Spectrophotometric Determination of p-Aminophenol Via Oxidative Coupling Reaction with N-(1-Naphthyl) ethylendiamine dihydrochloride in Presence of Potassium Iodate– Application to Paracetamol

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(Received on 7/8 /2008) **(Accepted for publication 25/1 /2009)**

Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of p-aminophenol is described. The method based on the oxidative coupling reaction of p-aminophenol with N-(1-naphthyl) ethylendiamine (N-NED) using potassium iodate as oxidizing agent in alkaline medium to form a water soluble product, that is stable and has a maximum absorption at 520 nm. Beer's law is obeyed in a concentration range 10 to 160 µg p-aminophenol / 25 ml with a molar absorptivity of 0.6002×10^4 $L.mol^{-1}.cm^{-1}$, a relative error of –0.59 to +4.92% and a relative standard deviation of ± 0.89 to ± 4.49% depending on the concentration of *p*-aminophenol. The proposed method has been successfully applied to the determination of paracetamol in various pharmaceutical preparations after acidic hydrolysis to *p*-aminophenol.

الخلاصة

يتضمن البحث طريقة طيفية بسيطة وسريعة وحساسة لتقدير بارا-أمينو فينول. تتـضمن الطريقـة تفاعل الأكسدة والاقتران للبارا-أمينو فينول مع ن-(1-نفثايل) اثيلين ثنـائي الأمـين (N-NED) باسـتخدام يوديت البوتاسيوم بوصفه عامل مؤكسد وفي وسط قاعدي. الناتج المتكون يكون ذائب بالماء ومستقر ويعطي أعلى شدة امتصاص عند الطول الموجي الأعظم 520 نانوميتر. كانت حدود قانون بير في مـدى التركيـز 10-160 مايكروغرام / 25 مل بارا أمينو فينول وقيمة الامتصاصية المولارية 0.6002×10^4 لتـر.مـول$^{-1}$.سم$^{-1}$. كان الخطأ النسبي بين 0.59- إلى 4.92+% وقيمة الانحراف القياسي النـسبي بـين 0.89± إلـى 4.49±% اعتماداً على مستوى التركيز للبارا-امينو فينول. الطريقة المقترحة تم تطبيقها بنجاح فـي تقـدير الباراسيتامول في مستحضراته الصيدلانية بعد اجراء عملية التحلل الحامضي للباراسيتامول.

Introduction

p-Aminophenol is used as a biological indicator when human being is exposed to aniline and is not considered as a cancerous compound [(1)], but it causes liver damage when the exposure to it lasts for longe period, also the nervous system and bone marrow are affected. *p*-aminophenol is consider as a metabolism product from paracetamol [(2)], thus is used in drugs product [(3)] and in dyes manufacturing specially hair dyes [(4)] and also in rubber manufacturing [(5)]. Several spectrophotometric meth-ods have been reported for the estimation of *p*-aminophenol using different reagents such as, *p*-xylenol in presence of sodium periodate [(6)], 2,3-dichloro-5,6-dicyano p-benzo-quinone(DDQ) in weak alkaline medium [(7)],resorcinol in alkaline medium[(8)], resorcinol in presence of manganese [(9)] and fluoroglucinol [(10)]. Other spectrophotometric methods included estimation of *p*-aminophenol result from acidic or basic hydrolysis of paracetamol using different reagents such as, vanillin [(11)], alkaline phenol in presence of potassium ferrocyanate [(12)], sodium sulphide in presence of Ce^{4+} or Fe^{3+} [(13)], 2,2`(1,4-phenylendivinylene) bis-8-hydroxy quinoline[(14)],ortho cresol[(15)] and chloranil [(16)].

Oxidative coupling organic rea-ctions are now a well established methods that when applied to the analysis of pharmaceutical prepar-ations [(17-19)],it can be considered to be an advantageous alternative to others normally used, owing to its simplicity, sensitivity and versatility of applications.

The present work aims mainly to develop a sensitive and accurate spectrophotometric method for the determination of *p*-aminophenol as pure and in paracetamol(after acidic hydrolysis) dosage.The method ba-sed on the reaction of *p*-ami-nophenol with N-(1-naphthyl)ethyl-enediamine in presence of potass-ium iodate in alkaline medium.

Experimental

Apparatus

All spectral and absorbance measurements were performed on Shimadzu UV-Visible-160 double beam recording spectrophotometer using 1 cm silica cell. pH meter type Philips PW 9420 was used for pH reading.

