



INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF IBUPROFEN USING CRYSTAL VIOLET DYE IN THE PRESENCE OF N-BROMOSUCCINIMIDE AS AN OXIDIZING AGENT

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Abstract. Ibuprofen in pure material and in pharmaceutical dosage forms was quantified by an indirect spectrophotometric procedure designed for simplicity, sensitivity, and accuracy. The analytical sequence begins with oxidation of ibuprofen by N-bromosuccinimide (NBS). Any NBS remaining after this step reacts with a fixed amount of crystal violet in hydrochloric acid and bleaches the dye. The loss in crystal-violet absorbance was monitored at 630 nm; its magnitude changed directly with the amount of ibuprofen present. Once the optimal reaction parameters were determined, the calibration response was linear over the range of 1.0–20.0 $\mu\text{g mL}^{-1}$ of ibuprofen. The value of molar absorptivity obtained was $1.05 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, which was found to be the most sensitive procedure. The detection limit and the quantification limit are 0.0797 $\mu\text{g mL}^{-1}$ and 0.2658 $\mu\text{g mL}^{-1}$, respectively, to be determined experimentally, and the accuracy and precision are 101.13% and 0.17%, respectively. The procedure was applied to marketed ibuprofen tablets of different manufacturers, satisfactory analytical performance was obtained.

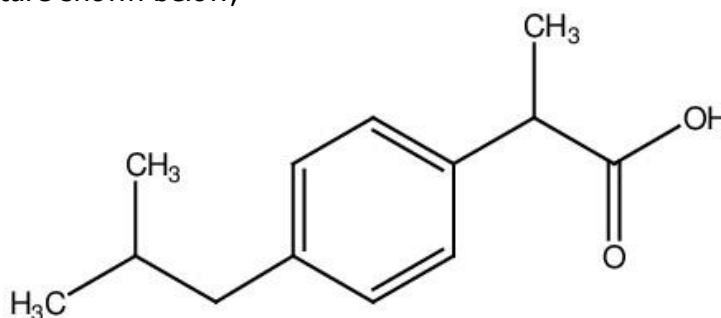
Keywords: Ibuprofen; Crystal Violet; N-Bromosuccinimide; Dye-Bleaching Reaction; Indirect Spectrophotometry

Introduction

Ibuprofen is the compound (RS)-2-(4-isobutylphenyl)propionic acid ($C_{13}H_{18}O_2$; 206.28 g mol⁻¹). Pharmacologically, it is not selective and inhibits the formation of prostaglandins due to pain, inflammatory response and fever by blocking both cyclooxygenase isoenzymes (COX-1 and COX-2). Because of its therapeutic effect and a safe margin, it has become one of the most used and over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs) in the world [1], [2].

Ibuprofen is used as a pain reliever, fever reducer and anti-inflammatory in the treatment of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, musculoskeletal pain, headache, dysmenorrhea, and other inflammatory conditions. The drug has been in use since the 1960s and still has a significant place in the therapeutic armamentarium due to its therapeutic effectiveness and good safety profile [3], [4].

Pharmaceutically, there are some physicochemical properties of ibuprofen that are of great importance. It is a weak carboxylic acid with its solubility being much lower in water than in several organic media affecting its formulation properties and bioavailability [5]. Since ibuprofen is found in many pharmaceutical products, there is a need for reliable quantitative methods which are rapid, sensitive, economical and applicable to dosage-form testing, biological-sample analysis, quality control and pharmaceutical pharmacokinetic studies. It has the chemical structure shown below;



Ibuprofen

There are some assays available for ibuprofen on various analytical platforms. The techniques reported include spectrophotometric methods [6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], chromatographic methods, especially high performance liquid chromatography (HPLC) methods [20], [21], [22], [23], [24], [25], [26], and electrochemical methods [27], [28], [29]. Spectrophotometry remains appealing to routine quality-control laboratories because it is fairly easy to operate, has quick turnaround and has moderate running costs.

In the present work, the indirect spectrophotometric method for quantification of Ibuprofen is established based on its bleaching effect on the crystal violet. Ibuprofen first consumes N-bromosuccinimide in hydrochloric acid; the fraction of oxidant that remains then reacts with a constant amount of crystal violet. The absorbance response is monitored at 630 nm for the resulting absorbance which is concentration dependent for ibuprofen. The process is to be a sensitive, simple, low cost, and relatively ecologically friendly method for the general analysis of drugs.

Experimental Instrumentation

The absorbance spectra and absorbance values were determined using a Shimadzu UV-1900i double-beam UV-Visible spectrophotometer with 1-cm quartz cells. The weight of the reagents was measured using an analytical balance (ADAM). For all times of the year when heat was needed, an Elektro-Mag thermostatically controlled water bath was used.

