ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 13, No. 1 (2012) 53-62

Simultaneous Spectrophotometric Determination of *o*-Aminophenol and *p*-Aminophenol Using H-Point Standard Addition Method -Application to Real Waters

Theia'a N. Al-Sabha* and Nagham N. Habeb

Chemistry Department, College of Education, Mosul University, Mosul, Iraq

Received 12 December 2011, Revised 24 April 2012, Accepted 10 May 2012

Abstract

H-point standard addition method (HPSAM) has been applied to the simultaneous determination of *o*-aminophenol (*oA*) and *p*-aminophenol (*pA*) isomers in binary mixtures. The method is based on the Schiff's base formation of aminophenols with 1,2-naphoquinone sulphonate (NQS) as a chromogenic reagent in the presence of micellar cityltrimethyl ammonium bromide (CTAB) and sodium carbonate. The results showed that simultaneous determinations could be performed with the ratios 0.4 : 2.0 and 4.0 : 2.0 measured at pair of wavelengths (480 and 610) nm with recovery % range 94.75-104.37 % and precision better than 4.0 % when *oA* considered as analyte and *pA* as interferent, and a ratio 0.4 : 0.2 and 4.0 : 2.0 measured at 455 and 540 nm with recovery % range 95.37-105.0 % and precision better than 4.0 % when *pA* considered as analyte and *oA* as interferent.

Keywords: HPSAM; Aminophenol isomers; NQS; Aqueous solution.

Introduction

Aminophenol isomers are primarily used as intermediates in the manufacture of dyes and pigments. These are crystalline solids of low volatility and cause contact dermatitis, which appears to be the greatest hazard arising from their use in industry [1]. These compounds are also the main metabolites of aniline both in vivo and in vitro [2].

Voltammetric method has been reported for determination of aminophenols in a mixtures based on the oxidation peak potential difference between *mA* and *oA* and *oA* and *pA* using single-wall carbon nanotubes modified glassy carbon electrode [3]. Sol-gel technique was used for separation of a mixture of isomeric aminophenols [4]. The only spectrophotometric method reported depends upon the kinetic determinations including the differential reaction-rate method for the simultaneous determination of aminophenols in a mixtures which is based on its oxidation by octacyanomolybdate(v). The data are evaluated using PLS to resolve binary and tertiary mixtures of the three aminophenols [5].

A number of spectrophotometric methods have been developed to determine the composition of a binary mixture. Most of these are directed at mixtures where one component can be isolated from the other or they require a Beer's law experiment to measure the molar absorptivity of each substance in the mixture.. In 1988, HPSAM was presented based on the principle of dual-wavelength spectrophotometry and the standard addition method [6-8]. This method precludes the interference of foreign matter and reagent blank even through the interfering species should be known [9].

*Corresponding Author Email: dr_theiaa@yahoo.com

In this work HPSAM was employed for the determination of *oA* and *pA* isomers in binary mixtures. The suggested method was successfully applied to the determination of these analytes in real waters.

Requirements for applying HPSAM

Consider an unknown sample containing an analyte X and an interferent Y. The determination of the concentration of X by HPSAM requires the selection of two wavelengths λ_1 and λ_2, at which the interferent species, Y, has the same absorbance [10]. A known amount of X is successively added to the mixture, and the absorbances are measured at the two wavelengths and expressed by the following equations:

$$A_{\lambda 1} = b_0 + b + M_{\lambda 1} C_i \quad (1)$$
$$A_{\lambda 2} = A_0 + A` + M_{\lambda 2} C_i \quad (2)$$

Where $A_{\lambda 1}$ and $A_{\lambda 2}$ are the analytical signals measured at λ_1 and λ_2, respectively. b_0 and A_0 ($b_0 \neq A_0$) are the original analytical signal of X at $A_{\lambda 1}$ and $A_{\lambda 2}$, respectively. b and $A`$ are the analytical signals of Y at $A_{\lambda 1}$ and $A_{\lambda 2}$, respectively. $M_{\lambda 1}$ and $M_{\lambda 2}$ are the slopes of the standard addition calibration lines at λ_1 and λ_2, respectively, and Ci is the added X concentration. The two straight lines obtained intersect at the so-called H-point ($-C_H$, A_H). If the component Y is a known interferent and, the analytical signal corresponding to Y, b (at λ_1 or λ_2), does not change with the addition of analyte X, A_H is only related to the signal of the interferent Y at the two selected wavelengths. To evaluate the interferent concentration from the ordinate value of the H-point (A_H), a calibration graph or the absorbance value of an interferent standard is needed. If the component Y is an unknown interferent, the Y analytical signals remain equal with the addition of analyte X. According to the above discussion, at the H-point, C_H is independent of the concentration of interferent and so A_H is also independent of the analyte concentration [11].

Experimental
Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell**,** Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**,** all calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

All reagents were of analytical grade (BDH, Fluka and Molekula companies). A standard solutions of 100 µg ml^{-1} of *oA* and *pA* were prepared separately in 100-ml volumetric flask by dissolving 0.01g in 2.0 ml of ethanol and diluting to the mark with distilled water. They were stored in dark and were found to be stable for at least 4 weeks. 5×10^{-3} M of NQS reagent was prepared by dissolving 0.065 g in distilled water in a 50 ml volumetric flask. 0.1 M sodium bicarbonate was prepared in a 500 ml volumetric flask. 0.1 % of CTAB was prepared in warm distilled water.

