Spectrophotometric Determination of Paracetamol in Pharmaceutical Preparations Via Arsenazo III - Cerium(III) Reaction

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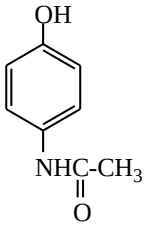
Abstract

A simple, rapid, accurate and precise spectrophotometric method is proposed for determining paracetamol in pure form and in its pharmaceutical preparations. The method is based on oxidation-reduction reaction between paracetamol and cerium(IV) ion, then reaction of cerium(III) with arsenazo III reagent in acidic medium to produce a green-blue complex which is stable, water soluble and has a maximum absorption at 654 nm with a molar absorptivity of 1.١٧٠٢×10^5 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range from 1 to 14 μg paracetamol in a final volume of 25 ml. The proposed method has been applied successfully to determine paracetamol in pharmaceutical preparations.

Keyword: Paracetamol, Arsenazo III, Oxidation-reduction.

Introduction

Paracetamol [acetaminophen, N-acetyl-*p*-aminophenol, 4-acetamidophenol] (1) is used as analgesic and antipyretic agents [1].



(1)

Various spectrophotometric methods have been utilised for determination of paracetamol, these include ultra-violet absorption spectrophotometriy [2], coupling with different diazotised such as sulphanlic acid [3], 2-nitroaniline [4], benzocaine [5] and 2,4-dinitroaniline [6]. Paracetamol can be determined by nitration and subsequent reaction with aceton as nucleophilic reagent [7] or complexation with Co(III) and Cu(II) ions, as a coupling agent [8].

Another femous colorimetric methods based on the hydrolysis of paracetamol to *p*-amino phenol followed by different types of analytical reactions for spectrophotometric determination such as diazotisation and coupling with different coupling agents such as 1-naphthol [9], oxidative coupling reaction has been used in determination of paracetamol based on reaction with *o*-cresol [10], or *p*-xylenol after hydrolysis to *p*-aminophenol [11] with an oxidizing agent sodium periodate.

Another method based on reaction of *p*-aminophenol at ambient temperatire with sodium sulphide in present of Ce(IV) or Fe (III) to produce a methylene blue-like dye [12]. Also a charge transfer complex formation has been used as indirect spectrophotometric determination of paracetamol using dichloro dicyano *p*-hydroquinon [13]. Another method based on the readuction of Fe(III) to Fe(II) by the reaction with acetaminophene in the present of 2,2`-bipyridyl, forming an orange-red complex [14].

Arsenazo III undergoes sensitive and selective reactions with several cations, such as reaction with cerium(III) ion in the presence of cerium(IV) ion. This reaction can be used in the determination of some organic compounds which have the ability to undergo oxidation-reduction reaction with cerium(IV) ion (15-17).

The present work is described a spectrophotometric method for assay of paracetamol in pharmacutical preparations based on the oxidation-reduction reaction of paracetamol with cerium (IV) ion, then a reaction of cerium(III) ion with arsenazo III reagent in acidic medium to yeild a couloured complex whose intensity is indirectly related to the concentration of paracetamol.

Experimental

All spectrophotometric measurements are performed on Shimadzu UV-visible recording spectrophotometer UV-160 using 1-cm silica cells. pH meter type Philips PW 9420 is used for pH reading.

Reagents

All chemicals used are of analytical– reagent grade.

Standard paracetamol solution, 100μg.ml^{-1}. This solution is prepared by dissolving 0.01 g of paracetamol (SDI- Iraq) in 10 ml ethanol to increase solubility and the volume is diluted to 100 ml with distilled water in a volumetric flask.

Working paracetamol solution, 10μg.ml^{-1}. This solution is prepared by appropriate dilution of standard solution.

Ammonium ceric sulphate [cerium(IV) ion solution], 6.6×10^{-5}M. This solution is prepared by dissolving 0.0042 g of ammonium ceric sulphate dihydrate (BDH) in 100 ml of distilled water in a volumetric flask, this solution is freshly prepared daily(15).

