HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF INDOMETHACIN AND ITS RELATED SUBSTANCES IN TABLET DOSAGE FORMS

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ABSTRACT
A reversed-phase high performance liquid chromatographic (RP-HPLC) method with UV detection was proposed for separation of indomethacin and its impurities from tablet dosage forms. The best separation was achieved on a LiChrosorb C18, 250 mm x 4.6 mm, 5 μm column at a detector wavelength of 240 nm. The utilization of mixture of 40 volumes 0.5 % v/v orthophosphoric acid, 20 volumes of methanol and 40 volumes of acetonitrile as mobile phase with a flow rate of 2ml/min enabled acceptable resolution of indomethacin, in large excess, from possible impurities, in a short elution time (9 min). Analytical parameters linearity, accuracy, precision and specificity were determined by validation procedure and found to be satisfactory. Overall, the proposed method was found to be simple, rapid, precise and accurate for quality control of indomethacin and its impurities in dosage forms and in raw materials.

In this work the kinetic investigation of the alkaline hydrolysis of indomethacin was also carried out. The degradation reaction was monitored by means of HPLC method developed and was found to follow first-order kinetics. The rate constant and half-life of the hydrolytic decomposition were estimated.

Keywords: Liquid chromatography, Validation, Indomethacin, Stability, Impurities.

INTRODUCTION

Indomethacin 1-[4-(chlorobenzyloxy)-5-methoxy-2-methyl-1H-indole-3-acetic acid], is a non-steroidal anti-inflammatory, analgetic and antipycritic drug. Despite its high toxicity, indomethacin is a primary medicine used for the treatment of rheumatoid arthritis, gout, and collagen disease. It is a potent inhibitor of cyclooxygenases, reducing prostaglandin synthesis, relieving pain and reducing fever in febrile patients. The drug is usually administered orally. It can also be administered as a suppository and topical gel. By decomposition indomethacin forms two degradation products: 4-chlorobenzoic acid, mentioned in European Pharmacopoeia as impurity A and 5-methoxy-2-methyldioleacetic acid. They have to be monitored together with an active substance both during manufacturing process and storage of pharmaceuticals with aim to control the quality and quantity of the active substance in capsules and suppositories.

The European Pharmacopoeia (Ph. Eur. 5) uses a titration method for the determination of indomethacin. The substance is titrated with 0.1 M sodium hydroxide and blank titration has to be carried out simultaneously. That procedure is time consuming and impractical for routine analyses of pharmaceutical samples, especially during stability studies and quality control in the manufacturing process, where there could be many samples to be controlled, often in replicates. The amount of indomethacin active substance in capsules and suppositories is determined by means of absorption spectrophotometry. The European Pharmacopoeia recommends thin-layer chromatography for determination of related substances in active substance and capsules as well as HPLC for purity assessment of suppositories. Some analytical methods have been reported for assaying indomethacin in pure as well as in pharmaceutical dosage forms. The methods include: densitometry, LC-MS, HPLC with UV detection, phosphorimetic method, polarography, potentiometry, fluorimetry, spectrophotometry. It is important to emphasize that there is a few analytical procedures for determination of indomethacin and its two degradation products in one analysis simultaneously. To achieve this aim there is a need to develop a new, simple and fast analytical method for the simultaneous determination of these substances in pharmaceutical formulations. In our study, HPLC with UV detection is chosen for separation, identification and quantitation of indomethacin active substance and its two degradation products 4-chlorobenzoic acid and 5-methoxy-2-indoleacetic acid in the tablet dosage form. The analytical method described is validated and also applied to monitor hydrolysis of indomethacin in alkaline media.

MATERIALS AND METHODS

Chemicals and Reagents

Indomethacin RS, 4-chlorobenzoic acid RS and 5-methoxy-2-indoleacetic acid RS were used as standards. The tablets containing 25 mg of indomethacin were obtained commercially. LC-grade methanol and acetonitrile were supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A fixed wavelength detector and communication bus module CBM-10A. A LiChrosorb C18, 250 mm x 4.6 mm, 5 μm column was used as a stationary phase. The components were separated isocratically with a mobile phase consisting of 40 volumes 0.5 % v/v orthophosphoric acid, 20 volumes of methanol and 40 volumes of acetonitrile at a flow rate of 2.0 ml/min. The analysis was carried out at an ambient temperature and injection volume was 20 μl. The UV detector was set at 240 nm.

