



Assay of Orphenadrine Citrate in Pharmaceuticals via Extraction-Spectrophotometric Method

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Abstract

An extraction-spectrophotometric technique has been applied and approved for the estimation of orphenadrine citrate in the presence of paracetamol in a binary synthetic mixture and in combined drugs. The procedure is built on the formation of a soluble red colour orphenadrine citrate – eriochrom black T (EBT) ion pair complex at pH 1.40, while the paracetamol not paired. The produced red colour ion-pair complex was extracted with chloroform and showed maximum absorption at 509 nm. For quantitative evaluation Beer's law applied to plot the absorbance against concentration, the relation was a linear in the concentration range of 0.10-6.00 µg/ mL with the molar absorptivity 4.4025×10^4 L / mol. cm. The limit of detection and limit quantification were 0.024 µg/ mL, and 0.100 µg/ mL respectively. The intra-assay precision evaluated in terms of % relative standard deviation (RSD) (< 2%). And accuracy was validated with % recovery (98.8-102.5%). The results showed that orphenadrine citrate could be determined successfully in the combined tablet without interference by paracetamol and other common co-formulated substance.

Keywords: Orphenadrine citrate, Paracetamol, Ion-pair complex, and Eriochrome black T.

تقدير سترات الاورفينادرين في الادوية بطريقة استخلاص - طيفية

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الخلاصة

تم تطبيق وتقييم طريقة استخلاص-طيفية لتقدير سترات الاورفينادرين بوجود الباراسيتامول في الخليط الثنائي. تضمن التقدير تكوين معقد الترابط الايوني احمر اللون ذائب في الماء بين سترات الاورفينادرين وصيغة ايروكروم بلاك تي عند درجة حموضة 1.4 بينما باراسيتامول لا يكون معقد الترابط الايوني. لقد تم استخلاص معقد الترابط الايوني كليا بواسطة الكلورفورم. وتم قياس اقصى امتصاص لها عند طول موجي 509 نانوميتر وان قانون بير ينطبق على مدى تراكيز تراوحت بحدود 0.10-6.00 مايكروغرام/ مللتر بدقة وتوافق جيدين اذ تراوح معدل نسبة الاسترجاع بين 98.8-102.5% مع انحراف قياسي نسبي اقل من 2%. وكانت الامتصاصية المولارية 4.4025×10^4 لتر. مول⁻¹.سم⁻¹ وبحد كشف وحد قياسي كمي 0.024 مايكروغرام/مللتر، و 0.0100 مايكروغرام/مللتر على التوالي. تعطي هذه النتائج امكانية تطبيق الطريقة بنجاح في تقدير المستحضرات الصيدلانية لسترات اورفينادرين بوجود الباراسيتامول والمتداخلات المصاحبه لمستحضر.

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1. Introduction

Orphenadrine citrate (RS)-dimethyl [2-(2-ethyl benzhydryloxy) ethyl] amine dihydrogen citrate, its molecular formula $C_{18}H_{23}NO \cdot C_6H_8O_7$, with molecular weight of (461.51). It is odourless white or almost white crystalline powder. Sparingly soluble in water, slightly soluble in alcohol, and insoluble in chloroform, ether, and benzene [1].

Orphenadrine citrate is diphenhydramine-mono methylated derivative of class tertiary amino ethers (ethanolamines or aminoalkyl ether), Figure-1 (A, B) with potent antihistamines muscle-relaxant and analgesic properties, and it has some usefulness in the treatment of Parkinsonism including that induced by drugs such as the phenothiazines [2, 3].

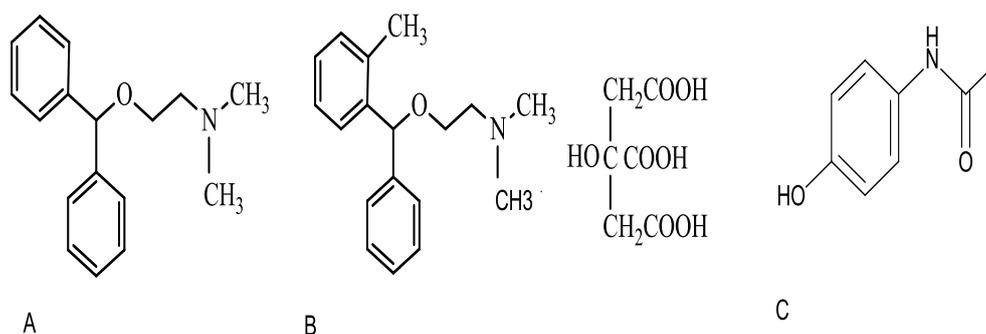


Figure 1- Structural formulae of (A) diphenhydramine (B) orphenadrine citrate (C) paracetamol.

