Research Article

Simultaneous Quantification of Paracetamol and Meloxicam in Tablets by High Performance Liquid Chromatography

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Abstract

Purpose: To develop and validate a simple, rapid and inexpensive RP-HPLC method for the simultaneous estimation of paracetamol and meloxicam in tablets.

Methods: For the analysis of the drugs, chromatographic analysis was performed on XTerra symmetry C18 column (100 x 4.6 mm, 5 μ particle size) with mobile phase consisting of methanol and phosphate buffer (pH 9.2) in the ratio of 50:50 v/v, at a flow rate of 0.8 mL/min and eluents monitored at 244 nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per International Conference on Harmonization (ICH) guidelines.

Results: The retention times of paracetamol and meloxicam were 2.467 and 4.971 min, respectively. The calibration curves of peak area versus concentration, which was linear from 5 - 60 μg/mL for paracetamol and 1 - 12 μg/mL for meloxicam, had regression coefficient ($r^2$) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of paracetamol and meloxicam in tablets.

Conclusion: The proposed method is simple, low-cost, accurate, precise and can be successfully employed in routine quality control for the simultaneous analysis of paracetamol and meloxicam in tablets.

Keywords: Paracetamol, Meloxicam, RP-HPLC, Simultaneous analysis, Tablets

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INTRODUCTION

Chemically, Paracetamol (PARA), shown in Figure 1A, is N-(4-hydroxy phenyl) acetamide. It is a well known analgesic drug, which is very effective for the relief of pain and fever. The mechanism of action of paracetamol is due to inhibition of cyclooxygenase (COX) enzyme and prostaglandin synthesis in the central nervous system (CNS). It acts directly on hypothalamus for the regulation of elevated body temperature [1,2]. Meloxicam (MEL), shown in Fig 1B, is chemically, 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1, 1-dioxide. It is used for the treatment of osteoarthritis, rheumatoid arthritis, and pauciarticular and polyarticular course juvenile rheumatoid arthritis. Its analgesic, antipyretic and anti-inflammatory activity is due to the inhibition of COX-2 enzyme [3,4].

![Chemical structures of paracetamol (A) and meloxicam (B)](image)

A survey of pertinent literature revealed that few HPLC [5-7], spectrophotometric [8] and LC-MS-MS [9] methods have been reported for the determination of paracetamol in combination with other drugs. Several analytical methods for the determination of meloxicam by flourimetry [10], capillary electrophoresis [11], HPLC [12-13], LC/MS [14] and spectrophotometry [10] have been reported. Ramesh S et al reported the simultaneous determination of paracetamol and meloxicam in tablets by spectrophotometry [15]. However, there is no liquid chromatographic method reported for the simultaneous estimation of paracetamol and meloxicam in tablet dosage form. The aim of the present work was to develop and validate a sensitive RP-HPLC method that can be applied for the simultaneous analysis of paracetamol and meloxicam in tablets.

EXPERIMENTAL

Instrumentation

The chromatographic system used comprised of Waters 2695 binary gradient pump, with in-built auto sampler, column oven and Waters 2487 dual wavelength absorbance detector (DAD). Data integration was carried out using Empower-2 software. Samples were injected into XTerra symmetry C18 column (100 x 4.6 mm, 5 µ particle size). A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Materials

Pure paracetamol (PARA) and meloxicam (MEL) used as working standards, were gifts from Aurobindo Pharma Ltd, Hyderabad, India. Tablets containing 7.5 mg of MEL and 325 mg of PARA (Melodol®) were obtained from Aristo Pharmaceuticals Pvt. Ltd, India and used within their shelf life period. Methanol and water (HPLC-grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck, India.

Chromatographic conditions

The high performance liquid chromatographic (HPLC) system used was operated isocratically with the column temperature maintained at 30 °C, using a mobile phase composition of methanol and phosphate buffer (pH adjusted to 9.2 with potassium hydroxide) in the ratio of 50:50 v/v at a flow rate of 0.8 mL/min within a run time of 7 min. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a millipore vacuum filter system equipped with a 0.45 µm high vacuum filter. Both drugs were detected and quantified at 244 nm.
Preparation of standard solutions

The standard solutions were prepared by transferring 10 mg of MEL and 100 mg of PARA working standards into separate 100 mL volumetric flasks. To each, 10 mL of 0.1M NaOH was added, and the mixture was sonicated to dissolve and made up to volume with methanol. Aliquots of these standard solutions were transferred using A-grade bulb pipettes into 100 mL volumetric flasks and the solutions made up to volume with mobile phase to give final concentrations of 1 - 12 µg/mL and 5 - 60 µg/mL of MEL and PARA, respectively.

