



## Validated Spectrophotometric Methods for the Determination of Oxybuprocaine Hydrochloride

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### Abstract

Simple, rapid, accurate and reliable spectrophotometric methods were developed and validated for determination of Oxybuprocaine hydrochloride (OXY) in pure form and in pharmaceutical preparation. The methods depend on charge transfer reaction of OXY as n-electron donor with *p*-chloranilic acid (*p*-CA), 2, 3 – dichloro 5, 6 – dicyano 1, 4 benzoquinone (DDQ) and iodine as  $\pi$  and  $\sigma$  acceptors, respectively. These reactions were studied under various conditions and the optimum parameters were selected. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9996, 0.9997, and 0.9998) were found between absorbance of the formed complexes and concentrations of OXY in the range of 20.0 - 220.0  $\mu\text{g/mL}$ , 10.0-80.0  $\mu\text{g/mL}$  and 4.0-44.0  $\mu\text{g/mL}$  for (*p*-CA), DDQ and iodine methods, respectively. The methods were successfully applied for the determination of OXY in pure form and in dosage form. Job's method was applied to determine the stoichiometry of the reactions. No significant difference was found at  $p = 0.05$  when the obtained results of the proposed methods were statistically compared with those obtained by an official method.

**Keywords:** Oxybuprocaine hydrochloride; *p*-CA; DDQ; Iodine; Charge transfer complex.

### 1. Introduction

Oxybuprocaine hydrochloride (OXY) or benoxinate hydrochloride chemically is 2-diethylamino ethyl 4-amino-3-butoxybenzoate hydrochloride (Figure 1). It is a para aminobenzoic acid ester local anesthetic used for surface anesthesia and is used as the hydrochloride form in 0.4% solution in short ophthalmological procedures. Solution of OXY (1%) is used for surface anesthesia of the ear, nose and throat <sup>(1)</sup>.

It has been analysed by spectroscopic methods <sup>(2-6)</sup>, high-performance liquid chromatographic methods (HPLC) <sup>(7-12)</sup>, thin layer chromatographic method <sup>(13)</sup>, gas chromatographic methods (GC) <sup>(14-15)</sup>, electrochemical methods <sup>(16-18)</sup> and thermal method <sup>(19)</sup>. Non-aqueous titration is the official method used for determination of benoxinate HCl <sup>(20-21)</sup>.

The aim of this work is to develop rapid, simple and accurate visible spectrophotometric methods for the determination of OXY in pure form and in dosage form by exploiting its basic nature and its electron donating property. Three electron acceptor compounds were used namely; *p*-chloranilic acid (*p*-CA), 2, 3- Dichloro-5, 6- dicyano-1, 4- benzoquinone (DDQ) and iodine.

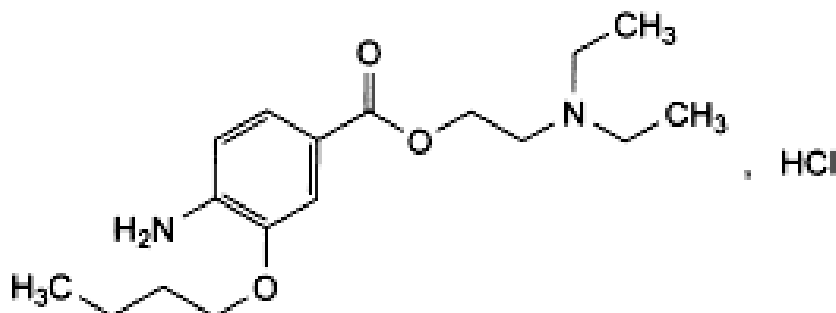


Figure (1): Chemical structure of oxubuprocaine hydrochloride.

## 2. Experimental

### 2.1. Apparatus

Shimadzu UV 2400 Series spectrophotometer connected to IBMPC computer and HP laser jet 1100 series printer, with two matched quartz cells of 1 cm optical length using the following spectral parameters; Band width = 2 nm; scan speed = fast; scan mode = single.

### 2.2. Samples

#### 2.2.1. Pure samples

Oxybuprocaine hydrochloride was kindly supplied by Egyptian International Pharmaceutical Industries Company (EPICO), 10<sup>th</sup> of Ramadan city, Egypt. Its purity was found to be  $99.79 \pm 0.619$  according to the official method <sup>(20)</sup>.

#### 2.2.2. Market sample

Benox eye drop ® (batch no.17468) was labeled to contain 0.4 % OXY and manufactured by Egyptian International Pharmaceutical Industries Company (EPICO), 10<sup>th</sup> of Ramadan city, Egypt.

### 2.3. Chemicals and Solvents

All chemicals and reagents used were of analytical grade.

- 2, 5- Dichloro-3, 6- dihydroxy-1, 4-benzoquinone, (p-Chloranilic acid, p-CA); (Merck, Darmstadt, Germany) was prepared as 0.4 % (w/v) solution in acetonitrile.
- 2, 3- Dichloro 5, 6- dicyano- 1, 4- benzoquinone (DDQ); (Acros, New jersey, USA) was prepared daily as 0.5 % (w/v) solution in acetonitrile.
- Iodine (Merck Limited, Mumbai, India) was prepared as 0.5 % (w/v) solution in dichloromethane.
- 1, 4- Dioxane (BDH Chemicals Ltd., England)
- 1, 2- Dichloromethane, isopropanol, ethanol, chloroform, carbon tetrachloride (Fisher scientific-UK)
- Acetone (lab-scan, Honil Limited, London, UK)
- Acetonitrile and methanol (Lab. Scan, Ireland).

### 2.4. Standard Solution of Oxybuprocaine Hydrochloride (1 mg/mL)

An accurately weighed amount about 100 mg of OXY was transferred to 100-mL volumetric flask and dissolved in 20 mL acetonitrile (for p-CA and DDQ methods) or in 20 mL 1, 2- dichloromethane (for iodine method) then the volumes were completed with the same solvent.

