

Novel colorimetric assay of indomethacin using 4-carboxyl-2,6-dinitrobenzene diazonium ion

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A simple, sensitive and direct colorimetric method for the determination of indomethacin either in pure form or in capsules has been developed. The method is based on the diazo coupling reaction between indomethacin and a highly reactive arenediazonium ion, 4-carboxyl-2,6-dinitrobenzene diazonium ion, with the consequent formation of an azo dye. The reaction is fast and gave an orange azo dye in ethyl acetate. The assay was carried out at 470 nm and the azo adduct was stable for three hours. Beer's law is obeyed in the concentration range of 3.3–11 $\mu\text{g mL}^{-1}$ of indomethacin. Optimization studies established an optimum reaction time of 20 minutes at 30 °C and the drug-to-reagent ratio of 1:2 for optimal detector response. The method developed has a low limit of detection of 0.90 $\mu\text{g mL}^{-1}$ and is precise (RSD 2.3%). The new method has been successfully applied to the determination of indomethacin in capsules and the method is of equivalent accuracy as the official (BP) spectrophotometric method. The new method could find application as a simple analytical method for the assay of indomethacin in capsules.

Keywords: indomethacin assay, colorimetry, 4-carboxyl-2,6-dinitrobenzene diazonium, diazo coupling reaction

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Indomethacin (INC), 1-(4-chlorobenzoyl)-5-methoxyl-2-methyl-1H-indole-3-acetic acid, is an indole derivative with prominent anti-inflammatory and analgesic-antipyretic properties similar to those of salicylates (1). The anti-inflammatory effects of indomethacin are evident in patients with rheumatoid and other types of arthritis, including acute gout. There is evidence of both a central and a peripheral action (2). INC is a potent inhibitor of cyclooxygenases, reducing prostaglandin synthesis, relieving pain and reducing fever in febrile patients.

Some analytical methods have been reported for assaying INC in pure as well as in pharmaceutical dosage forms. The methods include: densitometry (3), LC-MS (4), phosphorimetric method (5), polarography (6), spectrophotometric methods using Ehrlich's reagent (7), *m*-aminophenol-chloramine-T (8), 2-nitrophenylhydrazine (9), dimethylcin-

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namaldehyde in H_2SO_4 (10), NaNO_2 in H_2SO_4 (11) and diazotized *p*-phenylenediamine dihydrochloride (12). The official (BP) method (13) for the indomethacin pure form is titration in acetone using NaOH, while the capsules and suppositories are assayed by a UV-VIS spectrophotometric method. Many of the methods already described for the assay of indomethacin suffer from the need of heating, or extensive extraction, or require prior hydrolysis. All of these compromised accuracy and gave a higher Beer's law range.

We recently described the sensitive, simple and accurate determination of mefenamic acid capsules (14) and propranolol tablets (15) by colorimetry. The assay method employed a derivatization procedure (aromatic ring derivatization technique) using the newly developed diazotized 4-amino-3,5-dinitrobenzoic acid (16, 17) and 4-carboxyl-2,6-dinitrobenzene diazonium ion, CDNBD (18), as a chromogenic derivatizing reagent. In this paper, we describe the colorimetric assay of indomethacin *via* its azo adduct with CDNBD.

EXPERIMENTAL

Chemicals and reagents

Indomethacin capsule brands investigated are (A) Vital Vitacid® (Mission Pharmaceuticals Ltd, Mumbai, India), (B) IndoRich (Richy Gold International, Hamburg, Germany), (C) Flam (Formulations Plc Surrey, England), (D) Globa (Globa Pharmaceuticals GMBH, Germany), (E) Indocid (Maxheal Pharmaceutical India), all containing 25 mg of indomethacin per capsule. Indomethacin CRS (control No. 178078, WHO, Sweden), and ethanol, ethyl acetate, glacial acetic acid, concentrated sulfuric acid, sodium nitrite (all are analytical reagents from BDH, UK), pre-coated thin layer chromatographic plates GF₂₅₄, 0.2 mm (Merck, Germany) were used.

Equipment

UV/visible spectrophotometer (Unicam Aurora, Helio Scan software v 1.1, Pye Unicam, UK), digital colorimeter (6051, Spectronic Analytical Instruments, Gathforth, UK), ultrasonic bath (Langford Electronics Ltd, UK), nuclear magnetic resonance spectrometer (AMX 400 Bruker Daltonik, Germany), FTIR (Ati-Mattson Genesis Series FTIR, Mattson Instruments, USA), and mass spectrometer (FINNIGAN LCQMS, Thermo Electron Co., USA) were used.

