Asian Journal of Chemistry; Vol. 26, No. 20 (2014), 7016-7020

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17811

Spectrophotometric Determination of Paracetamol and Caffeine

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Received: 14 April 2014; *Accepted*: 27 July 2014; *Published online*: 25 September 2014; AJC-16057

Two UV-visible methods are described, method A to determine paracetamol and caffeine in tablet formulations by spectroscopic derivative. Paracetamol was determined after solving it in sodium hydroxide by taking the first order derivative spectroscopy at 314 nm and caffeine was determined after solving it in bidistilled water by taking the second order derivative spectroscopy at 280 nm. Beers law is obeyed in the concentration ranges 0.78-31.07 µg/mL and 1.51-30.23 µg/mL for caffeine and paracetamol, respectively. Method B to determine paracetamol in tablet and oral formulations. This method is based on the reduction of Fe^{3+} to Fe^{2+} by paracetamol, the resulting Fe^{2+} reacts with 1,10-phenanthroline to give a soluble orange-red complex in acetic acid medium. The maximal absorption is at the wavelength 510 nm. The complete reaction was achieved during heating time 25 min at 45 °C. Beers law is obeyed in the concentration range 1.21-7.56 µg/mL. The limit of detection, limit of quantification and linearity are calculated for both methods.

Keywords: Paracetamol, Caffeine, Spectrophotometric method.

INTRODUCTION

Paracetamol (acetaminophen) or 4-hydroxyacetanilide is one of the most popular non-steroidal antiinflammatory drugs and commonly used as an analgesic and antipyretic drug in pharmaceutical formulations alone or in the combination with a few contaminants, like caffeine, acetylsalicylic acid and some others. It is widely used in the treatment of pain and fever, available without a prescription[1] (Fig. 1a). Caffeine (1,3,7-trimethylxanthine) is mainly used to stimulate the central nervous and to the cardio vascular systems, having the effect of temporarily warding off and restoring alertness, is effective for the acute treatment of tension type headache[2] (Fig. 1b). Many methods like spectrofluorimetric determination in solid phase using partial least squares multivariate calibration[3], flow injection-solid phase spectrometry using C18 silica gel as an eluted support[4], flow-injection spectrophotometry[5], high performance liquid chromatography (HPLC)[6,7], quantitative thin-layer chromatography (TLC)[8], Reverse phase high performance liquid chromatography (RP-HPLC)[9], FTIR spectrometry[10], GC[11], fluorimetry[12], have been reported for the determination of paracetamol and caffeine in various biological and pharmaceutical preparations.

Direct UV-visible spectrometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, often demanding low-cost equipments. Up to now, several UV-visible spectrophotometric methods have been reported in the literature for the determination of paracetamol and caffeine[13-16]. However, these reported methods lack specificity, sensitivity, simplicity and less time for analysis. So, we present a new spectrophotometric method for determining paracetamol and caffeine in tablet or in oral formulations.

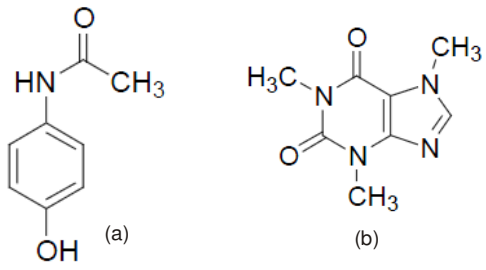

Fig. 1. Structures of paracetamol (a) and caffeine (b)

EXPERIMENTAL

UV-visible double beam spectrophotometer T80+ (England), quartz cells 1 cm, analytical balance TE64 Sartorius sensitivity 0.1 mg, Digital water bath, pH meter (Inolap-Germany). Pipettes product of HGB (Germany). All chemicals were of analytical grade, sodium hydroxide, iron(III) chloride, 1,10-phenanthroline, acetic acid produced by Merck Company (Germany), bi-distillated water, paracetamol produced by

Hebei Jiheng Pharmaceutical (China) and caffeine produced by Kores Pharmaceutical (India).

