SPECTROPHOTOMETRIC DETERMINATION OF SOME LOCAL-ANAESTHETICS BY USING PICRYL-CHLORIDE AND CHLORANILIC ACID

S. A. Hussein

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut-71526, Egypt

أستحدثت فى هذا البحث طريقتين طيفيتين لتعيين عشرة من المخدرات الموضعية. تعتمد الطريقة الأولى على قياس ناتج التفاعل بين كلوريد البكريل ومجموعة الأمين فى المخدر الموضعى فى وجود الأسيتون وإستعمال كربونات البوتاسيوم اللامائية كمستقبل للهيدروكلوريد. يتم التفاعل فى دقيقتين على حمام مائى عند درجة حرارة ٦٠ درجة منوية. وقد وجد أن ناتج التفاعل يعطى ذروتى امتصاص واحدة عند (٣٧٠-٣٧٥ ن.م) والثانية عند (٤١٠-٤٥٠ ن.م) تختلف بإختلاف تركيب العقار. أما الطريقة الثانية فتعتمد على قياس ناتج التفاعل بين العقار وحامض الباراكلورانيليك. يعطى ناتج التفاعل ذروة أمتصاص واحدة عند ٥٢٥ ن.م. وتتراوح قوة التركيز بين ٢,٥-٢٠ ميكروجـرام لكل مىليلتر فى الطريقة الأولى من ١٠-١٠٠ ميكروجرام لكل مىليلتر للطريقة الثانية. وقد أجريت دراسات عديدة على جميع العوامل المؤثرة على التفاعل. وقد طبقت الطرق المقترحة بنجاح لتعيين العقارات فى بعض المستحضرات الصيدلية بدقة وقورنت بالطرق الدستورية والمنشورة.

Two simple and sensitive spectrophotometric methods are described for the determination of ten local anaesthetics. The first one was based on measuring of coloured products resulting from using picryl chloride as a derivatization reagent for the quantification of the drugs. The cited reagent reacts with the amino group of local anaesthetic in acetone using anhydrous potassium carbonate as hydrochloride acceptor. The reaction was completed within 2 minutes on hot water-bath at 60°. The coloured products are quantified spectrophotometrically and exhibit two main absorption bands (370-375 nm) and (410-450 nm). The position of λ_{max} differ according to the structure of the drug. The second method was based on the interaction of the drug and p-chloranilic acid to yield stable and intensively coloured ion-pair salt. The reddish violet chromogen formed was measured at 525 nm. Beer's law was obeyed in range form 2.5-20 µg/ml for the first method and from 10-100 µg/ml for the second one. The optimization of the different experimental conditions is described. Applications of the suggested methods to representative pharmaceutical dosage forms are presented and compared with official methods with good accuracy and precision. The recoveries ranged from 98±1 to 100±0.6. Interferences from the commonly present additives or agents in pharmaceutical formulations were investigated. The proposed methods were found rapid and selective. The selectivity and specificity of picryl-chloride method is exhibited by the different position of maximum wavelength and can differentiate between the investigated drugs.

INTRODUCTION

Lidocaine, benzocaine, procaine hydrochloride, butacaine sulphate, dibucaine hydrochloride, bupivacine hydrochloride, propoxycaine hydrochloride, tetracaine hydrochloride, metacaine hydrochloride and amylocaine hydrochloride are widely used as local anaesthetics (Table 1). They penetrate tissues in the form of uncharged molecules and exert their action intracellulary as cations. This accounts for the fact that nearly all local anaesthetics are amines, with pKa values falling in a fairly narrow range.[1]

Bull. Pharm. Sci., Assiut University, Vol. 23, Part 1, June 2000, pp. 1-9.

