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Short communication

Sensitive spectrophotometric method for the determination of indomethacin in capsules

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Abstract

A simple, sensitive and selective spectrophotometric method for the determination of indomethacin (INM) either in pure form or in capsules is described. The method is based on the coupling reaction of hydrolyzed INM with diazotized *p*-phenylenediamine dihydrochloride (PPDD) in sulphuric acid medium to give a red coloured product having the absorption maximum at 510 nm. The product is stable for 20 h. Beer's law is obeyed in the concentration range of 0.2–10 μg/ml. Results of the proposed method compare favourably with those of the official methods and offer the merits of sensitivity and stability. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed method.

Keywords: Indomethacin; *p*-Phenylenediamine dihydrochloride; Spectrophotometry; Capsules

1. Introduction

Indomethacin (INM), chemically 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-IH-indole-3-acetic acid, is a indole derivative, known for its antipyretic and analgesic action [1]. It is a nonsteroidal anti-inflammatory agent used in the treatment of rheumatoid arthritis and a potent inhibitor of cyclo-oxygenase, reducing prostaglandia synthesis, relieving pain and reducing temperature in febric patients. It is also used topically in the eye to reduce local inflammation. Very few analytical techniques have been reported for the estimation of INM in pure as well as in pharmaceutical dosage forms. The methods include polarography [2], spectrofluorimetry [3], official titrimetric methods [4–6] and spectrophotometric methods [7–11]. The spectrophotometric methods already reported suffer from the disadvantages of heating or extraction, or require non-aqueous media and have higher Beer's law range. A comparison of some spectrophotometric methods for the analysis of INM are presented in Table 1.

The purpose of the present work is to offer a method for the determination of INM in the presence of excipients which provide the advantages of simplicity, sensitivity, stability and rapidity, without the need for heating or extraction,

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Table 1
Comparison of visible spectrophotometric methods for the determination of INM

Sl no.	Reagent	λ_{max} (nm)	B–L range (μg/ml)	ε (l/mol.cm)	Remarks	Reference
1.	Ehrlich reagent	545	20–60	Not reported	Heated on a water bath at 100 °C for 75 min	[7]
2.	Iron (III)	546	25–200	Not reported	Extraction with butanol	[8]
3.	4-Dimethylcinnamaldehyde+H_2SO_4	560	2.5–40	7.16×10^3	Carried out in propanol medium	[9,10]
4.	a. CAT-*m*-aminophenol	490	4–16	5.72×10^3	Stable only for 3 min	[9,10]
	b. Resorcinol–sodium hypochlorite	460	8–24	2.27×10^3	Takes 10 min to develop colour and stable for 5 min	
	c. Phloroglucinol–hypochlorite	460	5.5–24	3.18×10^3	Stable for 35 min	
5.	a. MBTH+iron(III)	660	1–10	1.7×10^4	Takes 15 min to develop colour	[10]
	b. $SbCl_3$ in presence of conc. HCl	600	2–16	8×10^3	Requires heating at 75 °C	
6.	$NaNO_2+H_2SO_4$	395	25–150	Not reported	Measured after 15 min	[11]
7.	PPDD	510	0.2–10	3.27×10^4	Stable for 20 h	Present method

besides having higher sensitivity range than any of the existing spectrophotometric methods.

In continuation of our work on the spectrophotometric determination of organic compounds of biological interest and pharmaceutical importance [12–21], the present paper reports a sensitive method involving azo coupling reaction of *p*-phenylenediamine dihydrochloride (PPDD) and hydrolyzed INM in acid medium. The red coloured product formed is highly stable.

2. Experimental

2.1. Instrument and materials

A JASCO model UVIDEC-610 UV–Vis spectrophotometer with 1.0 cm matched cells was used. INM (Fluka, Germany) and PPDD (Sigma, USA) were used as received. Sodium nitrite (BDH), HCl (AR), H_2SO_4 (AR) and sulphamic acid (BDH) were used. All other chemicals and solvents were used of analytical reagents grade. Deionized water was used to prepare all solutions and in all experiments. Commercial dosage forms were purchased from local resources.

2.2. Solutions

Accurately weighed (25 mg) INM was dissolved in 10 ml of 1 mol/dm^3 sodium hydroxide solution and then diluted to 250 ml in a calibrated flask. A 0.2% solution of PPDD was prepared by dissolving in 5 ml conc. HCl and then made up to 100 ml in a calibrated flask. A 0.5% aqueous solution of sodium nitrite, 3% aqueous solution of sulphamic acid and 1:1 sulphuric acid was used for the experiment.

