Anal. Bioanal. Electrochem., Vol. 5, No. 1, 2013, 99 - 108

Analytical & Bioanalytical Electrochemistry

Full Paper

Conductomtric Determination of Torasemide in Bulk Drug, in Formulations and in Plasma

Safa'a M. Riad

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, 11562- Cairo, Egypt

*Corresponding Author, Tel.: 00201068802592; Fax: 00235635145

E-Mail: s.mohany02@gmail.com

Received: 8 January 2013 / Received in revised form: 12 February 2013 / Accepted: 18 February 2013 / Published online: 28 February 2013

Abstract- Two simple and sensitive conductometric procedures were investigated for the determination of torasemide (TOS) using potasium tetraphenyl borate (K TPB) and ammonium reineckate (Amm. RNC) were described. Optimized conditions including temperature, solvent and reagent concentration were studied. The suggested methods were used for conductometric determination of (TOS) in its pharmaceutical preparations. Precision, measured as relative standard deviation was less than 1% and accuracy was 99.76 %.The obtained results were comparable with data using the reported method. The proposed procedures were successfully adapted for the determination of (TOS) in plasma. For comparison, some interference was also determined by the condutometric titrations. At equimolar concentration levels, some molecules of similar structure interfere with the original drug. A reduction in interferent concentration by a factor of 10 negated the interference.

Key words- Torasemide, Conductometry, Potasium Tetraphenyl Borate and Ammonium Reineckate

---

1. INTRODUCTION

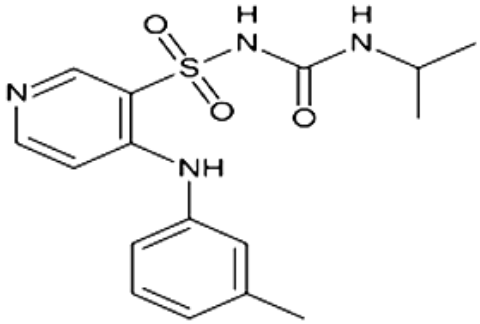

Fig. 1. Structural formula of Torasemide (TOS)

Torsemide (TOS) [1-2] is loop diuretic and is chemically known as 3-pyridine Sulfonamide N-[[(1-methylethyl) amino] - carbonyl]-4-[(3-methylphenyl) amino], whose structure is given in Fig. 1. It acts by inhibiting the Na^+/ K^+/ $2Cl^-$ carrier system (via interference of the chloride binding site) in the lumen of the thick ascending portion of the loop of Henle, resulting in the decrease in reabsorption of sodium and chloride. It's mainly used at low doses for the management of hypertension, where in large doses used for management of oedema associated with congestive heart failure [3] literature survey reveals that, several methods have been reported for the determination of (TOS), including colorimetry [4], differential–pulse adsorptive stripping voltammetry [5], capillary zone electrophoresis (CZE) [6-7], gas chromatography[8], micellar liquid chromatography [9], and HPLC [10-18] either alone or in combination. Few chromatographic methods [3-6] have been reported for the estimation of TOS in human plasma and urine. And just only one potentiometric method has been reported for the determination of TOS in pharmaceutical formulations and in Plasma [19]. To the best of our knowledge, there is no work in the literature reported about the conductometric method for the analysis of TOS either in biological fluids, in pharmaceutical formulations or in plasma.

Hence the author has made an attempt to develop precise, sensitive, cheap, maintenance-free and time saving (10 min) conductomtric procedures for the estimation of TOS in pure drug, in pharmaceutical formulations and in plasma without previous treatment.

The present study suggests two simple counductometric titrations which are successfully applied to some pharmaceutical formulations and in plasma without previous treatment. Analytes in coloured, turbid and viscous samples can be determined accurately. The proposed methods show rapid response to change in concentration. Furthermore, they may be used for measurements over wide concentration range; also the methods are generally tolerated to small change in pH.

2. EXPERIMENTAL

2.1. Apparatus

-A Cole- Parmer model 19050-00 conductance meter used with a minimum error of +0.2%. The Cole- Parmer model 4340 dip-type cell was cell constant, K cell of 1 cm^{-1}.
-pH glass electrode Jenway (UK), No. 924005-BO3-Q11C.
-Thermostatic shaker Schutzart DIN 40050-IP 20, 1 Nenn temp: 100 ºC, Type: WB 14.