Samiha A. Hussein

Several methods have been reported for the determination of studied drugs. These methods include titrimetric,[2-4] spectrophotometric,[5-12] fluorimetric,[13,14] polarographic,[15] and chromatographic determinations.[16-19] The official methods for these drugs and their dosage forms include nitrometric, UV, spectrophotometric and HPLC methods.[1-4]

Most of spectrophotometric methods published for the analysis of local anaesthetic cannot distinguish between the members of the studied drugs.[6,7] Some methods depend on the extraction with organic solvents, these methods are tedious and lengthy in their applications.[8,11] Others require several steps of calculation by using Orthoganal polynomials.[9]

This work presents the application of picryl chloride and p-chloranilic acid for the estimation of local anaesthetics.

In this work, simple, sensitive and selective methods were successfully applied for the determination of ten different local anaesthetics, either in pure or their dosage forms with good accuracy and precision. The selectivity and specificity of first method is exhibited by the different position of maximum wavelength and can differentiate between the drugs. We can analyze mixture of local anesthetic in the same dosage form.

EXPERIMENTAL

Apparatus

A Uvidec-320 spectrophotometer (Jasco, Tokyo, Japan) and a Perkin Elmer Lambda 3B UV/VIS (USA) spectrophotometer were used.

Materials and reagents

All solvents used were of analytical grade. Pharmaceutically pure procaine hydrochloride (E. Merck, Germany), tetracaine hydrochloride (Orgamol S.A., Swiss), Lidocaine (Medichein S.A., Swiss), Bupivacaine hydrochloride (Ciba Co., Swiss), benzocaine, dibucaine hydrochloride, Butacaine sulphate, propoxycain hydrochloride, amethocaine hydrochloride and amylocaine hydrochloride were donated by Merck (Damstadt, FRG), Lederle (Wagne, NJ,

USA) and were used as wokring standards.

Picryl chloride (PCC), Aldrich Chemical, Milwaukee, 0.1 mg ml^{-1} in acetone, the solution was found to be stable for one week at 4°.

Chloranilic acid (P-CA), BDH Chemicals, Poole, UK, 4 mg ml^{-1} in acetonitrile were prepared fresh daily.

All chemicals and solvents used were of analytical grade.

Pharmaceutical formulations

The following commercial dosage forms were subjected to the analytical procedures:

1- Xylocaine 2% injection, contains lignocaine hydrochloride 20 mg/ml (ASTRA, SÖdertälje, Sweden).

2- Lignocaine 5% cream (lidocaine hydrochloride 5%) (Nile Co. for pharmaceuticals and chemical industries, Egypt).

3- Marcaine 0.5% injection contains bupivacaine hydrochloride 5 mg/ml (ASTRA SÖdertälje, Sweden).

4- Neocaine cream 1% dibucaine hydrochloride (Alex. Co., Egypt).

5- Butacaine sulphate solution contains butacaine sulphate 200 mg/ml.

Procedures

Preparation of standard stock solutions

Dissolve 25 mg of each drug in about 10 ml of distilled water. Transfer to a separating funnel, add 0.5 ml of 33% ammonia solution. Extract the free base by shaking with three -10 ml portion of chloroform. Pass the combined organic layer through 5 g anhydrous sodium sulphate suitably supported in small funnel. Dilute the combined extracts quantitatively with chloroform to obtain the suitable final concentration listed in Table 2.

General procedure

Method A: using picryl-chloride solution (PCC). Transfer a volume of standard solutions containing 10-200 µg drug to 10-ml calibrated flasks. Add one ml of picryl chloride followed by 5 mg of anhydrous K$_2$CO$_3$ and 2-ml acetone, mix well. Heat the flask in hot water-bath for

two minutes, cool, dilute to volume with acetonitrile. Measure the absorbance of the solution at the specified maximum against a blank treated similarly.

Method B: Using chloranilic acid solution (P-CA). Place a volume of standard solution containing 50-1000 μg drug to 10-ml calibrated flask. Add 1 ml of chloranilic acid solution and the volume brought to 10 ml with acetonitrile. Measure the absorbance at 525 nm against a blank prepared simultanously.

Analysis of injection and solution

Measure a volume of injection or solution equivalent to 100 mg of the drug, dilute with about 20 ml distilled water into a separating funnel. Add 2-ml of 33% ammonia solution and extract the base quantitatively with three successive portions of 10-ml chlorform, filter over about 5 g anhydrous Na_2SO_4. Dilute the combined extracts with chloroform. Dilute the final solution to obtain the appropriate concentration.

