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Batch and Flow-Injection Spectrophotometric Determination of Paracetamol by Coupling with Diazotized 4-Aminoacetophenone

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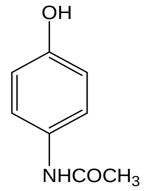
Abstract

Simple, rapid, inexpensive, and sensitive batch and flow injection spectrophotometric methods have been proposed for determination of paracetamol in some pharmaceutical preparations. The proposed methods are based on the coupling reaction between paracetamol and diazotized 4-aminoacetophenone (DAAPH) in a basic medium using sodium hydroxide to produce a pinkish-red azo dye with maximum absorptivity at 500 nm. The reaction conditions and other analytical parameters were studied and optimized for both batch and flow injection procedures. The calibration graphs resulting from measuring the absorbance were linear over the ranges 5.0 - 30 and 25 – 180 µg/ml of paracetamol with relative standard deviations 0.26% and 0.18%, and detection limits (0.30, 0.35) µg/ml for batch and flow injection methods respectively. The proposed methods were applied to the analysis of paracetamol in some commercial pharmaceutical preparations. The observed results have a reasonable agreement with those obtained by an HPLC method as a standard certified method from Awamedica company for drug in Erbil-Iraq which based on British pharmacopeia…

Introduction

Paracetamol the well-known pain killer which in the USA is called acetaminophen as its generic name or (nonproprietary name), and its trade name mostly is Tylenol, chemically named as N-acetyl-p-aminophenol or N-(4-hydroxyphenyl) acetamide, with a chemical formula ($C_8H_9NO_2$), molecular mass 151.163 g/mol, and chemical structure as shown below [1]. Paracetamol was first discovered to have both pain reliever and fever reducer properties in the late 19th century; these actions are known, respectively, as analgesic and antipyretic. Paracetamol was first used in medicine in 1893 by Von Mering [2] [3].

