Spectrophotometric Determinaiton of 4- Aminoantipyrine in Aqueous solution by coupling with Diazotised phenylephrine hydrochloride

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Abstract:

A simple, rapid and sensitive spectrophotometeric method for the determination of trace amounts of 4-amnoantiyrine in aqueous solution is described. The method is based on a coupling reaction between 4- amino antipyrine and diazotised phenyle phrine hydrochloride to form an intense violet, water-soluble dye that is stable and has a maximum absorption at 500 nm. A graph of absorbance versus concentration shows that Beer's law is obeyed over the concentration range 1-100 µg of 4-aminoantipyrine in a final volume of 25 ml (i.e., 0.04-4 p.p.m) with a molar absorptivity of 0.4024×10^{3} l mol^{-1} cm^{-1}, a sandell sensitivity of 539.26×10^{-3} µg cm^{-2}, a relative error -0.025 +0.028% and a relative standard deviation of 0.49-0.68% depending on the concentration. The optimum conditions for full colour development and the interference of foreign organic compounds have been investigated. The proposed method does not require either temperature control or a solvent extraction step.

Keywords: 4- aminoantipyrine determination, diazotization, phyenylephrine hydrochloride, spectrophotometry.

Introduction:

Aminoantipyrine is an important pyrazolone analgesic drug as it exerts an antipyretic effect in some situations in which a spirin is not completely effective, e.g., Hodgkin's disease, in which the fever is unresponsive to salicylates or chemotherapy(1). Among the various methods available for trace analysis, spectrophotometry continues to be one of the most popular because it is simple and cost-effective. There are very few spectrophotometric methods available for the determination of aminoantipyrine and these methods are not completely satisfactory(2-4) as they either require long reaction times or lack sensitivity.

This paper describes a spectrophotometric method for the determination of 4- amino antipyrine based on a coupling reaction with diazotized phenyle phrine hydrochloride in acidic medium, the proposed method has the advantage of simplicity and the complex formed is very stable.

Experimental

Apparatus:

All spectral and absorbane measurements were carried out on a Shimadzu uv-210-A digital double – beam recording spectrophotometer using 1-cm silica cells.

Reagents:

All chemicals used were of analytical-reagent grade.

4-aminoantipyrine stock solution 1mg ml^{-1}. A 0.250 g amount of 4-aminoantipyrine was dissolved in distilled water in a 250 ml calibrated flask and the solution was made up to volume with distilled water.

4- Aminoantipyrine working solution, 200 µg ml^{-1}, A 20- ml volume of the 4-aminoantipyrine stock solution was transferred into a 100ml calibrated flask and made up to mark with distilled water.

Phenylephrene hydrochloride reagent solution, 0.01 M, prepared by dissolving 0.2037g of phenylephrence hydrochloride in 100 ml of distilled water.

Hydrochloric acid 0.1 M: prepared by dilution of concentrated hydrochloride acid and standardized against sodium carbonate.

Interferent solutions, 3 mg ml^{-1}: prepared by dissolving the appropriate amount of the interferent in distilled water or ethanol and diluting to volume with distilled water.

Procedure:

In to a series of 25- ml calibrated flasks, transfer increasing volumes of the 4- ainoantipyrine working solution to cover the ranges 1-100 µg. Add 10 ml of distilled water and 1-5ml of diazotized phenylephrine-HCl and diluted to the mark with distilled water, measure the absorbance at 500 nm against a reagent blank prepared in the same way but containing no 4-aminoantipyrine The colour of the dye that is formed is stable for more than 24h.

The conditional molar absorptivity of the violet dye (formed with 4-aminoantipyrine) in the region of least photometric error and the wave length of maximum absorption was found to be 0.4024×10^{3} l mol^{-1} cm^{-1} and the sandell sensitivity was 539.26×10^{-3} µ g cm^{-2}.

In all subsequent experiments, 100µg of aminoantipyrine were used and the final volume was 25 ml.

