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Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF INDOMETHACIN IN PHARMACEUTICAL CAPSULE FORMULATION

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ABSTRACT:

Purpose : A new stability-indicating RP-HPLC assay method for estimation of indomethacin and its degradation products formed under various stress conditions was developed and validated for routine analysis of indomethacin in its marketed dosage forms.

Methodology : The method uses a volatile buffer and could be extended for LC-MS studies. The separation was carried out on Zorbax Eclipse Plus C18, 3.5 µm (4.6 mm × 100 mm) column with acetonitrile: 10 mM sodium acetate buffer pH 4, 60:40% v/v as the mobile phase at the flow rate of 0.5 ml/min with detection carried out using UV-Visible PDA detector at 226 nm. The retention time of indomethacin was 5 min and linearity was observed in the concentration range of 7.5-75 µg/ml. The developed method was validated as per the ICH guidelines. The drug was subjected to various stress conditions of acid and base hydrolysis, oxidation, photolysis, thermal degradation and neutral condition.

Findings: Considerable degradation was found under all stress conditions and the degradation products were well resolved from indomethacin in the proposed HPLC method. Conclusion: The new method could be used for routine analysis of indomethacin in its dosage forms. The model used for stress degradation studies is simple and could be extended for study of other drug molecules.

Keywords : *RP-HPLC, stability-indicating, Indomethacin, validation*

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INTRODUCTION

Indomethacin is used for short-term treatment of acute gouty arthritis, acute pain of ankylosing spondylitis and osteoarthritis. It belongs to the class of heteroarylacetic acid derivatives. The ability of indomethacin to potently inhibit prostaglandin biosynthesis accounts for its anti-inflammatory, antipyretic and analgesic actions. Aqueous solutions of indomethacin are not stable because of the ease of hydrolysis of p-chlorobenzoyl group[1]. The drug is official in Indian Pharmacopoeia[2], British Pharmacopoeia[3] and United States Pharmacopoeia[4]. Chemically indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-ylacetic acid (Fig 1). The estimation of indomethacin in capsules has been done by UV spectrophotometry[5-9], colorimetric methods[10,11] and RP-HPLC[12,13]methods. All the above methods were based on estimation of indomethacin alone and in combination in pharmaceutical capsules. As found from the literature there are two stability-indicating RP-HPLC methods for estimation of indomethacin. One of the methods evaluates stability of indomethacin under physiological conditions by subjecting it to hydrolysis under buffers of different pH[14]. Another

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method uses an environmental benign approach for estimation of indomethacin in a number of formulations[15]. Both the methods have failed to subject the drug to all the stress conditions and to resolve the degradants. The aim of the present work is to develop a novel validated stability-indicating RP-HPLC assay method for estimation of indomethacin and its degradation products formed when subjected under various stress conditions which can be also used for routine analysis of indomethacin in pharmaceutical formulations.

MATERIAL AND METHODS

Equipment

The study was performed using HPLC of Agilent 1260 make equipped with autosampler and auto injector with PDA detector and Double beam UV/VIS spectrophotometer of SHIMADZU make and model UV 2700. Indomethacin reference standard was supplied by Jagsonpal Pharmaceuticals Ltd.Capsule formulation Indocap® containing 25 mg of indomethacin per capsule was purchased from the local market. HPLC grade acetonitrile and water was purchased from Merck Specialities Private Limited, Mumbai. Sodium acetate and glacial acetic acid (AR grade) was obtained fromLobaChemiePvt. Ltd. and Thomas Baker, Mumbai respectively.

Chromatographic conditions

Zorbax Eclipse Plus C18, 3.5µm (4.6 mm × 100 mm) column was used for the separation. Acetonitrile : 10mM sodium acetate buffer pH 4, 60:40% v/v was delivered at a flow rate of 0.5 ml/min and detection carried out at 226 nm. The injection volume was 5 µl and the analysis was performed at ambient temperature.