Arsenazo III reagent solution, 2×10^{-4}M. This solution is prepared by dissolving 0.0411 g of arsenazo III (Fluka) in 250 ml distilled water in a volumetric flask.

Hydrochloric acid solution, 0.05 N. This solution is prepared by appropriate dilution of 4.2 ml of the concentrated hydrochloric acid (11.8 N) solution to 1000 ml with distilled water in a volumetric flask.

Buffer solution, pH= 3.0, This solution is prepared by mixing 50ml of 0.1N glycine solution (0.3852g glycine dissolved in 50ml distilled water) with 57ml of 0.2N

HCl, then the volume is completed to 100ml with distilled water(18).

Paracetamol tablets solution, 10μg.ml^{-1}. Weighted and finely powdered 10 tablets (each one contain 500mg paracetamol), an accurately weighed amount of powder equivalent to 0.01g paracetamol is dissolved in 5ml ethanol, then 50-75ml distilled water is added, shaking to increase the solubility, filtered into 100ml calibrated flask, then the solution is completed to the volume with a distilled water.

Paracetamol syrup solution, 10μg.ml^{-1}. A 0.4 ml of syrup (each 5ml contain 125 mg paracetamol) is transferred into a 100 ml calibrated flask and the total volume is dilluted with distilled water, then 10ml of the above solution is dilluted to 100ml with distilled water to prepared solution contain 10 μg paracetamol in each ml of solution.

paracetamol suppositories solution, 10μg.ml^{-1}. Weighed and mixed well 5 suppositories (each suppositories contain 250 mg paracetamol). An accurate weight amount of mixture equivalent to 0.01g paracetamol is dissolved in boiling distilled water, filtered, and the residues are washed with 5 ml ethanol and boiling distilled water and the volume is completed to 100 ml with distilled water, to prepared solution contain 10 μg paracetamol in each ml of solution 10 ml of the above solution is diluted to 100 ml with distilled water.

Procedure and calibration graph

To a series of 25 ml calibrated flasks an increasing volume (0.1-2.0 ml) of 10μg.ml^{-1} paracetamol solution are transferred to cover the range of calibration graph, followed by 5 ml of 6.6×10^{-5}M cerium (IV) ion solution and 0.5 ml of 1NHCl followed by 3ml of buffer solution (glycine-HCl), standing for 20 minutes, then 3 ml of 2×10^{-4}M arsenazo III reagent solution is added. After dilution the flasks with distilled water, the absorbances are measured at 654 nm against the reagent blank. Beer's law is obeyed over the range of concentration 1 to 14 μg paracetamol in 25 ml (Fig. 1). A negative deviation from Beer's law is occurred beyond the upper determination limits. The apparent molar absorptivity, referred to paracetamol, has been found to be 1.١٧٠٢×10^{5} l.mol^{-1}.cm^{-1}.

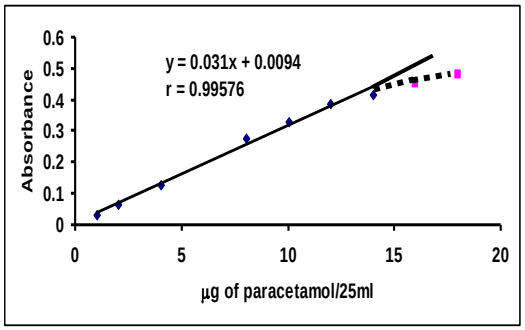


Fig. 1: The calibration graph for paracetamol determination using the reaction between arsenazo III and cerium(III).

Absorption spectrum

Absorption spectrum of the coloured complex formed from the reaction between cerium(III) ion with arsenazo IIII in acidic medium against its corresponding reagent blank shows maximum absorption at 654 nm contrast to the arsenazo reagent blank (Fig. 2).

