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ANALYSIS OF PARACETAMOL AND ASCORBIC ACID IN PHARMACEUTICAL BINARY MIXTURE

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تم استحداث اثنان من الطرق الطيفية البسيطة لتعيين كلا من الباراسيتامول وحمض الاسكوربيك في مخلوط صيدلي ثنائي. تعتمد الطريقة الأولى على استخدام المشتقة الأولى الطيفية للتعيين المتزامن لكلا من المركبين في مخلوط.

وتعتمد الطريقة الثانية على تفاعل المركبين مع مركب الديازوترايازول حمض الكربوكسيل (د.ت.ك.أ) لكي يعطى نواتج ملونة تقاس عند طول موجي قدره ٤٨٠ و ٣٨٠ نانوميتر لكل من الباراسيتامول وحمض الاسكوربيك على التوالي.

وقد تمت دراسة جميع المتغيرات التي تؤثر على التفاعلات واختيار انسبها. وقد تم تطبيق الطرق المقترحة بنجاح لتحليل المركبات المدروسة في أشكالها النقية والمستحضرات الصيدلية مع مقارنة نتائجها بنتائج الطرق المنشورة وقد وجد أن هذه النتائج متطابقة إحصائيا مما يدل على دقة وإحكام الطرق المقترحة.

Two simple and sensitive spectrophotometric methods were developed for the determination of paracetamol (I) and ascorbic acid (II) in pharmaceutical binary mixture. The first method depends on the use of the first-derivative spectrophotometric technique for the simultaneous determination of components of the mixture. The second method depends on the reaction of the studied drugs with 5-diazo-1, 2, 4-triazol-3-carboxylic acid (DTCA) reagent to give colored products measured at 480 nm and 380 nm for (I) and (II), respectively. All variables affecting reaction conditions were optimized. The proposed methods were successfully applied for the analysis of the studied drugs in their pure and commercial dosage forms and are in good agreement with those obtained from the reported methods. No significant difference in the accuracy and precision as revealed by the accepted values of t- and F-tests, respectively. Molar ratios of the drugs with the colorimetric reagent (DTCA) were determined and the reaction mechanisms were suggested.

INTRODUCTION

Ascorbic acid is used in combination with analgesic- anti-inflammatory drugs (e.g. paracetamol) for treatment of cold and influenza[1].

Different techniques were reported for the analysis of the studied drugs either simultaneously or for the determination of one drug in the presence of the other. These methods include derivative spectrophotometric[2-11], colorimetric[12-20], high performance liquid chromatography[21-24] and electrophoretic methods[25&26].

In the present article, the first derivitive (1D) spectrophotometric method was described for the simultaneous determination of both drugs in the binary mixture. Also a colorimetric method, which depends on the reaction of the studied drugs with (DTCA) reagent, which was previously used as a chromogenic reagent for many drug classes[27&28] to form colored products in alkaline media measured at different wavelengths.

EXPERIMENTAL

Apparatus

- An UV-1601 PC (Shimadzu, Tokyo, Japan) ultraviolet-visible spectrophotometer with matched 1 cm quartz cells; was used for all measurements.
- Ultrasonic cleaner (Cole-Parmer, Chicago, USA).

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Materials and reagents

Ascorbic acid (CID, Cairo, Egypt), and paracetamol (E.I.P.I.CO, Cairo, Egypt) were used as working standards without previous purification..

5-Diazo-1,2,4-triazol-3-carboxylic acid (DTCA) was synthesized in our laboratory from 5-amino-1,2,4-trizole-3-carboxylic acid according to reported method[29]. Sodium hydroxide and sodium carbonate (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt). All other chemicals and solvents used throughout this work were of analytical grade.

Pharmaceutical formulations

Cevamol tablets (CID, Cairo, Egypt), Cevilene drops (Kahira Co., Cairo, Egypt), C-retard capsules (ALKAN Pharma Co., Cairo, Egypt), Cetal drops and suspension (E.I.P.I.Co, Cairo, Egypt), Paramol tablets (Misr Co., Cairo, Egypt).

Preparation of standard solutions

An accurately weighed amount (50 mg) of each of the studied drugs was transferred into a 50-ml standard flask containing 30 ml distilled water. The contents were shaken well, sonicated for 10 minutes and completed to the mark with the same solvent. Further dilutions with distilled water were made to obtain the suitable concentrations.