Procedure
Individual calibration

Appropriate volumes containing 0.2-10 and 0.08-18 µgml^{-1} of *oA* and *pA* standard solutions, 0.4 and 0.8 ml of NQS reagent solution, 1.0 and 2.5 ml of sodium bicarbonate were added into 25ml volumetric flasks, followed by addition of 1.0 and 1.5 ml of CTAB respectively. The solution was made up to the mark with distilled water, and was left for 10 min at room temperature. A portion of the solution was transferred into a 1cm silica cell to measure the absorbance at 488 and 535 nm against their respective reagent blank for *oA* and *pA*, respectively.

H-point standard addition analysis

Standard solutions of synthetic samples containing different concentrations of *oA* and *pA* were prepared, and standard addition of *oA* or *pA* (up to 4.0 µg ml^{-1}) was made followed by addition of 0.6 ml NQS, 1.5 ml sodium bicarbonate and 1.5 ml CTAB. The volume was made up to 25 ml with distilled water in volumetric flask and left for 10 min at room temperature. Simultaneous spectrophotometric determination of *oA* and *pA* was made with HPSAM at 480 and 610 nm when

oA was considered as analyte and at 445 and 540 nm when *pA* was considered as analyte against their reagent blank for each sample solution. *oA* or *pA* can be determined simultaneously in the concentration range 0.4-4.8 μg ml^{-1}. The procedure was repeated for some synthetic mixtures to show applicability of the method.

Results and Discussion

Aminophenol isomers reacted with NQS reagent to give an orange color with λ_{max} at 488 nm for *oA* and, red color with λ_{max} at 535 nm for *pA* Schiff base in presence of CTAB (Figure1). Since the absorption maxima of the isomers are nearly identical it is difficult to determine them by classical technique. It is Therefore, necessary to use a chemometric method to solve this problem.

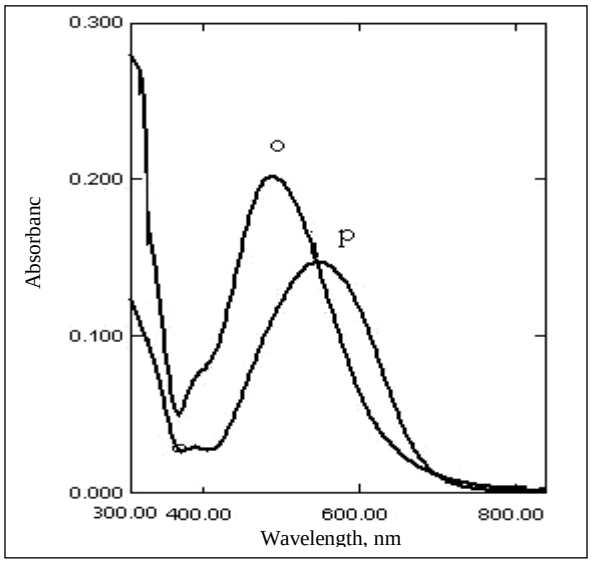

Figure 1. **Absorption spectra of NQS product with 4 μg ml^{-1} (o) *oA* and (p) *pA* at the optimum conditions.**

Effect of variables

To take full advantages of the procedures, the reagent concentrations must be optimized. The parameters were optimized by setting all parameters constant and optimizing one at a time.

Effect of pH

The effect of pH on the absorption spectra for solutions of *oA* and *pA* (4 μg ml^{-1}) was studied. To select a suitable pH for this study, a range between 2.0 and 11.0 pH value was examined. It was found that the products were formed in basic medium with maximum absorption in the ranges of 10.21-11.07 and 10.20-10.50 at 448 and 495 nm, in the absence of surfactant, for *oA* and *pA* respectively. A decrease in absorbance occurs above these range was noticed. The pH value of 10.3 was selected in this study.

Effect of bases and buffer solutions

To obtain high sensitivity for the products, the effect of bases like sodium hydroxide, sodium bicarbonate, sodium carbonate, potassium hydroxide and ammonium hydroxide have been examined. Potassium hydroxide gave maximum absorption at 475 and 525 nm for *oA* and *pA* respectively (Table 1), but with unstable color absorbance. Sodium carbonate gave more stable color at 475 and 495 nm for *oA* and *pA* respectively. Fig. 2 shows that concentration ranges of 1.0-3.0 ml and 1.5-5.0 ml of 0.1 M sodium carbonate yielded maximum absorption for *oA* and *pA* respectively. The effect of ammonium, borate, carbonate and phosphate buffers in above pH ranges were examined but no significant change was observed. Therefore, 1.5 ml of 0.1M sodium carbonate was selected in the subsequent experiments.

