# Colorimetric Assay Of Naproxen Tablets by Derivatization Using 4-Carboxyl-2,6-Dinitrobenzene Diazonium Ion

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**A colorimetric assay of naproxen tablets based on chemical derivatization with the chromogenic reagent 4-carboxyl-2,6-dinitrobenzene diazonium ion was developed. The optimal reaction time was found to be 5 min at 30 °C after vortex mixing of the drug/reagent mixture for 10 s. The optimal analytical wavelength was found to be 470 nm and linearity of response was obtained between 1-7 $\mu gml^{-1}$ of naproxen. The diazo coupling reaction occurred in a 2:1 reagent-drug stoichiometric ratio. The method is sensitive, accurate (mean recovery 101.06±3.06 %), reproducible (imprecision 3.03 % CV) and has a low limit of detection (0.95 $\mu gm^{-1}$). It was applied in the assay of naproxen tablets with equivalent accuracy (p>0.05) to the official (BP) UV spectrophotometric method but combined the advantages of speed and more affordable instrumentation. The method can be applied in the in-process quality control of naproxen tablets.**

**Keywords:** Naproxen assay, 4-carboxyl-2,6-dintrobenzene diazonium ion (CDNBD), colorimetry, diazo coupling reaction

## INTRODUCTION

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) which possesses analgesic, anti-inflammatory and antipyretic activity. It is indicated for the relief of symptoms of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [1]. A new derivative of naproxen, nitric oxide releasing naproxen (NO-naproxen) has been shown to markedly reduce the gastropathy associated with this drug [2].

The official method for the assay of the pure drug is titrimetry, using NaOH as the titrant, to a phenolphthalein end point [3]. The tablets can be assayed by a UV spectrophotometric method [4] or spectrofluorimetry [5]. Other pharmaceutical preparations may be analyzed by room temperature phosphorescence [6] and chemiluminescence [7]. A variety of methods have been used for the determination of the drug in biological fluids. Determination in serum and urine by liquid phosphorimetry [8] and in serum or plasma by isotachophoresis [9] and high performance liquid chromatography have been

reported [10].

In previous studies, the authors have described the accurate and precise determination of mefenamic acid [11], propranolol [12], diclofenac [13], nadolol [14] and reserpine [15] in bulk material and dosage forms by colorimetry. The assay methods employed aromatic ring derivatization using the newly developed 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) [16, 17] as the chromogenic derivatizing reagent. The unique combination of modern computing technology and low-cost afforded by modern digital colorimeters as well as the applicability of this reagent to a wide range of drugs [17] enables relatively easy colorimetric assay of drugs. This holds a promise of greater affordability for routine in-process quality control, especially in resource poor settings.

This paper reports a novel colorimetric assay of naproxen tablet formulations using the arenediazonium ion, CDNBD, as a derivatizing reagent through a diazo coupling reaction.

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## EXPERIMENTAL

### Apparatus

A UV/visible spectrophotometer (Unicam Aurora, Helio Scan software v 1.1, Pye Unicam, Cambridge, UK), digital colorimeter (Model 6051, Spectronic Analytical Instruments, Gathforth, Jenway, UK), ultrasonic bath (Langford Electronics Ltd., Langford, UK) and vortex mixer (Griffins & George Ltd, Sussex, UK) were used for all experiments.

### Reagents

Three brands of naproxen tablets were obtained from the market. Naproxen crystals were prepared in the laboratory. The solvents ethanol, ethyl acetate and glacial acetic acid as well as the reagents concentrated sulfuric acid and sodium nitrite sourced from BDH Chemicals (Poole, UK) were all of analytical grade.

### Procedure

A solution of CDNBD in concentrated sulfuric acid was diazotized as previously described [18]. Ten ml of a 1 mg/ml naproxen stock solution was prepared by dissolving naproxen crystals in glacial acetic acid.

