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Validated Spectrophotometric Methods for the Determination of Nabumetone in Tablets Dosage Form Using Three Dinitrobenzene Reagents

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ABSTRACT

Three spectrophotometric methods have been described for the determination of nabumetone (NAB) in its tablets dosage form. The methods are based on the reaction of nabumetone with three dinitrobenzene reagents, namely, m-dinitrobenzene (DNB), 1-chloro-2,4-dinitrobenzene (CDNB) and 1-fluoro-2,4-dinitrobenzene (FDNB) in alkaline medium (alcoholic potassium hydroxide solution). The studied reactions depend on the tendency of these dinitrobenzene reagents to react with the active methylene adjacent to the carbonyl group of the drug. Illustrative proposed pathways showing the reaction of NAB with the three dinitrobenzene reagents were presented. Spectrophotometric measurements were achieved by recording the absorbances at 580, 573 and 574 nm for the reaction with DNB, CDNB and FDNB respectively. Different experimental parameters affecting development and stability of the produced colors were optimized. The three methods were validated with respect to linearity, ranges, precision, accuracy and limits of detection and quantification. Beer's law was obeyed in the concentration ranges of 2-10, 40-240 and 10-50 µg/mL for DNB, CDNB and FDNB methods respectively with correlation coefficient values not less than 0.9994. In addition, detection limits of NAB were 0.27, 8.54 and 2.04 µg/mL for DNB, CDNB and FDNB methods, respectively. The proposed methods were successfully applied for assay of the drug in its tablets dosage form. Recovery data obtained by the proposed methods were favorably compared with those obtained by a reported spectrophotometric method.

Keywords: Nabumetone, m-dinitrobenzene, 1-chloro-2,4-dinitrobenzene, 1-fluoro-2,4-dinitrobenzene, spectrophotometric determination.

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INTRODUCTION

Nabumetone (NAB), chemically known as 4-(6-methoxy-2-naphthyl)-2-butanone, is a non-steroidal anti-inflammatory drug and it is almost devoid of activity itself; the real action is exerted by its active metabolite 6-methoxy-2-naphthylacetic acid, which more strongly inhibits the action of COX-2 than that of COX-1. The drug has proved to be effective in the treatment of rheumatoid arthritis, osteoarthritis and acute soft tissue injuries [1]. NAB is an official drug in BP 2013 [2] and USP 2013 [3] where assay of the pure form as well as different dosage forms has been carried out using liquid chromatography. However, reports found in the literature for the determination of NAB applied several techniques for its estimation in pharmaceuticals and biological fluids. Literature survey revealed HPLC with UV detection [4] and fluorometric detection [5], LC-MS/MS [6], gas chromatography [7] and HPTLC [8]. Some reports could be found in the scientific literature for the spectrophotometric determination of NAB in pharmaceutical preparations. Examples of the published analytical procedures were simple UV-spectrophotometric estimation of NAB in bulk powder and pharmaceutical dosage forms [9]. Very few methods suggested the use of color forming reagents such as Folin-Ciocalteau reagent [10] and the azo-dye forming reagent 4-carboxyl-2,6-dinitrobenzene diazonium [11]. Few phosphorimetric methods were carried out for the evaluation of NAB in pharmaceuticals [12] in addition to some electochemical techniques such as cyclic, differential pulse and square wave voltammetric techniques [13].

Ketones react through their active methylene groups with polynitro aromatic compounds including m-dinitrobenzene (DNB) in alkaline medium to yield a Meisenheimer complex showing an intense violet color. This reaction is traditionally known as Zimmermann reaction [14]. 1-chloro-2,4-dinitrobenzene (CDNB) and its fluoro analogue (FDNB) are useful reagents to introduce a chromophore group to amines, phenols, thiols and other compounds mostly by nucleophilic substitution reactions. In addition, both reagents can also react with ketones through their active methylene groups based on the same principle. These chromogenic reagents have been applied for the spectrophotometric determination of several pharmaceutical compounds [15-17]. The aim of this work is to develop simple, rapid, reliable and validated spectrophotometric methods for the analysis of NAB in bulk powder (drug substance) and in its tablets (drug product). The proposed methods utilized the ketone group and the readily ionizable adjacent active methylene in the structure of NAB for generation of intense colored products which are useful for the spectrophotometric measurement of the drug.

