

Simultaneous Spectrophotometric Determination of Nitrophenol Isomers in Environmental Samples Using First Derivative of the Density Ratio Spectra

F. Bagheban-Shahri^{1,*}, A. Niazi², A. Akrami¹

¹Young Researchers Club, Arak Branch, Islamic Azad University, Arak, Iran

²Department of Chemistry, Faculty of Science, Arak Branch, Islamic Azad University, Arak, Iran

Abstract: A new spectrophotometric method was developed for the simultaneous of ternary mixtures of nitrophenol isomers, without prior separation steps. This method is called the first derivative of the density ratio spectra. All factors affecting on the sensitivity were optimized and the linear dynamic range for determination of nitrophenol isomers was found. The method made use of a derivative of the double-divisor-ratio spectra of optical density. In this case, the linear determination ranges are 1.0-25.0 $\mu\text{g mL}^{-1}$ for m-nitrophenol, 1.0-25.0 $\mu\text{g mL}^{-1}$ for o-nitrophenol and 1.0-15.0 $\mu\text{g mL}^{-1}$ for p-nitrophenol. The RMSEP for m-nitrophenol, o-nitrophenol and p-nitrophenol by proposed method was 0.4907, 0.4779 and 0.2068, respectively. The method developed in this paper was rapid, easy to apply, not expensive and it was suitable for analyzing to overlapping signals of compounds in their mixtures without any chemical pre-treatment and also, the proposed method was satisfactorily applied to the rapid simultaneous determination of m-nitrophenol, o-nitrophenol and p-nitrophenol in synthetic and water samples.

Keywords: Nitrophenol isomers; Spectrophotometric; Derivative ratio spectrum; Water samples.

INTRODUCTION

Nitrophenol isomers belong to major phenolic pollutants that have been analyzed in the environment. Nitrophenols, coming from pesticide degradation products, car exhaust, and industrial waters are listed as priority pollutions by the US Environmental Protection Agency [1, 2]. They have great potential toxicities of carcinogenesis, teratogenesis, and mutagenesis [3]. Because of their detriment and vast scale distribution in the ecological environment, their separation and determination have been become one of the important study of environmental analysis. However, traditional spectrophotometry [4] and colorimetric method are easily interfered by related compounds. Gas chromatography [5] need beneficiation and derivatization before analysis, and it cannot be directly used to aqueous samples. High performance liquid chromatography [6-10] and capillary electrophoresis [3, 8] are a good alternative method, but it needs high cost to buy columns and waste more organic solvent. Spectral overlap and non-specific irrelevant absorption affect the interpretation of data for even

$$A_{M,\lambda_i} = \alpha_{X,\lambda_i} C_X + \beta_{Y,\lambda_i} C_Y + \gamma_{Z,\lambda_i} C_Z \quad (1)$$

Where A_{M,λ_i} denotes the absorbance of three-component mixture at λ_i , α_{X,λ_i} , β_{Y,λ_i} , γ_{Z,λ_i} denoted the absorptivity of X, Y and Z, respectively. A similar expression of one

the simplest-component organic systems, leading to variable intercepts on the absorbance axis and systematic errors in the graphs of absorbance versus concentration. These organic compounds present partially overlapping spectra. One elegant approach to the problem of solving spectral overlap that has received attention in pharmaceutical analysis is derivative spectroscopy [11]. Derivative methods have been seriously applied to the quantitative assay of drugs and organic compounds, and metals in mixtures [12-15]. Several workers have discussed the theory and application of derivative spectrophotometry [12-18] and ratio spectra derivative [11] methods. Therefore, the theory of used method in this study is given in brief in following.

