Portugaliae Electrochimica Acta 2011, 29(2), 79-90

DOI: 10.4152/pea.201102079

PORTUGALIAE ELECTROCHIMICA ACTA
ISSN 1647-1571

Microsized Graphite Sensors for Potentiometric Determination of Metronidazole and Spiramycin

F.I. Khattab, N.K. Ramadan, M.A. Hegazy,* and N.S. Ghoniem

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

Received 15 August 2010; accepted 14 March 2011

Abstract

Two microsized graphite-design sensors based on ionophore technique, polyvinyl chloride carboxylated (PVC-COOH) and β-cyclodextrin (β-CD), are used for fabrication of two membrane sensors for the two studied drugs, metronidazole (MZ), sensor 1, and spiramycin (SP), sensor 2. Fast and stable Nernstian responses near $1x10^{-5}$ - $1x10^{-3}$ M for MZ and $1x10^{-5}$-$1x10^{-2}$ M for SP over pH range 5.5-7.5 for the two electrodes reveal the performance characteristics of these electrodes which have been evaluated according to IUPAC recommendations. The aim of this work is to develop a new, simple, accurate and precise method for the determination of MZ and SP in their binary mixtures, which can be applied in routine quality control. The method is successively applied for the determination of the two drugs in their pharmaceutical formulations. Validation of the method according to the quality assurance standards shows suitability of the proposed electrodes for the use in the quality control assessment of these drugs. The recovery percentages for the determination of the two drugs by the two proposed selective electrodes are 99.86 ± 0.249 % and 99.69 ± 0.856% for sensors 1 and 2, respectively. Statistical comparison between the results obtained by this method and the reported one is done and no significant difference is found.

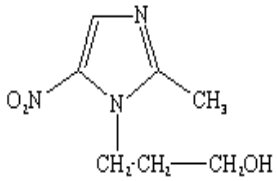
Keywords: metronidazole (MZ), spiramycin (SP), poly(vinyl chloride) carboxylated PVC-COOH, β-cyclodextrin (β-CD), pharmaceutical formulations.

Introduction

Metronidazole (MZ) is the reference agent of the nitroimidazole anti-infective family. It is chemically designated as 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol [1]. It is used mainly in the treatment of anaerobic bacteria and protozoa as it is reduced to its active form intracellularly. Besides, it has a radio-

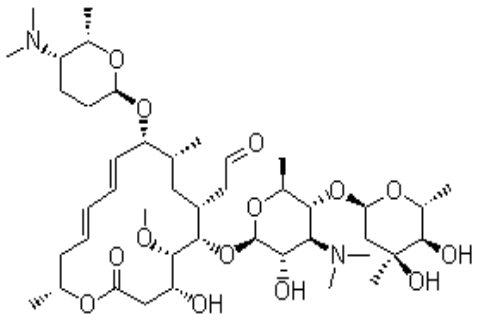
* Corresponding author. E-mail address: mahahgazy@yahoo.com

sensitizing effect on hypoxic tumor cells [2]. In addition of being a human medicine, MZ has also been used as antiparasitic in the veterinary field [3].



Metronidazole: mol. formula: $\underline{C_6}\underline{H_9}\underline{N_3}\underline{O_3}$; mol. weight:171.15g mol^{-1}.

Spiramycin (SP) is chemically designated as (6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-5,9,16-trimethyl-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside [1]. It is isolated from the streptomyces ambofaciens and is a natural mixture of three components: SP I together with its 3-acetyl (SPII) and 3-propanoyl (SPIII) [4] with minimum of 85% of SP I, and a maximum of 5% for SP II and 10% for SPR III [5]. It is well absorbed after oral administration and distributed in the tissues, especially lungs, liver and kidney [6].



Spiramycin: mol. formula: $C_{43}H_{74}N_2O_{14}$; mol. weight: 843.06 g mol^{-1}.

