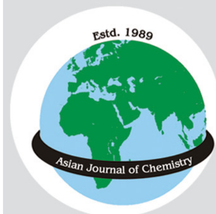

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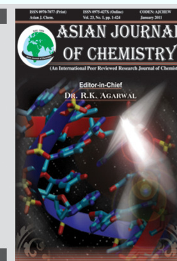

H-Point Standard Addition Method for Simultaneous Spectrophotometric Determination of Hydrochlorothiazide and Furosemide

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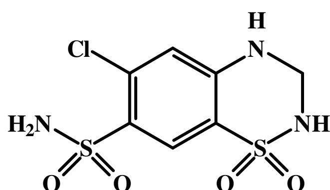
The applicability of H-point standard addition method for resolving of overlapping spectra corresponding to the hydrochlorothiazide and furosemide is verified. The results show that the H-point standard addition method is suitable for the simultaneous determination of hydrochlorothiazide and furosemide in aqueous media. The results of applying the H-point standard addition method showed that the two drugs could be determined simultaneously with the concentration ratios of hydrochlorothiazide to furosemide varying from 1:40-20:1 in the mixed sample. The proposed method has been successfully applied to the simultaneous determination of hydrochlorothiazide in the presence of furosemide in some synthetic samples. Moreover, the applicability of the method was demonstrated by the recovery of species in some biological fluids.

Key Words: Hydrochlorothiazide, Furosemide, UV-Vis spectrophotometry, H-Point standard addition method.

INTRODUCTION

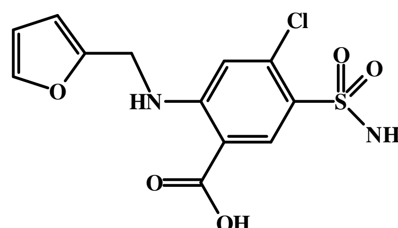
Diuretics are an important group of the drugs used in various clinical situations such as cardiac and renal insufficiency, nefrotic syndrome, edema, cirrhosis and hypertension[1].

Hydrochlorothiazide (6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide), is a popular diuretic drug of the thiazide class which acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Hydrochlorothiazide is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. It is effective for nephrogenic diabetes insipidus (paradoxical effect, which decreases urine formation) and is also sometimes used for hypercalciuria and Dent's disease.



Scheme-I: Chemical structure of hydrochlorothiazide

Furosemide (4-chloro-N-furfuryl-5-sulphamoyl anthranilic acid), belongs to the class of loop diuretics and it is prescribed mainly for the control of the hypertension associated to renal and cardiac insufficiency[2]. This drug is a potent diuretic that inhibits the reabsorption of electrolytes in the ascending limb of the loop of Henle and also in the renal tubules. While furosemide has no clinically significant effect on carbonic anhydrase, it enhances water excretion, increasing loss of sodium, chloride and potassium ions[3].



Scheme-II: Chemical structure of furosemide

Synergism effect between high-dose furosemide and hydrochlorothiazide in patients with severe congestive heart failure and impaired renal function shows diuretic resistance to a daily dose of furosemide of at least 250 mg[4]. The results also indicate that combined hydrochlorothiazide-furosemide is a potent diuretic regimen and is effective in many patients

with chronic renal failure who have a poor response to furosemide alone[5].

Being widely used drugs, several methods have been reported for their determination in pharmaceutical formulations. Hydrochlorothiazide have been determined by liquid chromatography/tandem mass spectrommetry[6,7], HPLC[8] and time resolved chemiluminescence[9] while furosemide includes spectrophotometry[10], fluorescence spectroscopy[11], coulometry[12] and HPLC[13].

According to our knowledge, there isn't any proposed method for simultaneous determination of hydrochlorothiazide and furosemide in pharmaceutical dosage forms. However, the lack of specificity of the UV-visible absorption usually hinders the application of this technique in case of mixtures of absorbing species, due to spectral overlap. In the present work, a very simple, sensitive, selective and low cost procedure for simultaneous spectrophotometric determination of hydrochlorothiazide and furosemide using H-point standard addition method (HPSAM) is described. The method is based on the determination of the absorbance of hydrochlorothiazide and furosemide complexes in an appropriate wavelength pair.

EXPERIMENTAL

All the chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) unless stated otherwise. Doubly distilled water was used throughout. Hydrochlorothiazide and furosemide were purchased from Darou Pakhsh and Iran Hormon pharmaceutical companies (Tehran, Iran), respectively.