### 2.5. Procedures

#### 2.5.1. Construction of calibration curves

Into a three separated series of 10-mL volumetric flasks, aliquots of OXY standard solutions (1 mg/mL) equivalent to 200.0 - 2200.0  $\mu\text{g}$  for p-CA, 100.0 - 800.0  $\mu\text{g}$  for DDQ and 40-440  $\mu\text{g}$  were transferred. 2 mL of 0.4 % p-CA, 1 mL of 0.5 % (w/v) of DDQ and 1 mL 0.5% iodine were added into the three separated series, respectively. The volumes were completed to the mark with acetonitrile for p-CA and DDQ while dichloromethane was used for iodine. The reactions were achieved instantaneously at ambient temperature ( $25 \pm 2$  °C). The absorbance of the resulting solutions was measured at the wavelengths of maximum absorbance (518, 588 nm and 365 nm for p-CA, DDQ and iodine, respectively) against reagent blanks treated similarly. The calibration curves relating the absorbances at the selected wavelengths to the corresponding concentrations ( $\mu\text{g/mL}$ ) were constructed and the regression equations were computed.

### 2.5.2. Determination of stoichiometry of the complex by Job's method.

For p-CA, into a series of 10-mL volumetric flasks 0.2-1.8 mL of  $3 \times 10^{-3}$  M OXY solution in acetonitrile were transferred, followed by 1.8 - 0.2 mL of  $3 \times 10^{-3}$  M p-CA solution in acetonitrile and the volumes were completed with acetonitrile. For DDQ, into a series of 10-mL volumetric flasks 0.1 - 0.9 mL of  $3 \times 10^{-3}$  M OXY solution in acetonitrile was transferred, followed by 0.9 - 0.1 mL of  $3 \times 10^{-3}$  M DDQ solution in acetonitrile and volumes were completed with acetonitrile. For iodine, into a series of 100-mL volumetric flasks 0.4 - 3.6 mL of  $3 \times 10^{-3}$  M OXY solution were transferred followed by 3.6 - 0.4 mL of  $3 \times 10^{-3}$  M of iodine and the volume was completed with dichloromethane. The absorbances of the resulting solutions were measured at the wavelength of maximum absorbance against reagent blanks treated similarly. The absorbances were plotted against mole fractions of the drug.

### 2.5.3. Analysis of pharmaceutical dosage form

A portion of the solution needed to obtain 0.5 mg/mL drug solution (12.5 mL) was transferred into a 100-mL volumetric flask then add 20 mL acetonitrile for p-CA and DDQ methods or 20 mL dichloromethane for iodine method. The content of the flask was sonicated for about 10 minutes and then made up to the volume with appropriate solvent. Aliquots of the drug solution were introduced and the general procedures were carried out.

## 3. Results and Discussion

### 3.1. Reaction with $\pi$ -acceptors

The interaction of OXY with  $\pi$ -acceptors such as p-CA and DDQ at room temperature was found to yield colored charge transfer complexes. In polar solvents, complete electron transfer from OXY (D), as an electron donor, to the acceptor moiety (A) takes place resulting in the formation of intensely colored radical anions<sup>(22)</sup>. The absorption spectra of OXY-  $\pi$ - acceptor reaction mixtures showed absorption peaks which were similar to the maxima of the radical anions of the  $\pi$ -acceptors<sup>(23)</sup>. Upon addition of OXY solution in acetonitrile to the golden yellow solution of p-chloranilic acid in acetonitrile, a purple color was obtained. This color was suggestive of a charge transfer complex formation, and was confirmed by appearance of  $\lambda_{\text{max}}$  at 518 nm as illustrated in Figure (2). The interaction of OXY with DDQ resulted also in the formation of colored charge transfer complex. The dissociation of charge transfer complex was promoted by the high ionizing power of the acetonitrile solvent where complete electron transfer from OXY to the DDQ moiety takes DDQ radical anion as predominant chromogen which absorbed maximally at 588 nm as shown in Figure (3). On the basis of our experimental findings and the literature background, the reaction mechanism is proposed and given in Scheme (1) and (2).

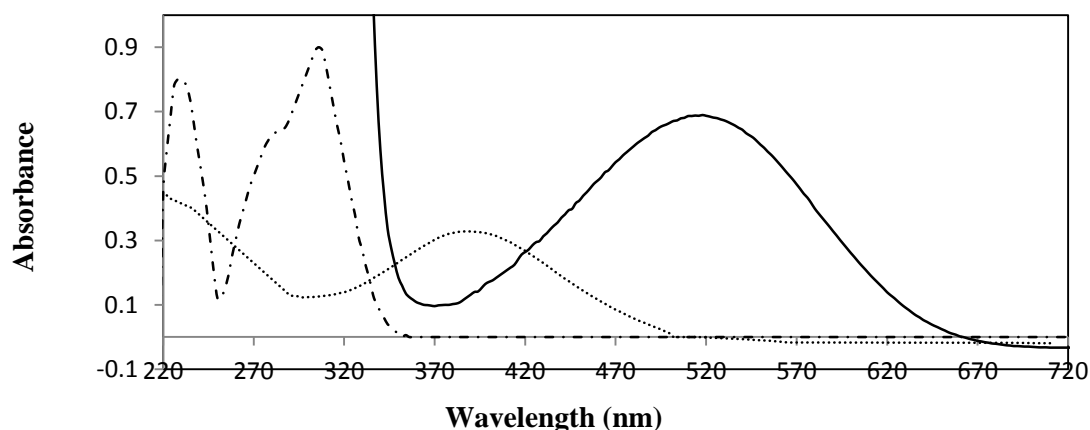


Figure (2): Zero order absorption spectra of:

- OXY *p*-CA charge transfer complex, 150 µg/ mL ( ——— ).
- OXY in acetonitrile, 20 µg/ mL (- - - - -).
- Blank reagent (.....).