Several methods have been reported for the determination of either MZ or SP each in its single pharmaceutical formulations. Several methods were reported for the determination of MZ in biological fluids, in pharmaceutical dosage forms, in combination with other drugs and in the presence of its metabolites, using spectrophotometry [7], thin layer chromatography (TLC) [8], liquid chromatography (LC) [9,10], high performance liquid chromatography (HPLC) [11], voltammetry [12], flow injection chemiluminescence analysis [13], nuclear magnetic resonance spectrometry (NMR) [14], capillary electrophoresis [15] and potentiometrically, using nanostructure thin film on the gold electrode through continuous pulsed-potential technique [16]. Also, different techniques have been described for the determination of SP, including titremetry [17], spectrophotometry [18], TLC [19], LC [20], HPLC [21,22], capillary electrophoresis [23], voltammetry [24], and immunological assay [25].

The combinations of MZ and SP have been developed on the basis of complementary of the antibacterial activity of both compounds in vitro. This binary mixture has been determined in plasma, saliva and gingival crevicular fluid by LC-MS/MS [10], and in fish muscle using HPLC with UV detection

[26]. Determination of MZ and SP by HPLC and HPTLC in tablets has been also reported [27]. A comprehensive literature search revealed the lack of any potentiometric techniques for the determination of MZ and SP in tablets.

The scientific novelty of the present work is that the method used is simple, rapid, selective and less expensive and less time consuming compared with other published LC, TLC and HPLC methods.

The focus of the present study is to develop and validate a potentiometric method for the determination of MZ and SP in their combined tablets dosage form.

Microelectrodes have been the subject of much research in recent years [28]. The advantages they offer over conventional electrodes are well known [29]. Their small physical size allows exploration of microscopic domains, such as biological systems. Their fast response time, due to the reduced diffusion layer, allows rapid scan rates to be used [30].

Metallic and graphite-based conductors of many geometric shapes have been suggested, such as wire, disc and cylinders [31-33]. These electrodes behave as two interface devices, membrane/electrolyte interface and membrane/metal interface [34]. Thus, the membrane potential in the cell regards as the electric potential difference between the two interfaces.

Membranes have been suggested to be prepared from polymer, ionophore and plasticizer [30]. The role of the polymer is to provide an inert solid support structure in which the rest of components are embedded. The ionophore can be viewed as a molecular receptor, because its chemical structure provides well defined inclusion cavities with a specific receptor function [35]. The ionophores can accommodate a wide variety of organic, inorganic, as well as biologic guest molecules to form stable host–guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity, showing high molecular selectivity and enantioselectivity [36]. The plasticizer plasticizes the membrane and affects the lipophilicity of the polymer membrane. It also alters the distribution coefficients (K) of different species, thus affecting the performance characteristics of the electrode [37].

The high selectivity of these electrodes imparts a great advantage over other techniques [38]. Analytes in colored, turbid and viscous samples can be determined accurately. They show rapid response to change in the concentration. Furthermore, they may be used for measurement over a wide concentration range. Ion selective electrodes are generally tolerant to small changes in pH. A further advantage is that they are relatively simple and not expensive to develop, set up and run. Moreover, the chemical design of the electrodes has been developed to give superior selectivity and response [38].

The present work includes microsized graphite-design sensors that based on ionophore technique, PVC-COOH and β-CD are used for fabrication of membrane sensors for the two studied drugs.

The microsized graphite rods are coated with thin films of PVC-COOH-β-CD-dioctylphthalate (DOP), and used as potentiometric sensors for MZ and SP. Upon soaking these sensors in MZ and SP test solutions, a homogenous electroactive layer from PVC-COOH-β-CD-DOP-Drug is formed which induces a potentiometric response for the two studied drugs.

Sensors 1 and 2 are simply fabricated without the need of ion association complex. They are only preconditioned by soaking in the corresponding drug solution where acid-base interactions take place between the dissociated COO^- group of the PVC and quaternary nitrogen atom of the drug in the test solution until chemical equilibrium is attained.

Experimental

Apparatus

- Jenway digital ion analyzer model 3330 (UK) with Ag/AgCl double junction reference electrode no. no Z113107-1EAPW (Aldrich Chemical Co.).
- Jenway (UK) pH glass electrode no. 924005-BO3-Q11C.
- Magnetic stirrer, Bandelin Sonorox, Rx510S (Hungarian).