Solutions and methods

The solution of CDNBD (0.918 mmol L⁻¹), was prepared in concentrated sulfuric acid (18).

Indomethacin stock solution (0.1%, *m/m*) was made by dissolving indomethacin CRS in glacial acetic acid.

Optimization studies. – Two critical response parameters (temperature and reaction time) were optimized using the method of steepest ascent. Aliquot (100 µL) of indomethacin stock solution containing 100 µg (2.8 mmol) of indomethacin was added to the reagent solution (500 µL, 459 mmol) in a test tube and the reaction mixture was mixed in a

Vortex mixer for 10 s, followed by incubation at 30 °C and 50 °C for 5 minutes and 20 minutes, respectively. Similar experiments were performed at 60 °C and 80 °C. Each determination was done in duplicate. The reaction was terminated by addition of ice-cold water (5 mL) to the reaction mixture kept in an ice-bath. Aqueous solution was extracted with ethyl acetate (10 mL) and kept in a vial wrapped with aluminum foil. A blank reagent solution was prepared in a similar way, but replacing the stock solution of indomethacin with glacial acetic acid. The absorption spectrum of the reaction mixture extract was determined against the absorption spectrum of the blank reagent extract, using the UV-Vis spectrophotometer, and the optimal absorption wavelength, 470 nm, was selected for sample determination on a digital colorimeter.

The optimal reaction time was determined by adding an aliquot of indomethacin stock solution (100 µg, 2.8 mmol) to the reagent solution (500 µL, 459 mmol) in seven tubes. Coupling reaction was carried out by incubation at 30 °C for 0, 5, 10, 15, 20, 25 and 30 minutes. Ethyl acetate extracts of the reaction mixture were prepared as usual after each reaction time and the absorbance was measured at 470 nm on the colorimeter. The optimal reaction time was then determined as the time corresponding to the maximal absorption of the samples. All determinations were done in duplicate.

Stoichiometric ratio of drug-reagent. – Solutions of equivalent molarities (0.918 mmol L⁻¹ CDNBD and 0.92 mmol L⁻¹ of indomethacin) were used. Into six different test tubes, 0, 0.25, 0.5, 0.67, 0.75, and 1.0 mL of the reagent solution were added, respectively. Each tube was then made up to 1.0 mL with the drug stock solution. A series of blank determinations were carried out in which the volume of the drug stock solution was replaced with glacial acetic acid. The mixture was mixed in a Vortex mixer for 10 s and kept at 30 °C for 20 minutes; extraction was carried out afterwards as usual. The absorbance was measured at 470 nm against the blank and the absorbance values obtained were plotted against the mole fraction of the reagent solution. Each determination was carried out in duplicate.

Stability of the azo adduct in ethyl acetate. – Standard test solutions containing 6.6 µg mL⁻¹ (0.02 mmol L⁻¹) indomethacin were prepared in six sample vials. Three of the vials were wrapped with aluminum foil, while the other three were left unwrapped. Both sets were kept on the laboratory bench. The absorbance readings of the extracts at 470 nm were taken at 30 min intervals for a period of three hours.

TLC analysis of azo adduct. – Standard test solution containing 6.6 µg mL⁻¹ (0.02 mmol L⁻¹) indomethacin was prepared as usual. The test solution containing the drug/reagent azo adduct was spotted on a precoated thin layer chromatography plate (5 × 10 cm). The reference sample of indomethacin was spotted alongside on the same plate and the blank reagent. The plates were developed separately in three different mobile phases/ethyl acetate/methanol, 9:1 (system I), chloroform/methanol, 8:2 (system II) and methanol/ammonia, 100:1.5 (system III), visualized under UV lamp at 254 nm, and the R_f values were computed.

Assay of CRS and dosage forms. – Preliminary INC identification was carried out by comparing R_f values of INC CRS with each capsule formulation using the TLC system I. The BP NaOH test (13) was also carried out.