Anaylsis of ointiment and creams

Transfer an accurately weighed amount of each preparation equivalent to about 100 mg of the drug into a separatory funnel. Add chloroform and 2 ml of 33% ammonia solution and 20-ml of distilled water. Complete as under injection.

RESULTS AND DISCUSSION

Preliminary investigations were carried out to test the derivatization reaction between the cited drugs and picryl chloride. Acetone was found to be an ideal solvent for the formation of the derivative. Dichloromethane, dichloroethane, chloroform and carbon tetrachloride produced lower absorbances. Acetonitrile decomposes on heating. So the reaction was more preferable to proceed in minimal amount of acetone (2 ml) in the presence of anhydrous K_2CO_3. Highly coloured yellow derivative was obtained which absorbed at two main wave-length bands (370-375 nm) and (410-450 nm). Wavelength above 400 nm was chosen as the nitro compounds absorb very intensely at wavelength below

400 nm and hence cannot be compensated for. Therefore an optimum selected wavelength above 400 nm were chosen at which the nitro compounds did not absorb at all. With picryl chloride a yellow colour (λ_{max} 410-450) was formed attributed to a nucleophilic substitution product.[20] This specificity of interaction permitted selective assay of different local anaesthetic drugs. The position of λ_{max} differ according to amine moiety and this also can differentiate between the drug which contain 1^{ry} amine group and the others (Table 1). Figure 1 shows the absorption spectra of lidocaine and procaine with picryl chloride reagent as a representative example. Spectral characteristics of studied compounds are summarized in Table 2.
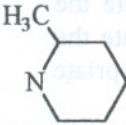
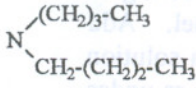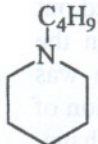## Factors affecting derivatization

The derivatization reaction conditions including K_2CO_3, picryl chloride concentration, dilution solvents, time of heating and stability of colour formed were investigated and optimized using procaine as a representative example.

The role of anhydrous K_2CO_3 is very important. It was found that anhydrous K_2CO_3 enhance the derivative formation in quantitative yield as hydrochloride acceptor. Potassium carbonate must be completely anhydrous to avoid formation of OH ion which cause hydrolysis of the reagent. The presence of traces of moisture in the solvents or reagents yield a mixture of different spectral absorbing compounds. The spectrum may be further complicated if picric acid is formed, because of the picrate anion which absorbs at 360 nm which adds up to fluctuation observed in the peak positions and intensities.[21,22] Use of 5-10 mg anhydrous K_2CO_3 was recommended from the results of this study. When various concentrations of picryl chloride were added to a fixed concentration of procaine, 1 ml of 0.1 mg/ml solution of picryl chloride was found to be sufficient for the production of maximum and reproducible colour intensity (Table 3).

The molar concentration of picryl chloride (1 ml, 0.1 mg/ml) must be five times more concentrated as procaine, which also sufficient for derivatization of the other drugs studied.

3

Table 1: Structure of different local anaesthetic drugs and peak position of the coloured product of these drugs with picryl-chloride and pKa values.

Agent	Aromatic-lipophylic	Amine hydrophilic	λ_{max}	pKa
Ester-linked				
1) Benzocaine	NH$_2$		420	2.8
2) Procaine hydrochloride	NH$_2$	N(Et)(Et)	435	9
3) Tetracaine hydrochloride	HN.C$_4$H$_8$	N(Me)(Me)	410	8.5
4) Propoxycaine hydrochloride	NH$_2$	N(Et)(Et)	445	8.6
5) Metacaine hydrochloride	H$_3$C (ring structure, N)		410	
6) Amylocaine hydrochloride		N(Me)(Me)	420	
7) Butacaine sulphate	NH$_2$	N, (CH$_2$)$_3$-CH$_3$, CH$_2$-(CH$_2$)$_2$-CH$_3$	445	9
Amide linked				
1) Lidocaine		N(Et)(Et)	410	7.9
2) Bupivacaine hydrochloride		C$_4$H$_9$ (N, piperidine)	430	8.1
3) Dibucaine hydrochloride		N(Et)(Et)	425	7.5

Table 2: Quantitative parameters for the reaction of different local-anaesthetics with picryl chloride and chloranilic acid.