Table 1. **Effect of bases on the absorption of *oA* and *pA*.**

Base (0.1M), ml	λ_{max} (nm)	*Ao* Absorbance (Sample)	*Ao* Absorbance (Blank)	λ_{max} (nm)	*Ap* Absorbance (Sample)	*Ap* Absorbance (Blank)
Without	480	0.163	0.030	480	0.195	0.019
NaOH	448	0.189	0.068	495	0.203	0.018
$NaHCO_3$	475	0.184	0.045	480	0.207	0.037
Na_2CO_3	475	0.182	0.040	495	0.239	0.032
KOH	475	0.198	0.071	525	0.283	0.018
NH_4OH	470	0.119	0.192	495	0.148	0.207

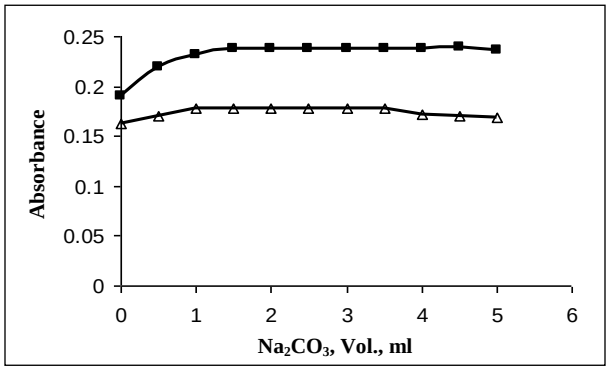

Figure 2. **Effect of Na_2CO_3 concentration on the absorption of reaction mixture of 4 μg ml^{-1} for each (Δ) *oA* measured at 475 nm and (■) *pA* measured at 495 nm in the presence of NQS reagent.**

Effect of NQS reagent

The effect of NQS reagent concentration was studied in the range 0.1-2.4 ml of 5×10^{-3} M. As shown in Fig. 3, it was found that the absorbance of both isomers increased by increasing NQS concentration up to 0.4 and 0.6 ml which remained constant at higher concentrations up to 0.6 and 1.2 ml for *oA* and *pA*, respectively, measured at their respective λ_{max} cited in Table 1, after which the absorbance decreased. Therefore, 0.4 ml of 5×10^{-3} M NQS was selected as optimum concentration.

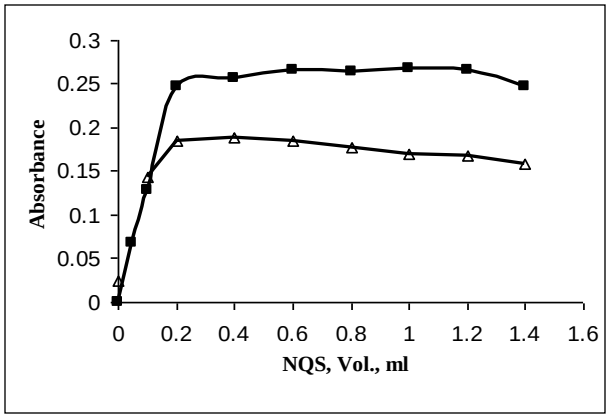

Figure 3. **Effect of NQS reagent concentration on the absorption of reaction mixture of 4 µg ml^{-1} for each (Δ) *oA* measured at 475 nm and (■) *pA* measured at 495 nm in the presence of Na_2CO_3 solution.**

Effect of surfactants

The effect of surfactants, cetyltrimethyl ammonium bromide (CTAB), cetylpyridinium chloride (CPC), Tween-80 (TW-80) and TritonX-100 (TX-100), of 0.1 % concentration, on the absorption spectra of products have been investigated. The cationic surfactants CTAB and CPC shift the maximum absorption to longer wavelength (Table 2) and increased the absorbance of both products, but the anionic SDS and nonionic TX-100 and TW-80 surfactants showed no positive effect. CTAB as a cationic surfactant was most effective, and the color was stable and reproducible. The absorbance increases with CTAB concentration up to 1.0 ml and remains constant up to 3.0 ml for both isomers (Fig. 4). Therefore 1.5 ml of 0.1% CTAB was selected for further investigation.

Table 2. **Effect of surfactants on the absorption of 4 µg ml^{-1} aminophenol isomer products.**

Surfactant	*oA*		*pA*	
	λ_{max} (nm)	Abs.	λ_{max} (nm)	Abs.
Without	475	0.188	495	0.262
CPC	475	0.189	525	0.285
CTAB	488	0.193	535	0.296
SDS	465	0.184	495	0.255
TX-100	465	0.187	500	0.259
TW-80	465	0.176	500	0.259

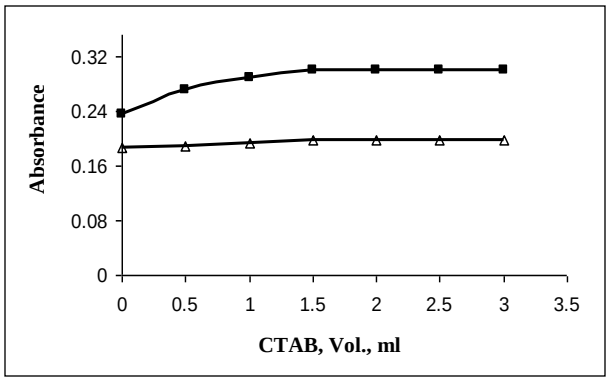

Figure 4. **Effect of CTAB concentration on the absorption of reaction mixture of 4 µg ml^{-1} for each (Δ) *o*A and (■) pA in the presence of NQS and Na_2CO_3 solution.**

Effect of temperature and developing time

The effect of temperature on the rate of reaction for isomers was studied at 25 and 40°C. The results indicated that products were formed after addition of reagents immediately at room temperature (25°C) and reached its maximum absorbance after 5.0 min at 488 and 535 nm for *oA* and *pA* respectively. It remains stable for more than 1h, although a decrease in absorbance with time was noticed at 40°C. Therefore, 10 min at 25°C was used in this work.