The capsule content containing 3.3 mg of indomethacin was weighed, dissolved in glacial acetic acid (6 mL) and mixed in an ultrasonicator for 5 min. The solution was fil-

tered through a cotton wool plug into a 10-mL volumetric flask. The cotton wool plug was rinsed with fresh glacial acetic into the flask and then made up with fresh glacial acetic acid, rinsing the filter aid in the process. An aliquot of the drug stock solution (100 μg , 2.8 mmol) was added to the reagent solution (500 μL , 459 mmol). The mixture was mixed in a Vortex mixer for 10 s and kept at 30 °C for 20 min; afterwards it was extracted with ethyl acetate (5 mL) as described above. Absorbance was measured at 470 nm. Determination of the indomethacin content in capsules was done upon the calibration line. The sample analysis was repeated using the BP procedure (13). Five formulations of indomethacin were used in the assays.

The assay results obtained by two methods were compared using the Student's *t*-test and *F*-ratio test. The 2-tailed probability values less than or equal to 0.05 were considered to be significant. Confidence interval was also determined for the result of the assay using the CDNBD method.

Figures of merit. – Calibration line was established upon 3 replicates. The assay precision was determined by a three-day precision assessment of reference INC recoveries as prescribed by USP (19). Two approaches were adopted for accuracy. The % recoveries of reference INC (3.3, 6.6 and 9.9 $\mu\text{g mL}^{-1}$) were calculated based on a three-day assessment. The second approach involved spiking 3.3 $\mu\text{g mL}^{-1}$ (0.009 mmol L⁻¹) of reference INC into capsule formulations and determining % recoveries. The limit of detection (LOD) was computed as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank (20), while the limit of quantitation was computed as 3xLOD.

Two approaches were adopted for investigating the method selectivity. In the first approach, aliquots of the stock solution (containing 6.6 $\mu\text{g mL}^{-1}$, 0.02 mmol L⁻¹) were spiked into each of lactose, magnesium stearate, starch as well as a mixture of these three excipients, commonly used in capsule formulation. Recoveries of indomethacin were determined using 500 μL (459 mmol) of the reagent and carrying out the procedure as usual.

In the second approach, samples of the five brands and the primary reference substance were kept at 100 °C in the oven for 5 hours. Afterwards, the methanolic extract of the samples and the reference substance were analyzed for possible degradation products. Three separate chromatographic systems were employed, as described earlier.

Synthesis, purification and characterization of azo adduct. – The CDNBD reagent (0.918 mmol L⁻¹) was prepared in concentrated sulfuric acid as reported previously (18). For each batch, 15 mg of indomethacin (reference substance) was weighed and dissolved in 10 mL glacial acetic acid. The solution was poured into the reagent solution and stirred continuously for 3 hours. At the end of the period, the reaction mixture was poured into ice cubes and allowed to separate overnight. The reddish-brown azo dye was recovered by filtration and dried at 70 °C. Several batches were similarly prepared and pooled together.

The crude indomethacin derived dye was purified by column chromatography using silica gel of flash chromatography grade (particle size 0.040–0.063 mm) by gradient elution. The crude product was dissolved in methanol, concentrated on a water-bath and then adsorbed on silica gel, and dried in the oven. Silica gel 60 (10 g) (Merck, Germany) was dispersed in *n*-hexane. The slurry was poured into the column and allowed to settle. Filter paper disc was dropped on the surface. The column was deaerated by positive pressure. The sample of indomethacin adduct adsorbed on silica gel was pour-

