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# Application of π receptors to the spectrophotometric determination of naftidrofuryl oxalate in pure form and its pharmaceutical preparation

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## ARTICLE INFORMATION

## ABSTRACT

Three different spectrophotometric methods are developed for the determination of naftidrofuryl oxalate (NAF) in pure form and its pharmaceutical preparation. The methods are based on charge transfer complexation reactions of NAF as n-electron donor with either *p*-chloranilic acid (PCA) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or tetracyano ethylene (TCNE) as π-receptors to give highly colored anion radicals species. The colored products were quantified spectrophotometrically at 515, 588 and 396 nm in PCA, DDQ and TCNE methods, respectively. Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 75.0-300.0, 25.0-150.0 and 15.0- 50.0 μg/mL NAF for PCA, DDQ and TCNE methods, respectively. The proposed methods were applied successfully to the determination of NAF in pure form and its commercial tablets with good accuracy and precision. Statistical comparison of the results was performed using Student's t-test and F-ratio at 95% confidence level, showing that there is no significant difference between the reference and the proposed methods with regard to accuracy and precision. Further, the validity of the proposed methods was confirmed by standard addition technique.

## 1. Introduction

Naftidrofuryl oxalate designated chemically as 2-(diethyl amino) ethyl 2-[(naphthalen-1-yl) methyl]-3-(tetrahydrofuran-2-yl) propanoate hydrogen oxalate (Figure 1), is a vasodilator drug used in the treatment of peripheral and cerebral vascular disorders. It is claimed to enhance the cellular oxidative capacity thereby protecting cells against ischemia [1]. NAF is an official drug in the British Pharmacopoeia [2] where a non-aqueous potentiometric titration and HPLC procedures were described for the assay of NAF bulk powder and capsules, respectively.
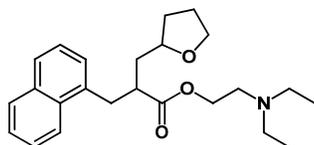


**Figure 1.** Chemical structure of naftidrofuryl oxalate (NAF).

Few analytical methods have been reported for the determination of NAF in biological fluids and/or pharma-ceutical preparations. Most of these studies focused on HPLC-UV detection [3-5], HPLC-fluorescence detection [6,7] and phosphorimetric analysis [8,9] Others included potentiometric methods using NAF ion-selective electrodes [10,11] flow injection analysis with fluorescence opti-sensor [12], spectrophotometric, spectrofluorimetric and voltammetric analyses in tablets [13], a spectrophotometric method using complexation reactions [14] and kinetic spectrophotometric method using alkaline potassium permanganate were described [15]. Also stability indicating methods were reported for the determination of the drug [16,17].

To the best of our knowledge, only two visible spectrophotometric methods have been reported for the quantification of NAF in pharmaceuticals. Visible spectro-photometry, because of its simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and easy access in most quality control laboratories, has remained competitive in an area of chromatographic techniques for pharmaceutical analysis. This paper describes, for the first time, the application of *p*-chloranilic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and tetracyano ethylene as π-acceptors to the spectrophotometric determination of NAF based on the interaction between these π-acceptors and the oxygen atom of tetrahydrofuran ring of NAF as good *n*-electron donor to form charge-transfer complexes. The purpose of this investigation was directed to develop simple, sensitive, precise and inexpensive procedures for the quantification of NAF in pharmaceutical and pure form.

## 2. Experimental

### 2.1. Apparatus

All absorption spectra and derivatives were recorded with a dual beam Shimadzu UV-visible spectrophotometer 1601PC with 1 cm quartz cuvettes, connected to an IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7 (Shimadzu), Shimadzu Corporation, Kyoto, Japan.

