

# Applications of New Validated RP-HPLC Method for Determination of Indomethacin and its Hydrolytic Degradants using Sodium Acetate Buffer

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## ABSTRACT

**Objective:** A New RP-HPLC method for estimation of indomethacin and its two degradant impurities 4-chlorobenzoic acid, 5-methoxy-2-methyl-indoleacetic acid was developed and validated as per ICH guidelines. **Background:** This new method is not only capable of identifying and quantifying the impurities but also can be used for the assay of indomethacin in marketed capsule formulations. **Methods:** The chromatographic conditions were optimized using Zorbax Eclipse Plus C18, 3.5  $\mu\text{m}$  (4.6 mm  $\times$  100 mm) column with methanol: acetonitrile: 10 mM sodium acetate buffer pH 3, 10:50:40% v/v as the mobile phase at the flow rate of 1 ml/min and detection was carried out using UV-Visible PDA detector at 254 nm. **Results:** The method was linear over concentration range of 25-70  $\mu\text{g/ml}$  for indomethacin, 0.25-2  $\mu\text{g/ml}$  for 4-chlorobenzoic acid and 0.25-2  $\mu\text{g/ml}$  for 5-methoxy-2-methyl-indoleacetic acid. The developed method was validated as per the ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity for indomethacin and its impurities.

**Key Words:** RP-HPLC, Indomethacin, 4-chlorobenzoic acid, 5-methoxy-2-methyl-indoleacetic acid, Validation.

## INTRODUCTION

Indomethacin belongs to the class of heteroarylacetic acid derivatives of NSAIDs and is used for the treatment of acute gouty arthritis, acute pain of ankylosing spondylitis and osteoarthritis. The anti-inflammatory, antipyretic and analgesic actions of indomethacin are due to the ability to inhibit prostaglandin biosynthesis.<sup>1</sup> The drug is official in Indian Pharmacopoeia,<sup>2</sup> British Pharmacopoeia<sup>3</sup> and United States Pharmacopoeia.<sup>4</sup> Chemically indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-ylacetic acid (Figure 1). The estimation of indomethacin in capsules has been done by UV spectrophotometry,<sup>5-9</sup> Colorimetric methods,<sup>10-11</sup> and RP-HPLC<sup>12-13</sup> methods. British Pharmacopoeia 2013 specifies 4-chlorobenzoic acid as an impurity of Indomethacin. Along with 4-chlorobenzoic acid (Figure 2), 5-methoxy-2-methyl-indole-

acetic acid (Figure 3) has been identified as another impurity by Tsvetkova B *et al*,<sup>14</sup> Novakova L *et al*,<sup>15</sup> Kwong E *et al*,<sup>16</sup> Sonja H *et al*,<sup>17</sup> Krzek J *et al*,<sup>18</sup> and Karima Fadhil Ali *et al*<sup>19</sup> and analyzed by RP-HPLC methods, UV spectrophotometric methods and Densitometric method in literature. Both these impurities are the degradation products of Indomethacin under hydrolytic conditions. The reported methods have a longer analysis time ranging from 5 to 10 min. The objective of the study was to develop newer RP-HPLC method for identification and analysis of indomethacin in presence of two possible degradant impurities 4-chlorobenzoic acid and 5-methoxy-2-methyl-indoleacetic acid in pharmaceutical capsule dosage formulation with emphasis on shortening the analysis time and validate in accordance with the ICH guidelines.<sup>20</sup>

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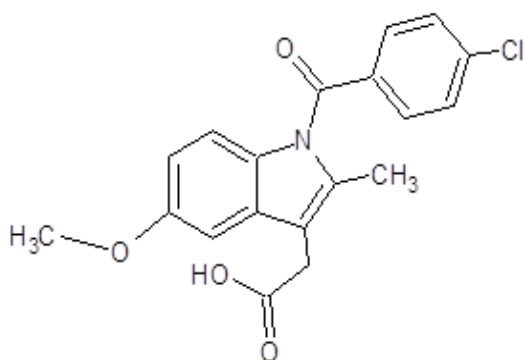