Results and Discussion:

Absorption Spectra:

When very dilute aqueous solution of 4-aminoantipyrine and diazotized pheylephrene-HCl are mixed, an intense violet dye forms immediately. This dye has a maximum absorption at 500 nm, in contrast to the reagent blank which shows no absorption over the range 400-600 nm,. Fig.1 shows the sprctra of the diazo aminoantipyrine dye formed and the reagent blank. The maximum absorption at 500 nm was used in all subsequent experiments.

Study of Optimum Reaction conditions

The effect of various parameters on the absorption intensity of the diazo dye were studied and the reaction conditions were optimized.

Effect of diazotized reagent:

To establish the optimum conditions (stability of the diazo dye resulting from the coupling reaction with 4-aminoantipyrine, intensity of the dye formed and relatively rapid coupling rates) a critical study of several diazotized reagents, most of which have been used previously, was carried out of the diazotized reagents examined, viz, diazotized anthranilic acid, 4-aminobenzoic acid, 4-amino salicylic acid, 4-nitro aniline, 2,4-dinitro aniline, aniline and phenylephrene hydrochloride, only phenylephrene-HCl satisfied the above requirements. This reagent gave a water-soluble dye with 4-aminoantipyrine under the experimental conditions, thus avoiding the need for a time consuming extraction process. The diazotized phenylephrene-HCl was studied further with respect to its effect on full colour development.

The formation of the violet dye reached a maximum when about 1.0 ml of 0.01M diazotized phenylephrene reagent solution were used and remained at this maximum when 2-10 ml of the prescribed reagent concentration were added. A 5-ml volume of 0.01 M diazotized reagent solution was therefore used in the procedure in order to ensure quantitative determination at the upper limit of the calibration graph. Finally, the diazotization of phenylephrene-HCl was carried out in various acids (HCl, H_2SO_4, HNO_3, H_3PO_4 and CH_3COOH) to establish which was the most suitable acid for the reaction.

The experimental data showed that hydrochloric acid (1ml of 0.1M Hcl in 100ml of diazotized reagent) was the most suitable of the acids tested. The recommended amount of the diazotized reagent in 25 ml of the reaction mixture gives a solution with a pH of 2.00 ± 0.01.

Effect of temperature:

The effect of temperature on the colour intensity of the dye was studied. In practice, the same absorbance is obtained when the colour is developed at room temperature (20^{o}C) or when the calibrated flask is placed in water-both at 50^{o}C. Therefore, it is recommended that the reaction be carried out at room temperature.

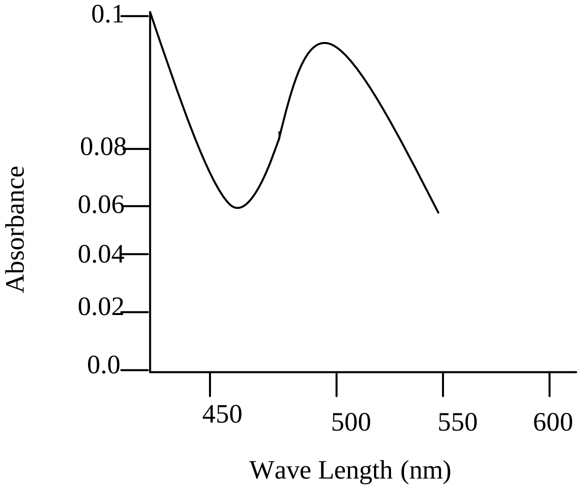


Fig.(1): Absorption spectrum of (4.A.A.P)

Effect of time on colour development:

Under the conditions described above, the intense violet colour dye develops immediately and remain stable for more than 24h.

Accuracy and precision:

To determine the accuracy and precision of the method, 4-aminoantipyrine was determined at three different concentrations. The result, shown in Table (1), indicates that satisfactory precision and accuracy could be attained with the proposed method.