EXPERIMENTAL

Instrumentation

All spectrophotometric measurements were performed using a thermospectronic Helios Alpha (UK) UV-VIS spectrophotometer (UV-1800, SPPC P001, Shimadzu Corporation, Japan) connected to Harvest computer system. The measurements were made in 1-cm quartz cells. The Harvest computer system is connected to Panasonic impact dot matrix printer KX-P3626.

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MATERIALS AND REAGENTS

Authentic sample of nabumetone (NAB) of 99.5 % purity was kindly provided by Marcyrl Pharmaceutical Industries, Cairo, Egypt. DNB (Fluka Chemie AG, Buchs, Switzerland), CDNB (Fluka Chemie AG, Buchs, Switzerland) and FDNB (Hopkin and Williams Co., Essex, UK) were used. Analytical reagent grade of potassium hydroxide (KOH), methanol, ethanol, dimethylformamide (DMF) and dimethylsulphoxide (DMSO) were used.

Preparation of Solutions

1. Preparation of Stock and Working Solutions

A standard stock solution containing 1 mg/mL (1000 µg/mL) of NAB was prepared by dissolving an accurate weight of 25.00 mg of the drug substance in DMF in a 25 mL volumetric flask, then diluted to volume with the same solvent. The solution was further diluted with the same solvent to obtain 0.1 mg/mL (100 µg/mL) working solution in **method I**.

A standard stock solution containing 1 mg/mL (1000 µg/mL) of NAB was prepared by dissolving an accurate weight of 25.00 mg of the drug substance in DMSO in a 25 mL volumetric flask, then diluted to volume with the same solvent for **methods II and III**. The prepared stock solutions were stored refrigerated at 4 °C.

2. Preparation of Sample Solution (Nabuxan® Tablets)

The pharmaceutical preparation assayed in this study is Nabuxan® tablets (UNI PHARMA, El Obour city, Cairo, Egypt, Batch No. 6538) labeled to contain 500 mg of NAB per tablet. Ten tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 50.00 mg NAB was extracted into 20 mL DMF with the aid of shaking for 15 min then filtered through Whatman filter paper No.1 into a 50 mL volumetric flask. The residue was washed with 2 × 10 mL portions of DMF and washings were added to the filtrate, then the solution was diluted to volume with DMF to reach a final concentration 1000 µg/mL for NAB. The solution was further diluted with the same solvent to obtain 0.1 mg/mL (100µg/mL) working solution. (working sample solution for the reaction with DNB)

Similarly, an accurately weighed portion of the powder equivalent to 50.00 mg NAB was extracted into 20 mL DMSO with the aid of shaking for 30 min then filtered through Whatman filter paper No.1 into a 50 mL volumetric flask. The residue was washed with 2 × 10 mL portions of DMSO and washings were added to the filtrate. Finally, the solution was diluted to volume with the same solvent to reach a concentration of 1000 µg/mL NAB (stock sample solution for the reaction with CDNB and FDNB).

3. Preparation of Reagents Solutions

DNB solution (10 mg/mL) and CDNB solution (10 mg/mL) were prepared by dissolving 250 mg DNB and CDNB separately in 25 mL methanol for methods I and II respectively. Fresh FDNB 1% solution was prepared for method III by diluting 0.1 mL FDNB in 10 mL DMSO. Ethanolic KOH (0.50 M) was freshly prepared by dissolving 0.70 g KOH in 25 mL ethanol.

General Procedure

1. Construction of Calibration Graphs

Method I (Reaction with DNB)

Accurate volumes (0.20-1.00 mL) of NAB working solution were transferred into a set of 10 mL volumetric flasks (to give the final concentrations within the range 2-10 µg/mL). A volume of 0.40 mL of DNB was added followed by 0.30 mL of 0.50 M ethanolic KOH solution. Dilution was made to volume with DMF and the absorbance of the resulting purple colored solutions was measured at λ_{max} 580 nm against reagent blank. The obtained absorbance readings were plotted against the corresponding concentrations to construct the calibration graph.

Method II (Reaction with CDNB)

Accurate volumes (0.40-2.40 mL) of standard NAB stock solution prepared in DMSO were transferred into a set of 10 mL volumetric flasks (to give the final concentrations within the range 40-240 µg/mL). A volume of 0.50 mL of CDNB was added followed by 0.70 mL of 0.50 M ethanolic KOH solution. Dilution was made to volume with DMSO and the absorbance of the resulting orange-red colored solutions was measured at λ_{max} 573 nm against reagent blank. The obtained absorbance readings were plotted against the corresponding concentrations to construct the calibration graph.

