Asian Journal of Chemistry; Vol. 25, No. 13 (2013), 7332-7336

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14624

HPLC Determination of Pregabalin in Bulk and Pharmaceutical Dosage Forms After Derivatization with 1-Fluoro-2,4-dinitrobenzene

E. SOURI[1,*], M. ESKANDARI[1], M. BARAZANDEH TEHRANI[1], N. ADIB[2] and R. AHMADKHANIHA[3]

[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14155-6451, Iran
[2]Department of Pharmaceutics, Food and Drug Laboratory Research Center, Ministry of Health, Tehran, Iran
[3]Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author: Fax: +98 21 66461178; Tel: +98 21 66959065; E-mail: souri@sina.tums.ac.ir; souri@tums.ac.ir

(*Received*: 28 September 2012; *Accepted*: 24 June 2013) AJC-13702

Pregabalin is an effective drug used for the treatment of epilepsy, neuropatic pain and anxiety. In this study, a simple and sensitive HPLC method was developed and validated for the determination of pregabalin after derivatization with 1-fluoro-2,4-dinitrobenzene and UV detection at 360 nm. Separation of reaction product was performed on a Nova-Pak C_{18} column using a mixture of acetonitrile and sodium dihydrogen phosphate 50 mM (pH 2.5) (60:40, v/v) and UV detection at 360 nm. The proposed method showed excellent linearity in the range of 1-100 µg mL^{-1} ($r^2 > 0.999$). The within-day and between-day precision values were in the range of 0.84-1.98 %. The validated method was successfully used for the determination of pregabalin in the presence of its degradation products under stress degradation, assay preparation of dosage form and dissolution medium without any interference.

Key Words: Pregabalin, 1-Fluoro-2,4-dinitrobenzene, HPLC, Derivatization, UV detection.

INTRODUCTION

Pregabalin (Fig. 1), (S)-3-(aminoethyl)-5-methyl hexanoic acid, is a structural analogue of γ-amino butyric acid (GABA) which is used for the treatment of epilepsy, neuropathic pain and also generalized anxiety. Pregabalin and gabapentin bind with high affinity to $\alpha_2\delta$ protein, an auxiliary subunit of Q-type voltage-sensitive calcium channels in the peripheral and central nervous system[1]. Binding to $\alpha_2\delta$ protein, results in calcium influx reduction at nerve terminals which leads to reduction of neurotransmitters such as glutamate and noradrenaline and abnormal neuronal excitability[2,3].

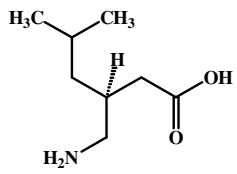

Fig. 1. Chemical structure of pregabalin

Determination of pregabalin in biological fluids has been reported using LC-MS-MS[4,5] or HPLC methods[6-8]. Few HPLC methods have also been used for analysis of pregabalin in pharmaceutical dosage forms[8-11]. Enantioselective methods were also used for determination of pregabalin enantiomers in bulk drug sample or biological samples[12-14].

As pregabalin has no significant UV, visible or fluorescence absorption, in most reported HPLC methods derivatization with a chromophore has been employed. Different derivatizating reagents such as *o*-phthaldialdehyde[6,10], picryl sulfonic acid[7] and fluorescamine[9] with fluorescence detection has been utilized in previous works. Spectrophotometric and spectrofluorimetric methods were also reported in the literature for determination of pregabalin in pharmaceutical dosage forms[15,16].

Pregabalin is not official in USP or BP, simple and validated methods are required for assay determination of the drug in dosage forms. In the present study, an HPLC method with UV detection is used for the determination of pregabalin after derivatization with 1-fluoro-2,4-dinitrobenzene (FDNB). This reagent has been successfully used before for determination of some other drugs[17-19]. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines.

EXPERIMENTAL

Pregabalin was from Sun Pharma, India (batch No: AHJPRGFL018) and was kindly provided by Bakhtar

Bioshimi Co., Kermanshah, Iran. Derivatizing reagent, 1-fluoro-2,4-dinitrobenzene, was from Fluka (Switzerland). All other chemicals and HPLC grade solvents were purchased from Merck (Darmstadt, Germany). Water was HPLC grade and prepared using a Milli-Q purification system (Millipore, Milford, MA, USA).