Reference samples

Metronidazole and spiramycin have kindly been supplied by El Pharonia Pharmaceuticals, new borg El-arab City, Alexandria, A.R.E. They are certified to contain 4412 IU mg^{-1} and 99.90 % w/w, respectively, according to the manufacturer's method.

Pharmaceutical formulations

Spirazole® tablets, batch no.1288009, are labeled to contain 125 mg of MZ and 750000 IU of SP, and are manufactured by El Pharonia Pharmaceuticals, New Borg El-Arab City, Alexandria, A.R.E.

Reagents

All chemicals and reagents used throughout this work were of analytical grade. Double distilled water was used.

Polyvinyl chloride carboxylated (PVC-COOH) and β-cyclodextrin (β-CD) were purchased from Fluka chemie (GmbH Germany). Tetrahydrofuran (THF) was purchased from BDH (limited Poole, England), while dioctyl phthalate (DOP) was purchased from Sigma/Aldrich (St. Louis, MO). Sodium hydroxide 0.1 M aqueous solution and hydrochloric acid 0.1 M aqueous solution were prepared and obtained from Prolabo (VWR International, West Chester, PA).

Standard solutions

- MZ standard stock solution (1×10^{-2} M): it was prepared by transferring 0.171 g of MZ into a 100 mL volumetric flask, and the volume was then completed to the mark with double distilled water.
- MZ working solutions (1×10^{-5} - 1×10^{-3} M): they were prepared by suitable dilution from their stock solution using double distilled water.
- SP standard stock solution (1×10^{-1} M): it was prepared by transferring 8.431 g of SP into a 100 mL volumetric flask, and the volume was then completed to the mark with double distilled water.
- SP working solutions (1×10^{-5} - 1×10^{-2} M): they were prepared by suitable dilution from their stock solution using double distilled water.

Procedures

Preparation of electroactive coating membrane: (β-CD / DOP / PVC-COOH)

In a glass Petri dish (5 cm diameter), a portion of 0.9 g PVC-COOH was thoroughly mixed with 0.35 g DOP and 0.3 g β-CD, then dissolved in 15 mL THF. The Petri dish was covered with a filter paper and left to stand for 1 h to allow slow solvent evaporation. A thick homogeneous solution was produced.

Sensor 1 fabrication (MZ-coated graphite electrode)

A rod of spectrographic graphite (5 mm in diameter and 15 mm long) was inserted in a polyethylene sleeve, and about 3 mm of the other end of the protruded rod served as a measuring surface. This end of the rod was washed with acetone, dried in air for 3 hours, and dipped rapidly into the previously prepared PVC-COOH-β-CD-DOP solution. The solvent was allowed to evaporate in air after each dipping, and the dipping process was repeated 6-8 times to produce a uniform membrane on the surface of the graphite rod. Drops of mercury were added in the polyethylene sleeve to ensure electrical contact with the connection cable. The coated graphite rod was conditioned by soaking in a 10^{-3} M MZ solution for 12 hours, and stored in the same solution when not in use.

Sensor 2 fabrication (SP-coated graphite electrode)

The procedure was followed as under *Sensor 1 fabrication (MZ-coated graphite electrode)* starting from "A rod of spectrographic graphite with the connection cable". The coated graphite rod was conditioned by soaking in a 10^{-2} M SP solution for 24 hours, and stored in the same solution when not in use.

Direct determination of MZ and SP in their pure powdered samples

The prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode were immersed in aqueous solutions of MZ and SP in the range of (1×10^{-5} to 1×10^{-3} M and 1×10^{-5} to 1×10^{-2} M), respectively. They were allowed to equilibrate while stirring and the emfs were recorded within ± 1 mV. The membrane sensors were stored in double distilled water between measurements. Calibration graphs were plotted relating the recorded potentials vs. –log drug concentration. These calibration graphs or the computed regression equations were used for subsequent measurements of unknown concentrations of MZ and SP.

Application of the proposed method for determination of MZ and SP in Spirazole® tablets

Ten tablets were accurately weighed and powdered. Amounts of the powdered tablets equivalent to 0.0171 g of MZ and 0.0843 g of SP were accurately transferred into two separate 100-mL measuring flasks, and the volume was completed to the mark to prepare a 10^{-3} M aqueous solution of MZ and SP, respectively. The emfs produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions were recorded, and the concentrations of MZ and SP were

then determined from the calibration curves of the corresponding drug or from the corresponding regression equation.