### 2.2. Chemicals and reagents

NAF was kindly supplied by Minapharm Pharmaceutical Industries Egypt. Its purity was found to be 99.4±0.76, according to its official procedure [2]. PRAXILENE® tablets (B.N.CCE0884) labeled to contain 200 mg NAF/tablet was purchased from the Egyptian market. PCA (Rolex Laboratory Reagent, Mumbai, India): 0.125% (*w/v*). Solution in acetonitrile (Merck, Mumbai, India) was prepared and kept in the dark when not in use. DDQ (Avra Synthesis Pvt. Ltd., Hyderabad, India): 0.2% (*w/v*) solution in acetonitrile (Merck, Mumbai, India), was prepared fresh just before use. TCNE (Sd. Fine Chem. India Ltd.): 0.2% (*w/v*) solution in acetonitrile (Merck, Mumbai, India), was prepared fresh just before use. All chemicals and reagents were of analytical reagent grade.

### 2.3. Standard solutions

Naftidrofuryl oxalate (NAF) stock solution 0.5 mg/mL was prepared in acetonitrile.

## 3. Procedures

### 3.1. Construction of calibration curves

#### 3.1.1. Method A (PCA)

Different aliquots (1.50, 2.00, ..., 6.00 mL) of a standard NAF (0.5 mg/mL) solution were accurately transferred into a series of 10 mL volumetric flasks followed by 2.5 mL of 0.125% PCA solution. The content was mixed well, diluted to the volume with acetonitrile and the absorbance was measured at 515 nm against a reagent blank prepared simultaneously. A calibration curve was constructed and the regression equation was computed.

#### 3.1.2. Method B (DDQ)

Different aliquots (0.50, 1.00, ..., 3.00 mL) of a standard NAF (0.5 mg/mL) solution were accurately transferred into a series of 10 mL volumetric flasks followed by 3.0 mL of 0.2% DDQ solution. The content was mixed well, kept aside for 15 minutes. Then diluted to the volume with acetonitrile. The absorbance was measured at 588 nm against a reagent blank prepared simultaneously. A calibration curve was constructed and the regression equation was computed.

#### 3.1.3. Method C (TCNE)

Different aliquots (0.30, 0.40, ..., 1.00 mL) of a standard NAF (0.5 mg/mL) solution were accurately transferred into a series of 10 mL volumetric flasks followed by 1.0 mL of 0.2% TCNE solution. The content was mixed well and kept aside for 25 minutes. The mixture was diluted to the volume with acetonitrile. The absorbance was measured at 396 nm against a reagent blank prepared simultaneously. A calibration curve was constructed and the regression equation was computed.

### 3.2. Application to pharmaceutical preparation

Ten tablets of "PRAXILANE" each containing 200 mg NAF were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50.0 mg of NAF was dissolved in about 25 mL of acetonitrile by shaking in ultrasonic bath for 1 hr. The solution was filtered and transferred quantitatively into 100 mL volumetric flask. The volume was then completed to the mark with acetonitrile. The necessary dilutions were made to reach concentrations of linear range. The procedures described under construction of calibration curves for the proposed methods were then followed and the concentration of NAF was determined from the corresponding regression equation.

### 3.3. Stoichiometric relationship

Job's method of continuous variations of equimolar solutions was employed to establish the stoichiometry of the formed complexes. The solutions equivalent to $1.00 \times 10^{-2}$, $5.00 \times 10^{-3}$ and $5.00 \times 10^{-4}$ M NAF and similar concentrations of PCA, DDQ and TCNE were prepared, respectively. A series of solutions was prepared in which the total volume of NAF and reagent was kept at 10 mL. The solutions were mixed in various proportions; in method A, the volume was completed to the mark immediately with acetonitrile while the volume was completed to the mark after 15 and 25 min with acetonitrile in case of method B and C, respectively. The absorbance of the resulting solutions was measured at the respective wavelengths of maximum absorbance ($\lambda_{max}$) against the reagent blank.

