International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 873-888, Oct-Dec 2009

DEVELOPMENT AND VALIDATION OF SIMULTANEOUS UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LEVOFLOXACIN AND AMBROXOL IN TABLETS

Makarand Avhad*, Dr. C.G. Bonde
Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, Shirpur, NMIMS University, Mumbai, Maharashtra, India.
Corres.author: makarand.avhad@indiatimes.com
Contact No. Mo: 09921242008

ABSTRACT: An accurate, specific and precise UV spectrophotometric method was developed for the simultaneous determination of levofloxacin (LVF) and ambroxol (AMB) in pharmaceutical dosage forms. The method involves formation of Q-absorbance equation at 219 (isoabsorptive point) and at 287 nm, using distilled water as a solvent. The linearity for both levofloxacin and ambroxol was in the range of 2-14 µg/ml and 5-35 µg/ml respectively. The % recovery was found to be 100-101% and 101-102% for levofloxacin and ambroxol respectively indicating proposed method is accurate and precise for simultaneous estimation of levofloxacin and ambroxol in tablets.
KEYWORDS: Levofloxacin Hemihydrate, Ambroxol Hydrochloride, UV Spectrophometry, Q analysis, Dosage Form

INTRODUCTION

Levofloxacin hemihydrate (LVF) (Fig. 1A) chemically, [(-)(s)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid is an optically L-isomer of ofloxacin.[1] It is a broad spectrum fluoroquinolone class of antibacterial agent and effective against many gram positive and gram negative bacteria.[2] It is a potent inhibitor of bacterial DNA gyrase enzyme (topoisomerase II & IV), which is necessary for negative supercoiling of DNA prior to replication.

Ambroxol hydrochloride (AMB) (Fig. 1B) chemically, 4-[(2-amino-3,5-dibromophenyl)-methyl]-amino] cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous secretions.[3]

A fixed dose combination of levofloxacin hemihydrate (LVF) and ambroxol hydrochloride (AMB) is available for the treatment of upper and lower respiratory tract infections.

Literature survey reveals that several methods have been developed for the quantitative determination of LVF in formulations as well as in plasma and urine. These include capillary electrophoresis and UV

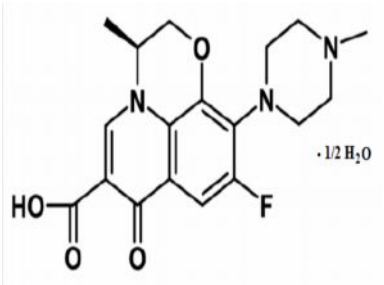

Fig. 1 (A) Chemical structure of Levofloxacin hydrochloride

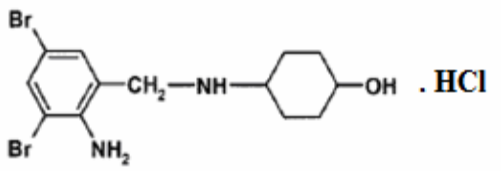

Fig. 1 (B) Chemical structure Ambroxol hydrchloride

spectrophotometry,[4] HPLC,[5,6] simultaneous HPTLC method with ornidazole[7] and flow injection analysis.[8]

It has been reported that ambroxol hydrochloride has been estimated by capillary electrophoresis,[9-10] spectrophotometry,[11] gas chromatography,[12] liquid chromatography with potentiometric estimation,[13] MS detection,[14] UV detection,[15-16] RP HPLC,[17] Raman spectroscopy,[18] liquid chromatography with roxithromycin[19] and derivative UV and HPLC.[20] Simultaneous reversed phase high performance liquid chromatographic method for determination of LVF and AMB in pharmaceutical formulations has been also reported.[21]

However, most of the analytical methods developed for the quantization of LVF and AMB involve analysis of single component, except HPTLC for LVF and HPLC for AMB, which are simultaneous and quite expensive.

This work was aimed to investigate the utility of UV spectrophotometric method in the simultaneous determination of LVF and AMB in pharmaceutical preparations. The method had sufficiently good accuracy, precision and permitted a simple and cost effective assay for these compounds in mixtures.

EXPERIMENTAL

INSTRUMENTATION

A Shimadzu UV spectrophotometer (LAMBDA 25, Perkin Elmer) with 1 cm matched quartz cells was used for the estimation.