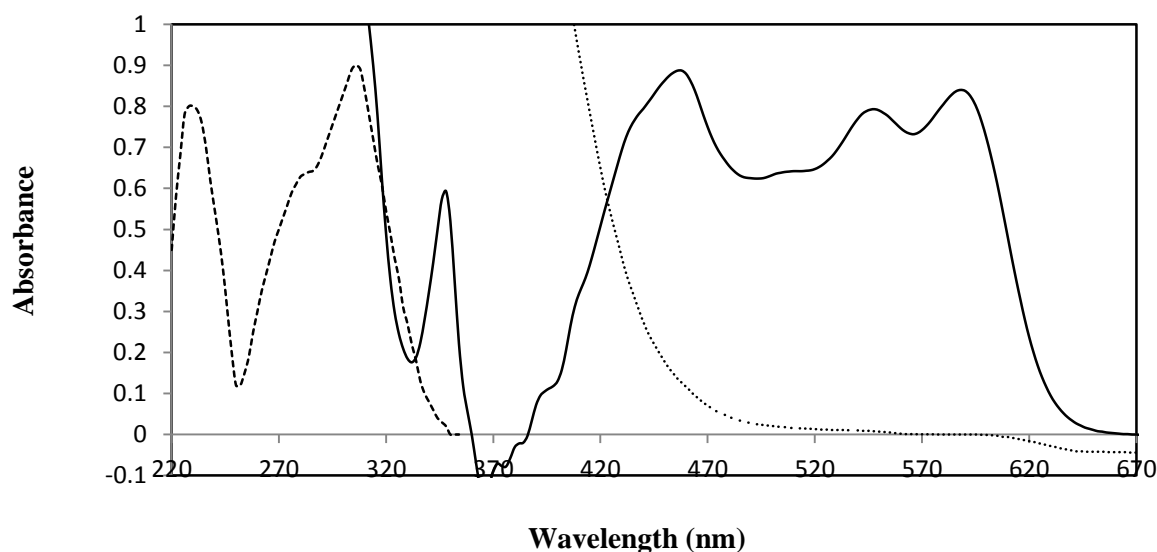
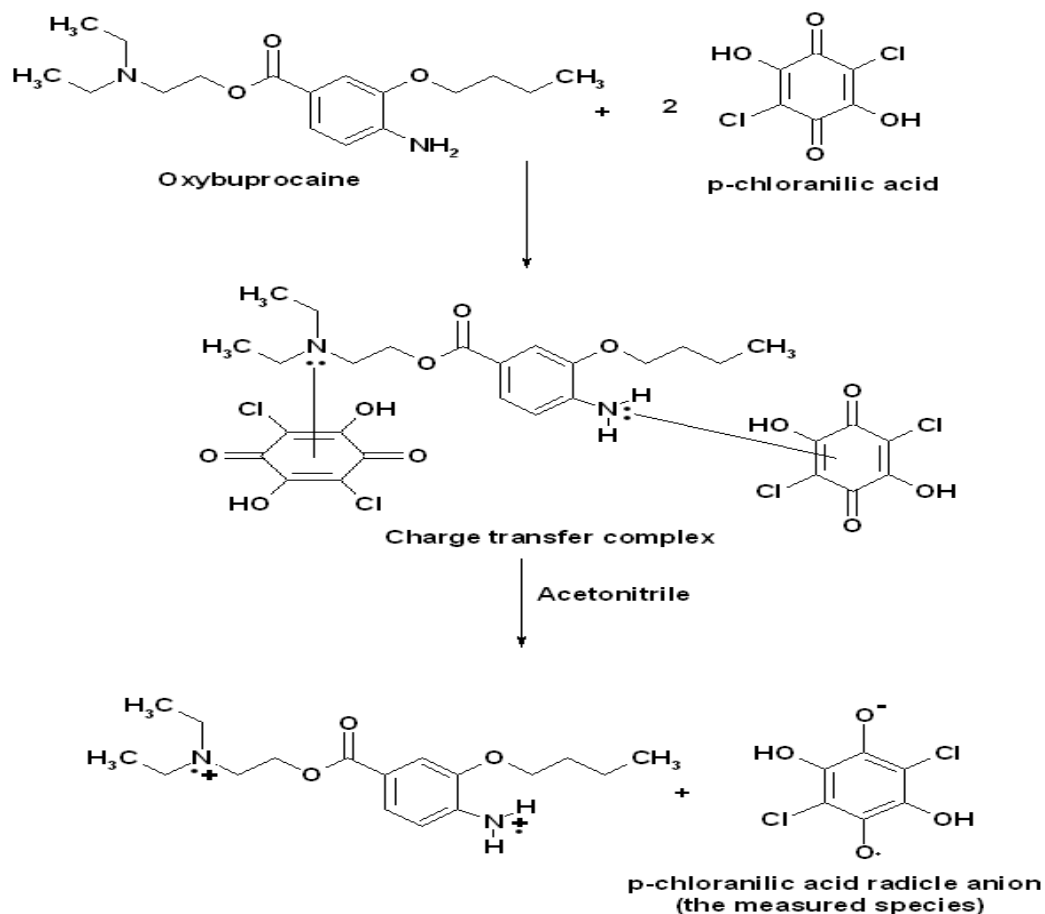
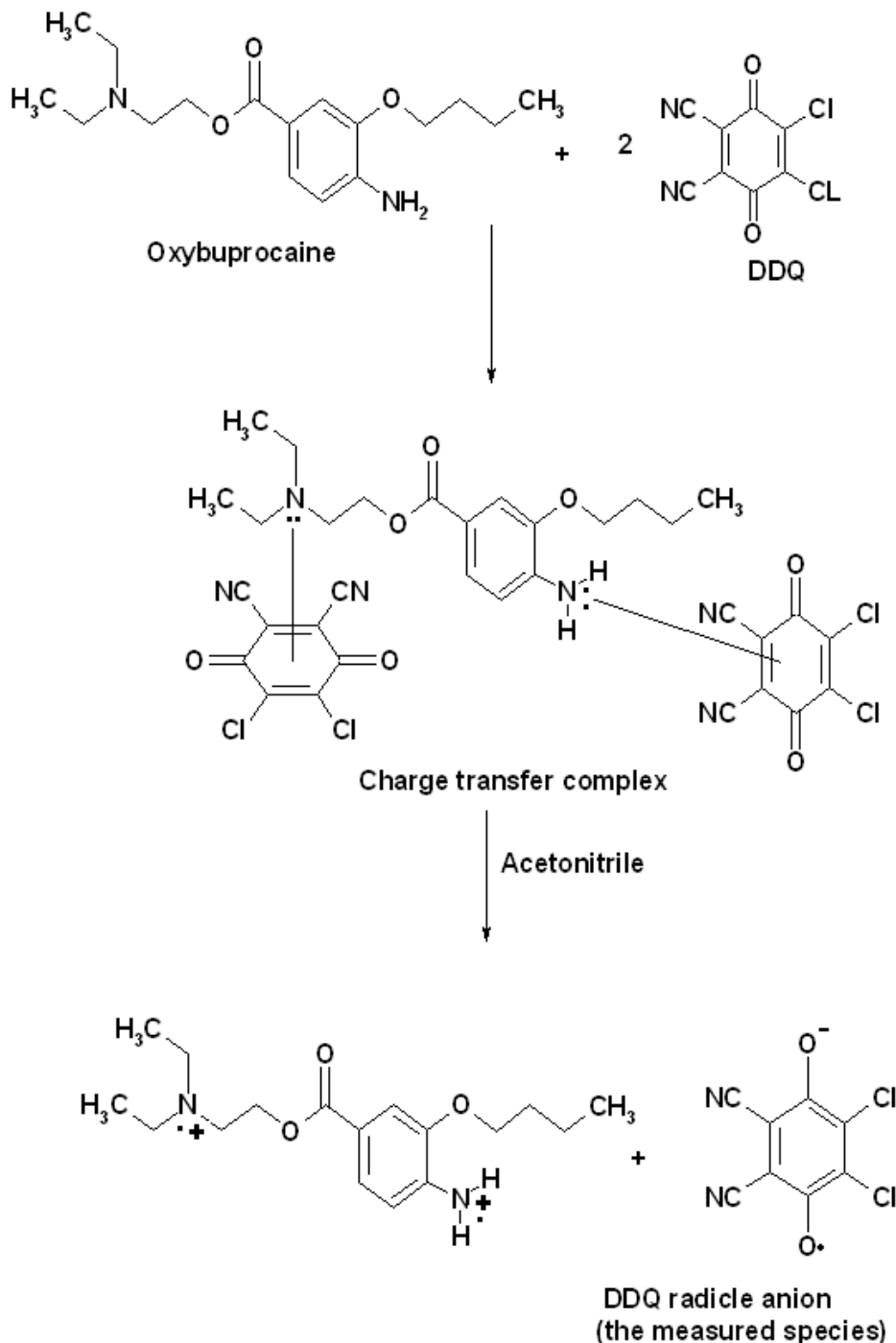