A 1.0×10^{-3} mol L^{-1} hydrochlorothiazide solution was prepared daily by dissolving 0.0298 g hydrochlorothiazide (99 %) in water and the solution was diluted to 100 mL with water in a 100 mL volumetric flask. The solution was kept in a refrigerator at 4 ºC and in dark. More dilute solutions were prepared by serial dilution with water.

A 1.2×10^{-3} mol L^{-1} furosemide solution was prepared daily by diluting a stock solution (10 mg mL^{-1}) by water to 50 mL in a 50 mL volumetric flask. The solution was kept in a refrigerator at 4 ºC in dark. More dilute solutions were prepared by serial dilution with water. Britton-Robinson (B-R) buffers (0.1 mol L^{-1} in phosphate, acetate and borate) in the pH range of 2-9 were used, throughout.

UV-Vis absorption spectra are measured on an Agilent UV-vis spectrophotometer, Perkin-Elmer (Lambda 25), with the use of 1.0 cm quartz cells. A Pentium IV (2.53 MHz) computer controlled all of the setting and data processing. A pH-meter (Metrohm, Model 691) with a double junction glass electrode was used to check the pH of the solutions.

Preparation of real samples: Urine samples were filtered through a cellulose acetate filter (0.2 μm pore size, Supelco) and collected in dark glass bottles previously cleaned with HCl and washed with deionized water. The samples were stored in darkness at 4 ºC until the analyses were performed.

Serum samples were obtained and stored frozen until the assay. In each of 10 centrifugation tubes containing a certain concentration of hydrochlorothiazide and/or furosemide, 0.10 mL of the human serum sample was transferred and then mixed well with 1.0 mL of methanol to precipitate the blood proteins. The precipitated proteins were separated by centrifuging of the mixture for 20 min at 4000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to produce protein-free human serum. The human serum sample was transferred into a 25 mL calibrated flask, completed to the volume with B-R buffer of pH 9.0 and then introduced to the optical cell.

Recommended procedure: An aliquot of the solution containing hydrochlorothiazide and/or furosemide and 1 mL Britton-Robinson buffer solution (pH 9.0) were added into a 10 mL volumetric flask and made up to the mark with water. The solution was then allowed to stand for 10 min at room temperature. After that a portion of the solution was transferred into a quartz cell to measure its absorbance at appropriate wavelength pairs (270 and 283 nm for determination of hydrochlorothiazide and 259 and 280.5 nm for determination of furosemide). The concentration ranges of hydrochlorothiazide and furosemide for construction of HPSAM calibration graph were 10-60 μmol L^{-1} for both species.

RESULTS AND DISCUSSION

The absorption spectra of hydrochlorothiazide and furosemide under certain experimental conditions are shown in Fig. 1. As can be seen in Fig. 1, the maximum wavelengths of two compounds are very close to each other and their spectra are highly overlapped. Therefore, determination of hydrochlorothiazide and furosemide in the presence of each other is impossible by classical spectrophotometry. Therefore, it is necessary to use a chemometrics method to solve this problem.

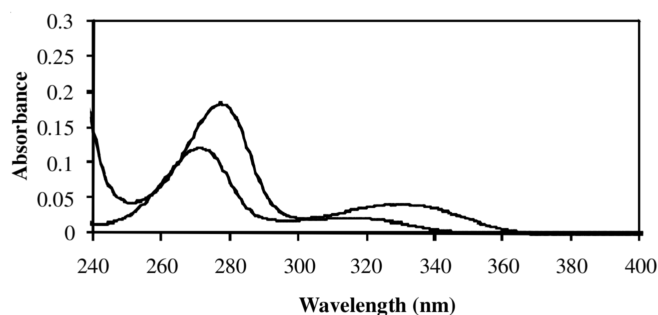


Fig. 1. Absorption spectra of (a) 10 μmol L^{-1} hydrochlorothiazide (b) 10 μmol L^{-1} furosemide at pH 5.0

Effect of operational parameters: In order to optimize the procedure for the simultaneous determination of hydrochlorothiazide and furosemide, we studied the effect of chemical parameters involving pH and KCl concentrations on the maximum absorbance of two species separately.

As it is shown in Fig. 2, there weren't significant changes in the absorption spectra of hydrochlorothiazide and furosemide in the pH range 2-10, but, at pH > 4.0 the overlap between two spectra decreased. Therefore pH of 9.0 was selected for obtaining higher selectivity.

