BATCH AND FLOW-INJECTION SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF PARACETAMOL IN PHARMACEUTICAL PREPARATIONS BY COUPLING WITH DIAZOTIZED 4-NITROANILINE

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Abstract

Two sensitive and fast spectrophotometric methods using batch and flow-injection procedures for the determination of paracetamol are proposed. The methods are based on the formation of a red dye between this drug and diazotized 4-nitroaniline in sodium carbonate medium. The reaction conditions are studied and optimized for both batch and flow injection procedures. The calibration graphs resulting from measuring the absorbance at 528 nm are linear over the ranges 0.5 – 20 and 1 – 150 μg mL^{-1} of paracetamol with relative standard deviations of 1.2420% and 1.5634% for batch and flow-injection methods, respectively. The methods are applied to the routine analysis of paracetamol in pharmaceutical preparations. The obtained results agree with those obtained by the British Pharmacopoeia method.

Keywords: Spectrophotometric; Flow-injection; Paracetamol; Diazotized 4-nitroaniline; Pharmaceutical preparations.

الخلاصة

اقترحت طريقتان حساستان و سريعتان لتقدير الباراسيتامول باستخدام طريقتي الدفعة و الحقن الجرياني. تعتمد الطريقتان على تكوين صبغة حمراء ناتجة عن ازدواج الدواء مع كاشف 4- نايترو أنلين المؤزوت في وسط كاربونات الصوديوم. تمت دراسة و تثبيت الظروف الفضلى للتفاعل لطريقتي الدفعة و الحقن الجرياني. إذ عطت قيم الامتصاصية عند الطول الموجي 528 نانومتر مدى خطي 0.5 – 20 و 1 – 150 مايكروغرام مل$^{-1}$ من الباراسيتامول مع انحراف قياسي نسبي 1.2420% و 1.5634% لطريقتي الدفعة و الحقن الجرياني على التوالي. طبقت الطريقتان في التحليل الروتيني للباراسيتامول في المستحضرات الصيدلانية. و كانت نتائج الطريقتين متوافقتين مع نتائج الطريقة المعتمدة في دستور الأدوية البريطاني.

Introduction

Paracetamol (4-acetamidophenol, acetaminophen) is an analgesic and antipyretic derived from phenacetin. It is widely used (alone or associated with other active substances such as caffeine) due to the lack of gastric upsets often associated with other analgesics such as acetyl salicylic acid[1].

Several batch methods for the determination of paracetamol in pharmaceutical preparations have been reported in the literature including spectrophotometry[2-6], reflectance near-infrared spectroscopy[7], chemiluminescence[8], liquid chromatography[4,9] and reversed-phase capillary electrochromatography[10]. A number of flow-injection (FI) methods have also been reported for the determination of paracetamol, such as FI-spectrophotometry, using different on-line derivatization reactions. However, the control of such reactions and / or manifolds is still complicated[11-14]. Some methods, such as FI-FTIR[15] and FI with a boron-doped diamond thin film electrode[16], involve relatively higher cost instruments.

FI is an easy and inexpensive way of automating analytical determinations and can be applied in

several situations to reduce reagent consumption, and to increase the repeatability, selectivity and accuracy of determinations.
In this paper two, batch and FI, methods using spectrophotometric detection at 528 nm are described for the determination of paracetamol. The methods are based on the formation of a red dye between this drug and diazotized 4-nitroaniline (DNAN) in sodium carbonate medium. The proposed methods have been successfully applied to the determination of paracetamol in pharma-ceutical preparations.

Experimental

Apparatus

A Shimadzu UV-VIS 260 (Tokyo, Japan) digital double-beam recording spectrophoto-meter was used for all spectral and absorbance measurements with matched 1-cm quartz cells.
The FI system comprised a peristaltic pump (Ismatec, Labortechnik-Analytic, CH-8152, Glatbrugg-Zurich, Switzerland, six channels) with polyvinyl chloride flow tubes of 0.8 mm i.d., an injection valve (Rheodyne, Altex 210, Supelco-USA), a 50 µL flow cells and a Shimadzu UV-VIS 260 spectrophotometer (Tokyo, Japan) as the detector. Flexible Teflon tubes of 0.5 mm i.d. were used for reaction coils and to transport the reagents solutions. T-link was also used to mix two streams of reagents.