Drug products: We determined the dosage of paracetamol and caffeine 500 and 65 mg/tablet, respectively in some Syrian trade names products: paracetamol extra (Barakat), cetagam extra (Gama), new cetamol (Al saad) by derivative spectroscopy and paracetamol alone 500 or 1000 mg/tablet in trade names products: paracetamol (Barakat), paracetamol (Thameco), paracetamol (Al razi), paracetamol extra (Barakat), cetagam extra (Gama), new cetamol (Al saad) and paracetamol 200 mg/5 mL in oral products: paracetamol pharmasyr (pharmasyr) and Ben-u-Ron (Avenzor) by reduction of Fe^{3+} to Fe^{2+}.

Paracetamol and caffeine stock solutions: Stock solutions of two drugs 1×10^{-3} mol/L were prepared separately by dissolving a suitable amount of drug powder in bi-distilled water in heated water bath at 45 ºC for 5-10 min then put in ultrasonic bath for 10 min until complete dissolution of the analyte.

Reagent stock solution: 1,10-Phenanthroline 2×10^{-3} mol/L was prepared by dissolving a suitable amount in bi-distilled water. Iron(III) chloride 1×10^{-3} mol/L was prepared by dissolving a suitable amount in bi-distilled water. Sodium hydroxide 1×10^{-1} mol/L was prepared by dissolving a suitable amount in bi-distilled water. Acetic acid 3×10^{-1} mol/L was prepared by taking a suitable volume in bi-distilled water.

Samples preparation:

Tablet: 20 tablets were weighed and ground to fine powder. An accurately weighed powder equivalent to 500 mg of paracetamol alone or combination with 65 mg of caffeine was transferred to 250 mL volumetric flask containing bi-distilled water in heated water bath at 45 ºC for 5-10 min then put in ultrasonic bath for 10 min until complete dissolution of the analyte.

Oral: 5 mL was taken, equal to 200 mg of paracetamol to 500 mL volumetric flask in bi-distilled water in heated water bath at 45 ºC for 5-10 min then put in ultrasonic bath for 10 min until complete dissolution of the analyte.

RESULTS AND DISCUSSION

Paracetamol and caffeine analysis (Method A): We determined paracetamol by taking the first order derivative spectroscopy, using 0.1 mol/L sodium hydroxide as a solvent. The wavelength 314 nm was adopted to determine the paracetamol, where zero absorption value for caffeine (Figs. 2 and 3).

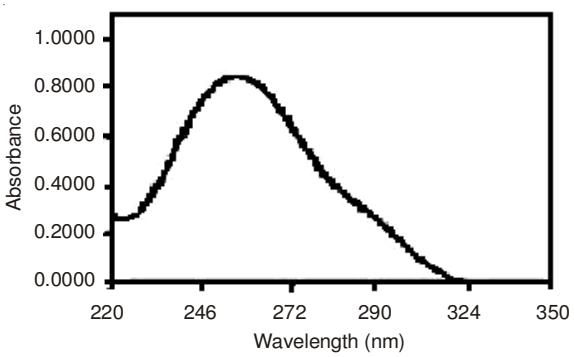

Fig. 2. Paracetamol, zero order spectrum C = 12.09 µg/mL

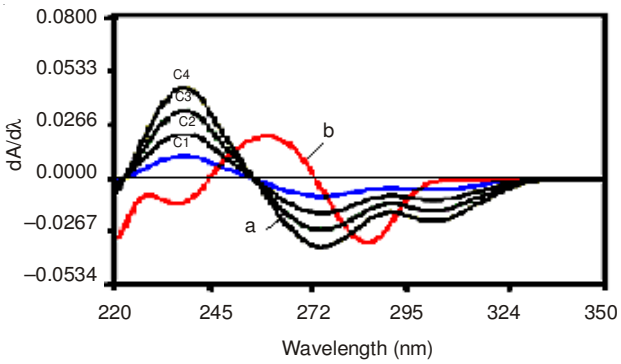

Fig. 3. First derivative order, a: Paracetamol spectra: C_1 = 6.05 µg/mL, C_2 = 12.09 µg/mL, C_3 = 18.14 µg/mL, C_4 = 24.19 µg/mL. b: Caffeine spectrum, C = 15.54 µg/mL