Reagents, Chemicals, and Solutions

Reagents and chemicals were of analytical grade and were used as received, with no additional purification. All solution preparations employed double-distilled water unless another solvent is stated.

Standard Ibuprofen Solution ($100 \mu\text{g mL}^{-1}$)

The stock solution of ibuprofen was prepared by dissolving 0.0100 g of ibuprofen in 5 mL of ethanol by gentle warming in a water bath to make a $100 \mu\text{g mL}^{-1}$ solution. The sample was quantitatively transferred to a 100 mL volumetric flask and then distilled water was filled to the calibration mark. This solution was kept in an amber bottle and was replaced every week.

Crystal Violet Solution ($250 \mu\text{g mL}^{-1}$)

A $250 \mu\text{g mL}^{-1}$ crystal violet solution was made by dissolving 0.0250 g of the dye in distilled water and adjusting the final volume to 100 mL in a volumetric flask.

N-Bromosuccinimide Solution ($500 \mu\text{g mL}^{-1}$)

N-bromosuccinimide (NBS) solution at $500 \mu\text{g mL}^{-1}$ was prepared from 0.0500 g of pure NBS; the reagent was dissolved in distilled water and diluted to a final volume of 100 mL in a volumetric flask.

Hydrochloric Acid Solution (1.0 M)

8.29 mL of concentrated hydrochloric acid (12.06 M) was pipetted into a 100-mL volumetric flask and then distilled water was added to the mark. 8.29 mL of concentrated hydrochloric acid (12.06 M) was pipetted into a 100-mL volumetric flask and distilled water was added to the mark.

Preparation and Analysis of Tablet Samples

Apifen Tablets (200 mg)

Ten tablets of Apifen containing 200 mg ibuprofen each (Ajanta Pharma Ltd., India) were weighed and broken down into an even fine powder. One tablet was dropped into a beaker, extracted with a small amount of ethanol, diluted and filtered through Whatman paper. The filtrate was quantitatively transferred to a 100 mL volumetric flask and made up to volume with water to create a stock solution of $2000 \mu\text{g mL}^{-1}$ of ibuprofen. This working solution was prepared by further diluting with distilled water to prepare the $100 \mu\text{g mL}^{-1}$ solution.

Piofen Tablets (400 mg)

Ten Piofen tablets (label claim 400 mg ibuprofen per tablet; Pioneer Co., Iraq) were handled in a similar way as Apifen. This preparation resulted in a stock solution with $4000 \mu\text{g mL}^{-1}$ of ibuprofen, the appropriate portion of which was then diluted in distilled water to prepare a working solution containing $100 \mu\text{g mL}^{-1}$ of ibuprofen.

Methods

Preliminary Experiments

Spectral Behavior and Calibration of Crystal Violet

The present initial experiments focused on the possibility of using crystal violet for a simple, sensitive, indirect spectrophotometric determination of ibuprofen.

Subsequent 0.05 - 1.20 mL aliquots of the $250 \mu\text{g mL}^{-1}$ crystal violet solution were added to each of the ten 10 mL volumetric flasks. 1.0 mL of 1.0 M hydrochloric acid was added to each flask and the distilled water was added to the mark.

The absorption peak (λ_{max}) of the spectrum for crystal violet at a concentration of $25 \mu\text{g mL}^{-1}$ was 630 nm as shown in Figure 1.

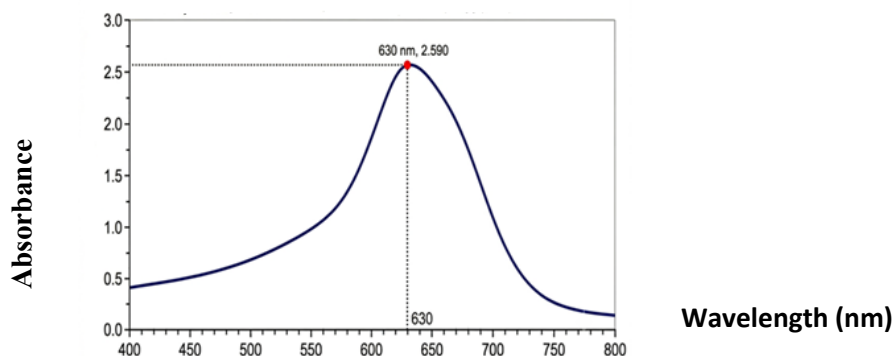


Figure 1. Visible absorption profile of crystal violet at 25 µg/mL.

