

This article was downloaded by:

On: 23 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

A Derivatization Reagent for Vigabatrin and Gabapentin in HPLC with Fluorescence Detection

Abdulrahman A. Al-Majed^a

^a Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

To cite this Article Al-Majed, Abdulrahman A.(2005) 'A Derivatization Reagent for Vigabatrin and Gabapentin in HPLC with Fluorescence Detection', *Journal of Liquid Chromatography & Related Technologies*, 28: 19, 3119 – 3129

To link to this Article: DOI: 10.1080/10826070500295229

URL: <http://dx.doi.org/10.1080/10826070500295229>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

A Derivatization Reagent for Vigabatrin and Gabapentin in HPLC with Fluorescence Detection

Abdulrahman A. Al-Majed

Department of Pharmaceutical Chemistry, College of Pharmacy,
King Saud University, Riyadh, Saudi Arabia

Abstract: A validated, selective, and sensitive chromatographic method for determination of two antiepileptic drugs, vigabatrin (VGA) and gabapentin (GBA) has been developed. Determination was obtained by pre-column reaction of the samples with fluorescamine and separating the corresponding derivatives with a reverse phase HPLC on a 5 micro ASMT BANSil CN column and fluorescence detection (λ_{ex} 390 nm, λ_{em} 472 nm). Acetonitrile-TBAH (20 : 80) containing phosphoric acid was used as mobile phase. A linear quantitative response curve was generated over a concentration range of 0.2–1.0 $\mu\text{g}/\text{mL}$ with a correlation coefficient of 0.999 and 0.995 for vigabatrin and gabapentin, respectively. The percentage recovery of vigabatrin from spiked plasma was 102.7 with RSD% of 4.51. Validation of accuracy and precision were satisfactory for both drugs within-and between-run assay. The method is specific for the intact drugs, and can be adopted in the presence of possible interferences.

Keywords: Vigabatrin, Gabapentin, Fluorescamine, Derivatization, HPLC, Fluorescence detection

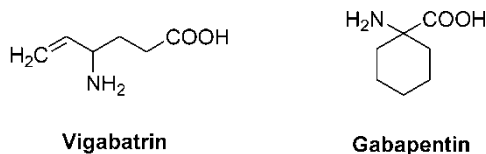
INTRODUCTION

Vigabatrin (γ -vinyl- γ -aminobutyric acid, γ -vinyl-GABA) is a catalytic inhibitor of brain GABA transaminase, the enzyme responsible for the degradation of the neurotransmitter, GABA. Consequently it produces an increase

Address correspondence to Abdulrahman A. Al-Majed, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia. E-mail: almajed99@yahoo.com

in brain GABA concentrations. This effect is presumed to be responsible for its antiepileptic action.^[1,2]

Gabapentin is a cyclic GABA analogue. Although, it is structurally related to GABA, gabapentin has no direct GABA mimetic effect, and its mechanism of action is essentially unknown.



Both drugs are licensed in numerous countries as add-on therapy for partial seizures. They have also been licensed to treat partial seizures with secondary generalization in patients in whom satisfactory control has not been achieved with standard antiepileptic drugs, or in patients who can not tolerate standard antiepileptic drugs.

There is a wide individual variation in the rate of clearance of these drugs and there is always a need to perform compliance testing, ascertain toxicity, and elucidate possible clinical interactions. Accordingly, the therapeutic monitoring of vigabatrin and gabapentin is highly desirable.^[3]

Numerous analytical methods have been reported in the scientific literature for the determination of vigabatrin and/or gabapentin in pharmaceutical preparations and human serum. These methods are based on GC,^[4,5] GC-MS,^[6] HPLC,^[7-12] capillary electrophoresis (CE),^[13,14] and fluorometry.^[15-17] The GC methods require complex sample preparation involving double derivatization of the drugs to improve the volatility and avoid column interactions. Comparing the CE and HPLC methods, the latter is more precise, reproducible, and sensitive than the former, although the many advantages of CE, such as smaller injection volume, simplicity, and wide applicability, should be taken into account. Fluorometric methods are less accurate and less specific than HPLC.

Most of the HPLC assay procedures for the determination of gabapentin (GBA) and/or vigabatrin (VGA) are based on the same approach, involving a simple automated o-phthalaldehyde (OPA) derivatization followed by HPLC separation in acidic mobile phases and fluorimetric detection. Although the derivatization step is simple and rapid, the OPA-derivative was only stable for 25 min and, therefore, less suitable for routine clinical monitoring.