Reagents

All chemicals used in this investigation are of analytical – reagent grade.

Solutions

p*-Aminophenol *solution, 100 $\mu g.ml^{-1}$. This solution is prepared by dissolving 0.01 g of *p*-aminophenol in 10 ml of ethanol to increase solubility and diluted to 100 ml in a volumetric flask with distilled water.

N-(1-naphthyl)ethylenediamine-dihydrochlorid (N-NED), 0.005M. This solution is prepared by dissolving 0.1295 g of N-NED (Fluka) in 100 ml distilled water.

Potassium iodate, 0.015 M. This solution is prepared by dissolving 0.321 g of potassium iodate (Fluka) in 100 ml distilled water.

Sodium hydroxide solution, 1N. This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to a plastic bottle

Paracetamol tablets solution, 100 $\mu g.ml^{-1}$. Weighted and finely powdered 10 tablets (each one contain 500 mg paracetamol), an accurately weighed amount of powder equivalent to 0.25 g paracetamol is dissolved in 10 ml ethanol, then 100-150 ml distilled water is added, shaking to increase the solubility, filtered into 250 ml calibrated flask, then the solution is completed to the volume with a distilled water(the solution is equivalent to 1000 $\mu g.ml^{-1}$ paracetamol), then 150 ml of 1000 $\mu g.ml^{-1}$ paracetamol is transferred into 250 ml round bottom flask provided with condenser,25ml of hydrochloric acid (11.8N) is added then reflux for 1 hour, after that the cold solution is neutralized by 20% sodium carbonate and diluted to 250 ml with distilled water in a volumetric flask. To prepared 100 $\mu g.ml^{-1}$ paracetamol (equivalent to 72.20$\mu g.ml^{-1}$ *p*-aminophenol).A 16.6 ml of above solution is diluted to 100 ml in a volumetric flask using distilled water.

Paracetamol syrup solution, 100 $\mu g.ml^{-1}$. A 10.4 ml of syrup (each 5ml contain 120 mg paracetamol) is transferred into a 250 ml calibrated

flask and the total volume is diluted with distilled water, and proceed the procedure as mentioned in preparation hydrolys-ed paracetamol solution from tablets.

Paracetamol suppositories solution, 100 μg.ml^{-1}. Weighed and mixed wells 4 suppositories (each suppositories contain 250 mg paracetamol). An accurate weight amount of mixture equivalent to 0.250 g paracetamol is dissolved in boiling distilled water, filtered, and the residues are washed with 10ml ethanol and boiling distilled water and the volume is completed to 250 ml in calibrated flask with distilled water, and proceeds the procedure as mentioned in preparation hydrolysed paracetamol solution from tablets.

Procedure and calibration graph

To a series of 25-ml calibrated flask transfer 0.1-2 ml of *p*-aminophenol solution (100 μg.ml^{-1}), then 3 ml of 5×10^{-3}M N-NED and 2 ml of 0.015M potassium iodate added. After that a 0.5 ml of 1 N sodium hydroxide added. Then the solutions are standed for 10 minutes before the volumes are completed to the mark with distilled water, the absorbance was read at 520 nm after 15 minutes from final addition against the reagent blank. A linear calibration graph is obtained over the concentration range of 10-160 μg *p*-aminophenol / 25 ml and a concentration above 160 μg / 25 ml gives negative deviation (Fig 1). The molar absorptivity has been found to be 0.6002×10^{4} l.mol^{-1}.cm^{-1}.

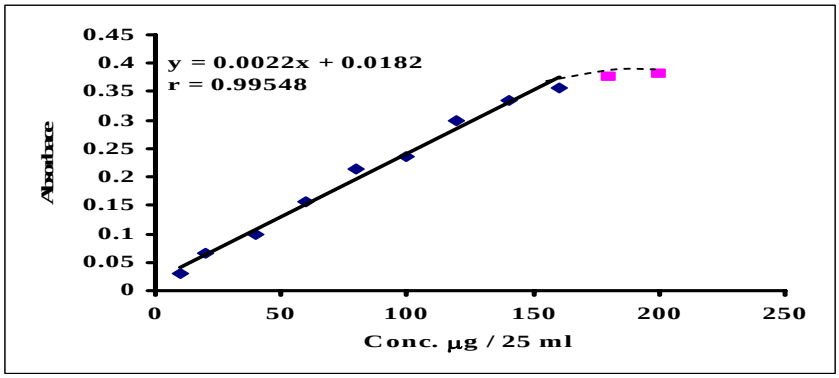


Fig. 1. Calibration graph of *p*-aminophenol determination

Results and discussion

The effect of various variables on the colour development is tested to establish the optimum conditions for the reaction of *p*-aminophenol with N-(1-naphthyl) ethylenediamine (N-NED) in presence of potassium iodate.For the subsequent experiments, 100μg of *p*-aminophenol is taken in 25 ml final volumes.