Caffeine was determined by taking the second order derivative spectroscopy, using bi-distilled water as a solvent, where we adopted the wavelength 280 nm to determine the caffeine, at zero absorption for paracetamol (Figs. 4 and 5).

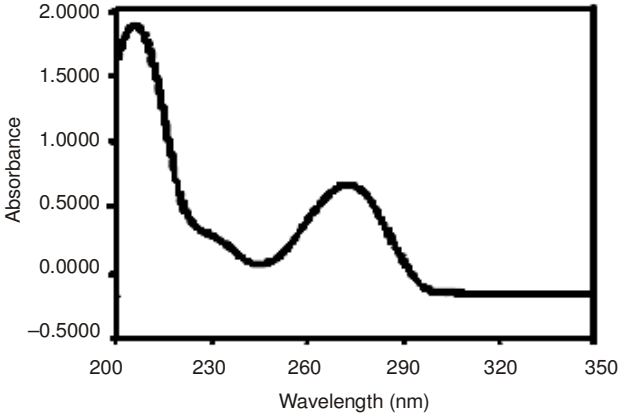

Fig. 4. Caffeine, zero order spectrum C = 15.54 µg/mL

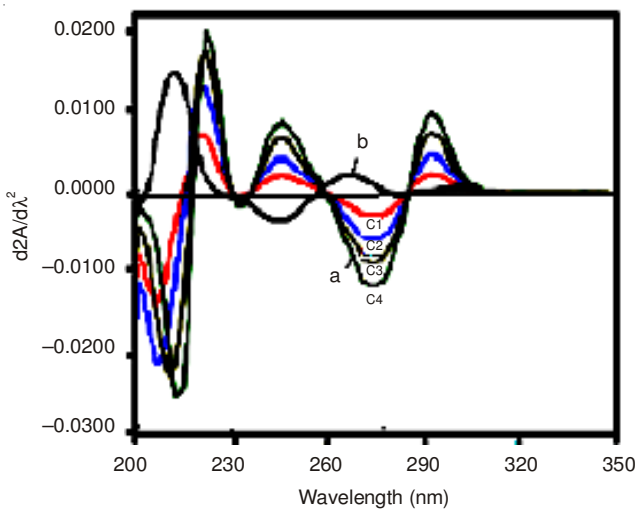

Fig. 5. Second derivative order, a: Caffeine spectra: C_1 = 7.77 µg/mL, C_2 = 15.54 µg/mL, C_3 = 23.30 µg/mL, C_4 = 31.07 µg/mL. b: Paracetamol spectrum, C = 12.09 µg/mL

Paracetamol and caffeine linearity: Paracetamol linearity at the wavelength 314 nm, of first derivative order by taking variable concentrations of paracetamol in NaOH solution was to be 1.51-30.23 µg/mL (Fig. 6).

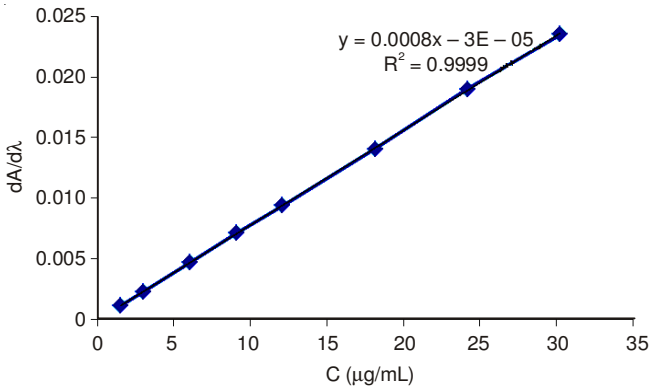

Fig. 6. Linearity of paracetamol

Where caffeine linearity at the wavelength of 280 nm of the second derivative order by taking variable concentrations of caffeine in bi-distilled water solution was to be 0.78-31.07 µg/mL (Fig. 7).