Reagent solutions

Aqueous solution of 5 mg/ml of DTCA was prepared and protected from light. Several dilutions were made to obtain the suitable concentrations.

Sodium hydroxide

Five molar solution of sodium hydroxide was prepared in previously boiled and cooled distilled water. Several dilutions were made to obtain the suitable concentrations.

Sodium carbonate

One molar solution of sodium carbonate was prepared in distilled water. Several dilutions were made to obtain the suitable concentrations.

Preparation of dosage forms

Capsules

The contents of ten capsules were carefully evacuated, mixed, and accurately weighed. An accurately weighed amount of the powder equivalent to about 10 mg of the drug was transferred into a 100-ml standard flask containing about 50 ml of distilled water. The contents of the flask were shaken well for 5 minutes, completed to the mark with methanol and sonicated for 10 minutes. The solution was filtered, and the first portion of the filtrate was rejected. The obtained filtrate was used as a stock sample solution for application of the general procedures.

Tablets

Twenty tablets were accurately weighed, finely powdered and mixed thoroughly. An accurately weighed quantity of the powdered tablets equivalent to 10 mg of the studied drug was transferred into a 100-ml standard flask and the procedure was completed as mentioned under capsules starting from "containing about 50 ml of distilled water …."

Effervescent tablets

An effervescent tablet was dissolved in about 200 ml of distilled water in a 250 ml-beaker. When all effervescence ceased, the solution was transferred quantitatively to a 250-ml standard flask and completed to the mark with the same solvent**.** Serial dilutions were made to obtain the suitable concentration for each drug for application of the general procedure.

Drops

An accurately measured volume of the drops equivalent to about 10 mg of the drug was transferred into a 100-ml standard flask. The procedure was completed as mentioned under tablets starting from "containing about 50 ml of distilled water …." without filtration.

Determination of molar ratio

Job's continuous variation method[30] was used for the determination of molar ratios between DTCA reagent and each of the studied

drugs. Master equal molar solutions of 1.135 x 10^{-3} M and 1.323 x 10^{-3} M from the reagent (DTCA) and each of ascorbic acid and paracetamol, respectively were prepared. A series of 10-ml portions of the master solutions of the drugs and reagent were made up comprising different complementary proportions (0.0:1.0, 0.1:0.9----------0.9:0.1, 1.0:0.0) in 10-ml standard flasks. The reactions were allowed to proceed for the optimal reaction conditions and then the absorbances of the resulting solutions were measured at the corresponding wavelengths of maximum absorbances (λ_{max}).

General assay procedures

I- First derivative method

One milliliter of standard or sample solution of ascorbic acid in the concentration range (20-250 µg/ml) and of paracetamol in the concentration range (10-150 µg/ml) were transferred into a 10-ml standard flask. The content of the flask was diluted to the mark with acetonitrile: methanol mixture (4:6). The absorption spectra of the drugs were recorded between 200-310 nm and first derivative spectra were recorded against blank. Ascorbic acid was measured at 246.5 nm while paracetamol was measured at 267.7 nm.

II- Colorimetric method

(a) For ascorbic acid

One milliliter of the working standard or sample solution in the concentration range (20-250 µg/ml) was transferred into a 10-ml standard flask. One milliliter of (0.7 mg/ml) DTCA reagent and one milliliter of 0.3 M sodium hydroxide were added.The solution was mixed well and allowed to stand at room temperature (25±5°C) for 10 minutes. The volume was made up with distilled water. The absorbance of the resulting solution was measured at λ_{max} 380 nm against a reagent blank treated similarly..

(b) For paracetamol

One milliliter of the working standard or sample solution in the concentration range (20-170 µg/ml) was transferred into a 10-ml standard flask. One milliliter of (0.4 mg/ml) DTCA reagent and one milliliter of 0.3 M sodium carbonate were added. The solution was mixed well and allowed to stand at room temperature (25±5°C) for 10 minutes. The volume was made up with distilled water. The absorbance of the resulting solution was measured at λ_{max} 480 nm against a reagent blank treated similarly.