Calibration solutions

Reference stock solutions of 4-chlorobenzoic acid (0.1 mg/ml), 5-methoxy-2-indoleacetic acid (0.2 mg/ml) and indomethacin (0.5 mg/ml) were prepared in the mobile phase and filtered through a 0.45-μm membrane filter. Calibration solutions for 5-methoxy-2-indoleacetic acid were prepared by diluting the reference stock solution to furnish concentrations in the range 25.00-200.00 μg/ml Calibration solutions for 4-chlorobenzoic acid were prepared by diluting the reference stock solution to obtain concentrations in the range 5.00-40.00 μg/ml Calibration solutions for 5-methoxy-2-indoleacetic acid were prepared by diluting the stock reference stock solution to achieve concentrations in the range 2.50-20.00 μg/ml Working standard solutions for analysis of related substances contained 4.00 mg/ml indomethacin, 20.00 μg/ml 4-chlorobenzoic acid and 10.00 μg/ml 5-methoxy-2-indoleacetic acid. Working standard solution for the test assay contained 10.00 μg/ml indomethacin.

Sample preparation

For the investigation indomethacin gastro-resistant tablets were used. One tablet contained 25 mg active substance. Sample solutions were prepared by first preparing stock solutions. Twenty tablets...
were weighed and finely powdered. An amount of the powder equivalent to 25.0 mg indomethacin for assay and 200.0 mg for analysis of related substances were weighed into 50.0 ml volumetric flasks and approximately 30 ml mobile phase were added to each. The samples were sonicated for 10 min and the solutions were then diluted to volume with mobile phase, mixed well, and filtered. For assay, 5.00 ml stock solution were diluted to 25.00 ml with mobile phase to give a solution containing 100 µg/ml indomethacin. The solution used for analysis of related substances contained 4.0 mg/ml indomethacin.

**Stability of indomethacin**

In the kinetic run, the reaction was initiated by adding 1.0 ml reference stock solutions of indomethacin to 50.00 ml of preheated sodium hydroxide buffer solution (pH 9.0; 1mM), the final concentrations being 100 µg/ml. The reaction flask was maintained at 25±0.2 °C. Samples were taken at suitable time intervals during 6 hr of incubation. The progress of hydrolysis was monitored by means of HPLC method developed. 20 µl of sample examined was analysed for remaining indomethacin. First order rate constant for the hydrolysis was determined from the slope of linear plot of the logarithm of residual indomethacin against time.

**RESULTS AND DISCUSSION**

Under the proposed chromatographic conditions the obtained retention times were 2.41 min for 5-methoxy-2-indoleacetic acid, 3.31 min for 4-chlorobenzoic acid and 7.42 min for indomethacin. From the chromatogram shown in Fig. 1, it is evident, that both of related substances were completely separated from each other (Rs = 0.95), which indicated that the method is selective and could be used for their simultaneously identification, quantification and in purity tests.

![Chromatogram of working standards: 4-chlorobenzoic acid RS, 5-methoxy-2-indoleacetic acid RS and indomethacin RS](image)

**Method validation**

The proposed method was validated with respect to specificity, linearity, precision, accuracy, limit of quantification (LOQ) and limit of detection (LOD).

**Specificity**

The specificity of the HPLC method was confirmed by injecting placebo as well as reference solutions. No other peaks were observed at the retention times of indomethacin and its degradation products, indicating that interfering substances were not present.

**Calibration and linearity**

The linearity of the method was determined at six concentration levels ranging from 25.00 to 200.0 µg/ml for indomethacin, 5.00 to 40.00 µg/ml for 4-chlorobenzoic acid and 2.50 to 20.00 µg/ml for 5-methoxy-2-indoleacetic acid.