H-Point standard addition method: Consider an unknown sample containing an analyte X and an interferent Y. In this special system, hydrochlorothiazide and furosemide were considered as the analyte and interferent, respectively. The determination of concentration of X by HPSAM under these conditions requires the selection of two wavelengths λ_1

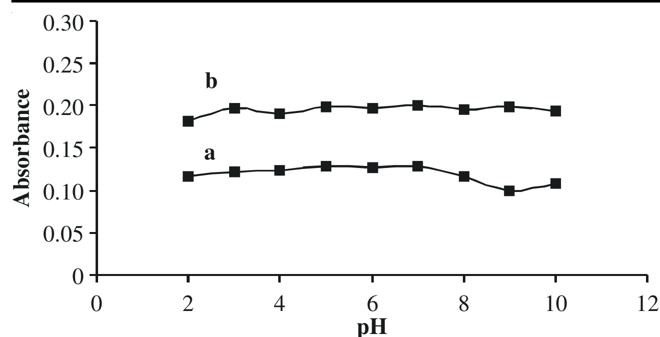


Fig. 2. Effect of pH on the maximum absorption spectra of (a) 10 μmol L^{-1} hydrochlorothiazide and (b) 10 μmol L^{-1} furosemide

and λ_2, at which the interferent species, Y, has the same absorbance[14,15]. Then, known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two wavelengths and expressed by the following equations:

$$A_{(\lambda_1)} = b_0 + b + M_{\lambda_1} C_i \quad (1)$$

$$A_{(\lambda_2)} = A_0 + A + M_{\lambda_2} C_i \quad (2)$$

where, $A_{(\lambda_1)}$ and $A_{(\lambda_2)}$ are the analytical signals measured at λ_1 and λ_2, respectively. b_0 and $A_0 (b_0 \neq A_0)$ are the original analytical signal of X at $A_{(\lambda_1)}$ and $A_{(\lambda_2)}$, respectively. b and A are the analytical signals of Y at $A_{(\lambda_1)}$ and $A_{(\lambda_2)}$, respectively. M_{λ_1} and M_{λ_2} are the slopes of the standard addition calibration lines at λ_1 and λ_2, respectively and C_i is the added X concentration. The two straight lines obtained intersect at the so-called H-point ($-C_H$, A_H).

At H-point, since $A_{(\lambda_1)} = A_{(\lambda_2)}, C_i = C_H$, from eqn. 1 and 2 it follows that:

$$b_0 + b + M_{\lambda_1}(-C_H) = A_0 + A + M_{\lambda_2}(-C_H) \quad (3)$$

$$-C_H = \frac{[(A_0 - b_0) + (A - b)]}{(M_{\lambda_1} - M_{\lambda_2})} \quad (4)$$

From eqn. 4, if the component Y, is the known interferent and the analytical signal corresponding to Y, b (at λ_1 or λ_2) do not change with the additions of analyte, X, that is, b = A = constant, so:

$$-C_H = \frac{(A_0 - b_0)}{(M_{\lambda_1} - M_{\lambda_2})} = -\frac{b_0}{M_{\lambda_1}} \quad (5)$$

$$= -\frac{A_0}{M_{\lambda_2}} \quad (6)$$

where, $C_H = C_X$ corresponds to the analyte concentration in the mixture, because $-C_H$ depends only on variables related to the analyte[16].

If the value of $-C_H$ is included in eqn. 1, A_H, the ordinate value of the intersection point, will be described as follows:

$$A_H = b_0 + b + M_{\lambda_1}(-C_H) \quad (7)$$

as $b_0 = M_{\lambda_1} C_H$,

then $A_H = b$

and similarly, $A_H = A$ (8)

Hence, A_H value is only related to the signal of the interferent Y at the two selected wavelengths. To evaluate the interferent concentration from the ordinate value of the H-point (A_H), a calibration graph or the absorbance value of an interferent standard is needed. Similarly, for determination of Y by HPSAM under these conditions, selection of two wavelengths λ_1 and λ_2, at which the species X, has the same absorbance is possible.

Wavelength selection: To select the appropriate wavelength pair for using HPSAM the following principles should be applied. At these selected wavelengths the analyte signals must be linear with concentrations and the interferent signal must be equal remains unchanged by changing the analyte concentration, the analytical signal obtained from a mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths (λ_1 and λ_2) must be as large as possible in order to get good accuracy and sensitivity[16-19].