The aim of the present study is to develop a new method of analysis that enhances the selectivity and sensitivity of the fluorescence methodology in HPLC for vigabatrin and gabapentin determination in dosage forms and biological fluids. The method is based on the reaction between these two drugs and fluorescamine, and the resultant fluorophore was separated by HPLC with fluorescence detection. Fluorescamine was chosen as a derivatization reagent in this study because of its fast reactivity and its high sensitivity

towards the studied drugs in mild reaction conditions. In addition to that, fluorescamine derivatizations provide highly stable derivatives.

Linearity, repeatability, and detection limit of the proposed method were examined. The applicability of the method to the determination of vigabatrin in human plasma was also demonstrated.

EXPERIMENTAL

Materials and Reagents

Vigabatrin, gabapentin, and fluorescamine were purchased from Sigma Chemicals, Saint Louis, MO. Stock solutions containing 0.5 mg/mL of vigabatrin or gabapentin were prepared as appropriate. The solutions are stable for at least 2 days if kept in the refrigerator.

Fluorescamine solution was prepared to contain 0.5 mg/mL in acetonitrile and it was stable for at least 7 days if kept in the refrigerator. Further dilution was made in acetonitrile as necessary. Borate buffer was prepared according to B.P. and the pH was adjusted to 8.2 by dropwise addition of sodium hydroxide solution. Copper acetate from BDH, Poole, UK was prepared as a 7% aqueous solution. Acetonitrile and methanol were of HPLC grade purchased from BDH, UK. Plasma was kindly provided by King Khalid University Hospital, Riyadh, K.S.A., and kept frozen until use after gentle thawing. Pharmaceutical preparations containing vigabatrin and gabapentin were purchased from the local market.

Equipment

The HPLC used was model LC-10ADVP, Shimadzu, Japan with mixing chamber model FCV-10 AL VP and a system controller model SCL-10A VP. The injector used was a Rheodyne-7725i (USA). The detector was a fluorescence detector model RF-10 XL. The system was driven by a Pentium-IV computer.

The HPLC column used was a CN nucleosil 5 μm (30 cm \times 3.9 mm, i.d.) from CapAnal HPLC 4d (USA). Guard columns of the same type were used during the analysis of the spiked plasma. The mobile phase filtration unit was a product of Millipore, type XX10-047-04, with filter papers type GC-0.22 μm from the same company. The degassing of the mobile phase was achieved by continuous bubbling of high purity helium gas during the course of analysis.

Calibration Curve

An accurately weighed amount of the reference standards vigabatrin or gabapentin was dissolved in water and a subsequent intermediate working

solution was prepared. Portions of this solution were transferred into a series of 10 mL volumetric flasks containing 3 mL of borate buffer (pH 8.2) followed by 1 mL fluorescamine solution (100 $\mu\text{g}/\text{mL}$ in acetonitrile). The volumes were adjusted by water to the mark. The final concentration range covered was 0.2–1.0 $\mu\text{g}/\text{mL}$. After a waiting time of 5 minutes, 20 μL portions of each solution were injected in replicates into the chromatograph. The eluents were detected by the fluorescence detector with the wavelength of excitation fixed at 390 nm and that of emission fixed at 472 nm. The signals emerging from the detector were integrated as peak area and a calibration graph of peak area against the concentration of vigabatrin or gabapentin was plotted. Alternatively, the regression equation was derived.

Assay of Dosage Forms

Accurate weights of powdered 20 tablets (VGA) or mixed contents of 20 capsules (GBA) equivalent to 50 mg of each drug were transferred quantitatively to 100 mL volumetric flasks with the aid of 50 mL of water. The contents of each flask were shaken for 10 min., the flasks were completed to volume with water, and filtered. The filtrates were then diluted with water to suit the procedure mentioned under the calibration curve. 20 μL portions were injected into the chromatograph in replicates, and the peak area obtained from each injection was compared to average peak areas of standard VGA and GBA treated similarly. The mean value (as % of the labeled amount) was calculated using the regression equation.