Results and discussion

The preparation and application of microelectrodes have attracted much interest in recent analytical chemistry studies [39]. This is part of a general trend in analytical chemistry towards miniaturization. The features of voltammetric microelectrodes have been investigated extensively during the last few years with the aim of improving the characteristics of electrochemical methods and performing studies under conditions not possible with conventional electrodes [40,41]. A variety of electrode materials of different shapes and sizes have been reported, but the most commonly used electrode materials are platinum [42], gold [43] and carbon fiber [44].

The replacement of the classical internal filling solution in potentiometric sensors is important because that design offers a number of advantages such as simplicity, lower cost, better mechanical flexibility and possibility of miniaturization. In ionselective electrode research there have been many attempts to replace the conventionally employed internal filling solutions for solid contacts [42].

Sensors fabrication

In the present work, the microsized graphite rods were coated with thin films of PVC-COOH-β-CD-DOP, and used as potentiometric sensors for MZ and SP. Upon soaking these sensors in 1×10^{-3} M MZ and 1×10^{-2} M SP test solutions, a homogenous electroactive layer from PVC-COOH-β-cyclodextrin-DOP-drug was formed, which induces a potentiometric response for the two studied drugs.

DOP (a non polar plasticizer) was found to be the optimum available mediator for the PVC-COOH membrane sensors. It plasticizes the membrane and adjusts the membrane permittivity to give the highest possible selectivity and sensitivity.

Cyclodextrins are optically active oligosaccharides that form inclusion compounds in aqueous and solid states with organic molecules. They were previously applied as sensor ionophores to potentiometric ISEs for the determination of protonated amines [45] and chiral molecules incorporating aryl rings [46].

In most cases reported, the interaction is classical. However, there have been rare reports of cooperative interactions in the field of cyclodextrins, concerning either natural cyclodextrins or synthetic cyclodextrin derivatives [28]. In the case of natural cyclodextrins, cooperative binding with certain guest molecules has been mostly attributed to intermolecular hydrogen bonding between the CD molecules, while intermolecular interactions between both the host and guest molecules, hydrogen bonds, hydrophobic interactions and Van der Waals forces, contribute to cooperative binding processes when synthetic CDs are used [26]. β-CD based sensors showed accurate results in both response and selectivity.

Once *PVC-COOH* has two recommended properties that are the partial dissociation and the high adhesion [47], sensors 1 and 2 were simply fabricated without the need of ion association complex. They were only preconditioned by

soaking in the corresponding drug solution for 12 or 24 hours where acid base interactions take place between the dissociated COO^- group of the PVC and the quaternary nitrogen atom of the drug in the test solution, until chemical equilibrium is attained.
PVC-COOH also acts as a regular support matrix as trap for the ion and as polymeric matrix to immobilize the sensor and to attain the formation of highly stable complex.

Sensors calibration and response time
Electrochemical performance characteristics of the proposed sensors were systematically evaluated according to IUPAC standards [48].
Table 1 shows the results obtained over a period of six months for two different assemblies of each sensor. Typical calibration plots are shown in Fig. 1. The sensors displayed constant potential readings within ± 2 mV from day to day and the calibration slopes did not change by more than ± 2 mV per decade over a period of 5 and 6 weeks for sensor 1 and sensor 2, respectively.

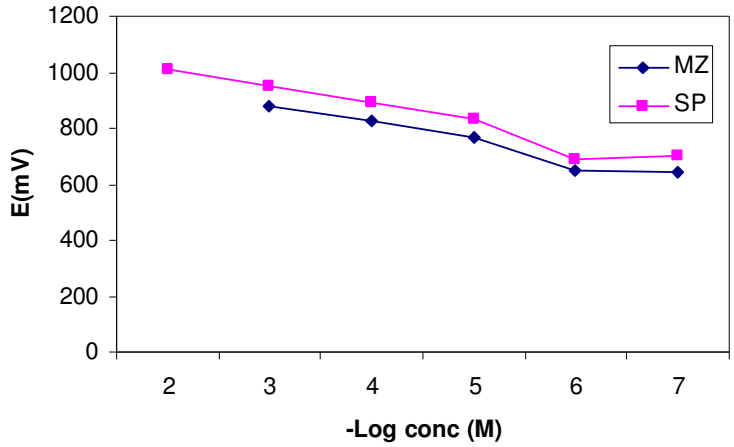


Figure 1. Profiles of the potential in mV/-log concentrations (M) of MZ and SP of sensors 1 and 2, respectively.

