INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SIMPLE AND ACCURATE RP-HPLC AND TLC-DENSITOMETRIC METHODS FOR DETERMINATION OF CARVEDILOL IN PHARMACEUTICAL FORMULATIONS

Fatma M Abdel-Gawad[1*], Yousry M Issa[2], Emad M Hussien[1], Magda M. Ibrahim[1] and Saadia Barakat[1]

[1]National Organization for Drug Control and Research (NODCAR) 6-Abou-Hazem St.,Pyramids Ave., P.O. Box 29 Cairo, Egypt.

[2] Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.

ABSTRACT

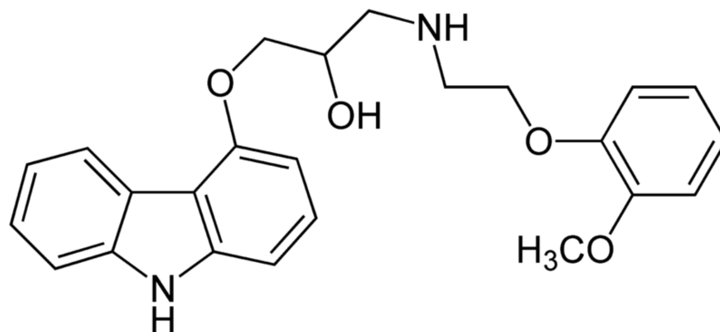
Simple and accurate two chromatographic methods were developed for the determination of carvedilol in raw material and in tablets. The first method uses isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method. Analysis was performed on Agilent C_{18} column using a mobile phase consisting of 0.05 M potassium dihydrogen phosphate (pH 2.5±0.1) and acetonitrile (60:40, v/v) with a flow rate of 2.0 mL min^{-1} and UV detection at 245 nm. The second method uses thin-layer liquid chromatography (TLC) separation of drug from its impurities followed by densitometric measurements of drug spots at 245 nm. The separation was carried out on silica gel 60 F_{254} using acetone-toluene-ethanol-ammonia solution 33% (45:45:10:1, v/v/v/v) as mobile phase. The methods were validated according to USP and ICH guidelines and the acceptance criteria for linearity, accuracy, precision, specificity and system suitability were met in all cases. The methods were linear in the range of 10-200 µg mL^{-1} and 2.0-37.4 µg/spot for HPLC and TLC, respectively. The proposed methods were successfully applied for the determination of carvedilol in bulk and tablets forms. The results were compared statistically at 95% confidence level with each other. There was no significant difference between the mean percentage recoveries and precision of the two methods.

Keywords: Carvedilol, RP-HPLC, TLC-densitometry, Tablets.

INTRODUCTION

Carvedilol(CRV), (±)-1-(9H-carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino }propan-2-ol (Scheme 1), is a non-selective beta adrenergic blocker with alpha-1 blocking activity. It has also antioxidant properties and it is reported to have no intrinsic sympathomimetic activity and only weak membrane stabilizing activity. CRV is used in the management of hypertension and treatment of congestive heart failure. It is also used to reduce the mortality in patients with left ventricular dysfunction following myocardial infarction[1-3].

CRV is the subject of monographic in British pharmacopoeia[4] and European pharmacopoeia[5] whereby a non-aqueous titrimetric method is recommended for its determination, as bulk powder. Several analytical methods have been published for its determination either in bulk powder or in pharmaceutical preparations and biological fluids, these methods include: spectrophotometry[6-11], fluorometry[12,13] chemiluminescence[14], electrochemical[15], gas chromatography[16], HPLC[17-24] and capillary electrophoresis[25-27].



Schem 1: Chemical structure of carvedilol (CRV).

In view of the fact that for the mentioned drug (CRV) there are few HPLC methods[7, 23,24], and one HPTLC method[23] in literature for analysis of CRV in pharmaceutical preparations. This led us to search for simple, accurate and reliable method that can be applied in quality control laboratories for determination of CRV. For this purpose, RP-HPLC and TLC-densitometric methods have been developed for determination of this drug in raw material and in tablets.