Reagents

All chemicals were of analytical reagent grade.
1- Paracetamol stock standard solution 500 µg mL^{-1} was prepared by dissolving 0.1000 g of pure paracetamol (SDI) in 20 ml of ethanol with carefully stir and diluting to the marked with distilled water in 200 mL volumetric flask. Working standard solutions were prepared by suitable dilution of the stock standard solution.
2- Sodium nitrite solution 0.05 M was prepared by dissolving 0.1725 g of sodium nitrite (Merck) in distilled water and diluting to the marked with the same solvent in 50 mL volumetric flask.
3- DNAN solution 3×10^{-3} M (for batch procedure) was prepared by dissolving 0.0207 g of 4-nitroaniline (Fluka) in 20 mL of ethanol with stir, then added 3 mL of 0.8 M of hydrochloric acid (BDH). The mixture was cooled to 0 ºC using ice-bath, then added 3 mL of 0.05 M of sodium nitrite (Merck) with stir. After 5 min, the mixture was transferred to 50 mL of volumetric flask and diluted to the marked with cooled distilled water.
4- DNAN solution 4×10^{-3} M (for FI procedure) was prepared by dissolving 0.0276 g of 4-nitroaniline in 10 mL of ethanol, 20 mL of heating distilled water and 3 mL of 0.8 M of hydrochloric acid (BDH) with heating on water-bath (40 ºC) for 2 min with stir. The mixture was cooled to 0 ºC using ice-bath, then added 3 mL of 0.05 M of sodium nitrite with stir. After 5 min, the mixture was transferred to 50 mL of volumetric flask and diluted to the marked with cooling distilled water.
5- Sodium carbonate solution 1 M was prepared by dissolving 10.5990 g of sodium carbonate (BHD) in distilled water and diluting to the marked with the same solvent in 100 mL volumetric flask.
More dilute solutions were prepared by appropriate dilutions using distilled water.

Pharmaceutical preparations of paracetamol

Pharmaceutical preparations were obtained from commercial sources.
1- Paracetamol tablets (Troge, Hamburg): 500 mg paracetamol for each tablet.
2- Paracetol tablets (SDI, Iraq): 500 mg paracetamol for each tablet.
3- Algesic tablets (SDI, Iraq): 350 mg paracetamol, 50 mg caffeine and 10 mg codien phosphate for each tablet.
4- Colden tablets (SDI, Iraq): 450 mg paracetamol, 5 mg promethazine hydrochloride and 5 mg phenylpherine hydrochloride for each tablet.
5- Emidol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet.
6- Kanagesic tablets (Kanawati Medical Products, Syria): 450 mg paracetamol and 35 mg orphenadrine citrate for each tablet.
7- Panatol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet.
8- Ultramol suppositories (Medico Labs. HOMS, Syria): 250 mg paracetamol for each suppository.
9- Hayamol injections (Ibn Hayyan Pharmaceutical HOMS, Syria): 375 mg paracetamol for each injection.

Recommended procedures for calibration

1- Batch procedure

To different volumes 0.1 – 5 mL of 100 µg mL^{-1} paracetamol, 4 mL of 3×10^{-3} M DNAN solution and 3 mL of 0.1 M sodium carbonate

solution were added and diluted with distilled water to 25 mL in calibrated flasks. After 30 min the absorbance of the dye formed was measured at 528 nm against reagent blank.

2- FI-procedure

The FI system is shown in Figure (1). 150 µL aliquots of paracetamol solutions prepared at different concentrations (1 – 150 µg mL^{-1}) were injected into carrier stream of DNAN solution of 4×10^{-3} M. The solution of sodium carbonate 0.05 M was mixed with the carrier stream at the down-stream confluence point. The total flow rate of the two channels was 2.4 mL min^{-1}. The reaction was carried out by passing the solution through a reaction coil (100 cm) and the absorbance of the resulting red dye was measured at 528 nm. Calibration graphs were prepared by plotting the absorbances of the peak maximum versus paracetamol concentration.