Preparation of Standard stock solution

Standard stock solution of indomethacin was prepared by dissolving 10 mg of indomethacin in 10 ml of methanol in a 10 ml volumetric flask to obtain concentration of 1000 µg/ml. Further dilutions were made with the mobile phase. The retention time of indomethacin was 5.0 min. Calibration curve was plotted and was found to be linear in the concentration range of 7.5-75 µg/ml with R^2 value being 0.998.

Assay of indomethacin in capsules

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 0.45µm filter to obtain sample stock solution. The sample stock solution was further diluted with mobile phase to obtain final concentration of 40 µg/mlfor sample analysis of indomethacin (Table 1).

Table 1: Assay results of Indomethacin

Name of the formulation	Label claim (indomethacin)	Amount present in the capsule*	Assay %	% RSD
Indocap	25 mg	24.705 mg	98.82	0.003

*Average of six determination, RSD is the relative standard deviation

VALIDATION

The method was validated for linearity, precision, specificity, accuracy, limit of detection, limit of quantification, ruggedness and robustness as per the ICH guidelines[16]. The linearity of calibration curves was determined over the concentration range of 7.5-75 µg/ml, and was found to be linear with R^2 value being 0.998. Accuracy was carried out by spiking standard to previously analyzed sample solution at three different levels of 80%, 100% and 120% of the target concentration. Precision was studied by performing six replicate assays of the capsule solution for repeatability and inter-day variation. The LOD and LOQ were calculated from the standard calibration curves based on standard deviation formula with equations LOD=3.3s /S and LOQ = 10s /S; where, s 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 ± 0.1 ml, change in the organic phase ratio by ± 2% v/v and pH by ± 0.2 units. Specificity study was carried out by evaluating adequate resolution between analyte and degradation products and ability of method to detect analyte in presence of other products. The solution stability was determined by performing the analysis upto 8 hr with the working solutions. System suitability was determined before sample analysis from six replicate injections of standard solution of 40 µg/ml of indomethacin.

Forced degradation studies

Stress studies[17,18] were performed at 1000 µg/ml of indomethacin and were analyzed at concentration of

70 µg/ml. All the solutions used for force degradation were prepared by dissolving the standard stock solution in equal volume of stressing agents. After degradation at set intervals, aliquot portion of these solutions were diluted with the mobile phase to yield the target concentration. The periodic time intervals are decided based on drug sensitivity to the stressing agents. Number of degradation products and % degradation under each condition identified and compared with respective chromatogram of blank or zero hour chromatogram.

The various conditions in which the stress studies were performed are described below.

Acid hydrolysis

It was performed using 0.1N HCl at standing and reflux conditions for 4 hr. 5 ml std stock solution of indomethacin (1000 µg/ml) was transferred into two 10 ml volumetric flasks each. To these, 5 ml each of 0.1N HCl was added. The first flask was refluxed at 60°C for 4 hr whereas, the second flask was kept standing for 4 hr and aliquot portions of sample were withdrawn from first flask at 4 hr and from second flask at 0, 1, 2 and 4 hr respectively and further neutralised with 0.1N NaOH and diluted with mobile phase to obtain final concentrations of 70 µg/mlof indomethacin each.

Base hydrolysis

It was performed using 0.1N NaOH at standing conditions for 4 hr. 5 ml std stock solution of indomethacin (1000 µg/ml) was transferred into a 10 ml volumetric flasks. To it, 5 ml of 0.1N NaOH was added. The flask was kept standing for 4 hr and aliquot portions of sample were withdrawn from the flask at 0 hr, 30 min, 1, 2 and 4 hr respectively and further neutralised with 0.1N HCl and diluted with mobile phase to obtain final concentrations of 70 µg/mlof indomethacin each.