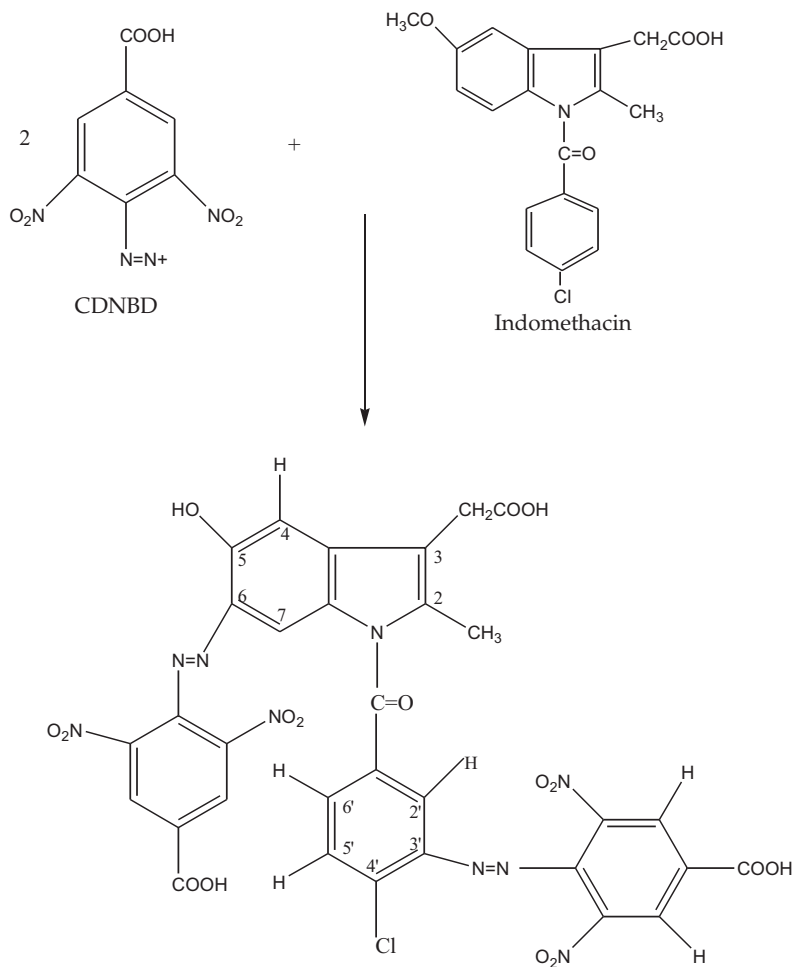
ed into the column containing a head of *n*-hexane. The column was packed under positive pressure. Gradient elution was carried out using hexane, ethyl acetate and methanol (from 100% hexane to 100% ethyl acetate to 100% methanol, with successive 10% addition of the next polar solvent until the final elution with 100% methanol). Column fractions were collected and spotted on a TLC plate developed in EtOAc:MeOH (9:1). The major fraction was the reddish-brown azo dye eluted by hexane/EtOAc (20:80) and this was collected and dried using the rotary evaporator. The dried sample was reconstituted in methanol and allowed to dry in a vacuum oven.

The ^1H NMR of the indomethacin azo adduct (Fig. 3, Table IV) was carried out in deuterated pyridine solution. Field strength of 400.14 MHz with 128 scans was applied. All chemical shift values were referred to the 8.68 signal of pyridine. The IR spectra of indomethacin (Fig. 2) and the azo adduct were recorded in KBr disk using the GENESIS FTIR machine fitted with WINFIRST[®] software for data acquisition and processing. Negative electrospray ionization technique (ESI) mass spectrometry was used for the analysis of the azo adduct.

RESULTS AND DISCUSSION

Diazo coupling reaction between the reagent and INC gave an instant purple colour indicating the formation of an azo dye (Scheme 1). The ethyl acetate extract of the adduct, however, is orange in colour. The absorption spectra of INC in ethyl acetate showed two maxima at 290 and 320 nm, and optimal absorbance when compared with the reagent was found at 470 nm. CDNBD exhibits absorption maxima at 260, 340 and 430 nm. The spectra of INC, CDNBD and the azo adduct are presented in Fig. 1. The absorption spectrum of the azo adduct shows a bathochromic shift with respect to INC while a hyperchromic shift was observed when compared with blank reagent. The peak at 470 nm was selected as the analytical wavelength, because at this wavelength the difference in absorptivity between the blank reagent and the adduct is maximal (21). Optimization studies revealed maximum absorbance of the adduct at 30 °C after 20 minutes reaction time. Incubation at elevated temperatures (higher than 30 °C) led to thermal degradation and lower detector response. The azo adduct formed by the coupling reaction was stable to light in the laboratory environment over a period of 3 h ($6.3 \mu\text{g mL}^{-1}$ was estimated for the analyte through a three hour period). Thin layer chromatographic analyses of the azo adduct in three mobile phase systems are presented in Table II, alongside those of capsules that were kept at elevated temperature for 5 hours.

Indomethacin is a methoxyl indole. Diazo coupling reaction fails between prototype arenediazonium ions and phenol ethers. Exceptionally reactive diazonium ion like 2,4-dinitrobenzenediazonium ion, was however reported to couple with some phenol ethers (anisole and phenetole), although it was not applied as a reagent (22). The instant colour obtained by reacting INC with CDNBD underscores the exceptional reactivity of this diazonium ion. CDNBD is also a dinitrobenzene diazonium analog. The stoichiometric ratio for the reagent and INC was the mole ratio of 2:1. The implication is that combining twice the molar concentration of the reagent with that of INC will produce the highest possible absorbance reading (23).