CHEMICALS AND REAGENTS

LVF and AMB were kindly supplied by Wockhardt Research Center, Aurangabad & Glenmark Research Center, Nashik as gift samples. Tablets containing LVF and AMB were procured from local pharmacy. All the reagents were of analytical grade. Double distilled water was used throughout the experiment.

STANDARD PREPARATION

Accurately weighed quantities (10 mg each) of LVF and AMB were dissolved separately in sufficient quantity of distilled water in a 100 ml volumetric flask. The solutions were sonicated and the volume was adjusted up to the mark with distilled water to obtain a stock solution of 100 µg/ml; each of LVF and AMB. For the selection of analytical wavelength for the Q absorbance method, the stock solutions of LVF and AMB were separately diluted in distilled water, to get concentrations of 10 µg/ml each, and scanned in the wavelength range of 200-400 nm. From the overlain spectra of both drugs, wavelengths 219 nm (isoabsorptive point) and 287 nm (λmax of LVF) were selected for the formation of Q-absorbance equation. For calibration curves, stock solutions of LVF and AMB were appropriately diluted to obtain concentration range of 2-16 µg/ml and 5-35 µg/ml respectively. The absorbance of LVF was measured at 287 nm and 219 nm, and calibration curves were plotted. Similarly the absorbance of AMB was measured at 219 nm and 287 nm, and calibration curves were plotted. The absorptivities (A1%, 1 cm) of each drug at both the wavelengths were also determined.

SAMPLE PREPARATION

For the estimation of drugs from the commercial formulations, twenty tablets of Mucosyn (Alembic Ltd., Vadodara, India) containing 500 mg of LVF and 75 mg of AMB were weighed, and finely powdered. For the analysis of drugs, a standard addition method was used. An accurately weighed 175 mg of pure AMB was added to finely powdered samples to bring the concentration of AMB in linearity range. With this addition, the ratio of LVF to AMB in samples was brought to 2:1. Quantity of powder equivalent to 20 mg of LVF and 10 mg of AMB was transferred to 100 ml volumetric flask, dissolved in sufficient quantity of distilled water, sonicated and the volume was adjusted up to the mark with distilled water to obtain a stock solution of 200 µg/ml of LVF and 100 µg/ml of AMB. The solution was then filtered through Whatman filter paper No. 41 and the filtrate was appropriately diluted to obtain final concentrations 10 µg/ml of LVF and 5 µg/ml of AMB. Absorbance of this solution was measured at appropriate wavelengths, and values were substituted in the respective formulae to obtain concentrations.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

LVF and AMB, both are sparingly soluble in water, hence double distilled water was chosen as a solvent for their determination in solid dosage forms. The UV spectra of standard solutions of LVF and AMB (10 µg/mL each) were determined separately in distilled water (Fig. 2A and 2B). The λmax of LVF was found to be 287 nm whereas the λmax of AMB was recorded at 245 nm.

Initially, simultaneous equation method was tried for the determination of drugs in their dosage forms, as AMB showed negligible absorbance at the λmax of LVF. However, LVF showed considerable absorbance at the λmax of AMB. Therefore, absorbance ratio (Q analysis) method was applied for the analysis of both the drugs in tablets.

The developed method for the simultaneous analysis of LVF and AMB was validated with respect to stability, linearity, sensitivity, precision, accuracy, specificity, robustness and ruggedness.[22-23]

The stability of both the drugs in distilled water was checked by recording their UV spectra at an appropriate time interval. They were compared with freshly prepared solutions and not any difference was found between them. This indicated that both these drugs were highly stable in solution phase. Further, a UV spectrum of standard solution containing LVF and AMB (mixture) was also recorded to check any chemical interaction between these drugs. The λmax of both the drugs in a mixture was found to be similar as compared to individual drugs indicating no chemical interference with each other (Fig. 2C).