Drug	Picryl chloride (Method A)					Chloranilic acid (Method B)				
	Linearity range μg/ml	εx10^3 mol^{-1}cm^{-1}	Intercept (a)	Slope* (b)	Correlation coefficient (r)	Linearity range μg/ml	εx10^3 mol^{-1}cm^{-1}	Intercept (a)	Slope* (b)	Correlation coefficient (r)
Benzocaine	2.5-20	6.14	0.0314	0.036	0.9941	-	-	-	-	-
Procaine hydrochloride	2.5-20	8.22	0.101678	0.0327	0.992145	10-80	2.88	-0.0247	0.0113	0.9960
Lidocaine	2.5-20	8.78	0.0251	0.035	0.9901	30-100	0.117	-0.045	0.005	0.9939
Bupivacaine HCl	2.5-20	10.98	-0.0032	0.0384	0.9995	10-80	2.989	-0.0082	0.0112	0.9979
Tetracaine hydrochloride	7.5-30	3.96	-0.003	0.0153	0.9996	30-100	0.1584	-0.039	0.0045	0.9900
Dibucaine HCl	2.5-20	15.24	0.0019	0.0442	0.9999	10-80	3.735	-0.0021	0.0111	0.9960
Butacaine sulphate	7.5-30	8.65	-0.0606	0.0343	0.9923	10-80	4.437	0.015	0.0130	0.9958
Metacaine hydrochloride	5-25	7.31	-0.0406	0.0321	0.9924	10-80	1.631	0.0615	0.0124	0.9970
Amylocaine hydrochloride	2.5-20	11.14	-0.0076	0.019	0.9973	10-80	3.466	-0.0121	0.0140	0.9881
Propoxycaine hydrochloride	2.5-20	12.48	-0.025	0.0449	0.9967	10-80	3.704	0.0160	0.0110	0.9968

* Calculated for 8 determinations for each compound.

Table 3: Effect of concentration of reagents on the intensity of coloured products.

Milliliters of 0.01% picryl chloride	Absorbance at λ_{max} 435 nm[*]	Milliliters of 0.4% chloranilic acid	Absorbance at λ_{max} 525 nm[+]
0.25	0.297	0.25	0.293
0.50	0.389	0.50	0.391
0.75	0.458	0.75	0.416
1.00	0.461	1.00	0.417
1.25	0.451	1.25	0.400
1.50	0.310	1.50	0.397
1.75	0.290	1.75	0.288
2.00	0.200	2.00	0.217

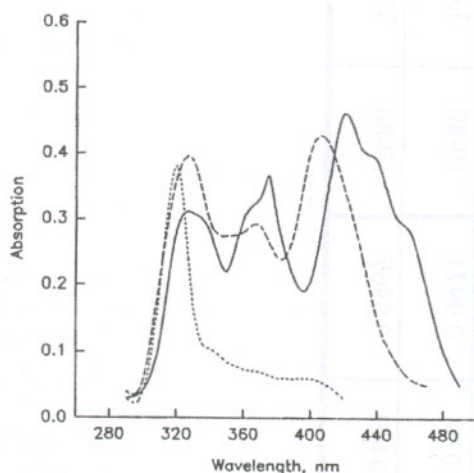[*] Conc. of procaine 10 μg/ml   [+] Conc. of procaine 40 μg/ml

Fig. 1: Absorption spectra of the coloured product of picryl—chloride with procaine (10 μg/ml) (——) lidocaine (10 μg/ml) (----) and reagent blank (·······).