For determination of hydrochlorothiazide and furosemide, we selected two pairs of wavelengths on the hydrochlorothiazide and furosemide spectra. In this case there were several pairs of wavelengths. As it is observed from Fig. 1, the best wavelength pairs were 270-283 and 259-280.5 nm for determination of hydrochlorothiazide and furosemide, respectively. Standard solutions of hydrochlorothiazide and furosemide were initially tested to validate the applicability of the chosen wavelengths. Figs. 3 and 4 are H-point standard addition calibration lines constructed at two selected wavelengths (259-280.5 nm) for determination of furosemide in the present of hydrochlorothiazide. According to the characteristics of HPSAM at the H-point, C_H is independent of interferent concentration, but A_H is dependent of the interferent.

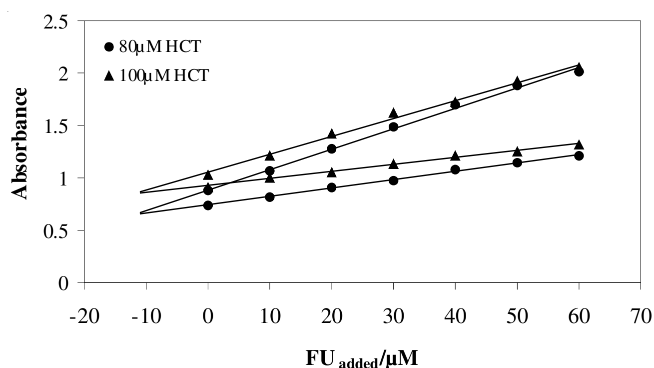


Fig. 3. H-Point standard addition plot for fixed furosemide concentration (10 μmol L^{-1}) and different concentrations of hydrochlorothiazide at pH 9.0

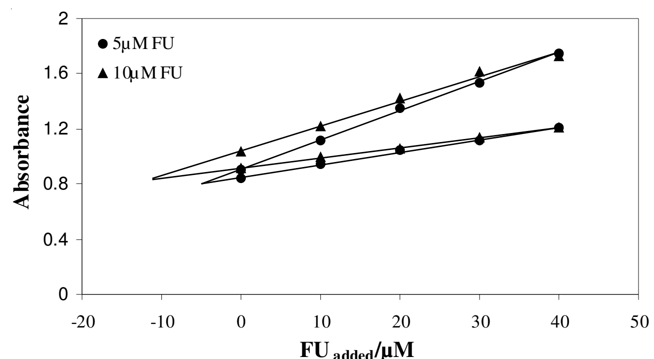


Fig. 4. H-Point standard addition plot for fixed hydrochlorothiazide concentration (100 μmol L^{-1}) and different concentration of furosemide at pH 9.0

TABLE-1
DETERMINATION OF HYDROCHLOROTHIAZIDE AND FUROSEMIDE IN SOME SYNTHETIC MIXTURES

A-C equation	R^2	Added (µmol L^{-1})		Found (µmol L^{-1})	
		Hydrochlorothiazide	Furosemide	Hydrochlorothiazide	Furosemide
A270 = 0.0117C + 0.2887 A283 = 0.0048C + 0.2157 A280.5 = 0.0146C + 0.244 A259 = 0.0065C + 0.1554	0.9990 0.9994 0.9947 0.9928	10	10	10.6	10.9
A270 = 0.0113C + 0.4503 A283 = 0.0043C + 0.3864 A280.5 = 0.0214C + 0.5363 A259 = 0.0074C + 0.2293	0.9960 0.9836 0.9976 0.9939	10	20	9.1	21.9
A270 = 0.0112C + 0.4194 A283 = 0.004C + 0.2771 A280.5 = 0.016C + 0.3049 A259 = 0.0066C + 0.219	0.9912 0.9570 0.9982 0.9836	20	10	19.8	9.1
A270 = 0.0123C + 1.3445 A283 = 0.0053C + 1.0196 A280.5 = 0.0101C + 0.5451 A259 = 0.0039C + 0.2328	0.9948 0.9836 0.9971 0.9829	50	50	46.4	50.4
A270 = 0.0153C + 0.9726 A283 = 0.006C + 0.773 A280.5 = 0.0177C + 0.9504 A259 = 0.0065C + 0.4374	0.9938 0.9874 0.9928 0.9893	20	40	21.5	44.6
A270 = 0.0159C + 0.9502 A283 = 0.0064C + 0.5693 A280.5 = 0.0193C + 0.7209 A259 = 0.0075C + 0.4418	0.9948 0.9856 0.9980 0.9946	40	20	40.1	23.6