Figure 1: Structure of Indomethacin

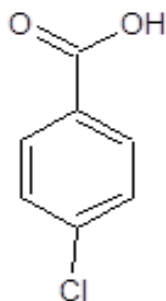


Figure 2: Structure of 4-Chlorobenzoic acid

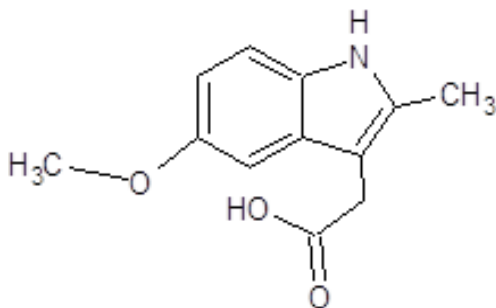


Figure 3: Structure of 5-Methoxy-2-methylindoleacetic acid

## MATERIAL AND METHODS

### Equipment

The studies were performed on two HPLC – Agilent 1260 and JASCO, Japan both equipped with autosampler and auto injector with PDA detector and Double beam UV/VIS spectrophotometer of Lab India® make of UV 3000+ Analytical Tech model. The reference standard of Indomethacin was supplied by Jagsonpal Pharmaceuticals Ltd., whereas, 4-chlorobenzoic acid and 5-methoxy-2-methylindoleacetic acid were purchased from Aldrich Chemicals. Indocap® containing 25 mg of indomethacin per capsule was purchased from the local market. HPLC grade methanol, acetonitrile and water was purchased from Merck Specialities Private Limited, Mumbai. Sodium acetate and glacial acetic acid (AR

grade) was obtained from Loba Chemie Pvt. Ltd. and Thomas Baker, Mumbai respectively.

### Chromatographic conditions

HPLC analyses for method development and validation was performed on Zorbax Eclipse Plus C-18, 3.5  $\mu\text{m}$  (4.6 mm  $\times$  100 mm) column was used for the separation. Isocratic system of Methanol: acetonitrile: 10 mM sodium acetate buffer pH 3, 10:50:40% v/v was delivered at a flow rate of 1 ml/min and detection carried out at 254 nm. The injection volume was 5  $\mu\text{l}$  and the analysis was performed at ambient temperature.

### Preparation of Standard stock solutions

Standard stock solutions of indomethacin and both the impurities were prepared by dissolving 10 mg of each separately in 10 ml of methanol in three separate 10 ml volumetric flasks to obtain concentration of 1000  $\mu\text{g/ml}$ . Further dilutions were made with the mobile phase to obtain final concentrations of 10-100  $\mu\text{g/ml}$  for indomethacin standard and 0.1- 2.5  $\mu\text{g/ml}$  for both the impurity working standard solutions respectively.

### Preparation of sample solution

Quantity of mixed contents of 20 capsules containing about 10 mg of indomethacin was weighed and transferred to 100 ml volumetric flask. About 10 ml of water was added and allowed to stand for 10 min with occasional stirring. 75ml of methanol was added and shaken well and sufficient methanol was added to make up the volume to 100 ml. The solution was filtered through 0.45 $\mu\text{m}$  filter to obtain sample stock solution. The sample stock solution was further diluted with mobile phase to obtain final concentration of 30  $\mu\text{g/ml}$  for sample analysis of indomethacin.