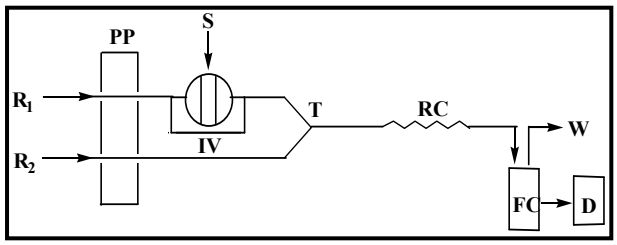


Figure (1): FI manifold for determination of paracetamol (R_1 = DNAN, R_2 = Na_2CO_3, S = Sample injection, PP = Peristaltic pump, IV = Injection valve, T = T-link, RC = Reaction coil, FC = Flow cell, D = Detector and W = Waste)

Procedure for the assay of pharmaceutical preparations

1- Tablets solution (500 µg mL^{-1})

The average tablet weight was calculated from the contents of 20 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 125 mg of paracetamol, was accurately weighed. The sample was shaken with 20 mL of ethanol and diluted with distilled water in a 250 mL volumetric flask. The solution was filtered twice into a 250 mL volumetric flask.

2- Suppositories solution (500 µg mL^{-1})

The contents of four suppositories were weighed. The accurately weighed amount of suppositories equivalent to 125 mg of paracetamol was dissolved in 10 mL of ethanol and a little amount of boiling distilled water. The solution was filtered into a 250 mL volumetric flask, the residue was washed with 10 mL of ethanol and boiling distilled water and diluted to volume with distilled water.

3- Injections solution (500 µg mL^{-1})

The contents of five injections were mixed. An aliquot corresponding to 125 mg of paracetamol (1.7 mL) was shaken with 20 mL of ethanol and diluted to 250 mL with distilled water in a volumetric flask.

Further appropriate solutions of pharmaceutical preparations for batch and FI procedures were made by using distilled water. Two different concentrations of each solution of pharmaceutical preparation were analyzed in five replicate by recommended batch and FI spectrophotometric procedures.

Results and discussion

Preliminary studies

Throughout the preliminary study on the reaction, between paracetamol with DNAN in sodium carbonate medium, a red colored product was obtained with a maximum absorbance at 528 nm [Figure (2)]. The absorbance of the colored product measured versus reagent blank which has minimum absorbance at the same wavelength.

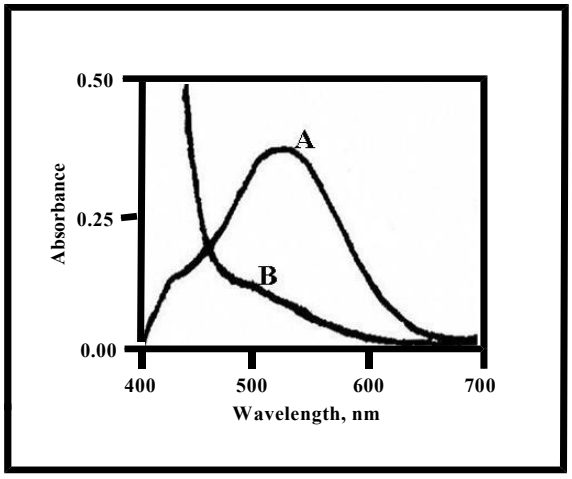


Figure (2): Absorption spectra of product against reagent blank (A) and reagent blank against distilled water (B)

Optimization of the experimental conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of paracetamol by batch and FI methods.

1- Batch method

In the subsequent experiments, 500 µg of paracetamol was taken in 25 mL final volume and the absorbance of a series of solutions were

measured by varying one and fixing the other parameters at 528 nm versus reagents blanks.

The effect of different volumes of 0.8 M hydrochloric acid (1 – 5 mL) (used for preparing the diazotized reagent), 3×10^{-3} M DNAN (1 – 7 mL) and 0.1 M sodium carbonate (1 – 5 mL) were examined on the maximum absorbance of the colored product. Figure (3) shows that 3 mL of hydrochloric acid (0.8 M), 4 mL of DNAN (3×10^{-3} M) and 3 mL of sodium carbonate (0.1 M) were enough to obtain the maximum absorbance.

