Sequential Injection Spectrophotometric Method for the Assay of Paracetamol in Drug Formulations

Salah M. Sultan, Abubakr M. Idris and Kamal E. E. Ibrahim
Chemistry Department, King Fahd University, KFUPM Box 2026, Dhahran 31261, Saudi Arabia
e-mail: smsultan@kfupm.edu.sa

Sequential injection analysis technique was employed as a successful tool for titrimetric determination of paracetamol in drug formulation. The potential aspect of paracetamol to reduce the absorbance of potassium permanganate in sulfuric acid media was applied to develop an ideal spectrophotometric data. The super modified simplex computer program was operated to optimize the main reaction parameters such as sulfuric acid and potassium permanganate concentrations and the (3^f) factorial design was employed for studying their interaction effects. This method was found to be optimum at $1.79X10^{-3}$ mol dm^{-3} potassium permanganate and 0.071 mol dm^{-3} sulfuric acid concentrations for the detection limit between 50 to 350 ppm. The results obtained showed an accurate outcome when statistically compared with the BP standard method. The method reveals no interferences from most of the added excipients in drug formulation.

Keywords: *titrimetry; sequential injection; permanganate; paracetamol and spectrophotometry.*

Introduction

Paracetamol or acetaminophen (N-acetyl-4-aminophenol) has been in use as an analgesic for home medication for over 30 years and is accepted as a very effective treatment for the relief of pain and fever. Several analytical methods have been reported in the literature for the determination of paracetamol using different techniques e.g. titrimetry[1], electroanalytical[2,3],chromatography[4-6] and IR spectrophotometry[7-16].

In the BP official method[17] paracetamol is determined by titrating the solution prepared in dilute hydrochloric acid versus ammonium and cerium sulphate using ferroin indicator to the yellow end point.

In the present method, the absorbance of $KMnO_4$ was measured at λ_{max} at 525nm after its oxudation with paracetamol in sulfuric acid media. The method is appropriate for routine pharmaceutical analysis with a throughput of 100 sample h^{-1}.

Experimental

Reagents and chemicals

Double-distilled deionised water was used throughout the preparation of stock and working solutions.

All inorganic chemicals used were of analytical reagent grade.

Paracetamol pure analytical grade sample was always freshly prepared from SPIMACO Saudi Arabia (in-house reference standard, Lot number (0109147) previously dried at 50°C in vacuum over magnesium perchlorate. This standard solution was used to give the appropriate concentrations by further dilutions.

Paracetamol tablet. From the proprietary drugs ten tablets were accurately weighed, crushed and powdered. The amount of powder containing the appropriate weight to give 200 ppm vitamin C was dissolved in about 40 ml of water; heated for 3 minutes then it was made up to volume in a 100 ml volumetric flask after cooling

Stock solutions

Paracetamol (0.04* mol dm^{-3}*).

A stock solutionwas directly prepared by dissolution in water with an appropriate amount of sulfuric acid priorly added to it.

Potasium permanganate (0.0199 mol dm^{-3}).

A standard solution was prepared by dissolving exactly about 3.5 g of dried potassium permanganate (P-279 Lot 746030 Fisher scientific company, USA) in 1000 ml. The stock solution was standardized with sodium oxalate (S-356 Lot 792406 Fisher scientific company, USA). This solution was used through all the experimental processes.

Sulfuric acid Solution (1.0 mol* dm^{-3}*).

A stock solution of H_2SO_4 (95-98% Specific gravity 1.84 kg / l, Merck, UK) was prepared the usual way. Working solutions were prepared by dilutions.

Apparatus

The FIALab 3000 (Medina, WA USA) has been used in this method. The apparatus consists of a syringe pump, a multi-position valve, a fiber optic spectrophotometer and a PC (Figure 1).