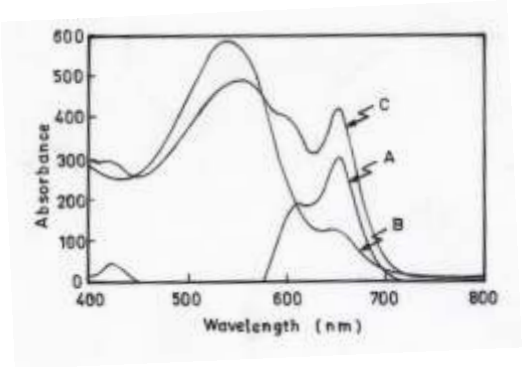


Fig.2: Absorption spectra of 10 μg paracetamol / 25 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Results and Discussion

During the investigation 10 μg of paracetamol is taken and the final volumes are brought to 25 ml with distilled water.

Optimization of variables

The effect of various parameters on the absorption intensity of the coloured complex is studied and the reaction conditions have been optimized.

Effect of pH

The effect of pH on intensity of the coloured complex is examined. Different volumes (0.05-5 ml) of 0.05M hydrochloric acid solution is added to an aliquot of solution containing 10 μg of paracetamol. The intensities of absorption are read against the reagent blank. The results are shown in Table 1.

Table 1. Effect of pH on absorbance

ml of 0.05M HCl	Absorbance	Final pH
0.05	0.175	3.96
0.10	0.217	3.65
0.30	0.272	3.23
0.50	0.303	3.05
0.70	0.238	2.91
1.00	0.234	2.80
3.00	0.207	2.39
5.00	0.166	2.23

The results shown in Table 1 indicate that the pH of 3.05 is considered optimum. A pH 3 is selected for subsequent investigation because of good sensitivity. Five buffer solutions of pH 3 with different compositions have been tested, tartaric acid-NaOH (B_1), citric acid-NaOH (B_2), KH phthalate-HCl (B_3), glycine-HCl (B_4) and formic acid-NaOH (B_5), although the experimental results indicated that the addition of different types with different amounts of buffer solutions give no useful effect(ie.decrease the intensity of complex). but it has been recommended to be used in the subsequent

experiments because using HCl only to get the appropriate pH give unstable complex (Table 2 and 3).

Table2: The stability of complex without buffer solution

Time (minute)	0	10	20	30	40	50	60
Absorbance	0.331	0.294	0.258	0.237	0.218	0.200	0.187

Table 3. Effect of buffer solutions on absorbance

ml of buffer solution	Absorbance/ml of Buffer added				
	B_1	B_2	B_3	B_4	B_5
1	0.259	0.214	0.324	0.303	0.245
2	0.228	0.166	0.325	0.310	0.242
3	0.220	0.160	0.333	0.333	0.234
4	0.168	0.103	0.279	0.287	0.194
5	0.151	0.088	0.301	0.294	0.202

The results in Table ٣ indicate that the buffer solution B_3 and B_4 give the highest intensity for the absorption of the coloured complex, and 3 ml of B_4 has been recommended for subsequent experiments.

Effect of oxidizing agent [cerium(IV) ion] amount

Different amounts of cerium(IV) ion solution are added and the optimum amount which gives higher intensity of complex and higher value of correlation coefficient (Table ٤) has been selected.

Table 4. The effect of ceric ion amount on absorbance

ml of 6.6×10^{-5}M cerium (IV) ion solution	Absorbance/μg paracetamol in 25ml					Absorbance of reagent blank	r
	2	4	8	10	12		
4	0.065	0.140	0.260	0.318	0.343	0.101	0.988933
5	0.064	0.135	0.283	0.340	0.405	0.107	0.997175
6	0.079	0.140	0.298	0.364	0.408	0.147	0.995815
7	0.045	0.127	0.285	0.327	0.401	0.156	0.993347
10	0.081	0.140	0.263	0.305	0.424	0.169	0.989621

The results shown in Table ٤ indicate that the volume of 5 ml of 6.6×10^{-5}M cerium(IV) ion solution is an optimum amount(high value of r and low absorbance of reagent blank compared with 6 ml), and therefore it recommended for subsequent experiments.

