April 2007 Volume 5 Issue 1-6

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 5(1-6), 2007 [1-7]

Construction Of Plastic Membrane Electrode And Its Applications For The Analysis Of Triamterene In Pharmaceutical Preparations And Biological Fluids

Corresponding Author

Abdalla Shalaby
Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, (EGYPT)

Co-Authors

Maha El-Tohamy, Magda El-Maamly
Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, (EGYPT)

Received: 31st December, 2006
Accepted: 15th January, 2007

Web Publication Date : 15th April, 2007

ABSTRACT

A PVC membrane electrode for triamterene hydrochloride and flavianic acid association complex was constructed. The basic electrode's performance characteristics were evaluated. The prepared electrode exhibits a nernstian response(19.6±0.2 mV per decade) over the concentration range of triamterene hydrochloride in solutions of pH 4-7. Common organic and inorganic cations showed negligible interference. Direct potentiometric determination of 1×10^{-1}-5×10^{-6}M aqueous triamterene hydrochloride using this membrane electrode system showed an average recovery of 99.91% with a mean standard deviation of ±0.390. The electrode gave a good stability reproducibility and fast response. These characteristics of the electrode enable it to be used successfully for the determination of triamterene hydrochloride in pure form, pharmaceutical preparations and biological fluids.

KEYWORDS

Ion-selective electrode;
Triamterene hydrochloride
Potentiometry.

INTRODUCTION

Triamterene(6-phenyl pteridine-2,4,7-triamine), (Figure 1) potassium sparing diuretic which is widely used. It is a natriuretic agent that is used for the treatment of various diseases such as oedema associated with congestive heart failure, liver cirrhosis, nephritic syndrome, idiopathic and drug-induced oedema[1].

It can also be applied as doping substance. In recent years, diuretics have been abused in sports mainly for two reasons to obtain a rapid diminution of corporal weight, important in sports which are divided in different weight categories, and to reduce concentration of medical drugs in urine by dilution by means of a rapid production of an elevated quantity of urine, thus trying to diminish the possibility

Full Paper

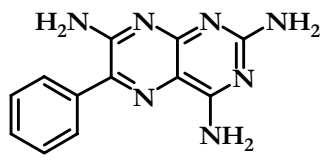

Figure 1: Chemical structure of triamterene

of detecting other doping substances[2]. Methods available for the determination of triamterene can be classified as follows: spectrophotometry[3-5], thin layer chromatography[6], liquid chromatography[7,8], liquid chromatography-mass spectrometry[9,10], high performance liquid chromatography[11-14], electrophoresis[15], potentiometric method[16] and fluorimetric methods[17-19].

EXPERIMENTAL

Materials and reagents

All chemicals used were of analytical or pharmacopeial grade. Doubly distilled water was used throughout the experiments. Triamterene hydrochloride (El-nasr Co. for pharmaceutical and chemical industries,(Cairo-Egypt). Poly(vinyl chloride)(PVC) (Aldrich, Germany), di-butyl sebacate(Fluka, Switzerland), flavianic acid(Memphis Delagrange, France) and tetrahydrofuran(THF)(Sigma, Austria).

Standard drug solution

Stock solution of triamterene hydrochloride, 1×10^{-2}M, was prepared with deionized water. Different standard solution(1×10^{-3}-1×10^{-6}M) were prepared by serial dilution of the stock solution. All solutions were stored in dark at 4^0C in airtight containers.

Apparatus

Jenway 3010 pH/mV meter(U.K.) with a triamterene-PVC membrane electrode in conjunction with double junction Ag/AgCl electrode(Orion 90-02)(Taiwan, R.O.C.) containing 10% w/v potassium nitrate in outer compartment. An orion 91-02 glass-calomel combination electrode, (Taiwan, R.O.C.) was used for pH adjustment. All potentiometric measurements were carried out at 25 ± 1^0C with constant magnetic stirring.

Recommended procedures

1. Preparation of triamterene-flavianate ion-pair

The ion-pair was prepared by mixing 50ml aliquots of 1×10^{-2} M triamterene hydrochloride and flavianic acid. The resulting precipitate was filtered through G_4 sintered glass crucible and washed thoroughly with deionized water then dried at room temperature for 24 hours.

2. Membrane composition

The membrane was prepared by dissolving 190 mg of powdered PVC, 0.35ml of the plasticized(di-butyl sebacate) and 10mg of the ion-pair in 5ml tetrahydrofuran(THF). The solution was poured into a petri dish(3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature.