Figure (3): Zero order absorption spectra of:  
 -- OXY - DDQ charge transfer complex, 80 µg/ mL ( ——— ).  
 -- OXY in acetonitrile, 20 µg/ mL ( - - - - - ).  
 -- Blank reagent (.....).

**Scheme (1): The Suggested Mechanism of Reaction between OXY and P-CA.**



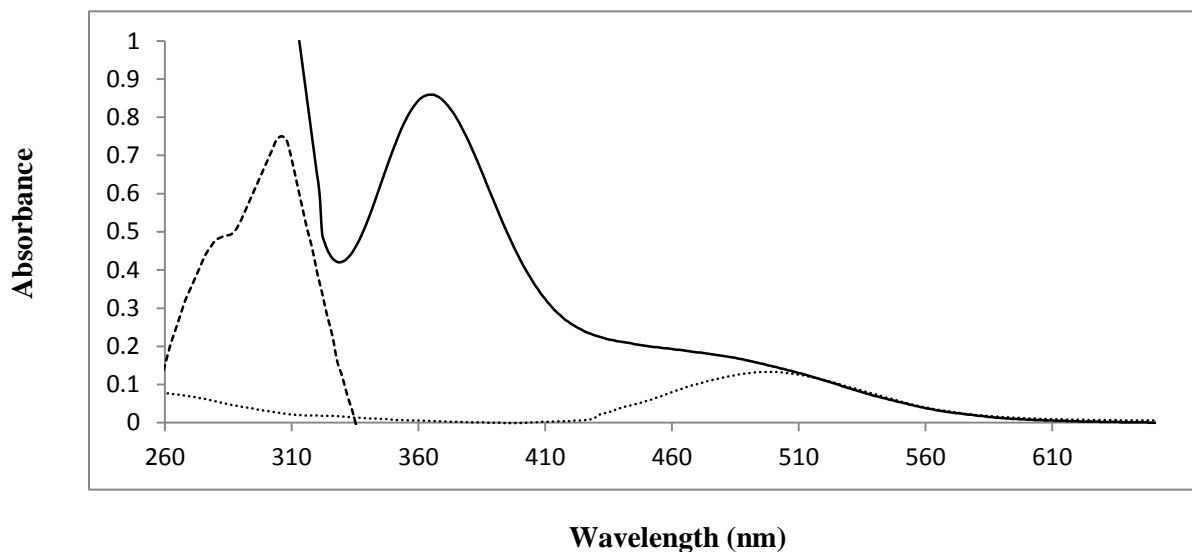
Scheme (2): The Suggested Mechanism of Reaction Between OXY and DDQ.



### 3.2. Reaction with $\sigma$ -acceptor (iodine)

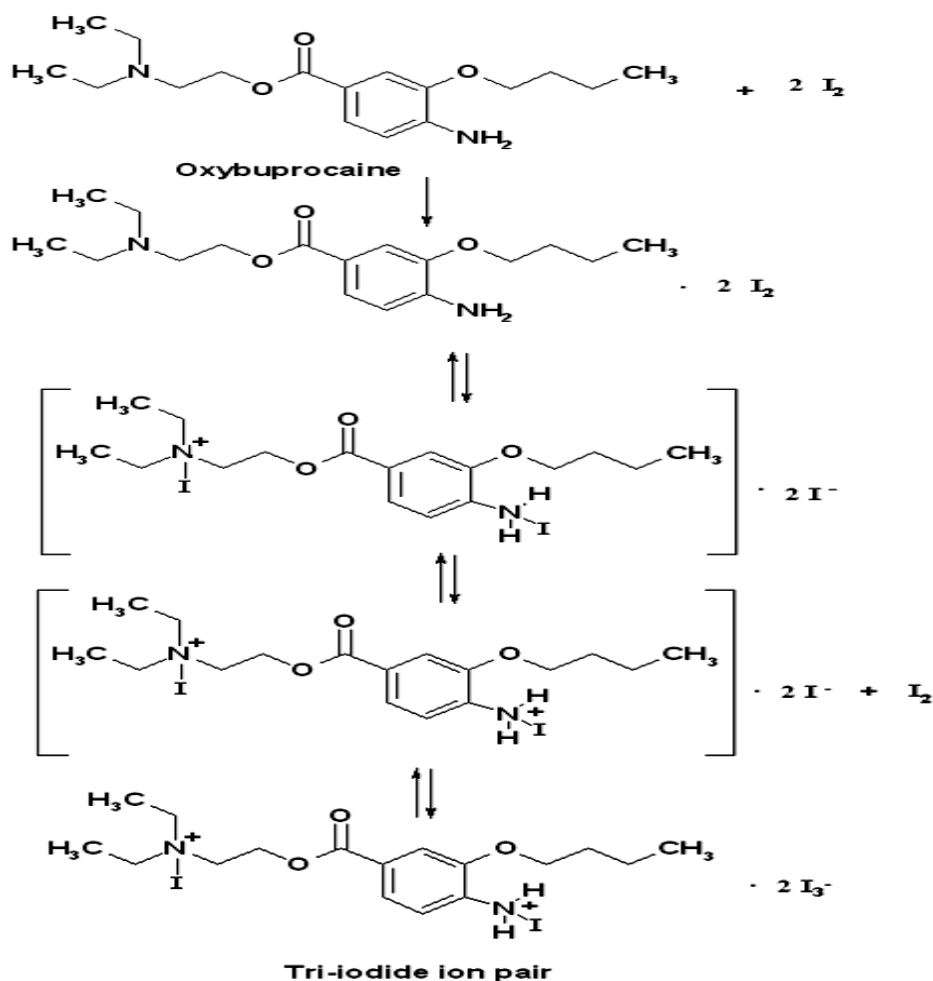
The immediate change of the violet color of iodine in dichloromethane to a lemon yellow upon reaction with OXY was taken as suggestive of charge transfer complex formation which justified scanning in the UV range for the new band (Figure 4). Further confirmation of the charge-transfer nature of the reaction was obtained on extracting the drugs from the complex by shaking with aqueous mineral acid, whereby the violet color of iodine in dichloromethane was restored<sup>(24)</sup>. The appearance of absorption peaks at 365 nm was attributed to the formation of