EXPERIMENTAL

Apparatus

The HPLC equipment consisting of an Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, auto sampler, photodiode-array (PDA) detector and HP ChemStation software.

The column used was a system C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size) from Waters (Milford, MA, USA), maintained at 25±1 ºC.

TLC aluminium plates (20 x 20 cm) precoated with 0.25 mm silica gel 60 F_{254} was purchased from E. Merck (Darmstadt, Germany). The samples were applied to the plates using 20 µL Hamilton microsyring. A Shimadzu dual wavelength flying spot densitometer model CS-9301 with video display, high speed, high quality and parallel head printer was used.

Instrumental conditions are: photo mode : reflection, scan mode : zigzag and swing width : 16 nm.

Materials and Reagents

All the reagents and solvents were of analytical grade and of highest purity available. Phosphate buffer was prepared by dissolving 3.4 g potassium dihydrogen phosphate (Merck) in about 400 mL distilled water and adjusted pH to 2.5±0.1 using orthophospheric acid (10%). The buffer solution was completed to 500 mL with water to obtain 0.05 M buffer solution. Water was always twice distilled from all glass equipment. Before use, mobile phase was filtered through 0.45 µm membrane filter paper and sonicated using ultrasonic bath prior to use.

Pharmaceutical grade CRV (Roche, Milan, Italy) was assayed for purity according to the official titrimetric method[4] to contain 99.93±0.35%. Dilatrend tablets labeled to contain 25 and 6.25 mg of CRV per tablet (Roche, Milan, Italy, under licence from F. Hoffmann-la Roche Ltd., Basel, Switzerland) were obtained from commercial sources.

Preparation of standard solution

Carvedilol stock solution was prepared by dissolving 100 mg of CRV reference standard in methanol and transferred to 100-mL or 25-mL volumetric flask. The solution was diluted to volume with methanol to obtain 1.0 or 4.0 mg mL^{-1} for HPLC or TLC method, respectively. Whenever, required diluted solutions were obtained by appropriate dilution with methanol

General procedures

RP-HPLC method

The chromatographic separation was performed on a 5 µm RP-agilent C_{18} column. The mobile phase was acetonitrile, 0.05 M phosphate buffer pH 2.5±0.1 (40:60, v/v) and the flow rate was 2.0 mL min^{-1}. The column was conditioned for at least 30 min and the injected volume was 10 µL. Standard CRV solutions were prepared separately in methanol by varying concentrations of drug in

the range 10-200 µg mL^{-1}. Triplicate 10 µL injections were made for solution. The detection was achieved with UV detection at 245 nm and peak area of each concentration was plotted against the corresponding concentration of drug to obtain the calibration graph and regression equation of CRV.

TLC-densitometric method
Working standard solutions in the concentration range 0.2-3.2 mg mL^{-1} of drug were prepared in methanol. Ten microleters (10 µL) from each solution was applied to TLC plate to obtain the concentration range 2-32 µg/spot. The plate was developed to 7±0.5 cm using the mobile phase, acetone-toluene-ethanol-ammonia solution 33% (45:45:10:1, v/v/v/v). The spots were determined densitometrically at 245 nm. The calibration curve and regression equation were obtained as in case of HPLC method.

Assay of CRV tablets
An accurately weighed amount of finely powdered tablets, equivalent to 100 mg of drug, was transferred to a 100 mL conical flask. CRV was extracted with four 20 or 5 ml portions of methanol. The combined extracts were filtered into 100 or 25-mL volumetric flask and diluted to volume with methanol. This solution contains 1.0 or 4.0 mg mL^{-1} of drug for HPLC or TLC method, respectively. Thereafter, the General procedures were followed.