The derivatization reaction was more preferable to proceed in a minimal amount of dry acetone than dimethylformamide or chloroform. However it was better to dissolve the final reaction mixture in acetonitrile. On other hand dimethylformamide and acetone are approximately useful and give maximum intensity but not stability of colour. Chloroform, methylene chloride, dichloroethane produced lower absorbance readings. Polar solvents were found to be unsuitable as their blanks with picryl-chloride give high absorbances[21,22] (Table 4). The optimum reaction time on hot water-bath

was determined by following the colour development for different time. The reaction was achieved after 2 min. for most of studied drugs. The colour remains stable for at least 20 min and then a gradual decrease occurs (Fig. 2).

Mechanism of reaction

Amine replaces halide attached to aromatic ring but only in fairly drastic conditions. Nucleophilic displacement is greatly enhanced, however, by the presence of nitro groups on the aromatic ring and such reaction have been extensively studied.[20] The formation of tetrahedral intermediate is reversible and its decomposition to products occur either spontaneously or in a base.[20] So the reaction of local anaesthetic contain terminal 1^{ry} amine moiety or 2^{ry} amine group is carried according to the mentioned equation:[20]

The reaction also happens in case if the drug contains only 3^{ry} amine moiety (e.g. bupvicaine, Table 1).

The λ_{max} was found to be dependent on the nitro acceptor (picryl chloride) and independent of the amine. The small difference in λ_{maxs} (Table 1) are due to different structures between these drugs. The evidence of the reaction of picryl chloride with terminal triethylamine moiety only is elucidated by carrying the experiment with triethylamine base.

Table 4: Effect of different solvents on the absorption intensity of the coloured products of procaine with picryl-chloride.

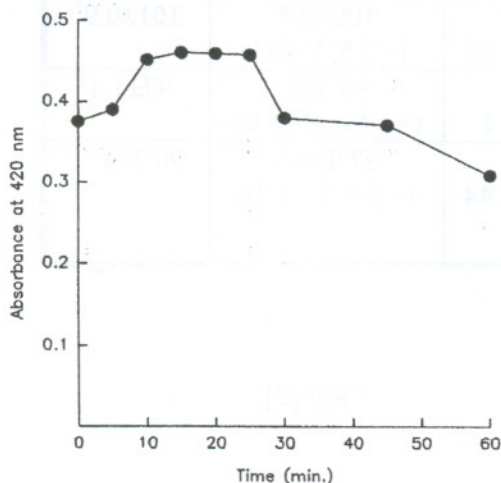|	Picryl chloride method[*]
Acetone	0.480
Chloroform	0.291
Dimethylformamide	0.390
Acetonitrile	0.461
Dichloroethane	0.290
Dichloromethane	0.281

[*] Conc. of procaine 10 μg/ml

Fig. 2: Effect of development time on the intensity of the coloured product of picryl chloride with procaine 10 μg/ml.
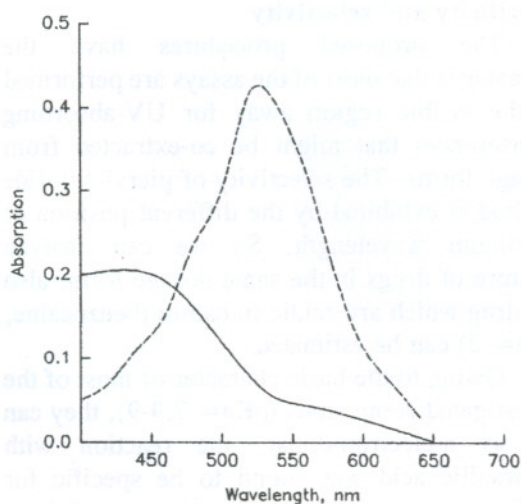

Fig. 3: Absorption spectra of procaine–PCA reaction product (- - - -) measured against blank and reagent blank (——) against acetonitrile.
- - - - conc. of procaine 10 μg/ml