2.3. Standard procedure

2.0 ml of 0.2% PPDD solution was transferred into each of a series of 25 ml calibrated flasks. 6.0 ml of 1:1 sulphuric acid was added and cooled in an ice bath for 5 min to attain the temperature around 5 °C. Then 4.0 ml of 5% aqueous sodium nitrite was added and left for 5 min with occasional shaking. 3.0 ml of 3% aqueous sulphamic

acid was added and the reaction mixture was allowed to stand for 5 min. Aliquots of standard solution of INM (5–250 μg) were added to the above and the resulting solution was made up to the mark with 1:1 sulphuric acid and mixed thoroughly. The absorbance of resulting red coloured solution was measured at 510 nm against the corresponding reagent blank, which has negligible absorption at this wavelength and the calibration graph was constructed.

2.4. Procedure for the assay of INM in capsules

Twenty capsules were emptied, pulverised and an amount equivalent to 50 mg of INM was taken and dissolved in sodium hydroxide as described above and filtered. An aliquot of this solution was treated as described under standard procedure for the pure sample.

3. Results and discussion

3.1. Spectral characteristics

The proposed method involves coupling reaction of diazotized PPDD with the hydrolyzed product of INM in sulphuric acid medium to give a red coloured product of λ_{max} 510 nm. This wavelength was used for all measurements. The absorption spectrum of the reaction product formed is shown in Fig. 1. The corresponding reagent blank has practically negligible absorbance at this wavelength.

3.2. Optimization of reagents

Various concentration and volume ranges for all the reagents were studied. However, the following are the optimum concentration and volume ranges. For the diazotization coupling reaction, use of sulphuric acid as the reaction medium was found to give more satisfactory results than hydrochloric acid. It was found that 1:1 sulphuric acid in the range of 3–6 ml, 0.5% aqueous sodium nitrite solution in the range of 1–5 ml, 3% aqueous sulphamic acid solution in the range of 1–4 ml were used to achieve maximum colour intensity. Hence, 6 ml of 1:1 sulphuric acid, 4 ml of sodium nitrite solution, 3 ml of sulphamic acid solution and 2 ml of PPDD were selected for diazotization. The excess of nitrite added during diazotization reaction was removed by the addition of sulphamic acid. An excess of sulphamic acid was found to have no effect on colour intensity.

Dilution of the coupled product produced by the interaction of hydrolytic product of INM and diazotized PPDD was studied with different solvents like water, ethanol, acetic acid, hydrochloric acid and sulphuric acid. Results proved that 1:1 sulphuric acid was found to give maximum colour intensity and stability of the resulting final product.

3.3. Quantification

Beer's law was obeyed over the INM concentration range of 0.2–10 μg/ml. Limit of quantification (LOQ) is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of the calibration curve (s) multiplied by a factor 10 ($10\sigma/s$). That means LOQ is approximately 3.3 times the limit of detection (LOD). That means LOD is $3\sigma/s$. Naturally, LOQ slightly crosses the lower limit of the Beer's law range. But, LOD is well below the lower limit of the Beer's law range. The upper limit of the Beer–Lambert range is determined by a plot of absorbance against concentration at the value of λ_{max}. Beyond this limit, the correlation results were really affected. Hence, the measurements were excluded above these limits to keep the relationship linear. The optical characteristics and precision data are given in Table 2.

3.4. Reaction sequence

INM when dissolved in sodium hydroxide undergoes hydrolysis to form 5-methoxy-2-methyl-IH-indole-3-acetic acid [22]. This couples with diazonium cation produced by the diazotization of PPDD in sulphuric acid medium to produce the red coloured product. For the diazotization process, it would be expected that two NH_2 groups in PPDD would be readily diazotized in acidic medium and that each diazonium group would