Effect of base on absorbance and colour contrast

The preliminary experiments have shown that *p*-aminophenol can give high intensity of coloured dye with (N-NED) in presence of potassium iodate in alkaline medium. So that different type and amounts of bases are examined (Table 1).

Table 1 The effect of base on absorbance and colour contrast

Base used (1 N)	Variable	Absorbance / ml base used					
		0	**0.5**	**1.0**	**1.5**	**2**	**3**
NaOH	A	0.171	0.224	0.218	0.217	0.215	0.216
	$\Delta\lambda^*$	51.5	99.0	100.0	99.5	101.0	103.0
	pH	3.76	11.61	11.82	12.05	12.78	12.8
KOH	A	0.164	0.220	0.209	0.209	0.207	0.209
	$\Delta\lambda$	49.5	98.0	99.0	99.0	99.5	100.0
	pH	3.53	11.67	11.90	12.09	12.19	12.44
Na_2CO_3	A	0.162	0.206	0.209	0.215	0.216	0.213
	$\Delta\lambda$	6	5	10	9	11	7.5
	pH	4.68	11.0	11.24	11.33	11.41	11.53
$NaHCO_3$	No colour contrast						

* $\Delta\lambda$ (colour contrast) = $\lambda_{max}S - \lambda_{max}B$ When S: the dye B = blank

The results in Table 1 indicated that 0.5 ml of 1N sodium hydroxide is the more suitable amount which gives the highest value of absorbance and good colour contrast.

Effect of N-(NED) amount

Various volume of N-NED are tested the final results indicated that 3 ml of N-NED with concentration 0.005M is the more suitable amount which gives the highest value of intensity to the coloured dye formed and the highest value of correlation coefficient upon 20-140 μg / 25 ml (Table 2).

Table 2. Effect of N-NED amount on absorbance

ml of N-NED (5×10^{-3}M)	Absorbance / μg of *p*-aminophenol in 25 ml							r
	20	40	60	80	100	120	140	
1	0.058	0.088	0.136	0.176	0.225	0.259	0.299	0.998802
2	0.058	0.099	0.145	0.203	0.238	0.287	0.327	0.9989.4
3	0.056	0.114	0.159	0.213	0.255	0315	0.356	0.999232
4	0.068	0.121	0.169	0.203	0.253	0.302	0.329	0.997641

Effect the amount of oxidizing reagent

The effect of different volumes (1-4 ml) of potassium iodate (0.015 M) on the colour intensity has been studied, it was observed that 2 ml of potassium iodate is the more suitable amount since it gives the highest colour intensity (Table 3), therefore the volume 2 ml was recommended for the subsequent experiments.

Table 3. The effect of oxidizing reagent on absorbance

ml of KIO_3 solution (0.015M)	Absorbance / μg of *p*-aminophenol in 25 ml							r
	20	40	60	80	100	120	140	
1	0.065	0.094	0.160	0.197	0.238	0.297	0.323	0.996171
2	0.065	0.101	0.154	0.216	0.235	0.296	0.336	0.996174
3	0.050	0.094	0.143	0.186	0.222	0.269	0.319	0.995351
4	0.095	0.104	0.144	0.192	0.231	0.265	0.294	0.992947

The effect of time on oxidative coupling reaction

The time required to give complete oxidation has been tested (Table 4).

Table 4. The effect of time on oxidative process.

Time minutes*	0	5	10	15	20	25	30
Absorbance	0.215	0.223	0.239	0.223	0.213	0.209	0.189

* Before the volumes are completed to the mark with distilled water

The results in Table 4 indicated that 10 minutes was needed to give complete oxidation process before dilution with distilled water.

Effect of reaction time

The effect of time on the development and stability period of the coloured dye was investigated under the optimum conditions of the reaction.

The stability of the colour intensity was reached after about 15 minutes and the absorbance of the coloured dye remaned constant for at least another 45 minutes. (Table 5).

The development time 15 minutes was selected as the optimum in the general procedure.