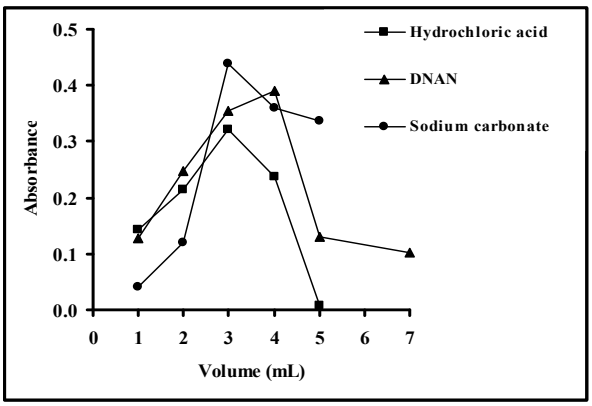


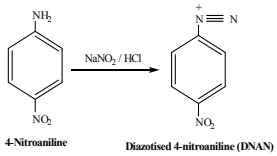
Figure (3): Optimum conditions of batch procedure for determination of paracetamol

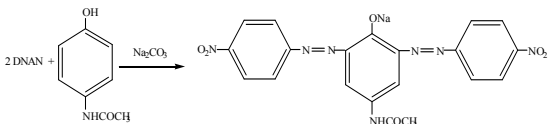
The red dye was only formed in alkaline medium. Therefore, the effects of different alkaline solutions were studied such as sodium carbonate, potassium hydroxide, sodium hydroxide and ammonium hydroxide. It was found that sodium carbonate is the most suitable alkaline medium to produce a maximum absorbance and was used in all subsequent experiments.

To obtain optimum results, the order of addition of reagents should be followed as given under the procedure, otherwise a loss in color intensity and stability were observed.

The stability of the dye was studied for 2 h following the mixture of the reagents. The absorbance of the dye was sharply increased 2 min after mixing and remained constant for at least 2 h.

The stoichiometry of the product was studied applying the mole ratio and continuous variation methods. The results obtained in Figure(4) and Figure (5) shows that a 1:2 product was formed between paracetamol and DNAN. Therefore, the formation of the product probably occurs as follows:





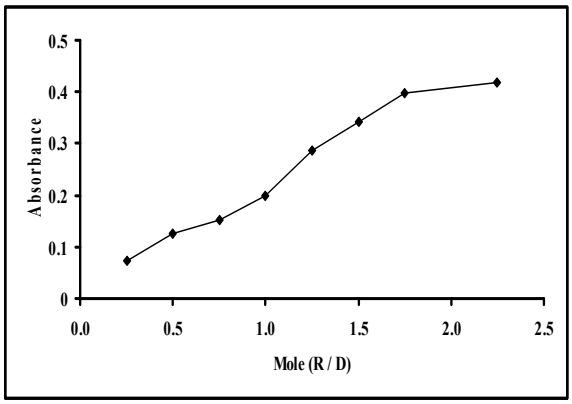


Figure (4): Mole ratio plot

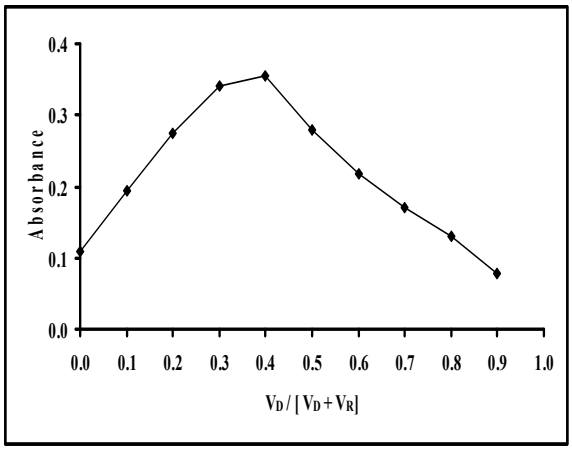


Figure (5): Continuous variation plot

The dye formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of paracetamol and DNAN with that of solution containing a five-fold excess of DNAN. The stability constant of the dye in water under the described experimental conditions was 1.43×10^{7} L^{2} mol^{-2}.

In order to assess the possible analytical applications of the proposed method. The effect of some common excipients frequently found with paracetamol in pharmaceutical preparations such as lactose, starch, talc, magnesium stearate and polyvinylpirrolidone (PVP) was studied by analyzing synthetic sample solutions containing 20 µg mL^{-1} of paracetamol and excess amounts

(10-fold excess) of each excipient, none of these substances interfered seriously [Table (1)].