a charge transfer complex between OXY and iodine, having an ionized structure  $DI^+ \cdots I_3^-$  in dichloromethane (Scheme 3).



**Figure (4):** Zero order absorption spectra of:  
 - OXY - iodine charge transfer complex, 32  $\mu\text{g}/\text{mL}$  ( ——— ).  
 - OXY in dichloromethane, 20  $\mu\text{g}/\text{mL}$  ( - - - - ).  
 - Blank reagent ( ..... ).

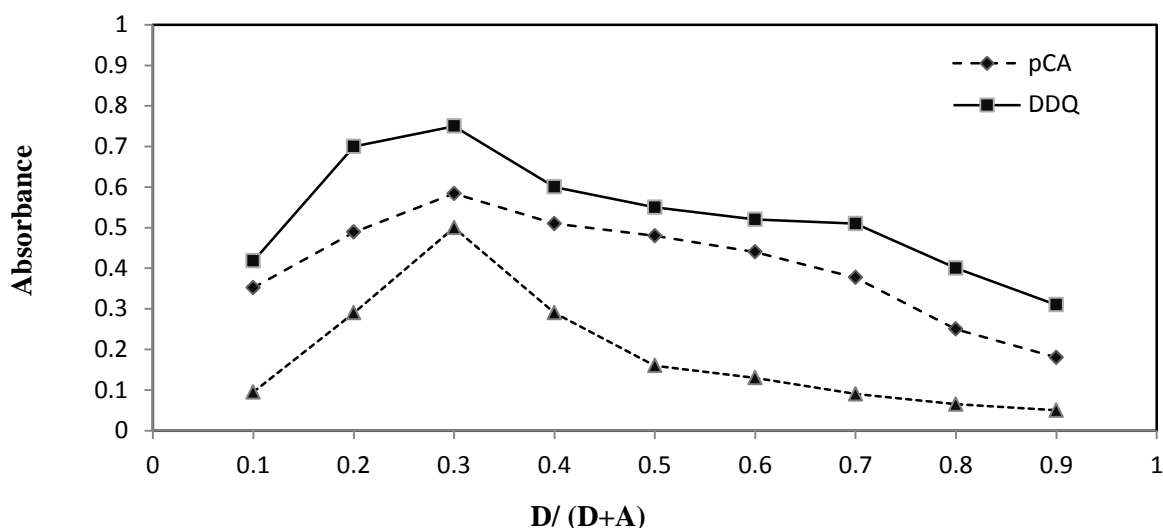
**Scheme (3): The Suggested Mechanism of Reaction Between OXY and Iodine.**



### 3.3. Stoichiometry of the reaction <sup>(25)</sup>

The stoichiometry of the produced charge transfer complexes was determined by applying Job's method of continuous variation. The absorbance of several solutions that having  $[D] + [A] = \text{constant}$ , but with varying  $D:A$  ratio was measured at 518 nm, 588 nm and 365 nm for *p*-CA, DDQ and iodine methods, respectively. These ratios were found to be 1:2 for drug to all acceptors, confirming the presence of two *n*-donating centers in OXY molecule as illustrated in Figure (5).

**Figure (5): Determination of Stoichiometry of the Reaction of OXY With *P*-CA, DDQ and Iodine by Continuous Variation Method ( $3 \times 10^{-3}$  M).**

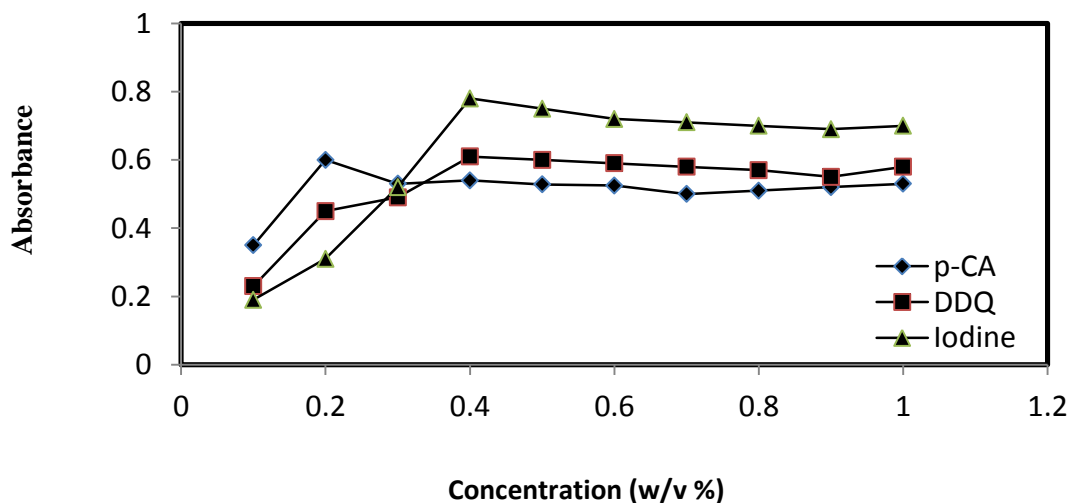


### 3.4. Optimization of reaction conditions

#### 3.4.1. Effect of reagents concentrations

The results of variations in the reagents concentrations indicated that 2 mL of 0.4% *p*-CA, 1 mL of 0.5% (w/v) of DDQ and 1 mL 0.5% iodine are the optimum ones. The higher concentrations of the reagents may be useful for rapidly reaching equilibrium, thus minimizing the time required to attain maximum absorbance at the corresponding wavelengths of maximum absorbance. The results of variations in the reagents concentrations are shown in (Figure 6).