## 4. Results and discussion

The reaction of π-acceptors with NAF as *n*-electron donor results in the formation of an intense orange-red product, which exhibits absorption maximum at 515 nm in case of PCA (Figure 2) due to the formation of the corresponding PCA radical anion. Intense reddish violet color was appeared which exhibits absorption maxima at 588, 545 and 455 nm in case of DDQ (Figure 3). For NAF-DDQ charge transfer complex the absorption band at 588 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product and blank absorbance. Intense yellow colored product was developed which exhibits absorption maximum at 396, 414 nm in case of TCNE (Figure 4). For NAF-TCNE charge transfer complex the absorption band at 396 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product.
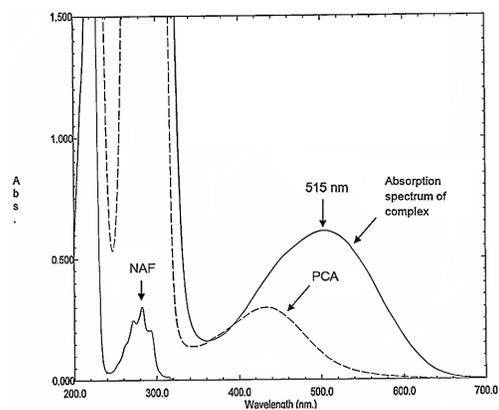


**Figure 2.** Absorption spectra of NAF, 25.0 μg/mL, that of its charge transfer complex with PCA, 200.0 μg/mL, and reagent blank in acetonitrile.
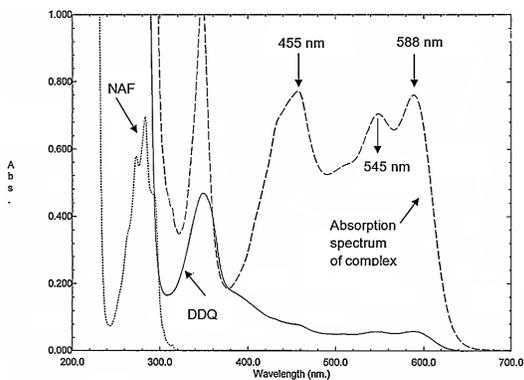
**Figure 3.** Absorption spectra of NAF, 50.0 μg/mL, that of its charge transfer complex with DDQ, 100.0 μg/mL and reagent blank in acetonitrile.
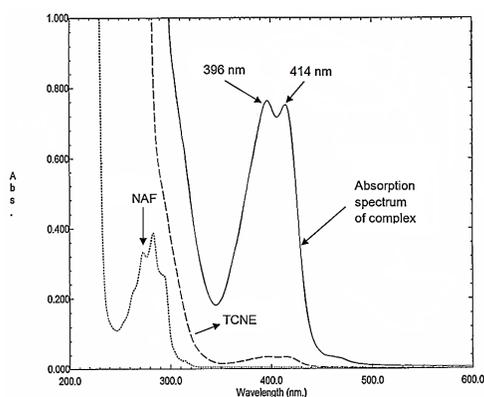


**Figure 4.** Absorption spectra of NAF, 25.0 μg/mL, and that of its charge transfer complex with TCNE, 50.0 μg/mL and reagent blank in acetonitrile.

The effect of the reagent concentration on the intensity of the color developed at the selected wavelengths was ascertained by adding different amounts of the reagents PCA, DDQ and TCNE to a fixed concentration of 200.0 μg/mL NAF in method A, 100 μg/mL in method B and 50.0 μg/mL NAF in method C. It was found that 2 mL of 0.125% PCA, 3.0 mL of 0.2% DDQ and 1.0 mL of 0.2% TCNE solutions were sufficient for the production of maximum and reproducible color intensity and the highest absorbance remained unaffected by further addition of these reagents (Figure 5).
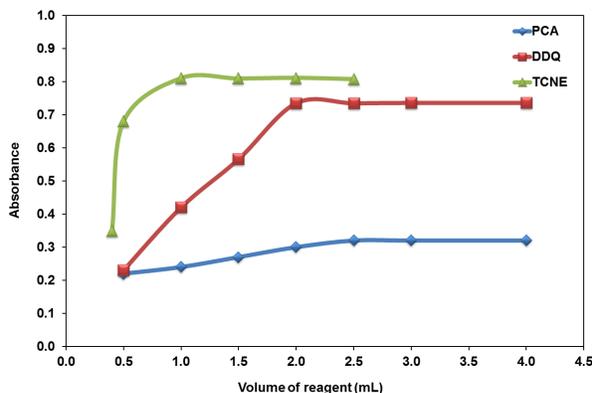


**Figure 5.** Effect of PCA (0.125%, *w/v*), DDQ (0.2%, *w/v*) and TCNE (0.2%, *w/v*) volumes on the absorbance of the colored complexes.