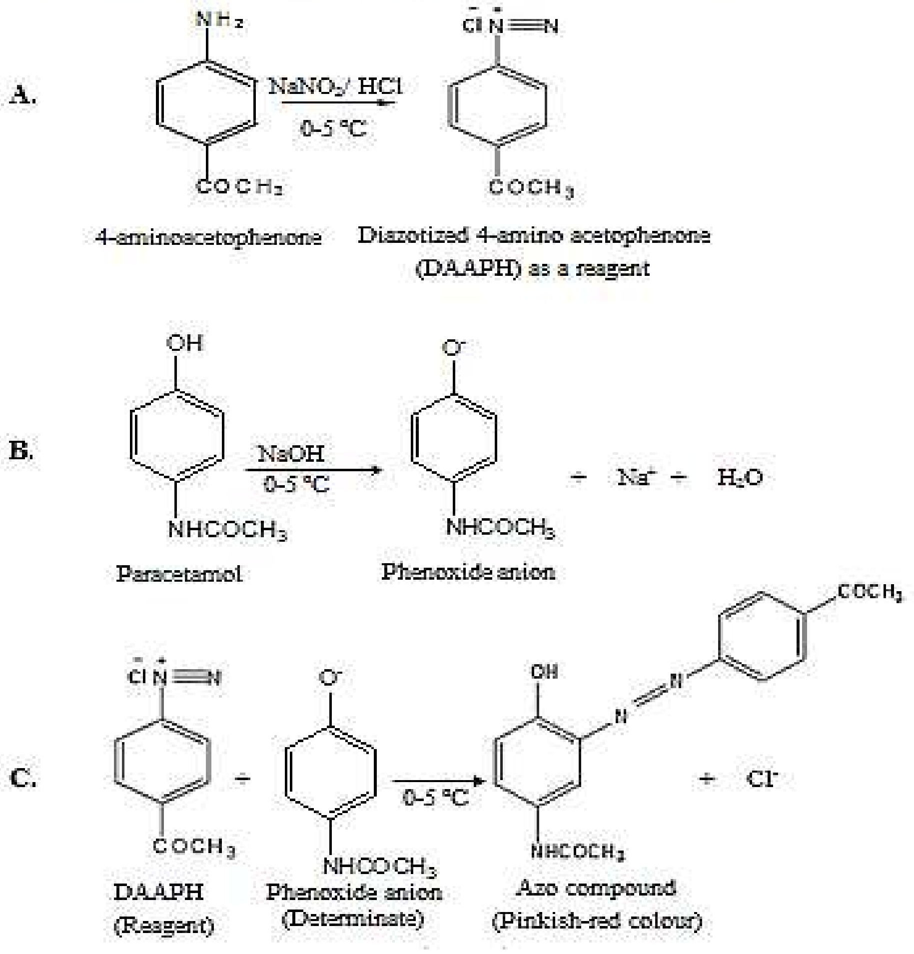


Because paracetamol is available in a great number of formulations in combination with a various drugs, hence it demands an analytical method that is sufficiently specific, rapid, and automatable for routine analyses and dissolution studies. Direct ultraviolet absorption spectrophotometry can be carried out for the determination of paracetamol in pharmaceutical products when there are no significant spectral interferences, otherwise when the drug formulated with other UV-absorbing substances, where spectral overlap is possible, separative techniques such as high performance liquid chromatography (HPLC) are usually necessary.

Several analytical techniques such as titrimetry, IR spectrometry, spectrofluorometry, colorimetry, capillary electrophoresis, chromatography, electrochemical methods and many other methods are proposed for the determination of paracetamol [4-16]. UV spectrophotometric methods are also available [17-19]. Spectrophotometric methods depend on the colour formation were used for determination of the drug [20-24]. Various flow-injection analyses (FIA) along with suitable detection technique have been investigated for the determination of paracetamol [25-29].

In this paper, batch and flow injection spectrophotometric methods are described for the determination of paracetamol. Both methods are based on the absorbance measurements of a pinkish-red azo dye formed between paracetamol and diazotized 4-aminoacetophenone (DAAPH) according to the proposed reactions shown below in sodium hydroxide at 500nm. The proposed methods have been successfully applied to the determination of paracetamol in some pharmaceutical preparations.

The proposed batch and FI methods were based upon the following reactions:



Experimental

Apparatus

Spectral and absorbance measurements for both batch and flow injection methods were carried out on a Bio-Tek Instrument spectrophotometer (model J643002, Vermont, USA), using 1.0 cm quartz cell, and a digital pH-meter: Hanna, pH 211. The FI system comprised a multi-channel peristaltic pump (Watson-Marlow 5012, USA), a 6-way injection valve with various sample loops, a quartz flow cell with 30µl and 10mm path length, T-links was used to mix two streams of reagents, and also developed as a degassing unit.

Reagents and solutions:

All chemicals were of analytical reagent grade.

1. Stock solution of paracetamol (500µg/ml): pure paracetamol solution (500µg/ml) was prepared by dissolving 0.05 g of paracetamol powder in 20 ml distilled water with carefully stir, then completed to 100 ml with distilled water and kept in refrigerator, working standard solutions were prepared by suitable dilution of the stock standard solution [30].

2. 4-aminoacetophenone (4-AAPH) solution (0. 1 M), was prepared by dissolving 1.3500g of 4-AAPH in 5.0ml ethanol with heating, then completed to 100 ml with distilled water.

3. Hydrochloric acid solution (~1.0M), was prepared by diluting concentrated hydrochloric acid (sp. gr =1.19, 37% and M.Wt =36.5 g/mol) with distilled water.

4. Sodium nitrite solution (0.1M), was prepared by dissolving 0.6900g of sodium nitrite in distilled water, and then completed to 100 ml with the same solvent.

5. Sodium hydroxide solution (~0.1M), was prepared by dissolving 0.4000g of sodium hydroxide in distilled water, and then completed to 100 ml with the same solvent.