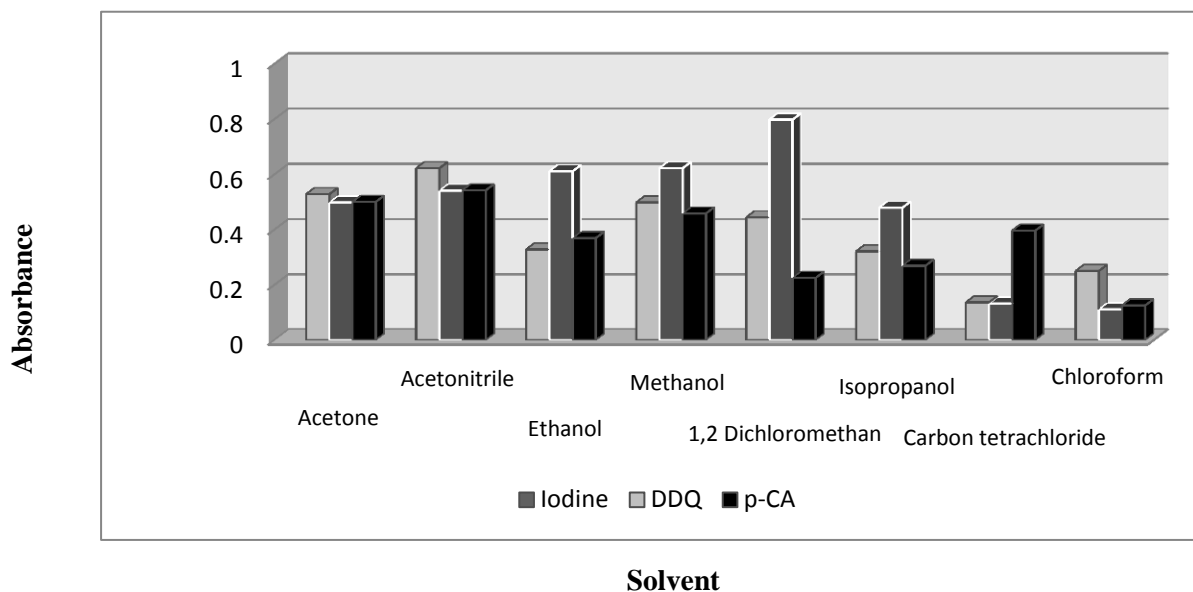
**Figure (6): Effect of the Reagent Concentrations on the Absorbance of Charge Transfer Complex (100.00  $\mu\text{g/mL}$  OXY for *p*-CA, 50.00  $\mu\text{g/mL}$  for DDQ and 28  $\mu\text{g/mL}$  for iodine).**



### 3.4.2. Effect of solvents

In order to select the most appropriate solvent, the reactions were carried out in different solvents as acetone, acetonitrile, methanol, ethanol, dichloromethane, methylene chloride 1, 4-dioxane and isopropanol. As represented in Figure (7) acetonitrile was found to be an ideal solvent in case of p-CA and DDQ and dichloromethane for iodine because it is favorable for the formation of tri-iodide ion pair (inner complex). Chloroform and carbon tetrachloride produced lower absorption readings. Polar solvents were found to be unsuitable as their blanks with iodine gave higher readings.

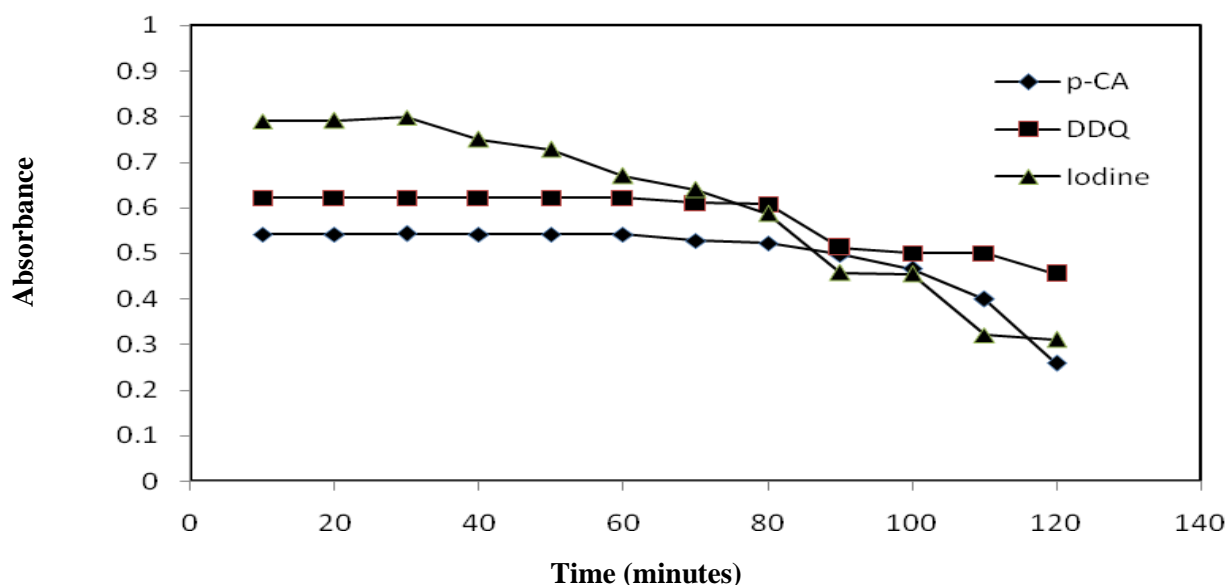
**Figure (7): Effect of Solvent on the Absorbance of the Charge Transfer Complex (100 µg/mL OXY for p-CA, 50 µg/mL for DDQ and 28 µg/mL for iodine).**



### 3.4.3. Effect of Reaction Time

The developed colors remained stable at room temperature for 1 hour for p-CA and DDQ and for 30 minutes for iodine as represented in Figure (8).

**Figure (8): Effect of the Time on the Stability of the Charge Transfer Complex (100 µg/mL OXY for p-CA, 50 µg/mL for DDQ and 28 µg/mL for iodine).**





### 3.5. Method validation<sup>(26)</sup>

ICH guidelines for the method validation were followed for validation of the suggested method.