### Optimization studies

In order to determine a suitable analytical wavelength for the reaction product, two critical response parameters (temperature and reaction time) were optimized using the method of steepest ascent [19]. A 50 µl aliquot of naproxen stock solution was added to 500 µl of the reagent solution 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 min and 10 min, respectively. Similar experiments were performed at 60 °C and 80 °C. Each determination was carried out in duplicate. The reaction was terminated by adding 5 ml of ice-cold water to the reaction mixture. The aqueous solution was extracted with 10 ml of ethyl acetate and kept in a vial wrapped in aluminum foil. A blank reagent solution was similarly prepared, replacing the drug stock solution 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 spectrophotometer. The optimal absorption wavelength was found to be 470 nm and was selected for sample determination.

The optimal reaction time was determined by adding a 50 µl aliquot of naproxen stock solution to 8 tubes each containing 500 µl of the reagent solution. The coupling reaction was carried out by incubation at 30 °C for 0, 2, 5, 8 and 10 min. Ethyl acetate extracts of the reaction mixture were prepared after each reaction time and the absorbance was measured at 470 nm on the digital colorimeter. An optimal reaction time was then determined as the time corresponding to the maximal absorbance of the samples. All determinations were done in duplicate.

### Stoichiometric ratio of drug-reagent adduct formation

Equimolar solutions ($9.18 \times 10^{-4}$ mol $l^{-1}$) of the reagent and the drug stock solution were prepared using the procedures described heretofore. Into 7 different test tubes, 0, 0.25, 0.33, 0.5, 0.67, 0.75 and 1.0 ml of the reagent solution were added. Each tube was then made up to 1.0 ml with the drug stock solution. A series of blank determinations were carried out by replacing the drug stock solution with glacial acetic acid. The mixtures were subjected to vortex mixing for 10 s and kept at 30 °C for 5 min. The absorbance of the ethyl acetate extracts obtained after the reaction was measured at 470 nm against the blank. 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 5 µg/ml naproxen were prepared in 6 sample vials. Three of the vials were wrapped in aluminium foil, while the others were left unwrapped. Both sets were kept on the laboratory bench. The absorbance of the extracts at 470 nm was read at 30 min intervals for a period of 3 h.

## TLC analysis of azo adducts.

A 5 µg/ml standard test solution of naproxen and the test solution containing the drug/reagent azo adduct were spotted on a precoated thin layer chromatography plate (5×10cm) against a secondary reference sample of naproxen and the blank reagent. The plates were developed separately in three different mobile phase systems, visualized under UV light at 254 nm and the $R_f$ values computed.

## Assay of dosage forms

Twenty naproxen tablets were weighed and powdered. The weight of powder equivalent to 10 mg of naproxen was dissolved in 6 ml glacial acetic acid and mixed in an ultrasonicator for 5 min. The solution was filtered through a cotton wool plug into a 10 ml volumetric flask and made up to volume with fresh glacial acetic acid, rinsing the filter aid in the process. A 50 µl aliquot of the drug stock solution was added to 500 µl of the reagent solution. The mixture was subjected to vortex mixing for 10 s and kept at 30 °C for 5 min. It was extracted with 10 ml of ethyl acetate and the absorbance measured at 470 nm. The content of naproxen in the tablets was determined by interpolation from calibration curves. The assay of the sample was repeated using the BP (1998) specifications. Three naproxen tablet formulations were used in the assays.

The assay results from the two methods were compared using the Student's t-test. A 2-tailed probability value less than or equal to 0.05 (95 % confidence interval) was considered significant.

## Assessment of method selectivity

***Stability of naproxen at elevated temperature:*** The three brands of naproxen tablets were subjected separately to ambient conditions and elevated temperature (100 °C) for 5 h in a simple stability test. The evolution of degradation products was monitored using TLC at 254 nm.

***Recovery from excipients:*** Standard solutions of naproxen stock (50 L) were spiked into the excipients gelatin, starch, magnesium stearate, lactose, talc and a mixture of these substances. The recovery was determined and compared with the recovery from reference samples alone

***Validation of method:*** Calibration lines using standard solutions of 0, 10, 30, 40, 50, 60 and 7 0µg/ml naproxen were prepared using the optimal analytical conditions. Linear regression analysis was used to calculate the slope, intercept and the coefficient of determination ($r^2$) of each calibration line. The assay precision and accuracy were determined as previously described [20]. The limit of detection was computed as previously described as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank [21].