The spectroscopic characterization of the azo adduct was done using IR, ^1H NMR and MS. IR spectrum of INC (Fig. 2a) and that of the azo adduct (Fig. 2b) showed differences at the following frequencies (cm^{-1}): 1700.62 (s) due to $\text{C}=\text{O}_{\text{str}}$ (CDNBD residue), 1550 (s) due to aromatic $\text{N}=\text{O}_{\text{str}}$ and 3428 (b) due to free phenolic OH_{str} , all present in the adduct and absent in INC. Absence of the band at 1235 (s) due to alkyl-aryl ether suggests the likely cleavage of the linkage on coupling. The proton NMR is presented in Fig. 3. One feature of the proton NMR recorded for the indomethacin adduct is the presence of solvent residues mainly *n*-hexane and ethyl acetate, which produced some complexities in the signals. However, a prominent observation is the complete disappearance of the signal due to methoxyl protons. The assigned signals (δ , ppm) are shown in

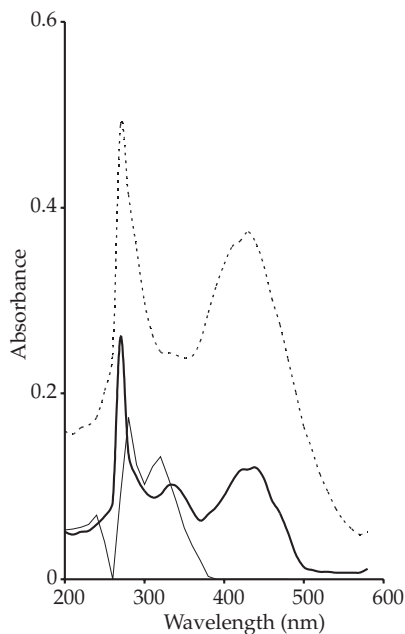


Fig. 1. Absorption spectra of indomethacin (—) ($10 \mu\text{g mL}^{-1}$ INC, 28 mmol L^{-1} INC), indomethacin-CDNBD azo adduct (- - -) (28 mmol L^{-1} INC, 459 mmol L^{-1} CDNBD) and CDNBD (—) in ethyl acetate.

Table IV. The assigned signals seem to reveal that indomethacin may have used up two moles of the reagent, as observed for the stoichiometric ratio assessment. The protons on the CDNBD residue were found at 9.34 as strongly intense singlets with an integral greater than 2, implying the presence of up to 3 or more protons (24). The 6th position on the indomethacin molecule appeared to be most favoured for the coupling with the first molecule of CDNBD since it is the most shielded. This, therefore, transforms the triplet on C-4 and doublet on C-7 to singlets, one of which may likely be the singlet occurring at 9.12 ppm. This can actually be H-7 since it will experience strong deshielding influence of the azo linkage and amide linkage. The second singlet however could not be assigned with such accuracy but could be either of 8.31 or 8.33 ppm. The singlet occurring at 9.23 ppm with integral an of about three protons may be due to H-5' and H-6'. Either of C-3' or C-5' on the indomethacin may have been favoured since both protons are *meta* to a carbonyl group, and *ortho* to the chlorine on C-4'. C-3' will most likely be preferred due to steric considerations. The C-2' proton could also be either of the singlets occurring at 8.31 and 8.33 ppm. The fact that two moles of CDNBD can be substituted in the indomethacin molecule can be attributed to the isolated nature of the rings, which may permit simultaneous electrophilic attack. The mass spectrum of the indomethacin adduct is presented in Fig. 4. Even though no molecular ion corresponding to the proposed adduct in Scheme 1 was obtained, an ion with an m/z of 581.50 is present, which corresponds to a 1:1 reaction product between indomethacin and CDNBD. This therefore implies that the expected molecular ion of 820.30 (corresponding to 1:2 mole ratio) is not stable during ionization and could not be detected. Other fragments in the mass spectrum are m/z (% relative abundance): 549.83 (100%), 445.28 (26.42%), 367.38 (28.30%), 337.23