The required time for the sensors to reach values within ± 2 mV of the final equilibrium potential after increasing drug concentration 10-folds, was found to be 45 and 30 seconds, for sensors 1 and 2, respectively. The slopes of the calibration plots were 58 and 59.3 mV/concentration decade for sensor 1 and 2, respectively, the typical value of monovalent substances, as these drugs behave as monovalent cations via their quaternary nitrogen atom.

Effect of pH and temperature
In the measurements with the two investigated sensors, the different factors affecting the response of the electrodes (emfs) were studied to reach the optimum experimental conditions. A pH value within the range 5.5-7.5 was found to be optimum from the point of view of both sensor function and the chemical form of the test solution; both MZ and SP were in the cationic form in acidic media. Fig. 2 shows the potential pH profile for 10^{-3} and 10^{-4} M drug solutions. Above pH 7.5, the potentials displayed by the sensors decrease due to the formation of non-

protonated drugs. Below pH 5.5, the potentials displayed by the sensors were noisy and unbalanced. It is apparent that the sensors responses are fairly constant in solutions of pH 5.5-7.5.

Table 1. Response characteristics of the investigated selective electrodes and validation parameters of the response and of the regression equations.

Parameters	MZ-coated graphite electrode	SP-coated graphite electrode
Validation of the regression equations		
Slope (mV/ decade) *	58	59.3
Intercept (mV)	1055	1127
SE of the slope	0.5774	0.9899
SE of the intercept	2.3570	3.6373
Correlation coefficient	0.9999	0.9996
Validation of the responses		
Concentration range (M)	10^{-5}-10^{-3}	10^{-5}-10^{-2}
LOD (M)**	6.8×10^{-7}	5.9×10^{-6}
Response time (Sec.)	45	30
Working pH range	5.5-7.5	5.5-7.5
Stability (weeks)	5	6
Accuracy (mean ± S.D.) *	99.86 ± 0.249	99.69 ± 0.856
R.S.D.	0.250%	0.860
Precision		
Repeatability % ***	100.03 ± 0.175	99.10 ± 0.276
Intermediate precision % ****	99.90 ± 0.343	99.07 ± 0.179

- Average of five determinations. ** Limit of Detection (LOD) defined as drug concentration obtained at the intersection of the extrapolated high concentration (linear segment) with the low concentration (zero slope segment) of the calibration plot. *** The intraday (n=5) mean values ± RSD of samples of concentrations 10^{-3} and 10^{-4} M of each MZ and SP, respectively. **** The inter-day (n=5) mean values ± RSD of samples of concentrations 10^{-3} and 10^{-4} M of each MZ and SP.

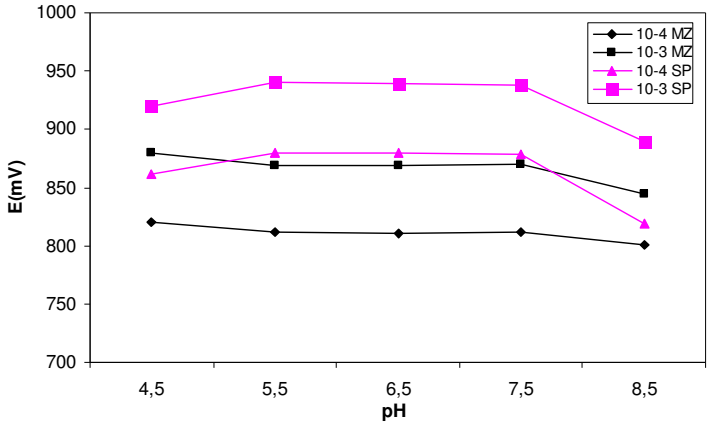


Figure 2. Effect of pH on the response of sensors 1 and 2.