3. Electrode construction

A punched circular membrane was attached to a polyethylene tube(8 mm in diameter) in an electrode configuration by means of PVC-THF solution. A mixture containing equal volumes of 1×10^{-3} M triamterene hydrochloride and potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The constructed electrode was preconditioned after preparation by soaking for at least 24 hours in 1×10^{-3} M drug solution and stored in the same solution. The electrochemical system is represented as follow: Ag/AgCl/inner solution/membrane/test solution/KCl salt bridge//SCE.

4. Electrode calibration

10ml aliquots of 1×10^{-1}-1×10^{-6} M standard triamterene hydrochloride solution were transferred into 50ml beaker and the sensor in conjunction with Ag/AgCl reference electrode were immersed in the solution. The measured potential was plotted against the logarithm of drug concentration. The electrode was washed with deionized water and dried with tissue paper between measurements.

5. Effect of pH

The effect of pH on the potential of the electrode was measured using two pH/mV meters. The combined glass calomel electrode was connected to one instrument and the PVC triamterene HCl membrane with the double junction Ag/AgCl reference electrode was connected to the second instrument. Thirty ml aliquots of 1×10^{-6}M, 1×10^{-5}M, 1×10^{-4}M

and 1×10^{-3}M drug solutions were transferred to a 100 ml beaker where the three electrodes were immersed, the potential readings corresponding to different pH values were recorded. The pH was gradually increased or decreased by the addition of small aliquots of dilute solutions of(0.1 or 1.0M) sodium hydroxide or 0.1N hydrochloric acid respectively and the pH-mV was measured and plotted.

6. Selectivity of the electrode

Selectivity coefficients were determined by the separate solution method[20] in which the following equation was applied.

$$\mathrm{Log}\,K^{pot}_{TA,J^{z+}} = (E_2 - E_1)/S + \log[TA] - \log[J^{z+}]^{1/z}$$

Where, E_1 is the electrode potential in 1×10^{-3}M triamterene HCl solution. E_2 is the potential of the electrode in 1×10^{-3}M solution of the interferent ion J^{z+} and S is the slope of the calibration plot. The selectivity of the electrode towards sugars, amino acids, certain cations and alkaloids was studied.

Analytical applications

1. Determination of triamterene in dosage forms

1.1. Tablets and capsules

Ten tablets were finely powdered shaken with 100 ml methanol and diluted with distilled water to obtain different concentrations in the range of 1×10^{-3} M-1×10^{-6}M. The prepared solutions were adjusted to pH 4 using 0.1N dilute hydrochloric acid. The PVC-triamterene-flavianate membrane electrode was immersed in the solution. The electrode system was allowed to equilibrate with stirring and the e.m.f. recorded and compared with the calibration graph. The standard addition(spiking technique) was also applied by recording the electrode potential after the addition of 0.1 ml of standard 1×10^{-2} M triamterene HCl solution to the above drug test solutions.

1.2. Content uniformity assay of triamterene tablets

Ten individual tablets were placed in separate 100ml beaker and dissolved in 90-100ml of distilled water. Concentration of the solutions was determined by addition method as described above.

1.3. Dissolution test

The test was carried out according to the USP XXV method[21], with the use of standard equipment for this purpose(pharmatest dissolution apparatus, Germany). One tablet was placed in the basket, and the dissolution medium(0.1N hydrochloric acid, 900 ml) was maintained at 37 ± 0.5^0C. The basket was rotated at 100 rpm. For the potentiometric determination, after an appropriate time interval(0.5-5min), the potential values were recorded, and the amount of the triamterene was calculated from the calibration graph.

2. Application to biological fluids

2.1. Determination of triamterene in serum and urine

Adjust urine pH 5(using 0.1N hydrochloric acid) and pH of serum to 6(using phosphate buffer). Add 0.1N hydrochloric acid to urine and phosphate buffer to serum dropwise until the suitable pH obtained. Transfer 5 ml previously adjusted urine or serum into small separatory funnels, and then separately add 5 ml, 10^{-3}, 10^{-4} and 10^{-5}M standard drug solution, followed by the addition of 20ml toluene for urine or 20 ml diethyl ether for serum. Shake each funnel for 5 min, and transfer aqueous layer to centrifuge tube. Centrifuge for 2 min at 1500 rpm, transfer each solution to a 50ml volumetric flask, and dilute to volume with deionized water. Apply above procedure as described under electrode calibration[22].