Method III (Reaction with FDNB)

Accurate volumes (0.10-0.50 mL) of standard NAB stock solution prepared in DMSO were transferred into a set of 10 mL volumetric flasks (to give the final concentrations within the range 10-50 µg/mL). A volume of 1.0 mL of FDNB was added followed by 0.50 mL of 0.50 M ethanolic KOH solution. Dilution was made to volume with DMSO and the absorbance of the resulting orange-red colored solutions was measured at λ_{max} 574 nm against reagent blank. The obtained absorbance readings were plotted against the corresponding concentrations to construct the calibration graph.

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2. Procedure for Pharmaceutical Tablets

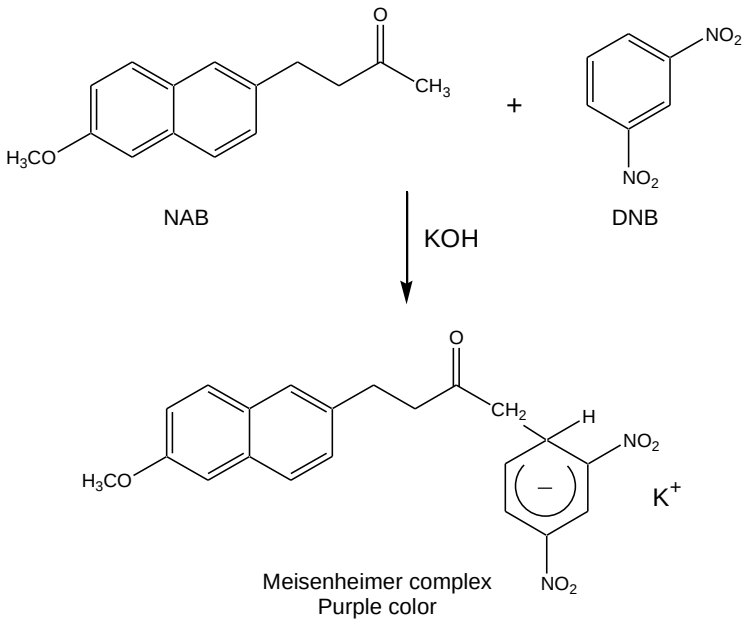
Accurate volumes of the working and stock sample solutions giving final concentrations within the specified linearity ranges were transferred into sets of 10 mL volumetric flasks and the general procedure was then followed. Recovery values were calculated from similarly treated external standard solutions.

For standard addition assay, sample solutions were spiked with aliquots of the appropriate standard NAB solution to obtain total concentrations within the previously specified ranges then were treated as under the general procedure. Recovered concentrations were calculated by comparing the analyte response with the increment response attained after addition of the standard.

RESULTS AND DISCUSSION

Spectral Characteristics and Pathways of the Reactions

Three spectrophotometric methods were developed to provide simple, rapid and reliable quality control analysis of NAB in its pure form as well as in its pharmaceutical preparation. Containing an active α–methylene adjacent to a carbonyl group, NAB reacts with DNB (method I), CDNB (method II) and FDNB (method III) in alkaline medium to form purple colored product measured at 580 nm for method I and orange-red products measured at 573 and 574 nm for methods II and III respectively. Figures 1-3 represent the absorption spectra of the reaction products of NAB with DNB, CDNB and FDNB in alkaline medium. The reaction of NAB with DNB (method I) in alkaline medium (alcoholic KOH) involves the formation of a colored Meisenheimer complex (anionic sigma complex) as illustrated in the following scheme.



Scheme 1: Proposed pathway for the reaction of NAB and DNB.