Assay of Spiked Human Plasma

A portion of spiked plasma prepared to contain a final vigabatrin concentration of 1.0 $\mu\text{g}/\text{mL}$ was mixed with a portion of 7% copper acetate solution, so that the final solution contains 20 mg/mL of copper acetate. The mixture was heated in a boiling water bath for 15 min and cooled.

An equal volume of acetonitrile was added to the above contents and centrifuged for 10 min at 3000 rpm. Portions of 100 μL of the supernatant solution (1 $\mu\text{g}/\text{mL}$) were transferred to each of a set of six vials labeled from 0 to 6 (i.e., all the vials will contain a final conc. of 0.2 $\mu\text{g}/\text{mL}$ vigabatrin from the plasma). Varying amounts of standard vigabatrin solution were added to the vials, so that vial zero has no added amount of vigabatrin, while the last vial has a final amount of 1 $\mu\text{g}/\text{mL}$.

All the vials were adjusted to have 200 μL volumes with water. Portions of 100 μL of fluorescamine, 0.5 mg/mL in acetonitrile, were added to all vials. Borate buffer (200 μL) pH 7.2 were added to all the vials and mixed (final volume 500 μL). Portions 20 μL were injected into the HPLC, and the area

for the peaks arising at a retention time around 3.0 minutes were plotted vs. the added amounts of vigabatrin.

RESULTS AND DISCUSSION

The maximum peak area of the derivatization reaction between VGA or GBA and fluorescamine was obtained when the reaction proceeded at room temperature in 5 minutes at pH 8.2 using borate buffer. The development and optimization procedures of the proposed method were achieved through several trials involving different types of columns including μ -Bondapack C₁₈ column, NH₂-column, and CN-column; all with different lengths and internal diameters.

The mobile phases tried with μ -Bondapack column included CH₃CN/H₂O, CH₃CN/H₂O/CH₃OH, CH₃CN/CH₃OH/H₃PO₄, CH₃CN/K₂HPO₄, and CH₃CN/H₃PO₄/TBAP (tetrabutylammonium phosphate), (Pic reagent) in different ratios. The peak of the reaction product of vigabatrin (or gabapentin) with fluorescamine was not resolved from that of the reagent peak. The addition of methanol worsened the resolution and, therefore, methanol was exempted from further trials.

The trials were then continued using other types of column, namely the NH₂-column. The mobile phases tried were combinations of CH₃CN and TBAP in different ratios. The results obtained using this type of column was not encouraging regarding the resolution factor.

The last type of column investigated was the CN-column. The mobile phase used was first, a combination of CH₃CN and H₃PO₄ in varying ratios, but the use of H₃PO₄ instead of TBAP was worse. Therefore, the trials were conformed to the use of CH₃CN/TBAP (20 : 80). When tetrabutylammonium hydroxide (TBAH) 0.005 M, pH adjusted to 7.2 with H₃PO₄, was used in the same ratio as above with CH₃CN, the shape of the peak and the resolution factor were as good as those obtained by using TBAP (Pic reagent). For this reason, it was concluded that the best column for this study was the CN column (3.9 × 30) with a mobile phase composed of CH₃CN:TBAH (0.005 M), adjusted to pH 7.2 with H₃PO₄, in the ratio of 20:80 and applying a flow rate of 1 mL/min. Typical chromatograms of VGA and GBA beside that of the reagent blank are shown in Figure 1.

The chromatograms obtained from a series of standard solutions and the calibration graph of the peak area vs. the concentration of the drug using the proposed method, were linear for both drugs over the ranges given in Table 1. The table shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear least squares treatment of the results along with standard deviation of the slope (S_b), intercept (S_a), on the ordinate, and the standard deviation of the residuals ($S_{y/x}$). The good linearity of the calibration graphs and the negligible scatter of the