Quantification

The Beer's law limits and molar absorptivity values were evaluated (Table 3). The corresponding correlation coefficient for the aminophenol isomers determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of six replicates of

each three different concentrations for the isomers indicated that the method is precise and accurate. Limit of quantitation (LOQ) is determined by taking the ratio of standard deviation of the blank with respect to water and the slope of calibration curve multiplied by a factor of 10. LOQ is approximately 3.3 times greater than LOD. Naturally, the LOQ slightly crosses the lower but LOD is well below the lower limit of Beer's law range.

Table 3. **Summary of optical characteristics and statistical data for the proposed method.**

Parameter	*oA*	*pA*
Beer's law limits (μg ml^{-1})	0.2-10	0.08-18.0
Molar absorptivity ($l.mol^{-1}. cm^{-1}$)	5167	7647
LOD (μg.ml^{-1})	0.05188	0.03480
LOQ (μg.ml^{-1})	0.1729	0.1160
Average recovery (%)**	100.23	102.50
Correlation coefficient	0.9994	0.9993
Regression equation (Y)*		
Slope, *a*	0.0474	0.0704
Intercept, *b*	0.0019	0.0145
RSD**	≤ 2.48	≤ 3.57

*$Y = a\,X + b$, where X is the concentration of analyte in μg ml^{-1}.
** Average of six determinations.

H-point standard addition method for simultaneous spectrophotometric determination of oA and pA

Based on the optimum conditions obtained above and cited in (Table 4), HPSAM has been applied for simultaneous determination of *oA* and *pA* isomers in binary mixtures.

Table 4. **Compromised reaction conditions for determination of aminophenol isomers.**

NQS		Na_2CO_3		CTAB		Tem. (°C)	Time (min)
Conc., M	Vol., ml	Conc., M	Vol., ml	Conc., %	Vol., ml		
5×10^{-3}	0.4	0.1	1.0	0.1	2.0	RT*	10

*RT-25°C

Wavelength selection

To select the appropriate wavelength pair for using HPSAM the following principles should be applied. At the selected wavelengths the analyte signals must be linear with concentration and the interferent signal must be equal and remaining unchanged by changing the analyte concentration. The analytical signal obtained from a mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths (λ_1 and λ_2) must be as large as possible in order to get good accuracy and sensitivity [12-15].

For determination of *oA* and *pA* in binary mixture, we selected two pairs of wavelengths for above spectra isomers. In this case there were several pairs of wavelengths (Table 5).

Table 5. **Amounts found of *oA* and *pA* by applying HPSAM at different pairs of wavelengths.**

Wavelengths (nm)	A-C Equation	R^2	Amount present (μg ml^{-1})		Amount found (μg ml^{-1})	
			oA	*pA*	*oA*	*pA*
480 - 610	Y_{480}=0.0488x+0.2161 Y_{610}=0.0157x+0.1505	0.9941 0.9753	2.0	2.0	1.98	1.86
490- 600	Y_{490}=0.0485x+0.2313 Y_{600}=0.0168x+0.1714	0.9933 0.9785	2.0	2.0	2.00	2.08
500- 590	Y_{500}=0.0477x+0.2427 Y_{590}=0.0217x+0.1906	0.9919 0.9790	2.0	2.0	2.00	2.29
465 - 520	Y_{465}=0.0432x+0.1618 Y_{520}=0.0764x+0.2303	0.9957 0.9974	2.0	2.0	2.01	2.06
470 - 515	Y_{470}=0.0460x+0.1717 Y_{515}=0.0739x+0.2282	0.9961 0.9978	2.0	2.0	2.14	2.02
455 - 540	Y_{455}=0.0380x+0.1383 Y_{540}=0.0826x+0.2294	0.9957 0.9969	2.0	2.0	2.18	2.04

The concentration of interferent was calculated in each sample by the calibration method with a single standard and the ordinate value of the H-point (A_H). As it is shown in Table 5, the best wavelength pairs were 610-480 and 455-540 nm for *oA* and *pA* as analytes, respectively (Fig. 5).

Standard solutions of two species were initially tested to validate the applicability of the chosen wavelengths. (Fig. 6, 7) are H-point standard addition calibration lines constructed at two selected wavelengths, 610-480 nm for determination of *oA* as analyte and *pA* as interferent and constructed at 455-540 nm for determination of *pA* as analyte and *oA* as interferent. According to the characteristics of HPSAM at the H-point, C_H is independent of interferent concentration, but A_H is dependent of the interferent. Several synthetic mixtures with different concentration ratios of *oA* and *pA* were analyzed by the proposed method (Tables 6, 7).