Oxidation

It was performed using 3% hydrogen peroxide at standing and reflux conditions for 8 hr. 5 ml std stock solution of indomethacin (1000 µg/ml) was transferred into two 10 ml volumetric flasks each. To these, 5 ml each of 3% hydrogen peroxide was added. The first flask was refluxed at 60°C for 8 hr whereas, the second flask was kept standing for 8 hr and aliquot portions of sample were withdrawn from first and second flask at 4 and 8 hr each and further diluted with mobile phase to obtain final concentrations of 70 µg/ml of indomethacin each.

Photolytic degradation

It was performed by keeping solid drug samples in an UV chamber (254 nm) for 4 and 8 hr. At the particular time interval, 10 mg of powder was weighed and transferred into 10 ml volumetric flask and diluted with mobile phaseto obtain final concentrations of 70 µg/ml of indomethacin.

Thermal degradation

Thermal degradation studies were carried out by subjecting it to heat in solid and in aqueous form to temperature of 60°C for 4, 8 hr and 1 hr respectively. Periodically, 10 mg of powder was weighed and transferred into 10 ml volumetric flask and diluted with mobile phase to obtain final concentrations of 70µg/ml of indomethacin. Thermal degradation in aqueous solution was carried out by subjecting standard solution (1000 µg/ml) of indomethacin to temperature of 60°C for 1 hr and aliquot portion of sample was withdrawn and diluted with mobile phase.

Neutral condition

This study was carried out as solution state stability studies at neutral pH. Aliquot portion of std stock solution of indomethacin (1000 µg/ml) was transferred into 10 ml volumetric flasks each at intervals of 0, 24 and 48 hr and further diluted with mobile phase to obtain final concentrations of 70 µg/ml of indomethacin each.

RESULTS AND DISCUSSION

The aim of this RP-HPLC method was to estimate indomethacin in marketed pharmaceutical formulations and to separate and quantitate indomethacin from its degradation products. Various trials were performed to develop and optimized the method. UV spectra of indomethacin in the optimized mobile phase show that the drug shows maximum absorbance at 226 nm and hence thissame wavelength was selected during degradation studies (Fig 2). The chromatographic separation was achieved using Zorbax Eclipse Plus C18, 3.5µm (4.6 mm × 100 mm) column. Acetonitrile : 10mM sodium acetate buffer pH 4, 60:40% v/v with flow rate of 0.5 ml/min was

Fig. 1: Structure of Indomethacin

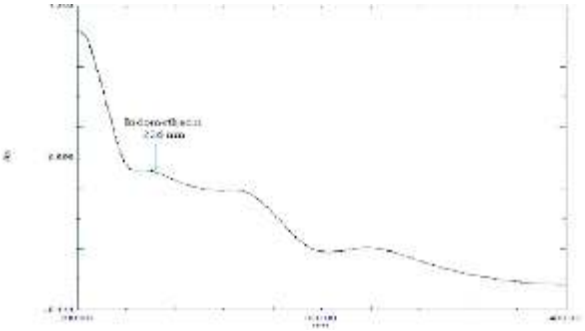

Fig. 2: UV spectra of indomethacin in the optimized mobile phase

selected as the optimized mobile phase after performing preliminary trials. The retention time of indomethacin was 5.0 min. Chromatogram of indomethacin is shown in the Fig 3. The system suitability parameters measured are shown in Table 2. The tailing factor was calculated statistically and found to be 1.23 for indomethacin. The assay results for indomethacin capsules are shown in the Table 2and % RSD of assay was found to be within limit of 2%.The standard calibration curve was plotted and the linearity range obtained was 7.5-75 µg/ml for indomethacin with R^2 value of 0.998. Accuracy was determined through recovery study of the standard at three levels ranging from 80-120% of target concentration and was found to be well within the acceptance limit indicating no interferences of the drug with the excipients present in the formulation and thus accuracy of the method was proven. The repeatability and inter-day precision was found to be less than 2% which is within the acceptance limit (Table 3). The low % RSD value indicates that the method is precise. The robustness of the method was determined on the sample solution by slightly varying the mobile phase ratio, pH and flow rate and