Orphenadrine citrate is an N-methyl-D-aspartate receptor antagonist, which is possible of analgesic efficacious in a variety of pain syndromes including headache, and low back pain. Associated side effects with orphenadrine citrate use are partially related to its anticholinergic action and include dry mouth, urinary retention, confusion, blurred vision, agitation, and restlessness [4, 5].

Orphenadrine citrate is available in a combined form with paracetamol Figure-1C, aspirin, and nonsteroidal anti-inflammatory drugs. The Combination of orphenadrine citrate and paracetamol has appeared predominant pain relieving impacts, compared to each fixing when utilized alone [6].

Several quantitative instrumental approaches have been constructed and developed for assay of orphenadrine citrate, zero-Crossing derivative spectrophotometry [7]. Three analytical methods (potentiometry, atomic absorption spectrometry, and spectrophotometry) built on reaction with ammonium reineckate to form ion-pair complexes [8]; ion-pair complexes with cobalt-thiocyanate in 2 M HCl medium [9], bromophenol blue, bromocresol green, and methyl orange as ion-pair reagents at pH 4 [10], alizarine, alizarine yellow G, alizarine red S or quinalizarin–drug ion-pair complexes [11], screening for basic drugs in 2 mL urine samples by dual-plate over pressured layer chromatography and comparison with gas chromatography-mass spectrometry [12], modelling of spectrophotometric data using partial least-squares method [13], 4-chloro-7-nitro-2,1,3-benzoxadiazole in nonaqueous medium as π -acceptor [14], simultaneous determination of orphenadrine citrate and paracetamol in dosage formulations and in human serum by RP-HPLC [15], eosin–drug ion pair complex [16], π -acceptors such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, and 3,6-dichloro-2,5-dihydroxy-p-benzoquinone [17], and application of normalized spectra in resolving a challenging orphenadrine citrate and paracetamol binary mixture [18].

In the present study, the chromogenic reagent is the acidic dye EBT [Sodium 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulphonate (NaH_2In)], Figure-2. It is Azo class ($-N=N-$) compound, and contains $-SO_3Na$ group allowing it to be soluble in water. At low pH (e.g. 1.4) the basic drug protonated (positively charged), and EBT is a partially ionized forming anion (H_2In). The electrostatic attraction between cationic drugs and the anionic dyes produce an ion-pair complex, which can be extracted using organic solvents [19].

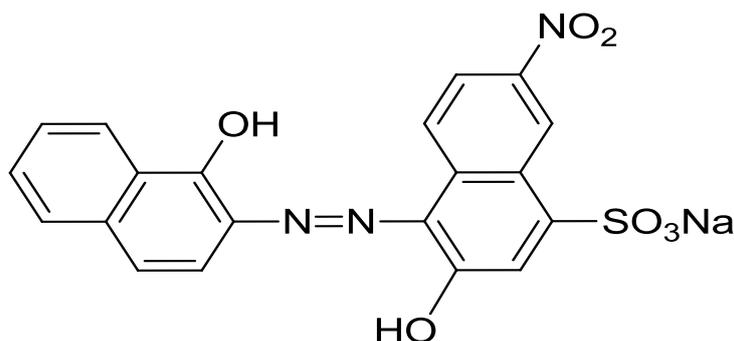


Figure 2- The Chemical Structure of EBT.

2. Experimental

2.1 Instruments

A double beam UV-visible spectrophotometer -Perkin Elmer Lambda 25 (USA) was used to carry out spectral runs, and JENWAY 6305 UV-visible spectrophotometer (UK) with glass cells (L=1cm) was used for all other absorbance measurements. A pH-meter HANNA (Portugal) was used for fast and steady pH measurements.

2.2 Material

Paracetamol and orphenadrine citrate were kindly provided by Awa medica pharmaceutical company, Hawler, Iraq. All other chemicals were pure analytical reagent grade.

The pharmaceutical products Myogesic (Na`ur- Jordan, Dar Al Dawa), Kanagesic (Syria, KANAWATI), and Muscadol (Ras Al Khaimah, U.A.E, Gulf pharmaceutical industries/Julphar) have been obtained from the local drugstores.

2.3 Reagents

2.3.1 Paracetamol and Orphenadrine Citrate Stock Solutions. (1000 µg/mL)

0.1 gm each paracetamol or orphenadrine citrate was dissolved in distilled water and diluted to 100 mL. Appropriate dilute working solutions were prepared from the stock solution. The standard solution stayed unchanged for 1 month when kept in dark bottle and refrigerated.