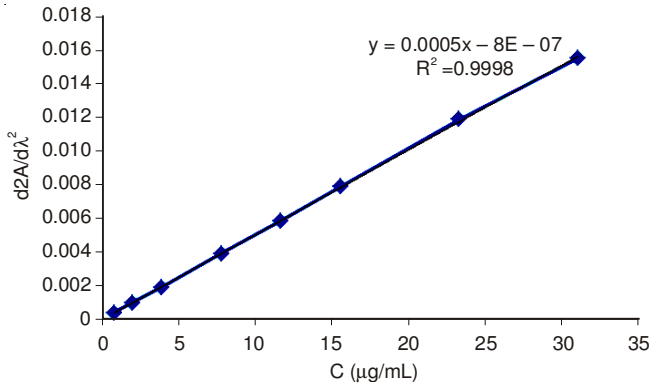

Fig. 7. Linearity of caffeine

Limit of detection (LOD), limit of quantification (LOQ) and linearity are presented in Table-1.

TABLE-1
LOD, LOQ, LINEARITY OF PARACETAMOL AND CAFFEINE

	LOD (µg/mL)	LOQ (µg/mL)	Linearity (µg/mL)
Paracetamol	0.42	1.26	1.51-30.23
Caffeine	0.32	0.97	0.78-31.07

Pharmaceuticals samples: Paracetamol and caffeine drugs were determined in three Syrian trade mark products: cetagam extra (Gama), paracetamol extra (Barakat), new cetamol (Al saad) by first spectroscopic derivative order for paracetamol and second spectroscopic derivative order for caffeine. The obtained results were presented in Table-2.

Paracetamol analysis (Method B): Determination of paracetamol formulations is based on the reduction of Fe^{3+} to Fe^{2+} by paracetamol, the result Fe^{2+} reacts with 1,10-phenanthroline to give a soluble orange-red complex in acetic acid medium. The maximal absorption is at the wavelength 510 nm.

The complete reaction was achieved in two steps during heating time 25 min at 45 ºC. At first step, Fe^{3+} was reduced by Paracetamol to Fe^{2+} as it is shown in the following equation:

$$2CH_3CONHC_6H_4\text{-}OH + 2Fe^{3+} \longrightarrow CH_3CONHC_6H_4\text{-}O\text{-}O\text{-}C_6H_4NHCOCH_3 + 2Fe^{2+} + 2H^+$$

At second step, Fe^{2+} was formed an orange-red complex by reacting between Fe^{2+} and 1,10-phenanthroline ($C_{12}H_8N_2$) = 180.2 g/mol. The indicator does not react with Fe^{3+}, as the suggested equation:

$$Fe^{2+} + 3C_{12}H_8N_2 \longrightarrow [Fe(C_{12}H_8N_2)_3]^{2+}$$

The result solution was scanned in the range of wavelengths 300-700 nm against a blank of all regents without paracetamol and then measured the absorbance at maximum wavelength 510 nm (Fig. 8).