RESULTS AND DISCUSSION

1. Nature and response characteristics of the electrode

Triamterene reacts with flavianic acid to form a stable triamterene-flavianate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as an active material with dibutyl sebacate as a solvent mediator in a poly(vinyl chloride) membrane response for triamterene HCl. The critical response characteristics of triamterene-flavianate PVC membrane electrode were determined and results are summarized in(TABLE 1).

The electrode exhibits a Nernstain response over the concentration range from 1×10^{-3}-1×10^{-6}M triamterene HCl with a cationic slope of 19.6±0.2 mV/

Full Paper

TABLE 1: Critical response characteristics of triamterene-flavianate-PVC membrane electrode

Parameter	Value
* Slope/mV per decade	19.59 ± 0.2
* Intercept/mV	317.34
* Correlation coefficient, r	0.9985
* Linear range/M	1×10^{-6}-1×10^{-3}
* Working pH range	4-7
* Response time for 10^{-3}M triamterene/sec	10
* Life time/day	45

decade change in concentration (Figure 2). The choice of membrane solvent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and its ability to dissolve ion-pair complex. The response time of the electrode was tested for 1×10^{-3}-1×10^{-6}M triamterene HCl solutions. The sequence of measurements was from low to high concentrations. This electrode exhibits a fast dynamic response of about 10 seconds. The electrode used for a period of 45 days without significant change in the electrode parameters.

2. Effect of pH

The effect of pH of the triamterene HCl solutions(10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M triamterene HCl) on the electrode potential was investigated. The solutions were acidified by the addition of very small volumes of HCl then the pH value was increased gradually using NaOH(0.1 or 1.0M) for each pH value, the potential was recorded and thus the potential-pH curves for four triamterene HCl concentrations were constructed(Figure 3).

As is obvious, within the pH range 4-7, the electrode potential is practically independent of pH, and in this range, the electrode can be safely used for triamterene determination. The increase in mV readings at pH less than 4 may be due to penetration of H^+ into the membrane surface or a gradual increase of protonated species and dependence of the e.m.f. on the pH of the solution.

At higher pH values(pH>7), free base precipitates in the test solution and consequently, the concentration of unprotonated species gradually increased. As a result, lower e.m.f. readings were recorded. The decrease in potential readings at (pH>7), on the other hand, can be probably attributed to penetration of OH^- ions into the gel layer of the membrane. During the operating life of the electrode(45 days), no significant change in the potential-pH behavior was observed.

3. Selectivity of the electrode

The selectivity of the ion-pair associates based membrane electrodes depends on the selectivity of the ion-exchange process at the membrane-test solution interface and the mobilities of the respective ions within the membrane. The selectivity coefficients obtained by the separate solution method[20]. TABLE 2, showed that the proposed triamterene-flavianate-

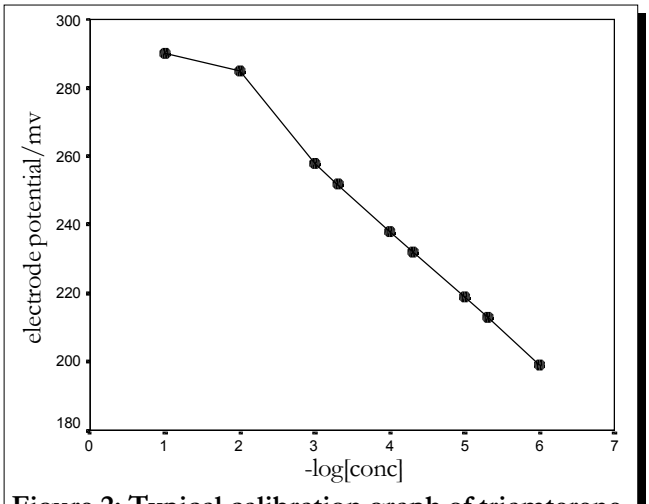

Figure 2: Typical calibration graph of triamterene-flavianate-PVC membrane electrode. (-log conc. of triamterene(M))

