

SPECTROPHOTOMETRIC DETERMINATION OF INDOMETHACIN USING PARTIAL LEAST SQUARE METHOD

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Received February 2007, accepted July 2007

Communicated by Dr. Manzur-ul-Haque

Abstract: Indomethacin reacts with ammonium oxalate in concentrated sulphuric acid to give a violet colour after heating for 17 min at 80 °C having maximum absorbance at 578 nm. The reaction is selective for indomethacin with a 0.05 mg/10 mL as visual limit of identification and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's Law from 0.05 mg to 4 mg/10 mL of indomethacin with inter-day precision of 0.98 %. The quantitative assessment of common interferences and tolerable amount of other drugs were also studied.

Keywords: Spectrophotometer, ammonium oxalate, sulphuric acid, analytical pharmacy.

Introduction

Indomethacin (Fig.1) is a derivative of indole. It has analgesic, anti-inflammatory and antipyretic action. The common adverse effects are gastrointestinal ulceration and bleeding, headache, depression, drowsiness, tinnitus, confusion, lightheadedness, insomnia, dizziness, convulsions, coma, hypertension and blood disorders [1].

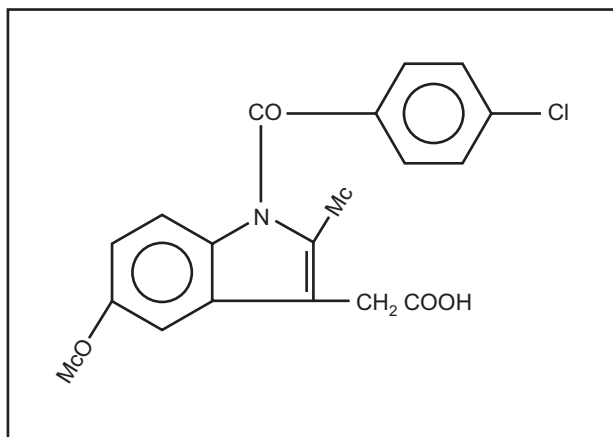


Fig. 1: Structural Formula of Indomethacin.

Many analytical techniques have been employed for the determination of indomethacin. In microscale HPLC [2], HPLC [3,4] and RP-HPLC [5], samples are prepared by plasma precipitation with acetonitrile (containing the methyl ester of indomethacin as the internal standard) [2] and analyzed using the shin-pack MAYI-ODS columns [3]. The UV detection is carried out at 250 nm [4] with higher RSD (relative standard deviation) value [5]. In the TLC [6] and RP-TLC [7] methods, a spectral mapping technique indicates that the eluent additives increase the apparent lipophilicity and specific hydrophobic surface area of non-steroidal anti-inflammatory drugs [1]. Capillary electrophoresis analysis is conducted by using a separation buffer consisting of 20 mM NaHPO₄, 20 mM β-cyclodextrin and 50 mM SDS at pH 9.0, at an applied potential of 20 KV and a temperature of 20 °C [8]. In the derivative spectrophotometry [9,10] and spectrophotometry [11,12], due to similar structure of indomethacin, acemetacin, piroxicam and tenoxicam their absorption spectra

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overlap making their resolution difficult [9] and the determination is carried out in UV region [10]. Only qualitative determination by wavelength coefficient method has been carried out for indomethacin suppositories [11], where the RSD of indomethacin iron (III) complex is 2.13% [12].

In another method, diazotized p-phenylenediamine dihydrochloride is coupled with hydrolyzed indomethacin [13]. Long and tedious methods are involved in chemometrical NIR spectroscopy and X-ray powder diffractometry [14], LC-APCI-MS [15] and LC electrospray [16].

The aim of present study was to develop a new spectrophotometric method for the determination of indomethacin in pure and pharmaceutical forms. The colour reaction has not been reported in the literature. It is shown that the present method is simple, precise, accurate and sensitive. Percentage of tolerable limits of common interferences and other drugs not interfering have also been studied.

Materials and Methods

Apparatus and reagents

Unicam Helios Alpha Spectrophotometer with 1 cm silica cells and a band width of 2.0 nm over a range of 190-1100 nm was used to measure the absorbance. Analytical grade chemicals and double distilled water were used. A 1 mg/mL standard solution (w/v) of indomethacin (Tabros Pharma Karachi, Pakistan) was prepared by dissolving 100 mg in 70 mL of ethyl alcohol (BDH) and the volume was made up to 100 mL with distilled water to give a stock solution. It was diluted further as required. A 10% ammonium oxalate (BDH) solution was prepared in distilled water. Concentrated sulphuric acid

(Merck) 36 N was used to make the medium acidic.