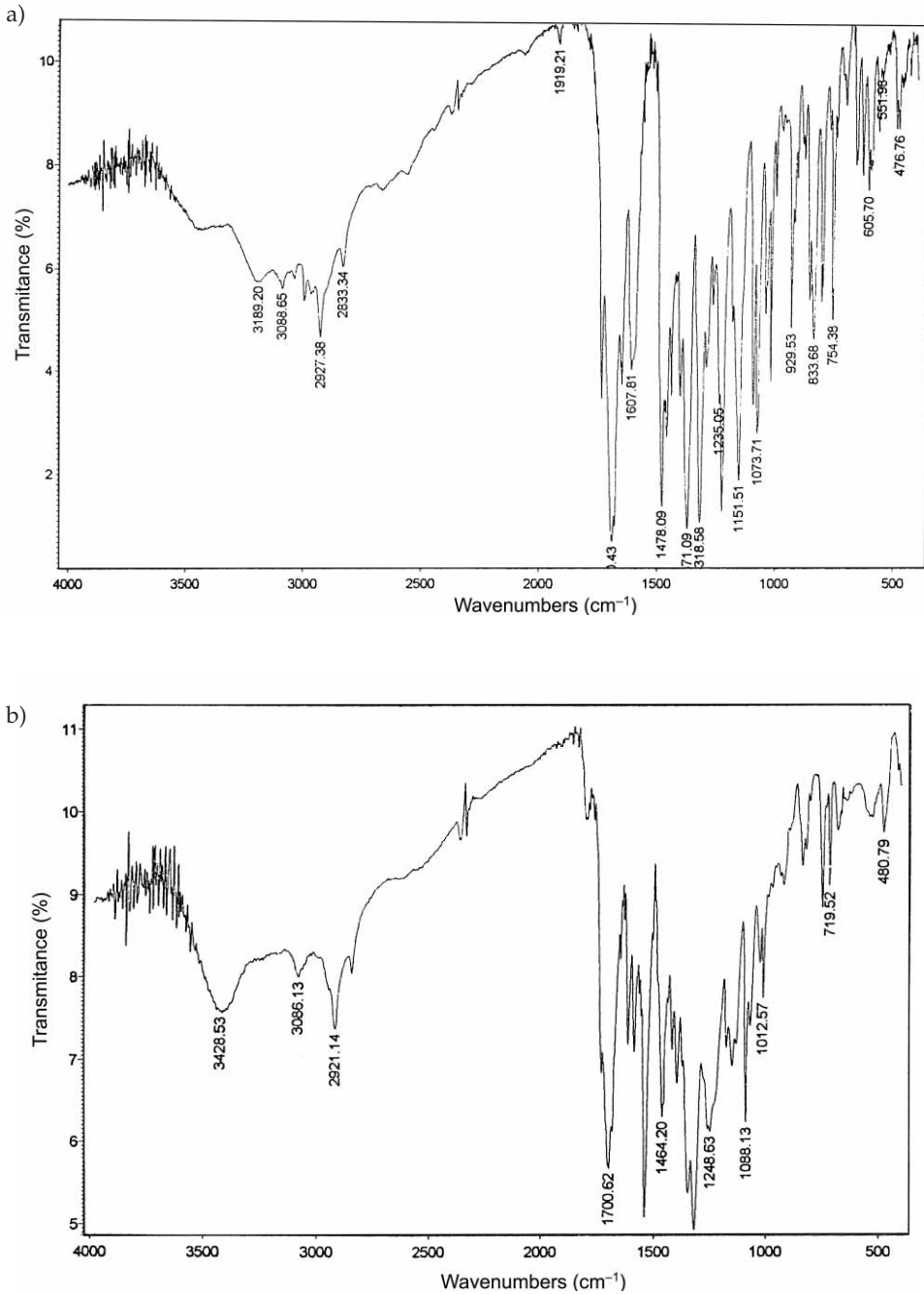


Fig. 2. IR spectra of: a) indomethacin and b) indomethacin-CDNBD azo adduct.