RESULTS AND DISCUSSION

First derivative method

The absorption spectra of the two drugs were overlapped as shown in Figure 1. So first derivative technique was used to solve this problem as in Figure 2. Each drug was measured at the zero crossing of the other compound. For the simultaneous determination of ascorbic acid and paracetamol, wavelengths at 246.5 and 267.7 nm were selected for the determination of both drugs respectively.

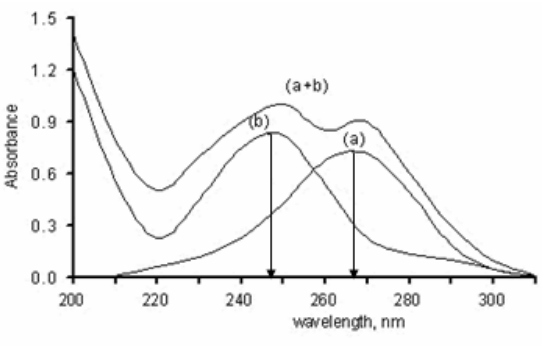


Fig. 1: Absorption spectra of: (a) 10 µg/ml of ascorbic acid, (b) 10 µg/ml of paracetamol in acetonitrile: methanol solvent mixture (4:6) and (a+b) mixture of them

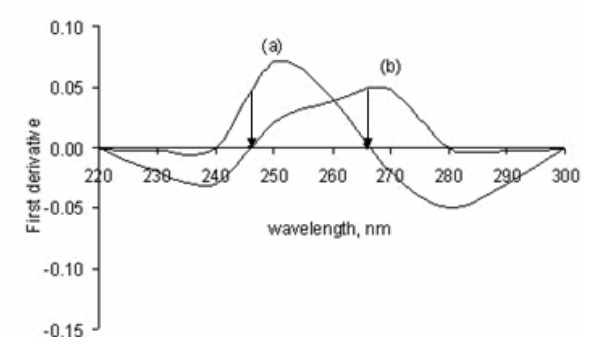


Fig. 2: First derivative spectra of: (a) 10 µg/ml of ascorbic acid and (b) 10 µg/ml of paracetamol in acetonitrile: methanol solvent mixture (4:6).

Effect of diluting solvents:

Different solvents were tested for the first derivative determination of the studied drugs in the mixture as. Ascorbic acid gave the highest reading value in acetonitrile, while paracetamol gave the heighest reading in methanol, so mixture of both solvents were tested using different ratios. That the best ratio was 4:6 (acetonitrile: methanol).

Colorimetric method

The reaction of the diazonium salt (DTCA) with the two studied drugs in alkaline medium gave colored products measured at different wavelengths. The reagent reacts with ascorbic acid in sodium hydroxide and with paracetamol in sodium carbonate at different concentrations to give a yellow product with ascorbic acid measured at 380 nm and an orange product with paracetamol measured at 480 nm (Fig. 3).

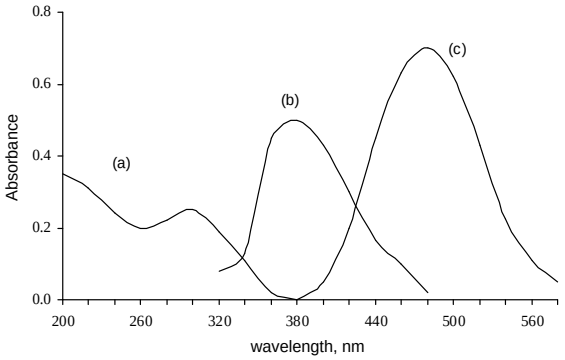


Fig. 3: Absorption spectra of: (a) DTCA (3 mg/ml), colored products of DTCA with both of (b) ascorbic acid (10 µg/ml) and (c) paracetamol (10 µg/ml).

Optimization of reaction variables in colorimetric method

Various parameters such as concentration of the diazonium salt (DTCA), type and concentration of alkali, diluting solvent, reaction and stability time were studied for their effect on the intensity and stability of the developed colored products.

1- Concentration of diazonium salt (DTCA)

Different concentrations of DTCA reagent were tested during this study. Absorption intensity reached its maximum value when the reagent concentration was between 0.6 and 0.8 mg/ml in case of ascorbic acid and between 0.3 and 0.6 mg/ml in case of paracetamol. So, concentrations of 0.7 and 0.4 mg/ml were selected for the determination of ascorbic acid and paracetamol, respectively (Fig. 4).