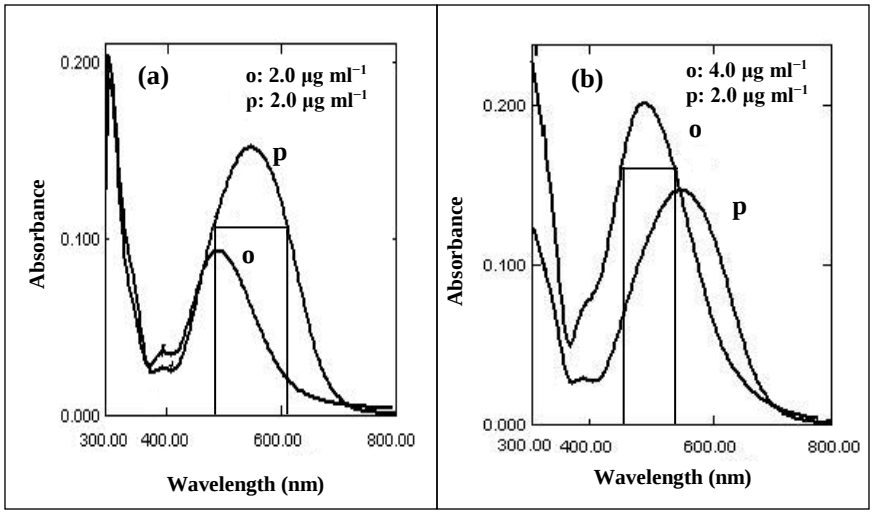

Figure 5. **Absorption spectra of (o) *oA* and (p) *pA* under the compromised conditions and wavelength selection when (a) *oA* as analyte and (b) *pA* as analyte.**

Table 6. **Results of several experiments for the analysis of *oA- pA* mixtures considering *oA* as analyte at different concentration ratios by HPSAM.**

A-C Equation	R^2	Present (µgm^{-1})		*oA*		*pA*	
		pA	*oA*	Found (µgml^{-1})	Recovery (%)	Found (µgml^{-1})	Recovery (%)
Y_{480}=0.0419x+0.4346 Y_{610}=0.0131x+0.3649	0.9833 0.9509	2.4	5.6	2.42	100.83	5.55	99.10
Y_{480}=0.0475x+0.3165 Y_{610}=0.0100x+0.1679	0.9861 0.9761	4.0	2.0	3.96	99.00	2.01	100.50
Y_{480}=0.0487x+0.2482 Y_{610}=0.0126x+0.2054	0.9908 0.9821	1.2	3.2	1.18	98.75	3.09	96.43
Y_{480}=0.0509x+0.2319 Y_{610}=0.0163x+0.1613	0.9899 0.9844	2.0	2.0	2.04	102.00	2.01	100.50
Y_{480}=0.0531x+0.1714 Y_{610}=0.0206x+0.1454	0.9958 0.9806	0.8	2.0	0.80	99.87	2.03	101.50
Y_{480}=0.0385x+0.3193 Y_{610}=0.0108x+0.2645	0.9852 0.9228	2.0	4.0	1.98	98.90	4.00	100.00
Y_{480}=0.0472x+0.3533 Y_{610}=0.0121x+0.2431	0.9678 0.9677	3.2	3.2	3.14	98.09	3.34	104.37
Y_{480}=0.0385x+0.3193 Y_{610}=0.0108x+0.2645	0.9852 0.9228	2.0	4.8	2.01	100.50	4.80	100.00
Y_{480}=0.0472x+0.3533 Y_{610}=0.0121x+0.2431	0.9678 0.9677	0.4	2.0	0.38	94.75	1.96	97.75
Y_{480}=0.0413x+0.2881 Y_{610}=0.0101x+0.2260	0.9903 0.9662	2.0	3.2	1.99	99.50	3.20	100.00

Table 7. **Results of several experiments for the analysis of *pA*-*oA* mixtures considering *pA* as analyte at different concentration ratios by HPSAM.**

A-C Equation	R^2	Present (μgm^{-1})		*oA*		*pA*	
		pA	*oA*	Found (μgml^{-1})	Recovery (%)	Found (μgml^{-1})	Recovery (%)
Y_{540}=0.0778x+0.1043 Y_{455}=0.0368x+0.0867	0.9993 0.9954	0.4	2.0	0.42	105.00	1.97	98.65
Y_{540}=0.0766x+0.1476 Y_{455}=0.0358x+0.0988	0.9981 0.9974	1.2	1.6	1.20	100.00	1.64	102.50
Y_{540}=0.0813x+0.2380 Y_{455}=0.0371x+0.1461	0.9987 0.9956	2.0	2.0	2.07	103.50	1.93	96.50
Y_{540}=0.0944x+0.3818 Y_{455}=0.0445x+0.2077	0.9996 0.9870	3.2	1.6	3.4	106.25	1.56	97.50
Y_{540}=0.0783x+0.1336 Y_{455}=0.0365x+0.1017	0.9986 0.9975	0.8	2.0	0.76	95.37	2.01	100.50
Y_{540}=0.0660x+0.3546 Y_{455}=0.0287x+0.2799	0.9961 0.9975	2.0	4.8	2.00	100.00	4.82	100.41
Y_{540}=0.0976x+0.2124 Y_{455}=0.0461x+0.1478	0.9980 0.9939	1.2	2.4	1.25	104.10	2.39	99.58
Y_{540}=0.0754x+0.3972 Y_{455}=0.0319x+0.2216	0.9994 0.9649	4.0	2.0	4.03	100.75	2.08	104.00
Y_{540}=0.0754x+0.0774 Y_{455}=0.0347x+0.0460	0.9966 0.9917	0.8	0.8	0.77	96.37	0.82	102.50
Y_{540}=0.0785x+0.2824 Y_{455}=0.0377x+0.1986	0.9989 0.9974	2.0	3.2	2.05	102.50	3.08	96.25