2.2. Materials

2.2.1. Pure samples

-Torasemide was kindly provided by Apex Pharma-Egypt and certified to contain 99.75% [6].

2.2.2. Pharmaceutical preparations

Examide® tablets (5, 10 and 20 mg, Batch no: MT 2480710, MT 3411010 and MT 0420112; respectively) manufactured in Apex Pharma-Egypt, Egypt Company and labeled to contain 25, 10 and 20 mg torsemide per tablet; respectively.

2.2.3. Chemicals and reagents

All reagents used were of analytical grade, the solvents of spectroscopic grade and water used throughout the procedures was distilled .
potassium tetraphenyl borate (KTPB) (BDH, England), Ammonium reinckate $NH_4[Cr(NH_3)_2(SCN)_4]$, (Amm. RNC)(BDH, England), Aqueous solutions of 10^{-2} MKTPB and Amm. RNC, Hydrogen peroxide 30% w/v (Adwic), Frozen human plasma was obtained from VACCERA.

2.2.4. Standard Solutions

-Stock standard aqueous solution (0.03 g ml^{-1}).
- Working solution (3 mg ml^{-1}), was freshly prepared by appropriate dilutions of the standard solution with distilled water.

3. PROCEDURES

3.1. Procedure for bulk powder

Volumes (0.5-5 ml) of the drug working standard solution (3 mg ml^{-1}) were accurately transferred into 100-ml volumetric flasks, the volume was made up to the mark with distilled water, the solutions were accurately transferred to beakers and the conductivity cell was immersed in each solution; respectively. Titration was carried out with 10^{-2} M KTPB or Amm. RNC using micro burette, the conductivity was measured subsequent to each addition

of reagent solution after through stirring. The conductivity reading 1 min after each addition was taken. Correction for dilution [20], by means of the following, equation was carried out:

$$\Lambda_{corr.} = \Lambda_{obs.} [V_1+V_2) /V_1]$$

Where V_1 is the initial volume, V_2 is the volume of the reagent added, Ω_{obs} is the observed conductivity and Ω_{corr} is the correct conductivity. A graph of corrected conductivity versus the volume of added titrant was constructed. Two intersecting lines will be obtained and the end point was the point of intersection.

Calculation: 1 ml of each of 10^{-2} M KTPB and 10^{-2} M Amm. RNC was theoretically equivalent to 3. 84 mg of torasemide.

3.2. Procedure for determining the drug ligand ratio

Ten ml aliquot of 10^{-2} M drug solution was transferred to a 100 ml volumetric flask and the volume was made up to the mark with distilled water. The content was transferred to a beaker and the conductivity cell was immersed. The solution was titrated with 10^{-2} M reagent solution using micro burette. The conductivity was measured subsequent to each addition of reagent solution after through stirring. A graph of conductivity versus volume was constructed and the end point was determined.

3.3. Procedure for tablets

Twenty tablets were powdered and mixed well, then a quantity of the powdered tablets equivalent to 30 mg of torasemide was accurately weighed and quantitatively transferred into a 100-ml volumetric flask, the volume was made up to distilled water. The procedure was followed as under procedure for bulk powder (3.1). A graph of conductivity versus volume was constructed and the end point can be determined.

3.4. Procedure for Plasma

Nine ml portion of drug free plasma was spiked with 10 ml of 10^{-2} M torasemide in 100-ml volumetric flask, mixed well, the content was diluted and reconstitute in 100 ml 0.1 M distilled water. The procedure was then followed as under procedure for bulk powder 3.1.

4. RESULTS AND DISCUSSION

Counductometry is a method of analysis based on measuring conductance of electricity through an electrolyte solution [21]. Conductometric measurements were used successfully in quantitative titration of system in which the conductance of the solution varies before and after the equivalence point. In this case, the titration curve can be represented by two lines intersecting at the end point. The present study showed a regular rise in conductance up to the equivalence point where a sudden change in the slope occurs, as shown in Fig. 2.