Quantification of paracetamol and meloxicam from tablets

Twenty tablets were accurately weighed and crushed to a fine powder in a mortar. An amount of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask and 10 mL of 0.1M NaOH was added to it. The mixture was sonicated to dissolve and then made up to volume with methanol. Following 15 min of mechanical shaking, it was kept in an ultrasonic bath for 5 min, and the solution filtered through a 0.45 µm filter paper. Suitable aliquots of the filtered solution were transferred to a volumetric flask and made up to volume with mobile phase to yield concentrations of MEL (1.0 µg/mL) and PARA (43.33 µg/mL). A 20 µL volume of the sample solution was injected into the chromatographic system, six times, under optimized chromatographic conditions. The peak areas were measured at 244 nm and concentrations in the samples were determined by interpolation from calibration plots of each drug previously obtained.

Method validation

The method was validated in accordance with ICH guidelines [16]. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility, robustness and system suitability.

Linearity

Twelve different concentrations of the mixture of PARA and MEL were prepared for linearity studies and injected into chromatographic system (n = 3). The responses were measured as peak areas.

Detection limit and quantitation limit

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to Eqs 1 and 2, respectively.

\[
\text{LOD} = 3.3(\text{SD})/S \quad \text{(1)}
\]

\[
\text{LOQ} = 10(\text{SD})/S \quad \text{(2)}
\]

where SD is the standard deviation of response (peak area) and S is the average of the slope of the calibration curve.

Accuracy

The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 100 and 150 %) of the labeled claim to the excipients. At each level, three determinations were performed and the results were recorded. Accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of an analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of injection, repeatability (intra-day), intermediate precision (inter-day) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for ten determinations of peak areas of PARA (32.5 µg/mL) and MEL (7.5 µg/mL), performed on the same day. For both intra-day and inter-day variation, standard solutions of PARA (16.25, 32.5 and 48.75 µg/mL) and MEL (3.75, 7.5 and 11.25 µg/mL) were injected in triplicate.
Robustness

The robustness of the proposed method was determined by carrying out the analysis, during which mobile phase composition (concentration of methanol was varied by ± 2 %), and buffer pH (varied by ± 0.1) were altered and the peak areas and retention times were noted.

RESULTS

A typical chromatogram recorded at 244 nm is shown in Figure 2. The retention times of PARA and MEL at a flow rate of 0.8 mL/min were 2.467 and 4.971 min, respectively. The analyte peaks were well resolved and free from tailing (< 1.5 for both analytes).

![Figure 2: A typical chromatogram of paracetamol (*RT = 2.467) and meloxicam (** RT = 4.971)](image)

Method validation

System suitability

To ensure the validity of the system and analytical method, system suitability test was performed. Percent relative standard deviation (%RSD) of the retention times (RT) and peak areas of PARA and MEL from the six consecutive injections of the standard solutions were 0.219 and 0.133, and 0.146 and 0.196, respectively. The tailing factor for PARA and MEL peaks were 1.38 and 1.12, respectively, thus reflecting good peak symmetry. The resolution (Rs) between PARA and MEL was 9.62, indicating good separation of both analytes from each other.

The theoretical plate no. for PARA and MEL were 3048 and 3637, respectively, thus indicating good column efficiency (Table 1).

Linearity

The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 5 - 60 µg/mL for PARA and 1 - 12 µg/mL for MEL (Table 1). The regression coefficients of PARA ($r^2 = 0.9991$) and MEL ($r^2 = 0.9997$) indicate a good linear relationship between peak area and concentration over a wide range.

Detection limit and quantitation limit

LOD for PARA and MEL was 0.13 and 0.03 µg/mL, respectively, while LOQ was 0.39µg/mL and 0.11µg/mL, respectively (see Table 1).

Accuracy

The mean recovery obtained for PARA and MEL was 100.28 and 99.82 %, respectively (Table 1).

Precision

Results for repeatability and intermediate precision, expressed as %RSD, results were given in Table 1. The low values of %RSD indicate that the method is precise. Injection repeatability values of PARA and MEL were 0.907 and 0.502, respectively. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the %RSD values, which indicates that the proposed method was reproducible.

Robustness

There was no significant change in the peak areas and retention times of PARA and MEL when the organic strength and pH of buffer were changed. The low values of %RSD indicate that the method was robust (Table 2).
Table 1: Validation parameters and data for proposed method