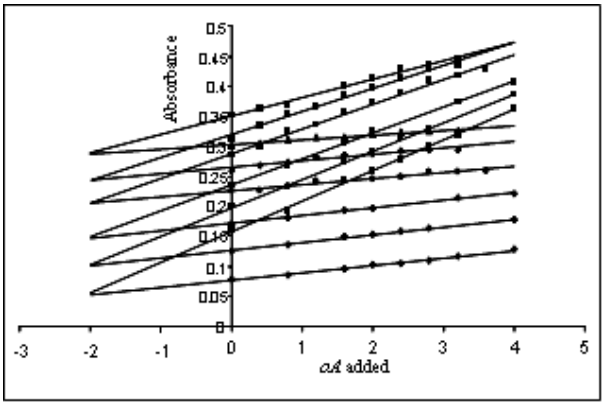

Figure 6. **Plots of H-point standard addition method for fixed amount *oA* (2.0 μg ml^{-1}) in the presence of 0.8, 1.6, 2.0, 3.2, 4.0 and 4.8 μg ml^{-1} of *p*A measured at (■) 480 nm and (♦) 610 nm.**

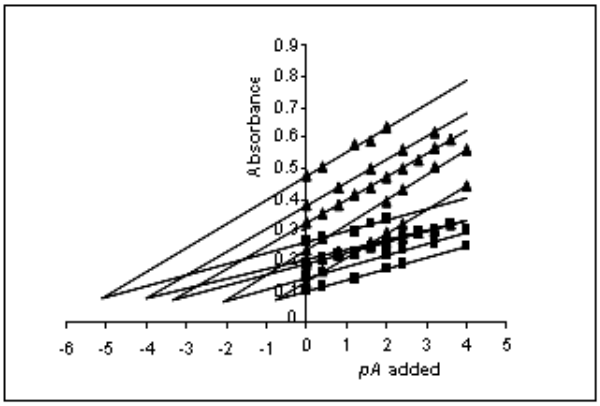

Figure 7. **Plots of H-point standard addition method for fixed amount *oA* (2.0 μg ml^{-1}) in the presence of 0.8, 2.0, 3.2, 4.0 and 5.0 μg ml^{-1} of *pA* measured at (♦) 531 nm and (■) 443 nm.**

Reproducibility and accuracy of the method

To check the reproducibility of the method four replicate experiments for the analysis of *oA* and *pA* were carried for a series of samples containing a fixed amount of *pA* in the presence of various amounts of *oA* or a fixed amount of *oA* in the presence of various amounts of *pA* by the addition of *oA* or *pA* standard solutions respectively (Tables 8, 9) show good accuracy. The relative standard deviations (RSD) for three replicate measurements of the mixture was < 4.0 % for each of both isomers.

Table 8. **Results of three replicates experiment of three concentrations for the analysis of *oA* in the presence of *pA* as interferent in mixtures.**

A-C Equation	R^2	Present (μgml^{-1})		Found (μgml^{-1})		RSD* (%)	
		oA	*pA*	*oA*	*pA*	*oA*	*pA*
Y_{480}=0.0531x+0.1714	0.9958	0.8	2.0	0.80	2.03		
Y_{610}=0.0206x+0.1454	0.9806						
Y_{480}=0.0552x+0.1703	0.9980	0.8	2.0	0.82	1.96	1.68	2.4
Y_{610}=0.0203x+0.1415	0.9825						
Y_{480}=0.0542x+0.1731	0.9961	0.8	2.0	0.83	2.01		
Y_{610}=0.0205x+0.1450	0.9856						
Y_{480}=0.0509x+0.2319	0.9899	2.0	2.0	2.04	2.01		
Y_{610}=0.0163x+0.1613	0.9844						
Y_{480}=0.0511x+0.2274	0.9954	2.0	2.0	1.934	2.01	2.41	0.51
Y_{610}=0.0146x+0.1564	0.9761						
Y_{480}=0.0510x+0.2306	0.9916	2.0	2.0	1.98	2.03		
Y_{610}=0.0151x+0.1595	0.9664						
Y_{480}=0.0465x+0.3229	0.9914	4.0	2.0	4.20	1.96		
Y_{610}=0.0093x+0.1650	0.9904						
Y_{480}=0.0471x+0.3204	0.9895	4.0	2.0	3.94	2.11	3.2	3.4
Y_{610}=0.0091x+0.1704	0.9902						
Y_{480}=0.0475x+0.3165	0.9861	4.0	2.0	3.96	2.01		
Y_{610}=0.0100x+0.1679	0.9761						