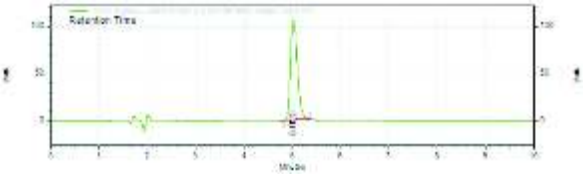

Fig. 3: Chromatogram of indomethacin

Table 2: Validation and system suitability parameters of the proposed method

Parameters*	Indomethacin
Retention time (min)	5 min
Linearity range (µg/ml)	7.5-75 µg/ml
Regression equation y=mx+c	y = 5,725,268x - 11,203,831
Slope (m)	5,725,268
Intercept (C)	11,203,831
Correlation coefficient	0.998
% Assay	98.82
Precision	
Intraday (n6) % RSD	0.003
Interday (n6) % RSD	0.002
Accuracy	
80 % of target concentration	98.40%
100 % of target concentration	99.12%
120 % of target concentration	99.66%
LOD (µg/ml)	3.33 µg/ml
LOQ (µg/ml)	10.11 µg/ml
System suitability	
Theoretical plates	5946
Resolution	-
Asymmetry	1.23
LOD (µg/ml)	3.33 µg/ml
LOQ (µg/ml)	10.11 µg/ml
System suitability	
Theoretical plates	5946
Resolution	-
Asymmetry	1.23

*Average of six determination, y is the area under curve and x is the concentration, RSD is the relative standard deviation, LOD- Limit of detection, LOQ- Limit of Quantitation

Table 3: Precision of the method

Parameter	Precision (Day 1)	Intermediate Precision (Day 2)
% assay	98.81	99.28
% RSD	0.003	0.002

*Average of six determination, RSD is the relative standard deviation

the percent assay found to be within limits and thus shows high degree of precision and reproducibility of the developed. The detection limit and quantitation limit was found to be 3.33 µg/ml and 10.11 µg/ml for indomethacin which indicates that the drug can be estimated accurately and method is sensitive_. The working solutions of sample and standard remained unchanged upto 8 hr indicating solution stability. There was no visible peak in the retention time upto 10min indicating high degree of specificity and also found to be specific in presence of degradants as shown in Fig 4(a), 4(b), 4(c), 5(a), 5(b), 6(b) and 6(c). The validation results are summarised in Table 1. Thus, the developed method is simple, accurate and precise and can be used for routine analysis of indomethacin in marketed dosage forms.

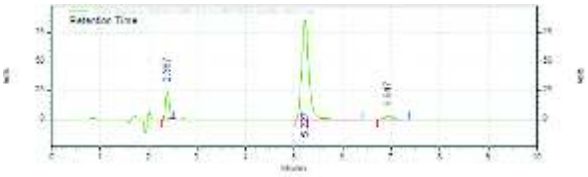

Fig. 4(a): Chromatogram of acid hydrolysis of indomethacin at 0 hr at room temperature

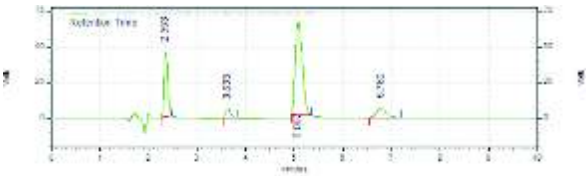

Fig. 4(b): Chromatogram of acid hydrolysis of Indomethacin after 4 hr standing at room temperature