6. Diazotize 4-aminoacetophenone (DAAPH) solution (0.01M) as a reagent (for batch procedure), was prepared by dissolving 0.1352g of 4-AAPH in 5.0ml ethanol, 5.0ml hydrochloric acid (0.8 M) and 20ml distilled water, the mixture was cooled to (0-5 Cº) in an ice bath for 5.0 minutes, after that a solution of 0.1725g sodium nitrite, dissolved in 10ml distilled water, was added dropwise drop in to the mixture with continuous stirring for 5.0 minutes. Finally the azo solution was transferred to 100 ml volumetric flask, diluted to the mark with cold distilled water [31]. Because diazonium salts are slowly decompose even at ice temperature, the solution must be kept in refrigerator, otherwise should be used immediately after preparation [32].

Sample preparation

Different pharmaceutical preparations from different sources containing paracetamol were obtained from local market Table (1).

1. Paracetamol tablets solution, 1000µg/ml: 10 tablets (each one contains 500 mg paracetamol) were weighed and grounded into a fine powder then mixed thoroughly. An accurately weighed amount of powder that contains 0.1 g paracetamol (0.1245 g powder brand A, 0.1114g powder brand B) is mixed with about 40 ml of distilled water and stirred for 10 minutes to increase solubility. The insoluble mass is filtered off on a Whatman No.41 filter-paper, washed with distilled water and the filtrate plus washings are diluted to 100 ml with cool distilled water in a calibrated flask [31].

2. Paracetamol syrup solution, 1000µg/ml: 4.17 ml from brand A (each 5.0 ml contains 120 mg paracetamol) and 4.0 ml from brand B (each 5.0 ml contains 125 mg paracetamol) was mixed with 40 ml distilled water in a 100 ml volumetric flask, the total volume is diluted to the mark with same solvent.

3. Paracetamol ampoule solution, 1000µg/ml: 0.835 ml from brand A (each 5.0 ml contains 600 mg paracetamol) and 1.0 ml from brand B (each 5.0 ml contains 500 mg paracetamol) was transferred into a100 ml volumetric flask and the total volume is diluted to the mark with distilled water.

The required volume of each sample solution was taken and further diluted to the range of calibration curve for the determination of paracetamol.

Recommended procedure for calibration:

1. Batch method:

Into a series of 25 ml volumetric flasks, 0.8ml of 0.1M sodium hydroxide, and increasing volumes of standard stock solution (500 µg/ml) containing (0.25-1.5) ml of paracetamol to cover the range of calibration curve (5.0-30) µg/ml were added, then 2.0ml of cold diazotized 4-aminoacetophenone (DAAPH) as a reagent was added. The solutions were shaken thoroughly and diluted to the mark with distilled water. After standing for 5.0 minutes in an ice bath, the absorbance of the pinkish-red azo dye formed was measured at 500 nm against reagent blank prepared in similar manner but without paracetamol, using 1.0 cm quartz cell.

2. FI method:

The schematic diagram of the flow injection analysis system employed for spectrophotometric determination of paracetamol using DAAPH as a reagent is given in Fig. (1): A multi-channel pesrtaltic pump was used for propelling four lines. Teflon tubes with same internal diameter (1.0 mm) were used for all lines in the manifold. Aliquots of paracetamol standard solution (100 µl) was prepared at different concentrations to cover the calibration curve in the range (25-180 µg/mL) were injected through the injection valve into the carrier stream. The final solution passed through the detector with total flow rate of (2.4ml/min). The absorbance of the resulting pinkish-red azo dye was measured at 500 nm. Calibration graphs were prepared by plotting the absorbance versus paracetamol concentration.

