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Determination of Paracetamol in Tablet by Difference Spectrophotometric Method

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A simple, rapid and sensitive difference spectrophotometric method was used for the determination of paracetamol in pharmaceutical dosage forms. The method based on the induced spectral changes upon changing the pH of the medium and measuring the difference in the absorbance at 268 nm. The calibration graph is linear over the concentration range of 2.5-45 μg/mL (r^2 = 0.9983), with a detection limit of 0.59 μg/mL. The proposed method applied to determine paracetamol in tablets, alone or associated with caffeine. The results statistically compared with those obtained by the reference method.

Key Words: Paracetamol, Difference spectrophotometry, Tablets, Caffeine.

INTRODUCTION

Paracetamol (Fig. 1), also known as acetaminophen, is widely used as an analgesic and antipyretic drug. It could obtain in different pharmaceutical formulations. It widely used as an alternative for patients susceptible to acetylsalicylic acid (aspirin) in treatment of pain and fever[1-3].

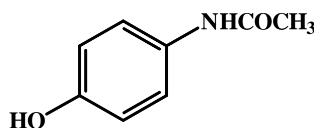


Fig. 1. Chemical structure of paracetamol

However, overdose of paracetamol could cause fatal hepatotoxicity and nephrotoxicity[2]. In this way, several methods have utilized for paracetamol determination in pure form, formulation and combination with other substances. The large attainment has given to the colorimetric and spectrophotometric methods[4-10]. Spectrophotometric procedure based on direct absorbance measurement in alkaline medium was reported for official method analysis of paracetamol in tables (British Pharmacopia)[11]. Spectrophotometic kinetic method also used for determination of paracetamol which oxidized with peroxydisulphate[12]. Literature survey show other methods such as chemiluminescence[13], electrochemical methods, especially with amperometric measurements[14,15].

Recently, various simultaneous methods for the determination of paracetamol with other components have reported. Colorimetry using tiron[16] was used for determination of paracetamol in combination of *p*-aminophenol. Sandulescu *et al.*[17] have developed a spectrophotometic and electroanalytical methods for both paracetamol and ascorbic acid in effervescent dosage forms. Vierordt`s method and ratio spectra derivative method have used to solve the overlapping of paracetamol with other active components[18-20]. First derivative spectrophotometry and difference spectrophotometry[21,22] and HPLC[23,24] are also used.

The idea of the present work is to provide simple sensitive and rapid spectrophotometric determination of paracetamol, the method is free from interference when excipient or other components such as caffeine are present. The essential feature of a difference specrophotometric assay is that the measured value is the difference absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms, which exhibit difference spectral characteristics. The simplest and most commonly employed technique for altering the spectral properties of analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers[25].

EXPERIMENTAL

A Jena Model UV-VIS spectrophotometer (Jena, Germany) with 1.0 cm matched quartz cells was used.

Pharmaceutical grade paracetamol was kindly provided by Prof. F. Belal (Faculty of Pharmacy, Mansoura University, Mansoura, Egypt), the drug was used without further purification. Sodium hydroxide and hydrochloric acid (were from Sigma, st Louis, MO, USA) (0.1M solution), potassium dihydrogen orthophosphate (Fluka-Garantie, Sigma-Aldrich, Milan, Italy), di-sodium hydrogen phosphate (East Anglia Chemicals). Water was always double distilled.

Phosphate buffer pH 7 (0.1 M): The buffer was prepared by dissolving 1.361 g of potassium dihydrogen orthophosphate in sufficient water to produce 100 mL and adjust the pH using a 3.5 % (w/v) solution of di-sodium hydrogen phosphate[11].

Commercial dosage forms, paracetamol extra (paracetamol P.B 500 mg and caffeine B.P 65 mg tablet, SQUARE Pharmaceuticals LTD), paracetamol caplets (paracetamol 500 mg tablets, The Wallis Laboratory LTD), Fevadol Plus (paracetamol 500 mg, caffeine 35 mg, codeine phosphate 8 mg tablet, SPIMACO), Amol Extra (paracetamol 500 mg, caffeine 65 mg tablet, Shaphaco Pharmaceutical Ind), were purchased from local drug stores.