2.3.2 Mixture Solutions

Paracetamol plus orphenadrine citrate lab-made mixtures have been prepared by mixing different volume from their particular stock standard solutions (1000 µg /mL) equivalent to (35, 40, and 45 µg/ mL) for orphenadrine citrate, and (400, 450 and 500 µg/ mL) for paracetamol, surrounding the dosage form ratio.

2.3.3 EBT Solution (0.01% W/V)

A fresh prepared EBT in small quantities was prepared by dissolving the correct weight in distilled water.

2.3.4 Buffer Solution

Different buffer solutions ranges between pH 1.2-6.0 were freshly prepared using standard methods [20]. The included buffer systems in this study are

- 1- 0.2 M HCl-0.2 M KCl (dissolve 14.91 g of KCl in 1000 mL water). The pH ranges are (1.2-2.2).
- 2- 0.2 M HCl-0.2 M potassium bi phthalate (dissolve 40.85 g of $\text{KHC}_6\text{H}_4(\text{COO})_2$ in 1000 mL). The pH ranges are (4.2- 5.8).
- 3- Specified amount of $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ in 1000 mL volumetric flask mixed with specified volume of acetic acid solution. The pH ranges are (4.1- 6.0).

2.4 Recommended Procedure

To found the maximum wavelength of detection in a volumetric flask, 3 mL of 25 µg/mL working solution of each paracetamol, orphenadrine citrate, and laboratory prepared mixture of both drugs (4.5 µg/ mL orphenadrine and 40 µg/ mL paracetamol)was mixed with 0.2 mL of 0.01% EBT solution, 3 mL buffer solution pH 1.4 and the volume filled up to 25 mL using distilled water. The solution mixture transferred to a separating funnel, the funnel was shaken twice by 5ml chloroform for 2-mints. Once a clear separation of the two-phases was obtained the chloroform layers were collected and decanted to a spectrophotometric cell. These solutions were scanned in the visible region between 400-800 nm. The blank was prepared by same method without drug solution.

3. Results and Discussion

3.1 Absorption Spectra

The current method depends on the formation of red colour ion pair complex by means of the reaction of orphenadrine citrate with EBT in the buffer pH 1.4, extracted ion pair complex was showed maximum absorbance at 509 nm, Figure-(3a), also the orphenadrine citrate in the presence of paracetamol in laboratory prepared mixture solutions, Figure-(3b). While the paracetamol alone was not react with EBT in the buffer pH 1.4, therefore the paracetamol aqueous layer, Figure-(3c), and the paracetamol organic chloroform layer, Figure-(3d) appear no absorbance.