#### 3.5.1. Linearity

Under the optimum experimental conditions, the calibration graphs were constructed by plotting absorbance measured as a function of OXY concentrations.

The regression equations were computed and found to be:

$$A = 0.004 C + 0.074 \quad r = 0.9996 \quad \text{p-CA method}$$

$$A = 0.009 C + 0.151 \quad r = 0.9997 \quad \text{DDQ method}$$

$$A = 0.025 C + 0.060 \quad r = 0.9998 \quad \text{iodine method}$$

Where, A = absorbance, C = Concentration in  $\mu\text{g/mL}$  and r = Correlation coefficient.

The corresponding concentration ranges, calibration equations, LOD, LOQ and other statistical parameters are listed in Table (1).

**Table (1): Results of Assay Validation Obtained by Applying the Proposed P-CA, DDQ and Iodine Methods for the Determination of OXY in Drug Substance**

Parameters	p-CA	DDQ	Iodine
Linearity ( $\mu\text{g/mL}$ )	20.00 – 220.00	10.00 – 80.00	4.00 – 44.00
LOQ ( $\mu\text{g/mL}$ )	10.75	7.55	1.545
Accuracy <sup>a</sup>			
-Mean	100.07	99.72	100.28
-R.S.D.	0.737	0.968	1.174
Precision <sup>b</sup>			
Repeatability R.S.D.	1.121	0.465	0.666
Intermediate precision R.S.D.	0.925	0.684	1.058
Regression equation			
-Slope	0.004	0.009	0.025
SE of the slope <sup>c</sup>	$5.97 \times 10^{-5}$	$8.93 \times 10^{-5}$	0.000287
Confidence limit of the slope <sup>d</sup>	0.00458-0.004911	0.009200-0.00963	0.02494-0.02564
-Intercept	0.074	0.151	0.060
SE of the intercept <sup>c</sup>	0.00824	0.00451	0.007936
Confidence limit of the intercept <sup>d</sup>	0.0513 – 0.0970	0.1404-0.1622	0.03818-0.082205
SE of estimation	00.99	0.00571	0.009617
-Correlation coefficient (r)	0.9996	0.9997	0.9998

<sup>a</sup> n = 5, <sup>b</sup> n = 3x3, <sup>c</sup> standard error, <sup>d</sup> 95% confidence limit

### 3.5.2. Accuracy

The accuracy of the investigated method was validated by analyzing pure samples of OXY in four different determinations. The concentrations of the active drugs were calculated from the corresponding regression equations. Good results were obtained as shown in (Table 2). The results of determination of OXY in pure powder form obtained from the proposed spectrophotometric method were compared with those obtained from the official method. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F-ratio at 95% confidence level and no significant difference was found (Table 3).

**Table 2: Accuracy of the Proposed *p*-CA, DDQ and Iodine Methods for the Determination of OXY in Pure and Dosage Form.**

<i>p</i> -CA			DDQ			Iodine		
Conc. Taken (µg/mL)	Drug substance	Benox <sup>®</sup> <sup>a</sup>	Conc. Taken (µg/mL)	Drug substance	Benox <sup>®</sup> <sup>a</sup>	Conc. Taken (µg/mL)	Drug substance	Benox <sup>®</sup> <sup>a</sup>
	Recovery % <sup>b</sup>			Recovery % <sup>b</sup>			Recovery % <sup>b</sup>	
20	99.07	98.03	10	98.53	98.60	4	101.74	99.20
80	99.65	98.67	30	99.15	98.70	16	98.51	99.50
120	100.12	98.94	50	100.98	100.30	20	100.13	98.70
160	100.56	100.05	60	100.34	99.11	36	100.78	100.50
220	100.94	99.03	70	99.59	99.19	44	100.23	98.90
Mean	100.07	98.94	Mean	99.72	99.18	Mean	100.28	99.36
R.S.D.	0.737	0.739	R.S.D.	0.968	0.681	R.S.D.	1.174	0.710

<sup>a</sup> Benox eye drop, Batch No.17468.

<sup>b</sup> Average of four different determinations.

**Table 3: Statistical Comparison between Results Obtained by Applying the Proposed *P*-CA, DDQ and Iodine Methods and the Official Method for the Determination of OXY in Pure Powder Form.**

Parameters	<i>p</i> -CA	DDQ	Iodine	Official method <sup>a</sup> (20)
Mean	100.06	99.72	100.28	99.79
SD	0.738	0.966	1.177	0.619
n	5	5	5	5
Variance	0.5439	0.9323	0.498	0.383
SE	0.329	0.301	0.315	0.276
Student's t -Test (2.306) <sup>b</sup>	0.627	1.490	1.024	
F-test (6.39) <sup>b</sup>	1.417	1.187	1.297	

<sup>a</sup> UV Spectrophotometric method (20),

<sup>b</sup> The values between parenthesis are the theoretical values of t and F at ( $p = 0.05$ ).

### 3.5.3. Precision

Precision was evaluated by calculating intra- and inter-day precision after repeating the assay of three different concentrations (40,100,160 µg/mL for p-CA, 20, 40, 60 µg/mL for DDQ and 12, 24, 36 µg/mL for Iodine) three times in the same day and assaying the samples in triplicate on three successive days using the proposed methods. The calculated R.S.D. was listed in Table (1) indicating satisfactory precision of the proposed method.

### 3.5.4. Application of the commercial dosage form

The proposed methods were successfully applied for the determination of OXY in commercial eye drops. The results shown in Table (2) were satisfactory with good agreement with the labeled amount. Moreover, to check the validity of the proposed methods, the standard addition technique was applied. The results obtained (Table 4) suggested that there is no interference from excipients, which are normally present in eye drops.

**Table (4): Application of Standard Addition Technique for the Determination of OXY by the Proposed P-CA and DDQ and Iodine Methods**

Drug product	Proposed methods	% found * of claimed amount ± RSD%	Claimed amount (µg/mL)	Standard added (µg/mL)	% found of standard Added
Benox eye drop (0.4 % OXY)	p-CA	98.94 ± 0.739	20	40	98.02
				60	99.36
				120	99.49
				180	100.36
				Mean ± R.S.D.	99.31 ± 0.973
	DDQ	99.18 ± 0.681	10	10	100.08
				30	98.45
				40	99.57
				60	101.36
				Mean ± R.S.D.	99.87 ± 1.208
	Iodine	99.36 ± 0.710	8	4	99.28
				12	98.16
				20	98.72
				28	98.08
				Mean ± R.S.D.	98.56 ± 0.566

<sup>a</sup> Average of four different determinations.