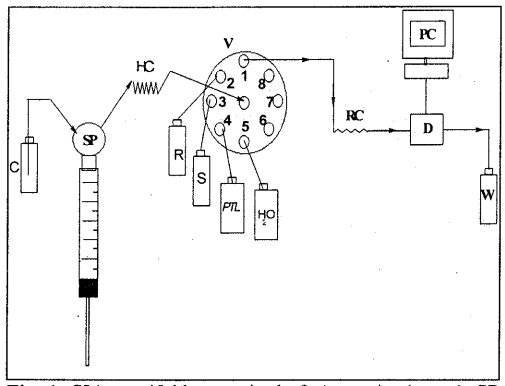

Fig. 1: SIA manifold comprised of; A. carrier (water); SP. 5 mL syringe pump; HC. holding coil; V. eight ports selector valve; RC. reaction coil; R. $KMnO_4$; S.sulfuric acid; PTL Paradetamol; RC. Reaction coil; D. spectrophotometer; PC. Computer and W. waste

The syringe pump is a 24,000 steps syringe pump with an optical encoder feedback; 1.5 seconds to 20 minutes per stroke of 5.0 mL size. It is >99% accuracy at full stroke.

The multi-position valve has eight(8) ports with a standard pressure of 250 psi(gas)/600 psi (liquid); zero dead volume; chemically inert; port selection by manual or software control.

The spectrophotometer is the S2000 miniature fibre optic spectrometer pre-set to 200-850 nm wavelength range, UV detector, multi-band pass coating, 25 micron entrance slit from ocean optics, Inc. USA.

Z-Flow cell is 10 mm path length Teflon and Plexiglas or high-grade stainless steel compatible with standard SMA terminated fibre optics

Pump tubing of 0.30 inch ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units, making the holding coil (400 cm long) and the reaction coil (100 cm Long).

Software Packages

FIALab software has been used for programming and controlling the SIA system. *Sigma plot, version 1.02* (Jandel Scientific, Erkrath, Germany) was employed for data-handling calculations, multiple regression analysis and constructing graphs.
The COPS i.e. "Chemometric Optimization by Simplex" programme was obtained from Elsevier Scientific Co., The Netherlands, and utilized for the optimization of variables using a compatible IBM personal computer.

Method and Procedure

In this method FIALab software is utilized for controlling different SIA components as shown in Fig 1.
For the sequential injection process, 5 ml syringe pump (SP) were used to perform both aspirating and dispensing operations with the following steps.
I- All working solutions of potassium permanganate (R), paracetamol (PTL) sulfuric acid (S) and water (for total volume adjustment) were linked to the selector valve through ports 2,3,4 and 5 respectively. Tubings were loaded with their respective reagents by an aspiration run.
II- The syringe pump was filled with water as a carrier (C) by directing the two way valve to the (in-position) mode.
III- 500 µl were dispensed to wash the holding coil (HC), Reaction coil (RC), the "Z" photo cell and to adjust the absorbance of the spectrophotometer to zero.
IV- With a 150 µl/s flow rate, 200 µl potassium permanganate, 30 µl sulfuric acid

and 50 µl water (equivalent to the reacted paracetamol volume) were sequentially aspirated into the holding coil.

V- A short reverse stroke was performed to allow all reagents to mix with a flow rate of 30 µl/s followed by continuous dispensing towards the detector (D) for 30 seconds and hence the absorbance (A_1) was recorded.

VI- Steps 4 and 5 were repeated with replacing water with 50 µl paracetamol and absorbance (A_2) was recorded.

VII- The absorbance equivalent to the paractetamol reacted with permanganate was calculated as the difference between (A_1) and (A_2).

Results and Discussion

The oxidation reaction of paracetamol with permanganate in sulfuric acid media is explored. The reaction route is suggested to take place similar to the reaction of paracetamol with other oxidants earlier reported[1-5]. Paracetamol is believed to be oxidised to its final oxidation product, parabenzoquinone.

The present SIA titration method is primarily based on this reaction performed by aspirating excess concentration of acidic permanganate and sequentially aspirating micro litre amounts of low concentrations of the drug into the holding coil. By spectrophotometric measurements of the absorbance of permanganate at its maximum wavelength at 525 nm before and after aspirating the drug, the absorbance equivalent to the paracetamol reacted with permanganate is calculated.