The calibration curves were constructed by plotting peak areas versus concentrations of compounds, and the regression equations were calculated. Each response was the average of three determinations. Linear regression data for calibration curves were shown in Table 1.

**Limit of quantification and limit of detection**

The limits of quantitation and limits of detection were calculated from the standard deviation of responses and slopes using signal-to-noise ratio. The quantitation limits for indomethacin, 4-chlorobenzoic acid and 5-methoxy-2-indoleacetic acid were 0.2 µg/ml, 0.2 µg/ml and 0.25 µg/ml, respectively, while detection limits were 0.05 µg/ml, 0.2 µg/ml and 0.25 µg/ml, respectively.

**Alkaline hydrolysis of indomethacin**

At constant pH value and temperature the formation of the degradation products 4-chlorobenzoic acid and 5-methoxy-2-indoleacetic acid was found to be linear function of the initial indomethacin concentration indicating first-order degradation kinetics. First-order rate constant for the hydrolysis was calculated

![Graph showing alkaline hydrolysis of indomethacin](image)

**Table 1: Linear regression data for calibration curves**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Indomethacin</th>
<th>4-Chlorobenzoic acid</th>
<th>5-Methoxy-2-indoleacetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (µg/ml)</td>
<td>25.00-200.0</td>
<td>5.00-40.00</td>
<td>2.50-20.00</td>
</tr>
<tr>
<td>Intercept</td>
<td>832.53</td>
<td>658.8</td>
<td>513.2</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9992</td>
<td>0.9986</td>
</tr>
</tbody>
</table>

**Precision and Accuracy**

The precision of the analytical system was investigated by performing six consecutive replicate injections of the same standard solution. The standard deviation (S_d) and relative standard deviation (RSD) obtained are listed in Table 2. The low RSD values indicated that the method is precise.

The accuracy of the method was investigated by determination of both impurities in the presence of indomethacin. A solution containing indomethacin (C = 4.0 mg/ml) with no detectable impurities was spiked with the reference substances at appropriate concentrations. The recovery and relative standard deviations (RSD) obtained (Table 3) confirmed the satisfactory accuracy of the method.

![Graph showing precision and accuracy](image)

**Limit of linear range**

The limits of linear range were found to be 2.00-20.00 µg/ml for 4-chlorobenzoic acid and indomethacin, 2.50-20.00 µg/ml for 5-methoxy-2-indoleacetic acid.
from the slope of semilogarithmic plot of percent indomethacin remaining versus time and was found to be $8.4 \times 10^{-3}$. On Fig. 2 the first order plot for degradation of indomethacin was presented. The corresponding half-life was 82.51 min.

### Table 2: Precision of the method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precision</th>
<th>S&lt;sub&gt;r&lt;/sub&gt;</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>99.77</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>4-Chlorobenzoic acid</td>
<td>19.61</td>
<td>0.34</td>
<td>1.73</td>
</tr>
<tr>
<td>5-Methoxy-2-indoleacetic acid</td>
<td>9.78</td>
<td>0.17</td>
<td>1.74</td>
</tr>
</tbody>
</table>

### Table 3: Results of recovery studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery (%)</th>
<th>S&lt;sub&gt;r&lt;/sub&gt;</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>99.66</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>4-Chlorobenzoic acid</td>
<td>99.14</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>5-Methoxy-2-indoleacetic acid</td>
<td>99.53</td>
<td>0.36</td>
<td>0.36</td>
</tr>
</tbody>
</table>

### Fig. 2: First-order plot for the degradation of indomethacin

**CONCLUSION**

HPLC procedure for quality control of indomethacin was performed. The method was validated in respect of purposes of pharmaceutical practices. The developed RP-HPLC procedure was suitable for simultaneous qualitative and quantitative determination of indomethacin and its related substances in tablet dosage forms and in raw materials.

**ACKNOWLEDGMENT**

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**REFERENCES**

10. Paula P, Lucia M, Saraiva M, Santos J, Lima J. A pulsed sequential injection analysis flow system for the fluorimetric