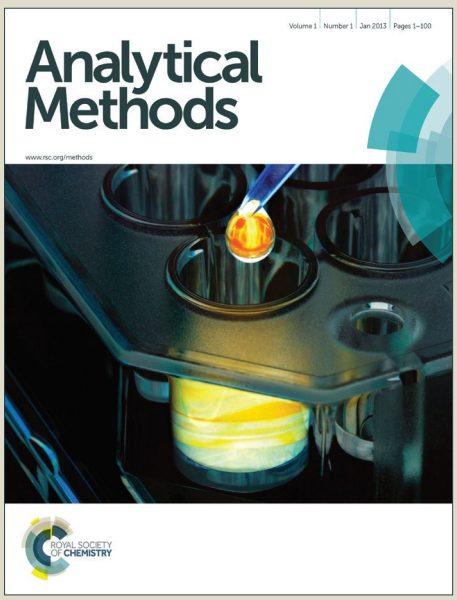
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Determination of metoprolol tartrate and bisoprolol fumarate by capillary electrophoresis coupled with tris(2, 2'-bipyridyl)-ruthenium(II) electrochemiluminescence detection and study on interaction between the drugs and human serum albumin

*Hong-Bing Duan, Jun-Tao Cao, Hui Wang, Yan-Ming Liu**

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A new method for separation and determination of metoprolol tartrate (ME) and bisoprolol fumarate (BF) by capillary electrophoresis (CE) coupled with tris(2, 2'-bipyridyl)-ruthenium(II) electrochemiluminescence (ECL) detection was developed. The parameters including pH and concentration of the running buffer, separation voltage and detection potential that affected CE separation and ECL detection were optimized. Under the optimized conditions, ME and BF were well separated and detected within 10 min, the limits of detection (LODs *S/N* = 3) are 1.9×10^{-8} mol L^{-1} for ME and 9.4×10^{-8} mol L^{-1} for BF, separately. RSDs of the peak area of ME and BF (n = 3) are 2.1% and 3.8% within a day (intraday), 3.1% and 5.5% in 3 consecutive days (interday). RSDs of the migration time of ME and BF are 4.5 and 6.7% for intraday, 7.4 and 7.9% for interday. The limits of quantitation (LOQs *S/N* = 10) in human urine sample are 3.3×10^{-7} mol L^{-1} for ME and 1.4×10^{-6} mol L^{-1} for BF. The recoveries (n = 3) of ME and BF in human urine are from 89.0 to 126.0% with less than 7.4% in RSD. This method was also successfully applied to the determination of ME and BF in human urine and study on interaction between ME or BF and human serum albumin (HSA). The number of binding sites of ME and BF with HSA are 1.2 and 1.1, and the binding constants are 2.8×10^{3} and 2.7×10^{3} L mol^{-1}, respectively.

Introduction

β-blockers such as ME and BF are a class of adrenergic drugs that block the action of endogenous catecholamine (adrenaline and noradrenaline) on β-adrenergic receptors.[1] They are widely used in the treatment of several diseases such as hypertension, arrhythmias, angina pectoris and other cardiovascular diseases.[2] However, an overdose of β-blockers may lead to bradycardia, hypotension, aggravation of cardiac failure, bronchospasm, hypoglycemia, and fatigue.[3] In addition, these drugs are also consumed in sports and other stressing activities as doping agents and excess of β-blockers is toxic.[4] Several methods have been reported for the determination of β-blockers such as spectrophotometry,[5] high performance liquid chromatography (HPLC),[6-8] gas chromatography (GC),[9,10] colorimetry,[11] and voltammetry.[12] Capillary electrophoresis (CE) coupled with electrochemiluminescence (ECL),[13,14] CE-diode array detection (DAD),[15,16] pressureassisted capillary electrochromatography-electrospray ionization mass spectrometry (pCEC-ESI-MS),[17] and heart cutting two dimensional CE-electrochemical detection (ED)[18] were also developed for β-blockers analysis.