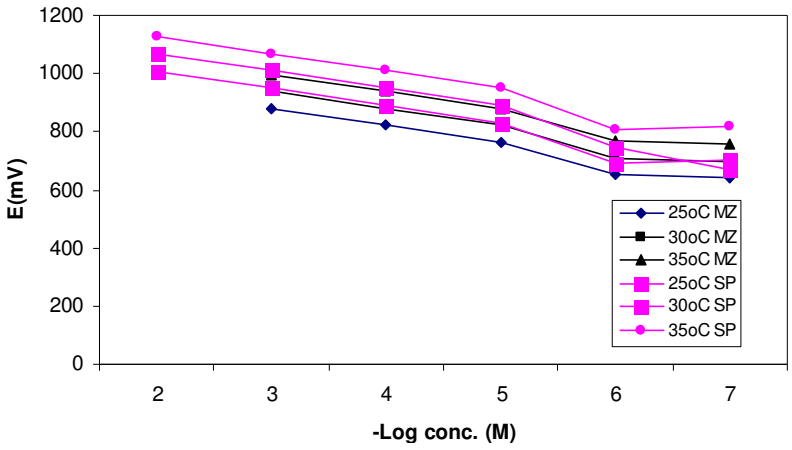


Figure 3. Effect of temperature on the response of sensors 1 and 2.

Upon studying the effect of temperature, the suggested sensors exhibit slight gradual increase in their potentials as the temperature increases in the range of 25-35 °C; however, the calibration graphs obtained at different temperatures were parallel, and the slope and response time did not significantly vary with variation of temperature, indicating reasonable thermal stability of the suggested sensors up to 35 °C (Fig. 3).

Sensor selectivity

The effect of interfering substances upon the performance of the sensors was studied by separate solutions method [48].

The performance of the two sensors in the presence of tablets excipients, organic and inorganic related substances and also some anti-infective drugs, was assessed by measuring and comparing the potentiometric selectivity coefficients. The results revealed that the proposed membrane sensors displayed high selectivity, and that no significant interference was observed from interfering species, Table 2.

Table 3 shows the results obtained for the determination of MZ and SP in Spirazole® tablets, proving the applicability of the method without prior treatment or extraction, using sensor 1 for the determination of MZ and sensor 2 for the determination of SP, as demonstrated by the accurate and precise percentage recovery; the results obtained were also compared with those obtained by using reported method [27]. No significant difference in results was found.

Table 1 shows all the validation parameters of the proposed method including linearity, range, accuracy and precision.

Table 2. Potentiometric selectivity coefficients ($K^{pot}_{1ry\ ion}$) of the two proposed electrodes.

Interferent**	Selectivity coefficient*	
	Sensor 1	Sensor 2
Spiramycin	7.24×10^{-6}	
Metronidazole		6.03×10^{-5}
Ampicillin	7.94×10^{-6}	6.30×10^{-5}
Amikacin	4.27×10^{-5}	8.71×10^{-5}
Cefoperazone	3.39×10^{-5}	9.12×10^{-6}
Ornidazole	1.70×10^{-5}	4.68×10^{-5}
Lactose	4.16×10^{-5}	3.63×10^{-5}
$MgSO_4$	4.90×10^{-5}	2.88×10^{-5}
Talc	1.82×10^{-5}	8.13×10^{-5}
Starch	4.37×10^{-5}	6.76×10^{-5}
NaCl	2.51×10^{-5}	5.89×10^{-5}

* Average of 3 determinations. ** All interferents are in the form of 1×10^{-3} M solution.

Table 3. Statistical comparison for the results obtained by the proposed electrodes and a reported HPLC method [27] for the analysis of MZ and SP in Spirazole® tablets.