In order to select the suitable solvent for charge transfer complex formation, the reaction of NAF with PCA, DDQ and TCNE was carried out in different solvents such as chloroform, acetone, methanol and acetonitrile. Acetonitrile was found to be optimum because it afforded the maximum sensitivity when compared with all other solvents and it possesses the highest dielectric constant of all solvents examined (Figure 6), a property which is known to promote the dissociation of the original charge-transfer complexes to the radical anions.
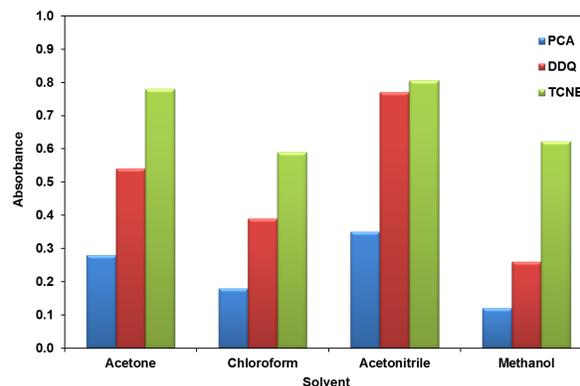


**Figure 6.** Effect of solvent type on the absorbance of the colored complexes formed between 100 μg/mL NAF with 0.2% DDQ and 0.125% PCA and 50 μg/mL NAF with 0.2%TCNE.

The optimum reaction time was determined by following the color development upon the addition of reagent solution to the NAF solution at room temperature. Complete color development was attained after 15 and 20 min with DDQ and TCNE, respectively, (Figure 7) while the reaction with PCA was instantaneous. The absorbance of these radical anions remained stable for at least 60 min.
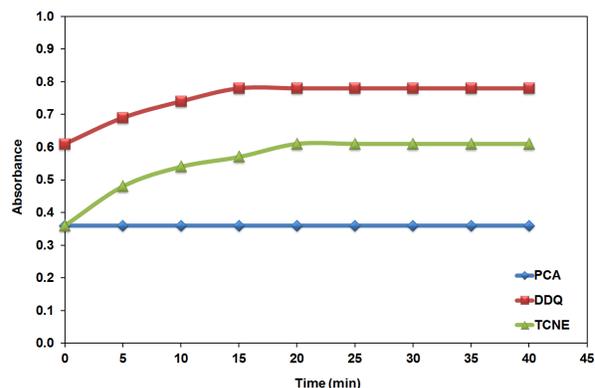


**Figure 7.** Effect of reaction time on the absorbance of the colored complexes formed between 100 μg/mL NAF with 0.2% DDQ and 0.125% PCA and 40 μg/mL NAF with 0.2%TCNE.

Job's continuous variations graph for the reaction between NAF and PCA or DDQ or TCNE shows that the interaction occurs on an equimolar basis via the formation of a charge-transfer complexes 1:1 (NAF:Reagent) (Figure 8) This finding was anticipated by the presence of one basic or electron donating center (-O-) in tetrahydrofuran ring of NAF.

Under optimum experimental conditions for determination of the drug under study, the absorbance versus concentration plots were found to be linear over the concentration ranges of 75.0-300.0, 25.0-150.0 and 15.0-50.0 μg/mL in the case of PCA, DDQ and TCNE, respectively, (Figure 9).