Procedure

Calibration: Stock paracetamol solution was prepared by dissolving 12.5 mg of pure paracetamol in 100 mL of water. Working standard solutions with concentration ranging from 2.5-45 μg/mL in water were prepared by transfer appropriate volumes of stock solution to 25 mL volumetric flasks in duplicate. The volume was then adjusted with 0.1 M HCl and 0.1M NaOH to give a series of equimolar solutions of paracetamol in different pH medium.

Assay of paracetamol in tablet: The average mass of 10 tablets was determined and were ground in a mortar. An amount of powder (accurately weighed) equivalent to 12.5 mg of paracetamol was transferred into 100 mL volumetric flask and made up to the mark with water. The content of the flask was stirred magnetically for 10 min and then the solution was filtered through Whatmann filter paper No. 1. The first filtrate was been removed and then 3 mL of the filtrate was transferred to 25 mL volumetric flask in duplicate. The volume was then adjusted with 0.1 M HCl and 0.1 M NaOH. The absorbance difference (ΔA) between the acidic solution and equimolar 0.1 M NaOH solution of pure drug and samples were measured at 268 nm using UV-Vis spectrophotometer with two 1.0 cm matched cells, by placing the acidic solution as reference and NaOH solution as sample.

The content of the tablets is calculated from the calibration curve or using the corresponding regression equation.

RESULTS AND DISCUSSION

This work describes a simple pH-induced difference spectrophotometric method for the determination of paracetamol in tablets (in presence of excipients).

The absorption spectra of equimolar solutions of paracetamol in 0.1 M HCl (pH 1), 0.1 M phosphate buffer solution (pH 7) and 0.1 M NaOH solution (pH 13) are shown in Fig. 2.

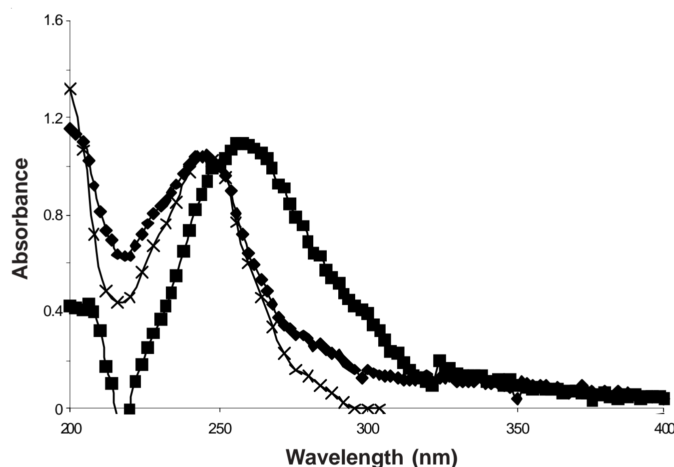
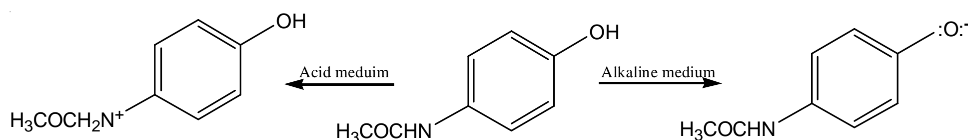


Fig. 2. Absorption spectra of equimolar solution of paracetamol (15 μg/mL) in (■) 0.1 M NaOH, (◆) 0.1 M HCl and phosphate buffer (pH 7) (×)

The ionization of the phenolic group in alkaline solution generates an additional n-(non-bonded) electrons that interacts with the ring π-electrons to produce a bathochromic shift of λ_{max} from 244 nm in acidic (or neutral) solution to 260 nm in alkaline solution. An increase in absorbance at the 260 nm (hyperchromic effect) is also shown (Fig. 2)[26,27].



The absorption spectrum of paracetamol in acidic medium is almost identical to that in buffer solution pH 7. Therefore, the further measurements will carry out in basic and acidic solution only.

Fig. 3 shows the difference absorption spectrum of paracetamol solution. It is generated by measure the absorbance of equimolar paracetamol solution at pH 13 (NaOH solution) in sample cell against the paracetamol at pH 1 (HCl) in the reference (blank) cell.