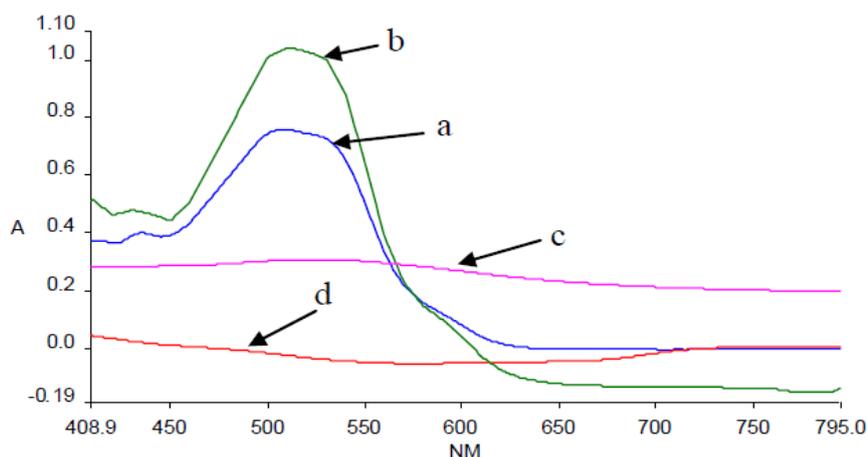
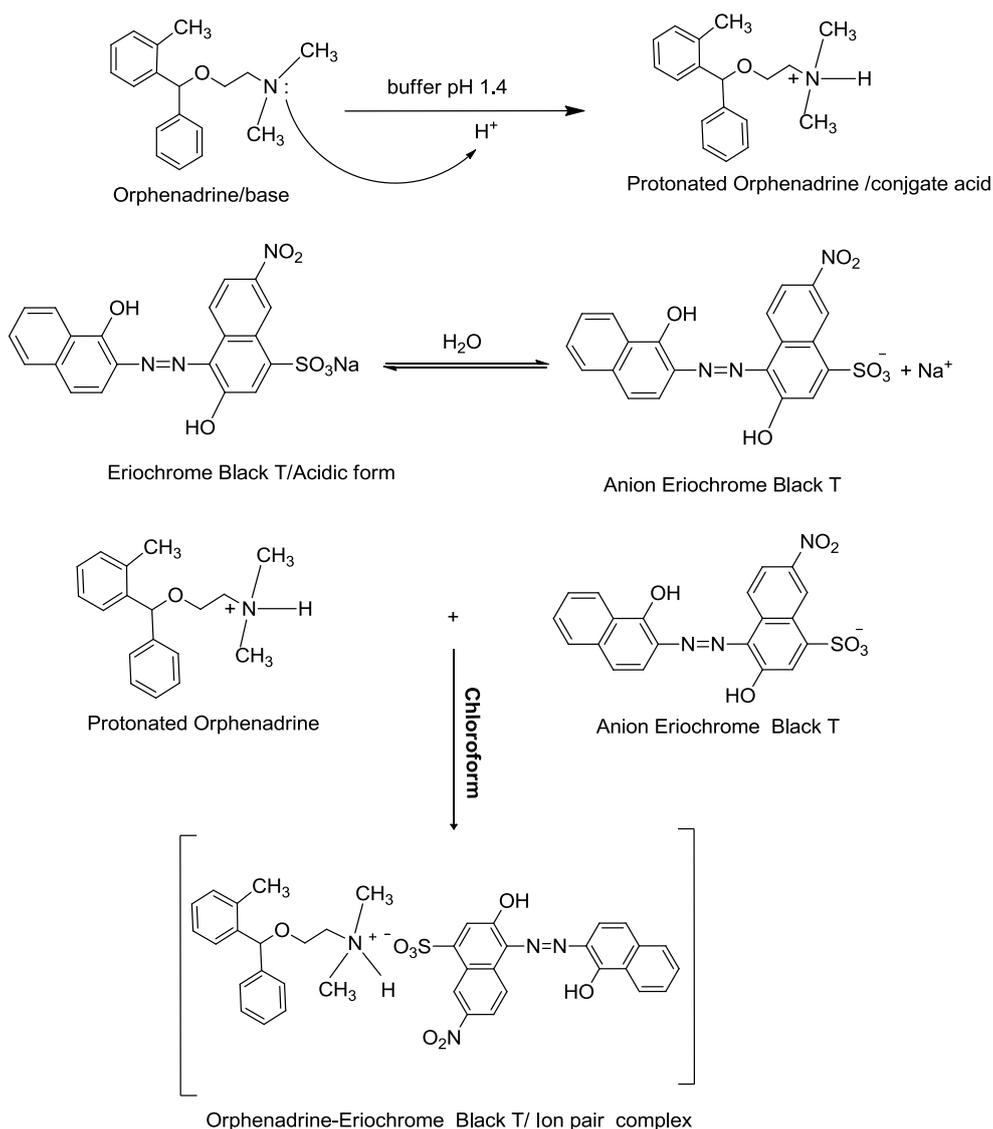


Figure 3- Absorption spectrum of a- 3 $\mu\text{g}/\text{mL}$ orphenadrine citrate- EBT ion pair complex b- mixture of paracetamol and orphenadrine citrate -EBT ion pair complex (4.5 $\mu\text{g}/\text{mL}$ orphenadrine and 40 $\mu\text{g}/\text{mL}$ paracetamol) c- paracetamol aqueous layer, and d- paracetamol organic chloroform layer.

3.2 Reaction Mechanism

Orphenadrine citrate is amino ether, the amine group is of a class tertiary amine (R_3N), that contain unshared electron pair on nitrogen, At low pH, a nonbonding pair of electrons on nitrogen is able to accept acidic proton forming protonated orphenadrine citrate charged positively. The anion of acidic dye attracts protonated orphenadrine citrate forming ion- pair neutral species. The orphenadrine ion-pair neutral species was extracted into chloroform phase consequently, the chloroform phase changes color from clear to red. Whereas paracetamol which is a phenolic drug at these conditions not paired, thus the color of the chloroform layer remains unchanged. The mechanism of the reaction and ion - pair formation described in scheme 1.



Scheme 1 - Reaction scheme of Orphenadrine-EBT ion pair formation.

3.4 Optimization of Variables

Parameters affecting on orphenadrine-EBT ion-pair formation were optimized through a number of elementary tests.