Human serum albumin (HSA), which is the most abundant protein in the human blood circulatory system, plays a critical role in the transport and deposition of a variety of endogenous and exogenous compounds.[19] As the most rich protein constituent in the human blood stream, HSA have been widely studied.[20,21] HSA can binds and carry through the blood-stream many drugs, which can increase drug solubility in plasma, decrease its toxicity and can affect the biological activity[22] and toxicity[23] of drugs. Understanding the interaction between HSA and drugs can facilitate the identification of novel drugs and the improvement of existing medicines. Knowledge of the binding parameters is helpful for better understanding of the absorption and distribution of drug molecules.

CE is considered as a relatively effective separation technique that can be used to separate almost all of the substance. It becomes an important analytical tool owing to its well-recognized advantages such as powerful separation efficiency, short analysis time, low consumption of sample and reagents, and ease of installation, operation, and maintenance, which become a particularly interesting candidate for drug analysis.[24-26] ECL detection is a special chemiluminescence where chemiluminescence emission correlates directly or indirectly with oxidation or reduction at an electrode surface. It is well known as a powerful analytical technique with the advantages of high sensitivity, wide linear range, and simple instrumentation and has become a sensitive and valuable detection tool.[27,28] Tris(2, 2'-

bipyridyl)ruthenium(II), namely $Ru(bpy)_3^{2+}$, may be the most efficient and widely used ECL reagent due to high luminescence efficiency and stability in aqueous media.[29] CE combining with ECL (CE-ECL) has the advantages of good resolution, high sensitivity, fast speed, low reagent consumption and wide linear range.[30-35] The method for the simultaneous separation and detection of ME and BF using CE-ECL has not been reported.

In this work, a new CE-ECL method for detection of ME and BF was described based on CE separation with end-column $Ru(bpy)_3^{2+}$ ECL detection. The conditions for CE separation and ECL detection were investigated. The proposed method was also used for the study of the interaction between HSA and ME and BF. The number of binding sites and binding constant of HSA and ME and BF were estimated.

Experimental

Apparatus

A MPI-A system was produced by Xi'an Remex Electronic Science-Tech Co., Ltd. (Xi'an, China) and employed for CE-ECL detection. The system include four main parts: a programmable high-voltage power supply (0-20 kV), a multifunction chemiluminescence detector, an electrochemical potentiostat and a multichannel data collection analyzer. All data collection was performed with MPI-A analysis software and recorded on a computer. CE separation was performed in a 50 cm uncoated fused-silica capillary with 50 μm i.d. and 375 μm o.d. obtained from Yongnian Reafine Chromatography Ltd. (Hebei, China). A conventional three-electrode system was situated in the end-column ECL detection cell, along with a Pt wire auxiliary electrode, a 500 μm diameter Pt disc working electrode and a Ag/AgCl (KCl saturated) reference electrode. UV-visible absorption spectra were recorded by an UVmini-1240 UV-vis spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Chemicals and solutions

Tris(2, 2'-bipyridyl)-ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2$), HSA (>96%, Mw = 65.3 kDa) were purchased from Sigma-Aldrich (St Louis, MO, USA). ME and BF were acquired from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Triton X-100, Triton X-114, Triton X-165, Triton X-305, Triton X-405, Triton X-705, Tween 20 and Sodium dodecyl sulfate (SDS) were achieved from Seebio Biotechnique Co. Ltd. (Shanghai, China). PVP (M_w = 1 300 000) was got from Alfa Aesar (A Johnson Matthey Company, Ward Hill, MA, USA). Ultrapure fresh water (18.2 MΩ cm^{-1}) used was processed with an ultrapure water system (Kangning Water Treatment Solution Provider, China). All solutions were stored under refrigeration at 4 °C and filtered through 0.22 μm cellulose acetate membrane filters (Shanghai Xingya Purification Material Factory, China) before use.