RESULTS AND DISCUSSION

RP-HPLC method
The developed RP-HPLC method has been applied for the determination of CRV in its tablets. To optimize the HPLC assay parameters, the effect of acetonitrile composition and the apparent pH of the mobile phase on the capacity factor ($k^{'}$) were obtained. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile and 0.05 M phosphate buffer (pH 2.5±0.1) in the ratio 40:60 (v/v) at ambient temperature (25±1 ºC) and the injection volume was 10 µL. Increasing acetonitrile concentration to 60% led to inadequate separation of CRV. At lower acetonitrile concentration, the retention time of drug increased, whereas at high or lower pH values resolution was poor. At apparent pH 2.5 improved resolution of the drug was observed.
Quantitation was achieved with UV detection at 245 nm. The retention time for CRV peak is about 4.25±0.10 min. 2 mL min^{-1} flow rate enable acceptable resolution of drug from possible impurities, in a short elution time (Fig. 1). This HPLC method does not need the addition of internal standard (IS) due to the purity of CRV peak.

TLC-densitometric method
Instrumental planar chromatography with precise application of the samples and computer- controlled chromatograms has been considered as reliable for purity control and quantitative drug testing[28].
Experimental conditions, such as mobile phase composition, scan mode, speed and detection wavelength were optimized to provide accurate, precise and reproducible results for CRV. The chosen scan mode was the zigzag mode and the wavelength of scanning was chosen to be 245 nm for CRV. The R_f value 0.79±0.02 was obtained by the system containing: acetone-toluene-ethanol-ammonia solution 33% (45:45:10:1, v/v/v/v). Fig. 2 is a scanning profile of the TLC chromatogram of CAR concentration (2.1, 6.6, 14.2, 18.7, 27.8, 32.0 and 37.4 µg/spot), the detection was carried out at 245 nm. The equilibration time required before development is important to achieve homogeneity of the atmosphere, thus minimizing the evaporation of the solvent from the TLC plate during the development; therefore, the saturation time of the tank has been optimized and found to be 30 min. The plate was developed by ascending chromatography to a distance about 7±0.5 cm with the developed mobile phase. The developed spot was visualized under UV lamp at 245 nm.

Method validation
Method validation was carried out under USP and ICH guidelines for validation of analytical procedures[29,30]. The assay was validated with respect to linearity, accuracy, precision, specificity, LOD, LOQ, robustness and ruggedness.

Linearity
Standard solutions containing 1.0 and 4.0 mg mL^{-1} of CRV were prepared in methanol, to nine different concentrations 10.0-200.0 µg mL^{-1} and 2.0-37.4 µg/spot of CRV for HPLC and TLC methods, respectively. Calibration curves of CRV concentration versus peak area were plotted and subjected to regression analysis using the least square method. The regression equations were computed and found to be:

$$Y=25.24X+183.65, r=0.9998 \quad \text{(HPLC method)} \quad (1)$$

$$Y=722.90X+2010.90, r=0.9995 \quad \text{(TLC method)} \quad (2)$$

Where Y is the integrated peak area at λ=245 nm and X is the concentration of CRV in µg mL^{-1} (HPLC method) or µg/spot (TLC method) and r is the correlation coefficient. The results are collected in Table 1 and the scanned chromatogram of TLC-densitometric method is shown in Fig. 3.

Accuracy

The previously mentioned procedures under linearity were repeated five times for three different concentrations of pure samples. The concentrations of CRV were calculated each from its corresponding regression equation. The mean recovery and SD percentages were calculated and tabulated in Table 2. The results of CRV were compared with those obtained by the BP method[4] using t- and F-test values at 95% confidence limit and found not exceed the theoretical values of 2.31 and 6.39 for t- and F-tests, respectively, indicating no significant difference between the performance of those methods regarding to accuracy and precision. The results are illustrated in Table 2.