Figure 2 shows a linear Beer response for crystal violet over 1.25–30.0 µg mL⁻¹. Consequently, subsequent experiments used 1.0 mL of the 250 µg mL⁻¹ dye solution, corresponding to 25 µg mL⁻¹ after dilution to the final volume.

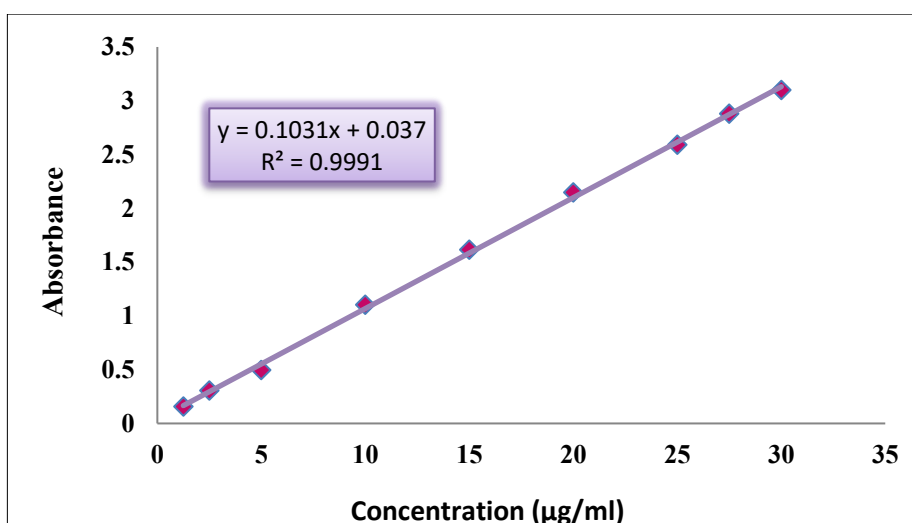


Figure 2. Calibration response of crystal violet.

Results and Discussion

Optimization of Reaction Variables

The variables controlling oxidation and subsequent crystal-violet bleaching were evaluated individually in the NBS/HCl system so that analytical sensitivity and color stability could be maximized.

Unless a particular experiment required otherwise, each optimization test contained 1.0 mL of 100 µg mL⁻¹ ibuprofen in a final volume of 10 mL. Measurements were taken at 630 nm at the prevailing laboratory temperature.

Optimization of the Oxidizing Agent

Selection of Oxidant Type

Candidate oxidants were compared to determine which one produced the most appropriate bleaching response with crystal violet. In each comparison, 1.0 mL of 250 µg mL⁻¹ crystal violet and 1.0 mL of 1.0 M hydrochloric acid were placed in a 10-mL flask, then 1.0 mL of the selected 500 µg mL⁻¹ oxidant was introduced. The mixture was brought to volume with distilled water, mixed, allowed to stand for 10 min, and finally read at 630 nm against its corresponding reagent blank.

N-bromosuccinimide produced the most pronounced bleaching among the oxidants examined, which is reflected by the smallest absorbance value in Figure 3. NBS was therefore retained for the subsequent experimental work.

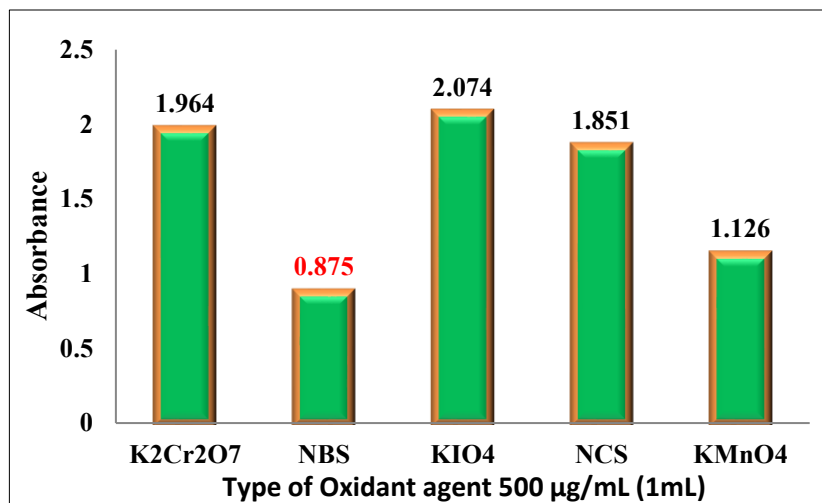


Figure 3. Comparison of oxidants according to their crystal-violet bleaching response.

Optimization of NBS Concentration

The concentration of NBS was varied between 100 and 700 $\mu\text{g mL}^{-1}$ to locate the concentration giving the desired bleaching behavior.