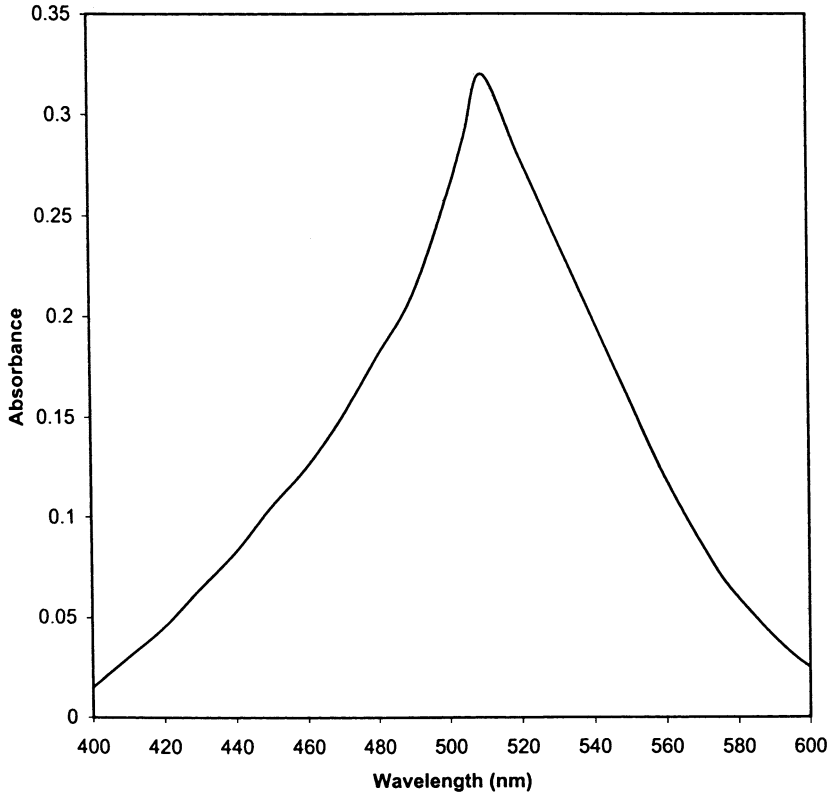


Fig. 1. Absorption spectrum of the reaction product. Initial concentration of INM = 5 μg/ml.

then react with a molecule of INM drug, by electrophilic substitution at the N-atom of indole moiety. Investigations of the continuous molar variation and mole-ratio methods showed that the diazotized PPDD interacts with INM in the ratio of 1:2. When the same reaction was carried out with INM dissolved in ethanol or methanol instead of alkali, it does not produce any colour, clearly indicating that it is the hydrolysis product of INM which is coupling with diazotized PPDD. However, indole does not undergo this coupling reaction, indicating the necessity of the presence of 5-methoxy group in the indole moiety. The reaction mechanism for the formation of the product is shown in Scheme 1.

3.5. Stability

The red coloured azo coupled product was found to be stable for 20 h. Reproducible results were produced in the temperature range of 20–55 °C. An increase in temperature above 55 °C decreased the absorbance readings indicating the decomposition of the product. However, a temperature of 30 °C is recommended for the absorbance measurements.

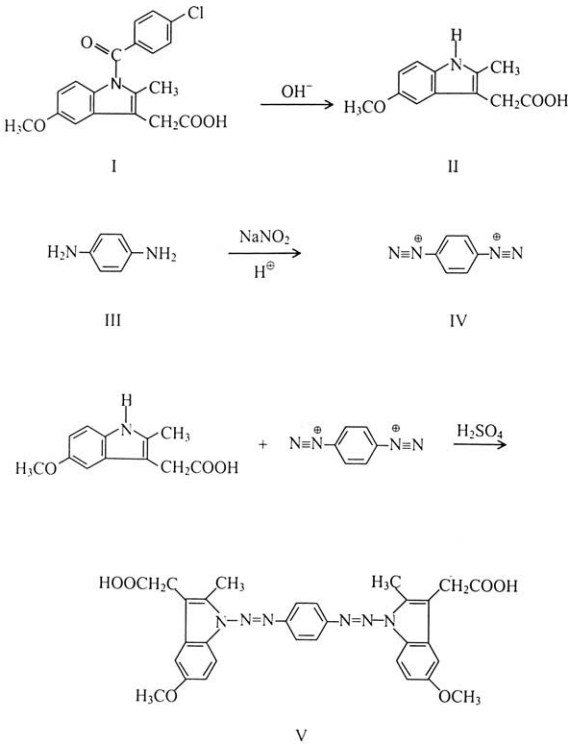
3.6. Interference

The effect of additives associated with the INM in its formulations were investigated using the present method. The extent of interference of common excipients and other concomitant substances were determined by measuring the absorbance of the solutions containing 5 μg/ml of the drug and various amounts of excipients. An error of ±2% in the absorbance readings was considered tolerable. The analysis of interference with the excipients individually was conducted from the initial step, i.e. the hydrolysis of INM. The ratio of

Table 2
Optical characteristics and precision data for the product

Parameters/characteristics	INM-PPDD
Colour	Red
λ_{max} (nm)	510
Stability (h)	20
Beer's law range (μg/ml)	0.2–10
LOD (μg/ml)	0.16
LOQ (μg/ml)	0.54
Molar absorptivity (l/mol.cm)	3.27×10^4
Sandell's sensitivity (μg/cm^2)	0.0109
Optimum photometric range (μg/ml)	0.5–9.1
Regression equation (y)[a]	
Slope (b)	0.0645
Intercept (a)	−0.0026
Correlation coefficient (r)[b]	0.9965
Relative standard deviation (%)[b]	0.24
Range of error (at 95% confidence level)	±0.33
Confidence limit (at 95% probability level)	0.36
Relative error	±0.29

[a] $y = bx + a$, where x is the concentration in μg/ml.
[b] $n = 5$.