If a three-component mixture containing three compounds (X, Y and Z) is considered, if the Beer-Lambert law is obeyed for three compounds at the whole wavelengths using the path-length of 1 cm, then the UV-V is spectra of three-component mixture at wavelength λ_i can be given by:

compound in three-component mixture can be written:

$$A_{M,\lambda_i} = \alpha_{X,\lambda_i} C_X + \beta_{Y,\lambda_i} C_Y \quad (2)$$

If Eq.(1) is divided by Eq.(2) and the resulting expression for the ratio spectra can be given as:

$$\frac{A_{M,\lambda_i}}{\alpha_{X,\lambda_i} C_X^0} = \frac{\alpha_{X,\lambda_i} C_X}{\alpha_{X,\lambda_i} C_X^0} + \frac{\beta_{Y,\lambda_i} C_Y}{\alpha_{X,\lambda_i} C_X^0} + \frac{\gamma_{Z,\lambda_i} C_Z}{\alpha_{X,\lambda_i} C_X^0} \quad (3)$$

Corresponding Author: F. Bagheban-shahri, Young Researchers Club, Arak Branch, Islamic Azad University, Arak, Iran

The ratio of $(\alpha_{X,\lambda_i} C_X)/(\alpha_{X,\lambda_i} C_X)$ is equal to constant (k) (in which k=1) with respect to λ_i in a certain range of wavelength, the given:

$$\frac{A_{M,\lambda_i}}{\alpha_{X,\lambda_i} C_X^0} = k + \frac{\beta_{Y,\lambda_i} C_Y}{\alpha_{X,\lambda_i} C_X^0} + \frac{\gamma_{Z,\lambda_i} C_Z}{\alpha_{X,\lambda_i} C_X^0} \quad (4)$$

The first derivation of Eq.(4) can be taken with respect to λ_i in the selected region of wavelength,

$$\frac{d}{d\lambda} \left[\frac{A_{M,\lambda_i}}{\alpha_{X,\lambda_i} C_X^0} \right] = \frac{d}{d\lambda} \left[\frac{\beta_{Y,\lambda_i}}{\alpha_{X,\lambda_i}} \right] \frac{C_Y}{C_X^0} + \frac{d}{d\lambda} \left[\frac{\gamma_{Z,\lambda_i}}{\alpha_{X,\lambda_i}} \right] \frac{C_Z}{C_X^0} \quad (5)$$

This derivative analytical signal of Y is dependent exclusively on the concentration C_Y , C_Z and C_X^0 , but is independent on the concentrations C_X in three-component mixtures.

In this method the concentrations C_Y and C_Z is proportional to the derivative signals in coincided points corresponding to the maximum and minimum of wavelength for pure Y , Z and their three-component mixture with X . As explained above, the analogue procedures can be used for estimation both of C_X or alone of each and the similar to this method can be using for estimation of C_X , C_Y and C_Z in three-component mixture. In this paper, a new method for the simultaneous spectrophotometric determination of nitrophenol isomers in synthesis and real samples such as water samples using ratio spectra derivative methods is proposed. This study is a preliminary step for further applications to the routine determination of nitrophenol isomers in water samples.

EXPERIMENTAL

Chemicals

All the chemicals used were of analytical reagent grade, sub boiling, distilled water was used throughout. Stock solutions of meta-nitrophenol, ortho-nitrophenol and para-nitrophenol were purchased from Fluka. Acetic acid, phosphoric acid, boric acid and sodium hydroxide were purchased from Merck. Standards of working solution were made by appropriate dilution daily as required. Adjusting the PH values of the working solutions was carried out using universal buffers (acetic acid-phosphoric acid-boric acid mixture) for this study [18].

Instrumentation and software

A Hewlett-Packard 8453 diode array spectrometer controlled by a Hewlett-Packard computer and equipped with a 1-cm path length quartz cell. Spectra were acquired between 300 and 480 nm (1 nm resolution). The pH was determined with a model 780 Metrohm pH-meter with combined

that the derivation of a constant (k) is zero and we can write the following Equation (5):

glass-calomel electrode was calibrated with at least two buffer solutions at pH 4.00 and 9.00. The data were treated in a personal computer (CPU 3.0 GHz and 2 Gb RAM) using MATLAB software, version 7.0 (The MathWorks). The derivative spectra were calculated using the Savitzky-Golay convolution method [19].