As summarized in Table 1, the optimum bleaching response corresponded to 250 $\mu\text{g mL}^{-1}$ NBS. This concentration was used in the experiments that followed.

Table 1. Crystal-violet bleaching response at different NBS concentrations.

Concentration $\mu\text{g / mL (1 mL) of NBS}$	Absorbance
100	0.481
250	0.422
400	0.619
500	0.875
600	0.911
700	0.874

Optimization of NBS Volume

At a fixed NBS concentration of 250 $\mu\text{g mL}^{-1}$, volumes from 0.25 to 3.00 mL were examined while every other experimental factor was kept unchanged.

The response shown in Figure 4 reached its optimum at 1.5 mL NBS. Accordingly, 1.5 mL was selected as the working oxidant volume for later measurements.

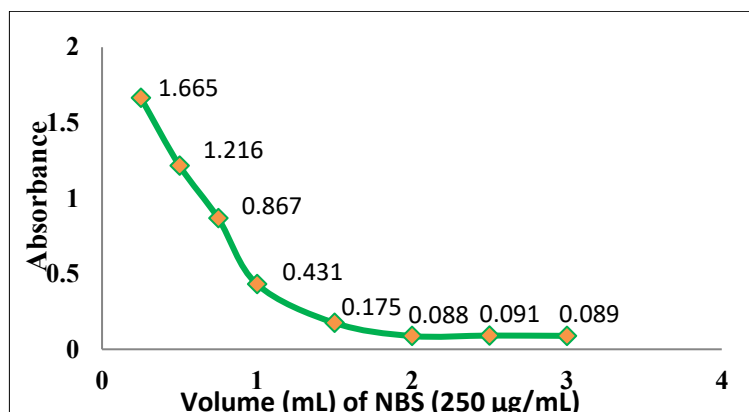


Figure 4. Response obtained when the volume of 250 µg/mL NBS was varied.

Optimization of the Acid Medium

Selection of Acid Type

Several inorganic and organic acids were assessed using 10 µg mL⁻¹ ibuprofen while all remaining reaction conditions were kept fixed.

Hydrochloric acid gave the greatest absorbance together with the most consistent response (Figure 5). On this basis, HCl was adopted as the reaction medium.

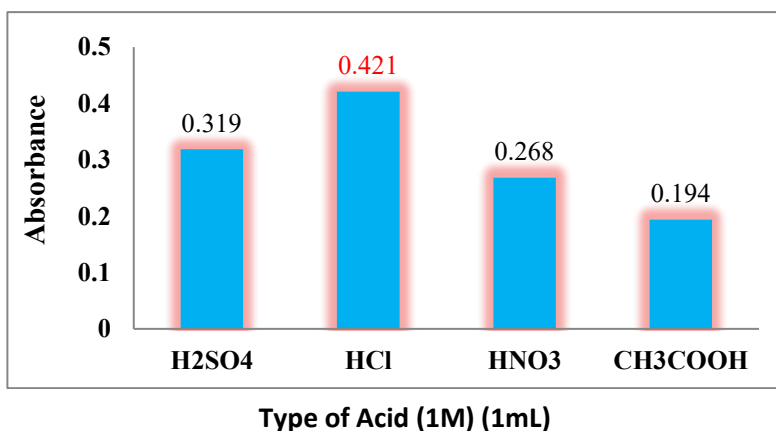


Figure 5. Comparison of acid media for the ibuprofen reaction.

Optimization of Hydrochloric Acid Concentration

HCl concentration was varied from 0.1 to 2.0 M to determine the concentration most favorable for the analytical reaction.

The maximum absorbance in Figure 6 occurs at 0.5 M HCl; this concentration was therefore used in the subsequent tests.

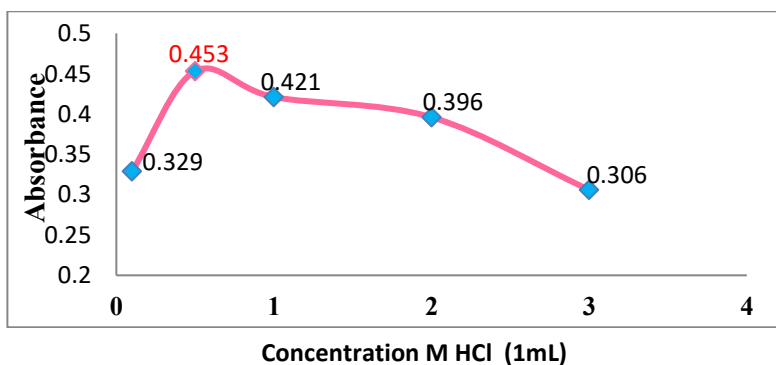


Figure 6. Absorbance response as a function of HCl concentration in the ibuprofen system.