Precision

The precision of the proposed methods was investigated with respect to intraday and interday precision. The intraday precision (repeatability) was evaluated by assaying freshly prepared drug solutions in triplicate at concentrations 50, 100 and 200 µg mL^{-1} (HPLC method) or 8, 16 and 32 µg/spot (TLC method) of pure drug. The specified chromatographic conditions were followed as described above. The mean recovery and the relative standard deviation (RSD) were 100.61±0.91 (%) and 100.98±0.87 (%) for HPLC and TLC methods, respectively. The interday precision (reproducibility) was calculated from assaying freshly prepared solution of drug (with the same formentioned concentrations) over a period of three days. The mean recovery and the relative standard deviation were 99.98±0.54 (%) and 100.35±0.66 (%) for HPLC and TLC methods, respectively. The RSD < 1% which indicates the good repeatability and reproducibility of the chromatographic methods. The relative standard deviation (RSD%) are grouped in Table 1.

Specificity

The specificity of the methods were investigated by observing any interference encountered from the common tablet excipients such as talc, lactose, glucose, sucrose, starch and magnesium stearate. These excipients did not interfere with the proposed methods. This fact indicates good selectivity of the methods to determine of this drug both in raw material and in tablets.

Detection and quantification limits

For HPLC method, the limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3:1 and the limit of quantification (LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of 10:1. The results are tabulated in Table 1.

For TLC method; according to ICH recommendation[30]**,** the approach based on the SD of the response and the slope (b) of the calibration curve of lower concentrations of CRV at 0.2-2.0 µg/spot, was used for determination the limit of detection (LOD = 3 x SD/b) and the limit of quantification (LOQ= 10 x SD/b). The results are included in Table 1.

Recovery study

The accuracy of HPLC and TLC methods was also checked by performing recovery experiments using the standard addition method. Known amounts of CRV 20, 60 and 100 µg mL^{-1} (for HPLC method) and 5, 10 and 15 µg/spot (for TLC method) were added to pre-analyzed tablets (100 µg mL^{-1} and 10 µg/spot for HPLC and TLC methods, respectively), and then determined the added concentration of CRV by the two methods. The results of the recovery analysis are tabulated in Table 3. It is included that the proposed methods are sufficiently accurate and precise in order to be applied to pharmaceutical dosage forms. High percentage recovery data show that the two methods are free from the interference of the excipients used in the formulations.

Robustness and ruggedness

The robustness of the method shows the reliability of an analytical method with respect to small, but deliberate variations in method performance parameters. Small deliberate variations of the experimental conditions for HPLC were applied in order to determine the effect on retention time and resolution. The comparison of different C_{18} columns showed that any stationary phase with strongly deactiviated silica could be used. Changes in mobile-phase composition (±2%) or the flow rate (±5%) did not affect significantly the chromatographic method. In TLC method, when changes in mobile-phase composition (±2%) and the plates were developed to 7±0.5 cm, did not have a significant effect on TLC chromatographic separation, illustrating the robustness of the method.

The ruggedness of the proposed methods was evaluated by applying the developed procedure to assay of CRV using the same instrument by two different analysts under the same optimized conditions at different days. Since there was no significant differences between the results obtained by the two analysts, the proposed methods may be considered rugged.

Analytical application

The developed and validated chromatographic methods are successfully applied to raw materials and commercially Dilatrend tablets at two different doses strength (25 and 6.25 mg CRV/tablet). The CRV content of tablets was determined using the calibration curve or regression equation method. The obtained amount of CRV and statistical analysis are given in Table 4. The results obtained by TLC method were statistically compared with the HPLC method which is included in this manuscript using t- and F-tests. HPLC method was chosen as the analytical reference method. At 95% confidence level, the calculated t-and F- test values did not exceed the theoretical ones, showing that there is no significant differences between TLC and HPLC methods (Table 4)

CONCLUSION

The suggested RP-HPLC and TLC-densitometric method can be used as stability-indicating methods for the determination of raw material or pharmaceutical formulations of the CRV without interference from its impurities and excipients. The two methods can be used to determine the purity of the drug available from the various sources by detecting the related impurities. Statistical analysis (Table 1) proves that the two methods are repeatable and selective for the analysis of CRV as raw material and in tablets. TLC-desitometric method is more sensitive than HPLC method and a large number of samples can be analyzed within a short time.

