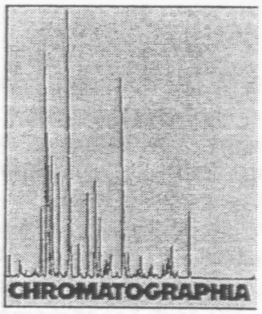
Determination and Validation of an LC Method for Fluvoxamine in Tablets



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Abstract

A new, simple, rapid and specific reversed-phase high-performance liquid chromatography (HPLC) method was developed and validated for the determination of fluvoxamine in pharmaceutical dosage forms. The HPLC separation was achieved on a C_{18} μ-Bondapack column (250 mm × 4.6 mm) using a mobile phase of acetonitrile–water (80:20, *v/v*) at a flow rate of 1 mL min^{-1}. Proposed method is based on the derivatization of fluvoxamine with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) in borate buffer of pH 8.5 to yield a orange product. The HPLC method is based on measurement of the derivatized product using UV-visible absorbance detection at 450 nm. The method was validated for specificity, linearity, precision, accuracy, robustness. The degree of linearity of the calibration curves, the percent recoveries of fluvoxamine, the limit of detection and quantification, for the HPLC method were determined. The assay was linear over the concentration range of 45–145 ng mL^{-1} ($r = 0.9999$). Limit of detection and quantification for fluvoxamine were 15 and 50 ng mL^{-1}, respectively. The results of the developed procedure (proposed method) for fluvoxamine content in tablets were compared with those by the official method. The method was found to be simple, specific, precise, accurate, reproducible and robust.

Keywords

Column liquid chromatography
Fluvoxamines in tablets
(E)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime
Dosage forms-tablets

Introduction

Fluvoxamine(Flu), (E)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime [1] (Fig. 1).

Flu facilitates serotoninergic neurotransmission via potent and selective inhibition of serotonin (5-hydroxytryptamine; 5-HT) reuptake into presynaptic neurons. Thus, Flu is an effective and well tolerated antidepressant agent that is becoming established as an alternative to older agents in patients with mild, moderate or severe depression [2].

There have been only a few articles published on the analytics of Flu. Determination of Flu in pharmaceutical preparations by capillary gas chromatographic methods has also been described [3, 4]. Skibinski et al. have studied determination of Flu in tablets densitometric and videodensitometric TLC [5] and HPLC [6] methods. An extractive spectrophotometric [7], capillary electrophoresis [8], polarographic [9], UV spectra derivative [10], HPLC [11] and UV-visible spectrophotometric and HPLC [12] methods have also been reported for determination of Flu in pharmaceutical preparations. Atmaca et al. [13] have studied spectrophotometric method for the determination of Flu in tablets. Furukori et al. [14] have determined by liquid–liquid extraction and column-switching HPLC method of fluvoxamine and its metabolite fluvoxamino acid by liquid–liquid extraction and column-switching HPLC. Titier [15] and Duverneuil [16] et al. have reported an HPLC method with diode array detection for the assay of Flu in human plasma. An HPLC methods with UV detection have also been reported for the determination of Flu in human plasma [17–21]. HPLC methods with fluorescence detection have also been reported for the determination of Flu in human plasma [22, 23].

According to the literature research, Flu has been derivatized with NQS and determined using a UV-visible absorbance detector for the first time. In the present study, a rapid, specific, precise and validated HPLC method.

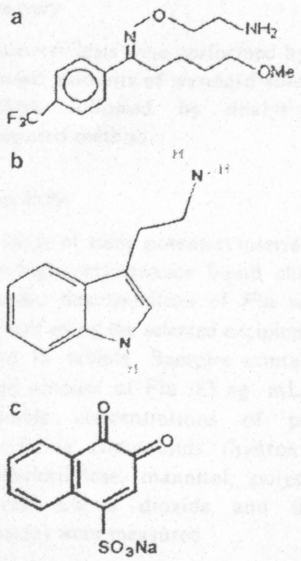

Fig. 1. Chemical structures of Flu (**a**) internal standard and (**b**) NQS (**c**)

Experimental

Materials and Reagents

Flu and its tablets (Faverin 50 mg) were a gift sample from Eczacıbası (Istanbul, Turkey). Internal standard (Tryptamine) and NQS were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of HPLC or analytical grade.

Apparatus

The chromatographic system used in this study consisted of a LC-Model P 4000 solvent delivery system (Thermo Separation, TX, USA), a Rheodyne injection valve with a 20 µL loop, a UV-Vis detector operated at 450 nm. Data acquisition was performed using Thermo Separation Products HPLC chromatography software. Shimadzu UV-160 spectrophotometer for scanning and selecting working wavelength of detection. Samples were analyzed on a 4.6 mm × 250 mm × 5 µm C_{18} column attached to a Phenomenex guard column (4 mm × 3 mm ID). All the assays were performed at room temperature and a flow rate of 1 mL min^{-1}. HPLC grade deionized water (by aquaMAX™ ultra, Young instrument (Korea) ultrawater purification system) was used throughout the analysis.