Optimization of Hydrochloric Acid Volume

Using 0.5 M HCl, the acid volume was changed from 0.5 to 3.0 mL to assess its effect on the analytical response.

The curve in Figure 7 shows that 1.0 mL HCl is sufficient to reach the maximum absorbance. Thus, 1.0 mL was fixed as the working acid volume.

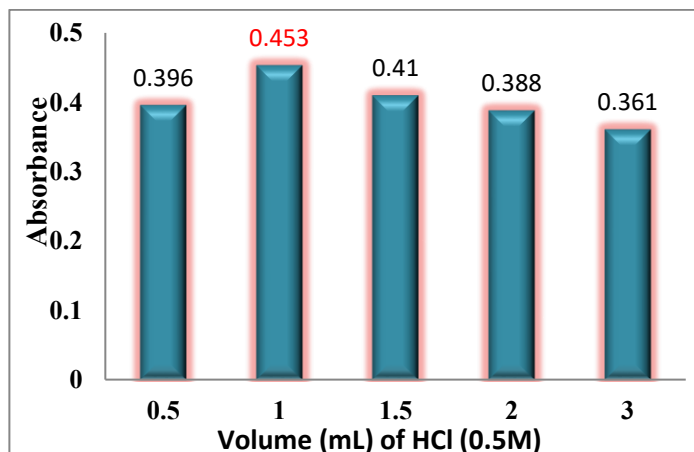


Figure 7. Dependence of the response on the volume of hydrochloric acid.

Optimization of Oxidation Time

Before adding crystal violet, the contact time between ibuprofen and NBS was set at values from 5 to 25 min in order to establish the oxidation period required by the method.

Table 2 records a steady increase in absorbance up to 20 min, at which the highest value was observed. Extending the reaction beyond 20 min did not yield an appreciable further increase.

Table 2. Effect of the pre-dye oxidation period on the analytical response.

Tim (min)	Absorbance
5	0.296
10	0.450
15	0.491
20	0.586
25	0.566

Accordingly, a 20-min oxidation interval was used for the remainder of the experiments.

Selection of the Diluting Solvent

Four diluents—distilled water, ethanol, methanol, and acetonitrile—were evaluated after the other experimental conditions had been optimized.

Distilled water generated the largest absorbance value in Table 3, whereas all three organic solvents produced substantially smaller responses.

Table 3. Analytical response obtained with the investigated diluting solvents.

Solvent	λ_{max}	Absorbance
Ethanol	760	0.026
Methanol	791	0.023
Acetonitrile	782	0.018
D.W	630	0.588

Choosing distilled water also limits the amount of organic solvent required by the assay and thereby improves its environmental profile.

Temperature Effect and Color Stability

The coloured response was developed and evaluated at the temperatures of 20, 40 and 50°C. There was no external heating, and an efficient reaction was performed at room temperature (20°C) as illustrated in Figure 8.

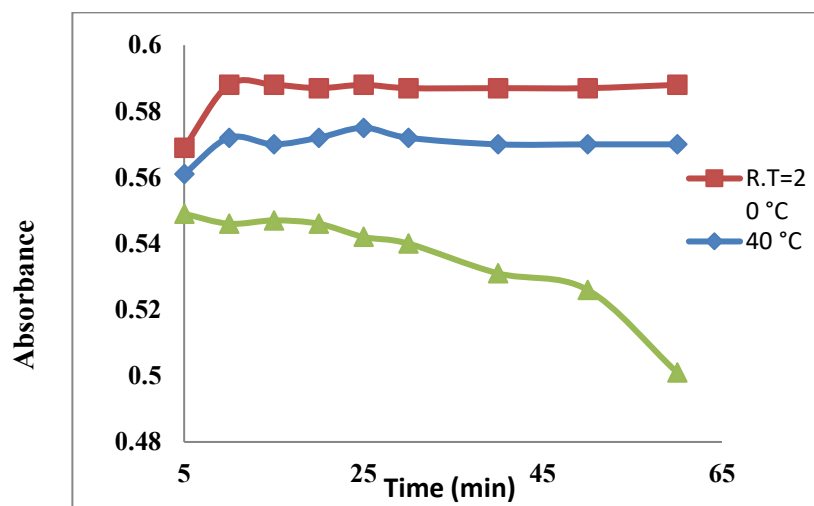


Figure 8. Temperature-dependent stability of the crystal-violet response during ibuprofen analysis.