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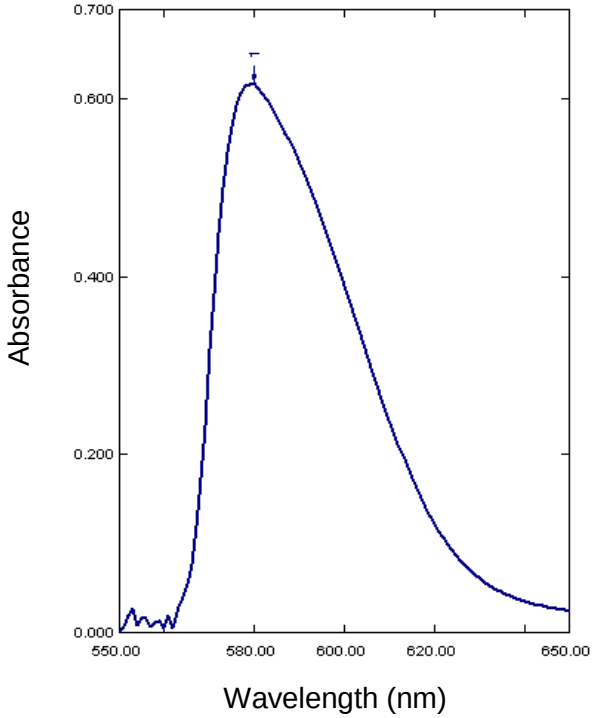

Figure 1: Absorption spectrum of the reaction product of 6 µg/mL NAB with DNB in DMF.

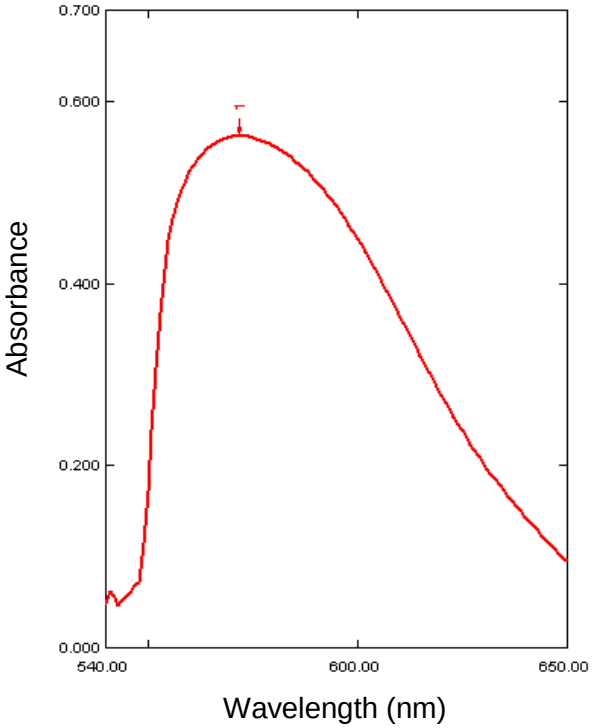

Figure 2: Absorption spectrum of the reaction product of 120 µg/mL NAB with CDNB in DMSO.

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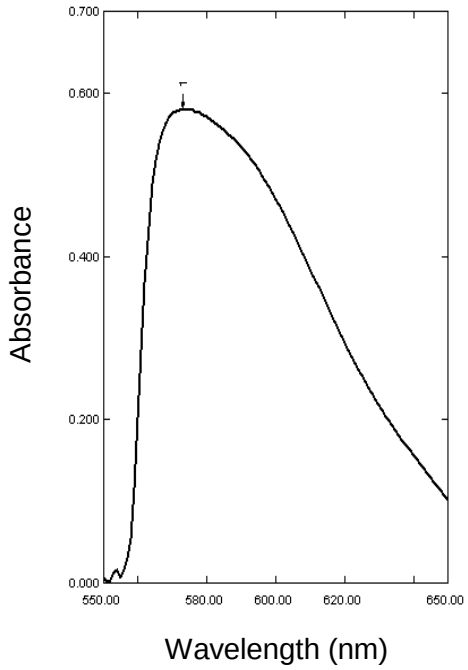
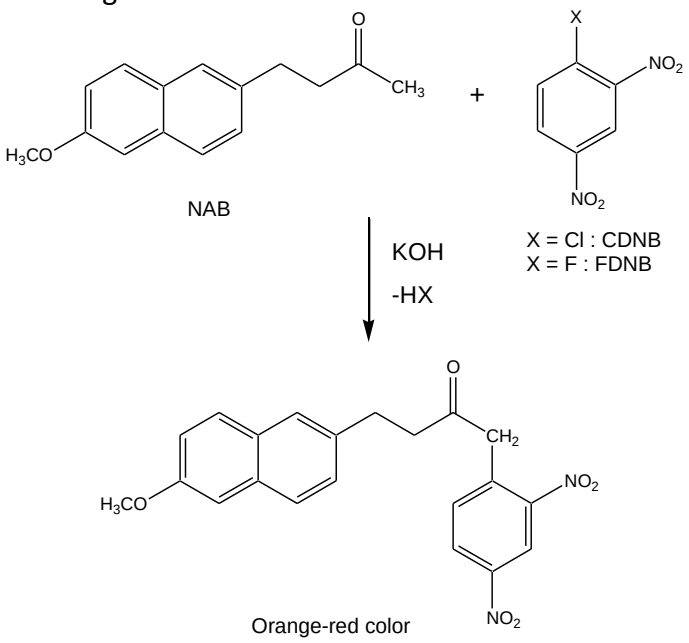