A Waters HPLC system consisted of a Model 515 isocratic pump, a Model 710 plus autosampler and a Model 480 UV-VIS detector was used. A multi-channel Chrom & Spec software for chromatography (version 1.5x) was used for data processing.

Standard solutions: To prepare the stock standard solution of pregabalin, 20 mg of drug was dissolved in 100 mL of distilled water to reach a concentration of 200 μg mL^{-1}. Calibration solutions of pregabalin (1, 2, 4, 10, 20, 40, 60, 80 and 100 μg mL^{-1}) were prepared by subsequent dilution.

A 16 mM solution of 1-fluoro-2,4-dinitrobenzene was prepared by dissolving 300 mg of the reagent in 100 mL of acetonitrile. The reagent is a skin irritant and should be handled carefully.

Borate buffer (0.25 M) was prepared by dissolving appropriate amounts of KCl and H_3BO_3 in distilled water and the pH was adjusted to 8.2.

Derivatization procedure: Aliquots of 500 μL of pregabalin standard solutions, 500 μL of borate buffer, 75 μL 1-fluoro-2,4-dinitrobenzene reagent and 2 mL of acetonitrile were transferred into a test tube. The mixture was vortexed for 5 s and kept at 60 ºC for 0.5 h. After cooling to the laboratory temperature, 75 μL of 1 M HCl solution was added and 20 μL of the solution injected to the HPLC system.

Chromatographic conditions: Separation of the reagent and reaction product was performed on a Nova-Pak® C_{18}, 4 μm column (150 mm × 3.9 mm, Waters, Milford, MA, USA) by using a mobile phase consisted of acetonitrile and 50 mM NaH_2PO_4 (pH 2.5) (60:40, v/v) at a flow rate of 1 mL min^{-1}. The mobile phase prepared daily and degassed by filtration through a 0.45 m Teflon membrane filter (Millipore, Milford, MA, USA) and also sonicated for 10 min. The maximum absorption of the pregabalin-1-fluoro-2,4-dinitrobenzene adduct (360 nm) determined by UV spectrophotometer was used for HPLC analytical detection wavelength at ambient temperature.

Optimization of reaction conditions: The reaction conditions were optimized by using different amount of the 1-fluoro-2,4-dinitrobenzene reagent ranging from 25-200 μL, different temperatures (25, 40 and 60 ºC) and reaction times (5-60 min).

Validation of the method: To find out the linearity of the proposed method, six series of pregabalin calibration solutions in the range of 1-100 μg mL^{-1} were prepared and derivatized according to the general procedure and injected to the HPLC system. The calibration curves were plotted and statistical analysis was performed.

Within-day and between-day accuracy and precision were evaluated by replicate analysis of standard solutions of pregabalin at 1, 40 and 100 μg mL^{-1} on one day and three separate days.

The robustness of the proposed method was evaluated by small changes in chromatographic conditions such as mobile phase composition and pH value.

Relative recovery: To find out the relative recovery of the proposed method, standard solution of pregabalin was added to an amount of capsule content equivalent to 50 % of a dosage form. Determination was performed according to the developed method and the obtained peak area compared with that of corresponding peak of the same concentration of standard solution of pregabalin and the relative recovery calculated.

Forced degradation studies: Degradation of pregabalin was studied at a concentration level of 1 mg mL^{-1} using 5 M HCl, 5 M NaOH and 30 % H_2O_2 according to Kasawar and Farooqui[11]. After 3 h exposure to these solutions, 500 μL of each solution was transferred to a 10 mL volumetric flask and appropriate amounts of NaOH or HCl was added for neutralization. The flasks were adjusted to the volume by distilled water and the samples were derivatized according to the general procedure. The peak area of the pregabalin-1-fluoro-2,4-dinitrobenzene product was compared with that of a standard solution of pregabalin derivatized by the same procedure and the percent of degradation was calculated.

Application of the method: The optimized method was used for the analysis of pregabalin in LYRICA (75 mg) capsules (Pfizer, GmbH Arzneimittelwerk Godecke, Freiburg, Germany). An amount of a mixture of 20 capsule contents equivalent to 75 mg of pregabalin was weighed and transferred to a 100 mL volumetric flask. About 70 mL of distilled water was added and the mixture sonicated for 15 min. After cooling, the flask made up to volume by distilled water and passed through a 0.45 μm syringe filter. After 20 times dilution and derivatization, the drug concentration was determined by using a standard solution of the same concentration level prepared by the same procedure.