Prior to the quantitative SIA titration procedure, thorough investigation on the reaction conditions was carried out. The reaction was found to be affected by both permanganate and sulfuric acid concentrations, therefore a full chemometrical optimization for these operating parameters was conducted. An experimental design approach[18-22] was employed and a 3^k factorial design was run where 3 stands for variable levels considering the upper, middle and lower values; and k is the number of factors studied. The upper, middle and lower values were determined by carefully studying the reaction conditions and assigned +1, 0 and –1 coded levels respectively as introduced in Table 1 with constant aspiration of 4.96 $x10^{-3}$ mol dm^{-3} of paracetamol. For permanganate, the higher concentration was 2.20 x 10^{-3} mol dm^{-3}, the middle was 1.73 $x10^{-3}$ mol dm^{-3}; and the lower was 9.44 x 10^{-4} mol dm^{-3}. For sulfuric acid, the higher concentration was 0.080 mol dm^{-3}, the middle was 0.055 and the lower was 0.030 mol dm^{-3}. Nine experiments were needed and the equivalent response function taken as peak absorbance difference between (A_1) and (A_2) as explained in the experimental part above.

The data obtained in Table 1 was treated by Sigma plot Software and the two factors were considered for a 3D response surface plot. Fig. 2 shows the response surface as a function of permanganate and sulfuric acid concentration levels. This plot reveals that the response increases as the concentration of both reagents increases. The increase behaviour of sulfuric acid shows levelling at higher concentrations and this could be attributed to the fact that at higher hydrogen ion concentration, the redox potential value for permanganate increases towards forming more products and hence higher response is obtained. The levelling effect is attributed to the limited amount of hydrogen ions required for reaction with oxygen of the permanganate forming water as one of the products.

Both variables, the permanganate and sulfuric acid concentrations were further more optimized employing the super modified simplex algorithm[18-22]. The simplex experiment was performed by injecting 50 µL paracetamol of 150 ppm. The step values for permanganate were 75 to 160 and for paracetamol concentrations were 0.02 to 0.053 mol dm^{-3}. The simplex was started by feeding the program with the initial simplex chosen on the light of experimental trials on the system. Nineteen experiments were found to be enough to judge on the suitable conditions for

maximum response value, which was obtained at experiment 14.

Table 1 Full treatment combinations in both their original and coded levels along with the responses obtained by reacting paracetamol with different concentrations of $KMnO_4$ in different H_2SO_4 concentrations.

Exp No	$KMNO_4$ Coded level	H_2SO_4 Coded level	$KMNO_4$ /moll^{-1}	H_2SO_4 /moll^{-1}	Response/ ml
1	-1	-1	$9.446x10^{-4}$	0.030	20.0
2	-1	0	$9.446x10^{-4}$	0.055	43.0
3	-1	+1	$9.446x10^{-4}$	0.080	52.0
4	0	-1	$1.73 x10^{-3}$	0.030	41.0
5	0	0	$1.73 x10^{-3}$	0.055	46.0
6	0	+1	$1.73 x10^{-3}$	0.080	53.0
7	+1	-1	$2.52 x10^{-3}$	0.030	50.0
8	+1	0	$2.52 x10^{-3}$	0.055	43.0
9	+1	+1	$2.52 x10^{-3}$	0.080	55.0

* The codes (–1), (0) and (+1) denote the lower, middle and upper value used for each factor respectively.

Table 2 Super-modified simplex optimization progress obtained by reacting 4.96 $x10^{-3}$ mol dm^3 paracetamol with different concentrations of $KMnO_4$ in different concentrations of H_2SO_4.

No	$KMnO_4$ /mol dm-3 (200µl injected)	H_2SO_4 /mol dm-3 (30µl injected)	Response
1	$1.05 x10^{-3}$	0.035	15
2	$1.37 x10^{-3}$	0.042	26
3	$1.13 x10^{-3}$	0.064	20
4	$1.46 x10^{-3}$	0.071	35 R
5	$1.55 x10^{-3}$	0.08	40
6	$1.70 x10^{-3}$	0.058	43
7	$1.89 x10^{-3}$	0.057	45 R
8	$1.50 x10^{-3}$	0.055	31 R
9	$1.85 x10^{-3}$	0.080	53
10	$2.20 x10^{-3}$	0.059	57
11	$2.20 x10^{-3}$	0.059	57 R
12	$2.14 x10^{-3}$	0.080	52
13	$1.89 x10^{-3}$	0.065	55 R
14	**$1.79 x10^{-3}$**	**0.071**	**66**
15	$1.99 x10^{-3}$	0.069	60 R
16	$2.19 x10^{-3}$	0.064	56
17	$2.05 x10^{-3}$	0.064	53
18	$1.98 x10^{-3}$	0.065	55 R
19	$2.12 x10^{-3}$	0.064	56