General procedure

To aliquots of indomethacin containing 0.05 mg to 4.0 mg/10 mL was added 1.5 mL of 10 % ammonium oxalate and 1.5 mL of concentrated sulphuric acid. The contents were heated for 17 min in a water bath at 80 °C and the volume was made up to 10 mL with ethyl alcohol (the drug is soluble in ethyl alcohol). The resulting absorbance of the violet colour was measured at 578 nm; the reagent mixture without indomethacin served as blank. The experiment was repeated with different volumes of standard indomethacin solution and a calibration curve was prepared (Fig. 2, %RSD shown in Table 1), and the results are reported as mg/10 mL of the drug. The amount of the drug in samples (pharmaceutical preparations) was calculated from the calibration curve. The colour reaction obeyed Beer's Law from 0.05 to 4.0 mg/10 mL of indomethacin.

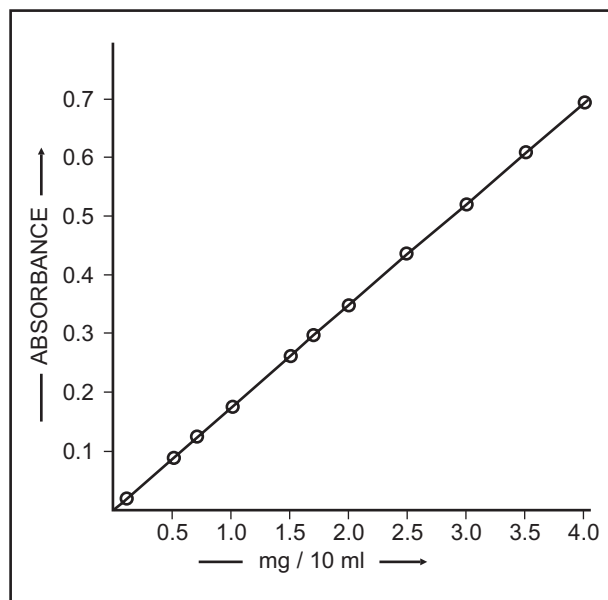


Fig. 2: Calibration Curve of Inomethacin with Ammonium Oxalate.

Table 1
Determination of indomethacin from pure solution.

Indomethacin (mg/10 mL)	Indomethacin Found* (mg/10 mL)	Inter-day precision (RSD %)
0.05	0.049	0.95
0.1	0.102	0.980
0.3	0.0301	0.332
0.50	0.47	0.212
1.00	1.01	0.099
1.5	1.51	0.66
2.0	1.99	0.079
3.0	2.95	0.053
3.5	3.512	0.044
4.0	3.95	0.040

*Every reading is a replicate of five independent measurements.

Procedure for studying the interfering compounds

Interferences were studied at low concentration of indomethacin because higher concentrations made the procedure uneconomical, requiring larger amount of reagents and solvents. Thus, to an aliquot containing 1 mg/mL of indomethacin different amounts of various organic compounds (1 mg/mL) having similar actions and common interferences in the analysis of indomethacin, were added individually until the solution showed the same (± 0.01) absorbance as that of pure indomethacin solution under the experimental conditions described in the general procedure above. The value was calculated as the percentage of organic compounds with respect to the amount of indomethacin. Other compounds (drugs having similar analgesic, antipyretic and anti-inflammatory properties such as acetaminophen, piroxicam, tenoxicam, aspirin, flurbiprofen, propranolol – HCl, mefenamic acid, paracetamol, ibuprofen) which are common interferences and have similar action did not interfere. Moreover, the excipients (lactose, sodium carbonate, sugar) did not interfere.

Procedure for the determination of indomethacin in pharmaceutical preparations

A 1 mg/mL solution of indomethacin was made by dissolving 100 mg of indomethacin (contents of 4 indomethacin capsules, each of 25 mg) in 70 mL ethyl alcohol, filtered and made up to 100 mL with distilled water. The above procedure (see general procedure above) was followed for aliquots containing 0.05 mg – to 4 mg/10 mL of indomethacin and the absorbance was measured at 578 nm. The quantity per capsule was calculated from the standard calibration curve.