Results and discussion

Absorption spectra

Using preliminary experimental concentrations of the chemicals used in the main reaction were as following**:** 1.0ml of 4-AAPH (0.01 M) and 0.5ml of hydrochloric acid (0.08 M) were added to a 25ml volumetric flask, the mixture was cooled to (0-5°C) in an ice bath for 5.0 minutes, after that 0.5ml of sodium nitrite (0.05 M) was added dropwise with continuous stirring, then a mixture of 0.5ml sodium hydroxide (0.1M) and an aliquot of standard paracetamol solution was added to give a final concentration of 30µg/ml paracetamol, a pinkish-red coloured product was obtained. The final solution was diluted to the mark with distilled water, and then mixed well. The absorption spectrum of the reaction product against the blank solution was recorded as shown in Fig. (2); which demonstrates that the best analytical wavelength is located at 500 nm, as in this wavelength the reagent blank has almost no absorbance, indicating that the blank does not interfere with the colour of the analyte.

Optimization of experimental parameters

1. Batch method

The effect of various variables on the colour development such as temperature, time of coupling reaction, amount of reactants, and addition sequence of reactants were studied to establish the optimum conditions for the determination of paracetamol. The results of optimization of each parameter were illustrated in Table (2).

2. FI method

The effect of various physical and chemical parameters was studied at ice temperature to establish the optimum conditions for the determination of paracetamol. The results of optimization of each parameter were illustrated in Table (3).

Quantitation

Calibration curve

Under optimum conditions, two calibration curves for each of batch and FI procedures were constructed, a straight line was achieved by plotting absorbance against concentration of paracetamol. The calibration curve for batch procedure as shown in Fig. (3); was obeyed Beer's law in the concentration range of 5.0 to 30 µg/ml paracetamol, on the other hand the range of linearity for FI procedure as shown in Fig. (4) was found to be 25 to 180 µg/ml paracetamol. Optical characteristics and statistical data for the proposed batch and FI methods are shown in Table (4).

Accuracy and precision

Under the optimum conditions and using the recommended procedure, the accuracy and precision of the proposed batch and FI methods were checked depending on the values of relative standard deviation (RSD %), relative error percentage (E_{rel} %), that were calculated by five replicate aliquots of three synthetic sample solutions (5.0, 20, and 30 µg/ml) and (25, 100, and 180 µg/ml) standard paracetamol for batch and FI methods respectively. The precision and accuracy data for both methods are shown in Table (5).

Study of interference

In order to assess the possible analytical applications of the proposed methods, the effect of some common excipients usually found with paracetamol drug in pharmaceutical formulations, were studied by analyzing synthetic sample solutions containing (20 and 100 µg/ml) pure paracetamol for batch and FI methods respectively and known amount of potentially interfering compounds. The tolerant limits of strange species were taken to be the amount of excipients that caused a relative error ≤ ±5.0% of the absorbance measurement of the standard paracetamol solution. The maximum amount of the interfering species for both methods is shown in Table (6).

Application of the methods

The proposed methods were successfully applied for the determination of paracetamol in various pharmaceutical products. The results obtained are shown in Table (7) for batch and FI methods. The data of the recommended procedures were compared with a standard HPLC method depending on British pharmacopeia which shows a reasonable agreement between them.

Conclusion

Two simple, accurate and sensitive batch and FI spectrophotometric methods have been developed for determination of paracetamol in pharmaceutical preparations. The developed procedures based on coupling reaction of the drug with diazotized 4-Aminoacetophenone reagent in presence of sodium hydroxide as an alkaline medium. The developed methods are very adequate for the determination of paracetamol in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (ppm) and without requiring any previous separation step and a temperature nor pH controlling. The proposed methods can be used for routine analysis of paracetamol in pharmaceuticals and in quality control, since there is no serious interference from the common excipients that might be found in commercial preparations. There is no significant difference between the two methods with respect to precision and accuracy**.** Moreover the proposed methods are very economical when compared to other methods such as those based on the use of HPLC.

Table (1): The trade names and composition of pharmaceutical products.