## RESULTS AND DISCUSSION

The diazo coupling reaction between the reagent solution and naproxen gave an instant violet colour that turned red within 5 min. The instant colour formation indicates the formation of an azo dye as shown in figure 1. The absorption spectrum of the adduct shows a bathochromic shift relative to the blank reagent and the underivatized pure drug (figure 2).

Naproxen belongs to the naphthylpropanoic acid class of NSAIDs. Owing to the 6-methoxy substituent, it is also a -naphthol ether. Diazo coupling reaction fails between prototype arenediazonium ions and phenol ethers. However, exceptionally reactive diazonium ions like 2,4-dinitrobenzenediazonium ion have been reported to couple with some phenol ethers (anisole and phenetole) [22]. The instant colour obtained by reacting CDNBD with naproxen underscores the exceptional reactivity of this reagent.

The propanoic acid and the methoxy substituents are both on -positions on the naphthalene ring. Typically, the electrophile (diazonium ion) attacks the -substituted naphthalene rings at the nucleophilic -position (ortho) to the substituent. This is corroborated by the TLC analysis of the reaction mixture that shows the presence of a single product, and the stoichiometric ratio

experiment suggesting a 2:1 reagent-drug adduct. This implies that for optimal azo dye formation, excess reagent is required. This has particularly been found to improve yield in the

synthesis of azo dyes [23]. The structures of the azo adduct formed between naproxen and CDNBD as elucidated by spectroscopic means are depicted in figure 1.
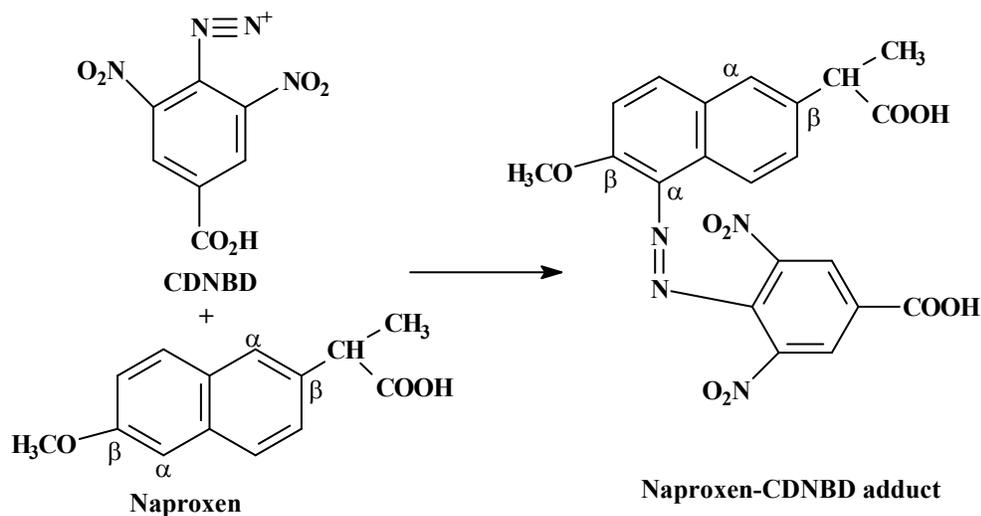