Solutions

Stock solution of Flu was prepared by dissolving accurately weighed 100 mg of the drug in 100 mL of methanol (final concentration, 1 mg mL^{-1}). Appropriate dilutions of Flu were made in methanol to produce working standard solution of 1 µg mL^{-1}. The prepared stock solution was stored at 4 °C protected from light. Tryptamine (IS) stock solution was made at an initial concentration of 1 mg mL^{-1}. The internal standard (IS) stock solution was diluted with methanol to a final concentration of 1 µg mL^{-1}.

A borate buffer (0.1 M) was prepared by dissolving 0.620 g of boric acid and 0.750 g of potassium chloride in 100 mL water. The pH was adjusted to 8.5 with 0.1 M sodium hydroxide solution and the volume was made up to 200 mL with water [24].

NQS was prepared as a 0.1 % (*w/v*) solution of distilled water.

Sample Solution

Twenty tablets were weighed, finely powdered and portions equivalent to 100 mg Flu were transferred into a 100 mL volumetric flask; 50 mL methanol was added, shaken thoroughly to dissolve, was brought to volume, mixed well and centrifuged the supernatant was used to prepare solutions of 1 µg mL^{-1} of Flu using the methanol as the diluent. The working sample solution (1 µg mL^{-1}) obtained by dilution of supernatant was used to set up the concentrations in the range of calibration studies.

Assay Procedure and Derivatization

Five hundred microliter of each of the Flu standard or sample solutions was transferred into 12 mL glass test tubes. To each tube, 500 µL of IS solution in methanol, 300 µL borate buffer pH 8.5 and 200 µL NQS solution were added. After 2-s vortexing the tubes were allowed to stand in a water bath at 70 °C for 30 min. After cooling, under tap water, the solution in each tube was acidified with 100 µL of 0.1 M hydrochloric acid solution, The NQ-derivatives were extracted three times with 1.5 mL dichloromethane: *n*-butanol (4:1, *v/v*). The organic phases were separated by centrifugation at 2,000*g* for 2 min, transferred quantitatively to a 5 mL calibrated flask and diluted to volume with dichloromethane: *n*-butanol (4:1, *v/v*). A portion (100 µL) of the supernatant organic phase was transferred into a 0.5 mL centrifuge tube and dried with a N_2 stream. The residue was redissolved in 200 µL of the mobile phase, and a portion (20 µL) was injected into the HPLC system.

Method Validation

Linearity (Calibration Curve)

The calibration curves were constructed with nine concentrations including the LOQ ranging from 45 to 145 ng mL^{-1}. The peak area ratio of the drug to the IS was considered for plotting the linearity graph. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Detection and Quantification Limits (Sensitivity)

Limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. The limit of detection was calculated by LOD = $3.3\sigma/S$, where σ is the standard deviation of the response of the blank and S is the slope of the calibration curve. The limit of quantification was calculated by LOQ = $10\sigma/S$ under the ICH guidelines [24, 25]. The calibration curves consisted of nine points and six replicate injections of standards at each concentration level were performed.

Accuracy and Precision

Accuracy of the assay method was determined for both intra-day and inter-day variations using different samples of Flu. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples of same concentration during same day. The precision was studied by comparing the assays on different days (3 days). Accuracy of the analytical method was determined by analyzing Flu of different known concentrations.

Recovery

Recovery tests were performed by adding known amounts of standard solutions to sample followed by analysis using proposed method.

Specificity

A study of some potential interference in the high performance liquid chromatographic determination of Flu was performed using the selected excipients often used in tablets. Samples containing a fixed amount of Flu (85 ng mL^{-1}) and variable concentrations of potential interfering compounds (hydroxypropyl methylcellulose, mannitol, polyethylene glycol, silicon dioxide and titanium dioxide) were measured.

System Suitability

The specificity of the HPLC method was determined by the complete separation of Flu along with other parameters like retention time (t_r), capacity factor (k), tailing or symmetrical factor (T), area, height, plates, resolution, HETP (mm) [25, 26].

Stability

The stability of the Flu-NQ derivatives solution was determined for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing at 4 °C for 30 days.

Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the percentage of mobile phase acetonitrile and water, in the mobile phase flow rate and in the wavelength.

Results and Discussion

Method Development and Optimization

The reaction of NQS with Flu that own a free primary amine group results in the formation of coloured products.

It has been previously reported that derivatization reactions with NQS are pH-dependant with optimum pH around 7–10 for derivatization of amines. A systematic study was performed to optimize the pH of the reaction. A solution of Flu was derivatized under different pH values in the range 7–10. Maximum absorption was observed at pH value of 8 and 8.5. Therefore, it was decided to carry out further experiments at pH 8.5 borate in order to obtain the highest sensitivity.

The effect of temperature on the color intensity was studied in the range from 50 to 80 °C for different periods of time. The color intensity increased on increasing the applied temperature up to 70 °C. The effect of the reaction time on the reaction course was studied by measuring the corresponding absorbance at constant temperature for different periods of time. It was found that the optimum reaction time is 30 min.