PROCEDURE

Standard calibration set

In this method, a series of ten solutions for each nitrophenol isomers with sufficient amount of m-nitrophenol, o-nitrophenol and p-nitrophenol were made from the working solutions as follow; known amount of the standard solution nitrophenol isomers were placed in a 10-ml volumetric flask and sufficient amount buffer solution added to it and then diluted to the final volume with double distilled water (final pH 9.0). The concentrations of m-nitrophenol, o-nitrophenol and p-nitrophenol were between 1.0-25.0, 1.0-25.0 and 1.0-15.0 $\mu\text{g mL}^{-1}$ varied, respectively. The mixed standard solutions were placed in a 10-ml volumetric flask and completed to the final volume with deionized water (final pH 9.0). The absorption spectra were recorded between 200 and 500 nm at a step of 1.0 nm against a blank of universal buffer.

Prediction set and analysis of real samples

For prediction set, 10 mixtures prepared that these not included in the previous set were employed as an independent test. The real samples in this study were collected in different waters. The range concentrations were added to be 1.0-25.0, 1.0-250.0 and 1.0-15.0 $\mu\text{g mL}^{-1}$ for m-nitrophenol, o-nitrophenol and p-nitrophenol, respectively. Tap water, river water and waste water samples were collected from Arak (Arak, Iran). Prior to the preconcentration procedure, all the water samples were filtered through a 0.45 μm pore size membrane filter to remove suspended particulate matter and then stored at 4 °C in the dark.

RESULTS AND DISCUSSION

Spectrophotometric study

Figure 1 shows the absorption spectra in aqueous solution of the individual nitrophenol isomers, and

their mixture at pH 9.0, which were measured in the spectral range 200-500 nm with a step of 1.0 nm. As can be seen, nitrophenol isomers cannot be determined using the traditional spectrophotometric method, because of an almost

complete overlap of the signals of components for various concentration ratios. For this reason, the three-component solution was analyzed using the derivative of ratio spectra method described above.

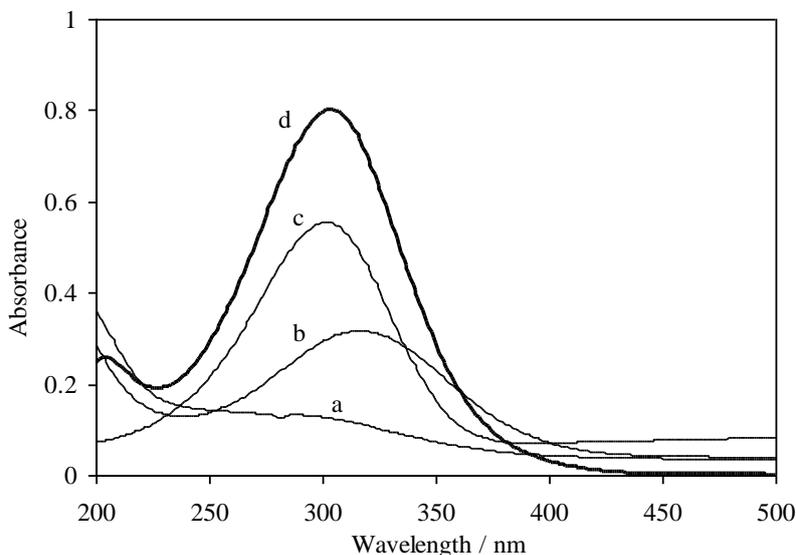


Fig 1. Typical spectrum of the individual nitrophenol isomers at pH 9.0. (a): m-nitrophenol ($9\mu\text{g mL}^{-1}$), $\lambda_{\text{max}}=280\text{nm}$ (b): o-nitrophenol ($9\mu\text{g mL}^{-1}$), $\lambda_{\text{max}}=322\text{nm}$, (c): p-nitrophenol ($4\mu\text{g mL}^{-1}$), $\lambda_{\text{max}}=301\text{nm}$ and (d): their ternary mixture, $\lambda_{\text{max}}=302\text{nm}$.

Optimization of experimental condition

For the finding the optimum conditions, the influence of pH values on the spectrum of each nitrophenol isomers at a constant concentration of each isomers was studied. The formed isomers were affected differently with pH. In order to select the optimum pH value at which the minimum overlap occurs, influences of the pH of the medium on the absorption spectra of nitrophenol isomers were studied over the pH range 1.0-12.0. However, pH 9.0 was chosen as the optimum pH for this work because nitrophenol isomers have maximum absorbance and minimum overlap at this pH.