**Table 1.** Assay validation sheet for the proposed spectrophotometric methods for the analysis of naftidrofuryl oxalate.

| Parameters | Naftidrofuryl Oxalate | | |
|---|---|---|---|
| | PCA | DDQ | TCNE |
| Range (µg/mL) | 75.0-300.0 | 25.0-150.0 | 15.0-50.0 |
| *Regression equation* | | | |
| Slope | 0.0025 | 0.0047 | 0.0192 |
| Intercept | 0.0104 | 0.2138 | -0.1169 |
| Correlation coefficient (*r*) | 0.9994 | 0.9996 | 0.9994 |
| Accuracy | 99.36±0.87 | 99.78±1.35 | 100.78±1.83 |
| LOD (µg/mL) | 10.50 | 5.57 | 5.40 |
| LOQ (µg/mL) | 11.5 | 19.7 | 18.4 |
| Repeatability ᵃ | 1.400 | 1.537 | 1.940 |
| Intermediate precision * | 1.650 | 1.368 | 1.815 |

ᵃ Intra-day (n = 3) relative standard deviations of samples of concentrations (100, 150.0, 200.0 µg/mL), (50.0, 100.0, 150.0 µg/mL) and (20.0, 40.0, 50.0 µg/mL) for PC-A, DDQ and TCNE methods, respectively of naftidrofuryl oxalate by colorimetric methods.
ᵇ Inter-day (n = 3) relative standard deviations of samples of concentrations (100.0, 150.0 µg/mL), (50.0, 100.0 µg/mL) and (40.0, 50.0 µg/mL) for PC-A, DDQ and TCNE methods respectively of naftidrofuryl oxalate by colorimetric methods.

**Table 2.** Analysis of naftidrofuryl oxalate in pharmaceutical preparation by the proposed PCA, DDQ and TCNE methods and application of standard addition technique.

| Dosage form | Found (%) * | Added standard (µg/mL) | Found (µg/mL) * | Recovery (%) of added standard |
|---|---|---|---|---|
| Praxilene ® 200 mg tablets | 99.71±0.14 | 75.0 | 75.81 | 101.08 |
| (batch no. CCE0884) | (PCA method) | 100.0 | 101.17 | 101.17 |
| | | 100.0 | 98.37 | 98.37 |
| Mean±S.D | | | | 100.21±1.59 |
| Praxilene ® 200 mg tablets | 102.87±1.06 | 25.0 | 24.42 | 97.68 |
| (batch no. CCE0884) | (DDQ method) | 50.0 | 50.54 | 101.08 |
| | | 75.0 | 74.54 | 99.38 |
| Mean±S.D | | | | 99.38±1.7 |
| Praxilene ® 200 mg tablets | 101.55±0.66 | 15.0 | 15.10 | 100.69 |
| (batch no. CCE0884) | (TCNE method) | 20.0 | 19.73 | 98.65 |
| | | 25.0 | 24.99 | 99.94 |
| Mean±S.D | | | | 99.76±1.03 |

**\*** Average of three determinations.
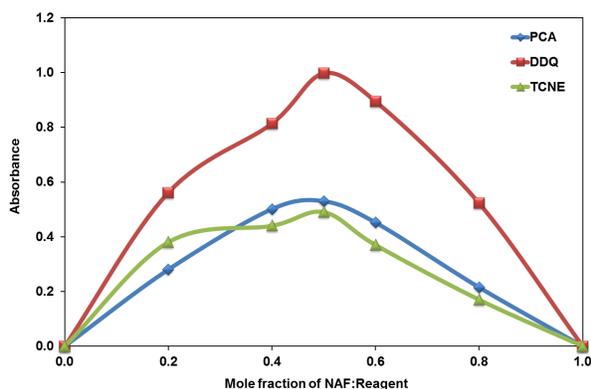


**Figure 8.** Determination of the stoichiometry of the reaction between $(1.00 \times 10^{-2}$ M) PCA, $(5.00 \times 1^{-3}$ M) DDQ, $(5.00 \times 10^{-4}$ M) TCNE and the corresponding concentrations of NAF by continuous variation method.
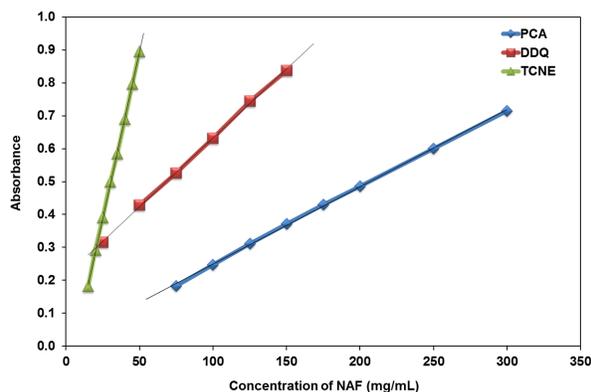