Effect of time on reduction of cerium(IV) ion

The effect of time needed to complete the reduction of cerium(IV) ion to cerium(III) ion is studied by standing of the solutions after adding cerium(IV) ion solution for different times, then the other reagents are added and the absorbances measured against the reagent blank (Table ٥).

Table 5. Effect of time on reduction process

Time (minute)	0	٥	١٠	١٥	٢٠	٢٥	٣٠
Absorbance	0.093	٠,٢٠٠	0.300	٠,311	0.333	0.332	0.329

The results indicate that complete reduction of cerium(IV) ion occurred after 20 minutes and the intensity decreased above 20 minutes, because the intensity of reagent blank solutions increased. Therefore, the standing time 20 minutes is recommended for the subsequent experiments.

Effect of arsenazo III reagent amount

The effect of the amount of arsenazo III reagent on maximum formation of the coloured complex is investigated. The results is shown in Table ٦.

Table 6. Effect of arsenazo III reagent amount on absorbance

ml of 2×10^{-4}M arsenazo III reagent	Absorbance / μg paracetamol in 25ml						r
	2	4	6	8	١٠	١٢	
٢	0.063	0.130	0.176	0.231	0.299	0.318	0.993075
٣	0.068	0.128	0.214	0.278	0.331	0.390	0.997255
4	0.052	٠.106	0.164	0.212	0.291	0.358	0.99708

The results shown in Table 6 indicate that 3 ml of arsenazo III reagent solution give the higher sensitivity and higher value of correlation coefficient therefore it has been selected for subsequent experiments.

Effect of order of addition of reagents

The effect of order of addition of reagents on intensity of coloured complex is investigated (Table 7).

Table 7. The order of addition of reagents

Reaction components	Order number	Absorbance
Paracetamol (P) + cerium (IV) ion (O) + HCl + buffer solution (B) + arsenazo III (R)	I	0.333
P+ O+ B+H + R	II	0038
P+ O+ R+H+ B	III	0.155
P+ O+ R +B+H	IV	0.041

The results indicate that the order of addition of reagents should be followed as given under procedure (order I).

Effect of Time

The effect of time on the development and stability of the coloured complex for different amounts of paracetamol is investigated under the optimum experimental conditions established. The colour formation occurs immediately after all reaction mixtures are added and the absorbance of the complex remains constant for at least 1 hour (Table 8).

Table 8. Effect of time on the absorbance of complex

µg of SDA in 25 ml	Absorbance*/min. standing time												
	0	5	١٠	15	20	25	30	35	40	45	50	55	60
2	0.093	0.095	0.096	0.098	0.099	0.100	0.101	0.102	0.104	0.106	0.107	0.107	0.108
5	0.193	0.197	0.198	0.199	0.200	0.201	0.202	0.206	0.205	0.209	0.211	0.213	0.213
10	0.334	0.334	0.334	0.335	0.335	0.338	0.339	0.338	0.339	0.340	0.343	0.343	0.343

*After 20 minutes reaction time of paracetamol with Ce(III)

Although the intensity of the complex slightly increase with time the above stability period is sufficient to allow several measurements to be performed sequentially.

Accuracy and precision

To check the accuracy and precision of the calibration graph, paracetamol is determined at three different concentrations. The results shown in Table 8 indicate that the calibration graph is satisfactory.

Table 8. Accuracy and precision

Amount of SDA taken, µg/25 ml	Relative error*,%	Relative standard deviation*, %
2	-2.3	2.1
8	+1.4	1.6
10	-0.6	1.4

* Average of five determinations

Nature of the reaction between paracetamol and cerium(IV) ion

Job's method is used in the determination of reaction ratio of paracetamol with cerium(IV) ion.