3.4.1 Effect of Type, pH, and Volume of Buffer Solution

Buffers of a type HCl-KCl (pH 1.2-pH 2.2), HCl- KHphthalate (pH 4.2 -pH 5.8), and $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ (pH 4.1- pH 6.0) at different pH ranges were examined. The maximum color intensity and highest absorbance values were recorded in potassium chloride- hydrochloric acid (pH1.4) Figure-4.

Effect of buffer volume was also tested by addition of different volumes of HCl- KCl buffer solution (pH 1.4) in the range of 1- 4 mL; it was found that 2 mL gave highest absorbance value Figure-4.

3.4.2 Effect of EBT Volume

The volume range 0.05 to 0.4 mL of 0.01 % solution of EBT has been selected to mix with the drug to investigate the colour intensity of Orphenadrine-EBT ion-pair complex. The results, Figure-4 showed the absorbance of orphenadrine-EBT complex was directly proportional to the volume EBT up to 0.3 mL, which remained approximately constant by further addition. More addition make the colour opaque, therefore 0.3 mL of the EBT was carefully chosen for the determination of orphenadrine.

3.4.3 Effect of Shaking Time

To extract the ion-pair complex from aqueous layer to chloroform layer. Shaking times ranging from 0.5 to 3.5 mints was studied, Figure-4. Between 1.0 to 3.5 minutes, the absorbance stayed fairly constant, and the designated optimum time for maximum extraction was 2 minutes.

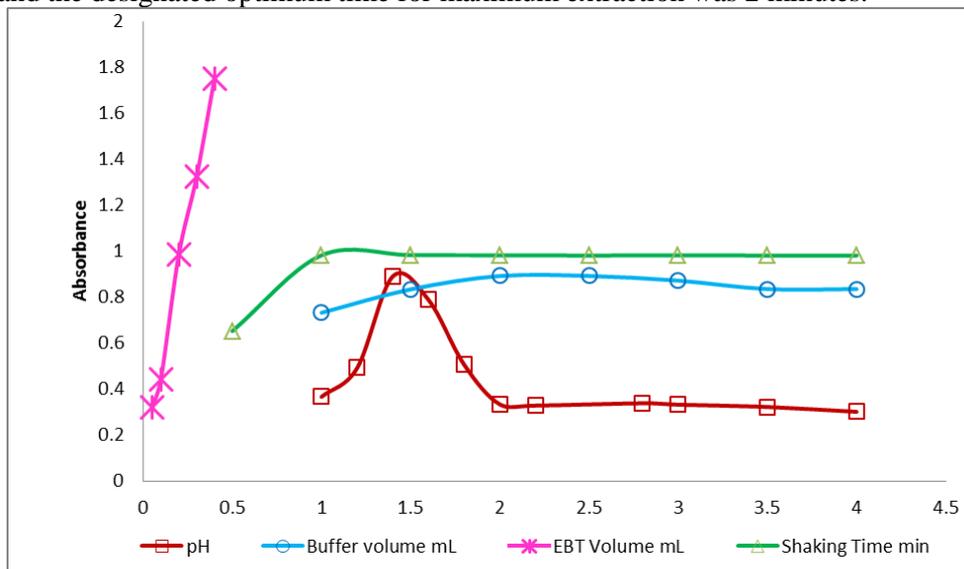


Figure 4- Effect of pH, buffer volume, EBT (0.01%) volume, and shaking time on absorbance of the orphenadrine –EBT ion-pair.

3.4.4 Effect of Temperature and Stability of the Ion-Pair

In this method, the red colour ion-pair complex of orphenadrine-EBT was created directly after mixing the reagents with the drug at room temperature. The temperature effect on ion –pair complex was studied at 25, and 30 °C, negligible change in the absorbance values were found and the results were constant at room temperature. After extraction, the ion pair complex remained stable for at least 1.5 hours, Figure-5.

