZN in method I; 0.06 and 0.54 µg mL\(^{-1}\) for PCM, 0.42 and 0.91 µg mL\(^{-1}\) for NIMS, 0.1 and 0.2 µg mL\(^{-1}\) for TZN in method II.

**Robustness**

The robustness of developed method was checked by performing the method on another spectrophotometer and results was found to be satisfactory.

**Assay of tablet formulations**

The results of the analysis of tablet formulation (Niciflex T) for both methods are reported in Table 1. The assay values for PCM, NIMS, and TZN were 99.44, 99.35, and 99.36, respectively, for method I, and 99.75, 99.45 and 99.73, respectively for method II. The standard deviation for both the methods was found to be <1.0. The assay values indicate that interference of the excipient matrix is insignificant in the estimation of PCM, NIMS, and TZN by proposed methods.

**Conclusion**

The proposed UV spectrophotometric methods for simultaneous estimation of PCM, NIMS and TZN are accurate and precise. The proposed methods are simple, rapid and easy to perform. These are applicable for estimation of PCM, NIMS and TZN in pure and combined dosage form in quality control laboratories.

**Acknowledgements**

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**References**