Chloranilic acid method

The absorption spectra of the reaction products of p-chloranilic acid (P-CA) in acetonitrile with different local anaesthetic derivatives were studied. The formation of purple coloured chromogen exhibit a broad absorption maxima in the vicinity 520-530 nm but with different intensities (Fig. 3). The colour reaction products of P-CA with local anaesthetics has been studied to determine optimal reaction conditions. It has been found that P-CA gives immediate colour with the drug (base) at room temperature. For quantitative purposes a volume of 1 ml (0.4%) w/v P-CA in acetonitrile for a final volume of 10 ml was found to be optimal concentration that gives maximum colour formation (Table 3). The colour was highly stable for at least 24 hours. Since the reaction product is purple so, we may consider that HA⁻ is the form the P-CA involved in the reation with the chosen local anaesthetics. This may lead to an assumption that by the interaction of P-CA with the local-anaesthetic base, a proton transfer from P-CA to the basic center of the drug take place (scheme 1). The obtained ion-pair salt is dissociated to the purple HA⁻ anion.[23] This dissociation is promoted by the high dielectric constant of solvent. Acetonitrile was considered as an ideal solvent and a good solvent for P-CA.

$$H_2A \rightleftharpoons H^+ + HA^- \text{ (purple)}$$
$$B + H^+ \rightleftharpoons BH^+ \text{ (colourless)}$$
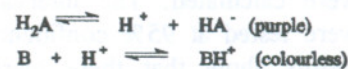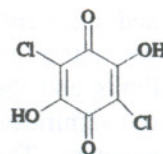
Scheme 1

Table 5: Determination of some local-anaesthetic drugs in commercial dosage forms using the proposed method and some reported methods.[a]

Formulation	Composition	Claim-ed	Recovery% ± S.D. (n= 5)		
			Picryl chloride	Chloranilic acid	Official methods
Xylocaine injection	Licocaine hydrochloride	2%	99.2±2 t= 2.2[b], F= 1	100±1 t= 0.2, F= 4	100.3±2[o]
Lignocaine cream	Lidocaine hydrochloride	2%	98±1.4 t= 2.7, F= 1.16	99.4±1 t= 0.8, F= 1.69	100±1.3[c]
Marcaine injection	Bupivacaine hydrochloride	0.5%	100.1±0.7 t= 2.08, F= 1.29	100±0.4 t= 2.5, F= 3.9	101±0.9[+]
Neocaine cream	Dibucaine hydrochloride	1.0%	98.1±0.7 t= 1.57, F= 4	98.3±1 t= 1.69, F= 1.96	97±1.4[c]
Butacaine sulphate solution	Butacaine sulphae	100 mg/ml	98±1 t= 1.86, F= 1.44	97.4±1.5 t= 0.8, F= 1.56	96.7±1.2[d]

[a] Average ± standard deviation of five determinations.
[b] Theoretical values   t= 2.306, F= 6.39
[o] USP XXI   [+] B.P. 1993   [c] Ref [6]   [d] Ref [7].

Quantification

A fixed experimental conditions, the intensity of absorption at the specified wavelength was found a function of the concentration of the investigated drugs. In all cases studied, Beer's low plots were linear with very small intercept in the general concentration range 2.5-20 $\mu g\ ml^{-1}$ for the method A and 20-100 $\mu g\ ml^{-1}$ for the method B (Table 2). Under the specified reaction conditions, the molar absorptivity at the λ_{max} was found to be a function of concentration of the investigated drugs. In all cases studied, Beer's law plots (n= 8) were linear with very small intercepts, slopes ranged from 0.01 to 0.04 in the concentration ranges given in Table 2. The correlation coefficient ranged from 0.9881 to 0.9999. The confidence intervals for slopes and intercepts were calculated. The intervals for intercepts were tested at 95% confidence limits and the results show that there were no significant difference between zero and intercepts. The mean of five replicate analyses of solution of the studied compounds at the concentration limits listed in Table 5. The level of precision of the method is adequate for the quality control analysis of the studied drugs.

Specificity and selectivity

The proposed procedures have the advantages that most of the assays are performed in the visible region away for UV-absorbing interferences that might be co-extracted from dosage forms. The selectivity of picryl-chloride method is exhibited by the different position of maximum wavelength. So we can analyze mixture of drugs in the same dosage form, also the drug which are acidic in nature (benzocaine, pKa= 2) can be estimated.