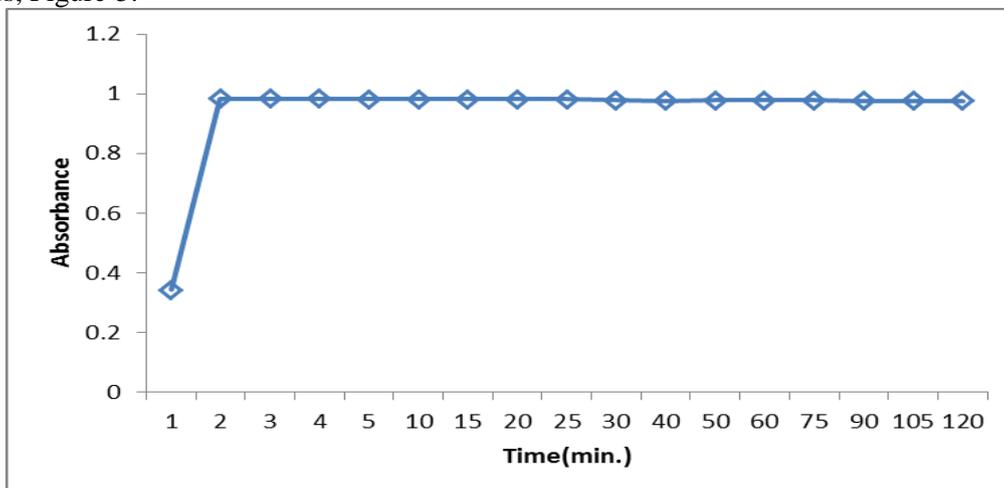


Figure 5- Effect of the time on the stability of ion pair complex.

3.4.5 Effect of Extracting Solvent

To find efficient extraction solvent, the organic solvents such as ethyl acetate, butanol, propanol, chloroform, carbon tetrachloride, and dichloromethane, were tested for extraction of the ion pair complex of Orphenadrine-EBT. Chloroform was chosen because it gave maximum absorbance of the extracted complex Table-(3.1).

Table 3.1- Solvent effect on orphenadrine-EBT ion-pair complex absorbance.

Solvent	Ethyl acetate	Butanol	Propanol	Chloroform	Carbon tetra chloride	Dichloro-methane
Absorbance	0.125	-0.063	-0.038	0.882	0.296	0.116

3.5 Justification of Recommended Method

3.5.1 Analytical Parameters

A standard graph of the ion-pair complex was constructed by plotting absorbance against concentration, under the optimum conditions, Figure-6.

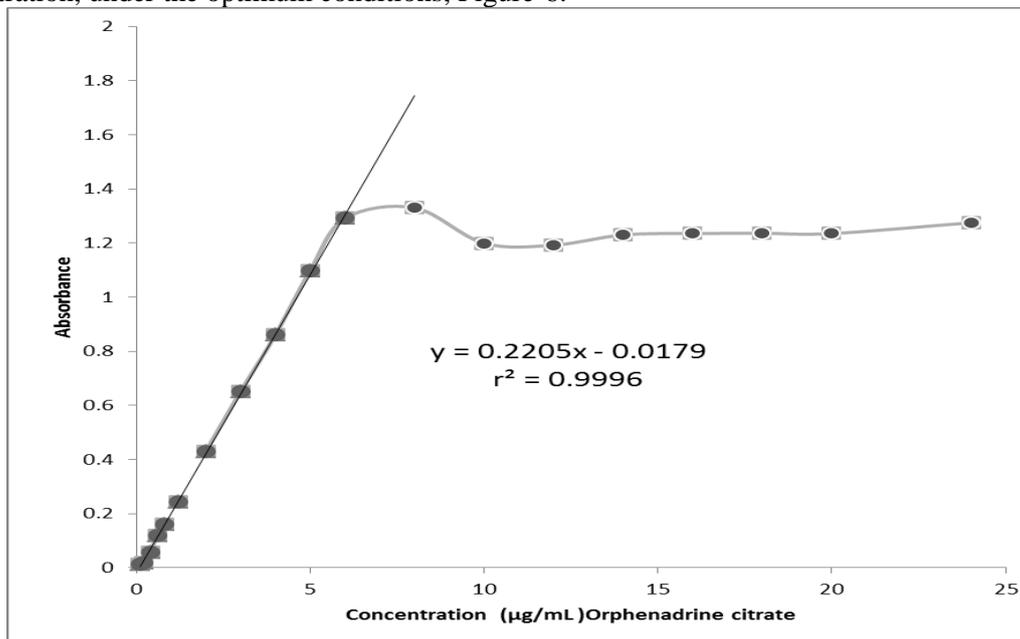


Figure 6- Calibration graph of the orphenadrine-EBT ion-pair complex.

The standard graph indicates that the extracted ion- pair was followed Beer's Law in the concentration range of 0.1- 6 µg/ mL, with high correlation coefficient value $r^2 = 0.9996$. At high concentrations (greater than 6µg/mL) there is a deviation from Beer's Law and the line loses its linearity. The Sandell's sensitivity, and molar absorptivity were 0.0122 µg / cm², 4.4025 x 10⁻⁴ L/mol.cm respectively. The limits of detection and quantification were found to be 0.024 µg/mL and 0.100 µg/mL respectively; these low values designate a good sensitivity of the method. The analytical parameter of the standard graph was shown in Table- (3.2).