Procedure

New capillary was activated before use by 2.0 mol L^{-1} CH_3OH-NaOH (2 g NaOH dissolved in 25 mL 4:1/methanol:water solution, v/v), 1.0 mol L^{-1} NaOH, 1.0 mol L^{-1} HCl, H_2O, and finally with running buffer for 30 min. At the beginning use of each day, the capillary was flushed with 0.1 mol L^{-1} NaOH, water, and equilibrated with the running buffer for 5 min successively to maintain an active and reproducible capillary inner surface. The voltage of photomultiplier tube (PMT CR105, Beijing Binsong Photonics, China) for collecting the ECL signal was set at -850 V in the process of detection. The detection potential applied to the working electrode was fixed at 1.25 V. Electrokinetic injections were performed at 10 kV for 10 s. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ with 50 mmol L^{-1} phosphate buffer solution (PBS) was added in the detection cell. The peak area was used for the analysis.

Preparation of the human urine sample

Urine samples of a healthy male volunteer were from Xinyang Normal University. The complete ethical approval had been obtained, and the male volunteer gave written informed consent. The study was performed in compliance with the relevant laws and institutional guidelines and also approved by the Institutional Review Board of Xinyang Normal University. Prior to analysis, the fresh urine samples were immediately centrifuged for 10 min at 2000 rpm to remove deposit. The urine sample was diluted 100-fold with pure water and then filtered through 0.22 μm cellulose acetate filters.

Results and discussion

Cyclic voltammetry of $Ru(bpy)_3^{2+}$ and ME or BF

In the CE-ECL system, the ECL intensity depends upon the rate of the light-emitting chemical reaction which is turning dependent on the potential applied to the electrode. Fig. 1A shows cyclic voltammograms of 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ in 50 mmol L^{-1} PBS (pH 8.0) before and after adding BF and ME, respectively. The oxidation peak currents of BF and ME could be distinguished from the background current. The corresponding ECL curves were illustrated in Fig. 1B. Compared with the background curve (curve a in Fig. 1B), curve b and c in Fig. 1B reveal that there was an obvious increase of ECL intensity. The observations indicate that BF and ME could react with $Ru(bpy)_3^{2+}$ and enhance the ECL intensity. The results suggest that the proposed CE-ECL method could be used for the sensitive detection of ME and BF.

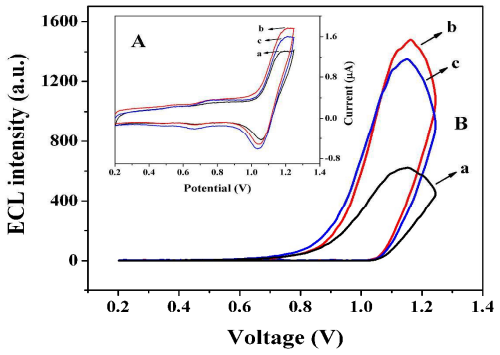


Fig. 1. Cyclic voltammograms (A) and their corresponding ECL curves (B). (a) 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ + 50 mmol L^{-1} PBS (pH 8.0); (b) a + 2 μmol L^{-1} ME ; (c) a + 2 μmol L^{-1} BF; scan rate, 100 mV/s.

Choice of the additive in running buffer

Since the physico-chemical properties and structures of ME and BF are similar, simultaneous separation of them is an obstacle. The preliminary experimental results show that the baseline separation and detection of ME and BF was not achieved when single PBS was used as running buffer. In order to obtain good separation and high ECL intensity, we choose 5 mmol L^{-1} SDS, 0.01% PVP, 0.05% Tween 20 and 0.05% different kinds of Triton (Triton X-100, Triton X-114, Triton X-165, Triton X-305, Triton X-405 and Triton X-705) (v/v) as additive respective into the running buffer. It can be found that there were no obvious improvements in resolution for all of these additives except for Triton X-100. The effect of Triton X-100 concentration was investigated in the range of 0.03-0.07%. The ECL intensity increased with the Triton X-100 concentration increasing in the range 0.03-0.05%, and reached the largest signal intensity at 0.05%. Further increasing concentration of Triton X-100 decreased ECL intensity. So, 0.05% Triton X-100 was chosen.

Optimization of pH and concentration of the running buffer

The influence of the running buffer pH was tested within the pH range from 6.0 to 10.0. The ECL intensity increased as pH value changed from 6.0 to 8.0, and then decreased when the pH value exceeded 8.0. The effect of concentration of the running buffer from 10 to 30 mmol L^{-1} was researched and found that the highest ECL intensity was acquired as the buffer concentration was 20 mmol L^{-1}.