However, in pharmaceutical analysis where the analyte concentration levels are fairly high, so TLC method is more suitable compared to the reported HPTLC method[23].

Table 1: Results of assay validation of the proposed HPLC and TLC-densitometric methods for the analysis of CRV

	Method	
Parameter	HPLC	TLC-densitometric
Linearity	10.0-200. 0 μg mL^{-1}	2.0 - 37.4 μg/spot
λ_{max} (nm)	245	245
Retension time min^{-1} (R_t)	4.25±0.10	
Flow rate (R_f)	2 mL min^{-1}	0.79±0.02
Regression equation (y)[a]		
Slope (b)	25.54	722.90
Interecept (a)	183.65	2010.90
Correlation coefficient	0.9998	0.9995
RSD% (n=5)	0.72	0.77
Intraday precision (n=9)[b]	0.91	0.87
Interday precision (n=9)[b]	0.54	0.66
LOD	1.29 μg mL^{-1}	0.05 μg/spot
LOQ	3.92 μg mL^{-1}	0.17 μg/spot

[a] $Y = bX + a$, where Y is the integrated peak area at 245 nm and X is the concentration of CRV in μg mL^{-1} (HPLC method) or μg/spot (TLC method).
[b] Relative standard deviation (RSD%).

Table 2: Accuracy data for the analysis of pure samples of CRV by the proposed methods and compared with the official PB method (non-aqueous titration)

Item	method		
	HPLC	TLC-densitometric	BP[4]
mean±SD (%)[a]	99.73±0.39	99.90±0.98	100.23± 0.87
Variance	0.15	0.96	0.76
t-test	1.17	0.56	(2.31)[b]
F-test	5.07	1.26	(6.39)[b]

[a] Mean±standard deviation of five determinations.
[b] Theoretical values of t- and F-tests at p=0.05.

Table 3: Standard addition method for the determination of CRV by HPLC and TLC-densitometric methods

Concentration of CRV (µg mL^{-1})				Concentration of CRV (µg/spot)			
HPLC method				TLC-densitometric method			
Taken	Added	Found	Recovery (%)[a]	Taken	Added	Found	Recovery (%)[a]
Dilatrend tablets (25 mg CRV/tablet)							
100.0	20.0	19.98± 0.18	99.90	10.0	5.0	5.02± 0.04	100.40
100.0	60.0	60.20± 0.46	100.33	10.0	10.0	10.12±0.09	101.20
100.0	100.0	99.80± 0.73	99.80	10.0	15.0	14.82±0.14	98.80
Mean			100.01				100.13
RSD (%)			0.80				0.88
Dilatrend tablets (6.25 mg CRV/tablet)							
100.0	20.0	20.09± 0.12	100.45	10.0	5.0	4.98± 0.03	99.60
100.0	60.0	60.70± 0.57	101.20	10.0	10.0	10.04±0.07	100.40
100.0	100.0	99.51± 0.80	99.51	10.0	15.0	15.02±0.11	100.13
Mean			100.39				100.04
RSD (%)			0.93				0.68

[a]Mean of three determinations.

Table 4: Application of HPLC and TLC-densitometric methods for determination of CRV in raw material and tablet.

Sample	Recovery ±SD (%)[a]	
	TLC-densitometric	HPLC
Raw material	99.92±0.87	99.88±0.61
	t= 0.08	(2.31)[b]
	F=2.03	(6.39)[b]
Dilatrend tablets (25 mg CRV/tablet)	100.11±0.83	100.31±0.69
	t= 0.41	
	F=1.45	
Dilatrend tablets (6.25 mg CRV/tablet)	100.29±0.97	100.01±0.56
	t=0.56	
	F=3.00	

[a] Mean±standard deviation of five determinations.
[b] Theoretical values of t- and F-tests at p=0.05.