## 4. Conclusion

The developed method has the advantages of being accurate, sensitive and suitable for routine analysis in control laboratories also it can be applied in quality control laboratories for quantitative determination of OXY in pure form and in pharmaceutical dosage form.

## References

- [1] S. C. Sweetman, Martindale: The complete drug reference”, 36<sup>th</sup> ed., 2008, The pharmaceutical press, London, pp. 1868.
- [2] F.M. Abdel-Gawad and N. M. El Guindi, Spectrophotometric Determination of Metoclopramide and Oxybuprocaine Through Ion Pair Formation with Thiocyanate and Molybdenum(V) or Cobalt(II), Anal. Lett., 1995, 28(8),1437-1447.
- [3] F.M. Abdel-Gawad, Spectrophotometric determination of trace amounts of oxybuprocaine hydrochloride with halofluorescein derivatives, Egypt J. Anal. Chem., 1994, 3(1), 168-172.
- [4] B. S. Al Farhan and H. M. Khalil, J. Saudi Chem. Soc., Sensitive spectrophotometric determination of benoxinate hydrochloride using different aldehydes through condensation reactions, 2011, 15, 89-93.

- [5] A. El Gindy, J. Pharm. Biomed. Anal., First derivative spectrophotometric and LC determination of benoxinate hydrochloride and its degradation products, 2000; 22(2), 215-234.
- [6] R. Wintersteiger, G. Guebitz and A. Hartinger, Fluorimetric determination of local anaesthetics with primary amino-groups in pharmaceuticals and biological material, *Mikrochim Acta.*, 1979, II (3-4), 235-244.
- [7] O. Kuhlmann, G. Stoldt, H. G. Struck and G.J. Krauss, J. Pharm. Biomed. Anal., Simultaneous determination of diclofenac and oxybuprocaine in human aqueous humor with HPLC and electrochemical detection, 1998, 17(8), 1351-1356.
- [8] R. Grouls, E. Ackerman, H. Korsten, L. Hellebrekers and D. Breimer, Partition coefficients (*n*-octanol/water) of *N*-butyl-*p*-aminobenzoate and other local anesthetics measured by reversed-phase high-performance liquid chromatography, *J. Chromatogr. B. Biomed. Appl.*, 1997, 694(2), 421-425.
- [9] T. Arinobu, H. Hattori, A. Ishii, T. Kumazawa, X. Lee, O. Suzuki and H. Seno, Comparison of sonic spray ionization with atmospheric pressure chemical ionization as an interface of liquid chromatography-mass spectrometry for the analysis of some local anesthetics, *Chromatographia*, 2003, 57(5-6), 301-307.
- [10] M. Chorny, D. Levy, I. Schumacher, C. Lichaa, B. Gruzman, O. Livshits and Y. Lomnický, Development and validation of a stability-indicating high performance liquid chromatographic assay for benoxinate, *J. Pharm. Biomed. Anal.*, 2003, 32(1), 189-196.
- [11] F. Kasuya, K. Igarashi and M. Fukui, Oxybuprocaine and five metabolites simultaneously determined in urine by gas chromatography and gas chromatography - mass spectrometry after extraction with Extrelut, *J. Chromatogr. Biomed. Appl.*, 1987, 60(1 (J. Chromatogr., 416)), 189-195.
- [12] M. Banicerua, C. V. Mandaa and S. M. Popescub, Chromatographic analysis of local anesthetics in biological samples, *J. Pharm. Biomed. Anal.*, 2011, 54, 1-12.
- [13] D. Spiegeleer, V. d. Bossche, D. Moerloose and P. Massart, A strategy for two-dimensional, high-performance thin-layer chromatography, applied to local anesthetics, *Chromatographia*, 1987, 23(6), 407-411.
- [14] F. Kasuya, K. Igarashi and M. Fukui, Oxybuprocaine and five metabolites simultaneously determined in urine by gas chromatography and gas chromatography-mass spectrometry after extraction with Extrelut, *Clin. Chem.*, 1987, 33(5), 697-700.
- [15] H. Seno, O. Suzuki, T. Kumazawa and H. Hattori, Positive and negative ion mass spectrometry and rapid isolation with Sep-Pak C18 cartridges of ten local anaesthetics, *Forensic. Science Int.*, 1991, 50, 239 - 253.
- [16] A. K. Attia, Electrochemical Determination of Anaesthetic Drug Benoxinate Hydrochloride, *Sensing in Electroanalysis*, 2010, 5, 209-219.
- [17] A. F. Shoukry, Y. M. Issa, R. El-Shiekh and M. Zareh, New Ion-Selective Electrodes for Determination of Bupivacaine and Oxybuprocaine, *Anal. Lett.*, 1991, 24(9), 1581-1590.
- [18] E. S. Elzanfaly and M. Nebsen, Ion Selective Membrane Electrodes for Stability Indicating Determination of Benoxinate Hydrochloride in Pure form and in Drug Product, *Anal. Bioanal. Electrochem.*, 2013, 5(2), 166 – 177.
- [19] J. Zheng, Z. Piao, Y. Hou and J. Yang, Determination of the purity of oxybuprocaine by DSC, *Zhongguo Yiyao Gongye Zazhi*. 1989, 20(12), 543-545.
- [20] The British pharmacopeia, (2012), The stationary office, London.
- [21] The United States Pharmacopeia, The National Formulary, 2012, USP 35 – NF 30, Twinbrook Parkway, Rockville.
- [22] H. Salem, Analytical Study for the Charge-Transfer Complexes of Pregabalin, *E-Journal of Chemistry*, 2009, 6(2), 332-340.
- [23] H. Wu and L. M. Du, Spectrophotometric determination of anilines based on charge-transfer reaction, *Spectrochim. Acta (A)*, 2007, 67(3-4), 976-979.

- [24] N. Rahman and S. M. Haque, Optimized and validated spectrophotometric methods for the determination of enalapril maleate in commercial dosage forms, *ACI*, 2008, 1(3), 31– 43.
- [25] J. Rose, *Advanced Physico Chemical Experiments*, 1964, Pitman, London, UK, pp. 54.
- [26] ICH, *Stability Testing of New Drug Substances and Products Q1A (R2)*, International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2003.