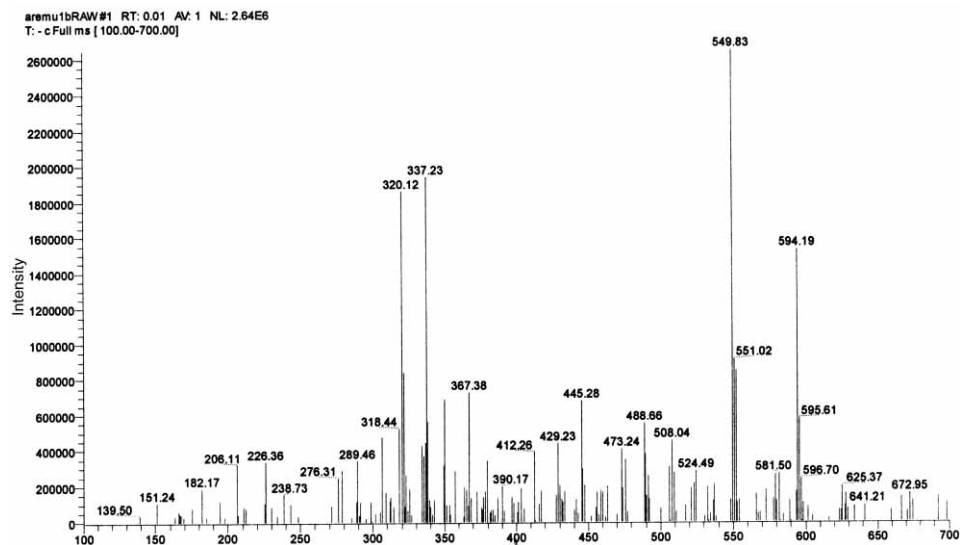


Fig. 4. Mass spectrum of indomethacin-CDNBD azo adduct (negative ESI).

All the brands of indomethacin capsules assayed were found to contain INC using the TLC procedure described above and the BP (13) NaOH test. Comparative analysis of the five brands of indomethacin capsules by the official spectroscopic method (13) and the new colorimetric method is shown in Table III. There is no significant difference between the accuracy of both methods. The active ingredient content ranged between 92.45 and 102.12% of the declared value. The BP requires that indomethacin capsule should contain between 90 and 110% of indomethacin.

While the advantage of simplicity of this new method is self-evident, the method also provides a novel procedure for the assay of indomethacin capsules. The initial purple colour produced when a solution of indomethacin in glacial acetic acid reacts with the reagent and the eventual orange colour in ethyl acetate could serve as additional qualitative tests for the capsules.

Table I. Inter-day accuracy and precision of the indomethacin assay

INC ($\mu\text{g mL}^{-1}$) ^a	Day 1		Day 2		Day 3		Between-day statistics	
	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)	Mean \pm SD	RSD (%)
3.3	96.5 \pm 4.7	4.9	93.8 \pm 0.0	0.0	93.8 \pm 0.0	0.0	94.7 \pm 1.3	1.4
6.6	96.6 \pm 2.0	2.1	98.7 \pm 3.9	3.9	99.7 \pm 4.7	4.7	99.7 \pm 1.3	1.3
9.9	99 \pm 3.1	3.2	95.6 \pm 1.4	1.4	98.3 \pm 4.1	4.1	98.3 \pm 1.8	1.8

^a $n = 9$ at each concentration level. Regression equation: $y = 0.0187x + 0.102$ ($R^2 = 0.9974$). Between-day statistics for all concentrations: $96.86 \pm 2.20\%$ (RSD = 2.3%).

Table II. Thin layer chromatographic analysis of indomethacin capsules and indomethacin/CDNBD reaction mixture on silica gel GF₂₅₄

Mobile phase	R _f						Adduct	CDNBD
	Capsule brand							
	INC	A	B	C	D	E		
EtOAc : MeOH (9:1)	0.81	0.81	0.81	0.81	0.81	0.81	0.22	0.55
CHCl ₃ : MeOH (8:2)	0.82	0.82	0.82	0.82	0.82	0.82	0.55	0.87
MeOH : NH ₃ (100 :1.5) ^a	0.85	0.85	0.85	0.85	0.85	0.85	0.65	0.59

^a Plate was coated with 0.1 mol L⁻¹ methanolic KOH before spotting the samples.

The new method gives comparable reproducibility to the use of *m*-aminophenol-chloramine-T and resorcinol-sodium hypochlorite by Sastry *et al.* (8). However, the procedure of Abdel-Hay *et al.* (9) required the use of dicyclohexylcarbodiimide as a condensation catalyst for hydrazide formation with 2-nitrophenylhydrazine. Similarly, the use of 4-dimethylaminocinnamaldehyde in H₂SO₄ requires propan-2-ol to stabilize the colour (10). Both methods work in a calibration range higher than for CDNBD method reported here, thereby conferring the advantage of sensitivity to the latter. Since other carboxylic acid-containing substances and indole nucleus may react, respectively, in the two methods, the selectivity will obviously be affected. The use of nitrosyl sulphuric acid as described by Chowdary *et al.* (11) has the main disadvantage of cross reaction with any other aromatic nucleus, which may be present since it is not a specific reaction. The concentration range of 25–150 µg mL⁻¹ used is almost 80–120% that of the CDNBD new method. A procedure based on diazo-coupling reaction was, however, recently reported by Nagaraja *et al.* (12). The method, which involves an initial hydrolysis of INC before diazo coupling is effected with *p*-phenylenediamine dihydrochloride in sulphuric acid medium. This hydrolytic step could compromise accuracy, especially when handled by an inexperienced analyst. The utilization of CDNBD does not require heating, extensive extraction or prior hydrolysis.