Derivative of ratio spectra method

The optimum wavelength for the quantitative determination of nitrophenol isomers was chosen using the following three-step procedure: (I) First derivatives of the ratio spectra of pure m-nitrophenol and its three-component mixture with o-nitrophenol and p-nitrophenol, were normalized using the double divisor (DD) for the pure o-nitrophenol and p-nitrophenol (DD (I)). Then, a working point for determining m-nitrophenol was chosen at 358 nm, where the double divisor-ratio derivative (DDD) spectrum exhibits a maximum (Figure 2 (I)).

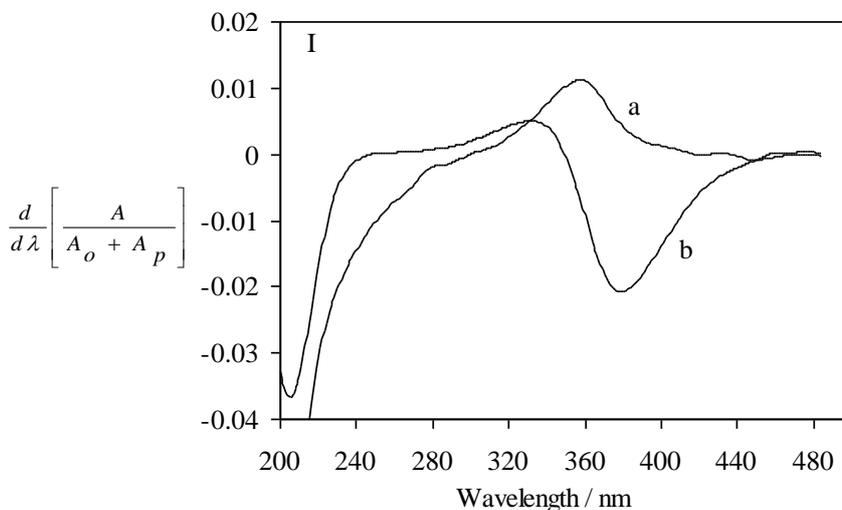


Fig 2 (I). First derivatives of the ratio spectra normalized to the double dividers: (a) m-nitrophenol ($9 \mu\text{g mL}^{-1}$), (b) o-nitrophenol ($9 \mu\text{g mL}^{-1}$) + m-nitrophenol ($9 \mu\text{g mL}^{-1}$) + p-nitrophenol ($4 \mu\text{g mL}^{-1}$) normalized to DD(I) for o-, p-nitrophenol.

(II) First derivatives of the ratio spectra for pure o-nitrophenol and its three-component mixture with m-nitrophenol and p-nitrophenol, were normalized using the double divisor for the pure m-nitrophenol and p-nitrophenol (DD (II)). Then a working point for determining o-nitrophenol was chosen at 370 nm, where the DDD spectrum exhibits a maximum (Figure 2 (II)).

(III) First derivatives of the ratio spectra for pure p-nitrophenol and its three-component mixture with m-nitrophenol and o-nitrophenol, were normalized using the double divisor for the pure m-nitrophenol and o-nitrophenol (DD (III)). Then

a working point for determining p-nitrophenol was chosen at 412 nm, where the DDD spectrum exhibits a maximum (Figure 2 (III)). The DDD spectra of model solutions were measured in the spectral range from 300 to 480 nm at a 1.0 nm step and smoothed over a 10-nm interval. The obtained calibration graphs for nitrophenol isomers (determined using the working points chosen as described above) were linear in the concentration range 1.0– 25.0 $\mu\text{g mL}^{-1}$ for m-nitrophenol, 1.0– 25.0 $\mu\text{g mL}^{-1}$ for o-nitrophenol and 1.0–15.0 $\mu\text{g mL}^{-1}$ for p-nitrophenol.