Optimization of separation voltage

The separation voltage directly influences the migration time, resolution and the ECL intensity of analyte. The effects of separation voltage were examined varying from 12 to 18 kV. For both of ME and BF, both the ECL intensity and the resolution reached a maximum value at 14 kV and then decreased. So 14 kV was selected.

Optimization of detection potential

The effects of detection potential on the ECL intensity of ME and BF were considered. When detection potential changed from 1.10 to 1.35 V, the ECL intensity of both ME and BF reached a maximum at 1.25 V. So 1.25 V was used.

Performance characteristics of the method

Under the optimized conditions: ECL separation at 14 kV, 20 mmol L^{-1} PBS containing 0.05% Triton X-100 at pH 8.0 as running buffer, 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ with 50 mmol L^{-1} PBS at pH 8.0 in the detection cell, electrokinetic injection for 10 s at 10 kV, detection at 1.25 V, ME and BF were well separated within 10 min. Fig. 2 presents the typical electropherogram of the mixed standard solution of ME and BF.

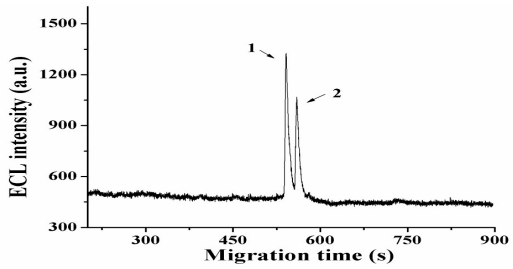


Fig. 2. Electropherograms of standard solution. Peak 1: 1 μmol L^{-1} ME; Peak 2: 1 μmol L^{-1} BF. Conditions: running buffer, 20 mmol L^{-1} PBS containing 0.05% Triton X-100 at pH 8.0; injection, 10 kV 10 s; separation voltage, 14 kV; detection potential, 1.25 V; ECL detection solution, 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ with 50 mmol L^{-1} PBS at pH 8.0.

The calibration curves were calculated by plotting the peak area values against the concentration of analytes. The results were listed in Table 1. It can be seen from Table 1 that the regression coefficients of the calibration curves are greater than 0.996. The LOD was considered the minimum analyte concentration in standard solution yielding an S/N ratio equal to three. The LOQ[36] was defined as the lowest analyte concentration in real sample (human urine) yielding an S/N of ten.

The precisions (measured by RSD %, $n = 3$) of the peak area of 0.5 μmol L^{-1} ME and 10 μmol L^{-1} BF are 2.1% and 3.8% within a day (intraday), 3.1% and 5.5% in 3 consecutive days (interday, three consecutive days and three consecutive times per day), RSDs of the migration time of ME and BF are 4.5% and 6.7% for intraday, 7.4% and 7.9% for interday, respectively.

Table 1. The performance characteristics of the proposed method.

Analytes	Linear range (μmol L^{-1})	Calibration curves			LOD (mol L^{-1})	LOQ (mol L^{-1}) in urine
		Slope	Intercept	r		
ME	0.05-60	2271	19570	0.998	1.9×10^{-8}	3.3×10^{-7}
BF	0.5-40	1904	21973	0.996	9.4×10^{-8}	1.4×10^{-6}

Analytical applications

The proposed CE-ECL method was utilized for the determination of ME and BF in human urine samples. The electropherograms of blank healthy person urine sample and urine sample spiked with 10 μmol L^{-1} BF and 10 μmol L^{-1} ME are illustrated in Fig. 3 (a, b). The results show that ME and BF were not detected in the blank urine sample because the two drugs are exogenous substances and could be well separated and detected in spiked urine samples. In addition, the unknown peaks X in the urine sample appeared in the electropherogram. The recoveries of the ME and BF at three different spiked concentration were also determined by injecting the standard samples into urine samples and the results were listed in Table 2. It can be seen that the recoveries of ME and BF in urine samples were from 89.0 to 126.0% with less than 7.4% in RSD.