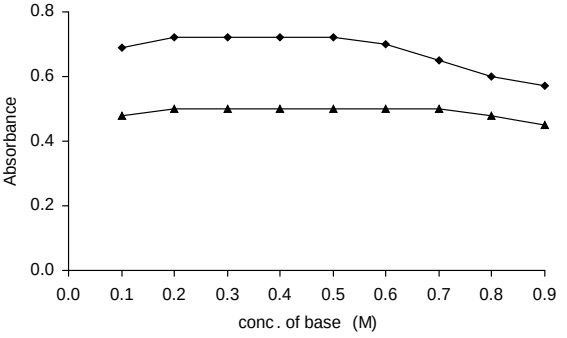


Fig. 4: Effect of DTCA concentration on absorbance intensity of the colored product of: (a) 10 µg/ml of ascorbic acid and (b) 10 µg/ml of paracetamol

2- Type and concentration of alkali

Different types of alkali were tested for the reaction of the diazonium salt with the studied drugs e.g.; sodium hydroxide, sodium carbonate, sodium bicarbonate and sodium acetate. It was found that, the most intense and stable color was developed in the presence of sodium hydroxide in case of ascorbic acid. Sodium carbonate and sodium hydroxide were equally useful in case of paracetamol. Sodium carbonate was preferred because the colored product was measured at higher wavelength (at 480 nm) and there was no interference with the colored product of ascorbic acid as shown in Figure 3. While in presence of sodium hydroxide, the colored product was measured at 463 nm. So, concentrations of 0.3 M of sodium hydroxide and 0.3 M of sodium carbonate were selected for determination of ascorbic acid and paracetamol, respectively as shown in Figure 5.

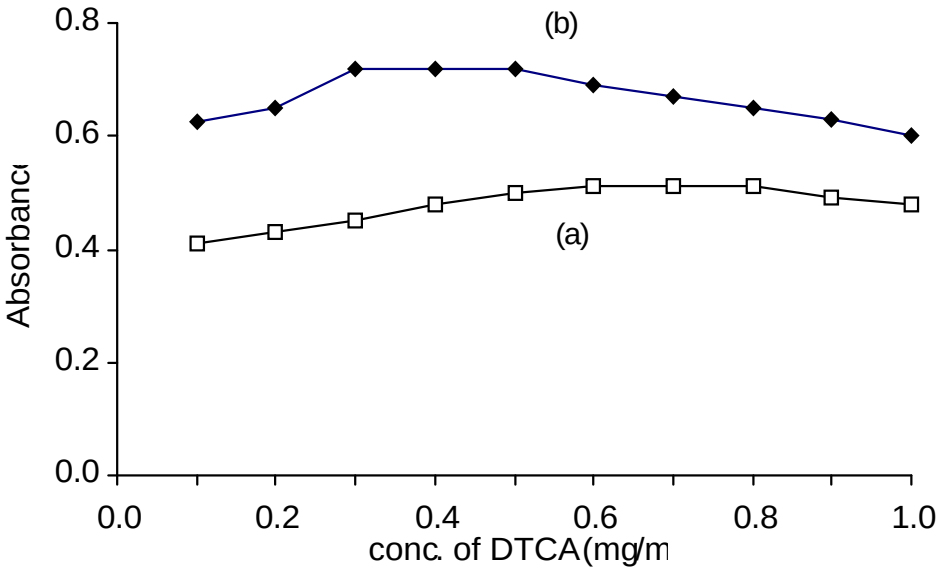


Fig. 5: Effect of base concentration on absorbance intensity of the colored product of: (a) 10 µg/ml of ascorbic acid and (b) 10 µg/ml of paracetamol with DTCA

3- Reaction time at room temperature

The effect of time on the absorption intensities of the colored products of the studied drugs with DTCA was studied at room temperature (25±5 ºC). It was found that higher absorbance was obtained after 5 minutes and remains stable till 20 minutes in case of ascorbic acid and till 30 minutes in case of paracetamol. Further increase of time shows gradual decrease in the absorbance, so the reaction products were allowed standing for 10 minutes before dilution with the suitable solvent in both cases.