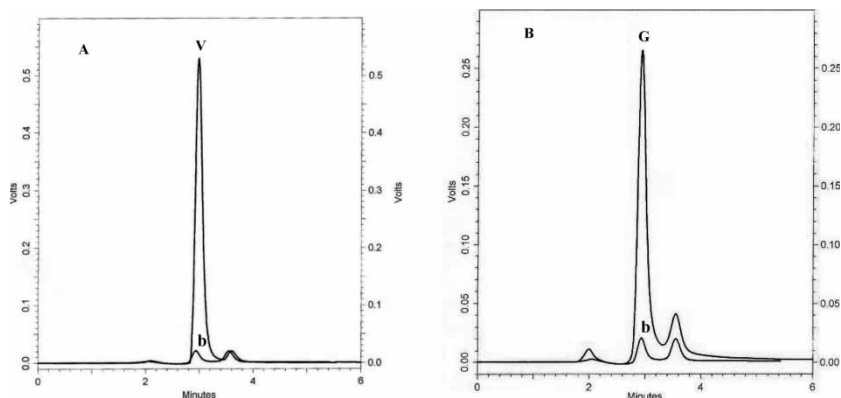


Figure 1. Typical chromatograms of the reaction product of vigabatrin (1.0 $\mu\text{g/mL}$) (A) or gabapentin (0.2 $\mu\text{g/mL}$) (B) with fluorescamine. V = VGA, G = GBA, b = reagent blank.

experimental points are clearly evident by the values of the correlation coefficients and standard deviations. The Table also shows the detection limits of the drugs. As the table shows, the proposed method is more sensitive for vigabatrin than gabapentin.

Table 1. System suitability criteria and performance data for the proposed HPLC method

Parameter	VGA	GBA
Retention time (min)	2.94	2.92
Resolution (from the reagent peak)	1.13	
Tailing factor	1.3	
Number of theoretical plates	2153	
Height equivalent to theoretical plate (HETP)	4.6×10^{-4} m	
Regression equation		
Intercept (a)	0.75×10^5	2.67×10^5
Slope (b)	5.3×10^5	7.15×10^5
Correlation coefficient (r)	0.9995	0.9954
Linearity range	0.2–10 $\mu\text{g/mL}$	0.2–10 $\mu\text{g/mL}$
Limit of detection	0.03 $\mu\text{g/mL}$	0.1 $\mu\text{g/mL}$
	1.74×10^{-7} M	(2.38×10^{-7}) M
$S_{y/x}$ (peak area unit)	5.7×10^3	2.52×10^4
S_a (peak area units)	5.99×10^3	2.64×10^4
S_b (peak area units μg^{-1}).	4.03×10^3	3.98×10^4

The stability of the reaction products was estimated in the reaction mixture, prior to injection into the HPLC system, it was found that the fluorophore is stable for at least two days at room temperature.

The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets or capsules. It is shown that these additives do not interfere with the proposed method. It was also found that the degradation product of VGA, 5-vinylpyrrolidin-2-one, and co-administered drugs such as lamotrigine, phenobarbitone, valproic acid, clopazam, carbamazepine, clonazepam, and cimitidine do not interfere with the proposed method, since they do not react with the derivatizing reagent and are not detected under these chromatographic conditions.

The validity of the proposed method was assessed by the determination of VGA and GBA in pure form. The values of the percentage recoveries are shown in Table 2. The ruggedness of the procedure, repeatability (within-day precision) and reproducibility (between-day precision) were evaluated by replicate analysis of varying concentrations of vigabatrin during the same day, and for four consecutive days. The results are abridged in Table 3. In all cases, the mean values and the standard deviations were satisfactory.

The sensitivity of the method allowed the determination of the two drugs in their dosage forms. The mean % content and the standard deviation are presented in Table 4. The results were compared with those obtained by a reference method^[17] calculating the *t* and *F* values at *P* = 0.05, and comparing them with the tabulated values. It was also successfully applied for the determination of vigabatrin (as a representative example) in spiked plasma. A typical chromatogram of spiked plasma is shown in Figure 2. The interference arising from the endogenous amino acids has been

Table 2. Percentage recoveries from added amounts of vigabatrin and gabapentin pure samples using the proposed method

	Amount added ($\mu\text{g/mL}$)	Amount found ($\mu\text{g/mL}$)	Recovery (%)	Mean \pm S.D. (%)
Vigabatrin	0.400	0.390	97.50	$\bar{X} = 100.20$ S.D. = 2.34
	0.400	0.412	103.00	
	0.800	0.823	102.88	
	0.800	0.784	98.00	
	1.000	0.996	99.60	
Gabapentin	0.400	0.397	99.25	$\bar{X} = 99.87$ S.D. = 1.87
	0.400	0.402	100.50	
	0.800	0.807	100.88	
	0.800	0.817	102.13	
	1.000	0.966	96.60	

Table 3. Within-day and between-day recovery (%) of VGA using the proposed method