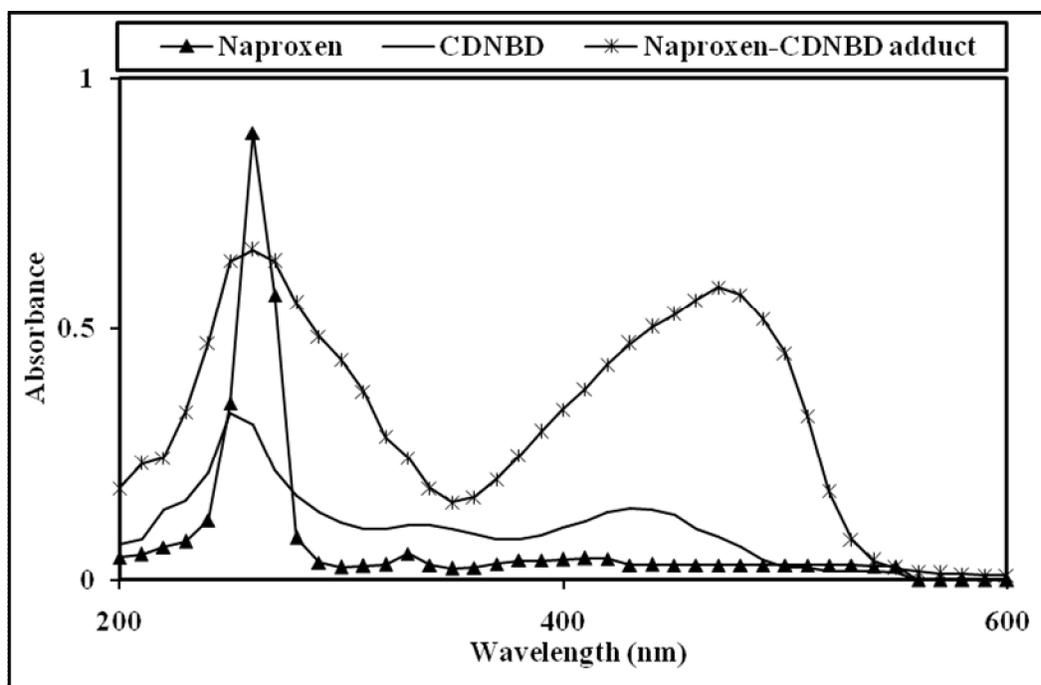**Figure 1: Coupling reaction between CDNBD and naproxen.**



**Figure 2: Overlaid absorption spectra of naproxen, CDNBD and naproxen-CDNBD dduct in ethyl acetate**

## Optimization

The absorption spectra of naproxen showed absorption maxima at 260 nm and 330 nm while the ethyl acetate extract of the azo adduct was orange in colour with an optimal absorbance at 470 nm in the visible region but also shared an absorption maximum with the reagent at 260 nm. The blank reagent exhibited absorption maxima at 260 nm, 340 nm and 430 nm (figure 2). The peak at 470 nm was selected as the analytical wavelength on account of the maximal difference in absorptivity between the blank reagent and the adduct at this wavelength [24]. Absorbance of the reaction mixture (adduct) was highest at 30 $^{o}$C and 5 min reaction time (figure 3). Maximum absorbance of the adduct was obtained at a mole fraction of 0.67 for the reagent solution (figure 4). For two interacting species, at a constant total concentration, the complex (adduct) is at its greatest concentration at a point where the two species are combined in the ratio in which they occur in the complex [25]. The azo adduct was stable to light in the laboratory environment over 3 h.

## Validation studies

The regression equation for the calibration line under the optimal conditions was $y=0.054x+0.069$ with $r^2=0.995$. The 95 % confidence limit for the slope and intercept are $0.054\pm 0.008$ and $0.069\pm 0.032$, respectively. The limit of detection was determined as 0.95 gml$^{-1}$. The overall recovery of extracted samples was $101.06\pm3.06$ % and the coefficient of variation was 3.03 % (table 1). The molar absorptivities of CDNBD-naproxen adduct were found to be $1.51\times10^7$ and $1.34\times10^7$ mol l$^{-1}$cm$^2$ at 260 and 470nm, respectively.
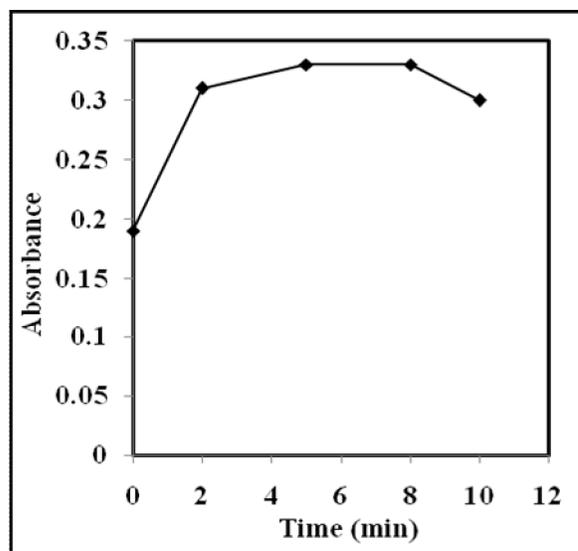