Table (1): Determination of 20 µg mL^{-1} of paracetamol in the presence of excipients

Excipient (200 µg mL^{-1})	Concn. of paracetamol (µg mL^{-1})* Found	E**,%	Recovery,%
Lactose	20.365	+1.825	101.825
Starch	19.863	-0.685	99.315
Talc	19.910	-0.450	99.550
Mg stearate	19.954	-0.230	99.770
PVP	20.543	+2.715	102.715

*** Average of four determinations.**
**** E is relative error**.

2- FI method

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configurations and the approximate ranges of the tested parameters. The design of the manifold selected is shown in Figure (1) using total flow rate of 2.4 mL min^{-1} for two-channel. A two-channel FI assembly was adopted, in which the sample (100 µL) was injected into the DNAN stream, which was then mixed with a stream of sodium carbonate in the reaction coil (100 cm). The reagent and the sodium carbonate stream were pumped at the same flow rate to achieve effective mixing of the sample and reagent solutions. The DNAN reacted with paracetamol to produce a dye, whose absorbance was measured at 528 nm. The presence of the paracetamol caused an increase in the absorbance, which was proportional to its concentration.

According to the results of the preliminary spectrophotometric studies concerning the effect of alkaline medium on the absorbance of the product, a sodium carbonate was used for the FI method.

The effect of the concentration of sodium carbonate was studied in the range 0.01 – 0.70 M with fixed paracetamol concentration of 50 µg mL^{-1}. As can be observed from Figure (6) the absorbance was increased as the concentration of sodium carbonate was increased up to 0.05 M, thus 0.05 M sodium carbonate was found to be the most suitable concentration for a maximum absorbance and was chosen for further use.

The effect of different volumes of 0.8 M hydrochloric acid (used for preparing the diazotized reagent) was studied in the range 1 – 7 mL [Figure (6)]. The results show that 3 mL of hydrochloric acid enough to obtain the maximum absorbance and was chose for further use.

It was found that the reaction between paracetamol and DNAN in sodium carbonate medium depends on the DNAN concentration. Therefore, the effect of different concentrations of DNAN ($1 \times 10^{-3} - 7 \times 10^{-3}$ M) was studied [Figure (6)]. The result obtained indicated, that the absorbance increased with the increasing concentration of DNAN up to 4×10^{-3} M, thus a concentration of 4×10^{-3} M gave the maximum absorbance and was chosen for further use.

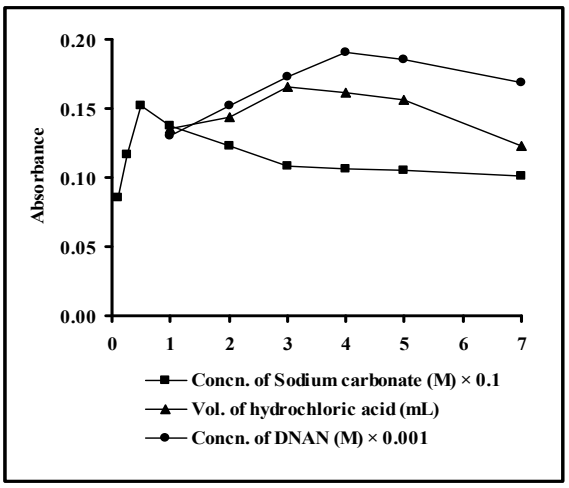


Figure (6): Chemical conditions of FI procedure for determination of paracetamol

The use of FI as an alternative to existing methods for paracetamol determination is dependent on optimization of the system to achieve maximum absorbance. As a consequence, several experiments were conducted in order to establish the best experimental conditions for operating the FI manifold.

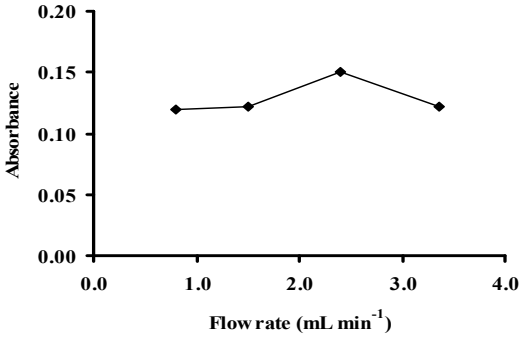
Figure (7) shows the effects of flow rate, reactor length and sample injection volume on the absorbance. The effect of flow rate on the absorbance was studied over the range 0.8 – 3.36 mL min^{-1}. Figure (7) shows that, with increasing flow rate, maximum sensitivity was obtained at 2.4 mL min^{-1}, which was selected, as a compromise between reproducibility and sampling rate. Above this value, the absorbance decreased slightly owing to dispersion effects.