Also the drug release was determined using a dissolution apparatus (Erweka, Heusenstomm, Germany) at 37 ± 0.1 ºC. The paddle apparatus at 50 rpm and 900 mL of 0.06 M HCl as medium were used. Samples were drawn at 10, 20, 30 and 45 min. The solutions were passed through a 0.45 μm Millipore filter, derivatized according to the general procedure after adding appropriate amounts of 0.06 M NaOH to neutralize the HCl and injected to the HPLC system. The peak area of the pregabalin-1-fluoro-2,4-dinitrobenzene product was compared with a standard solution at maximum dissolved concentration (75 mg per 900 mL) and the percent drug released was calculated.

RESULTS AND DISCUSSION

Chromatographic conditions: Optimized chromatographic conditions was achieved by utilizing a Nova-Pak C_{18} column and a mixture of acetonitrile and sodium dihydrogen phosphate 50 mM (pH 2.5) (60:40, v/v) as mobile phase. In this chromatographic system acceptable peak shape and retention time for the reaction product was observed without any interference from the reagent by-products or pharmaceutical dosage form excipients. Representative chromatograms are presented in Fig. 2.

The system suitability parameters calculated for replicate injections were within the acceptable criteria (Table-1).

Derivatization reaction: As pregabalin exhibit a low UV absorption, derivatization with a chromophore is needed to