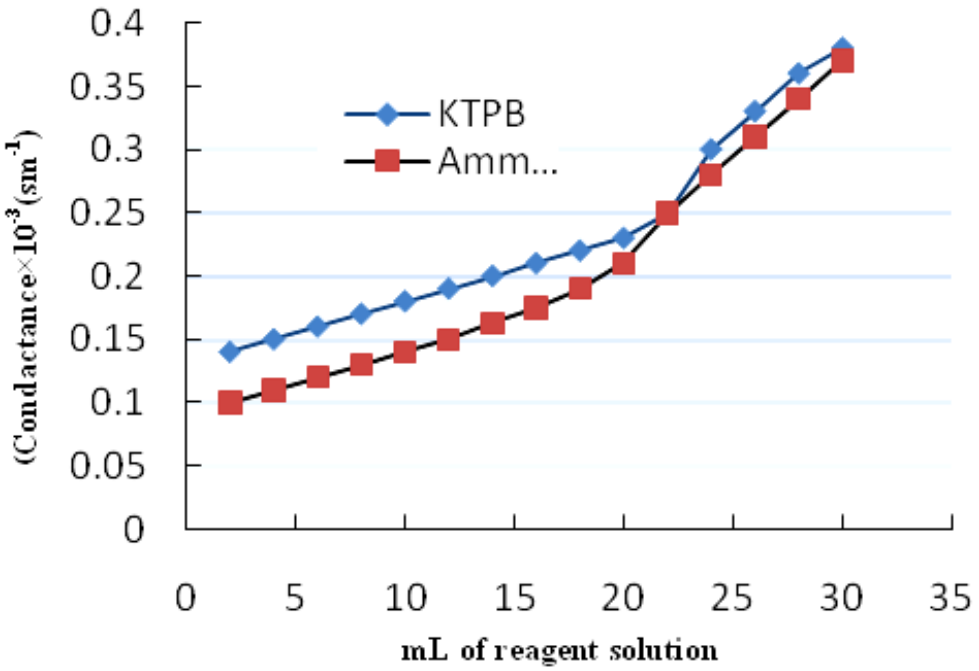

Fig. 2. Conductometric titration of 10 mg of cilostazol with 10^{-2} M KTPB and 10^{-2} M Amm. RNC

On adding KTPB or Amm.RNC, the ion-associates were formed by replacing the TPB and RNH^+ ions by the mobile NH^{4+} or K^+ causing an increase in the conductance [22].

After the equivalence point, more reagents were added and the conductivity increases more rapidly. Equivalence points were reproducible within ±1% at 1:2 drug: KTPB or Amm.RNC stoichiometry. The optimum conditions for performing the proposed titrations in a quantitative manner were elucidated as described below.

Effect of temperature was studied on raising the temperature to 35°C, no change in the conductivity readings was observed, whereas above 40°C the conductivity value changed leading to changes in the shape of the titration curve .

The reagent concentration in each titration must not be less than 10 times that of the drug solution in order to minimize the dilution effect on the conductivity throughout the titration. The optimum concentration of the reagents was 1.0×10^{-2} M KTPB and Amm.RNC to achieve a constant and highly stable conductance reading within 1-2 min. of mixing. Concentration less than these limits leads to unstable readings and more time was needed to obtain constant conductance values. The optimum concentrations for determining torasemide were in the range 15-150 μg ml^{-1}, at which sharp inflections and stable conductivity values were obtained. The suggested conductometric titrations display constant conductance readings within ±1 ms from day to day, and its offer the advantages of time saving (10 min.), needs no preliminary treatment (compared with the reported method[6], direct application to turbid and

coloured drug solution and adequate selectivity in presence of many additives found in the studied pharmaceutical formulations. The results for the determination of torasemide by the two procedures over a period of four weeks were summarized in Table 1, and show good recoveries and standard deviations. Independent analyses of a suitable number of samples known to be near the detection limit, under the specified conditions of the proposed procedures were undergoing. The detection limits, of 13.5 µg ml^{-1} for the two procedures was the minimum level at which the studied drug can be reliably detected.