**Figure 9.** Linearity of the absorbance of the colored PCA complex, DDQ complex and TCNE complex to the corresponding concentration of NAF (µg/mL).

The regression equations were computed and found to be:

$$A = 0.0025\ C + 0.0104\ r = 0.9994\ \text{(for PCA (method A)} \quad (1)$$

$$A = 0.0047\ C + 0.2138\ r = 0.9996\ \text{(for DDQ (method B)} \quad (2)$$

$$A = 0.0192\ C - 0.1169\ r = 0.9994\ \text{(for TCNE (method C)} \quad (3)$$

where A is the absorbance at 515, 588 and 396 nm in case of PCA, DDQ and TCNE, respectively, C is the concentration of NAF in µg/mL and *r* is the correlation coefficient.

The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope ($S_b$) and the intercept ($S_a$) showed good results. The linearity of the calibration graphs was demonstrated by the high values of the correlation coefficients (*r*) (Table 1). Where the accuracy of the proposed methods was checked by under construction of calibration curves were repeated for the determination of different concentrations of NAF. The concentrations were calculated from the corresponding regression equations. The sensitivity of proposed methods was checked by through the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ are calculated and recorded for the proposed methods in (Table 1).

In order to determine the precision of the proposed methods, solutions containing three different concentrations of NAF were prepared and analyzed in three replicates and the analytical results are summarized in (Table 1). The low values of the relative standard deviation (% R.S.D) indicate the high precision and the good accuracy of the proposed methods. RSD (%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over three days to evaluate intermediate precision (inter-day precision).

The proposed methods were successfully applied to the determination of NAF in the representative tablets Praxilene 200 mg. The results obtained are showed in (Table 2) and were compared with those obtained by the official method [2] by means of Student's t- and F-tests at 95% confidence level.

**Table 3.** Statistical comparison for the results obtained by the proposed methods and the official one for the analysis of NAF in pure powder form.

| Item | PCA method | DDQ method | TCNE method | Official method ** |
|---|---|---|---|---|
| Mean | 99.31 | 99.78 | 100.78 | 99.66 |
| S.D. | 0.87 | 1.35 | 1.83 | 1.62 |
| Variance. | 0.75 | 1.82 | 3.34 | 2.62 |
| N | 6 | 6 | 6 | 6 |
| F [5.05] * | 3.49 | 1.44 | 1.27 | |
| t [2.23] * | 0.47 | 0.139 | 1.128 | |

\* The figures in parenthesis are the corresponding tabulated values at p = 0.05.

\*\* Potentiometric titration with acetous perchloric acid in non-aqueous titration.

**Table 4.** Analysis of naftidrofuryl oxalate in the presence of common excipients by the proposed spectrophotometric methods.

| Ingredient | Recovery % ± RSD * | | |
|---|---|---|---|
| | PCA method | DDQ method | TCNE method |
| Na alginate | 98.7±3.90 | 101.2±1.20 | 99.9±0.52 |
| Talc | 99.7±1.30 | 100.0±0.09 | 98.2±0.33 |
| Mg stearate | 100.4±1.38 | 100.5±0.78 | 101.4±1.60 |
| Ca gluconate | 101.5±1.89 | 100.2±0.69 | 101.5±1.19 |
| Lactose | 99.0±3.11 | 100.4±1.36 | 100.6±0.61 |
| Starch | 100.1±0.15 | 100.3±1.07 | 98.5±1.11 |
| Glucose | 100.3±1.54 | 100.4±0.55 | 100.3±1.07 |

**\***Average of three determinations.

The values of the calculated t and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods and the official one (Table 3).