TABLE-2
RESULTS OF THE REPLICATE MEASUREMENTS FOR DETERMINATION OF HYDROCHLOROTHIAZIDE AND FUROSEMIDE IN SYNTHETIC MIXTURES

A-C equation	R^2	Present in the sample (µmol L^{-1})		Found (µmol L^{-1})	
		Hydrochlorothiazide	Furosemide	Hydrochlorothiazide	Furosemide
A270 = 0.0164C + 0.2476 A283 = 0.0064C + 0.1655 A280.5 = 0.0191C + 0.2069 A259 = 0.0073C + 0.1105	0.9990 0.9989 0.9996 0.9993	8.0	8.0	8.2	8.2
A270 = 0.0165C + 0.2370 A383 = 0.0062C + 0.1570 A280.5 = 0.0189C + 0.2132 A259 = 0.0072C + 0.1135	0.9985 0.9957 0.9997 0.9994	8.0	8.0	7.8	8.5
A270 = 0.0161C + 0.2477 A283 = 0.0060C + 0.1620 A280.5 = 0.0188C + 0.2162 A259 = 0.0074C + 0.1193	0.9997 0.9988 0.9992 0.9979	8.0	8.0	8.5	8.5
A270 = 0.0159C + 0.2449 A283 = 0.0059C + 0.1689 A280.5 = 0.0187C + 0.2127 A259 = 0.0073C + 0.1174	0.9990 0.9985 0.9974 0.9960	8.0	8.0	7.6	8.3
Average				8.0	8.4
Standard deviation				0.4	0.1
A270 = 0.0147C + 1.7917 A283 = 0.0060C + 1.2792 A280.5 = 0.0191C + 1.5873 A259 = 0.0080C + 0.8876	0.9927 0.9988 0.9963 0.9972	60.0	60.0	58.9	63.0
A270 = 0.0168C + 1.8898 A283 = 0.0068C + 1.2909 A280.5 = 0.0193C + 1.6078 A259 = 0.0081C + 0.8930	0.9967 0.9964 0.9935 0.9923	60.0	60.0	59.4	61.1
A270 = 0.0167C + 1.9369 A283 = 0.0064C + 1.3340 A280.5 = 0.0188C + 1.5990 A259 = 0.0077C + 0.8909	0.9941 0.9777 0.9996 0.9985	60.0	60.0	58.5	63.8
A270 = 0.0144C + 1.8146 A283 = 0.0060C + 1.2936 A280.5 = 0.0196C + 1.5771 A259 = 0.0083C + 0.8852	0.9977 0.9936 0.9945 0.9982	60.0	60.0	62.0	61.2
Average				59.7	62.3
Standard deviation				1.6	1.3

TABLE-3
DETERMINATION OF HYDROCHLOROTHIAZIDE AND FUROSEMIDE IN SOME BIOLOGICAL FLUIDS (n = 3)

Sample	Added (μmol L^{-1})		Found (μmol L^{-1})		Recovery (%)	
	HCT	FU	HCT	FU	HCT	FU
Whole blood	–	–	< Detection limit	< Detection limit	–	–
	10	10	9.9 ± 1.3	9.8 ± 1.4	99.0	98.0
Plasma	–	–	< Detection limit	< Detection limit	–	–
	20	20	20.3 ± 2.5	31.5 ± 1.9	101.5	105.0
	50	50	50.3 ± 3.9	50.8 ± 4.1	100.6	101.6
Urine	–	–	< Detection limit	< Detection limit	–	–
	30	10	30.2 ± 1.2	11.3 ± 0.7	100.7	113.0
	50	20	49.2 ± 1.9	21.5 ± 2.2	98.4	107.5

*Detection limit.

Accuracy: Several synthetic samples with a different concentration ratio of hydrochlorothiazide and furosemide were analyzed by using the suggested method. As can be seen from Table-1, the accuracy of the results is satisfactory in all cases, when the concentration ratio of hydrochlorothiazide and furosemide vary from 1:40-20:1.

Reproducibility of the method: To check the reproducibility of the method four replicate experiments for the analysis of hydrochlorothiazide and furosemide mixtures were done (Table-2). A good standard deviation was obtained for two pharmaceutical compounds.