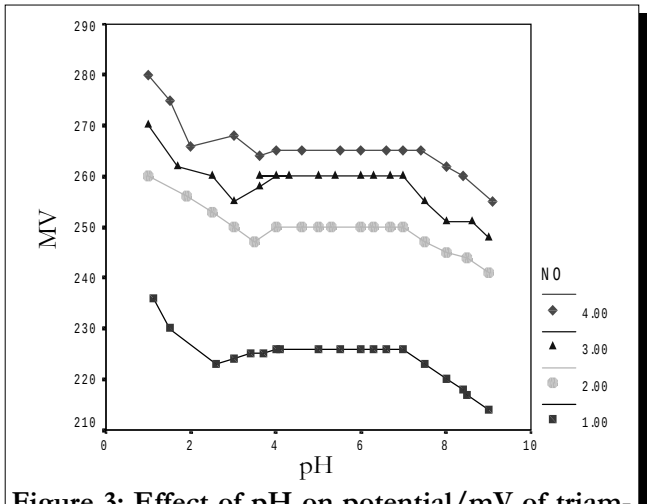

Figure 3: Effect of pH on potential/mV of triamterene-flavianate-PVC membrane electrode. 1×10^{-3}M (1), 1×10^{-4}M(2), 1×10^{-5}M (3), 1×10^{-6}(4)

PVC membrane is highly selective toward triamterene ion. The inorganic cations did not interfere due to the differences in their mobilities and permeabilities as compared with triamterene. In the case of sugars and amino acids, the high selectivity is mainly attributed to the difference in polarity and lipophilic character of their molecules relative to triamterene hydrochloride.

4. Quantification, accuracy and precision

Direct potentiometric determination of triamterene HCl using the triamterene-flavianate-PVC membrane electrode was performed and calculated from the calibration curve. The statistical data of the analytical results obtained by the proposed method(direct and standard addition techniques) for the pure drug was compared with the reference method[21] and are listed in(TABLE 3).

Furthermore, the results obtained were encouraging so the proposed method was applied for the determination of triamterene HCl in some of its pharmaceutical preparations, the results obtained were compared with the reference method[21], (non-aqueous titration of triamterene HCl using 0.1N perchloric acid as titrant and the crystal violet as indicator), as in (TABLE 4).

TABLE 2: Selectivity coefficients of the triamterene-flavianate-PVC membrane electrode calculated by the separate solution method(1.0×10^{-3}M of both triamterene and the interferent) at 25^0C

Interferent	$K^{pot}_{TA}+Cl^-$	Interferent	$K^{pot}_{TA}+cl^-$
Glucose	1.4×10^{-3}	Zinc chloride	2.5×10^{-3}
Lactose	6.8×10^{-4}	L-Valine	6.8×10^{-4}
Sucrose	1.8×10^{-3}	Tryptophan	4.3×10^{-4}
Ammonium chloride	3.8×10^{-4}	Atropine sulphate	1.3×10^{-3}
Calcium chloride	1.3×10^{-4}	Quinidine	8.6×10^{-4}
Potassium chloride	2.4×10^{-4}	Urea	2.3×10^{-3}
Sodium chloride	5.1×10^{-5}	Aminophylline	5.6×10^{-5}
Barium chloride	5.4×10^{-4}	Xipamide	1.4×10^{-5}
Nickel chloride	8.3×10^{-5}	Hydrochlorothiazide	1.8×10^{-2}
Sodium chloride	4.9×10^{-5}		
Magnesium sulphate	3.4×10^{-4}		

TABLE 3: Determination of triamterene HCl in pure form using triamterene-flavianate-PVC membrane electrode in comparison with reference method[21]

Statistical parameters	Reference method	Direct potentiometry	
		Calibration graphs	Standard addition method
Mean % Recovery	99.83	99.91	99.87
N	6	7	7
Variance	0.114	0.152	0.292
S.D.	0.338	0.390	0.540
S.E.	0.138	0.147	0.204
R.S.D.	0.339	0.390	0.541
"t"		$(0.397)(2.201)^+$	$(0.162)(2.201)^+$
		$(1.33)(4.39)^+$	$(2.56)(4.39)^+$

*** Theoretical values of "t" and F at P=0.05**

5. Electrode response in pharmaceuticals and biological fluids

The uses of triamterene drug in various fields, from clinical to abuse in sports has necessitated an accurate and rapid quantitative analysis in various matrices(dosage forms, urine and serum). This work proposed a fast, simple, easy, sensitive and straightforward potentiometric method to determine triamterene in biological fluids without the need for prior separation and preconcentration or derivati-zation procedures. The potential of the triamterene-flavianate-PVC membrane electrode showed no significant dif-