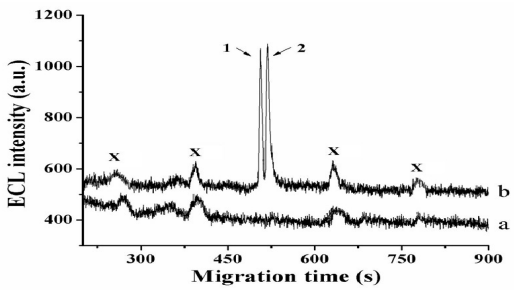


Fig. 3. Electropherograms of the blank urine sample (a) and the urine sample spiked with ME and BF (b). Peak 1: ME; Peak 2: BF; Peak X: unknown compounds. Other conditions are the same as in Fig. 2.

Table 2. Recoveries of ME and BF in human urine at different spiked levels.

Analytes	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	RSD (%) (n = 3)
ME	0.5	0.56	112.0	5.2
	10	12.6	126.0	4.7
	40	35.6	89.0	4.2
BF	1.0	1.05	105.0	7.4
	5.0	4.84	96.8	5.9
	20	24.2	121.0	5.4

Study on the binding of HSA with ME and BF

It is significant to study the interaction of HSA, the most abundant and important protein in the blood, with drugs. Therefore, the binding behavior between HSA and ME or BF was studied in this work. In order to obtain the equilibrium time, a mixture solution of 100 μL 200 μmol L^{-1} HSA and 100 μL 20 mmol L^{-1} ME or BF in the dialysis bag was incubated at 37 °C. The solution outside of the dialysis bag was 4 mL PBS and the ECL intensity of ME or BF outside of the bag was detected every half an hour (a total of about 8 h) until no obvious change in the ECL intensity was found, which indicates that the reaction had already reached its equilibrium. The results obtained show that the equilibrium time is 5 h.

UV-visible absorption spectroscopy technique is a simple and useful method and can be used to explore whether ME or BF combine with HSA. In this experiment, 100 μL 20 mmol L^{-1} ME or BF, 100 μL 200 μmol L^{-1} HSA, and a mixture of 100 μL 20 mmol L^{-1} ME or BF with 100 μL 200 μmol L^{-1} HSA balance from start to finish were examined by UV-vis spectrophotometry for comparison, separately, as shown in Fig. 4. The facts in Fig. 4 confirmed the binding of HSA with ME or BF under the experimental condition.

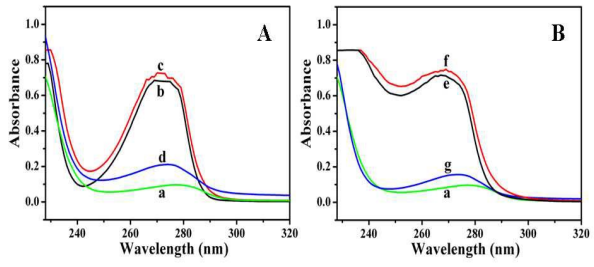


Fig. 4. The UV-visible absorption spectroscopy of the solution inside the dialysis bag. Curve a: HSA; Curve b: ME; Curve c: HSA + ME; Curve d: after ME combination with HSA. Curve e: BF; Curve f: HSA + BF; Curve g: after BF combination with HSA.

In most cases, it is supposed that the drug (C) is bound to m types of independent binding sites on the protein (P). The fraction (r) of bound drug molecules per protein molecule can be represented as[37]:

$$r = \frac{[C_{bound}]}{[P_{total}]} = \sum_{i=1}^{m} n_i \frac{K_i[C_{free}]}{1 + K_i[C_{free}]} \qquad (1)$$

where $[C_{bound}]$, $[P_{total}]$, $[C_{free}]$ stand for the concentrations of bound drug, total HSA and free drug, respectively; r is the fraction of bound drug molecules per protein molecule; n represents the binding site number while K denotes the binding constant,[38] n_i represents the number of sites of class i and K_i is the binding constant. Drug protein data analysis often assumes one type of binding sites on the protein and eq. (1) can therefore be simplified to (2):