The selectivity of proposed methods was checked by the effect of the presence of common excipients, such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate and magnesium stearate was tested for possible interference in the assay by placebo blank and synthetic mixture analyses whereas no significant interference was observed from these excipients as shown in Table 4.

## 5. Conclusion

The proposed methods in this study are based on well-characterized charge-transfer complexation reaction, and have the advantages of simplicity, speed, accuracy and precision, and use of inexpensive equipment compared to the reported HPLC and electrochemical methods. The TCNE method is more sensitive than the PCA and DDQ methods as seen from the lower LOD value. The highlight of the proposed methods is their ability to quantify naftidrofuryl oxalate in pharmaceutical formulation without any interference from the common excipients. Thus, the methods are useful for the quality control and routine analysis of NAF in pharmaceutical and commercial formulation.

### *Acknowledgement*

## References

[1]. Reynolds, J. E. F. (Ed.), Martindale: The Extra Pharmacopoeia, 35th edition, the Pharmaceutical Press, London, 2007.

[2]. British Pharmacopoeia-2009, Her Majesty Stationary Office, London, Vol: I, 2009.

[3]. Brodie, R. R.; Chasseaud, L. F.; Taylor, T.; Hunter, J. O.; Ciclitira, P. J. *J. Chromatogr.* **1979**, *164*, 534-540.

[4]. Waaler, P. J.; Müller, B. W. *Inter. J. Pharm.* **1992**, *1(3)*, 223-227.

[5]. Sabry, S. M.; Belal, T. S.; Barary, M. H; Ibrahim, M. E. *J. Drug Test Anal.* **2012**, *5(6)*, 500-508.

[6]. Stehlik, P.; Houbova, H. *Cesk. Farm.* **1990**, *39(9)*, 394-399.

[7]. Walmsley, L. M.; Wilkinson, P. A.; Brodie, R. R.; Chasseaud, L. F. *J. Chromatogr.* **1982**, *338(2)*, 433-437.

[8]. Segura, C. A.; Cruces, B. C.; Canabate, D. B.; Fernandez, S. J. F.; Fernandez, G. A. *Anal. Chim. Acta* **2000**, *417*, 19-30.

[9]. Munoz, P. A.; Espinosa, M. A.; Murillo, P. J. A.; Alanon, M. A.; Fernandez, L. P. *Analyst* **1998**, *123*, 2285-2290.

[10]. Ionescu, M. S.; Badea, V.; Baiulescu, G.; Cosofret, V. V. *Talanta* **1986**, *33*, 101-103.

[11]. Abbas, S. S.; Zaazaa, H. E.; El-Ghobashy, M. R.;Fayez, Y. M.; Fattah, S. A. *Port. Electr. Acta* **2012**, *30(3)*, 177-188.

[12]. Fernandez, S. J. F.; Segura, C. A.; Cruces, B. C.;Fernandez, G. *Anal. Chim. Acta* **2002**, *462*, 217-224.

[13]. Suzy, M. S.; Tarek, S. B.; Magda, H. B.; Mohammed, E. A. I. *J. Biomed. Sci.* **2009**, *5(3)*, 283-292.

[14]. Ayad, M.; Abdellatef, H.; Hosny, M.; Sharaf, Y. *Asian J Pharm.  Clinic. Res.* **2013**, *6(1)*, 92-102.

[15]. Belal, T. S.; Barary, M. H.; Sabry, S. M.; Abdellatef, M. E. *Food Drug Anal.* **2009**, *17(6)*, 415-423.

[16]. Abbas, S. S.; Zaazaa, H. E.; El-Ghobashy, M. R.; Fayez, Y. M.; Fattah, S. A. *Anal. Chem., An Indian J.* **2012**, *11(11,12)*, 329-334.

[17]. Abbas, S. S.; Zaazaa, H. E.; El-Ghobashy, M. R.; Fayez, Y. M.; Fattah, S. A. *Anal. Chem., An Indian J.* **2009**, *8(4)*, 613-620.