3.5.2 Repeatability (Precision)

Repeatability is the precision of the method measured in the similar working conditions over a short interval of time. Furthermore, repeatability is named intra assay precision and is usually calculated in terms of % relative standard deviation RSD. A set of six replicate measurements at three different concentrations (2µg/mL, 4µg/mL, and 6µg/mL within Beer's law limit) of orphenadrine citrate performed by the same person, at the same time on the same standard solution, using the same method. An intraday precision of the orphenadrine citrate are shown in Table- (3.2).

Table 3.2- Analytical parameters and precision of extraction of orphenadrine-EBT ion pair complex.

Parameter	Value
λ nm	509.00
Linear range ,µg/ mL	0.10-6.00
Detection Limit ,µg/mL	0.024
Quantification Limit ,µg/mL	0.100
Correlation Coefficient, r^2	0.9996
Sandell's Sensitivity ,µg/cm ²	0.0122
Molar absorptivity, L/mol. cm	4.4025x10 ⁴
RSD%	1.095*
	0.5041**
	0.732***

RSD% : Average of six determination.

*** 2µg/mL, ** 4 µg/mL, and ***6 µg/mL of orphenadrine citrate.**

3.5.3 Trueness (Accuracy)

The trueness of the method was evaluated by addition of known amounts of standard orphenadrine citrate solution to pre-quantified orphenadrine citrate solution. The analysis was done in a set of six replicate measurements at three different spiking, Table-(3.3). Percent recovery % calculated using the following formula.

$$\text{Recovery\%} = (\text{amount found} / \text{total amount}) \times 100$$

Table 3.3- Accuracy evaluation of the determination orphenadrine citrate

% Spiking	Actual concentration, $\mu\text{g/mL}$	Added concentration, $\mu\text{g/mL}$	Found concentration, $\mu\text{g mL}$	% Recovery \pm S.D
50	2	1	2.964	98.8 \pm 0.011
100	2	2	4.101	102.5 \pm 0.032
150	2	3	4.973	99.4 \pm 0.121

3.5.4 Interference

To study the efficiency and selectivity of the suggested method, a procedure of the analysis under the optimum conditions was carried out for the effect of excipients type (binders, disintegrate and diluents) as Cellulose-micro crystalline, Mg stearate, Silica-colloidal, Starch, dextrose, mannitol that are present in a tablet dosage. In this study, the solutions containing 3 $\mu\text{g/mL}$ of orphenadrine citrate and 10-fold of excipients were used following the recommended procedure. Experimental results achieved were nearly the same as those obtained for orphenadrine citrate solutions without excipients (error not more than $\pm 1\%$), Table- (3.4).

Table 3.4- Determination of 3 $\mu\text{g/mL}$ of orphenadrine citrate in the presence of excipients.

Excipients (30 mg L ⁻¹)	Orphenadrine citrate (3 $\mu\text{g/ mL}$) found*	Error %	Recovery %
Cellulose-microcrystalline	2.985	-0.500	99.5
Magnesium stearate	2.976	-0.800	99.2
Silica-colloidal anhydrous	3.012	0.400	100.4
Starch	3.025	0.833	100.8
Dextrose	3.014	0.467	100.4
Mannitol	3.008	0.266	100.26

* Mean value of three repeated measurements.

3.6 Analysis of pharmaceutical formulation

Three different pharmaceutical samples of orphenadrine citrate were estimated by the suggested method. The analysis outcomes were compared with reference HPLC quantification method [20], by using stationary phase intersil C8 phase (5 μm), column size: ($\varnothing = 4.6$ mm, I= 0.25 m), flow rate 2.0 ml/min, mobile phase (A: 0.1% v/v H₃PO₄ in water, B: Acetonitrile 100%), and spectrophotometer detector at λ 215 nm. Table-(3.5), shows that the results obtained by the two methods were in close agreement.

Table 3.5- Determination of orphenadrine citrate in a pharmaceutical preparation.

Sample	Labl claim mg orphenadrine citrate	Official method		Proposed method	
		Found	Recovery %	Found	Recovery%
Myogesic	35	33.43	95.5	34.53	98.6
Kanagesic	35	33.04	94.4	34.12	97.4
Muscadol	35	33.01	94.3	34.16	97.6

4. Conclusion

In the suggested method, orphenadrine citrate in the presence of paracetamol reacts directly with EBT in acidic medium forming stable ion pair complex. Under the optimized extraction conditions, the method was validated, and applied successfully to estimate orphenadrine citrate in single- drug or in combine drug in the presences of paracetamol without any interference from paracetamol or the commonly used excipients. The result shows that the method was selective, accurate, precise, rapid, and economical.