Table 5. Effect of time on absorbance

***p*-Aminophenol (μg / 25 ml)**	**Absorbance* / minutes**								
	0	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**
40	0.113	0.111	0.107	0.103	0.100	0.098	0.098	0.097	0.097
100	0.245	0.241	0.224	0.213	0.207	0.204	0.202	0.202	0.202

*After the volumes are completed to the mark with distilled water

Order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in colour intensity was observed.

Effect of temperature

The reaction between *p*-aminophenol with N-NED in the presence of potassium iodate was studied at three different temperature (0°C, room

temperature and 40°C). The results indicated that neither lower temperature(0°C) nor higher temperature(40°C)as compared to the laboratory temperature(25±3°C) could achieve faster(compared to 0°C)and higher sensitivity (compared to 40°C) reaction. Therefore, laboratory temperature(25±3°C) has been recommended in the subsequent experiments .

Final absorption spectra

The absorption spectra of the red dye formed by reaction of *p*-aminophenol with N-NED in presence of potassium iodate in alkaline medium shows a maximum absorption at 520 nm. The reagent blank gives no absorption at this wavelength (Fig 2).

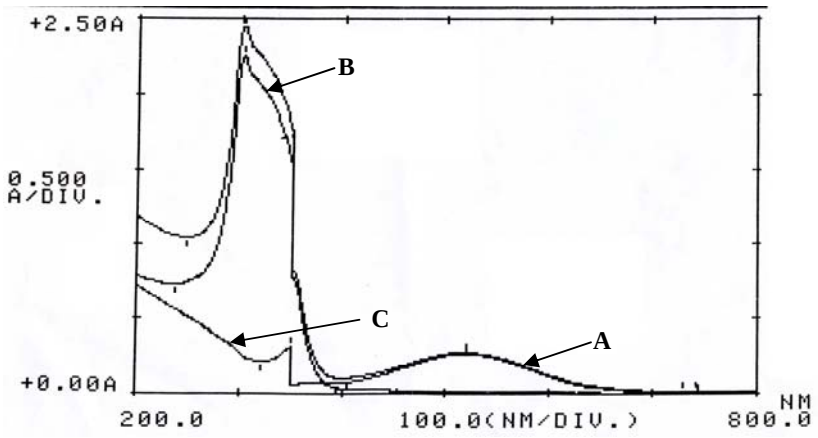


Fig. 2. Absorption spectra of 100 μg of *p*-aminophenol / 25 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Nature of the dye product

The stoichiometry of the reaction was studied adopting Job's method of continuous variation. The results obtained (Fig 3) show that 1:1 *p*-aminophenol to N-NED reagent was formed.

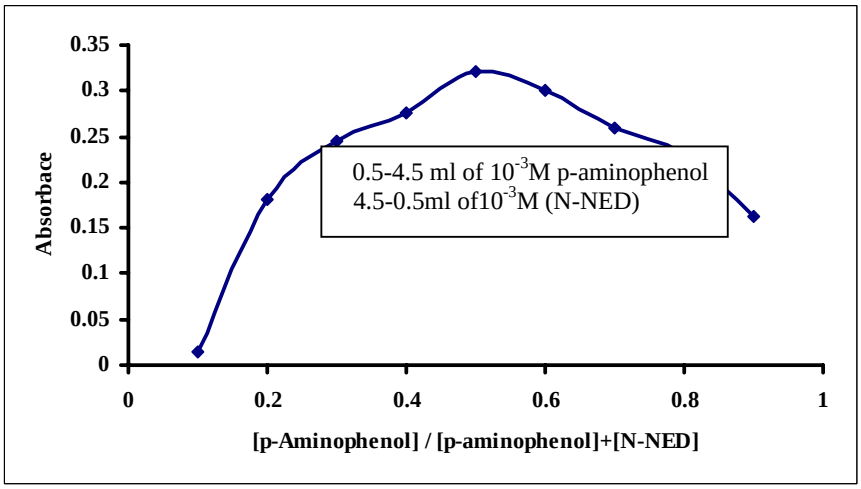
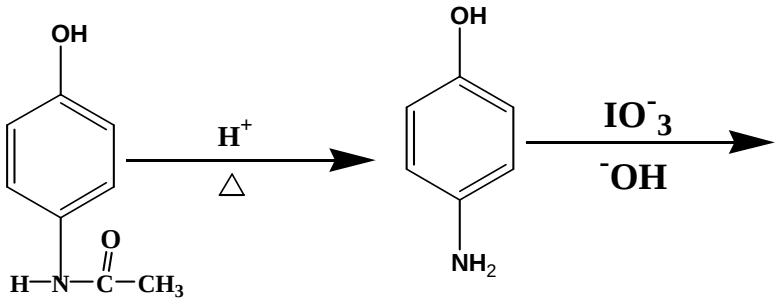
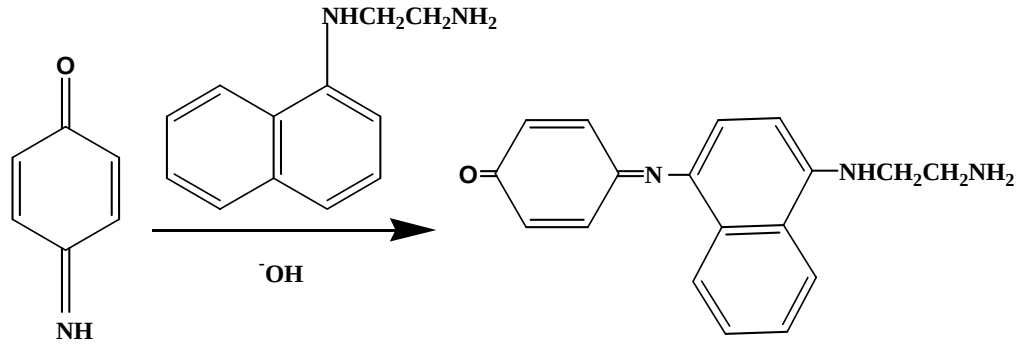