Figure 3: Absorption spectrum of the reaction product of 30 µg/mL NAB with FDNB in DMSO.

On the other hand, the reactions of NAB with CDNB (method II) and FDNB (method III) in alkaline medium most probably proceed through nucleophilic substitution reactions. It has been reported that diethyl malonate which has an active methylene group reacts with FDNB in DMSO to yield a red product. The reaction mechanism was explained as a nucleophilic substitution reaction [18]. Similarly, one molecule of the reagent (CDNB or FDNB) condenses with one molecule of NAB through its nucleophilic center (active methylene group adjacent to the carbonyl moiety) with the liberation of HX. A proposed pathway for this reaction is demonstrated in the following scheme.



Scheme 2: Proposed pathway for the reaction of NAB and CDNB or FDNB

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Optimization of Spectrophotometric Conditions

Different experimental factors were optimized in order to achieve maximum absorbance and reproducible measurements. Such factors were changed individually while keeping the others constant. These factors include: reagent concentration, volume of 0.50 M KOH, diluting solvent and reaction time. The optimized experimental parameters for the three reactions are presented in Table 1.

Table 1: Optimum experimental conditions for the proposed spectrophotometric methods

Reagent	Volume of reagent (mL)	Volume of 0.5 M KOH (mL)	Diluting Solvent	Reaction Time	λ_{max} (nm)
DNB	0.4	0.3	DMF	Zero time	580
CDNB	0.5	0.7	DMSO	Zero time	573
FDNB	1.0	0.5	DMSO	Zero time	574

1- Effect of Reagent Concentration

The influence of reagent concentration was studied in terms of volume of stock reagent added (10 mg/mL DNB, 10 mg/mL CDNB and 1% v/v FDNB). It was found that 0.40 mL of DNB was sufficient to achieve the highest color intensity, after which no more increase in absorbance was recorded. In case of CDNB, maximum color intensity was obtained upon using 0.50 mL reagent. Finally, increasing the volume of FDNB produced obvious increase in absorbance of the color product up to 1.00 mL reagent above which a decrease in absorbance was recorded. Therefore, 1.00 mL FDNB was selected. The effect of concentration of the three reagents on absorbance of the formed color products is illustrated in Figure 4.