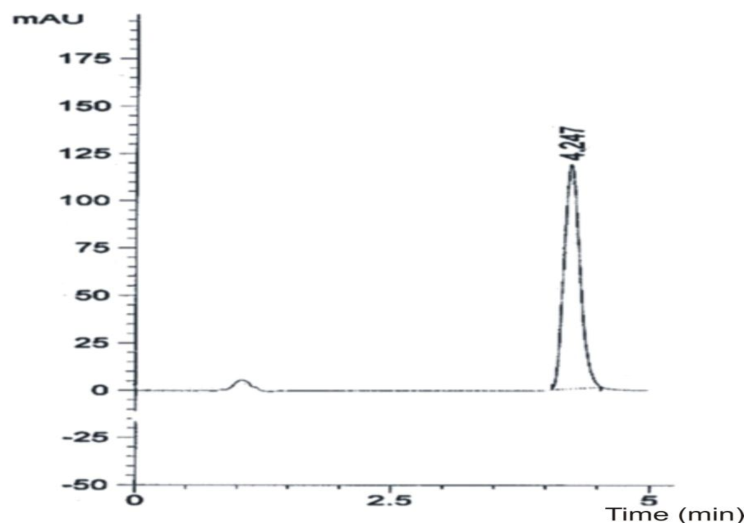


Fig. 1: Chromatogram obtained for carvedilol at 50.0 µg mL^{-1} using Agilent C_{18} and mobile phase composed of acetonitrile:phosphate buffer pH 2.5 (40:60, v/v) at 25 °C and flow rate of 2 mL min^{-1}. Detection was performed at 245 nm

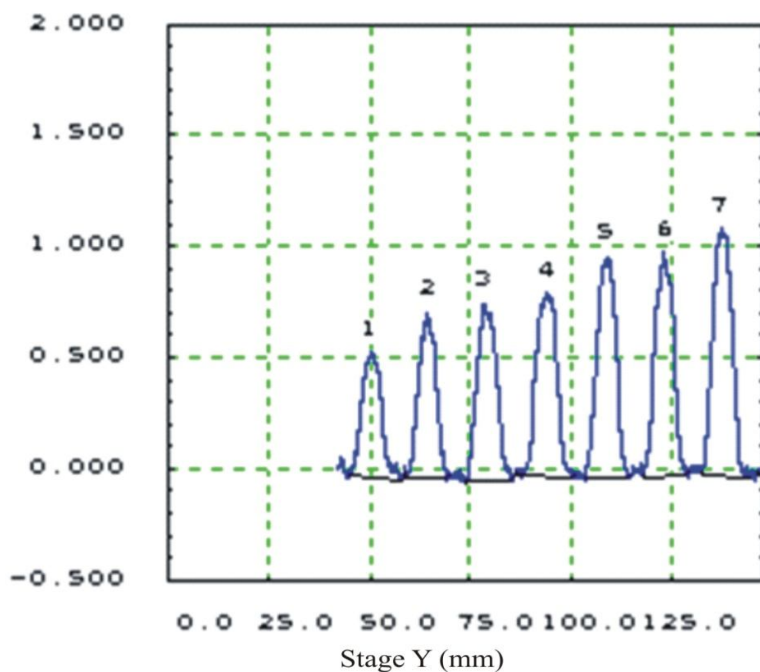


Fig. 2: Scanning profile of the TLC chromatogram of CRV concentration (2.1, 6.6, 14.2, 18.7, 27.8, 32.0 and 37.4 µg/spot) at 245 nm.

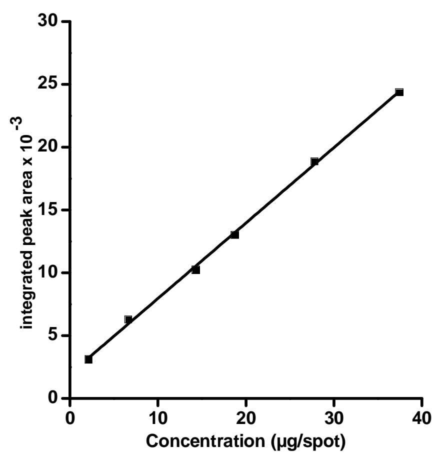


Fig. 3: Calibration curves of CRV concentration (2.1, 6.6, 14.2, 18.7, 27.8 and 37.4 µg/spot) versus peak area.