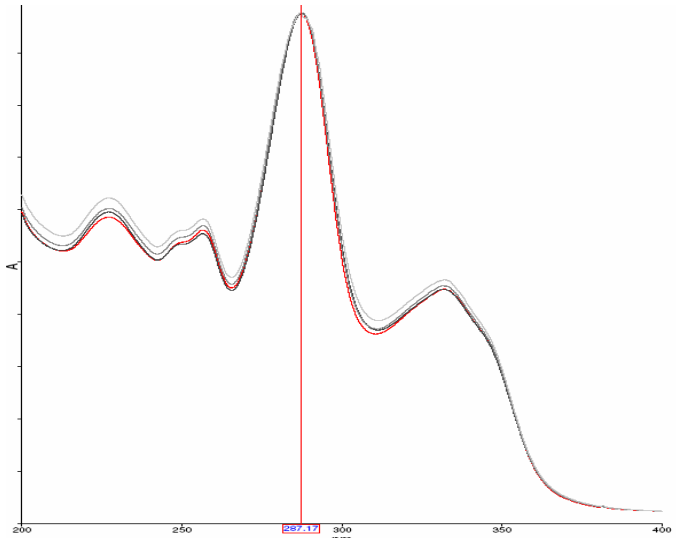

Fig. 2A UV Spectra of LVF

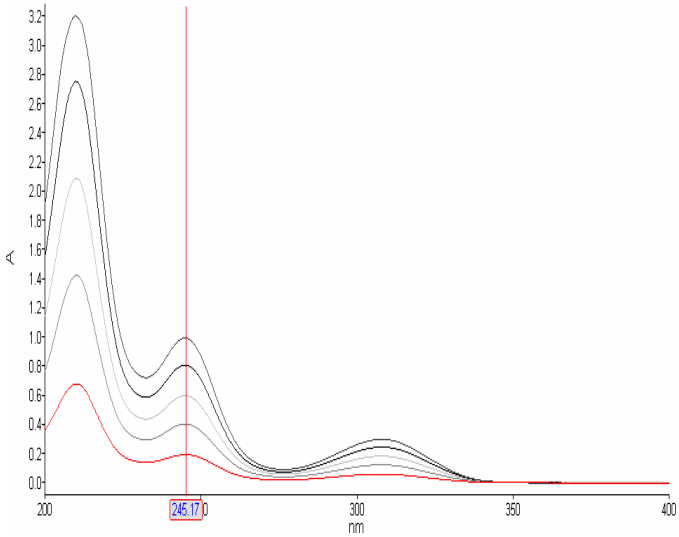

Fig. 2B UV Spectra of AMB

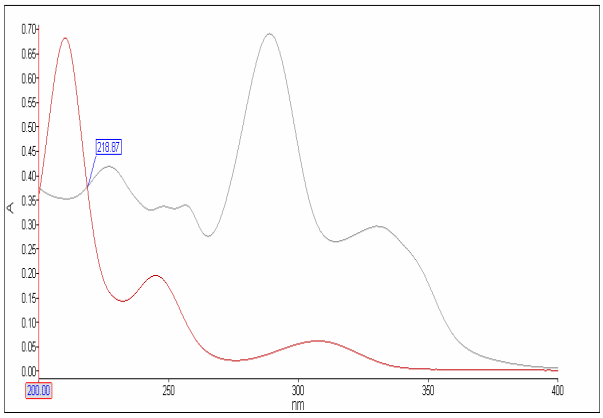

Fig.2C UV Spectra of Mix. (LVF+AMB)

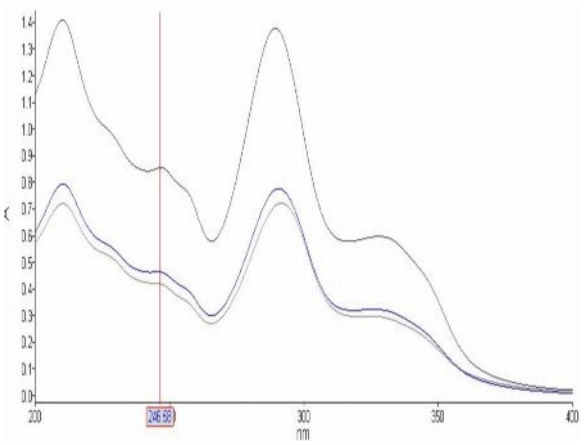

Fig.2D Overlain spectra of Mix. (LVF+AMB)

Q-ANALYSIS METHOD

The ratio of two absorbance determined on the two solutions at two different wavelengths is constant. This constant is termed as Q value. The Q value is independent of concentration and thickness of solution and therefore is used to access the purity of compounds. The absorbance ratio method is a modification of the simultaneous equation procedure. Graphical absorption ratio method uses the ratio of observed absorbance at two selected wavelengths, one of which is isoabsorptive point. It depends on property for that substance which obeys Beer's law at all wavelengths. The ratio of absorbance at any wavelength is constant value independent of concentration or path length.