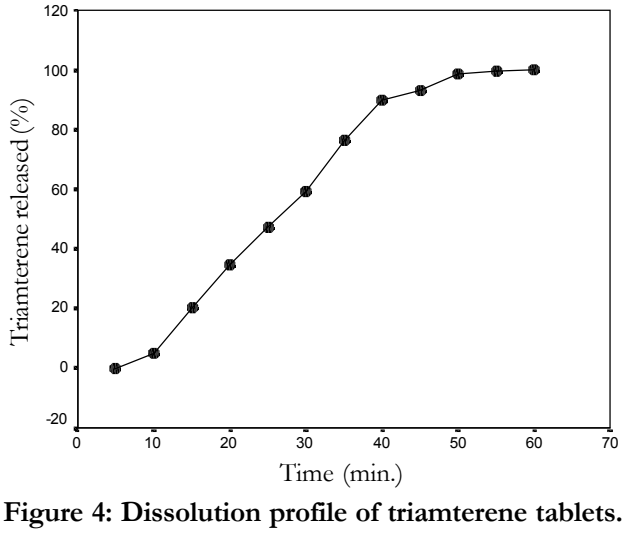

Figure 4: Dissolution profile of triamterene tablets. All values are the average of six determinations

Full Paper

TABLE 4: Determination of Dyrenium® capsules, Epitens®, Maxzide®, and Dyazide® tablets using triamterene-flavianate-PVC membrane electrode in comparison with reference method[21]

Sample	Statistical parameters	Direct potentiometry		
		Calibration graphs	Standard addition	Official method
Dyrenium® Capsules (100 mg triamterene per capsule) (Wellspring U.S.A)	* Mean % recovery	99.13	99.38	99.40
	* (P =0.05)			
	* N	6	6	6
	* Variance	0.483	0.654	0.217
	* S.D.	0.695	0.809	0.466
	* S.E.	0.284	0.330	0.190
	* R.S.D.	0.701	0.814	0.469
	* t	(0.790)(2.228)*	(0.053)(2.228)*	
	* F	(2.23)(5.05)*	(3.01)(5.05)	
Epitens® tablets (40mg triamterene per tablet) (Eipico Co. Egypt)	* Mean % recovery	99.26	99.50	99.37
	* (P =0.05)			
	* N	6	6	5
	* Variance	0.429	0.455	0.207
	* S.D.	0.655	0.667	0.455
	* R.S.D.	0.267	0.272	0.203
	* t	(0.328)(2.262)*	(0.383)(2.262)	
	* F	(2.07)(5.19)*	(2.15)(5.19)*	
Maxzide® tablets (75mg triamterene per tablet) (Mylan Bertek, U.S.A)	* Mean % recovery	99.61	99.70	99.42
	* (P =0.05)	6	6	6
	* N	0.174	0.264	0.554
	* Variance	0.417	0.514	0.744
	* S.D.	0.170	0.210	0.304
	* S.E.	0.419	0.516	0.748
	* R.S.D.	(0.545)(2.228)*	(0.758)(2.228)*	
	* t	(3.18)(5.05)*	(2.10)(5.05)*	
	* F			
Dyazide® tablets (50mg triamterene per tablet) (GlaxoSmithkline Beecham, U.S.A)	* Mean % recovery	99.10	99.35	99.19
	* (P =0.05)	6	6	5
	* N	0.295	0.095	0.199
	* Variance	0.543	0.308	0.446
	* S.D.	0.222	0.126	0.199
	* S.E.	0.548	0.310	0.450
	* R.S.D.	(2.302)(2.262)*	(0.679)(2.262)*	
	* t	(1.48)(5.19)*	(2.09)(5.19)*	
	* F			

ference of response time between aqueous solution and diluted urine and serum. The results obtained from the determination of triamterene HCl in biological fluids are listed in(TABLES 5,6).

The proposed method shows good accuracy for content uniformity assay of this drug with relative standard deviation ± 0.788. Also, figure 4, shows the dissolution profile of triamterene in tablets.