$$r = n \frac{K[C_{free}]}{1 + K[C_{free}]} \qquad (2)$$

A series of different volumes (range from 40-160 μL, increment of 20 μL) of 20 mmol L^{-1} ME or BF were mixed with 100 μL 200 μmol L^{-1} HSA in the dialysis bag and incubated in 4 mL PBS at 37 °C. After the equilibrium, the ECL intensity outside the dialysis bag was measured and then the concentration of free drug could be estimated by the CE-ECL. According to formula (2) and a non-linear fit using Origin 8.0, the number of binding sites and binding constant can be achieved. Under the calculated results, the number of binding sites of ME and BF with HSA are 1.2 and 1.1, and the binding constant are 2.8×10^3 and 2.7×10^3 L mol^{-1}.

Comparison with reported methods

The validated CE-ECL method has high sensitivity compared to majority of the methods developed for analysis of ME and BF.[5-12, 17] The sample consumption of the proposed method is only nanoliter level, which is considerably less than that of other procedures[5-12, 17] except the similar method.[13, 14] Additionally, the proposed method could become an effective tool to study the interaction between protein and drugs.

Conclusions

In this work, a sensitive method of CE-ECL featured simple instrumentation, high sensitivity, wide linear range and excellent analytical performance was developed for the simultaneous separation and detection of ME and BF. The use of Triton X-100 as an additive in CE could improve separation efficiency. The proposed method was successfully applied to the determination of ME and BF in human urine. The interaction of between HSA and the two drugs was studied by the developed strategy. The number of binding sites and binding constant of HSA with ME or BF were estimated. This work demonstrates that the CE-ECL technique has great potential in the biomedicine analysis and interaction study between drug and protein.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (21375114, 21405129), the Project of Science and Technology Development of Henan Province (142300410197), the Foundation of Henan Educational Committee (14A150013), the Program for Innovation Research Team (in Science and Technology) in University of Henan Provience (15IRTSTHN001) and the Scientific Research Foundation of Graduate School of Xinyang Normal University (2014KYJJ32).

Notes and references

College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, China. E-mail: liuym9518@sina.com; Tel and Fax: +86-376-6392889.