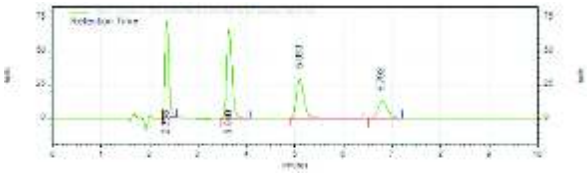

Fig. 4(c): Chromatogram of acid hydrolysis of indomethacin after 4 hr reflux

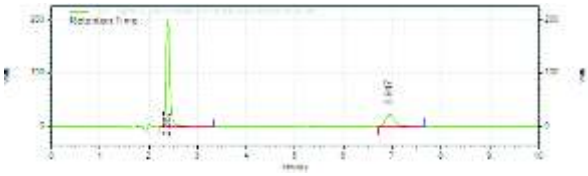

Fig. 5(a): Chromatogram of base hydrolysis of indomethacin at 0 hr at room temperature

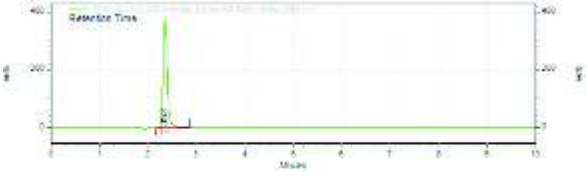

Fig. 5(b): Chromatogram of base hydrolysis of indomethacin after 4 hr at room temperature

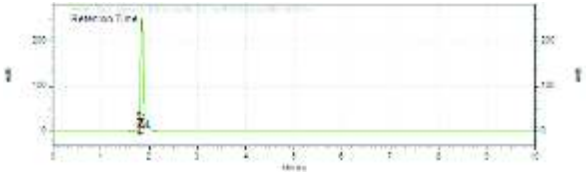

Fig. 6(a): Chromatogram of oxidation blank

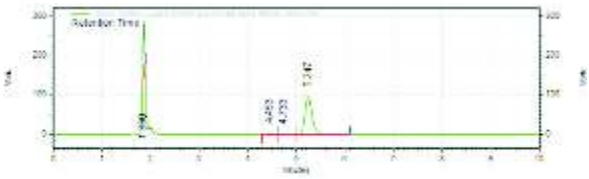

Fig. 6(b): Chromatogram of oxidation of indomethacin after 8 hr at room temperature

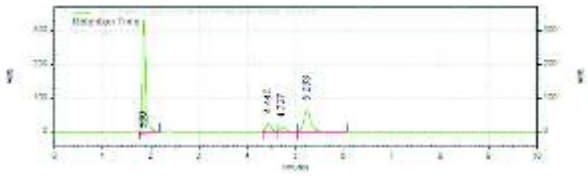

Fig.6(c) : Chromatogram of oxidation of indomethacin after 8 hr reflux

The method was validated as per the ICH guidelines. Force degradation studies were carried out by subjecting the drug to various stress conditions of acid and base hydrolysis, oxidation, photolytic degradation, thermal degradation and under neutral conditions. Acid hydrolysis showed three degradation products at retention times 2.353 min, 3.633 min, 6.780 min after 4 hr at room temperature and at retention times 2.353 min, 3.640 min and 6.793 min after 4 hr reflux [Fig 4(a), 4(b) and 4(c)]. The chromatogram of base hydrolysis showed two peaks at 2.387 min and 6.947 min at 0 hr and one degradation peak at 2.353 min after 4 hr at room temperature [Fig 5(a) and 5(b)]. On subjecting the drug to chemical oxidation the drug formed two degradation products at retention times 4.453 min and 4.733 min after 8 hr at room temperature and at 4.440 min and 4.727 min after 8 hr reflux whereas, the hydrogen peroxide peak was seen at retention time of 1.8 min [Fig 6(a), 6(b) and 6(c)]. In photolytic and thermal stress conditions the drug did not form degradation products even after 8 hr [Fig 7(a), 8(a) and 8(b)]. Under neutral conditions the drug did not degrade and no additional peak was seen in the chromatogram, thus indomethacin is found to be stable even after 48 hr [Fig 9(a)]. The study indicates that the drug is very susceptible to acid and base hydrolysis and oxidation. The retention times of the degradation products and the percent degradations under various stress conditions is presented in Table 4 and 5 respectively. The degradants formed were well resolved from the drug peak. The proposed method can be conveniently used to detect the possible degradation products in the dosage form during the stability studies.