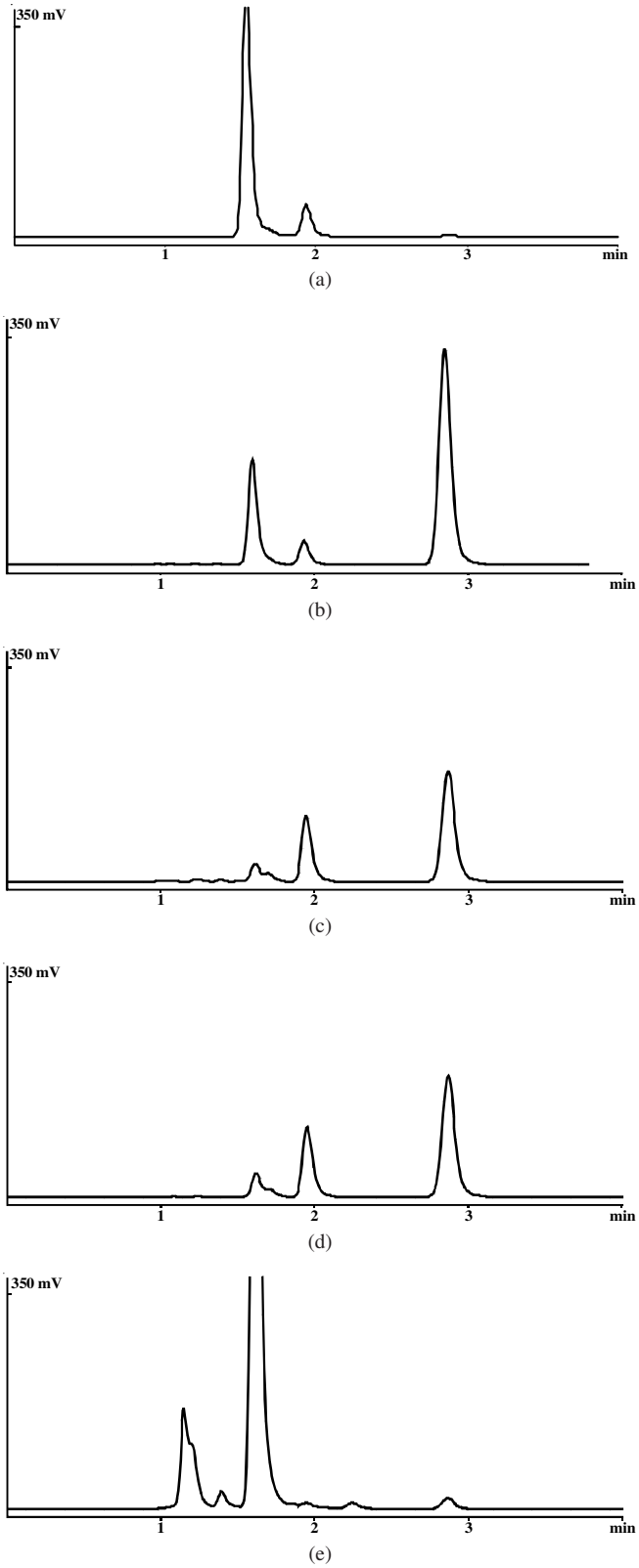

Fig. 2. HPLC chromatograms of pregabalin-1-fluoro-2,4-dinitrobenzene product. (a) 1-fluoro-2,4-dinitrobenzene reagent (b) Pregabalin standard solution (50 µg mL^{-1}); (c) Pregabalin in 5 M HCl solution after 3 h in room temperature; (d) pregabalin in 5 M NaOH solution after 3 h in room temperature; (e) pregabalin in 30 % H_2O_2 solution after 3 h in room temperature

increase the sensitivity of the determination method. The derivatization reaction between 1-fluoro-2,4-dinitrobenzene and primary amines proceeded under mild basic medium using

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Parameters	Found	Acceptable limits
USP theoretical plates (n = 6)	3800	N > 1500
USP tailing factor (n = 6)	1.25	T < 1.5
Repeatability (t_R) (n = 6)	0.16	RSD < 1 %
Repeatability (peak area) (n = 6)	0.62	RSD < 1 %

t_R: Retention time (min); N: Theoretical plate; T: Tailing factor; RSD: Relative Standard Deviation

borate buffer (pH 8.2). A nucleophilic substitution reaction proceeds and the unreacted 1-fluoro-2,4-dinitrobenzene undergoes hydrolysis to 2,4-dinitrophenolate which turns to 2,4-dinitrophenol under acidic conditions.

The derivatization process leads to the formation of a highly absorbing UV derivative at 360 nm which could be determined with acceptable sensitivity. Different experimental parameters affecting the derivatization reaction such as reagent amount, reaction temperature and time were studied and optimized. The derivatization reaction was performed at different temperatures and tested at different time intervals to find out the best temperature and time for reaction. According to the results (Fig. 3), the optimum reaction conditions were obtained at 60 ºC for 0.5 h. At lower temperatures, the reaction rate was slow, whereas at higher concentrations, solvent evaporation was encountered. For stable results reaction temperature of 60 ºC for 0.5 h was selected.

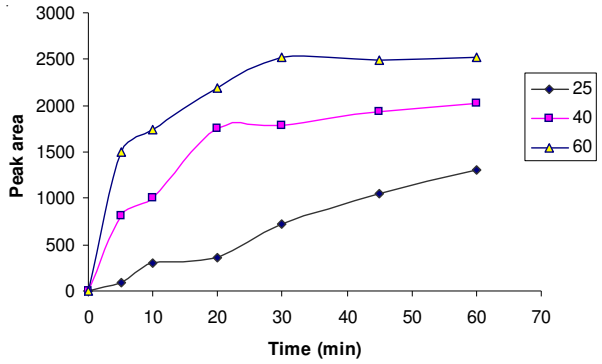

Fig. 3. Effect of time and temperature on the derivatization reaction of 1-fluoro-2,4-dinitrobenzene and pregabalin (100 µg mL^{-1}) (n = 4)

By using varying amounts of the reagent, it was shown that the peak area intensity increases by increasing the reagent volume from 25 µL up to 200 µL (Fig. 4). It seems that the reaction was completed by using 75 µL of the reagent. More reagent volumes did not increase the peak area.

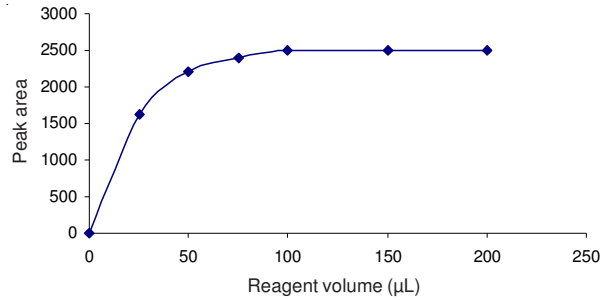

Fig. 4. Effect of reagent amount on the derivatization reaction of 1-fluoro-2,4-dinitrobenzene and pregabalin (100 µg mL^{-1}) (n = 4) (60 ºC for 0.5 h)

Method validation: The linearity of the proposed method was investigated by constructing six calibration curves using the general procedure for sample preparation. The results demonstrated good linearity with an acceptable correlation coefficient in the range of 1-100 μg mL^{-1} (Table-2).