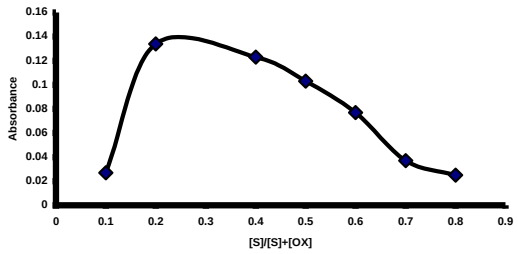


Fig.3: Job's plot for paracetamol -cerium (IV) ion

Analytical applications

The proposed method is applied to determine paracetamol in different pharmaceutical preparations(tablets and suppository) ,the results indicated good recovery is obtained as shown in Table 10.

The results obtained (Fig. 3) showed that a 1:4 paracetamol to cerium(IV) ion ratio is obtained.

Nature of arsenazo III-cerium(III) ion complex

The stoichiometry of the reaction is investigated using the Job's method under the optimized conditions. The results obtained (Fig. 4) showed that a 1:1 arsenazo III to cerium(III) ion ratio is obtained.

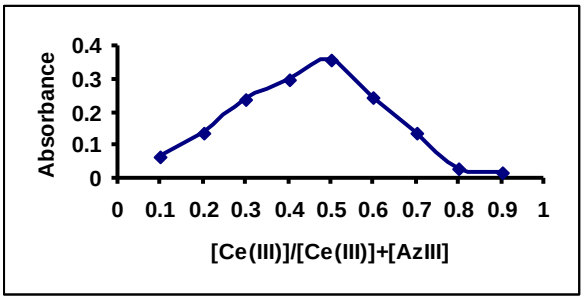
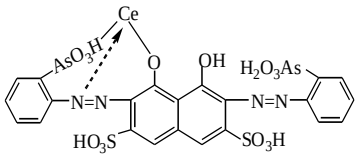
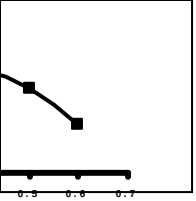


Fig. 4: Job's plot for arsenazo III-cerium (III) ion complex

Therefore, the structure of the formed complex may be written as follows(19):



Greenish-bluecomplex



[Az]

Table 10. Analytical applications

Pharmaceutical preparation	µg paracetamol present/25ml	µg paracetamol measured/25ml	Recovery (%)
Paracetamol tablets 500 mg S.D.I-Iraq	4	3.87	96.75
	8	7.76	97.00
	10	9.62	96.20
Antipyrol-suppository 250mg Delta- Syria	4	4.12	103.00
	8	8.11	101.37
	10	10.10	101.00

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التقدير الطيفي للباراستامول في المستحضرات الصيدلانية بواسطة تفاعل ارسينازو (III) – سيريوم (III)

نبيل صبيح عثمان و صفاء عبد العليم

قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

الخلاصة

تم اقتراح طريقة طيفية بسيطة، سريعة، دقيقة ومتوافقة لتقدير الباراسيتامول بشكله النقي وفي مستحضراته الدوائية. اعتمدت الطريقة على تفاعل الأكسدة والاختزال بين الباراسيتامول و أيون السير يوم الرباعي يتبعها مفاعلة ناتج التفاعل أيون السيريوم الثلاثي مع الكاشف آرسين آزو III في وسط حامضي ليعطي معقداً اخضر مزرق اللون مستقر وذائب بالمحلول المائي يقاس شدة امتصاصه عند الطول الموجي 654 نانوميتر وكانت قيمة الامتصاصية المولارية ١,١٧٠٢ ×١٠^{٥} لتر.مول$^{-١}$.سم$^{-١}$ وانطبق قانون بير في مدى التركيز بين ١ و١٤ مايكروغرام من الباراسيتامول في حجم نهائي ٢٥ مل ، تم تطبيق الطريقة المقترحة بنجاح في تقدير الباراسيتامول في مستحضراته الدوائية.