Fig. 3. Job's plot method of *p*-aminophenol-N-NED in the presence of IO_3

The probable mechanism of the reaction might be the following:



Paracetamol **p-Aminophenol**



p-Iminobenzoquinone **Red-dye**

Accuracy and precision

The accuracy and precision of the method were evaluated by performing five replicate analyses of *p*-aminophenol in pure form at three

different concentration levels (20, 40 and 100 µg / 25 ml). the recovery range between 95.08% to 100.59% and relative standard deviation (< 4.5) can be considered to be very satisfactory (Table6).

Table 6. Accuracy and precision of the proposed method

***p*-aminophenol (µg / 25 ml**	**Relative error*, %**	**Relative standard deviation*, %**
20	- 4.92	± 4.49
40	+ 0.59	± 1.64
100	- 1.02	± 0.89

Interferences studied

In order to investigate the analytical application of the proposed method on the determination of paracetamol (after hydrolysis) in different pharmaceutical preparations, the effect of some excipients usually present in the pharmaceutical preparations were investigated by carrying out the determination of 100 µg / 25 ml *p*-aminophenol in the presence of the different excipients. Experimental results showed that there was no interference from excipients for the examined method up to 10 fold excess except starch at high amount ratio (Table 7).

Table 7. Effect of excipients for assay of *p*-aminophenol

Exipient	Recovery (%) of 100 μg of *p*-aminophenol per μg excipient added		
	400	**800**	**1000**
Glucose	96.69	96.3	97.4
Lactose	99.6	96.6	94.7
Gum arabic	94.9	95.8	95.5
Starch	97.4	88	89

Analytical applications

The proposed method was successfully applied to determine paracetamol after (acid hydrolysis to *p*-aminophenol) in different pharmaceutical preparations (tablet, syrup and suppository). On applying proposed procedure good recovery was obtained as shown in Table 8.

Table 8. Application of the proposed method to the determination of paracetamol in pharmaceutical preparation.

Pharmaceutical preparation	μg paracetamol present / 25 ml	μg paracetamol found / 25 ml	Recovery* (%)
Paracetamol tablets,500 mg (S.D.I. Iraq)	40	38.91	97.28
	80	78.46	98.07
Antipyrol syrup,120 mg / 5 ml (S.D.I. Iraq)	40	39.92	99.80
	80	80.16	100.20
Antipyrol suppositories,250 mg (Medico laboratory Syria)	40	39.31	98.28
	80	79.35	99.19

* Average of five determinations

Conclusion

A simple and sensitive spectrophotometric method for the determination of trace amount of *p*-aminophenol in aqueous solution based on the reaction of *p*-aminophenol with N-NED in the presence of potassium iodate and sodium hydroxide, has been developed. The proposed method has

been successfully applied to the assay of paracetamol (after hydrolysis to *p*-aminophenol) in various pharmaceutical preparations.

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