For Q analysis method, the overlain spectra of LVF and AMB were recorded in the range of 400 to 200 nm. It showed that (Fig. 2D) the peaks were well resolved, satisfying the criteria for obtaining maximum precision, based on absorbance ratios.[24] The criteria being the ratios, $(A_2/A_1)/(ax_2/ax_1)$ and $(ay_2/ay_1)/(A_2/A_1)$, should lie outside the range 0.1-2.0 for the precise determination of X (LVF) and Y (AMB), respectively. Where A_1, A_2 represents the absorbance of the mixture at λ1 (wavelength at isoabsorptive point) and λ2 (λmax of LVF), ax_1 and ax_2 denote absorptivities of X at λ1 and

λ2, and ay_1 and ay_2 denote absorptivities of Y at λ1 and λ2, respectively. In the present work, the above criteria was found to be satisfied for LVF (X) and AMB (Y), where λ1 was 219 nm and λ2 287 nm for Q-absorbance method.

In the quantitative assay of LVF and AMB in an admixture by absorbance ratio method, absorbances were measured at any two wavelengths, one being iso-absorptive point (λ1) and the other being λmax of one of the component i.e. LVF (λ2). Two equations were constructed as described below (Eq. 1 and Eq. 2), using the relationship $ax_1 = ay_1$ at λ1 and b = 1 cm. Equations are

$A_1 = ax_1C_X + ax_1C_Y$ at λ1 > $ax_1 = ay_1$ at λ1…… (1)

and

$A_2 = ax_2C_X + ay_2C_Y$ at λ2 …… (2)

Dividing Eq. 2 by Eq. 1

$$\frac{A_2}{A_1} = \frac{ax_2C_X + ay_2C_Y}{ax_1C_X + ax_1C_Y}$$

Dividing each term by $C_X + C_Y$ and let $F_X = C_X/(C_X+C_Y)$ and $F_Y = C_Y/(C_X + C_Y)$ where, F_X and F_Y are the fractions of X and Y respectively in the mixture of LVF and AMB.

$$\frac{A_2}{A_1} = \frac{ax_2F_X + ay_2F_Y}{ax_1F_X + ax_1F_Y}$$

But $F_Y = 1 - F_X$

$$\frac{A2}{A1} = \frac{Fxax_2 - F_Xay_2 + ay_2}{ax1}$$

$$\frac{A_2}{A_1} = \frac{F_Xax_2}{ax_1} - \frac{Fxay_2}{ay_1} + \frac{ay_2}{ay_1} \quad > ax_1 = ay_1 \text{ at } \lambda1$$

Let

$$Q_X = \frac{ax_2}{ax_1} \quad Q_Y = \frac{ay_2}{ay_1} \quad Q_M = \frac{A_2}{A_1}$$

$$Q_M = F_X(Q_X - Q_Y) + Q_Y$$

$$F_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \quad \text{…… (3)}$$

Eq. (3) gives the fraction of X in the mixture of LVF and AMB. For the determination of absolute concentration of X and Y the equation 5 was rearranged.

$A_1 = ax_1 (C_X + C_Y)$

$$C_X + C_Y = \frac{A_1}{ax_1} \quad \text{……..(4)}$$

From Eq. 3

$$\frac{C_X}{C_X + C_Y} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \quad > F_X = C_X\ ?(C_X + C_Y)$$

$$\frac{C_X}{A_1/ax_1} = \frac{Q_M - Q_Y}{Q_X - Q_Y}$$

$$C_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{ax_1} \quad \text{…… (5)}$$

Similarly,

$$C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_2}{ay_1} \quad \text{…….. (6)}$$

Where, C_X and C_Y are concentrations of LVF and AMB, respectively.[24]

LINEARITY AND PRECISION

In quantitative analysis the calibration curve was constructed for both LVF and AMB after analysis of consecutively increased concentrations. To check the precision and reproducibility of the method, six samples of the same concentration (n=6) of LVF and AMB were prepared and analysed. The low % RSD values obtained for LVF (0.54) and AMB (0.10) indicated that the method had high precision and reproducibility. The regression equation, slope, intercept, correlation coefficient, precision and linearity range are given in Table 1.