Scheme 1.

excipients and the active ingredient (INM) really does not matter. The results are given in Table 3 for various excipients and the percentage recovery of the drug varied from 99.5 to 100.3.

Only amines like aniline, piperidine, morpholine, etc. interfere in the analysis because of diazotization reaction. As these amines do not form part of pharmaceutical preparations, their interference in the analysis is not considered. However, indole was found not to interfere.

3.7. Application

The reproducibility of the proposed method was checked by five replicate determinations of 5 μg/ml of the drug and the maximum relative standard deviation (%) was found to be 0.3 for the pure drug. Since Beer's law range is 0.2–10 μg/ml for making validation studies, the central value, i.e. 5 μg/ml was chosen. Since the analysis of INM was carried out on different days at different assay temperatures, it not only amounts to repeatability, but also reproducibility. Even for 4, 6, 7 μg/ml of INM, studies on repeatability and reproducibility have been carried out and the results were excellent. The applicability of the method for the assay of pharmaceutical preparations was examined. The proposed method was applied to INM capsules of different pharmaceutical laboratories. The results of the assay of available capsules of INM are summarised in Table 4. The results are highly reproducible and the assay of capsules were

Table 3
Determination of INM (5 μg/ml) in the presence of excipients

Excipients added	Amount (mg)	% Recovery ± % RSD[a]
Talc	30	99.8 ± 0.25
Glucose	40	99.6 ± 0.21
Lactose	35	99.8 ± 0.19
Sodium chloride	45	99.5 ± 0.20
Starch	30	99.9 ± 0.24
Sodium alginate	30	100.2 ± 0.22
Magnesium stearate	40	100.1 ± 0.20
Gumacacia	40	99.9 ± 0.18
Carboxymethyl cellulose	35	100.3 ± 0.19

[a] Average of five determinations.

Table 4
Analysis of INM in pharmaceutical preparations

Capsule	Label claim (mg)	Amount of drug found[a] in mg			t-Value	F-value
		Proposed method	U.S.P. method	I.P. method		
Idicin[b]	25	24.97±0.325	24.89±0.285	24.92±0.313	0.53	1.31
Indocap[c]	25	24.87±0.312	24.85±0.325	24.75±0.285	0.50	1.07
Inmecin-R[d]	75	74.90±0.298	74.85±0.302	74.82±0.278	0.54	1.07
Microcid[e]	25	24.89±0.255	24.94±0.259	24.90±0.279	0.63	1.00
Artisid[f]	25	24.75±0.335	24.79±0.325	24.80±0.326	0.97	1.06
Indoflam[g]	25	25.13±0.310	25.20±0.303	25.25±0.284	0.52	1.07

[a] Average of five determinations±standard deviation.
[b] Marketed by: Indian Drugs and Pharmaceuticals Limited.
[c] Marketed by: Jagsonpal Pharma Limited.
[d] Marketed by: Sterfillab.
[e] Marketed by: Micro Laboratories.
[f] Marketed by: Sun Pharma.
[g] Marketed by: Recon.

cross checked by the reported method [9,10] and the official methods [5,6]. The values of student's t-test and variance ratio F-test are also shown in Table 4. The performance of the proposed method was compared statistically in terms of student's t-test and variance ratio F-test. At 95% confidence level, the calculated t-values and F-values do not exceed the theoretical values for the present method. The theoretical t-value was 2.77 (for $n = 5$) and F-value was 6.37 (for $n = 5$). It is found from Table 4 that there is no significant difference between the proposed method and the official method (USP method), indicating that the proposed method is as accurate and precise as the official method.

4. Conclusions

The method is found to be simple, selective and more sensitive compared to the methods reported already and therefore can compete with other methods. Precision and recovery data clearly indicate the reproducibility and accuracy of the method. Analysis of authentic samples of the drug has shown the non-interference of common excipients and additives. Hence this approach could be considered as the best alternative to the existing methods for the determination of INM in pure form as well as in pharmaceutical preparations.

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