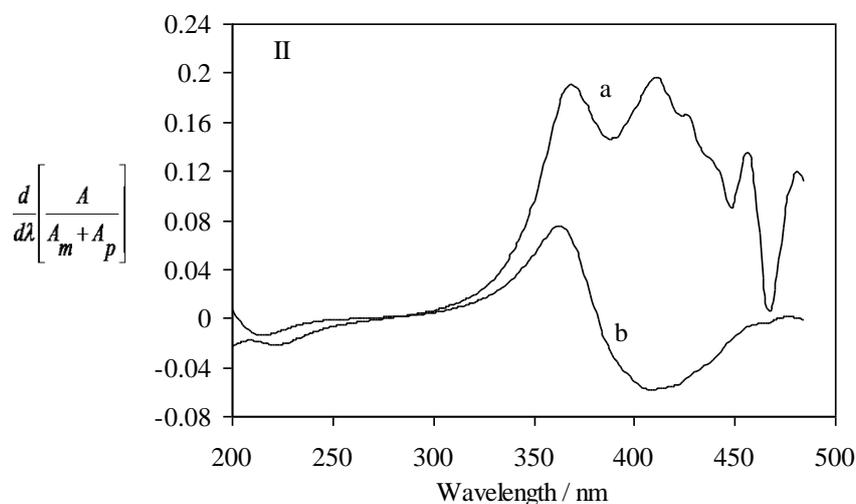


Fig 2 (II). First derivatives of the ratio spectra normalized to the double dividers: (a) o-nitrophenol ($9 \mu\text{g mL}^{-1}$), (b) m-nitrophenol ($9 \mu\text{g mL}^{-1}$) + o-nitrophenol ($9 \mu\text{g mL}^{-1}$) + p-nitrophenol ($4 \mu\text{g mL}^{-1}$) normalized to DD(II) for m- and p-nitrophenol.

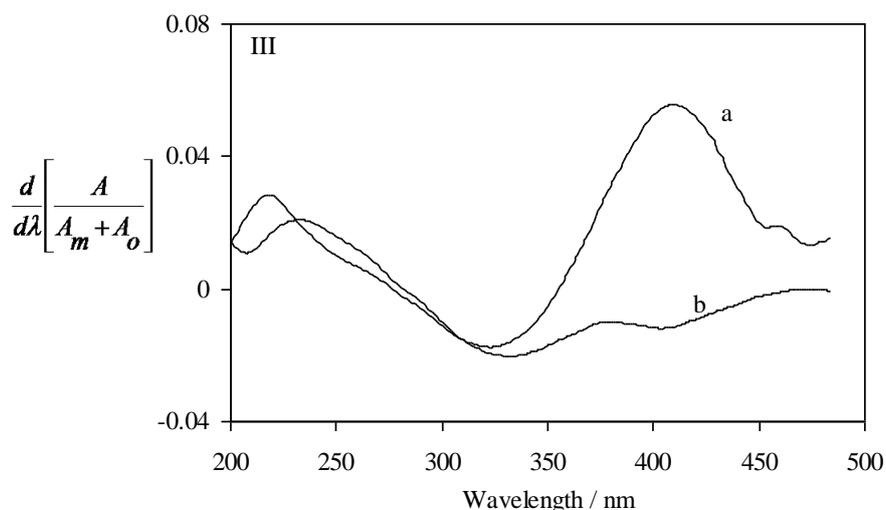


Fig 2 (III). First derivatives of the ratio spectra normalized to the double dividers: (a) p-nitrophenol ($4 \mu\text{g mL}^{-1}$), (b) m-nitrophenol ($9 \mu\text{g mL}^{-1}$) + o-nitrophenol ($9 \mu\text{g mL}^{-1}$) + p-nitrophenol ($4 \mu\text{g mL}^{-1}$) normalized to DD(III) for m- and o-nitrophenol.

Figure 3 (I) shows the derivative of ratio of spectra curves for a series of m-nitrophenol solutions with increasing concentrations normalized to the optical density (DD (IV)) of a standard o-nitrophenol and p-nitrophenol solution. In these spectra, one

maximum (347 nm) and one minimum (386 nm) were found suitable for the quantitative determination of m-nitrophenol in three-component mixtures with o-nitrophenol and p-nitrophenol.