Table III. Assay of indomethacin capsules by the official method and the new method

Capsule brand ^a	Mean ± SD		Recovery (%) ^d	CI ^e
	BP (reference) ^b	CDNBD ^c		
A	92.45 ± 0.71	95.56 ± 3.38	96.6 ± 1.3	6.5 ± 0.2
B	93.89 ± 0.58	95.62 ± 5.74	96.3 ± 2.9	6.4 ± 0.4
C	95.38 ± 0.95	98.87 ± 4.45	97.9 ± 2.4	6.5 ± 0.2
D	92.77 ± 0.30	94.00 ± 2.22	93.2 ± 1.8	6.2 ± 0.1
E	97.61 ± 0.21	102.12 ± 3.36	102.4 ± 1.6	6.7 ± 0.2

^a *n* = 5. Capsule is expected to contain between 90 and 110% of declared drug amount (25 mg INC) (BP).

^b Official spectrophotometric method.

^c CDNBD – new colorimetric assay.

^d Spiked with 3.3 µg mL⁻¹ (concentration assayed = 9.9 µg mL⁻¹).

^e Confidence interval for 6.6 µg mL⁻¹ analyte at *p* = 0.05.

Table IV. ^1H NMR signals for indomethacin-CDNBD azo adduct

Proton	Chemical shift (δ , ppm)	Integral	Multiplicity/coupling constant (J , Hz)
CH_3	2.18	9	singlet CH_3 's of EtOAc included
CH_2	4.55	approx. 2	quartet, $J = 7.12$
	7.45	approx. 3	triplet ($J = 16$ and 4 ; <i>ortho</i> and <i>meta</i>)
	7.51		triplet ($J = 4$ and 8 ; <i>meta</i> and <i>ortho</i>)
4'	8.31	1	triplet ($J = 4$ and 4 Hz; <i>meta</i> splitting)
2'	8.33	1	triplet ($J = 4$ and 4 Hz; <i>meta</i> splitting)
7	9.12	1	singlet
5', 6'	9.26	3	singlet
CDNBD residual protons	9.34	approx. 3	singlet

The CDNBD method, when compared with the official (BP) spectrophotometric method (13), is sensitive with low limits of detection. The new method does not require strict control of pH unlike the official method that uses methanol/phosphate buffer pH 7.2 as solvent. Low analyte levels of INC can be also determined with sufficient accuracy in the CDNBD method.

CONCLUSIONS

The CDNBD method described in this work is a simple, direct and sensitive colorimetric assay procedure. The new method is of the same accuracy as the official (BP) spectrophotometric assay. The advantages of the new method reported here are its simplicity, more affordable instrumentation (digital colorimeter) and the lower analyte levels compared to the BP method. It may find application in the in-process quality control of indomethacin capsules.

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S A Ž E T A K

**Nova kolorimetrijska metoda određivanja indometacina pomoću
4-karboksil-2,6-dinitrobenzen diazonija**

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Razvijena je jednostavna, osjetljiva i izravna kolorimetrijska metoda za određivanje čistog indometacina i indometacina u kapsulama. Metoda se temelji na reakciji diazo kopulacije između indometacina i vrlo reaktivnog arendiazonijevog iona, diazonijevog iona 4-karboksil-2,6-dinitrobenzena, pri čemu nastaje azo obojeni spoj. Reakcija je brza i daje azo spoj narančaste boje u etil-acetatu, koji je stabilan tri sata. Određivanje se izvodi na 470 nm. Beerov zakon vrijedi u koncentracijskom području od 3,3 do 11 $\mu\text{g mL}^{-1}$. Na temperaturi 30 °C optimalno vrijeme reakcije je 20 minuta, a optimalni omjer analita i reagensa 1:2. Granica detekcije je niska (0,90 $\mu\text{g mL}^{-1}$), a metoda precizna (RSD 2,3%). Metoda je uspješno primijenjena i za određivanje indometacina u kapsulama s jednakom točnošću kao i oficijalna spektrofotometrijska metoda (BP).

Ključne riječi: indometacin, kolorimetrija, 4-karboksil-2,6-dinitrobenzen diazonij, diazo kopulacija

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