ANALYSIS IN TABLET FORMULATIONS

For the determination of LVF and AMB from pharmaceutical tablet formulations by Q analysis method, the absorbance of sample solutions and absorptivity values at the particular wavelengths were calculated and substituted in the following equation (equations 4 and 5) to obtain the concentrations of two components.

$C_{LVF} = (Q_M-Q_Y)\times A_1/(Q_X-Q_Y)\times ax_1$, $C_{AMB} = (Q_M-Q_X)\times A_1/(Q_Y-Q_X)\times ay_1$ where, C_{LVF} and C_{AMB} are concentrations of LVF and AMB, respectively, A_1 is the absorbance of sample at 219 nm, ax_1 is the absorptivity of LVF at 219 nm, ax_2 is the absorptivity of LVF at 287 nm, ay_1 is absorptivity of AMB at 219 nm, ay_2 is absorptivity of AMB at 287 nm, Q_X was obtained by using the equation, (absorptivity of LVF at 287 nm ax_2)/(absorptivity of LVF at 219 nm ax_1). Similarly, Q_Y was obtained from (absorptivity of AMB at 287 nm ay_2)/(absorptivity of AMB at 219 nm ay_1) and Q_M from, (absorbance of sample at 287 nm A_2)/(absorbance of sample at 219 nm A_1). The respective absorptivity values for LVF and AMB at λ1 and λ2 are represented in Table 2. The results obtained from analysis of dosage forms are given in Table 3.

REPRODUCIBILITY

The accuracy and specificity of the proposed method was tested by recovery experiments. Recovery studies were carried out at 100 % level by adding a known quantity of pure drug to the preanalyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated (Table 4). The % recovery for LVF and AMB were found to be in the range of 100.58-101% (% RSD ± 0.21) and 101.57-102.00% (% RSD ± 0.88) respectively for both the formulations tested. The high recovery rate with low

% RSD values indicated that the method had a good accuracy and specificity, as there was no interference from the excipients present in formulations.

Intra-day precision and accuracy were evaluated by analyzing three samples of two different concentrations, prepared on same day. Inter-day variability was assessed by analyzing two concentrations on three different days, over a period of one week. No significant difference was found in these experiments, indicating accuracy and reproducibility of the assays. The % RSD values reported in Table 4 shows that proposed method provides acceptable intra-day and inter-day variation of LVF and AMB.

Ruggedness of the proposed methods was determined by analyzing LVF and AMB by different analysts, using similar operational and environmental conditions; the % RSD values are reported in Table 4 and found to be less than 2 %.

Table 1: Validation parameters for standard LVF and AMB.

Parameter	LVF	AMB
Linearity range (µg/ml)	2-14	5-35
Correlation coefficient	(r2) 0.999a	0.9998a
	0.999b	0.9995b
Intercept	0.004a	0.009a
	0.002b	0.003b
Slope	0.044a	0.048a
	0.074b	0.027b
Regression equation		
	y = 0.044x + 0.004a	y = 0.048x + 0.0095a
	y = 0.074x + 0.002b	y = 0.027x + 0.0038b
Precision (% RSD)*	0.56	0.68

LVF: Levofloxacin; AMB: Ambroxol hydrochloride; a: at 219 nm; b: at 287 nm; *Indicates mean of six determinations (n=6).

Table 2: Absorptivity values at 219 nm (isoobsorptive wavelength) and 287 nm (λmax of LVF)

Absorptivity at 219 nm* (Mean ± S.D.)		Absorptivity at 287nm* (Mean ± S.D.)	
LVF	**AMB**	**LVF**	**AMB**
ax_1	ay_1	ax_2	ay_2
41.48 ± 0.512	47.41 ± 0.812	74.28 ± 0.597	3.35 ± 0.983

LVF: Levofloxacin; AMB: Ambroxol hydrochloride; * Indicates mean of three experiments; S.D.: Standard deviation.

Table 3: Analysis of dosage forms and recovery studies

Product	Drug	Label claim	% Estimated	* % RSD	% Recovery
Mucosyn	**LVF**	500 mg	100.02	0.56	100.58
	AMB	75 mg	98.87	0.68	101.57

LVF: Levofloxacin hemihydrate; AMB: Ambroxol hydrochloride; * Indicates mean of six determinations (n=6).