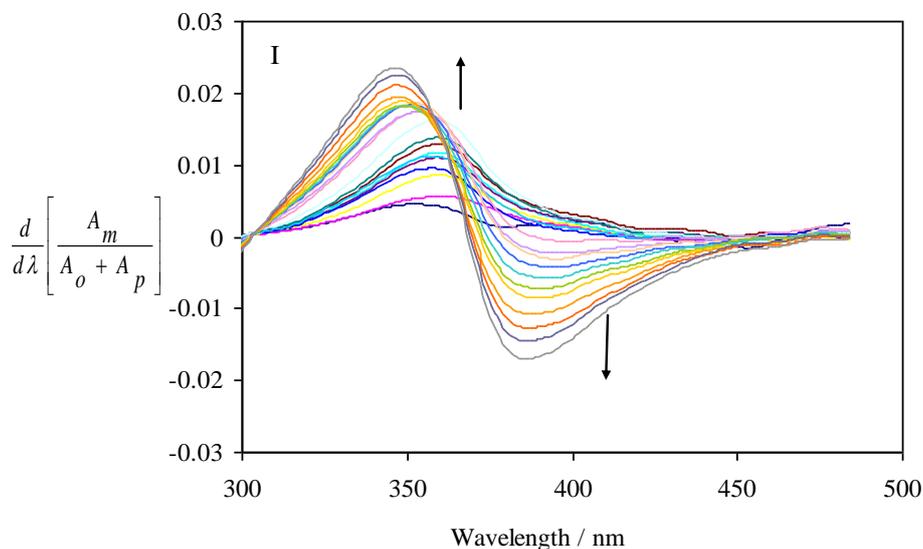


Fig 3 (I). First derivatives of the ratio spectra: m-nitrophenol ($1.0\text{-}25.0 \mu\text{g mL}^{-1}$) relative to o-nitrophenol ($9 \mu\text{g mL}^{-1}$) + p-nitrophenol ($4 \mu\text{g mL}^{-1}$).

Figure 3 (II) shows the derivative of ratio of spectra curves for a series of o-nitrophenol solutions, which are normalized to the optical density (DD (V)) of a standard m-nitrophenol and p-nitrophenol solution. As can be seen, there are

several maxima and minima in these curves. It was found that one maximum (365 nm) and two minimum (410 and 468 nm) can be used for the determination of o-nitrophenol in three-component mixtures with m-nitrophenol and p-nitrophenol.

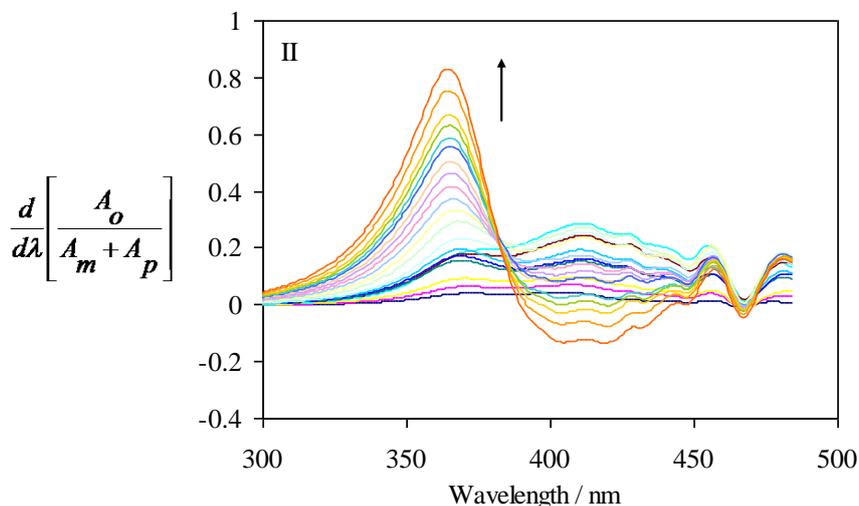


Fig 3 (II). First derivatives of the ratio spectra: o-nitrophenol ($1.0\text{-}25.0 \mu\text{g mL}^{-1}$) relative to m-nitrophenol ($9 \mu\text{g mL}^{-1}$) + p-nitrophenol ($4 \mu\text{g mL}^{-1}$).

Figure 3 (III) shows the derivative of ratio of spectra curves for a series of p-nitrophenol solutions, which are normalized to the optical density (DD (VI)) of a standard m-nitrophenol and o-nitrophenol solution. In these spectra, one maximum (409 nm) and one minimum (330 nm) were found suitable for the quantitative determination of p-nitrophenol in three-component mixtures with m-nitrophenol and o-nitrophenol.