Amount expected ($\mu\text{g/mL}$)	Within-day recovery (%)				Between-day mean recovery (%) + S.D. \bar{X}
	Day 1	Day 2	Day 3	Day 4	
0.2	100.15	98.18	—	—	100.92
0.4	97.68	103.05	103.45	100.20	100.56
0.6	107.48	101.91	103.95	103.87	101.42
0.8	101.91	98.23	101.51	103.81	101.24
1.0	96.41	97.62	96.69	99.51	—
r	0.9972	0.9971	0.9971	0.9985	
\bar{X}	100.92	100.56	101.42	101.24	101.4–04
S.D \pm .	3.87	2.37	2.56	2.61	0.33

removed by the addition of copper acetate solution.^[17] The amino acids precipitated as their copper salts were then removed by centrifugation.

The linearity in the spiked plasma adopting a standard addition method was satisfactory over the range 0.2–1.0 $\mu\text{g/mL}$ (Figure 3). The regression equation obtained from the standard addition series of spiked plasma was:

$$P = 2.855C - 0.593 \quad (r = 0.9932)$$

where P is the peak area ($\times 10^6$) and C is the concentration of VGA in $\mu\text{g/mL}$ added to the spiked plasma already containing 0.2 $\mu\text{g/mL}$ of vigabatrin.

Table 4. Application of the proposed HPLC method to the determination of vigabatrin and gabapentin in dosage forms

Pharmaceutical preparation	Recovery ^a (%) \pm SD	
	Proposed method	Reference method [17]
Sabril tablets ^b (500 mg vigabatrin)	101.97 \pm 1.65 t = 2.12 (2.22)* F = 1.83 (6.16)*	100.21 \pm 1.22
Neurontin capsules ^c (400 mg gabapentin)	100.04 \pm 0.83 t = 0.81 (2.20)* F = 1.4 (6.09)*	99.61 \pm 0.98

^a Mean recovery of 7 separate determinations.

^b Product of Marrion Merrell (france), Batch no. 1184.

^c Product of Pak Davis (Germany), Batch no. 0061033.

* The values between brackets are the tabulated values for t and F at P = 0.05.

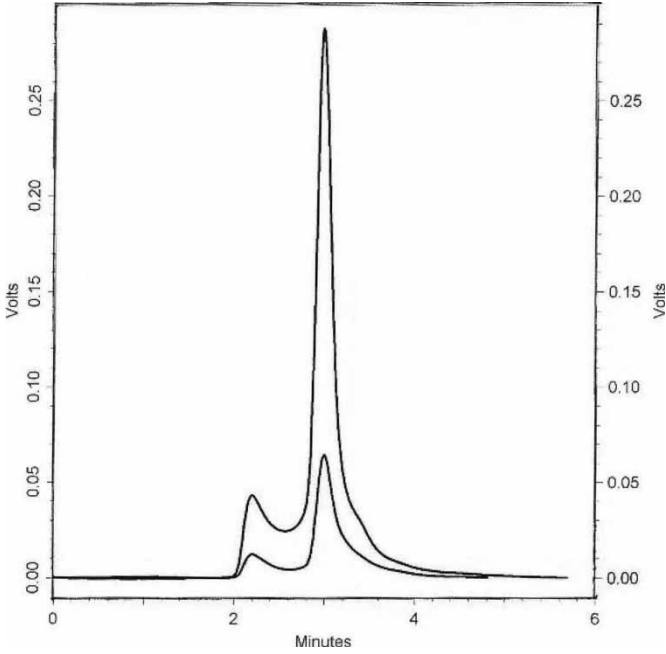


Figure 2. Typical chromatograms of vigabatrin spiked plasma. A: Plasma spiked with vigabatrin (0.2 $\mu\text{g}/\text{mL}$). B: The same as A but containing an additional 1.0 $\mu\text{g}/\text{mL}$ of vigabatrin.