TABLE-2
STATISTICAL DATA OF CALIBRATION CURVES OF PREGABALIN IN STANDARD SOLUTIONS (n = 6)

Parameters	Results
Linearity range	1-100 μg mL^{-1}
Regression equation	y = 23.89x – 2.43
Standard deviation of slope	0.15
Relative standard deviation of slope (%)	0.64
Standard deviation of intercept	0.37
Correlation coefficient (r^2)	0.9995

The within-day and between-day precision and accuracy were determined at three different concentrations on three separate days. The results summarized in Table-3 showed very low coefficients of variation in the range of 0.84-1.98 %. The accuracy of the method was also in the range of 0.06-1.00 %. The limit of quantification with a CV < 2 % was 1.00 μg mL^{-1}. Assay results for LYRICA capsules by two analysts using different HPLC systems showed CV values less than 2 %.

TABLE-3
PRECISION AND ACCURACY OF METHOD FOR DETERMINATION OF PREGABALIN IN STANDARD SOLUTIONS (n = 9; 3 SETS FOR 3 DAYS)

Concentration added (μg mL^{-1})	Concentration found (μg mL^{-1})	CV (%)	Error (%)
Within-day (n = 3)			
1	1.01 ± 0.02	1.98	1.00
40	39.87 ± 0.52	1.30	-0.33
100	100.06 ± 0.84	0.84	0.06
Between-day (n = 9)			
1	1.01 ± 0.01	0. 99	1.00
40	39.84 ± 0.45	1.13	-0.40
100	100.42 ± 0.88	0.88	0.42

Robustness: The effect of small variations in optimal method parameters on chromatographic parameters was studied. The results are shown in Table-4. Ten per cent changes in the mobile phase composition and buffer pH did not influence the peak area values significantly (CV < 1.2 %). Although significant variations of the retention time were observed by changing the mobile phase composition, no influence on sensitivity and selectivity of the method was observed.

TABLE-4
INFLUENCE OF SMALL CHANGES IN MOBILE PHASE COMPOSITION (METHOD ROBUSTNESS)

Mobile phase composition	Retention time	Peak area
Acetonitrile-NaH_2PO_4 50 mM (pH 2.5) (60:40)	2.81	492.62
Acetonitrile-NaH_2PO_4 50 mM (pH 2.5) (64:36)	2.63	492.54
Acetonitrile-NaH_2PO_4 50 mM (pH 2.5) (56:44)	3.49	499.59
Acetonitrile-NaH_2PO_4 50 mM (pH 2.2) (60:40)	2.74	509.35
Acetonitrile-NaH_2PO_4 50 mM (pH 2.2) (64:36)	2.35	506.78
Acetonitrile-NaH_2PO_4 50 mM (pH 2.2) (56:44)	3.42	507.38
Acetonitrile-NaH_2PO_4 50 mM (pH 2.8) (60:40)	2.80	503.49
Acetonitrile-NaH_2PO_4 50 mM (pH 2.8) (64:36)	2.34	503.73
Acetonitrile-NaH_2PO_4 50 mM (pH 2.8) (56:44)	3.38	501.86

Stability: Derivatization of stock standard solutions of pregabalin kept at 4 ºC, showed that the solutions are relatively stable for at least 7 days. Also the derivatization product was stable for 24 h (recovery > 99 %).

Specificity: The specificity of the proposed method was checked in the presence of excipients and also degradation products of stress stability tests. The relative recovery of pregabalin by using standard addition method showed excellent results (99.92 ± 0.58 %) and no interfering peak from excipients was observed using the proposed method for assay determination and dissolution test. Also, using acidic, alkaline and oxidative degradation conditions according to the previously reported article[11], no interferences from the degradation products were observed (Fig. 2). The results of the forced degradation of pregabalin upon different conditions were in good agreement to the previously reported work.

Assay and dissolution test for pregabalin: The proposed method was used for assay determination of pregabalin capsules and the results showed to be in good agreement with the label claim (73.41 ± 0.14 mg). The dissolution profile of LYRICA capsules is shown in Fig. 5.