Table 1. Accuracy and precision of the proposed conductometric methods for the analysis of Torasemide in pure form

Taken (µg.ml^{-1})	*KTPB*		Amm.RNC	
	Found [a] (µg.ml^{-1})	Recovery (%)	Found [a] (µg.ml^{-1})	Recovery (%)
15	14.90	99.33	15.05	100.33
30	29.85	99.00	30.00	100.00
45	44.50	98.89	44.60	99.11
60	60.20	100.33	59.50	99.17
75	74.60	99.47	75.00	100.00
90	90.00	100.00	89.75	99.72
105	105.90	100.86	105.2	100.19
120	121.00	100.83	119.50	99.85
135	143.00	99.26	134.90	99.93
150	149.50	99.67	148.90	99.27
Mean ± RSD		**99.76±0.715**		**99.76±0.431**

[a]average of five determinations

Validations of the investigated methods were carried out, (Table 2) and satisfactory results were obtained. The suggested procedures displayed constant conductance readings within±1 min. from day to day. The proposed conductometric titrations were applied to the

Table 2. Validation for the analysis of Torasemide by the investigated procedures

Parameter	***KTPB***	***Amm.RNC***
Linear range (µg.ml^{-1})	15-150	15-150
Regression equation	Λ =0.985C+0.082	Λ =0.862C+0.075
Mean ± RSD	99.76±0.715	99.76±0.431
Intercept (a)	0.082	0.075
Slope (b)	0.985	0.862
Correlation coefficient (r)	0.9995	0.9998
LOD (µg.ml^{-1})	13.5	13.5
LOQ (µg.ml^{-1})	15	15
Student's t (2.31)^{a}	2.01	2.12
RSD% (intraday, n=4)	0.495-0.888	0.490-0.899
RSD% (interday, n=5)	0.582-0.892	0.579-0.890

Λ= corrected conductivity
a= Tabulated 95% confidence limit

determination of torasemide in pure form and in pharmaceutical preparations. Equivalence points were reproducible within +1% at 1:2 drug: KTPB or Amm. RNC stoichiometry.

The results in Table 3, were compared with those from the reported method [6]. This comparison indicated that both methods were of equal confidence and accuracy. The proposed conductometric titrations, however offer the advantages of time saving, needs no preliminary treatment, direct application to turbid and coloured drug solution.

Furthermore no interference was observed when the drug was spiked with the human plasma samples without prior removal of the protein as shown in Table 4. Upon adopting the proposed conductomtric titrations on determination of (TOS) in presence of additives found in the studied pharmaceutical formulations e.g. mannitol, propyl parapen and ascorbic acid, no interferences were obtained. The results show that torasemide can be directly determined in its pharmaceutical preparation without separation of these excipients.

Table 3. Application of the standard addition technique for the determination of torasemide in its pharmaceutical preparationby the proposed procedures

Tablet	*Taken (μg ml^{-1})*	*Authentic Added (μg.ml^{-1})*	*Authentic Found[a] (μg ml^{-1})*			Recovery (%)		
			KTPB	*Amm.RNC*	**Published method[6]**	*KTPB*	*Amm.RNC*	**Published method[6]**
	50	10	10.00	9.95	9.95	100.00	99.50	99.50
Examide 5 mg	50	20	19.90	19.95	19.90	99.50	99.95	99.50
(B.N.MT 2480710)	50	40	40.05	39.90	40.05	100.13	99. 75	100.13
	50	80	79.50	79.90	80.00	99.38	99.88	100.00
Mean ± RSD						**99.75±0.368**	**99.78±0.242**	**99.78±0.330**
Examide	50	10	9.99	10.00	9.95	99.90	100.00	99.50
10 mg	50	20	20.00	19.80	19.90	100.00	99.00	99.50
(B.N.MT	50	40	39.80	40.00	40.20	99.95	100.00	100.50
3411010)	50	80	79.50	79.50	80.00	99.38	99.83	100.00
Mean ± RSD						**99.81±0.280**	**99.71±0. 478**	**99.88±0.479**
	50	10	9.90	9.93	10.00	99.00	99.30	100.00
Examide	50	20	19.90	20.00	19.90	99.50	100.00	99.50
20 mg	50	40	39.95	40.00	39.90	99.88	100.00	99. 75
(B.N.MT 0420112)	50	80	80.00	79.40	79.90	100.00	99.25	99.88
Mean ± RSD						**99.59±0.450**	**99.64±0. 419**	**99.79±0.261**