Owing to the basic character of most of the investigated compounds (pKa= 7.9-9), they can act as n-electron-donar, the reaction with chloranilic acid was found to be specific for basic drugs, so we can estimate some of these drugs in the presence of benzocaine (pKa= 2).

Analysis of pharmaceutical formulations

The proposed and official methods were applied to the determination of ampoules, vials, ointment and cream. The obtained mean value ($\pm$S.D.) of the labelled amounts ranged from $0.90\pm$ to $1.9\pm$ (Table 5). In "t-test" = 2.306 and "F-test" = 6.39 no significant differences were found between the calculated and theoretical values (95% confidence) of both the proposed and official methods. This indicates similar precision and accuracy.

Conclusion

The proposed spectrophotometric methods are simpler than Pharmacopeial method, time saving and selective. Picryl-chloride exhibiting the highest ϵ values wtih all studied compounds could be considered the most suitable one for further work.

REFERENCES

1- S. Wiedling and C. Tegnir in "Progress in Medicinal Chemistry", Vol. 3, Edited by G.P. Ellis and G.B. West, p. 332 (1963).

2- British Pharmacopeia, HMSO, London, pp. 74, 91, 382, 544 (1993).

3- The United States Pharmacopeia XXII, National Formulary XVII United States Pharmacopeal Convention, Rockville, MD, pp. 147, 196, 1147, 766 (1990).

4- The United States Pharmacopeia XX, National Formulary XV, US Pharmacopeial Convention, Inc., MD, pp. 72, 103, 660, 776 (1980).

5- J. Novakovic, Boll. Chim. Farm., 128 (12), 370 (1989).

6- G. A. Saleh and H. F. Askal, Analytical Letters, 28 (15), 2663 (1995).

7- A. I. Mohamed, H. Y. Hassan, H. A. Mohamed and S. A. Hussein, Journal of Pharmaceutical and Biomedical Analysis, 9 (7), 525 (1991).

8- L. Zivanovic, S. Agtonovic, M. Vasiljeric, L. Markovic and I. Nemcova, Pharmazie, 49 (12), 927 (1994).

9- A. M. Wahbi, S. Belal, M. Bedair and H. J. Abdine, Assoc. Off. Anal. Chem., 64, 1179 (1981).

10- M. A. Korany, A. M. Wahbi and I. I. Hewala, Arch. Pharm. Chem. Sci., Ed. 12, 26 (1984).

11- F. A. Mohamed, A. I. Mohamed, H. A. Mohamed and S. A. Hussein, Talanta, 43, 1931 (1996).

12- K. A. Kovar, W. Mayer and H. Auterhoff, Arch. Pharm., 314, 447 (1981).

13- J. T. Stewart and R. E. Wilkin, J. Pharm. Sci., 61, 432 (1972).

14- L. J. Dombrowski and E. L. Pratt, Anal. Chem., 43, 1042 (1971).

15- H. A. Dingijan, W. P. Bennekom and A. Bult, Pharm. Weekbl. Sci., 9 (4), 198 (1987).

16- Z. H. Mo, J. Luo and M. L. Li, Analyst, 122 (2), 111 (1997).

17- S. Emara, A. Khedr and H. Askal, Biomedical Chromatography, 10, 131 (1996).

18- V. D. Gupta, J. Pharm. Sci., 72, 205 (1983).

19- P. K. Narang, G. Bird and W. G. Gronthamel, J. Pharm. Sci., 69, 1384 (1980).

20- S. Patai, "The chemistry of the amino group", Interscience Publishers, London, New York, Sydney, Chapter 6 (1968).

21- T. Abe, Nature, 187, 234 (1960).

22- V. Gold and C. H. Rochester, J. Chem. Soc., 1722 (1964).

23- M. A. El-Sayed, M. E. Abdel Hamid, M. A. Korany, M. H. Abdel-Hady and S. M. Galal, Spectroscopy Letters, 17 (12), 803 (1984).