Calibration Graph:

Employing the conditions described under procedure, a linear calibration graph (Fig. 2) for 4-aminoantipryine is obtained, which shows that Beer's law is obeyed over the concentration range 1-100 µg per 25 ml (0.04-4 p.p.m.) with a correlation coefficient of 0.9764 and an intercept of 0.0114.

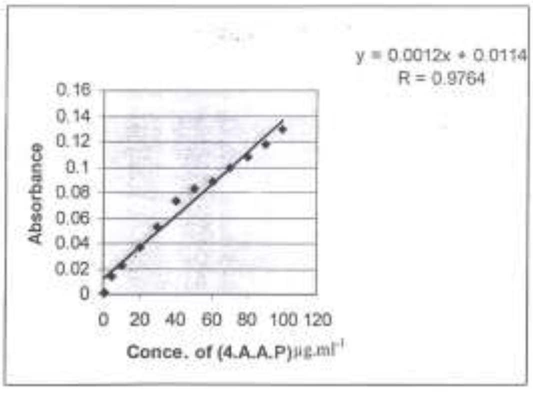


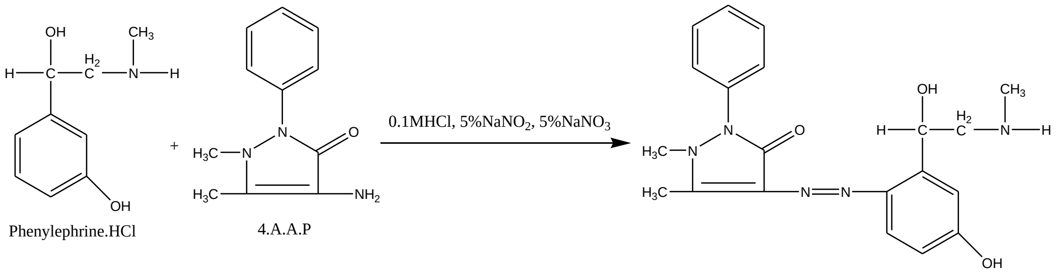
Fig.(2): Calibration graph of (4.A.A.P)

Interferences :

To demonstrate the selectivity of the proposed method, the interfering effects of various compounds were examined by determining 100 µg of 4-aminoantipyrine in the presence of each of the interferets using the recommended procedure. The results obtained are summarized in Table (2); it was found that indole and 1-naphthal interfered seriously.

Structure of the Dye :

The Stoicheiometry of the reaction between 4-aminoantipyrine and the diazonium salt derived from phenylephrene-HCl was investigated using the molar ratio method(5), the results obtained (Fig. 3) shows that a 1:1 complex is formed between 4-aminoantipyrine and the diazotized phenylephrene-HCl reagent at 500 nm. Therefore , the formation of the dye probably occurs as follows:



Fig(4) schematic diagram of phenylephrine.HCl and 4.A.A.P

The dye formed is soluble in water, methanol, ethanol, acetone, and N,N-dimethylformamide. Table (3) shows the spectrophotometric characteristics of the dye in these organic solvents. Although the molar absorptivity of the dye is higher in most of the organic solvents tested, water was used because it is more cost-effective.

The apparent stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amounts of 4-aminoantipyrine and diazotized phenylephrene-HCl with that of a solution containing a five fold excess of the diazotized reagent. As shown in schematic diagram of phenylephrine.HCl and 4.A.A.P fig (4).

The average conditional stability constant of the dye in water, under the described experimental conditions, is 1.2×10^5.

Conclusion:

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of trace amount of 4-aminoantipyrine in aqueous solution, based on the coupling of 4-aminoantipyrine with diazotized phenylephrene-HCl reagent. The proposed method does not require either temperature control or a solvent extraction step.