Addition of crystal violet led to a jump to the highest signal after about 10 min, which remained stable for over 60 min. The absorbance can be measured routinely at this time.

Influence of Reagent-Addition Order

To determine whether reagent sequence affected the signal, six addition orders were evaluated under the optimized reaction conditions. Their absorbance values are listed in Table 4.

Table 4. Absorbance obtained for the six investigated reagent-addition sequences.

Sequence number	Order of addition	Absorbance
I	D + H + O + Cr	0.587
II	D + O + H + Cr	0.612
III	H + O + D + Cr	0.421
IV	O + H + D + Cr	0.336
V	Cr + D + H + O	0.007
VI	Cr + H + O + D	0.302

D = Drug, H = Acid, O = Oxidant, Cr = Crystal Violet

Among the six sequences, sequence II produced the largest absorbance. In this order, ibuprofen undergoes complete oxidation before crystal violet enters the mixture, so the residual NBS can subsequently react with the dye in a consistent manner.

For all subsequent tests, therefore, Sequence II was used.

Visible Absorption Spectra

The working conditions were defined and spectra of the reaction system were taken in the visible wavelength range.

The maximum was observed at 630 nm for crystal violet. The first oxidation with NBS in hydrochloric acid after consumption of the drug caused partial decolourisation of the dye and this gave rise to a measurable decrease at the same wavelength.

Therefore, 630 nm was chosen for all subsequent absorbance to be measured since this gave the highest analytical response sensitivity.

The spectra thus obtained are plotted in Figure 9.

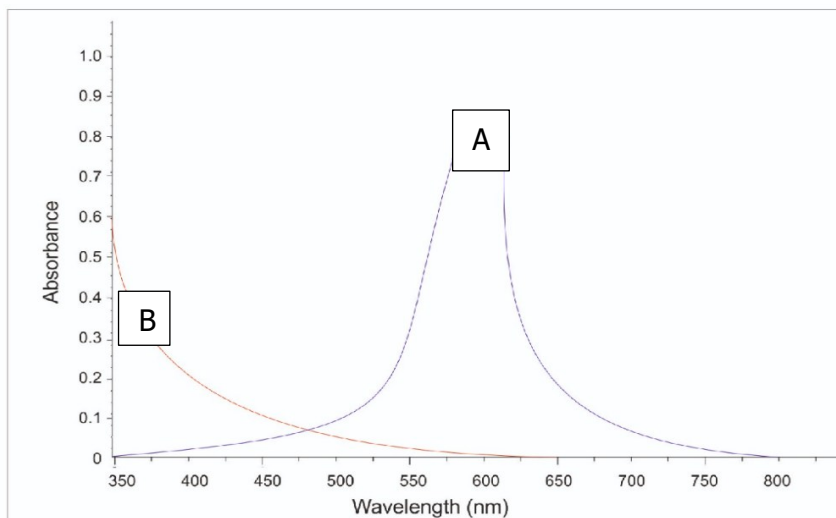


Figure 9. The following visible spectra were produced for the analytical system:

- A. With crystal violet and ibuprofen as reagent blank.
- B. Reagent blank (contained in crystal violet, HCl, NBS) and distilled water as reference.

Ibuprofen Calibration

Ibuprofen standards covering a final concentration range of 1.0–20.0 $\mu\text{g mL}^{-1}$ were prepared in separate 10-mL volumetric flasks.

The following reagents were added to each flask:

- a. 1.5 mL of 250 $\mu\text{g mL}^{-1}$ N-bromosuccinimide solution,
- b. 1.0 mL of 0.5 M hydrochloric acid.

These mixtures were allowed to stand for 20 min with the aim of oxidizing ibuprofen to completion.

After this oxidation period,

- a. mL of 250 $\mu\text{g mL}^{-1}$ crystal violet solution was added,
- and distilled water was used to bring each mixture to its final volume.

The absorbance of each of the prepared solutions was then determined using the reagent blank.

Across 1.0–20.0 $\mu\text{g mL}^{-1}$, the measured absorbance showed a linear dependence on ibuprofen concentration. The derived molar absorptivity was

$$1.05 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1},$$

which confirms the high analytical sensitivity of the assay.

Figure 10 displays the calibration relationship obtained for ibuprofen.