***** Average of three determinations

Table 9. **Results of three replicates experiment of three concentrations analysis of *pA* in the presence of *oA* as interferent in mixtures.**

A-C Equation	R^2	Present (μgml^{-1})		Found (μgml^{-1})		RSD* (%)	
		pA	*oA*	*pA*	*oA*	*pA*	*oA*
Y_{540}=0.0886x+0.1464	0.9986	0.8	2.0	0.81	2.04		
Y_{455}=0.0415x+0.1081	0.9935						
Y_{540}=0.0783x+0.1336	0.9986	0.8	2.0	0.76	2.01	3.88	1.16
Y_{455}=0.0365x+0.1017	0.9975						
Y_{540}=0.0853x+0.1459	0.9985	0.8	2.0	0.83	2.06		
Y_{455}=0.0398x+0.1081	0.9947						
Y_{540}=0.0816x+0.2398	0.9975	2.0	2.0	2.03	2.03		
Y_{455}=0.0389x+0.1530	0.9921						
Y_{540}=0.0800x+0.2373	0.9972	2.0	2.0	2.05	2.01	0.87	2.37
Y_{455}=0.0374x+0.1498	0.9867						
Y_{540}=0.0813x+0.2380	0.9987	2.0	2.0	2.07	1.93		
Y_{455}=0.0371x+0.1461	0.9956						
Y_{540}=0.0751x+0.3242	0.9984	3.2	2.0	3.33	2.03		
Y_{455}=0.0352x+0.1911	0.9926						
Y_{540}=0.0751x+0.3149	0.9985	3.2	2.0	3.17	2.08	3.07	1.31
Y_{455}=0.0331x+0.1815	0.9868						
Y_{540}=0.0788x+0.3202	0.9987	3.2	2.0	3.12	2.03		
Y_{455}=0.0366x+0.1883	0.9821						

Interference study

The influence of the presence of several cations and anions on the determination of 2.0 μg ml^{-1} of *oA* and *pA* have been investigated by HPSAM at wavelengths 480 and 610 nm. The tolerance limit was defined as the concentration of the added ion causing less than a ±3 % relative error. The results showed that 2000 μg ml^{-1} Na^+, K^+, Mg^{2+}, SO_4^{2-}, NO_3^-, Cl^-, and 1000, 250 and 100 μg ml^{-1} Al^{3+}, Fe^{2+} and Ca^{2+} respectively did not interfere with the determination of aminophenols. Some aliphatic amines as interferents have been investigated to examine the selectivity of the method. It was found that 40 μg ml^{-1} dibutylamine and tributylamine and 20 μg ml^{-1} butylamine did not interfer.

Application of HPSAM for determination of oA and pA isomers in real water samples

To evaluate the applicability of the proposed method to real samples, simultaneous determination of *oA* and *pA* in real water samples was done. The samples tested were found to be free from *oA* and *pA*. Synthetic samples were prepared by adding known amounts of *oA* and *pA* to the water samples. The absorbance was measured at pairs of wavelengths (480-610) and (455-540) nm (Tables 10, 11). Since the recoveries are between 93.5 and 110.0 %, there is no serious interference in such water samples. The proposed method is therefore, suitable for simultaneous determination of *oA* and *pA* in water samples.

Table 10. **Simultaneous determination of *oA* and *pA* in water samples considering *oA* as analyte.**

Types of water	A-C Equation	R^2	*oA*		*pA*	
			Found (μgml^{-1})	Recovery (%)	Found (μgml^{-1})	Recovery (%)
Tap water	Y_{540}=0.0623x+0.0877	0.9949	1.0	98.90	1.0	98.00
	Y_{455}=0.0325x+0.0582	0.9880				
	Y_{540}=0.0591x+0.1967	0.9979	2.0	100.50	2.0	105.00
	Y_{455}=0.0302x+0.1385	0.9904				
	Y_{540}=0.0530x+0.3771	0.9793	4.0	103.75	4.0	96.75
	Y_{455}=0.0301x+0.2820	0.9848				
River water	Y_{540}=0.0812x+0.1069	0.9970	1.0	97.30	1.0	101.90
	Y_{455}=0.0397x+0.0665	0.9843				
	Y_{540}=0.0704x+0.2204	0.9945	2.0	103.50	2.0	102.00
	Y_{455}=0.0350x+0.1469	0.9857				
	Y_{540}=0.0498x+0.3752	0.9937	4.0	102.75	4.0	104.00
	Y_{455}=0.0252x+0.2740	0.9808				
well water	Y_{540}=0.0770x+0.1050	0.9962	1.0	99.50	1.0	103.00
	Y_{455}=0.0343x+0.0625	0.9922				
	Y_{540}=0.0768x+0.2246	0.9952	2.0	97.60	2.0	102.00
	Y_{455}=0.0386x+0.1500	0.9870				
	Y_{540}=0.0629x+0.4159	0.9794	4.0	102.75	4.0	96.75
	Y_{455}=0.0346x+0.2994	0.9769				
Sea water	Y_{540}=0.0841x+0.1084	0.9955	1.0	98.50	1.0	96.90
	Y_{455}=0.0421x+0.0670	0.9967				
	Y_{540}=0.0841x+0.2389	0.9988	2.0	100.00	2.0	97.85
	Y_{455}=0.0436x+0.1577	0.9787				
	Y_{540}=0.0674x+0.4308	0.9847	4.0	103.50	4.0	93.50
	Y_{455}=0.0432x+0.3305	0.9908				