Tablets containing 50 mg of indomethacin were powdered, weighed, dissolved in 70 mL ethyl alcohol and filtered. The filtrate was made up to 100 mL with distilled water to get a 1 mg/mL solution of indomethacin. Aliquots containing 0.05 mg to 4.0 mg/mL indomethacin were subjected to the same procedure as described above (general procedure) and the

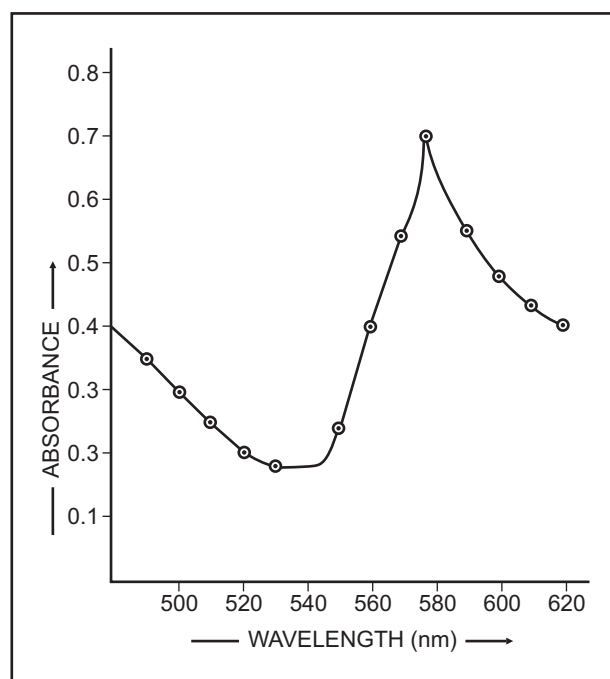


Fig. 3: Absorption Spectrum of Indomethacin with Ammonium Oxalate.

absorbance was measured at 578 nm. The quantity per tablet was calculated from the standard calibration curve.

Results and Discussion

Absorption spectrum of the coloured complex

Indomethacin reacts with ammonium oxalate when heated for 17 min at 80 °C in acidic medium to give a violet complex (when sulphuric acid is used to acidify) the absorbance of which under the optimum conditions lies at 578 nm (Fig. 3). Acids other than sulphuric acid were also studied for the production of colour; little or no colour was obtained with acetic acid, phosphoric acid and nitric acid or hydrochloric acid.

Effect of colour producing reagent

Ammonium oxalate was used as colour producing reagent. It was found that 150 mg/10 mL of 10 % ammonium oxalate gave maximum

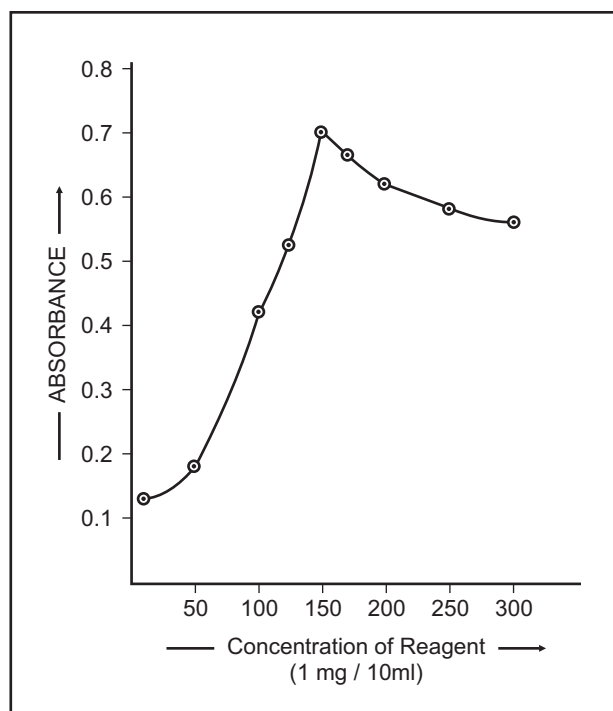


Fig. 4: Effect of Ammonium Oxalate.