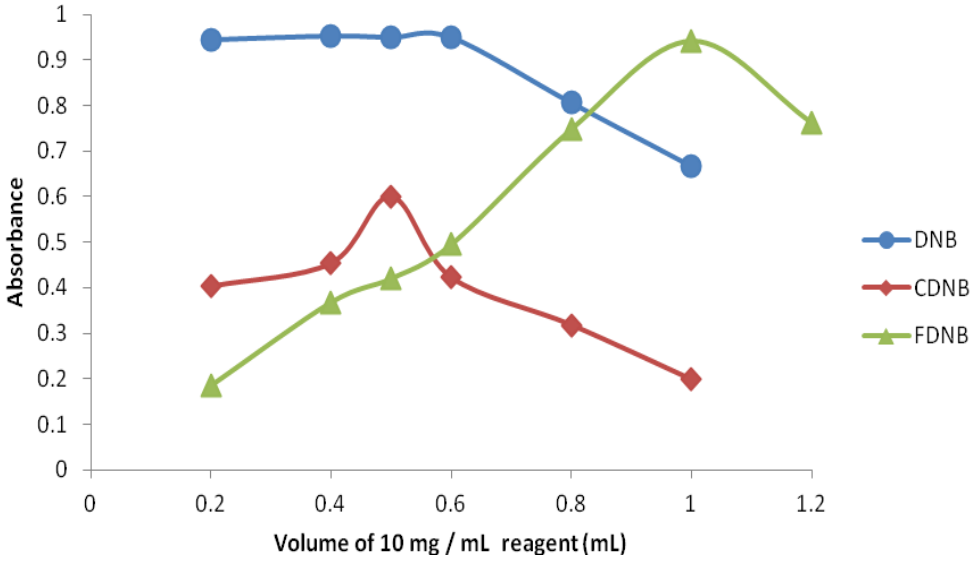

Figure 4: Effect of volume of reagents on the absorbance intensity of the reaction products of 10 µg/mL NAB with 10 mg/mL DNB, 120 µg/mL NAB with 10 mg/mL CDNB and 50 µg/mL NAB with 1% v/v FDNB.

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2- Effect of 0.50 M Potassium Hydroxide Solution Concentration

Ethanolic potassium hydroxide (0.50 M) solution was used as a source of basic medium for the three proposed reactions. Reaction mixtures were prepared using different volumes of KOH solution then absorbances of the color products were recorded. In all three reactions, increasing volume of KOH solution produced increases in color intensity until a maximum was reached above which a decline in absorbance was observed. This maximum was reached at 0.30, 0.70 and 0.50 mL KOH for method I (DNB), method II (CDNB) and method III (FDNB) respectively. Figure 5 demonstrates the effect of KOH on the proposed colorimetric methods.

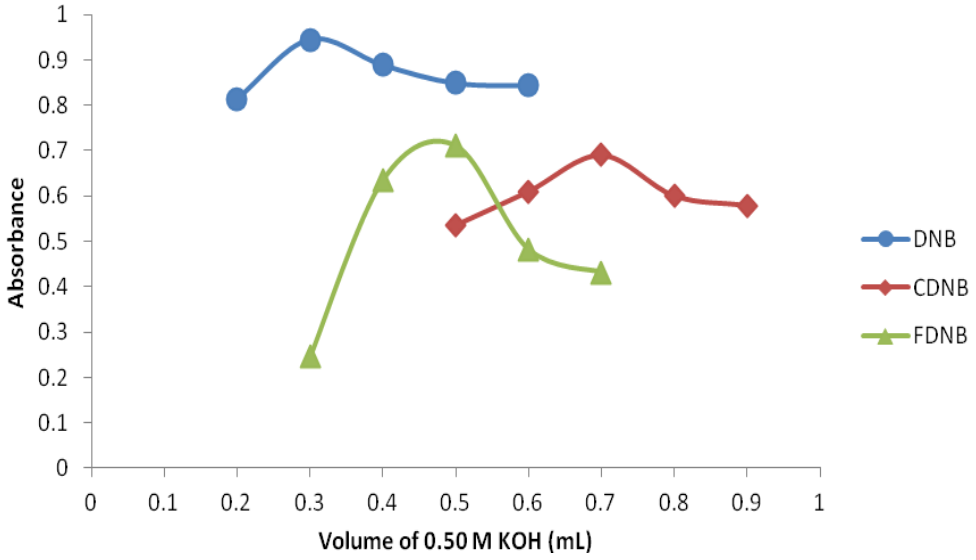

Figure 5: Effect of volume of 0.50 M ethanolic KOH on the absorbance intensity of the reaction products of 10 µg/mL NAB with 10 mg/mL DNB, 160 µg/mL NAB with 10 mg/mL CDNB and 30 µg/mL NAB with 1% v/v FDNB.

3- Effect of Solvent

In order to select the most appropriate diluting solvent, the reactions were carried out in different organic solvents such as methanol, ethanol, DMF, DMSO, isopropyl alcohol and acetonitrile. DMF was considered as an ideal solvent for the reaction with DNB (method I). On the other hand, DMSO was found to be the best solvent regarding intensity of the color formed with CDNB (method II) and FDNB (method III). Other solvents showed much lower absorbance readings.

4- Effect of Time

Effect of waiting time was optimized to achieve optimum sensitivity by monitoring the color development at room temperature (25 ± 5 °C). Complete color development was attained instantaneously with the three studied reagents, consequently, the color products can be measured at zero time.

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Validation of the Proposed Methods

The proposed spectrophotometric methods were validated according to the International Conference on Harmonization guidelines (ICH) [19].