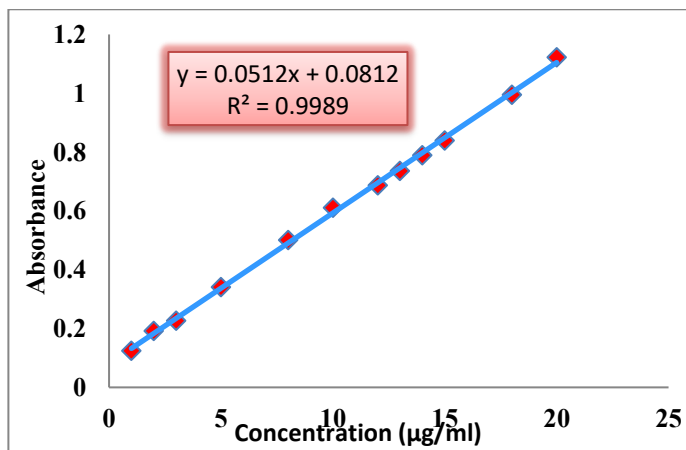


Figure 10. Ibuprofen calibration relationship under the selected conditions.

Proposed Basis of the Reaction

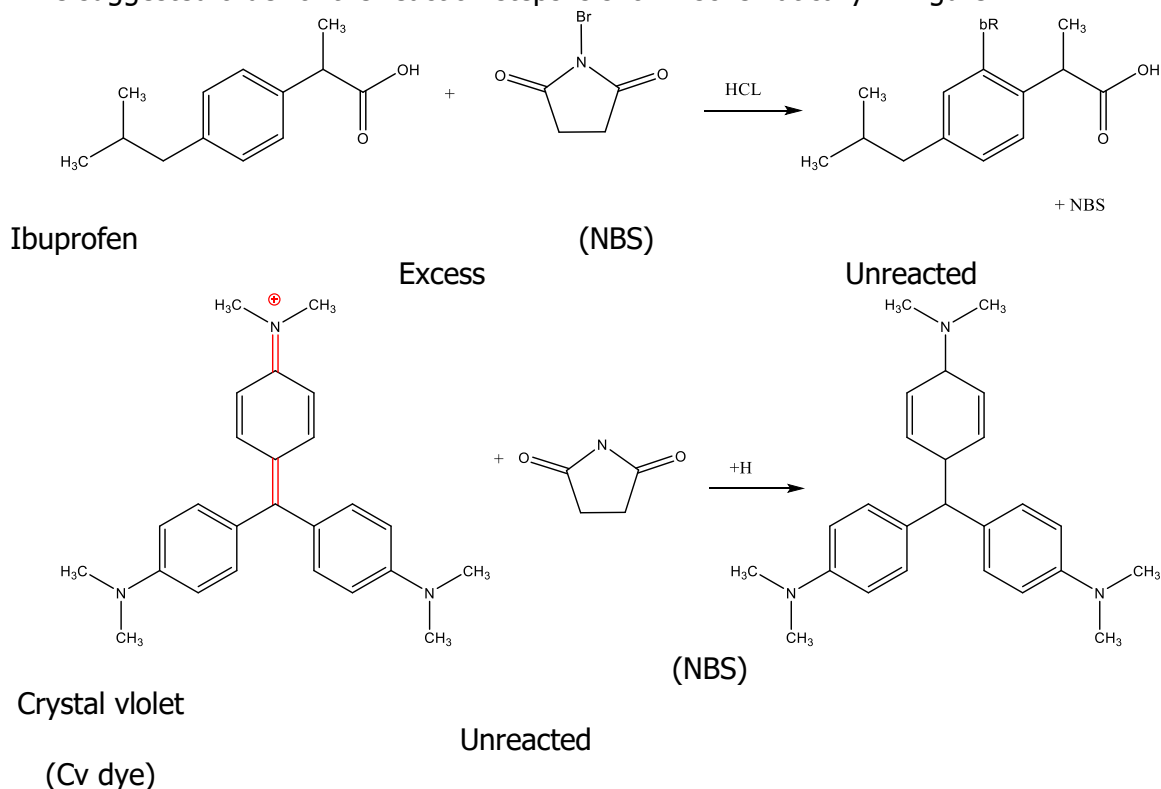
The measurement principle is based on the oxidative bleaching of the crystal violet under controlled conditions.

Ibuprofen is first exposed to N-bromosuccinimide (NBS) in hydrochloric acid, where oxidation of the drug consumes part of the oxidant.

After the drug oxidation is finished, the remaining amount of NBS will oxidize the fixed amount of crystal violet resulting in partial decolourisation of the crystal violet.

In the first oxidation step there is a higher consumption of NBS with increasing concentration of ibuprofen. This will result in a high absorbance of the dye and less NBS will absorb the dye causing less bleaching. So, absorbance of crystal violet is directly proportional to the concentration of ibuprofen.

The suggested order of the reaction steps is shown schematically in Figure 11.