Pharmaceutical preparations		**Trade name**	**Manufacture**	**Composition**	**Total amounts**
Tablet	Brand A	Paracetol	N.D.I – IRAQ	Paracetamol	500 mg/Tablet
	Brand B	Parazar	Awamedica- Iraqi Kurdistan/ Erbil	Paracetamol	500 mg/Tablet
Ampoule	Brand A	Paracemol	Shanghai – China	Paracetamol	600 mg/5ml
	Brand B	Apmol	Ajanta – India	Paracetamol	500 mg/5ml
Syrup	Brand A	Adol	Julphar – Ras Alkhaimah, U.A.E	Paracetamol	120 mg/5ml
	Brand B	Parazar	Awamedica- Iraqi Kurdistan/ Erbil	Paracetamol	125 mg/5ml

Table (2): Results of optimization of analytical variables for batch method.

Optimized Variables		**Measurements**										
Temp.	C^{o}	Room temperature (17 C^{o})					**Ice temperature (0-5 C^{o})#**					
	Abs.	0.125					**0.234**					
Time	min.	At once	**5#**	10	15	20	25	30	35	40	45	50
	Abs.	0.127	**0.235**	0.235	0.234	0.235	0.236	0.233	0.235	0.234	0.235	0.235
Vol. of (0.01 M) 4-AAPH	mL	0.5	1.0	1.5	**2.0#**	2.5	3.0					
	Abs.	0.219	0.235	0.281	**0.338**	0.298	0.259					
Vol. of (0.08 M) HCl	mL	0. 2	0.4	0.6	0.8	**1.0#**	1.2	1.4				
	Abs.	0.167	0.205	0.251	0.285	**0.339**	0.287	0.262				
Vol. of (0.05 M) $NaNO_2$	mL	0.25	0.5	0.75	**1.0#**	1.25	1.5	1.75	2.0			
	Abs.	0.158	0.234	0.297	**0.341**	0.290	0.244	0.222	0.189			
Vol. of (0.1 M) NaOH	mL	0. 2	0.4	0.6	**0.8#**	1.0	1.2					
	Abs.	0.135	0.195	0.288	**0.340**	0.288	0.269					
Sequence of addition	Seq.	Reagent + Drug + Base				Reagent + Base + Drug			**Base + Drug + Reagent#**			
	Abs.	0.338				0.285			**0.435**			

Table (3): Results of optimization of analytical parameters for FI method.

Optimized parameters	Variables		Measurements								
Physical	Total flow rate	ml/min	0.4	0.8	1.2	1.6	2.0	**2.4#**	2.8	3.2	3.6
		Abs.	0.129	0.177	0.215	0.255	0.296	**0.321**	0.297	0.275	0.260
	Mixing coil	cm	10	**20#**	30	40	50	60	70	80	90
		Abs.	0.288	0.335	0.335	0.334	0.335	0.336	0.335	0.334	0.335
	1^{st} Reaction coil	cm	20	30	40	50	60	70	**80#**	90	100
		Abs.	0.085	0.101	0.153	0.215	0.274	0.313	0.336	0.337	0.336
	2^{nd} Reaction coil	cm	20	30	40	**50#**	60	70	80	90	100
		Abs.	0.216	0.275	0.291	0.335	0.263	0.241	0.228	0.204	0.181
	3^{rd} Reaction coil	cm	20	**30#**	40	50	60	70	80	90	100
		Abs.	0.251	0.335	0.285	0.255	0.211	0.186	0.162	0.148	0.122
	Sample volume	µl	25	50	75	**100#**	125	150	175		
		Abs.	0.189	0.242	0.319	0.405	0.375	0.353	0.323		
Chemical	Conc. of 4-AAPH	M	0.01	0.02	0.03	0.04	**0.05#**	0.06	0.07	0.08	
		Abs.	0.199	0.376	0.488	0.568	0.625	0.586	0.535	0.485	
	Conc. of HCl	M	0.02	0.03	0.04	**0.05#**	0.06	0.07	0.08		
		Abs.	0.275	0.409	0.528	0.627	0.513	0.422	0.378		
	Conc. of $NaNO_2$	M	0.02	0.03	0.04	0.05	**0.06#**	0.07	0.08		
		Abs.	0.224	0.365	0.477	0.558	0.628	0.553	0.372		
	Conc. of NaOH	M	0.02	0.03	0.04	**0.05#**	0.06	0.07	0.08		
		Abs.	0.214	0.334	0.477	0.625	0.501	0.411	0.285		

Table (4): Optical characteristics and statistical data for the proposed batch and FI methods.