References

1. L. Poirier and Y. Lacourcière, *Can. J. Cardiol.*, 2012, **28**, 334-340.
2. P. M Vanhoutte and Y. S Gao, *Curr. Opin. Pharmacol.*, 2013, **13**, 265-273.
3. J. S. Huang, J. Y. Sun, X. G. Zhou and T. Y. You, *Anal. Sci.*, 2007, **23**, 183-188.
4. A. C. Anderson, *Clin. Pediatr. Emerg. Med.*, 2008, **9**, 4-16.
5. S. M. Al-Ghannam, *J. Pharm. Biomed. Anal.*, 2006, **40**, 151-156.
6. S. Morante-Zarcero and I. Sierra, *J. Pharm. Biomed. Anal.*, 2012, **62**, 33-41.
7. I. Baranowska, S. Magiera and J. Baranowski, *J. Chromatogr. B*, 2011, **879**, 615-626.
8. S. Fan, J. H. Zou, H. Miao, Y. N. Wu and Y. F. Zhao, *Chinese J. Anal. Chem.*, 2011, **39**, 1153-1158.
9. S. Magiera, C. Uhlschmied, M. Rainer, C. W. Huck, I. Baranowska and G. K. Bonn, *J. Pharm. Biomed. Anal.*, 2011, **56**, 93-102.
10. M. Caban, P. Stepnowski, M. Kwiatkowski, N. Migowska and J. Kumirska, *J. Chromatogr. A*, 2011, **1218**, 8110-8122.
11. A. S Amina, G. H Ragabb and H Salehb, *J. Pharmaceut. Biomed.*, 2002, **30**, 1347-1353.
12. R. N. Goyal, V. K. Gupta, M. Oyama and N. Bachheti, *Electrochem. Commun.*, 2006, **8**, 65-70.
13. Y. C. Wang, Q. Wu, M. R. Cheng and C. Cai, *J. Chromatogr. B*, 2011, **879**, 871-877.
14. Y. M. Liu, Y. Yang, J. Li and J. J. Du, *Anal. Methods*, 2012, **4**, 2562-2568.
15. R. Ariasa, R. Jiménez, R. Alonso, M. Télez, I. Arrieta, P. Flores and E. Ortiz-Lastra, *J. Chromatogr. A*, 2001, **916**, 297-304.
16. D. Dogrukol-Ak, A. G. Dal and M. Tuncel, *Chromatographia*, 2007, **66**, 159-163.
17. M. H. Lu, L. Zhang, B. Qiu, Q. Feng, S. F. Xi and G. N. Chen, *J. Chromatogr. A*, 2008, **1193**, 156-163.
18. X. W. Zhang and Z. X. Zhang, *Chin. J. Anal. Chem.*, 2010, **5**, 648-652.
19. G. W. Zhang, Q. M. Que, J. H. Pan and J. B. Guo, *J. Mol. Struct.*, 2008, **881**, 132-138.
20. S. S. Kalanur, J. Seetharamappa and V. K. L. Kalalbandi, *J. Pharm. Biomed. Anal.*, 2010, **53**, 660-666.
21. A. Sivertsen, J. Isaksson, H. K. S. Leiros, J. Svenson, J. S. Svendsen and B. O. Brandsdal, *BMC Struct. Biol.*, 2014, **14**, 1-14.
22. N. Seedher, *Indian J. Pharm. Sci.,* 2000, **62**, 16-20.
23. D. Silva, C. M. Cortez, J. Cunha-Bastos and S. R. W. Louro, *Toxicol. Lett*., 2004, **147**, 53-61.
24. L. W. Shao, C. Q. Dong, X. Y. Huang and J. C. Ren, *Chinese Chem. Lett.*, 2008, **19**, 707-710.
25. Y. M. Liu, L. Mei, L. J. Liu, L. F. Peng, Y. H. Chen and S. W. Ren, *Anal. Chem.*, 2011, **83**, 1137-1143.
26. Y. Li, J. M. Liu, F. Han, Y. Jiang and X. P. Yan, *J. Anal. Atom. Spectrom.*, 2011, **26**, 94-99.
27. G. F. Shi, J. T. Cao, J. J. Zhang, K. J. Huang, Y. M. Liu, Y. H. Chen and S. W. Ren, *Analyst*, 2014, **139**, 5827-5834.
28. Y. M. Liu, J. J. Zhang, G. F. Shi, M. Zhou, Y. Y. Liu, K. J. Huang and Y. H. Chen, *Electrochim. Acta*, 2014, **129**, 222-228.
29. X. B. Yin, B. B. Sha, X. H. Zhang, X. W. He and H. Xie, *Electroanal.*, 2008, **20**, 1085-1091.
30. X. Wang and D. R. Bobbitt, *Anal. Chim. Acta*, 1999, **383**, 213-220.
31. Y. Gao, Y. L. Tian, X. B. Yin and E. K. Wang, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.*, 2006, **832**, 236-240.
32. Y. H. Xu, Y. Q. Liu, J. B. Zhu and E. K. Wang, *Talanta*, 2013, **116**, 308-314.
33. S. J. Sun, C. J. Long, C. Y. Tao, S. Meng and B. Y. Deng, *Anal. Chim. Acta*, 2014, **851**, 37-42.
34. Y. F. Hu, *J. Chromatogr. B*, 2015, **986-987**, 143-148.
35. G. M. Zhu, S. H. Long, H. Sun, W. Luo, X. Li and Z. B. Hao, *J. Chromatogr. B*, 2013, **941**, 62-68.
36. S. Dziomba, P. Kowalski and T. Bączek, *J. Pharm. Biomed. Anal.*, 2012, **62**, 149-154.
37. M. H. A. Busch, L. B. Carels, H. F. M. Boelens, J. C. Kraak and H. Poppe, *J. Chromatogr. A*, 1997, **777**, 311-328.
38. A. V. Rudnev, S. S. Aleksenko, O. C. Semenova, G. Hartinger, A. R. Timerbaev, B. K. Keppler, *J. Sep. Sci.*, 2005, **28**, 121-127.