The effect of reactor length was studied in the range 25 – 200 cm in the same experimental

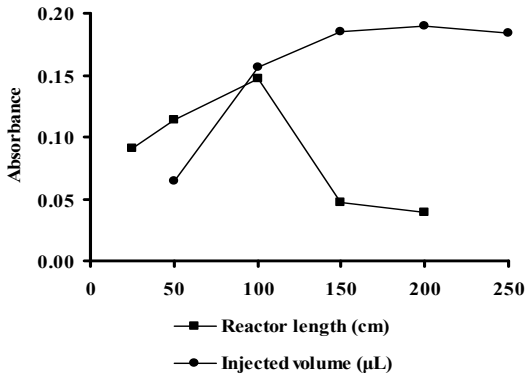
conditions selected above. As can be seen from Figure (7), maximum absorbance value was obtained at 100 cm and was selected for further use.

The volume of sample injected was varied in the range 50 – 250 µL by changing the length of the sample loop in the injection valve, while the other variable remained fixed. The absorbance increased with increasing volume of sample injected up to 150 µL [Figure (7)] which was selected.

The flow system selected provided a sampling rate of 173 samples h^{-1}



Figure(7): Physical conditions of FI procedure for determination of paracetamol



Analytical characteristics of the batch and FI spectrophotometric methods

For the batch and FI methods, the calibration graphs were obtained by the procedures described previous and a series of standard solutions were analyzed in triplicates to test the linearity. The slope (a), the intercept (b), the correlation coefficient (r) and the correlation of determination (r^2) were evaluated by a least-squares regression analysis and are included in Table (2).

Table (2): Data for the calibration graphs for paracetamol using the proposed methods

Parameter	Value	
	Batch method	FI method
Linearity range (µg mL^{-1})	0.5 – 20	1 – 150
r	0.9992	0.9995
r^2	0.9984	0.9990
a (mL µg $^{-1}$)	0.0183	0.0019
b	0.1016	0.1017
$S_{y/x}$	5.7412×10^{-3}	3.5633×10^{-3}
S_a	2.9405×10^{-4}	2.3292×10^{-5}
S_b	3.0932×10^{-3}	1.8262×10^{-3}
E%	0.3641*	0.2255**
RSD%***	1.2420	1.5634

*** For 12 µg mL^{-1} of paracetamol.**
**** For 70 µg mL^{-1} of paracetamol.**
***** Average of five determination.**

Statistical evaluation[17] of the regression line gave the values of standard deviations for residuals ($S_{y/x}$), slope (S_a) and intercept (S_b) at 95% confidence are shown in the same Table.

These small figures point out to the high precision of the proposed methods**.**

Accuracy and precision of the batch and FI spectrophotometric methods

The accuracy and precision of the two methods were tested by analyzing five replicate samples of paracetamol by batch and FI spectrophotometric methods. The low values of the percentage errors (E%) are summarized in Table (2). The percentage relative standard deviation (RSD%) was found to be less. These values indicate the high accuracy and precision of the two methods.

Pharmaceutical applications

In order of demonstrate the applicability of the proposed methods to the determination of paracetamol, the methods was applied to the analysis of paracetamol in various samples of pharmaceutical preparations.

The two proposed methods were successfully applied to the analysis of different pharmaceutical preparations containing paracetamol and the results are summarized in Table (3). When different pharmaceuticals of paracetamol were analyzed by the proposed methods, interference from the sample matrix

posed no problem. For all the formulations examined, the assay results of both methods were in good agreement with the declared content. In two methods, quantitative recoveries between 98.400 and 102.916% were obtained.

The results obtained by the two proposed methods were compared with British Pharmacopoeia (BP) method [Table (4)] by applying the F-test and the t-test at 95% confidence level. The calculated values for F and t for batch and FI methods (1.612, 0.496 and 1.274, 0.787 respectively), did not exceed the critical values of F= 4.033 and t = 2.101 ($n_1 + n_2 - 2 = 18$). These confirming that there are no significant differences between the two proposed methods with BP method[18] with respect to precision and accuracy in the determination of paracetamol in pharmaceutical preparations.