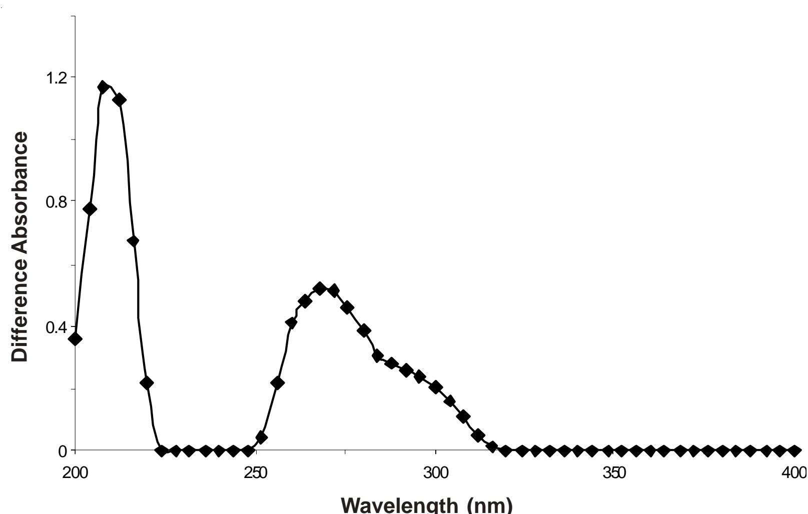


Fig. 3. Difference absorption spectrum of equimolar solution of paracetamol (15 μg/mL) in 0.1 M NaOH *vs.* 0.1 M HCl

At 250 and 320 nm both acidic and alkaline solutions of paracetamol have identical absorbance and consequently exhibit zero difference absorbance. Such wavelengths of equal absorptivity of the 2 species called isobestic or iso-absorptivity points[26,27]. Above 250 nm the alkaline solution absorbs more intensely than the acidic solution and the ΔA is therefore positive. Below 250 nm the acidic solution absorbs more intensely than alkaline solution so the ΔA is negative. The suitable wavelength for quantitative difference spectrophotometric measurements of paracetamol is at 268 nm (λ_{max}).

Calibration plot: Under the above conditions, a plot of difference absorbance (at 268 nm) *versus* paracetamol concentrations were linear over the concentration range 2.5-45 μg/mL ($r^2 = 0.9983$) with a slop of 3.55×10^{-3} and intercept of 3.79×10^{-3} (Fig. 4).

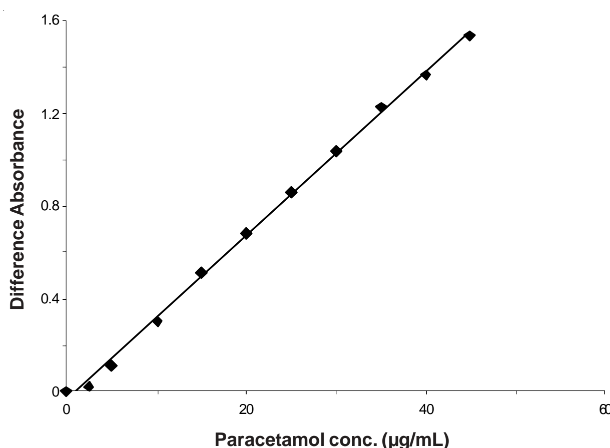


Fig. 4. Difference absorption calibration curve of paracetamol in 0.1 M NaOH and 0.1 M HCl. The linear regression equation is: Y = 0.0355X + 0.0379 (r^2 = 0.9983)

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be detected. The LOD was found to be 0.59 μg/mL, according to the 3 s/m definition[28], where s is the standard deviation (n = 6) of the signal from 15 μg/mL paracetamol aliquots and m is the slop of the calibration graph.

Interference studies: The possible interference effect of caffeine (co-formulated drug) at 3 different concentrations (7.5, 15, 30 μg/mL), were tested by adding to paracetamol standard solution (15 μg/mL). Table-1 illustrated that, the difference absorbance values obtained from the paracetamol-caffeine mixture was undistinguishable from the difference absorbance values produce by paracetamol alone. According to these results, it is concluded that caffeine produced no measurable interference in commercial samples containing paracetamol and caffeine.

TABLE-1
EFFECT OF CAFFEINE ON THE PERFORMANCE OF THE PROPOSED METHOD, USING 15 μg/mL PARACETAMOL STANDARD SOLUTIONS

Caffeine concentration (μg/mL)	Difference absorbance (ΔA)*
0.0	0.522
7.5	0.525
15.0	0.533
30.0	0.516

*Each value is the average of 2 values.