Parameters	Characteristics	
	Batch method	FI Method
λ max (nm)	500	500
Linearity (µg/ml)	5.0-30	25-180
Molar absorptivity ((l/mol.cm)	$0.23x10^4$	$0.11x10^4$
Coefficient of Linearity (r^2)	0.9989	0.999
Correlation coefficient (r)	0.9994	0.9995
Detection limit (µg/ml)	0.3	0.35
Sample frequency (s/h)	-------	120

Table (5): Accuracy and precision results for batch and FI methods.

Method	Paracetamol Conc. (μg/ml)	SD	RSD%	$E_{rel}\%^*$
Batch method	5.0	0.0015	2.32	-3.5
	20	0.0011	0.26	2.7
	30	0.0070	2.26	-2.6
FI method	25	0.0008	0.81	2.6
	100	0.0011	0.18	-2.3
	180	0.0150	1.20	3.1

* Average of five determinations

Table (6): The influence of foreign species on determination of.

Excipients	Batch method		FI method	
	Maximum conc. (μg/ml)	Error %*	Maximum conc. (μg/ml)	Error %*
Citric acid	40	3.7	40	-4.4
Fructose	130	-4.9	220	4.8
Glucose	50	-1.0	150	-3.0
Lactose	70	4.5	70	-4.0
Polyvinylpyrrolidone (PVP)	70	-4	150	-4.3
Potassium chloride	100	2.2	150	2.3
Sodium chloride	50	4.3	120	3.6
Starch	50	-2.7	100	3.7
Sucrose	70	3.3	140	2.7

* Average of three determinations

Table (7): Results of analysis of commercial drugs by batch and FI spectrophotometric methods using (DAAPH) as a regent and standard certified method (HPLC) from Awamedica company for drug.

Methods	Pharmaceutical preparations	Composition	Content (mg) declared	Found (mg)		$E_{rel}\%^*$
				Proposed method	Standard method	
Batch method	Paracetol tablets	Paracetamol	500	477.9	468.07	2.10
	Adol tablets	Paracetamol	500	510.1	499.62	2.10
	Apmol ampoule	Paracetamol	600	584.2	572.17	2.10
	Paracetamol ampoule	Paracetamol	500	488.5	476.47	2.50
	Adol syrup	Paracetamol	120	112.5	115.95	-2.98
	Parazar syrup	Paracetamol	125	122.2	125.80	-2.86
FI method	Paracetol tablets	Paracetamol	500	474.3	468.07	1.33
	Adol tablets	Paracetamol	500	506.99	499.62	1.48
	Apmol ampoule	Paracetamol	600	583.4	572.17	1.96
	Paracetamol ampoule	Paracetamol	500	485.2	476.47	1.83
	Adol syrup	Paracetamol	120	113.4	115.95	-2.2
	Parazar syrup	Paracetamol	125	123.7	125.80	-1.7

* Average of four determinations

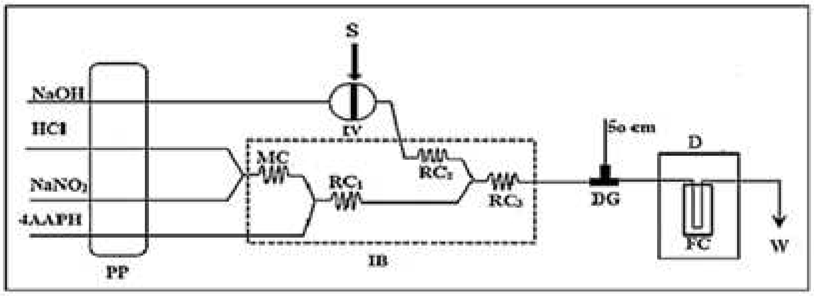
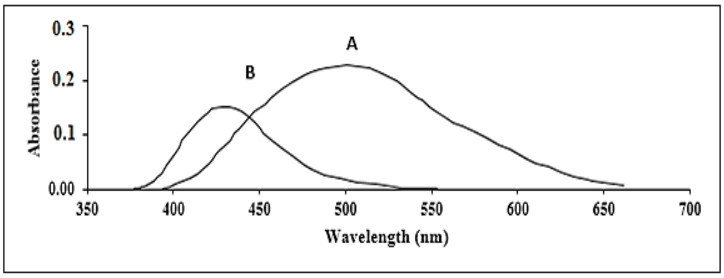