Conclusions

The dye is stable in sodium carbonate medium and has spectrophotometric characteristics suitable for application to spectrophotometric determination of the drug by batch and FI techniques.

The FI spectrophotometric methods proposed for the determination of paracetamol in pure and pharmaceutical forms has the advantages of simplicity, speed, accuracy and the use of inexpensive equipment.

The batch and FI methods are useful for the quality control and routine analysis of paracetamol in pharmaceuticals since there is no 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**.**

Table (4): Comparison of the two methods with BP method for determination of pharmaceutical preparations

Pharmaceutical Preparation	Recovery, %*		
	Batch method	FI method	BP method
Pure paracetamol	101.476	99.839	100.000
Paracetamol Tablets	100.444	99.818	99.627
Paracetol Tablets	100.761	100.098	100.192
Algesic Tablets	100.220	99.152	100.777
Colden Tablets	99.757	99.713	98.978
Emidol Tablets	101.280	100.642	100.813
Kanagesic Tablets	100.606	100.501	100.000
Panatol Tablets	99.200	101.445	100.000
Ultramol Suppoistor.	100.444	100.069	100.186
Hayamol Injections	99.095	101.205	101.128

***Average of five determinations.**

Table (3): Pharmaceutical applications for paracetamol using the proposed methods

Method	Pharmaceutical Preparation	Concn. of paracetamol (µg mL^{-1})*		E,%	Recovery,%	RSD,%
		Present	Found			
Batch	Paracetamol Tablets	10.000	9.968	– 0.314	99.686	1.400
		20.000	19.990	– 0.050	99.950	0.680
	Paracetol Tablets	10.000	10.006	+ 0.062	100.062	0.458
		20.000	20.026	+ 0.133	100.133	0.825
	Algesic Tablets	10.000	9.875	– 1.250	98.750	1.137
		20.000	19.910	– 0.446	99.554	0.354
	Colden Tablets	10.000	9.870	– 1.220	98.780	1.340
		20.000	20.129	+ 0.645	100.645	1.790
	Emidol Tablets	10.000	10.060	+ 0.615	100.615	1.226
		20.000	20.133	+ 0.668	100.668	1.200
	Kanagesic Tablets	10.000	9.960	– 0.311	99.689	1.809
		20.000	20.260	+ 1.310	101.312	1.407
	Panatol Tablets	10.000	10.091	+ 0.909	100.909	1.720
		20.000	20.390	+ 1.980	101.980	0.570
	Ultramol Suppoistories	10.000	10.160	+ 1.689	101.689	3.890
		20.000	20.090	+ 0.450	100.450	1.651
	Hayamol Injections	10.000	10.000	0.000	100.000	0.910
		20.000	20.480	+ 2.410	102.410	1.500
FI	Paracetamol Tablets	70.000	70.000	0.000	100.000	1.953
		120.000	121.066	+ 0.888	100.888	1.393
	Paracetol Tablets	70.000	70.454	+ 0.649	100.649	1.329
		120.000	121.048	+ 0.873	100.873	0.838
	Algesic Tablet	70.000	70.933	+ 1.333	101.333	2.348
		120.000	118.928	– 0.893	99.107	1.388
	Colden Tablets	70.000	70.503	+ 0.719	100.719	3.443
		120.000	118.554	– 1.205	98.795	2.413
	Emidol Tablets	70.000	70.451	+ 0.645	100.645	1.013
		120.000	123.500	+ 2.916	102.916	0.535
	Kanagesic Tablets	70.000	69.014	– 1.408	98.592	2.387
		120.000	123.144	+ 2.620	102.620	1.258
	Panatol Tablets	70.000	68.880	– 1.600	98.400	2.112
		120.000	120.000	0.000	100.000	1.283
	Ultramol Suppoistories	70.000	70.000	0.000	100.000	1.944
		120.000	121.066	+ 0.888	100.888	1.572
	Hayamol Injections	70.000	69.034	– 1.379	98.620	1.398
		120.000	119.482	– 0.431	99.568	1.140

***Average of five determinations.**

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