Table 11. **Simultaneous determination of *oA* and *pA* in water samples considering *pA* as analyte.**

Types of water	A-C Equation	R^2	*oA*		*pA*	
			Found (μgml^{-1})	Recovery (%)	Found (μgml^{-1})	Recovery (%)
Tap water	Y_{480}=0.0447x+0.1167	0.9924	1.0	102.50	1.0	101.30
	Y_{610}=0.0174x+0.0887	0.9893				
	Y_{480}=0.0483x+0.2274	0.9969	2.0	100.50	2.0	101.50
	Y_{610}=0.0122x+0.1545	0.9905				
	Y_{480}=0.0473x+0.4395	0.9830	4.0	99.50	4.0	103.25
	Y_{610}=0.0160x+0.3148	0.9754				
River water	Y_{480}=0.0600x+0.1264	0.9922	1.0	96.60	1.0	98.60
	Y_{610}=0.0157x+0.0836	0.9838				
	Y_{480}=0.0578x+0.2411	0.9986	2.0	97.65	2.0	100.90
	Y_{610}=0.0172x+0.1618	0.9847				
	Y_{480}=0.0433x+0.4056	0.9971	4.0	100.50	4.0	95.25
	Y_{610}=0.0139x+0.2873	0.9624				
well water	Y_{480}=0.0431x+0.1148	0.9842	1.0	102.50	1.0	101.30
	Y_{610}=0.0152x+0.0862	0.9704				
	Y_{480}=0.0455x+0.2233	0.9975	2.0	101.50	2.0	102.50
	Y_{610}=0.0125x+0.1561	0.9846				
	Y_{480}=0.0435x+0.4345	0.9836	4.0	103.25	4.0	104.75
	Y_{610}=0.0145x+0.3146	0.9531				
Sea water	Y_{480}=0.0446x+0.1128	0.9963	1.0	100.00	1.0	98.20
	Y_{610}=0.0124x+0.0806	0.9236				
	Y_{480}=0.0428x+0.2144	0.9985	2.0	98.20	2.0	102.50
	Y_{610}=0.0058x+0.1417	0.9917				
	Y_{480}=0.0401x+0.4169	0.9805	4.0	110.00	4.0	94.25
	Y_{610}=0.0091x+0.2792	0.9387				

Conclusion

The HPSAM has been applied for simultaneous determination of *oA* and *pA* isomers. The method is based on the color of the product in the reaction with NQS in the presence of sodium carbonate in micellar media. It offers good selectivity, accuracy and precision and has been applied successfully for simultaneous determination of these isomers in real water samples. The proposed method needs no extraction steps. It is simpler than the other spectrophotometric method [5].

References

1. J. M. Stellman, Encyclopaedia of occupational health and safety, fourth ed., International labour Office, Vol. 5, USA, (1998).
2. D. V. Parke, *J. Biochem*, 77 (1960) 493.
3. Z. Wang, H. Zhu, H. Zhang, G. Gao, H. Suna, Z.; Liu and X. Zhao, *Electrochimica Acta*, 54 (2009) 7531.
4. Y. C. Wang, Z. R. Zeng, C. H. Xie, N. Guan, E. Q. Fu and J. K. Cheng, Chromatographia, 54 (2001) 475.
5. G. Lopez-Cueto, S. Maspoch, J. F. Rodriguez-Medina and C. Ubide, *Analyst*, 121 (1996) 407.
6. F. Bosch-Reig and P. Campins-Falco, *Analyst*, 113 (1988) 1011.
7. F. Bosch-Reig and P. Campins-Falco, *Analyst*, 115 (1990) 111.
8. P. Campins-Falco, J. Verdu-Andres, F. Bosch-Reig and C. Molins-Legua, *Anal. Chim. Acta*, 270 (1992) 253.
9. J. Yang, G. Zhou, N. Jie, R. Han, C. Lin, and Hu, *J, Anal. Chim. Acta*, 325 (1996) 195.
10. F. Bosch-Reig and P. Campins-Falco, *Analyst*, 113 (1988) 1011.
11. A. Safavi and H. Abdollahi, *Talanta*, 54 (2001) 727.
12. P. Campíns-Falcó, F. Bosch-Reig, R. Herráez-Hernández, A. Sevillano-Cabeza, *Anal. Chim. Acta*, 257 (1992) 89.
13. E. Shams, H. Abdollahi, M. Yekehtaz, R. Hajian, *Talanta*, 63 (2004) 359.
14. E. Shams, H. Abdollahi and R. Hajian, Electroanalysis, 17 (2005) 1589.
15. A. A. Ensafi and R. Hajian, *Anal. Chim. Acta*, 580 (2006) 236.