1- Linearity and Concentration Ranges

Linearity of the proposed methods was evaluated by analyzing series of different concentrations of NAB. Linear regression equations were generated by the least-squares treatment of the absorbance data versus the corresponding concentrations. Under the optimal experimental spectrophotometric conditions, linear relationships existed between the absorbance readings of the reaction products and the corresponding concentrations of NAB. Table 2 presents the performance data and statistical parameters for the proposed methods including intercepts and slopes of the regression equations, concentration ranges, apparent molar absorptivity values (ε), correlation coefficients (r), standard deviations of the intercept (S_a), slope (S_b) and standard deviation of residuals ($S_{y/x}$). An important statistical parameter for indicating the random error in the estimated values of "y" is the standard deviation of residuals, $S_{y/x}$. The smaller its value the closer the points are to the straight line. Regression analysis for the calibration curves of the drug showed good linear relationships over the concentration ranges 2-10 µg/mL, 40-240 µg/mL and 10-50 µg/mL for methods I (DNB), II (CDNB) and III (FDNB), respectively. Correlation coefficients were greater than 0.9994 with RSD% of slope values (S_b%) less than 2%.

The analysis of variance (ANOVA) test for the regression lines reveals that, for equal degrees of freedom, an increase in the variance ratio (F values) means an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the steeper is the regression line. The smaller the mean of squares due to residuals, the less is the scatter of experimental points around the regression line. Consequently, regression lines with high F values (low significance F) are much better than those with lower ones. Good regression lines show high values for both r and F statistical parameters [20].

2- Limits of Detection and Quantification

The limit of detection (LOD) is the lowest concentration of the analyte that can be detected but not necessarily quantified under the stated experimental conditions while the limit of quantification (LOQ) is the lowest concentration that can be determined with acceptable precision and accuracy. LOD was defined as 3.3σ/S where σ is the standard deviation of the intercept of regression line and S is the calibration graph slope. LOQ was defined as 10σ/S. The LOD and LOQ values were calculated and presented in Table 2. Obviously, the LOD, LOQ and apparent molar absorpitivity (ε) values emphasize that the reaction with DNB (method I) provides the best sensitivity of measurement of NAB among the three proposed methods.

Table 2: Analytical parameters for the determination of NAB using the proposed spectrophotometric methods

Parameter	DNB (method I)	CDNB (method II)	FDNB (method III)
Concentration range (µg/mL)	2 – 10	40 – 240	10 – 50
Molar absorptivity (L mol^{-1} cm^{-1})	24633	979	3881
Intercept (a)	-0.0253	0.0260	0.0575
S_a	0.0087	0.0111	0.0105
Slope (b)	0.1079	0.00429	0.0170
S_b	0.0013	7.12×10^{-5}	0.0003
RSD% of the slope (S_b%)	1.20	1.66	1.76
Correlation coefficient (r)	0.99978	0.99945	0.99948
$S_{y/x}$	0.0083	0.0119	0.0100
F value	6778	3630	2897
Significant F	3.90×10^{-6}	4.54×10^{-7}	1.41×10^{-5}
LOD (µg/mL)	0.27	8.54	2.04
LOQ (µg/mL)	0.81	25.87	6.18

3- Accuracy and Precision

The accuracy and within-day precision (repeatability, intra-day precision) for the proposed methods were examined at three concentration levels of the analyte within its linearity range (4, 6 and 8 µg/mL for method I, 80, 160 and 200 µg/mL for method II and 20, 30 and 40 µg/mL for method III) with three replicate determinations for each concentration in the same day. Similarly, the accuracy and between-day (inter-day) precision were tested by analyzing the same three concentrations using three replicate determinations on three different days. The recovered concentrations were calculated using the corresponding regression equations. The analytical results obtained from this investigation are summarized in Table 3. The low values of percentage relative standard deviation (RSD %) and the percentage relative error (Er %) values (less than 2%) indicate the high precision and good accuracy of the proposed methods for the estimation of NAB in bulk form.

4- Stability

The stability of the colored products at room temperature was examined. No spectrophotometric changes were observed within 30 min after measurement. Also, the stock standard solutions of NAB prepared either in DMF or DMSO were stable for at least 3 days when stored refrigerated at 4 ºC.