colour (Fig. 4) (different amounts of 10% solution of ammonium oxalate were used and the amount corresponding to 150 mg gave maximum colour). This final amount was 1.5 mL of 10% solution corresponding to 150 mg. It is mg/10ml of ammonium oxalate (Fig. 4). At low concentration of indomethacin the colour was stable and above 4.0 mg/mL of indomethacin Beer's law was not obeyed. Colour did not diminish during measurement. Other oxidizing reagents such as sodium nitrate, hydrogen peroxide, sodium nitrite, potassium dichromate, potassium iodate and potassium iodide were tested for the production of colour and it was observed that none of them reacted with indomethacin to give a coloured complex. Only 1.5 mL of concentrated sulphuric acid (36 N) gave maximum colour. When indomethacin was added to ammonium oxalate without the addition of sulphuric acid no colour developed. The probable mechanism is that the electron of nitrogen from indomethacin is taken up by the H^+ ion from H_2SO_4 forming a nitrogen ion [17] which in turn reacts with oxalate ion giving a charge transfer stable violet complex having a maxima at 578 nm.

Effect of temperature & heating time

The effect of temperature (Fig. 5) shows that with the rise of temperature the colour intensity increased and was maximum and stable at 80 °C. The colour did not develop at room temperature. The absorbance of the developed colour intensity remained stable for more than 48 hours. The samples were heated for 17 min in a water bath at different temperatures and the contents of the test tube were immediately diluted to 10 mL with ethyl alcohol before measuring the absorbance. Fig. 6 shows that heating for 17 min at 80 °C gave maximum colour intensity. Above and below this time (17 min) and temperature (80°C) the colour intensity decreased.

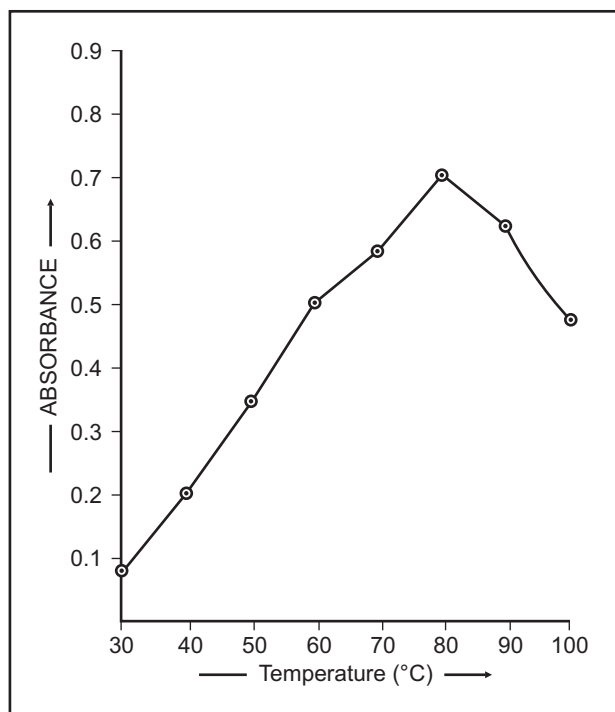


Fig. 5: Effect of Temperature.

Effect of organic solvents

Colour extraction was used to check whether the stability can be increased. Different organic solvents such as acetone, xylene, n-hexane, carbon tetrachloride, isopropyl alcohol were tested for colour extraction and for stability but none was effective. Colour was stable for more than 48 hours without any solvent. Therefore no organic solvent was employed.

Table 2
Optical characteristics, precision and accuracy of the proposed methods.

Parameters	Values
λ_{max} (nm)	578
Beer's Law Limit/Range (mg/10 mL, C)	0.05 mg – 4.0 mg
Molar Absorptivity ($\text{mole}^{-1} \text{cm}^{-1}$)	0.626×10^3
Regression Equation (Y)*	
Slope (b)	0.3247
Intercept (a)	0.0464
Correlation coefficient	0.8435
Inter-day precision (RSD)** (%)	0.98
% Range of error (Confidence limit at 95 % confidence level)	49.96 ± 0.02

* $Y = ab+c$ where C is the concentration of analyte in mg/10 mL and Y is the absorbance unit

** Calculated from five independent measurements

Analytical figures of merit

The results for the determination of indomethacin are shown in Tables 1 and 2, indicating the sensitivity, validity and repeatability of the method. The method was reasonably precise and accurate as the amount taken from identical samples was known and the amount found by the above procedure did not exceed the relative standard deviation (RSV) of 0.98 % which is the replicate of five determinations (Table 1). The calibration graph was linear in the range of 0.05-4.0 mg/10 mL. The apparent molar absorptivity calculated was $0.6 \times 10^3 \text{ mole}^{-1} \text{ cm}^{-1}$ and the regression equation [17] was calculated by the method of least squares from eleven points, each of which was the average of five determinations. The correlation coefficient (r^2) between absorbance and concentration was 0.8435 (the value of r^2 for good correlation ranges from zero to +1, thus $r^2=0.8435$ showed good correlation)

Table 3
Quantitative Assessment of Tolerable Amounts of Other Drugs.