4- Diluting solvent

Different solvents such as distilled water, methanol, ethanol, acetonitrile, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO) were tested as a diluting solvent. Distilled water was selected in both cases, because it gave the highest intensities, safe, cheap and avaliable.

5- Stability time

The colored products of the studied drugs with DTCA remain stable for about 20 minutes after dilution, and then gradual decrease in the absorption intensities was observed. So measurements must be done within this time.

Stoichiometry and suggested reaction mechanism

Job's continuous variation method for the determination of the molar ratio between DTCA reagent and both of the studied drugs by the colorimetric method was applied. The results appear in Figure 6 indicating the ratio of 1:1 between the reagent and both of the studied drugs. The method is based upon coupling of the diazonium salt DTCA with phenolic group to yield azo dyes[31]. The normally preferred site of coupling is para to the activating group, however, ortho- substitutions are observed when the para position is occupied.

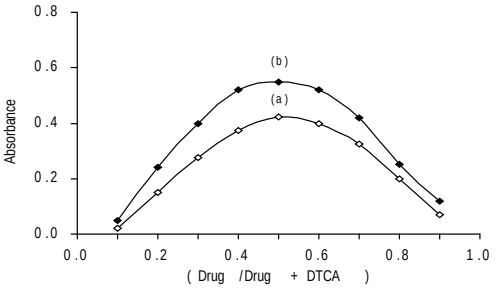
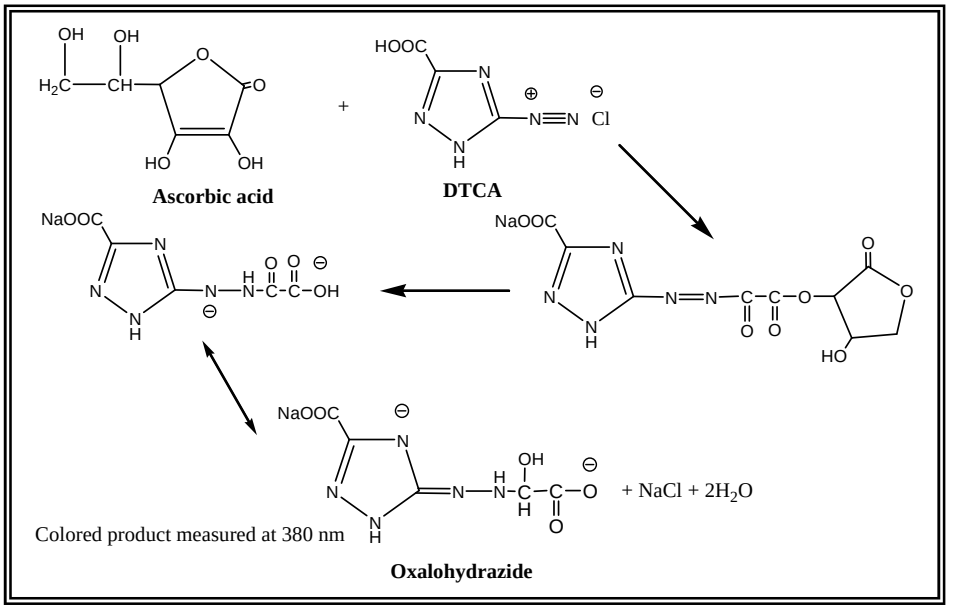