The parameters of calibration graphs used for the determination of nitrophenol isomers using the working points indicated above are presented in Table 2. The predictive ability of this method was determined using 10 three-component mixtures with the compositions given in Table 3. As can be seen, the recovery was also quite acceptable. The parameters of calibration graphs for nitrophenol

isomers at the working wavelengths are given in Table 1.

Validation of calibrations

In order to validate the above calibration graphs different composition mixtures were prepared. We applied the above signal analyzing procedure on

the synthesis mixture for determination of nitrophenol isomers and we observed that the derivative of ratio of spectra method gave us satisfactory results (see Table 2). As can be seen from Table 2, the recovery was also quite acceptable.

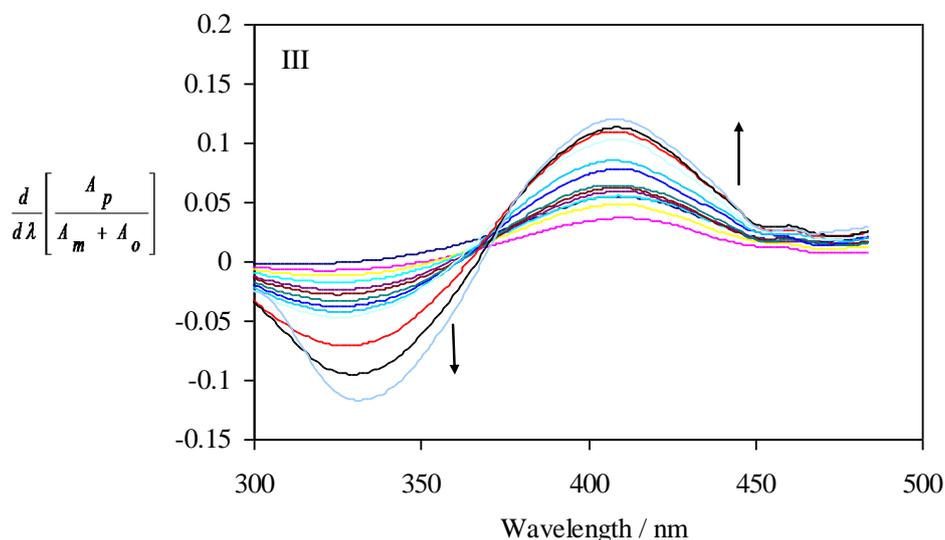


Fig 3 (III). First derivatives of the ratio spectra: p-nitrophenol ($1.0\text{-}15.0 \mu\text{g mL}^{-1}$) relative to m-nitrophenol ($9 \mu\text{g mL}^{-1}$) + o-nitrophenol ($9 \mu\text{g mL}^{-1}$).

Table 1. Parameters of the calibration graphs for the determination of nitrophenol isomers using derivative ratio of spectra method.

Working wavelength, nm	Nitrophenol isomers	Derivative ratio of spectra	Regression equation	r^2	S_f
358	m-nitrophenol	DD(I)	$I=0.0041+0.0013C_m$	0.9933	0.0052
370	o-nitrophenol	DD(II)	$I=0.1741+0.0071C_o$	0.9960	0.0048
412	p-nitrophenol	DD(III)	$I=0.0421+0.0032C_p$	0.9921	0.0038
347	m-nitrophenol	DD(IV)	$I=0.0039+0.001C_m$	0.9929	0.0042
386	m-nitrophenol	DD(IV)	$I=0.0066-0.0002C_m$	0.9965	0.0023
365	o-nitrophenol	DD(V)	$I=0.1555+0.0085C_o$	0.9982	0.0011
410	o-nitrophenol	DD(V)	$I=0.2995-0.0051C_o$	0.9961	0.0012
468	o-nitrophenol	DD(V)	$I=0.0217-0.0007C_o$	0.9946	0.0016
330	p-nitrophenol	DD(VI)	$I=0.0022-0.0048C_p$	0.9993	0.0006
409	p-nitrophenol	DD(VI)	$I=0.0263+0.0065C_p$	0.9928	0.0039

Table 2. Added and found results of synthetic mixtures of nitrophenol isomers by derivative ratio of spectra method ($\mu\text{g mL}^{-1}$).