Analytical Appraisals

Series of standard solutions of paracetamol were sequentially aspirated into the holding coil already loaded with acidic permanganate of the same optima above. A calibration plot of absorbance versus paracetamol concentration

The response value calculated in mm as a difference of absorbance before and after aspirating the drug versus experiment number is shown in Fig. 3. The figure demonstrates the progress of the simplex gradually towards the maximum. Experiment 14 gave the highest response and considered for the optimum experimental conditions for quantitative analysis of paracetamol, which were, $1.79x10^{-3}$ mol dm^{-3} permanganate and 0.071 mol dm^{-3} sulfuric acid.

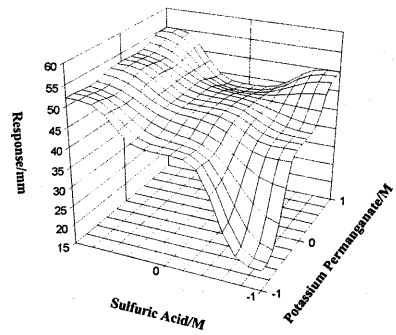

Fig.2 Surface plot of the response in mm versus permanganate and sulphuric acid concentration levels

was computed and the following calibration equation was obtained: $A = -0.0126 + 0.0012\ C$ where C is the concentration of paracetamol in ppm. The above calibration equation was found to be linear over the concentration range 50 to 350 ppm with a correlation coefficient of 0.994 and a relative standard deviation of 1.33 % for 5 aspirations.

3.2 Application

The SIA titrimetric method was applied to the determination of paracetamol in proprietary drugs. Table 3 shows the results of analyses of these drugs containing paracetamol with a statistical comparison comparing with those results obtained when the official (BP)[17] was applied to the same batch of samples. The statistical results

Table 3: The results obtained by the SIA method for paracetamol in proprietary drugs Statistically compared with the results obtained by the BP method

Drug	Supplier	Contents/mg	Mean rrecovery ± RSD(%)*		t
			SIA method	method	
Aspirine	Bayer, Germany	Aspirin 400mg Vitamin C 240 mg	100.3 ± 5.3	100.7 ± 0.3	1.2
Sedergine	UPSA, France	Aspirin 300mg Vitamin C 200 mg	99.8± 2.1	101.3± 1.9	1.8
Anacine	Whitehall Labs Corp., USA	Aspirin 400mg Caffeine 32 mg	102 ± 1.3	99.8 ± 3.0	2.3
Aspro	Nicholas, UK	Aspirin 320mg Caffeine 32.4 mg Chlorpheniramin e maleate 2.0 Phenylphrine base 10.0	102 ± 2.0	99.9 ± 2.7	1.7
Coricidin-D	Schering, USA	Aspirin 388.8 mg Caffeine 32 mg	100.9 ± 1.4	102.2 ± 2.6	1.6

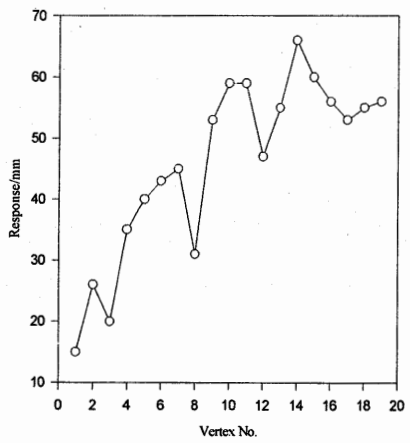

Fig.3 Response function progress of the simplex

clearly indicate that the proposed SIA titration method is accurate and precise. In addition, results indicate that excipients added such as starch, CMCS, magnesium stearate, etc. has no interference effects. It is concluded that the present method is quite suitable for the determination of paracetamol in drug formulations.

3.3 Conclusion

The present method is considered a new positive, successful and a potential application of SIA tirimetry. The use of permanganate for the first time for paracetamol determination is considered an excellent success and advantage over other methods using different mild oxidants such as dichromate and cerium(IV), which were found to react slowly. The present method is fast and economical with respect to time of analysis and consumption of reagents.

3.4 Acknowledgement

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