Item	MZ	Reported HPLC method**	SP	Reported HPLC method**
Mean	98.19	98.3	99.41	99.05
SD	1.132	1.481	0.860	1.082
RSD%	1.269	1.500	0.865	1.090
n	5	5	5	5
Variance	1.281	2.193	0. 740	1.171
Student's t-test	0.089(2.31)*		0.393 (2.31)*	
F value	1.712(6.3382)*		1.582 (6.3382)*	

* The values in parentheses are the corresponding tabulated values at p=0.05. ** HPLC method (using phosphate buffer pH 2.4: acetonitrile, 70:30 v/v, C-18 column, flow rate 1 mL min^{-1}, at 232 nm).

References

1. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biological, 13th Ed., Merck & Co., Inc. (2002).
2. A.G. Gilman, L.E. Limbird, J.G. Hardman, Goodman & Gilman's, The Pharmacological Basics of Therapeutics, 9th ed., McGraw-Hill, New York. (1996).
3. K.C. Lamp, C.D. Freeman, N.E. Klutman, M.K. Lacy, *Clin. Pharmacokinet*. 36 (1999) 353.

4. British pharmacopoeia through internet communications, http://www.pharmacopoeia.co.uk/ixbin/bp.cgi (2008).
5. P. Mourier, A. Brun, *J. Chromatogr. B* 704 (1997) 197. 10.1016/S0378-4347(97)00477-5
6. K. Ninomiya, Antibiotics for Animals, Youkendo Press, Tokyo (1987) 307.
7. F. Li, Y.Y. Ye, W.P. Jia, *Chin. J. Pharm. Anal.* 26 (2006) 1311.
8. H. Yao, X.Z. Zhang, X.Z. Li, *Shaanxi Yaowu Fenxi Zazhi* 20 (2000) 198.
9. X. Xia, X.W. Li, J.Z. Shen, S.X. Zhang, S.Y. Ding, H.Y. Jiang, *J. AOAC Int.* 89 (2006)1116.
10. C. Sagan, A. Salvador, D. Dubreuil, P.P. Poulet, D. Duffaut, I. Brumpt, *J. Pharm. Biomed. Anal.* 38 (2005) 98. 10.1016/j.jpba.2004.12.033
11. D.K. Bempong, R.G. Manning, T. Mirza, L. Bhattacharyya, *J. Pharm. Biomed. Anal.* 38 (2005) 776. 10.1016/j.jpba.2005.02.019
12. Q.Y. Jiang, J. Liu, H.Y. Gu, *Yaowu Fenxi Zazhi* 26 (2006) 777.
13. Z.F. Fu, H. Chen, Z.J. Zhang, *Fenxi Shiyanshi* 23 (2004) 1.
14. A.A. Salem, H.A. Mossa, B.N. Barsoum, *J. Pharm. Biomed. Anal.* 41 (2006) 654. 10.1016/j.jpba.2005.12.009
15. W.R. Jin, W. Li, Q. Xu, Q. Dong, *Electrophoresis* 21 (2000) 1409. 10.1002/(SICI)1522-2683(20000401)21:7
16. B. Rezaei, S. Damiri, *Electrochim. Acta* 55 (2010) 1801. 10.1016/j.electacta.2009.10.070
17. X.M. Du, N.Y. Sun, L. Zhao, *Yaowu Fenxi Zazhi* 14 (1994) 56.
18. Y. Hua, F. Pan, *Zhongguo Yiyao Gongye Zazhi* 24 (1993) 179.
19. C. Sun, R. Yu, Q. Yang, S. Sheng, X. Zhao, *Yaoxue Xuebao* 22 (1987) 515.
20. M. Pendela, C. Govaerts, J. Diana, J. Hoogmartens, A. van Schepdael, E. Adams, *Rapid Comm. Mass Spectrometry* 21 (2007) 599. 10.1002/rcm.2868
21. W. Li, Y.M. Chu, H.M. Wen, G.Y. Liu, J. Zhang, *Yaowu Fenxi Zazhi* 26 (2006) 1465.
22. H.K. Chepkwony, A. Vermaelen, E. Roets, J. Hoogmartens, *Chromatographia* 54 (2001) 51. 10.1007/BF02491832
23. H.R. Gonzalez, Y.M. Li, A. Schepdael, E. Roets, J. Hoogmartens, *Electrophoresis* 20 (1999) 2407. 10.1002/(SICI)1522-2683(19990801)20:12<2412::AID-ELPS2412>3.0.CO;2-F
24. Y.F. Yang, B. Wang, *Electrophoresis* 17 (1996) 359. 10.1002/elps.1150170215
25. C. Situ, C.T. Elliott, *Anal. Chim. Acta* 529 (2005) 89. 10.1016/j.aca.2004.08.013
26. H.M. Maher, R.M. Youssef, R.H. Khalil, S.M. El-Bahr, *J. Chromatogr. B* 876 (2008) 175. 10.1016/j.jchromb.2008.10.033
27. H.M. Maher, R.M. Youssef, *Chromatographia* 69 (2009) 345. 10.1365/s10337-008-0865-2
28. E.E. Sideris, G.N. Valsami, M.A. Koupparis, P.E. Macheras, *Eur. J. Pharm. Sci.* 7 (1999) 271. 10.1016/S0928-0987(98)00035-9
29. M.S. Stanley, K.L. Busch, A. Vincze, *J. Planar. Chromatogr. Mod. TLC* 1 (1988) 76.
30. K. Prasad, R. Kala, T.P. Rao, G.R.K. Naidu, *Anal. Chim. Acta* 566 (2006) 69. 10.1016/j.aca.2006.02.064
31. M. Shamsipur, F. Mizani, M.F. Mousavi, N. Alizadeh, K. Alizadeh, H. Eshghid, H. Karami, *Anal. Chim. Acta* 589 (2007) 22. 10.1016/j.aca.2007.02.030
32. X. Ji, B. Jin, J. Ren, J. Jin, T. Nakamura, *J. Electroanal. Chem*. 579 (2005) 25. 10.1016/j.jelechem.2005.01.020