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>PARA</th>
<th>MEL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td>5-60 µg/mL</td>
<td>1-12 µg/mL</td>
</tr>
<tr>
<td>Regression line</td>
<td>y = 137893 x + 580387</td>
<td>y = 72394 x + 28467</td>
</tr>
<tr>
<td>Regression coefficient ($r^2$)</td>
<td>0.9991</td>
<td>0.9997</td>
</tr>
<tr>
<td>Limit of detection (µg/mL)</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Limit of quantitation (µg/mL)</td>
<td>0.39</td>
<td>0.11</td>
</tr>
<tr>
<td>Accuracy (% recovery)*</td>
<td>100.28</td>
<td>99.82</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability of injection (%RSD)**</td>
<td>0.907</td>
<td>0.502</td>
</tr>
<tr>
<td>Intra-day precision (%RSD)*</td>
<td>0.666</td>
<td>0.687</td>
</tr>
<tr>
<td>Inter-day precision (%RSD)*</td>
<td>0.718</td>
<td>0.766</td>
</tr>
<tr>
<td><strong>Reproducibility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day precision (%RSD)*</td>
<td>0.809</td>
<td>0.533</td>
</tr>
<tr>
<td>Inter-day precision (%RSD)*</td>
<td>0.897</td>
<td>0.452</td>
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<tr>
<td>System suitability parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak area (%RSD)</td>
<td>0.133</td>
<td>0.196</td>
</tr>
<tr>
<td>Retention time (%RSD)</td>
<td>0.219</td>
<td>0.146</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.38</td>
<td>1.12</td>
</tr>
<tr>
<td>Number of theoretical plates</td>
<td>3048</td>
<td>3637</td>
</tr>
<tr>
<td>Resolution</td>
<td>9.62</td>
<td></td>
</tr>
</tbody>
</table>

*Replicates of three concentration levels (in three determinations); ** Ten repetitive injections of same homogeneous sample

Table 2: Results for robustness of the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Original</th>
<th>Used</th>
<th>Analyte</th>
<th>Peak area</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean±SD (n=3)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Methanol</td>
<td>50</td>
<td>PARA</td>
<td>49</td>
<td>3241676±14588</td>
<td>0.458</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>3293060±3890</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>3252060±33738</td>
<td>1.037</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>MEL</td>
<td></td>
<td>310362±1452</td>
<td>0.468</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
<td>50</td>
<td>312570±822</td>
<td>0.263</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>310130±1827</td>
<td>0.589</td>
</tr>
<tr>
<td>pH (Buffer)</td>
<td>9.2</td>
<td>PARA</td>
<td>9.1</td>
<td>3247733±22101</td>
<td>0.681</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.2</td>
<td>3294380±3694</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.3</td>
<td>3254849±17345</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>9.1</td>
<td>MEL</td>
<td>9.1</td>
<td>310683±545</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>9.2</td>
<td></td>
<td>9.2</td>
<td>312400±742</td>
<td>0.238</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.3</td>
<td>310656±814</td>
<td>0.262</td>
</tr>
</tbody>
</table>
Table 3: Assay results for paracetamol and meloxicam in tablets

<table>
<thead>
<tr>
<th>Product</th>
<th>Analyte</th>
<th>Label claim per tablet (mg)</th>
<th>% analyte estimated (Mean±SD)*</th>
<th>RSD (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melodol</td>
<td>Paracetamol</td>
<td>325</td>
<td>100.14±0.33</td>
<td>0.3302</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>Meloxicam</td>
<td>7.5</td>
<td>99.84±0.24</td>
<td>0.2395</td>
<td>0.0976</td>
</tr>
</tbody>
</table>

* n = 6; SEM = standard error of mean

Quantification of paracetamol and meloxicam in tablets

The proposed method was applied to the simultaneous determination of paracetamol and meloxicam in tablets. The results of the assay yielded 100.14 ± 0.33 % for PARA and 99.84 ± 0.24 % for MEL, of label claim of the tablets. The assay results show that the method was selective for the simultaneous determination of PARA and MEL without interference from the excipients used in the tablet dosage form. The results are shown in the Table 3.

DISCUSSIONS

In order to achieve simultaneous elution of the two components, initial trails were performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested in varying proportions, including acetonitrile, methanol, water and buffers with different pH values. Finally, a mixture of methanol and phosphate buffer (adjusted to pH 9.2 with potassium hydroxide) in the ratio of 50:50 v/v was selected as the optimum mobile phase.

Generally, assay methods are relatively time-consuming and involve expensive instrumentation not readily accessible to many groups. Therefore, our goal was to develop a relatively rapid and low-cost assay that could be performed at any laboratory with adequate HPLC instrumentation. The RP-HPLC method, as described, was validated and successfully employed for the simultaneous quantification of PARA and MEL in tablets. There is need to consider the successive steps for the development of RP-HPLC method. In particular, the problems relating to the standardization of sample preparations and selection of mobile phase needs to be emphasized. The optimized chromatographic conditions were selected based on sensitivity, retention time, peak shape and baseline drifts. The method was selective for the determination of PARA and MEL since no interfering peaks appeared near the retention time of the compounds of interest.

The method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines. Adequate resolution between PARA and MEL peaks showed the efficiency of the method based on its capacity to identify and determine each analyte at the same time with no interference. The LOD and LOQ values were low which indicates that the method is sensitive. The accuracy data show that the method is accurate within the desired range. The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and retention time.

CONCLUSION

The developed method for the simultaneous determination of paracetamol and meloxicam has the advantages of sensitivity, accuracy, precision and low cost. The non-interference
of tablet excipients makes the method suitable for the simultaneous determination of these drugs in tablets, and hence can be used for routine quality control of paracetamol and meloxicam in this dosage form.

ACKNOWLEDGEMENT

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