Application: In addition for showing the applicability of the method, biological fluids with complex matrixes were spiked with hydrochlorothiazide and furosemide and proposed method was applied for determination of the species. The results are shown in Table-3. It can be seen that the results are all satisfactory.

Limit of detection: Limit of detection was calculated as LOD = $3S_{CH}$[17], were S_{CH} is the standard deviation of several (n = 6) replicated measurements of zero concentration of analyte with the HPSAM. The corresponding values obtained for hydrochlorothiazide and furosemide were 0.81 and 0.45 μmol L^{-1}, respectively.

Interferents: For analyzing interference, the influence of several species was tested, including those that most frequently accompany with hydrochlorothiazide and furosemide in real samples. The effect of interfering species at different concentrations on the current response of a solution containing 10 μmol L^{-1} of each analyte was studied. An ion was considered to interfere when its presence produced a variation in the absorbance of the sample (at two wavelengths) greater than ± 10 %. The ions Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Li^+, K^+, Br^- and Cl^- did not interfere at the 1000 times more than pharmaceutical compounds. Other antihypertension drugs such as nalidixic acid, captopril and propranolol, did not interfere up to 10 times. Among the pharmaceutical drugs examined, most did not interfere at high level except losartan, hydralazine, methoprolol and ciprophеloxacin which would be tolerated at low concentration levels but these drugs do not uses in a combination form with hydrochlorothiazde or furosemide.

Conclusion

The suggested method shows that application of HPSAM can be well adopted for resolving binary mixtures of hydrochlorothiazide and furosemide. The proposed method provides satisfactory results in synthetic and real mixtures. The method also offers good selectivity, accuracy and precision that can be applied for varying hydrochlorothiazide and furosemide concentration ratio.

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REFERENCES

1. J.M. Wright, C.H. Lee and E.K. Chamber, *Can. Med. Assoc. J.*, **161**, 25 (1999).
2. L.S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, edn. 10 (2001).
3. W. Martindale and W. Wynn, The Extra Pharmacopeia, The Pharmaceutical Press, London (1989).
4. T.P.J. Dormans and P.G.G. Gerlag, *Eur. Heart J.*, **17**, 1867 (1996).
5. G.L. Wollam, R.C. Tarazi, E.L. Bravo and H.P. Dustan, *Am. J. Med.*, **72**, 929 (1982).
6. F. Liu, Y. Xu, S. Gao, J. Zhang and Q. Guo, *J. Pharm. Biomed. Anal.*, **44**, 1187 (2007).
7. H. Li, Y. Wang, Y. Jiang, Y. Tang, J. Wang, L. Zhao and J. Gu, *J. Chromatogr. B*, **852**, 436 (2007).
8. T. Huang, Z. He, B. Yang, L. Shao, X.I. Zheng and G. Duan, *J. Pharm. Biomed. Anal.*, **41**, 644 (2006).
9. J.A.M. Pulgarin, A.A. Molina and G.P. Olivares, *Anal. Chim. Acta*, **518**, 37 (2004).
10. A.S. Cabeza, P.C. Falco and M.C.S. Garcia, *Anal. Lett.*, **30**, 91 (1997).
11. F.G. Sanchez, A.F. Gutierrez and C.C. Blanco, *Anal. Chim. Acta*, **306**, 313 (1995).
12. K.I. Nikolic and K. Velasevic, *J. Pharm. Belg.*, **44**, 387 (1989).
13. M.B. Barroso, R.M. Jimenez, R.M. Alonso and E. Oritz, *J. Chromatogr. Biomed. Appl.*, **675**, 303 (1996).
14. F.B. Reig and P.C. Falco, *Analyst*, **113**, 1011 (1988).
15. F.B. Reig, P.C. Falco, B.H. Hernandez and A.S. Cabeza, *Anal. Chim. Acta*, **257**, 89 (1992).
16. A.Safavi and H. Abdollahi, *Talanta*, **54**, 727 (2001).
17. E. Shams, H. Abdollahi, M. Yekehtaz and R. Hajian, *Talanta*, **63**, 359 (2004).
18. E. Shams, H. Abdollahi and R. Hajian, *Electroanalysis*, **17**, 1589 (2005).
19. A.A. Ensafi and R. Hajian, *Anal. Chim. Acta*, **580**, 236 (2006).