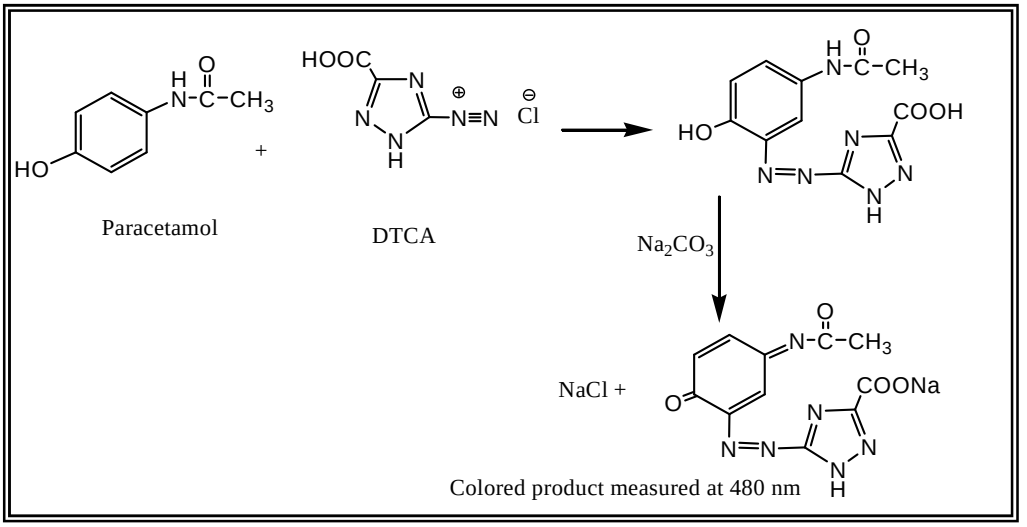
Fig. 6: Continuous Job's plots of DTCA ($1.135x10^{-3}$ and $1.323x10^{-3}$ M) with (a) ascorbic acid ($1.135x10^{-3}$) and (b) paracetamol (1.323×10^{-3} M).

Ascorbic acid react actively with DTCA reagent to form lactone which in alkaline medium form oxalohydrazide after resonance, the colored product was measured at 380 nm[13].

For paracetamol, para position is occupied, so coupling with DTCA may be occur at any ortho position to the phenolic OH. The phenolic OH group in paracetamol loses hydrogen atom in presence of alkaline medium and become quinone, this conjugated system makes the colored product measured at higher wavelength as shown in Scheme 2.



Scheme 1



Scheme 2

Validation and application of the proposed methods

Linearity, detection and quantitation limits[32]

The total derivative spectra taken at the selected optimal wavelengths by the first derivative method and the colored chromogen obtained by the reaction with DTCA in the second colorimetric method afforded the best linear responce to the drug concentration. Parameters of the regression curves evaluated by the least square method are presented in Table 1. Beer's plots were linear over concentration ranges listed in Table 1 with good correlation coefficients (0.9993-0.9997). The limit of detection (LOD) and limit of quantitation (LOQ) values were determined[33] using the formula:

$$LOD \text{ or } LOQ = k.\delta/S$$

where k=3.3 for LOD and 10 for LOQ, δ is the standard deviation of the responce and S is the slope. The ranges of LOD were [0.33 – 0.61 µg/ml] and [0.50 – 0.66 µg/ml] for the first and second method respectively. While the values of LOQ were [1.00 – 1.80 µg/ml] and [1.51 – 2.00 µg/ml] for the first and second methods respectively indicating higher sensitivity of the proposed procedures.

Accuracy and analysis of pharmaceutical formulations

The commercially available pharmaceutical formulations of the studied drugs were subjected to analysis by the two proposed and reported methods[13&16]. The obtained results were then statistically compared with each other. The mean percentages label claim, relative to the labeled amounts, obtained by the proposed methods ranged from 101.0 -101.5 ± 0.02–0.15 by the first derivative method and ranged from 98.4–100.7±0.02-0.28% by the colorimetric method (Table 2). With respect to t- and F-tests, no significant differences were found between the calculated and theoretical values of the proposed and the reported methods at 95% confidence level this indicated similar accuracy and precision in the analysis by the proposed and reported methods.

Precision

The precision of the developed procedures was confirmed by analyzing six replicate samples at three concentration levels for all the studied drugs by the two suggested methods. The relative standard deviations by the first derivative method were found to be ranged 1.11-1.31 and 1.01-1.10 for I and II respectively. By the colorimetric method the relative standard deviation was ranged 1.00 – 1.42 and 0.90-1.60 for I and II respectively. The values of RSD were less than 2 and this level of precision is adequate for the routine analysis in quality control laboratories.

Robustness

Robustness of the proposed colorimetric method was study by small variations in some of the reagents concentration and reaction time. It was found that, this small variation has no significant effect on the absorption intensities of the studied drugs, so the proposed method can be considered robust (Table 3).

Table 1: Quantitative parameter for the analysis of the studied drugs by first derivative spectrophotometric and c olorimetric methods.