33. W. Peng, E. Wang, *Anal. Chim. Acta* 281 (1993) 663. 10.1016/0003-2670(93)85028-I
34. H.J. James, G. Carmack, H. Freiser, *Anal. Chem*. 44 (1972) 856. 10.1021/ac60312a046
35. M. Bender, M. Komiyama, In Cyclodextrin Chemistry; Springer: Berlin, Hiedelberg, Germany, 1978.
36. B. Kieser, C. Fietzek, R. Schmidt, G. Belge, U. Weimar, V. Schurig, G. Gauglitz, *Anal. Chem*. 74 (2002) 3005. 10.1021/ac015689k
37. D. Diamond, "Principles of Chemical, Biological Sensors", in: Chemical Analysis; Wiley, 150, (1998).
38. B.A. Conway, "Ion Selective Electrodes"; Ottawa Press (1995).
39. T. Isoda, N. Takahara, H. Imanaga, R. Imamura, S. Hasegawa, K. Noguchi , T. Kimura, *Sensors Actuators B: Chem.* 120 (2006) 1. 10.1016/j.snb.2006.01.039
40. R.M. Wightman, *Anal. Chem*. 53 (1981) 1125A. 10.1021/ac00232a004
41. A.M. Bond, *Analyst* 119 (1994) 1R. 10.1039/AN994190001R
42. A.M. El-Kosasy, M.Y. Salem, G.M. El-Bardicy, M.K. El-Rahman, *Chem. Pharm. Bull.* 56 (2008) 753.
43. L. Wang, J. Wang, F. Zhou, *Electroanalysis* 16 (2004) 627. 10.1002/elan.200302849
44. T.E. Edmonds, *Anal. Chim Acta* 175 (1985) 1. 10.1016/S0003-2670(00)82713-0
45. M.C.B.S.M. Montenegro, J.L.F.C. Lima, *Microchim. M. Acta* 131 (1999) 187. 10.1007/PL00010030
46. J.L.F.C. Lima, M.C.B.S.M. Montenegro, A.M.R da Silva, *J. Pharm. Biomed. Anal*. 8 (1990) 701. 10.1016/0731-7085(90)80106-Y
47. S.S. Hassan, M.M. Amer, S.A. Abd El-Fatah, A.M. El-Kosasy, *Talanta* 46 (1998) 1395. 10.1016/S0039-9140(98)00008-3
48. Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, S. Amemiya, *Pure Appl. Chem.* 72 (2000) 1851. 10.1351/pac200072101851