Added			Found			Recovery (%)		
meta	ortho	para	meta	ortho	para	meta	ortho	para
2.0	2.0	2.0	1.86	1.92	2.01	93.0	96.0	100.5
20.0	20.0	10.0	18.93	21.14	10.43	94.7	105.7	104.3
2.0	12.0	6.0	1.89	11.72	6.04	94.5	97.7	100.7
7.0	15.0	2.0	7.64	14.85	2.03	109.1	99.0	101.5
12.0	11.0	9.0	11.52	10.51	9.25	96.0	95.5	102.8
2.0	2.0	8.0	1.85	1.89	8.13	92.5	94.5	101.6
18.0	18.0	8.0	17.47	18.02	7.79	97.1	100.1	97.4

8.5	8.5	6.0	8.65	7.88	6.13	101.8	92.7	102.2
17.0	8.0	8.0	16.51	7.83	8.31	97.1	97.9	103.9
2.0	15.0	8.0	1.84	14.54	8.06	92.0	96.9	100.8

Statistical parameters

The predictive applicability of a regression model can be defined in various ways. The root mean square error of prediction (RMSEP) and relative

standard error of prediction (RSEP) can be used [20]:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (y_{pred} - y_{obs})^2}{n}} \quad (6)$$

$$RSEP(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^n (y_{pred} - y_{obs})^2}{\sum (y_{obs})^2}} \quad (7)$$

where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed value of the concentration in the sample and n is the number

of samples in the validation set. The value of RMSEP and RSEP(%) for nitrophenol isomers summarized in Table 3.

Table 3. Statistical parameters of the optimized matrix using proposed method in synthesis samples (Table 2).

Nitrophenol isomers	RMSEP	RSEP (%)
m-nitrophenol	0.4907	4.3136
o-nitrophenol	0.4779	3.7985
p-nitrophenol	0.2068	2.8759

Analysis of water samples

The accuracy and precision of the proposed methods as applied to the determination of the nitrophenol isomers in the real matrix samples were checked via a recovery study. To this end, aliquots of solutions of the nitrophenol isomers were supplied with variable amounts, between 1.0-25.0, 1.0-25.0 and 1.0-15.0 $\mu\text{g mL}^{-1}$ for m-nitrophenol, o-nitrophenol and p-nitrophenol, respectively, and their absorbance recorded. As can be seen from Table 4, the results obtained in

the determination of nitrophenol isomers in water samples (river, waste and tap water) were quit good. In fact, the recoveries ranged for m-nitrophenol, o-nitrophenol and p-nitrophenol by proposed method were 85.3 to 96.4 %, 95.0 to 100.5 %, 94.8 to 102.8 %, respectively. Therefore, the derivative ratio of spectra method is able to predict the concentrations of each nitrophenol isomers in the real matrix samples such as water samples.

Table 4. Simultaneous determination of nitrophenol isomers in real matrix samples ($\mu\text{g mL}^{-1}$).

Samples	Added			Founded*			Recovery (%)		
	meta	ortho	para	meta	ortho	para	meta	ortho	para
River	4.0	8.0	6.0	3.41	7.75	6.17	85.3	96.9	102.8
Waste	8.0	10.0	4.0	7.23	10.05	3.86	90.4	100.5	96.5
Tap	10.0	4.0	8.0	9.64	3.86	7.58	96.4	96.5	94.8

* Mean of three measurements.

CONCLUSION

The m-nitrophenol, o-nitrophenol and p-nitrophenol mixture is an extremely difficult complex system due to the high spectral overlapping observed between the absorption for these nitrophenol isomers. This difficulty can be surmounted using differential spectroscopy techniques, in particular, the derivative ratio of spectra method. The results obtained using this method for a series of model mixtures and ready-to-use preparations showed good predicting ability

of the proposed methods. Both procedures can be used for the simultaneous spectrophotometric determination of nitrophenol isomers, without any primary chemical reaction or separating steps in synthetic and natural water samples.

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