Compound	Calibration Range	Intercept ± SD	Slope ± SD	ε Molar absorbtivity x 10^3 (mole^{-1} cm^{-1} liter)	Correlation coefficient (r)	LOD (µg/ml)	LOQ (µg/ml)
First derivative method							
Ascorbic acid	2.0-25.0	0.001 ± 0.0010	0.006 ± 0.0003	-----	0.9996	0.61	1.80
Paracetamol	1.0-15.0	0.001 ± 0.0005	0.005 ± 0.0002	-----	0.9997	0.33	1.00
Colorimetric method							
Ascorbic acid	2.0-20.0	0.015 ± 0.0100	0.050 ± 0.0000	9.080	0.9994	0.66	2.00
Paracetamol	2.0-17.0	-0.015 ± 0.0110	0.073 ± 0.0010	10.820	0.9993	0.50	1.51

LOD: Limit of Detection LOQ: Limit of Quantitation

Table 2: Analysis of the studied drugs in their pharmaceutical formulations by the two proposed and reported methods (n= 6).

Pharmaceutical dosage form	Drug (content, mg)	% Recovery*± SD (n=6)		F-value	t-value
		Proposed method	Reported method		
1- Cevamol (eff. tablets)	Ascorbic acid (250)	101.5 ± 0.15	101.7 ± 0.25 [13]	2.767	1.469
	Paracetamol (400)	101.0 ± 0.02	101.1 ± 0.01 [16]	4.870	1.610
2- Cevilene (drops)	Ascorbic acid (100/ml)	100.6 ± 0.02	100.7 ± 0.24 [13]	1.119	0.729
3- C-retard (capsules)	Ascorbic acid (200)	99.3 ± 0.21	99.2 ± 0.24 [13]	1.268	0.178
4-Cetal (drops)	Paracetamol (100/ml)	100.7 ± 0.19	100.4 ± 0.35 [16]	3.338	1.423
5- Cetal (suspension)	Paracetamol (250/5ml)	100.7 ± 0.16	100.6 ± 0.25 [16]	2.294	0.828
6- Paramol (tablets)	Paracetamol (500)	98.4 ± 0.28	98.3 ± 0.18 [16]	2.428	0.799

* Theoretical values of F and t at 95 % confidence limit are 5.050 and 2.228.

Table 3: Influence of small variations in the assay conditions of colorimetric method on the suitability test parameters and sensitivity.

Variation	% Recovery ± SD*	
	Ascorbic acid	Paracetamol
No variation**	100.3 ± 0.9.0	99.8 ± 0.4.0
DTCA conc. ± 0.2 mg /ml	(0.5) 99.8 ± 0.4.0 (0.9) 100.3 ± 0.9.0	(0.2) 99.7 ± 0.10 (0.6) 99.8 ± 0.20
Base ± 0.2 M	(0.1) 100.9 ± 0.60 (0.5) 100.0 ± 0.60	(0.1) 99.3 ± 0.90 (0.5) 99.2 ± 0.30
Reaction time ± 2 (min)	(8) 98.9 ± 0.40 (12) 99.9 ± 0.10	(8) 99.1 ± 0.10 (12) 99.8 ± 0.90

* Mean of three determinations.
** No variation in the assay conditions of the proposed method.

Conclusion

The present study developed two simple and accurate spectrophotometric methods for the analysis of ascorbic acid-paracetamol binary mixture. The first method depends on the use of first derivative technique to separate the overlapped spectra of the studied drugs. The second method depends on the coupling of the DTCA reagent with the studied drugs giving different colored products measured at different wavelengths. The methods are reliable for the accurate determination of the studied drugs in bulk and dosage forms without interference from the common additives in dosage forms. Therefore, these methods can be recommended for the routine analysis of binary mixture of ascorbic acid-paracetamol in quality control laboratories. .

REFERENCES

1- G. A. Gilman, W. T. Rall, S. A. Nies and P. Tylor, "The Pharmacological Basis of Therapeutics" 9th Ed., McGraw-Hill, New York, 1996, pp. 1568, 673.
2- A. Dimitrovska, S. Trajkovic-Jolevska and K. Stojanoski, Anal. Lab., 7, 32 (1998).