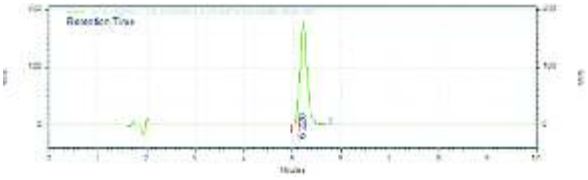

Fig.7(a): Chromatogram of photolytic degradation of indomethacin after 8 hr

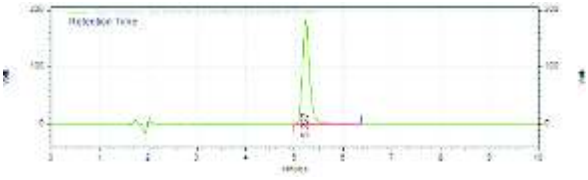

Fig. 8(a): Chromatogram of thermal degradation of indomethacin after 8 hr

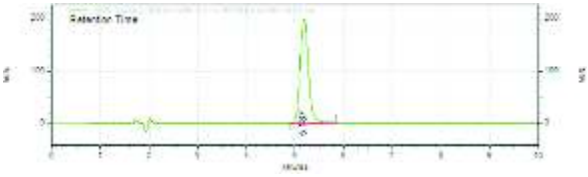

Fig. 8(b): Chromatogram of thermal degradation of indomethacin aqueous solution after 1 hr

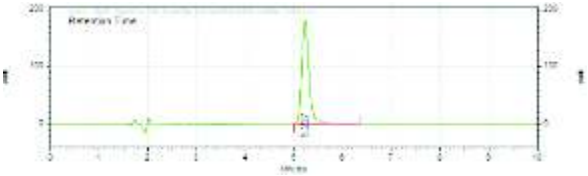

Fig. 9(a): Chromatogram of neutral hydrolysis of indomethacin after 48 hr standing

Table 5: Total percentage of degradation

Stress conditions	Time (hr)	Retention time (min)	% degradation
0.1N HCL	0 hr	5.227	67.49
	4 hr at room temperature	5.087	74.77
	4 hr reflux	5.093	85.74
0.1N NaOH	0 hr	0	100
	4 hr at room temperature	0	100
Oxidation	0 hr		0
	8 hr at room temperature	5.247	68.95
	8 hr reflux	5.223	76.56
Photolytic degradation	0 hr		0
	8 hr under UV 254 nm	5.22	36.02
Thermal degradation	0 hr		0
	8 hr at 60°C	5.227	34.49
	Aqueous solution for 1 hr at 60°C	5.187	6.49
Neutral condition	0 hr		0
	48hr at room temperature	5.227	0

Table 4: Retention times of the degradation products formed under different stress conditions

Stress conditions	Time (hr)	Retention time of degradation products (min)
0.1N HCL	0 hr	2.387, 6.947
	4 hr at room temperature	2.353, 3.633, 6.780
	4 hr reflux	2.353, 3.640, 6.793
0.1N NaOH	0 hr	2.387, 6.947
	4 hr at room temperature	2.353
Oxidation	0 hr	-
	8 hr at room temperature	4.453, 4.733
	8 hr reflux	4.440, 4.727
Photolytic degradation	0 hr	-
	8 hr under UV 254 nm	-
Thermal degradation	0 hr	-
	8hr at 60°C	-
	Aqueous solution for 1 hr at 60°C	
Neutral condition	0 hr	-
	48hr at room temperature	-

CONCLUSION

A novel, simple and reproducible stability-indicating RP-HPLC assay method was developed which can be used for routine analysis in pharmaceutical dosage forms. The method was found to be accurate, precise, robust and separation of the degradants from the drug was achieved in a shorter duration of time. It can be used to examine the stability of indomethacin capsules.

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