References

1. Sean, C.S. **2007**. *Martindale: The complete drug reference*. 35th Edition, vol 1. Pharmaceutical press.UK, 730.
2. Beale, Jr. J. M. and Block, J. H. **2011**. *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 12th. ed . Philadelphia: Lippincott Williams & Wilkins, 592.
3. Goth, A., G. and Vesell, E.S. **1984**. *Medical pharmacology: principles and concepts*, 11thed. St. Louis, Toronto: Mosby,pp: 136-137.
4. McCleane, G. **2009**. Muscle relaxants. In: Smith H.S. (ed), *Current Therapy in Pain*. Saunders, USA. pp: 470-475.
5. Wallace, M.S. and Staats, P.S. **2004**. *Medicine and Management: Just the Facts*. McGraw-Hill. 76.
6. McGuinness, B.W. **1983**. A double-blind comparison in general practice of a combination tablet containing orphenadrine citrate and paracetamol (norgesic) with paracetamol alone. *Journal of International Medical Research*, **11**(1): 42-45.
7. Mohie, K., S., Abuirjeie, M.A. and Abdel-Hay, M.H. **1991**. Simultaneous determination of acetaminophen with orphenadrine citrate, ibuprofen or chlorzoxazone in combined dosage forms by zero-crossing derivative spectrophotometry. *Analytical letters*, **24**(12): 2187-2206.
8. Elnemma, E.M., El Zawawy F. M. and Hassan, S. **1993**. Determination of amitriptyline, imipramine and orphenadrine in antidepressant drugs by potentiometry, spectrophotometry, and atomic absorption spectrometry. *Microchim. Acta*, **110**(1): 79-88.
9. Revanasiddappa, H.D. and Ramappa, P.G. **1995**. Extractive spectrophotometric determination of pentazocine, orphenadrine, and doxepin hydrochloride. *Indian J Pharm Sci.*, **57**(2): 85–87.
10. Nagaraja, P. and Murthy, K.C.S. **1995**. Extractive spectrophotometric determination of antihistaminic drugs, *Ind. J. Pharm. Sci.*, **5**: 139-141.
11. Shama, S.A. **2002**. Spectrophotometric determination of phenylephrine HCl and orphenadrine citrate in pure and in dosage forms. *J. Pharm. Biomed. Anal.*, **30**(4): 1385-1392.
12. Pelander, A., Ojanperä, I., Sistonen, J., Rasanen, I. and Vuori, E. **2003**. Screening for basic drugs in 2-mL urine samples by dual-plate overpressured layer chromatography and comparison with gas chromatography-mass spectrometry. *Journal of Analytical Toxicology*. **27**(4): 226–232.
13. Srathaphut, L. and Ruangwises, N. **2007**. Determination of paracetamol and orphenadrine citrate in pharmaceutical tablets by modeling of spectrophotometric data using partial least-squares and artificial neural networks. *Yakugaku zasshi journal of the Pharmaceutical Society of Japan*. **127**(10): 1723-1729.
14. Saleh, H.M., El-Henawee, M.M., Ragab, G.H. and Abd El-Hay, S.S. **2007**. Utility of NBD-Cl for the spectrophotometric determination of some skeletal muscle relaxant and antihistaminic drugs. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. **67**(5): 1284-1289.
15. Arayne, M.S., Sultana, N. and Siddiqui, F.A. **2013**. Simultaneous determination of paracetamol and orphenadrine citrate in dosage formulations and in human serum by RP-HPLC. *Journal of the Chinese Chemical Society*. **56**: 169-174.
16. Walash, M.I., Belal, F.F., Eid, M.I. and Abo-ElAbass, S.M. **2011**. Spectrophotometric determination of tizanidine and orphenadine via ion pair complex formation using eosin Y. *Chem. Central J*. **5**(1): 60-69.
17. Mohamed, G.G., Frag, E.Y.Z. and Awad, Y.H.H. **2012**. Spectrophotometric studies using charge-transfer complexes of orphenadrine citrate in pure and in dosage forms with π -acceptors. *J. Pharmacy Research*. **5**(3): 1774-1779.

- 18.** Yehia, A.M. and Abd El-Rahman, M.K. **2015.** Application of normalized spectra in resolving a challenging orphenadrine and paracetamol binary mixture. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy.* **138**(5): 21-30.
- 19.** Ham, H.B. and MaHam, A. **2015.** *Analytical Chemistry: A Chemist and Laboratory Technician's Toolkit.* Wiley, 193.
- 20.** United States Pharmacopeial Convention. **2009.** *The United States pharmacopeia: USP 29,* Rockville, MD. United States Pharmacopeial Convention, 1587.