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Table 3: Precision and accuracy for the determination of NAB in bulk form using the proposed spectrophotometric methods

Methods		Nominal value (µg/mL)	Found ± SD* (µg/mL)	RSD(%)	E_r(%)
Within-day	DNB	4	3.94 ± 0.019	0.48	-1.50
		6	6.08 ± 0.045	0.74	-1.33
		8	7.90 ± 0.098	1.24	-1.25
	CDNB	80	80.02 ± 0.72	0.90	-0.03
		160	156.94 ± 0.81	0.52	-1.91
		200	201.32 ± 1.17	0.58	-0.66
	FDNB	20	19.72 ± 0.14	0.71	-1.40
		30	30.42 ± 0.37	1.22	-1.40
		40	39.49 ± 0.25	0.63	-1.28
Between-day	DNB	4	3.97 ± 0.077	1.94	-0.75
		6	6.08 ± 0.094	1.55	-1.33
		8	7.88 ± 0.131	1.66	-1.50
	CDNB	80	81.25 ± 1.09	1.34	-1.56
		160	157.04 ± 2.54	1.62	-1.85
		200	202.44 ± 2.67	1.32	-1.22
	FDNB	20	19.70 ± 0.16	0.81	-1.50
		30	30.04 ± 0.41	1.36	-0.13
		40	40.76 ± 0.52	1.28	-1.90

*Mean ± standard deviation for three determinations

Assay of Tablets Dosage Form

The developed spectrophotometric procedures were applied for the assay of NAB in its tablets dosage form available in the local market, namely Nabuxan® tablets (labeled to contain 500 mg NAB per tablet). Recoveries were calculated using both external standard and standard addition methods. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 4). The good recoveries indicated the absence of any interference from commonly encountered inactive ingredients that may be present in the tablets.

Furthermore, a simple reported spectrophotometric method was adopted as a reference method for the estimation of NAB in its tablets. The reported method was based on measurement of the absorbance of the drug in methanol at 330 nm [21]. The recoveries obtained from the proposed methods were statistically compared with those of the reference method using the one-way analysis of variance (Single factor ANOVA) [22]. The ANOVA test is a

useful statistical tool for comparing recovery data obtained from more than two methods of analysis. The calculated F-value did not exceed the critical value, indicating that there were no significant differences between the proposed methods together with the reported method (Table 4). It is evident from these results that the proposed spectrophotometric methods are applicable to the assay of NAB in commercial tablets with a satisfactory level of accuracy and precision.

Table 4: Application of the proposed spectrophotometric methods for the determination of NAB in Nabuxan® tablets

Using external standard analysis				
Parameter	**DNB (method I)**	**CDNB (method II)**	**FDNB (method III)**	**Reference method [21]**
%Found ± SD *	98.80 ± 1.85	100.19 ± 1.61	100.22 ± 1.39	99.25 ± 1.20
RSD%	1.87	1.61	1.39	1.21

ANOVA (single factor)

Source of Variation	SS	Df	MS	**F**	P-value	**F critical**
Between Groups	7.455859	3	2.485286	**1.060528**	0.393353	**3.238872**
Within Groups	37.49509	16	2.343443			
Total	44.95095	19				

Using standard addition analysis			
Parameter	**DNB (method I)**	**CDNB (method II)**	**FDNB (method III)**
%Recovery ± SD *	99.34 ± 1.72	99.28 ± 1.27	100.71 ± 1.52
RSD%	1.73	1.28	1.51

* Mean ± standard deviation for five determinations.

CONCLUSION

Three simple and rapid spectrophotometric methods were described for the estimation of the analgesic anti-inflammatory drug nabumetone in bulk form and in tablets dosage form. The proposed methods made use of the carbonyl and active methylene groups in the drug for the formation of intense color products upon the reaction with several dinitrobenzene reagents. The proposed methods are advantageous to the previously reported UV-based spectrophotometric methods [9,21], as absorbance measurements are performed in the visible region, away from any possible interfering UV-absorbing excipients that might be co-extracted from the tablets. The developed methods are direct, simple and time saving as they do not require elaborate pretreatment of the samples and/or tedious extraction of the chromophores, additionally, the color products are formed instantaneously at room temperature. The developed methods used only a spectrophotometer, which is readily available in all quality control laboratories. The proposed spectrophotometric methods were validated and their

applicability was evaluated through determination of the drug in commercial tablets with good accuracy and precision and without any interference from commonly encountered tablets additives.

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