Drugs	Maximum Amount Not Interfering*(%)
Flubiprofen	200
Aspirin	200
Propranolol – HCl	120
Mefenamic acid	100
Paracetamol	190
Ibuprofen	300
Phenytoin sodium	100
Triprolidine HCl	100
Cyclizine – HCl	100
Diclofenac sodium	295
Metamizol sodium	175
Naproxen	220
Piroxicam	210
Buscopan	100
Fluoxetine	150
Chlorpromazine	300
Zyrtec	320
Tenoxicam	240
Atarax	100
Acemetacin	200

*The value is the percentage of the drugs with respect to 1 mg/10 mL of indomethacin that causes ± 0.01 change in absorbance.

Interferences

The quantitative assessment of different organic compounds (w/v) under the experimental conditions is given in Table 3. Various amounts of diverse interfering compounds having similar actions were added to a fixed amount of indomethacin (1 mg/10 mL) and the recommended procedure for the spectrophotometric determination was followed.

Application

The spectrophotometric method for the determination of indomethacin turned out to be reliable, simple, sensitive and reproducible. The results of statistical analysis are in good agreement with that of the UV spectrophotometric official British Pharmacopoeia 1988 Procedure [19]. The colour reaction does not require many reagents or solvents. It is selective for indomethacin. The method can be successfully applied to the microdetermination of indomethacin. The literature indicates that this colour reaction has not been reported previously. The advantage of the present procedure is that [1] it does not require extraction nor many reagents or solvents [2], whereas in the

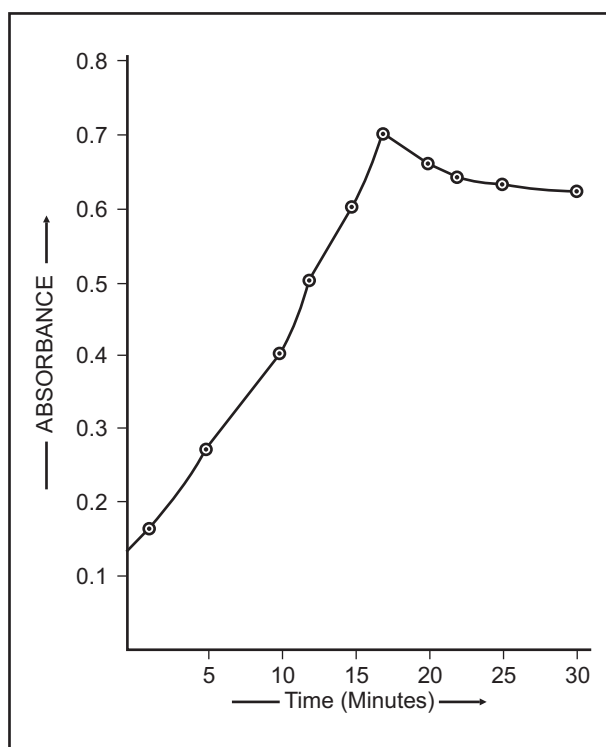


Fig. 6: Effect of Time.

spectrophotometric procedure [9] overlapping of the absorption spectra hinders the resolution of indomethacin, acemetacin, piroxicam and tenoxicam. While in the present procedure, these drugs did not interfere 200 folds in the determination of indomethacin. The method can

Table 4
Determination of indomethacin from available pharmaceutical preparations.

Drug	Trade Name	Pharmaceutical preparation	Amount present manufacturer specification (mg)	Amount found* by the present method (mg)	Amount found by the B.P. uv-method (mg)	Recovery By the present method (%)
Indomethacin	Indomethacin (Tabros – Pharma, Karachi, Pakistan)	Capsules	25	24.98	25	99.92
Indomethacin	Indomethacin (Chong qing Medicines 2 Health Products China)	Tablets	50	50.1	50.1	100.2

*Every reading is an average of five independent measurements. Excipients did not interfere.

also be used to analyze *in vivo* samples; indomethacin is largely converted to inactive metabolites [20].

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