The effect of foreign substances, inactive excipient materials, that commonly accompanying the drug in pharmaceutical formulation (tablets) (starch, mannitol, cellulose, polyvinylpyrrolidone, calcium phosphate, magnesium stearate, hydroxypropyl-methylcellulose, polyethylene glycol, titanium dioxide) was studied by comparing

the difference absorption spectra of paracetamol in standard solution and in solution of tablets extract (for example: paracetamol caplets, fevadol plus, amol extra and paracetamol extra tablets). The obtained absorption spectra of paracetamol tablets extract and paracetamol standard solution are undistinguishable, Fig. 5, confirmed that tablet excipients have no interference effect on the measurements of the ΔA values at λ_{max}.

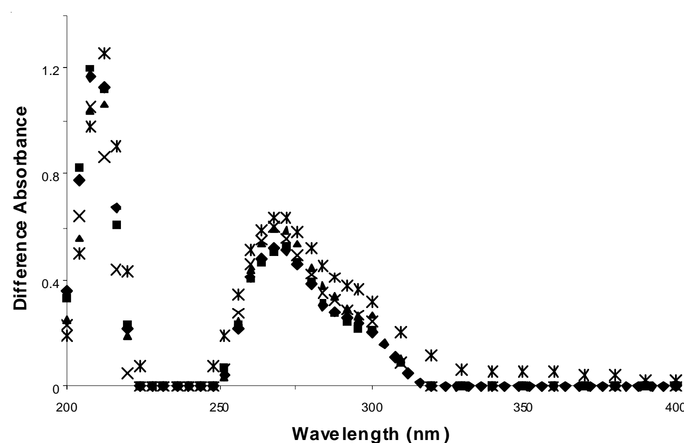


Fig. 5. Difference absorption spectra of equimolar solution of paracetamol (15 µg/mL) in 0.1 M HCl *vs.* 0.1 M NaOH, using standard solution (◆) and using the solution of 4-dosage forms *e.g.*, paracetamol caplets extract (■), fevadol plus extract (▲), amol extra extract (×) and paracetamol extra (✱)

Analysis of commercial tablets: Difference spectrophotometric method applied to 4 brands of paracetamol tablets well known in the market. The results of analysis are reported in Table-2. The tablets were found to be within the British Pharmacopia acceptable range (100.40-104.38 %). The reproducibility of the method was checked by 7 replicate determinations and then the relative standard deviation (RSD) was calculated. The results obtained were compared with those obtained from a reference method (direct absorbance measurement)[11]. When the student's t-test (measure of accuracy) was applied, the t-values at the 95 % confidence level, for the four preparations, were smaller than tabulated value. When the variance ratio-F-test (measure of precision) was applied, F-values were also smaller than the tabulated value. It is concluded that there is no significant difference between the proposed method and official method in terms of accuracy and precision[29].

Conclusion

A rapid, practical and sensitive difference spectrophotometric method is described for determination of paracetamol in tables. The method dose not required stringent conditions nor any specific reagent or buffer. It neither needs preliminary extraction process. The statistical analyses show that the data from the proposed method are in good agreement with those of the reference method.

TABLE-2
DETERMINATION OF PARACETAMOL IN COMMERCIAL TABLETS USING THE PROPOSED PROCEDURE AND OFFICIAL METHOD

	Difference spectrophotometric method (Recovery %)			
	Fevadol plus	Paracetamol caplets	Paracetamol extra	Amol extra
Mean[a] ± SD	102.93 ± 1.07	100.40 ± 1.20	103.71 ± 0.64	104.38 ± 0.62
RSD[b]	1.0400	1.200	0.620	0.590
t-Test[e]	0.0365	1.589	1.400	1.420
F-Test[e]	0.7160	0.954	0.078	0.159
	Official method[d]			
	Fevadol plus	Paracetamol caplets	Paracetamol extra	Amol extra
Mean[a] ± SD	102.78 ± 0.88	99.68 ± 1.13	103.38 ± 1.44	103.83 ± 1.18
RSD[b]	0.86	1.13	1.39	1.14
t-Test[e]	–	–	–	–
F-Test[e]	–	–	–	–

[a]Mean of 7 values; [b]RSD = Relative standard deviation; [c]Recovery (%) = [Amount found/ Amount taken] × 100; [d]Tablets Analyzed by direct measurement of absorbance [B.P., 1998]. [e]t-Test and F-test statistical analyses were performed using SPSS10 programme.

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