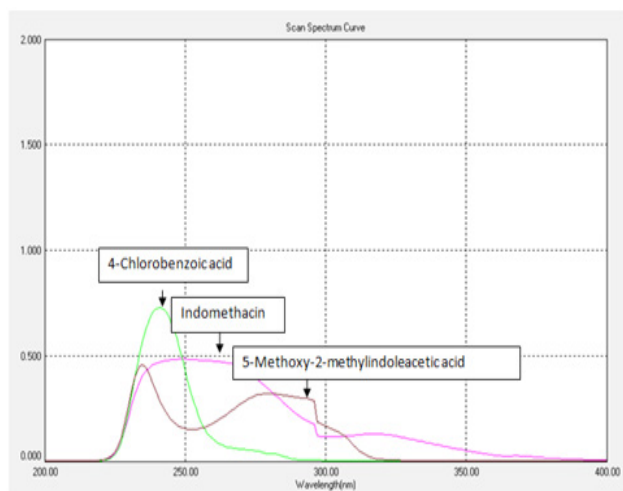
## VALIDATION

The method was validated for linearity, precision, specificity, accuracy, limit of detection, limit of quantification, ruggedness and robustness as per the ICH guidelines<sup>20</sup>. The linearity of calibration curves of indomethacin was determined over the concentration range of 25-70  $\mu\text{g/ml}$ . For both the degradation products linearity was tested from 0.1-6.6% of the active substance indomethacin and was found to be linear in the concentration range of 0.25-2  $\mu\text{g/ml}$ . Accuracy was determined by spiking standard solutions in the previously analysed sample solutions at three different levels of i.e. 80%, 100% and 120% of the target concentration and calculating the % recovery. Precision was calculated on six replicate injections of the sample solution at two levels 0.25 and 2  $\mu\text{g/ml}$ , being the lower and higher concentration of linear-

ity range. Specificity study was carried out by comparing chromatograms of blank, standard solutions and sample solution spiked with both the impurities. The LOD and LOQ were calculated from the standard calibration curves based on standard deviation formula with equations  $LOD=3.3\sigma/S$  and  $LOQ = 10\sigma/S$ ; where,  $\sigma$  is the standard deviation of the response and  $S$  is the slope of the calibration curve. Robustness of the method was determined by making slight changes in the operating conditions viz. flow rate  $\pm 0.2$  ml, change in the organic phase ratio by  $\pm 2\%$  v/v and pH by  $\pm 0.2$  units. System suitability was determined before sample analysis from six replicate injections. The solution stability was determined by performing the analysis up to 8 hr with the working solutions.

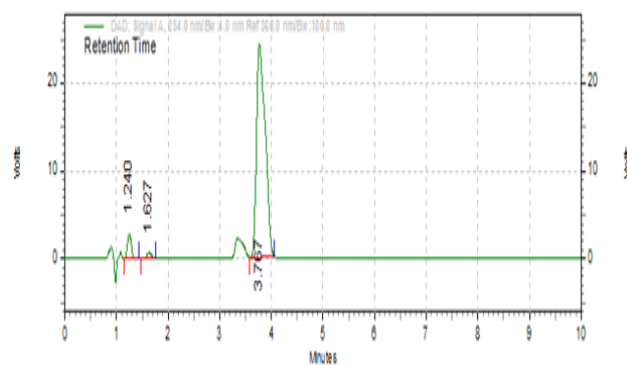
## RESULTS AND DISCUSSION

The aim of this RP-HPLC method was to estimate indomethacin and its two impurities in capsule formulation and validate it in accordance with the ICH guidelines. This method can also find an application in assay of indomethacin in its solid dosage form. As both the impurities are present in very low concentrations as compared to indomethacin there was a need to select the detection wavelength, where impurities showed significantly higher absorbance than the active substance; hence 254 nm was selected as the detection wavelength. UV overlain spectra of indomethacin and both impurities in the optimized mobile phase is shown in Figure 4. The chromatographic separation was achieved using Zorbax Eclipse Plus C-18, 3.5  $\mu$ m (4.6 mm  $\times$  100 mm) column. After performing exploratory trials methanol: acetonitrile: 10 mM sodium acetate buffer pH 3, 10:50:40% v/v with flow rate of 1 ml/min was selected



**Figure 4: UV overlain spectra spectra of indomethacin, 4-chlorobenzoic acid and 5-methoxy-2-methyl-indoleacetic acid in the optimized mobile phase**