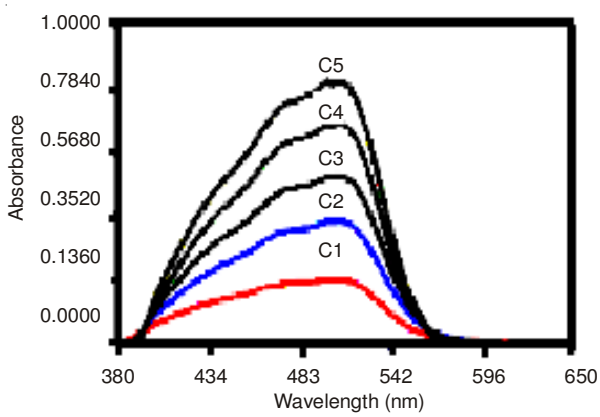

Fig. 8. Complex spectra of variable concentrations of paracetamol: C_1 = 1.21 µg/mL, C_2 = 2.11 µg/mL, C_3 = 3.02 µg/mL, C_4 = 3.93 µg/mL, C_5 = 4.84 µg/mL

Effect of temperature and time: We studied the effect of the temperature degree between 25-50 ºC during interval heating time 5 min of paracetamol C = 6.05 µg/mL. It was found that when the temperature is rising, the absorbance continued rising and the time being shorter, till the absorbance stayed constant (Fig. 9). So the temperature 45 ºC for 25 min was to be chosen as analysis condition (Fig. 10).

Effect of iron(III) chloride volume: To study the iron(III) chloride volume influence on the coloured solution, we made a series of 25 mL separation volumetric flask solution by adding

TABLE-2
PARACETAMOL AND CAFFEINE TABLETS

Trade mark	Active ingredient	Dose (mg/tab.)	$\overline{X}$ n = 5 (mg/dose)	Percent	RSD (%)	Recovery (%)
Cetagam extra	Paracetamol	500	483.34	96.67	2.86	98.89
	Caffeine	65	68.45	105.31	2.51	102.94
Paracetamol extra	Paracetamol	500	493.24	98.65	1.73	98.15
	Caffeine	65	68.62	105.57	3.22	103.68
New cetamol	Paracetamol	500	493.84	98.77	2.01	97.92
	Caffeine	65	67.92	104.49	2.13	103.53