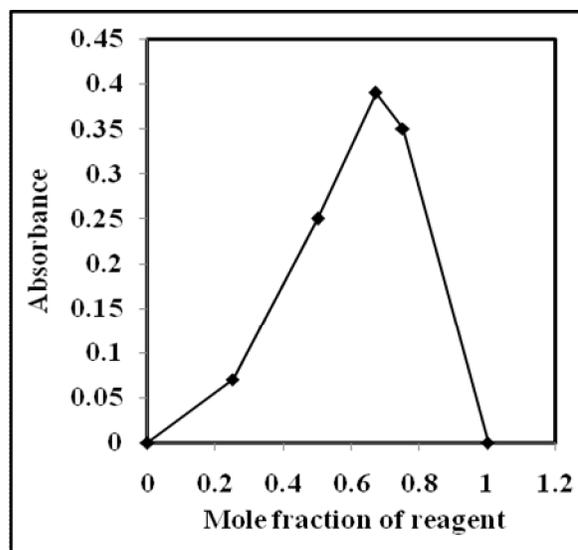**Figure 3: Optimization of reaction time at 30 $^{o}$C**



**Figure 4: Determination of stoichiometric ratio of drug/reagent adduct.**

**Table 1: Three day assessment of accuracy and repeatability of the colorimetric method.**

| Concentration (µg ml $^{-1}$) | Day 1 Mean± S.D (CV%) | Day 2 Mean± S.D (CV%) | Day 3 Mean± S.D (CV%) |
|---|---|---|---|
| 3 | 106.54± 5.19 (4.87) | 99.25± 5.52 (5.56) | 101.0± 2.75 (2.72) |
| 5 | 103.9± 4.93 (4.74) | 98.84± 4.93 (4.98) | 98.72 ±2.03 (2.06) |
| 7 | 104.6± 2.78 (2.66) | 97.64± 2.22 (2.27) | 99.08± 1.49 (1.49) |

Single extraction of the adduct with ethyl acetate was routinely performed because previous work [11] showed that multiple extraction of a CDNBD-derived azo dye gave similar absorbance as single extraction. Complete extraction after a single extraction process was clearly demonstrated by the orange aqueous solution becoming clear. This process also afforded a shorter time of analysis.

### Method selectivity

Thin layer chromatographic analyses of the tablet brands investigated confirmed the absence of any impurity or degradation product in the tablets under ambient conditions and at elevated temperature (100 $^{o}$C) in addition to confirming the presence of a single adduct after the coupling reaction. Furthermore, comparable accuracy was observed when naproxen was spiked into tablet excipients implying a lack of interference from the commonly utilized tablet excipients and conferring a measure of selectivity on the new method described in this work (table 2).

**Table 2: Interference studies with common tablet excipients**

| Excipient | % recovery of naproxen from matrix |
|---|---|
| Lactose | 99.26 ±1.26 |
| Starch | 101.12 ±1.05 |
| Gelatin | 98.52 ±1.86 |
| Magnesium stearate | 101.49 ±1.11 |
| Talc | 101.49 ±1.11 |
| Mixture of excipients | 101.05 ±0.94 |

### Analysis of dosage forms

The method was applied to the assay of naproxen in three brands of naproxen tablets. Results of the assay of the three brands by the official BP (1998) spectrophotometric method and the new colorimetric method is shown in table 3. There was no significant difference ($p > 0.05$) between the official method and the developed method.

**Table 3: Assay of different naproxen tablet brands by the official and the developed CDNBD methods.**

| Tablet Brand | Naproxen content (Mean ± S.D) | |
|---|---|---|
| | BP (1998) | CDNBD |
| I | 98.45±0.91 | 98.96±1.91 |
| II | 101.0± 1.19 | 101.1± 1.85 |
| III | 103.3± 2.06 | 102.42 ± 2.17 |

### CONCLUSION

The colorimetric method developed in this study is comparable in accuracy with the official BP (1998) spectrophotometric method for the assay of naproxen tablets, and shares other typical features of instrumental methods. It has the advantages of speed and the use of more affordable instrumentation. It could therefore find application in the in-process quality control of naproxen tablets.

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