as the optimized mobile phase. The retention times of indomethacin, 4-chlorobenzoic acid and 5-methoxy-2-methylindoleacetic acid were 3.767 min, 1.627 min and 1.240 min respectively. Resolution of the method was found to be highly satisfactory as indicated by good separation of compound peaks. The chromatogram of indomethacin sample solution spiked with the two impurities is shown in Figure 5. The system suitability tests were carried out and parameters are summarized in Table 1. The assay results for indomethacin capsules are shown in the Table 1 and % RSD of assay was found to be within limit of 2%. The linearity range for indomethacin was found to be 25-70  $\mu$ g/ml, for impurities 4-chlorobenzoic acid 0.25-2  $\mu$ g/ml and 5-methoxy-2-methyl-indoleacetic 0.25-2  $\mu$ g/ml. The accuracy was determined by calculating % recovery at 3 levels of 80, 100 and 120% and was found to be well within the acceptable limits. RSD for repeatability and inter-day precision was found to be less than 2% which is within the acceptance limit and indicates that the method is precise. The detection limit and quantitation limit of indomethacin, 4-chlorobenzoic acid and 5-methoxy-2-methyl-indole acetic acid was found to be 1.036  $\mu$ g/ml, 0.104  $\mu$ g/ml, 0.104  $\mu$ g/ml and 3.141  $\mu$ g/ml, 0.308  $\mu$ g/ml, 0.308  $\mu$ g/ml respectively. There was no other visible peak at retention time up to 10 min indicating high degree of specificity of the method. The method validation results are summarised in Table 1. The robustness of the method was determined on the sample solution by slightly varying the mobile phase ratio, pH, flow rate and the percent recovery was found to be within limits. It was observed that the retention time and peak area of all the working standard and sample solutions remained unchanged and no significant degradation observed up to 8 hr at ambient temperature. Thus, the new method is found to be accurate, precise and specific and could be used for routine analysis of indomethacin in pres-



**Figure 5: Chromatogram of Indomethacin spiked with 4-chlorobenzoic acid and 5-methoxy-2-methyl-indoleacetic acid in the optimized mobile phase**

**Table 1: Method validation results**

	Indomethacin	4-Chlorobenzoic acid		5-methoxy-2-methyl-indoleacetic acid	
<b>SYSTEM SUITABILITY TESTS</b>					
Theoretical plates	2457	1758		1672	
Asymmetry	1.26	0.00		1.25	
Resolution	9.69	2.90			
<b>VALIDATION</b>					
Linearity (µg/ml)	25-70 µg/ml	0.25-2 µg/ml		0.25-2 µg/ml	
Regression equation	$y = 1,630,399.06x - 2,204,584.44$	$y = 385776x + 140995$		$y = 739706x + 84634$	
Correlation coefficient (R <sup>2</sup> value)	0.9992	0.9985		0.999	
Accuracy (%)					
80%	99.70%	100.31%		101.6%	
100%	100.06%	100.49%		101.4%	
120%	99.28%	101.18%		99.63%	
Precision (n=6)	30 µg/ml	0.25 µg/ml	2 µg/ml	0.25 µg/ml	2 µg/ml
Repeatability (% RSD)	0.003	1.624	0.449	1.708	0.448
Intermediate Precision (% RSD)	0.005	1.860	0.336	1.651	0.480
Limit of Detection (µg/ml)	1.036 µg/ml	0.104 µg/ml		0.104 µg/ml	
Limit of Quantitation (µg/ml)	3.141 µg/ml	0.308 µg/ml		0.308 µg/ml	
Assay (%)	98.60 %	-		-	
Retention time (min)	3.767	1.627		1.240	

ence of its hydrolytic impurities 4-chlorobenzoic acid and 5-methoxy-2-methyl-indoleacetic acid.

## CONCLUSION

A new RP-HPLC method was developed and validated for estimation of indomethacin and its two impurities using methanol, acetonitrile and a volatile buffer. All the compounds eluted within 5 min and thus required shorter time of analysis. The method is simple, accurate, precise and robust and can be used for routine analysis of indomethacin in its marketed formulation.

## ACKNOWLEDGEMENT

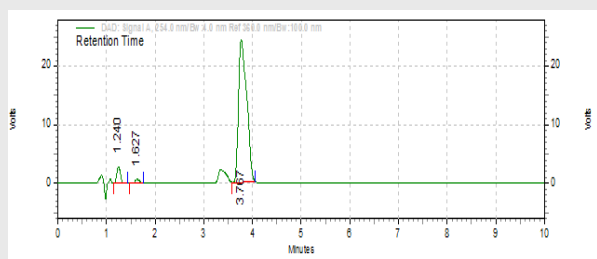
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## PICTORIAL ABSTRACT



## SUMMARY

- Indomethacin degradants 4-chlorobenzoic acid and 5-methoxy-2-methyl indoleacetic acid are reported as hydrolytic impurities.
- New validated RP-HPLC method is developed on an ODS column to identify Indomethacin and its two hydrolytic degradants within short run time of 5 min.
- The new method can be successfully applied for analysis of Indomethacin in marketed capsule dosage forms and for impurity profiling experiments

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