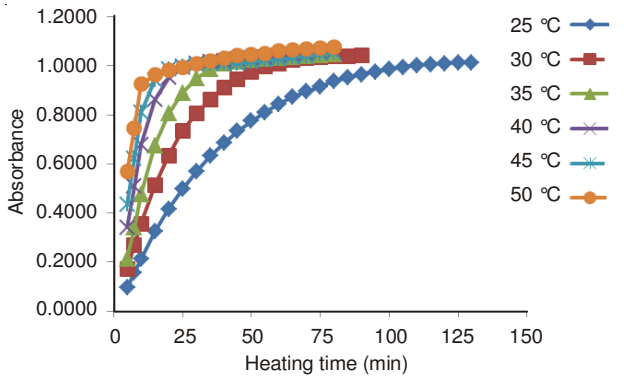

Fig. 9. Temperature effect on the complete formation complex

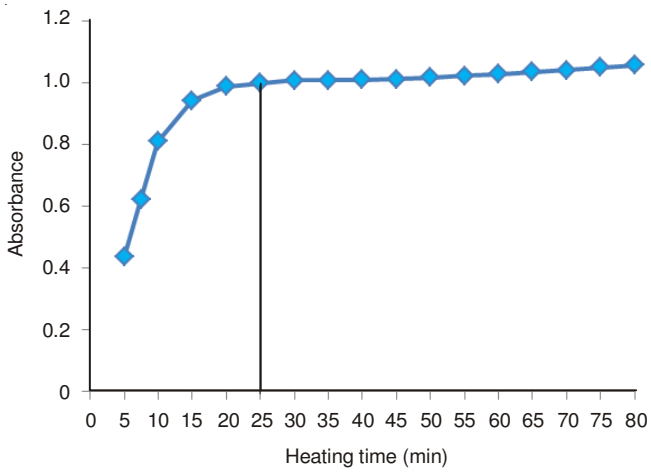

Fig. 10. Analysis condition temperature 45 °C and time 25 min

1 mL of paracetamol 1×10^{-3} mol/L, 2 mL of acetic acid 3×10^{-1} mol/L and between (0.5-6.0 mL) of iron(III) chloride 1×10^{-3} mol/L, then added 10 mL of 1,10-phenanthroline 2×10^{-3} mol/L and completed to 25 mL by bi-distillated water. The result solutions were put at 45 ºC for 25 min in water path. The absorbance at 510 nm for every added iron(III) chloride volume, against the blank was determined. It was found that the completed coloured complex formation was after 3.5 mL of iron(III) chloride solution as shown in Fig. 11.

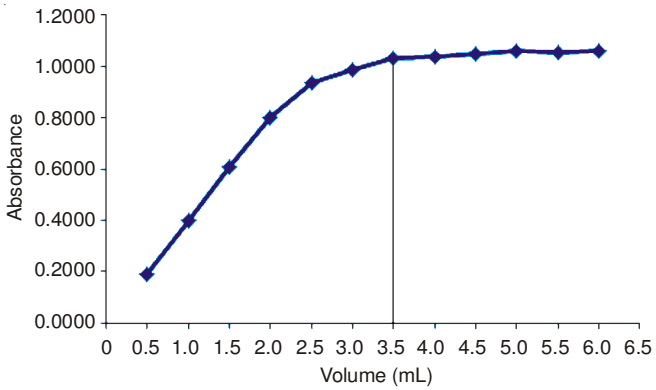

Fig. 11. Effect of iron(III) chloride volume

Effect of 1,10-phenanthroline volume: To study the 1,10-phenanthroline volume influence on the coloured solution, we made a series of 25 mL separation volumetric flask solution by adding 1 mL of paracetamol 1×10^{-3} mol/L, 2 mL of acetic acid 3×10^{-1} mol/L, 5 mL of iron(III) chloride 1×10^{-3} mol/L and then added between (0.5-10 mL) of 1,10-phenanthroline 2×10^{-3} mol/L and completed to 25 mL by bi-distillated water. The result solutions were put at 45 °C for 25 min in water path. The absorbance at 510 nm for added of 1,10-phenanthrolin volume, against the blank was determined. It was found that the completed coloured complex formation was after 7 mL of 1,10-phenanthroline solution as it is shown in Fig. 12.

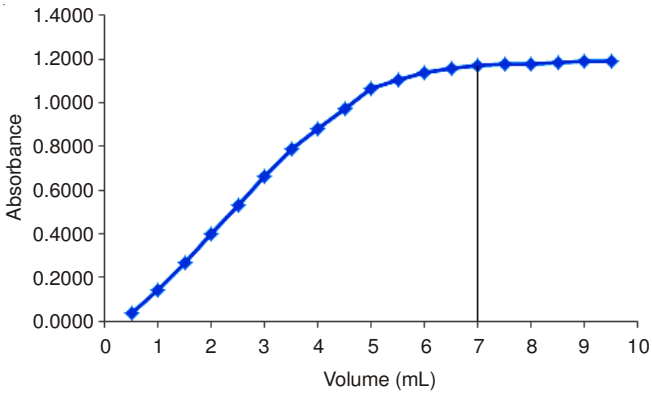

Fig. 12. Effect of 1,10-phenanthroline volume

Effect of acetic acid: We studied the complex in acid medium because the iron ions precipitated in alkaline medium. The absorbance complex was constant in acetic acid for all assays, where the uses of HCl, H_3PO_4, H_2SO_4, HNO_3 solutions in place of acetic acid did not lead to form the complex.

Addition sequence: The best sequence to give higher absorbance was: paracetamol, acetic acid, iron(III) chloride, 1,10-phenanthroline.