Table 4: % RSD values for repeatability, intra- day, inter-day variation and ruggedness (n=3).

Parameter	LVF	AMB
Repeatability	0.56	0.28
Precision		
Intra-day	0.60	0.10
Inter-day	0.54	0.20
Ruggedness		
Analyst1	0.54	0.20
Analyst 2	0.56	0.24

LVF: Levofloxacin hemihydrate; AMB: Ambroxol hydrochloride; n: No. of experiments.

CONCLUSION

The proposed method was successfully applied to the simultaneous determination of LVF and AMB from bulk and pharmaceutical tablet formulation. The presented method was found to be simple, accurate, precise and rugged. It can be directly and easily applied to the analysis of the combined pharmaceutical tablet formulation of LVF and AMB. Moreover, the present method is quick and cost effective as compared to chromatographic techniques. Therefore, it can be concluded that the proposed method provides an alternative procedure for the quality control of LVF and AMB in pharmaceutical formulations.

ACKNOWLEDGEMENT

We are grateful to Wockhardt Research Center, Aurangabad & Glenmark Research Center, Nashik for providing gift samples of drug for my work.. We are thankful to Associate Dean, School of Pharmacy & Technology Management, Shirpur for providing laboratory facilities and constant encouragement.

REFERENCES

1. Sweetman, S. C., Martindale The Complete Drug Reference; 33rd ed., Pharmaceutical Press: London, 2002; p. 219.
2. Bertino, J.; Fish, D., Clin. Ther. 2000, 22, 798.
3. Budavari, S., The Merck Index; 12th ed., Merck and Co. Inc.: Whitehouse Station, NJ, 1996; p. 404.
4. Belal, F.; Al-Majed, A.; Al-Obaid, A. M. Talanta. 1999, 50, 765.
5. Ines, M.; Santoro, R. M.; Singh, A. K.; Erika, R. M.; Hackmam, K. J., Pharm. Biomed. Anal. 2006, 40, 179.
6. Neckle, U.; Joukhadar, C.; Mayer, B. X., Anal. Chim. Acta. 2002, 463, 199.
7. Chepurwar, S. B.; Shirkhedkar, S. B.; Bari, S. B.; Fursule, R. A.; Surana, S. J., J. Chromatogr. Sci. 2007, 45, 531.
8. Altiokka, G.; Atkosar, Z.; Can, N. O., J. Pharm. Biomed. Anal. 2002, 30, 881.
9. Pospíilová, M.; Poláek, M.; Jokl, V., J. Pharm. Biomed. Anal. 2001, 24, 421.
10. Perez-Ruiz, T.; Martínez-Lozano, C. C.; Sanz, C.; Bravo, E., J. Chromatogr. B. 2000, 742, 205.
11. Reddy, N. M.; Kanna Rao, K. V.; Swapna, M.; Sarkar, D. G., Indian J. Pharm. Sci. 1998, 60, 249.
12. Colombo, L.; Marcucci, F. M.; Marini, G. M.; Pierfederici, P.; Mussini, E., J. Chromatogr. B. 1990, 530, 141.
13. Bazylak, G.; Nagels, L. J., J. Pharm. Biomed. Anal. 2003, 32, 887.
14. Kim, H.; Yoo, J. Y.; Han, S. B.; Lee, H. J.; Lee, K. R., J. Pharm. Biomed. Anal. 2003, 32, 209.
15. Heinänen, M.; Barbas, C., J. Pharm. Biomed. Anal. 2001, 24, 1005.
16. Koundourellis, J. E.; Malliou, E. T.; Broussali, T. A., J. Pharm. Biomed. Anal. 2000, 23, 469.
17. Indrayanto, G.; Handayani, R., J. Pharm. Biomed. Anal. 1993, 11, 781.
18. Hwang, M. S.; Cho, S.; Chung, H.; Woo, Y. A., J. Pharm. Biomed. Anal. 2005, 8, 210.
19. Qi, M.; Wang, P.; Cong R.; Yang, J., J. Pharm. Biomed. Anal. 2004, 35, 1287.
20. Dinçer, Z.; Basan, H.; Göger, N. G., J. Pharm. Biomed. Anal. 2003, 5, 867.
21. Kothekar, K. M.; Balasundaram, J.; Khandhar, A. P.;Mishra, R. K., Eurasian J. Anal. Chem. 2007, 2, 21.

*****