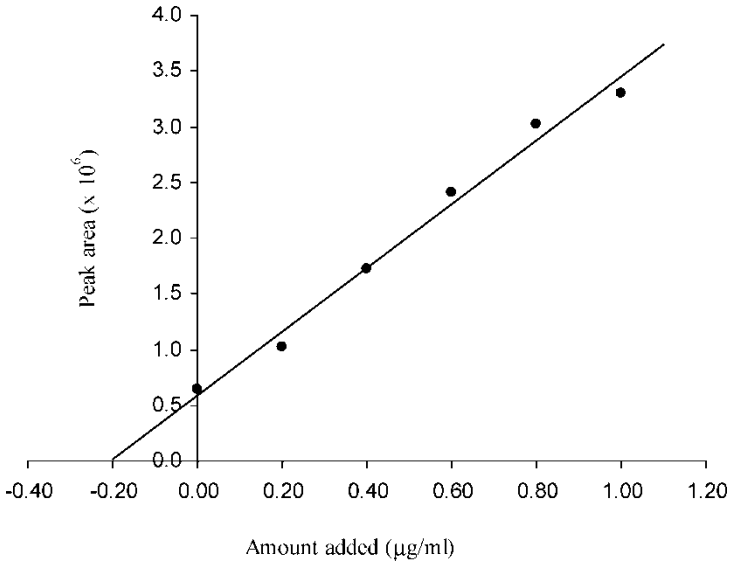


Figure 3. Standard addition graph for determination of vigabatrin in spiked plasma.

Table 5. Percentage recovery of vigabatrin from spiked plasma

Amount added	Amount found	Recovery (%)
0.20	0.207	103.50
0.40	0.395	98.75
0.60	0.634	105.67
0.80	0.853	106.63
1.00	0.949	94.90
$\bar{X} = 102.70$		
S.D. = 4.63		
RSD = 4.51		

Percentage recoveries from added amounts in plasma are abridged in Table 5.

CONCLUSION

A simple, specific, and precise HPLC method has been developed, using a single isocratic system, for the determination of VGA and GBA in bulk material and their dosage forms. The determination of VGA is extended to the in vitro analyses in a spiked human plasma sample. No interference was encountered from the possible degradation products and, therefore, it could be used as a stability-indicating method.

ACKNOWLEDGMENTS

The author gratefully acknowledges Mr. Nasr Khalil for his technical assistance.

REFERENCES

1. Saber, A.; Gram, L. *Pharmacol. Toxicol.* **1992**, *70*, 237–243.
2. Grant, S.M.; Heel, R.C. *Drugs* **1991**, *41*, 889–926.
3. Patsalos, P.N. *Seizure* **1994**, *3*, 163–170.
4. Kushnir, M.M.; Crossett, J.; Brown, P.I.; Urry, F.M. *J. Anal. Toxicol.* **1999**, *23*, 1–6.
5. Wolf, C.E.; Saady, J.J.; Poklis, A. *J. Anal. Toxicol.* **1996**, *20*, 498–501.
6. Borrey, D.C.R.; Godderis, K.O.; Engerrelst, V.I.L.; Bernard, D.R.; Langlois, M.R. *Clin. Chim. Act.* **2005**, *354*, 147–151.

7. Chen, T.M.; Contario, J.J. *J. Chromatogr.* **1984**, *314*, 495–498.
8. Chen, T.M.; Fike, R.R. *Chromatography* **1990**, *11*, 4–5.
9. George, S.; Gill, L.; Braithwaite, R.A. *Clin. Biochem.* **2000**, *37*, 338–342.
10. Zhu, Z.; Neirinck, L. *J. Chromatogr.* **2002**, *779*, 307–312.
11. Cetin, S.M.; Atmaca, S. *J. Chromatogr.* **2004**, *1031*, 237–242.
12. Vermeij, T.A.C.; Edelbroek, P.M. *J. Chromatogr. B* **2004**, *810*, 297–303.
13. Garcia, L.L.; Shihabi, Z.K.; Oles, K. *J. Chromatogr. B* **1995**, *669*, 157–162.
14. Lin, F.-M.; Lin, H.-S.; Kou, H.S.; Wu, S.-M.; Chen, S.-H.; Wu, H.-L. *Anal. Chim. Act.* **2004**, *523*, 9–14.
15. Al-Zehouri, J.; Al-Madi, S.; Belal, F. *Arzneim. Forsch.* **2001**, *51*, 97–103.
16. Olgun, N.; Erturk, S.; Atmaca, S. *J. Pharm. Biom. Anal.* **2002**, *29*, 1–5.
17. Belal, F.; Abdine, H.; Al-Majed, A.; Khalil, N.Y. *J. Pharm. Biom. Anal.* **2002**, *27*, 253–260.

Received May 12, 2005

Accepted June 13, 2005

Manuscript 6660