TABLE 5: Determination of triamterene HCl in pure form 'spiking technique' in human urine using triamterene-flavianate-PVC membrane electrode

Calibration method		Standard addition method
Mean ± S.D.	99.58 ± 0.349	99.47 ± 0.554
(P=0.05)		
N	6	6
Variance	0.122	0.307
S.D.	0.349	0.554
S.E.	0.142	0.226
R.S.D.	0.350	0.557

***Average of three experiments**

TABLE 6: Determination of triamterene HCl in pure form 'spiking technique' in human serum using triamterene-flavianate-PVC membrane electrode

Calibration method		Standard addition method
Mean ± S.D.	99.62 ± 0.422	99.34 ± 0.618
(P=0.05)		
N	6	7
Variance	0.178	0.382
S.D.	0.422	0.618
S.E.	0.172	0.234
R.S.D.	0.424	0.622

***Average of three experiments**

CONCLUSION

The proposed method has some important advantages: the electrode proved to be successful, providing a rapid, simple and low cost potentiometric method for the determination of triamterene in pure solutions, pharmaceutical preparations, and biological fluids; it ensures a good accuracy for the triamterene assay due to the possibility to control the ion activity continuously.

REFERENCES

[1] M.D.Vicker, P.S.J.Spencer, M.S.Read; 'Drug in Anesthetic and Intensive Care Practice', 18th Ed., Butterworth Heinmann, 415 **(1999)**.

[2] A.Murillo, A.Alanon, P.Fernandez; Anal.Chim.Acta, **449**, 179 **(2001)**.

[3] K.Kargosha, A.H.Sarrafi; J.Pharm.Biomed.Anal., **26**, 273 **(2001)**.

[4] A.Jonzyk, Z.Nowakowska; Acta Pol.Pharm., **58**, 339 **(2001)**.

[5] I.D.Meras, A.E.Mansilla, F.S.Lopez, M.J.R.Gomez; J.Pharm.Biomed.Anal., **27**, 81 **(2002)**.

[6] W.Jacke, H.Horacee, L.B.Haroldskrdlant, S.Harvey; J.Chromatogr., **138**, 111 **(1977)**.

[7] S.Carda-Boch, J.S.Esteve Romeri, M.C.Garcia Alvarez Coque; Anal.Chim.Acta, **375**, 143 **(1998)**.

[8] M.L.Luis, S.Corujedo, D.Blanco, J.M.G.Fraga, A.I. Jimenez, F.Jimenez, J.J.Aria; Talanta, **57**, 223 **(2002)**.

[9] L.M.Cabalin, A.Ruperez, J.J.Laserna; Anal.Chim.Acta, **306**, 203 **(1996)**.

[10] K.Devebter, F.T.Delbeke, K.Roels, P.Van Eenoo; Biomed.Chromatogr., **16**, 529 **(2002)**.

[11] V.White, D.V.Canfield, J.Hordinsky; J.Anal.Toxicol., **18**, 52 **(1994)**.

[12] R.Oertel, K.Richter, T.gramatte, W.Kirch; J.Chromatogr. A, **797**, 203 **(1998)**.

[13] Y.Qin, X.B.Wang, C.Wang, M.Zhao, M.T.Wu, Y.X.Xu, S.Q.Peng; J.Chromatogr. B, **794**, 193 **(2003)**.

[14] B.Q.Che; Yao.Xue.Bao., **39**, 618 **(2004)**.

[15] F.J.Rodriguez, J.J.Berzas Nevado, I.Duran Meras, M.J. Gomez Rodriguez; J.Sep.Sci., **28**, 658 **(2005)**.

[16] M.Aevand, M.F.Mousavi, M.A.Zanjanchi, M.Shamsipur; J.Pharm.Biomed.Anal., **33**, 975 **(2003)**.

[17] A.Dominguez Vidal, P.Ortego Barrales, A.Molina Diaz; J.Pharm.Biomed.Anal., **28**, 721 **(2002)**.

[18] M.L.Luis, J.M.G.Fraga, A.I.Imenez, F.Jimenez, O. Hernanddez, J.J.Arias; Talanta, **62**, 307 **(2004)**.

[19] E.J.Llorent Martinez, P.Ortega Barrales, A.Molina Diaz; Anal.Bioanal.Chem., **383**, 797 **(2005)**.

[20] T.S.Ma, S.S.M.Hassan; 'Organic Analysis using Ion Selective Electrodes', **I** and **II**, Academic Press, London, **(1982)**.

[21] United States, Pharmacopoeia, 25 NF 20, 1747, **(2002)**.

[22] M.K.Khadiga, J.AOAC, **87**, 1309 **(2004)**.