Figure (1): FIA manifold: (PP) peristaltic pump, (S) sample injected, (MC) mixing coil, (RC_1) first reaction coil, (RC_2) second reaction coil, (RC_3) third reaction coil, (IB) ice bath, (IV) injection valve, (DG) degassing, (D) detector, (FC) flow cell, (W) waste.



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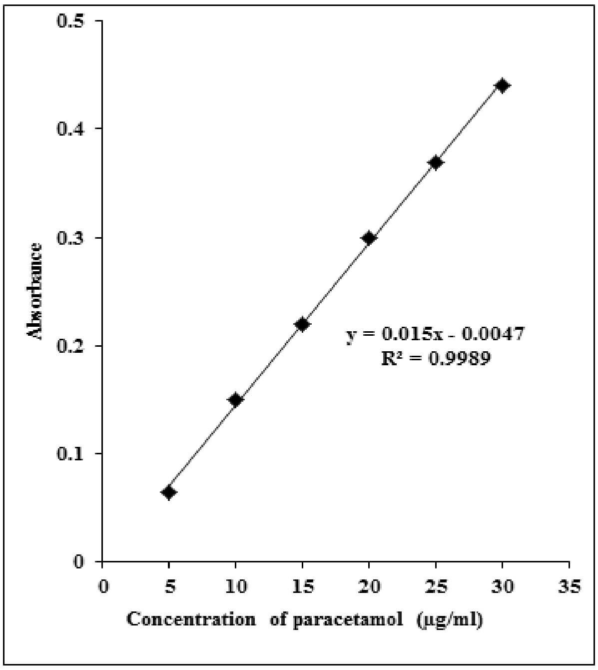

Figure (3): Calibration curve for spectrophotometric determination of paracetamol using DAAPH as a reagent.

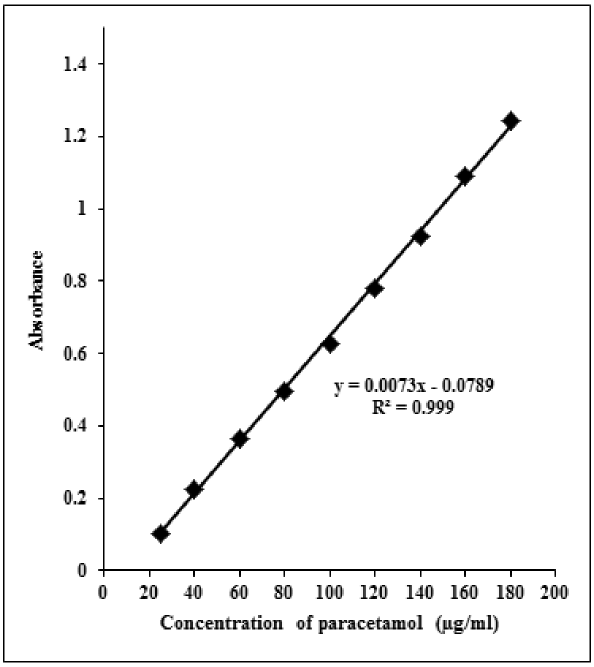

Figure (4): Calibration curve for FI spectrophotometric determination of paracetamol using DAAPH as a reagent.

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