REFERENCES

1. Morgan T. Pharmacokinet, 1994;26:335-346.
2. Keating GM and Jarvis B. Drugs. 2003;63:1697-1741.
3. Martindale. The Complete Drug Reference, thirty fifth edition, Pharmaceutical Press, London, 2007; p. 1114.
4. British Pharmacopoeia, the Stationary Office, London, 2009; p. 374.
5. European Pharmacopoeia, Council of Europe, 67075 Strasbourg Codex, France, 2007; p. 1427.
6. Cardoso SG, Ieggli CVS and Pomblum SCG. Die Pharmazie. 2007; 62:34-37.
7. Leggli CVS, Cardoso SG, Belle LP. J AOAC. 2005;88:1299-1303.
8. Imran M, Singh RS and Chandran S. Pharmazie. 2006;61:766-769.
9. Sreevidya TV and Narayana B. Indian J ChemTechnol. 2009;16:74-78.
10. Sultan M. Asian J. Chem., 2008; 20:2283-2292.
11. Verma JK and Syed HA. Indian J Pharm Sci. 2007;69:303-304.
12. XU LX, Hui N, Ma LY and Wang HY. Spectrochim Acta A. 2005; 61:855-859.
13. Wang HY, Xiao Y, Han J and Chang XS. Anal Sci. 2005;21:1281-1285.
14. Pires CK, Marques KL, Santos JLM, Lapa RAS, Lima J and Zagatto EAG. Talanta. 2005;68:239-244.
15. Dogan B and Ozkan S. Electroanalysis. 2005;17:2074-2083.
16. Myung SW and Jo CH. J. Chromatogr B Analyt Technol Biomed Life Sci. 2005; 822: 70-77.
17. Galanopoulou O, Rozou S and Antoniadou-Vyza E. J Pharm Biomed Anal. 2008; 48:70-77.
18. Gomes NA, Vaidya VV, Pudage AM, Joshi SS, Parekh SA and Tamhankar AV. Chromatographia. 2009;69:19-25.
19. Jeong DW, YH Kim, HY Ji, YS Youn, KC Lee and HS Lee. J Pharm Biomed Anal. 2007;44:547-52.
20. Leung GNW, Leung DKK, Wan TSM and Wong CHF. J Chromatogr A. 2007; 1156:271-279.
21. Saito M, Kawana J, Ohno T, Kaneko M, Mihara K, Hanada K, Sugita R, Okada N, Oosato S, Nagayama M, Sumiyoshi T and H Ogata. J Chromatogr B Analyt Technol. Biomed Life Sci. 2006;843:73-7.
22. Stojanovic J, Marinkovic V, Vladimirov S, Velickovic D and Sibinovic P. Chromatographia. 2005;62:539-542.
23. Patel IJ, Suhagia BN, Shah PB and Shah RR. Indian J Pharm Sci. 2006;68:790-793.
24. Zarghi A, Foroutan SM, Shafaati A and Khoddam A. J Pharm Biomed Anal. 2007;44:250-253.
25. Chankvetadze B, Lomsadze K, Burjanadze N, Breitkreutz J, Pintore G, Chessa M, Bergander K and Blaschke G. Electrophoresis. 2003;24:1083-1091.
26. Clohs L and McErlane KM. J Pharm Biomed Anal. 2001;24:545-554.
27. Koppenhoefer B, Epperlei UB, Christian B, Lin Y Ji and Y Chen. J Chromatogr A. 1996;735:333-343.
28. Renger B. In Proceedings of the sixth international symposium on instrumental planner chromatography, Bad Duerkheim. 1999; 291.
29. The united States Pharmacopoeia, Uited States Pharmacopoeial Convention, INC, USA, 2005; pp. 2748-2751.
30. The Eurpean Agency for the Evaluation of Medical Products ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures; Methodology, 1996, CPMP/ICH/281/95