3- H. N. Dogan and A. Duran, Pharmazie, 53, 781 (1998).
4- T. Aburjai, B. I. Amro, K. Aiedeh, M. Abuirjeie and S. Al-Khalil, ibid., 55, 751 (2000).
5- I. H. Refaat, Bull. Pharm. Sci, Assiut University, 27, 269 (2004).
6- F. Garcia Montelongo, M. J. Sanchez, J. C. Garcia Castro and A. Hardisson, Anal. Lett., 24, 1875 (1991).
7- M. U. Ozgur and S. Sungur, Talanta, 42, 1631 (1995).
8- N. Erk, J. Pharm. Biomed. Anal., 21, 429 (1999).
9- C. Yucesoy and K. Tulek, Egypt. J. Anal. Chem., 3, 156 (1994).
10- M. I. Toral, P. Richter, E. Araya and S. Fuentes, Anal. Lett., 29, 2679 (1996).
11- N. Erk, Y. Özkan, E. Banoğlu, S. A. Özkan and Z. Senturk, J. Pharm. Biomed. Anal., 24, 269 (2001).
12- K. Güclü, K. Sözgen, E. Tütem, M. Özyürek and R. Apak, Talanta, 65, 1226 (2005).
13- E. Y. Backheet, K. M. Emara, H. F. Askal and G. A. Saleh, Analyst, 116, 861 (1991).
14- O. H. Abdel-Mageed, P. K. Khashaba, H. F. Askal, G. A. Saleh and I. H. Refaat, Talanta, 42, 573 (1995).
15- H. M. Abd El-Wadood, H. A. Mohamed and F. A. Mohamed, Boll. Chim. Farmac.–Anno., 141, 52 (2002).
16- F. A. N. Nour El-Dien, M. A. Zayed and G. G. Mohamed, Fenxi Ceshi Xuebao, 18, 31 (1999).
17- F. A. Mohamed, M. A. Abdallah and S. M. Shammat**,** Talanta, 44, 61 (1997).
18- P. Nagaraja, K. C. Srinivasa and K.S. Rangappa, J. Pharm. Biomed. Anal., 17, 501 (1998).
19- S. P. Arya and M. Mahajan, Ann. Chim., 88, 429 (1998).
20- K. Srividya and N. Balasubramanian, Chem. Anal., 44, 689 (1999).
21- S. S. Zarapkar and A. A. Dhanvate, Indian Drugs, 32, 405 (1995).
22- M. W. Dong and J. L. Pace**,** LC-GC, 14, 794 (1996).
23- S. Sun, G. Liu and Y. Wang, Chromatogr., 64, 719 (2006).
24- H. Maeda, K. Katayama, R. Matsui, Y. Yamauchi and H. Ohmori, Anal. Sci.,16, 293 (2000).
25- J. Olsson, O. Nordstrom, A-C Nordstorm and B. J. Karlberg, J. Chromatogr. A, 826, 227 (1998).
26- S. Heitmeier and G. J. Blaschke, J. Chromatogr. B: Biomed. Appl., 721, 93 (1999).
27- N. A. El-Koussi, A. I. Mohamed, H. A. Mohamed and Z. S. Fargaly, Anal. Lett., 29, 2487 (1996).
28- N. A. El-Koussi, H. A. Mohamed and A. I. Mohamed, Bull. Pharm. Sci., Assiut University, 20, 17 (1997).
29- G. I. Chipen and V. Ya Grinshtein, Khimiya Geterotsiklicheskikh Soedinenii, 1, 624 (1965).
30- P. Job, Ann. Chem., 16, 97 (1963), in: "Advanced Physicochemical Experiments", 2nd Ed., Oliner and Boyd, Edinburgh, 1964, p. 54.
31- S. Pattai, "The Chemistry of Diazo Groups and Diazonium Salts", Part 2, John Wiley & Sons, Chichester, UK, 1978, p.546.
32- The United States Pharmacopeia 25, the National Formulary 20, 2002, (U.S. Pharmacopeial Convenion Inc., Rockville, MD), pp. 2256-2259.
33- Topic Q2B: "Validation of Analytical Procedures", Methodology, International Conference on Harmonization (ICH), (2005).