Application to Pharmaceutical Tablets

The real dosage forms method performance was studied by the quantitative determination of Ibuprofen in market purchased tablet.

Standard addition procedure was used to minimize the contribution of formulation excipients in the assay of Apifen 200 mg and Piofen 400 mg tablets.

The recoveries of these products were in the range of 99.1% to 102.0%, revealing good agreement with the declared amounts.

Under the chosen conditions, common ingredients present in the tablets did not cause detectable interference with the ibuprofen determination.

The standard-addition relationships are shown in Figures 11 and 12, whereas the corresponding numerical results are compiled in Table 5.

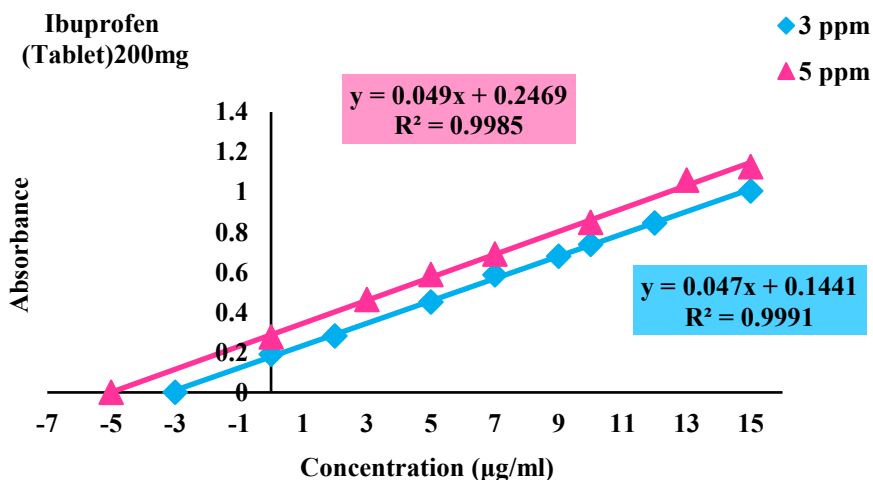


Figure 11. Standard-addition response obtained for the 200 mg ibuprofen tablets.

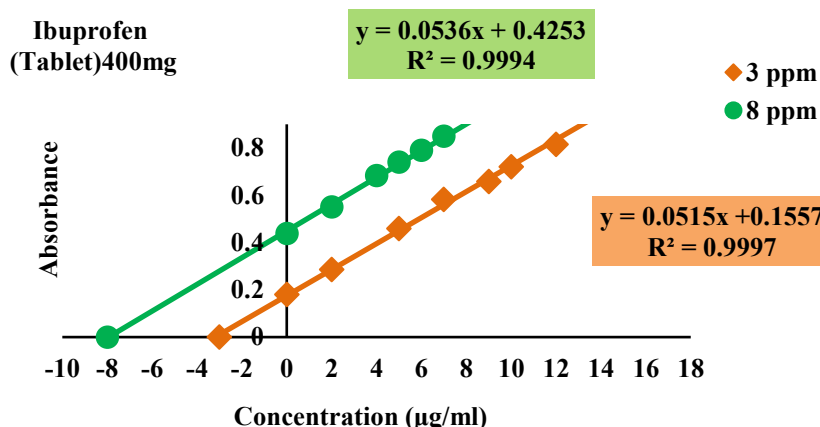


Figure 12. Standard-addition response obtained for the 400 mg ibuprofen tablets.

Table 5. Results for ibuprofen tablets obtained by the standard-addition procedure.

Pharmaceutical preparation	Validated value (mg)	Amount present (µg/ml)	Recovery%	Drug content found (mg)
Apifen Tablets	200	3	102.00	204.00
		5	100.60	201.20
piofen Tablets	400	3	100.66	402.64
		8	99.12	396.48

Conclusion

A sensitive and accurate indirect spectrophotometric assay was established for ibuprofen in both pure material and tablet dosage forms using a simple procedure with a favorable environmental profile.

The assay couples NBS oxidation of ibuprofen in hydrochloric acid with subsequent bleaching of a fixed amount of crystal violet by the oxidant that remains. Recording the response at 630 nm provided the required analytical sensitivity.

The calibration was linear from 1.0 to 20.0 µg mL⁻¹ and gave a molar absorptivity of 1.05×10^4 L mol⁻¹ cm⁻¹.

Commercial ibuprofen tablets gave satisfactory recoveries and the common formulation excipients were non-interfering under the chosen conditions.

The procedure is simple, cheap, sensitive and is performed with water as the final diluent, thus making it a possible alternative method for routine quality-control analysis of ibuprofen in pharmaceutical laboratories.

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