Range and linearity of paracetamol: The same work for studying paracetamol linearity was done by adding the same precedent volumes of acetic acid, iron(III) chloride, 1,10-phenanthroline and variable concentration of paracetamol stock solution, then put in temperature 45 ºC for 25 min and measured at 510 nm. It is found that linearity was obeyed Beers law in concentration 1.21-7.56 µg/mL and the linearity is presented in Fig. 13.

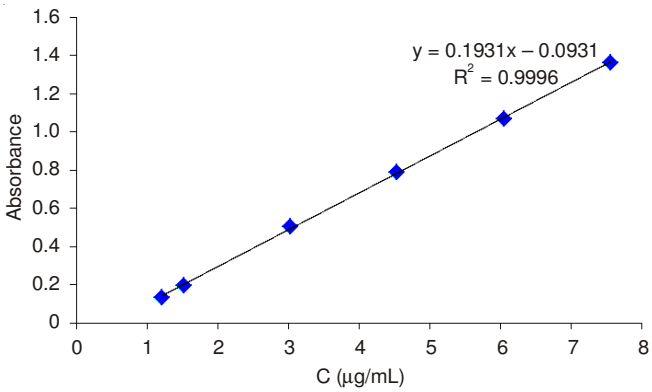

Fig. 13. Linearity of paracetamol

Limit of detection (LOD) and limit of quantification (LOQ) are presented in Table-3.

TABLE-4
PARACETAMOL TABLETS AND SUSPENSION

Trade mark	Dose	$\overline{X}$ n = 5 (mg/dose)	Percent	RSD (%)	Recovery (%)
Paracetamol (Thamico)	500 mg/tab.	490.10	98.02	1.31	98.10
Paracetamol (Al razi)	500 mg/tab.	508.24	101.65	0.61	101.01
Paracetamol 500 (Barakat)	500 mg/tab.	518.04	103.61	0.86	102.38
Paracetamol 1000 (Barakat)	1000 mg/tab.	1020.92	102.09	0.69	102.22
Cetagam extra (Gama)	500 mg/tab.	483.40	96.68	0.88	98.37
Paracetamol extra (Barakat)	500 mg/tab.	492.10	98.42	0.61	98.89
New cetamol (Al saad)	500 mg/tab.	487.17	97.43	0.69	99.04
Paracetamol pharmasyr (pharmasyr)	200 mg/5 mL	191.86	95.93	0.65	96.23
Ben-u-Ron (Avenzor)	200 mg/5 mL	191.16	95.58	0.62	96.65

TABLE-3
LOD, LOQ AND LINEARITY

	LOD (µg/mL)	LOQ (µg/mL)	Linearity (µg/mL)
Paracetamol	0.12	0.37	1.21-7.56

Pharmaceuticals samples: Paracetamol dosage was determined in nine trade mark products: paracetamol (Thameo), paracetamol (Al razi), paracetamol 500 or 1000 (Barakat), cetagam extra (Gama), paracetamol extra (Barakat), new cetamol (Al saad) and paracetamol 200 mg/5 mL in oral products: paracetamol pharmasyr (pharmasyr) and Ben-u-Ron (Avenzor). Table-4 presents the analytical results.

Conclusions

The developed UV estimation method (A) reported here is rapid, simple, sensitive and specific. The method was also successfully used for quantitative estimation and analysis of paracetamol and caffeine in combined dosage form.

Determination of paracetamol by proposed method (B) is based on oxidation-reduction reaction by iron(III) salts. The proposed method is found to be simple, economical and highly sensitive than some of the reported methods.

Such simple methods based on spectrophotometry have become an accepted analytical tool for the assay and evaluation of drugs. The spectrophotometric methods proposed in this study can be applied for direct determination of paracetamol and caffeine in drug control laboratories as a rapid, reliable, sensitive and low cost.

ACKNOWLEDGEMENTS

This work was financially and technically supported by Ministry of High Education through University of Aleppo, Faculty of Science, Department of Analytical Chemistry in the University of Aleppo, Aleppo, Syria.

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