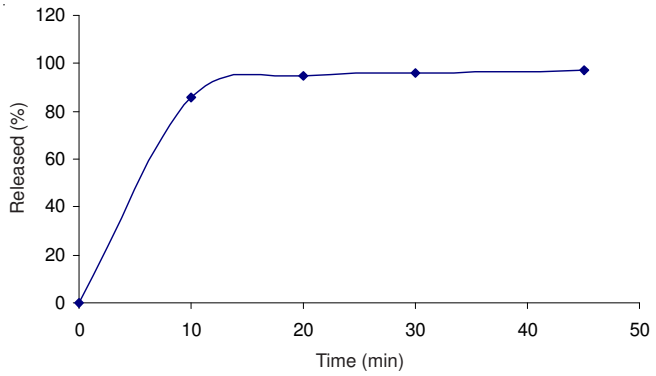

Fig. 5. Dissolution profile of 75 mg LYRICA capsules (n = 6) using 0.06 M HCl as dissolution medium and paddle at 50 rpm

Conclusion

The results showed that the developed method is simple, reliable, practical and economic for determination of pregabalin in dosage forms. Although the reaction time is *ca.* 0.5 h, because of the stability of the product, the procedure could be applied in a great number of samples simultaneously and as the retention time of the chromatographic separation is 3 min, the overall analysis time would be acceptable and more convenient than other reported methods. The proposed method is superior to other methods with respect to its simplicity, rapidity and cost effectiveness. The proposed method could be possibly improved to be used in plasma samples, because of the suitable determination limit.

ACKNOWLEDGEMENTS

This study was part of a Pharm D. thesis and has been supported by Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences.

REFERENCES

1. C. P. Taylor, T. Angelotti and E. Fauman, *Epilepsy Res*., **73**, 137 (2007).
2. K. Fink, W. Meder, D.J. Dooley and M. Gothert, *Br. J. Pharmacol*., **130**, 900 (2000).
3. J.A. Van Hooft, J.J. Dougherty, D. Endeman, R.A. Nichols and W.J. Wadman, *Eur. J. Pharmacol*., **449**, 221 (2002).
4. V.V. Vaidya, S.M. Yetal, S.M.N. Roy, N.A. Gomes and S.S. Joshi, *Chromatographia*, **66**, 925 (2007).
5. R. Nirogi, V. Kandikere, K. Mudigonda, P. Komarneni and R. Aleti, *J. Chromatogr. B*, **877**, 3899 (2009).
6. T.A.C. Vermeij and P.M. Edelbrock, *J. Chromatogr. B*, **810**, 297 (2004).
7. D. Berry and C. Millington, *Ther. Drug Monit*., **27**, 451 (2005).
8. R.S. Gujral, S.K.M. Haque and S. Kumar, *Afr. J. Pharm. Pharmacol*., **3**, 327 (2009).
9. B. Martinc, I. Grabnar, A. Mrhar and T. Vovk, *J. AOAC Int*., **93**, 1069 (2010).
10. M. Dousa, P. Gibala and K. Lemr, *J. Pharm. Biomed. Anal*., **53**, 717 (2010).
11. G.B. Kasawar and M.N. Farooqui, *Indian J. Pharm. Sci*., **72**, 517 (2010).
12. S. Jadhav, D.B. Pathare and M.S. Shingare, *Chromatographia*, **65**, 253 (2009).
13. X. Chen, D. Zhang, J. Deng and X. Fu, *J. Chromatogr. Sci*., **46**, 42 (2008).
14. Y. Zhang, Ch. Holliman, D. Tang, D. Fast and S. Michael, *J. Chromatogr. B*, **875**, 148 (2008).
15. A. Onal and O. Sagirli, *Spectrochim. Acta A*, **72**, 68 (2009).
16. H. Salem, *E-J. Chem*., **6**, 332 (2009).
17. M. Zhou, G. Wei, Y. Liu, Y. Sun, S. Xiao, L. Lu, C. Liu and D. Zhong, *J. Chromatogr. B*, **798**, 43 (2003).
18. S. Nicoli and P. Santi, *J. Pharm. Biomed. Anal*., **41**, 994 (2006).
19. E. Souri, H. Jalalizadeh and A. Shafiee, *Chem. Pharm. Bull*., **55**, 1427 (2007).