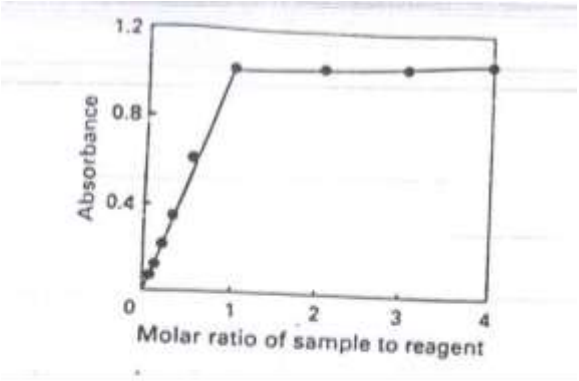


Fig.(3): Molar ratio of sample to reageat for the diazotized phenylephrene-HCl- 4-aminoantipyrine complex. Intitial concentration of phenyle phrene-HCl= $1x\ 10^{-4}$ M

Table(1): accuracy and precision of the proposed method

Amount of 4-aminoantipyrine taken/µg per 25 ml	Relative error, %*	Relative standard deviation, %*
4	+1.2	1.4
20	+1.0	0.5
30	+0.5	0.1

* Average of five determinations.

Table (2): Effect of foreign compounds on the determination of 200 µg of 4-aminoantipyrine.

Interferent	Amount added / µg	Interference %
Antipyrine	500	0.0
Antipyrine	2000	-0.5
4-aminobenzoic acid	500	-6.5
Benzoic acid	2000	+0.9
Tryptophan	500	-1.5
Alanine	500	0.0
Imidazole	2000	+1.6
Uracil	2000	+0.5
Phenol	2000	-1.2
Salicylic acid	2000	+1.00
Arginine	2000	+1.00
Indole	400	Violet Precipitate
1-Naphtol	400	
Urea	2000	0.00
Saccharin	2000	+0.3
EDTA+	2000	0.00
Nitrate ++	2000	0.00
Hydroxylamine +++	2000	-1.1
NH_4^+ *	2000	0.00

+ Added as EDTA sodium salt dihydrate.

++ Added as sodium nitrate

+++ Added as hydroxylamine hydrochloride

- Added as ammonium chloride.

Table(3): Spectrophotometric characteristics of the diazo dye in various organic solvents:

Solvent	λmax/ nm	Molar absorptivity/ lmol^{-1} cm^{-1}
N,N-Dimethyl formamide	510	1.6273×10^3
Ethanol	505	1.5853×10^3
Methanol	500	0.5321×10^3
Water	500	0.4024×10^3
Acetone	500	0.3122×10^3

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التقدير الطيفي لـ ٤– أمناينو انتي بايرين في المحاليل المائية بازدواج مع فنيل فرين هايدروكلورايد المؤزوت

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الملخص

تتضمن البحث تطوير طريقة طيفية للتقدير الكمي لمقادير ضيلة من ٤– أمناينو أنتي بايرين في المحاليل المائية بأستخدام المطياف الفوتومتري. تعتمد الطريقة على تفاعل الازدواج بين ٤– أمينو أنتي بايرين و كاشف فينايل فرين هيدروكلورايد المؤزوت حيث تكون صبغة بنفسجي مستقرة وذائبة في الماء و تعطي أعلى أمتصاص عندطول موجي 500 نانوميتر. ويشير الرسم البياني الخطي للأمتصاص مقابل التركيز بأن قانون بير ينطبق ضمن مدى التركيز 1-100 مايكروغرام من المادة في حجم نهائي 25 مل (4-0.04) جزء بالمليون. وكانت قيمة معامل الامتصاص المولاري للطريقة مساوي الى 0.4024×10^{4} لتر مول$^{-1}$ سم$^{-1}$ وقيمة حساسية سندل 539.26×10^{-3} مايكروغرام سم$^{-2}$ و الخطأ النسبي 0.028+0.025% مع انحراف القياسي النسبي أقل من 0.49-0.65% اعتماداً على مستوى التركيز المراد تقديره.تم دراسة الظروف المثلى للتفاعل و هذه الطريقة لا يحتاج الى أما ضبط درجة الحرارة أو ظروف أستخلاص المذيب.