[a]Average of five measurements

Table 4. Precision and recovery of torasemide in spiked human plasma using the proposed procedures

Plasma added[*] (mg)	*Torasemide Recovery** % ± RSD*	
	KTPB	*Amm.RNC*
30	99.40±0.123	99.91±0.205
60	98.98±0.095	100.01±0.187
90	99.75±0.282	100.19±0.165

*Using 0.3 mg.ml^{-1} standard solutions

**Average of three determinations

5. CONCLUSIONS

The described novel counductometric procedures were sufficiently simple, cheap and selective for the quantitative determination of torasemide in pure form, plasma and in pharmaceutical formulations. The use of the proposed methods offers advantages of fast response and elimination of drug pretreatment or separation steps. They can therefore, be used for routine analysis of torasemide in quality control laboratories.

REFERENCES

[1] J. L. Blose, K. F. Adams, and J. H. Patterson. Ann. Pharm. Ther. 29 (1995) 396.
[2] S. F. Flower, and K. M. Murray, Am. J. Health Syst. Pharm. 52 (1995) 1771.
[3] S. Engelhardt, I. Meineke, J. Brockmöller. J. Chromatogr. B. 831 (2006) 31.
[4] M. V. Krishna, and D. G. Sankar. E J. Chem. 5 (2008) 473.
[5] M. Fernandez, R. M. Alonso, R. M. JimCnez, M. J. Legorburu. Analyst 119 (1994) 319.
[6] U. Akesolo, L.Gonzalez, R. M. Jimenez, and R. M. Alonso. J. Chromatogr. A. 990 (2003) 271.
[7] U. Akesolo, L. Gonzalez, R. M. Jimenez, and R. M. Alonso. Electrophoresis 23 (2002) 230.
[8] S. Sharma, M. C. Sharmab, and D. V. Kohlic, Der Pharm. Lett. 2 (2010) 374.
[9] E. Besenfelder. J. Pharmaceut. Biomed. 5 (1987) 259.
[10] M. B. Barroso, R. M. Alonso, and R. M. Jiménez. J. Chromatogr. Sci. 39 (2001) 491.
[11] I. J. Khan, P. Loya, and M. N. Saraf. Ind. J. Pharm Sci. 70 (2008) 519.
[12] S. Engelhardt, I. Meineke, and J. Brockmöller. J. Chromatogr. B. 831 (2006) 31.
[13] R. Xiaowen, L. Hongqi, L. Xiaoyan, L. J. Zhang1, Y. Wang, W. Chunlong, and W. Xu, Asian., J. Pharmacokinet. Pharmacodyn. 8 (2008) 43.
[14] D Gowri Sankar, M Vamsi Krishna, N Sujatha, LA Rama Prasad, B Sneha Latha, Development, 3 (2007).
[15] D. Y. Lee, J. L. Shin, G. L. Myung. Int. J. Pharm. 298 (2005) 38.
[16] L. Kwang-Hyeon, L. Yun-Kyeong, R. L. Ji-Young, Dong-Jun, K. Wonku, L. S. Sang, Y. Young -Ran, and S. Jae-Gook. Chromatogr. 60 (2004) 639.
[17] M. Clark, D. Farthing', B. Wells, E. Besenfelder, and H. Th. Hrnes. J. Pharm. Sci. 79 (1990) 453.
[18] Y. Qin, X. B. Wang, C. Wang, M. Zhao, M. T. Wu, Y. X. Xu, and S. Q. Peng. J.Chromatogr. B. 794 (2003) 193.
[19] S. M. Riad, J. Anal. & Bioanal. Electrochem. 4 (2012) 417

[20] S. L. Bramer, P. N. V. Tata, S. S. Vengurlekar. And J. H. Brisson. J. Pharm. Biomed. Anal. 26 (2001) 637.
[21] K. J. Yeon, Y. J. Park, E. Ban, Y. B. Kim, C. K. Kim. J. Liq. Chromatogr. Relat. Technol. 28 (2005) 109.
[22] M. S. Bahbouh, A. A. Salem, and Y. M. Issa. Mikrochim. Acta, 128 (1988) 57.