The molar ratio between Flu and the derivatizing reagent (NQS) required to obtain maximum absorbance (sensitivity) and precision was also investigated at the following ratios: 1:0.5, 1:1, 1:2, 1:5, 1:6, 1:10. An equimolar ratio (1:1) was found to be enough to give maximum yield (as indicated by absorbance).

The solvents used for NQS reagent as well as for dilution of the reaction mixture were carefully studied using different solvents: water, methanol, ethanol, and acetonitrile. Water was found to be the optimum solvent as the highest absorbance values were obtained.

This isocratic-mode method with absorbance detection was developed for the determination of Flu. To develop a rugged and suitable HPLC method for the quantitative determination of Flu, different mobile phases and stationary phases were employed. Our preliminary trails using different compositions of mobile phases consisting of water, methanol and buffer systems on different reversed-phase stationary phases did not give good peak shape and resolution. The mobile phase consisted of acetonitrile–water at various ratios (75:25, 80:20, 85:15, v/v) was tested as starting solvent. The variation at the mobile phase leads to considerable changes in the chromatographic parameters. However, the proportion acetonitrile–water at a ratio of 80/20 (v/v) yielded the best results.

The preliminary investigations were directed towards the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of the organic modifier, the

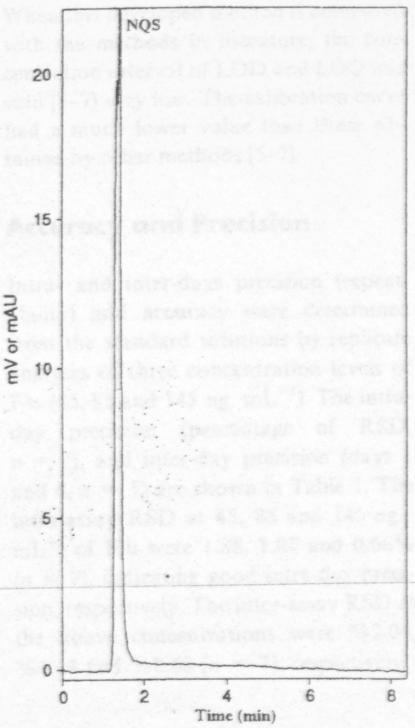

Fig. 2. Representative HPLC chromatogram of a blank (placebo) solution

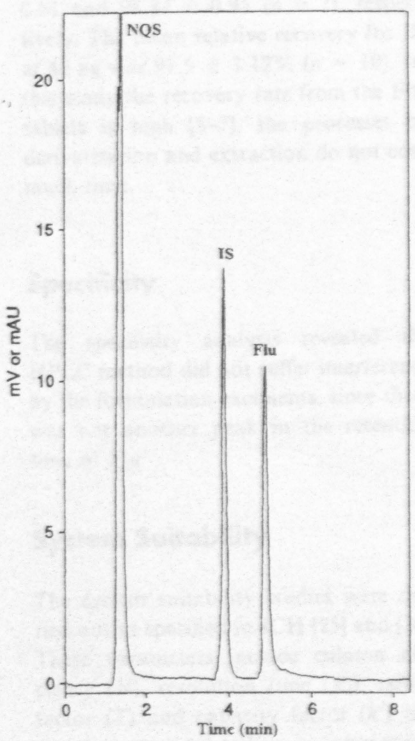

Fig. 3. Typical chromatogram obtained from separation of fluvoxamine (4.807 min), internal standard (4.079 min) and NQS (1.891) on a C_{18} μ-Bondapack column with mobile phase of acetonitrile–water (80:20, v/v) at a flow rate of 1 mL min^{-1}

Table 1. Intra- and inter-day precision and accuracy of the assay for Flu

Nominal concentration (ng mL^{-1})	No. of replicates (n)	Estimated concentration (ng mL^{-1}) mean ± SD	Precision (RSD%)	Accuracy (RME%)
Intra-day				
45	7	45.77 ± 0.86	1.88	2.26
85	7	85.87 ± 0.92	1.07	0.83
145	7	146.1 ± 0.97	0.66	0.76
Inter-day				
45	7	45.98 ± 0.94	2.04	2.18
85	7	86.12 ± 0.98	1.14	1.32
145	7	146.5 ± 0.99	0.68	1.03

Table 2. System suitability study

Parameter	Data
Retention time	4.807
RSD (%)	0.92
Capacity factor	7.1421
Area (μAU-s or μV)	94,533
Height (μAU or μV)	12,955
PW at 50% (min)	0.110
PW at 10% (min)	0.217
PW at 5% (min)	0.256
PW at base (min)	0.192
USP tailing, EP symmetry factor	1.3269
CFR asymmetry factor (10%)	1.2670
Plates (USP, EP)	7,217, 7,596
Plates/meter (USP, EP)	72,173, 75,960
Resolution (USP, EP)	2.21,2.67
Reduced plate height (USP, EP)	2.77, 2.61

USP calculations done by tangent method
EP calculations

column, the mobile phase. The first trial was carried out by using reversed phase C_{18} column (4.6 mm × 250 mm × 5 μm) and a mixture of acetonitrile-water (80:20, v/v). This system was found to be suitable to elute Flu but the retention time was short and the column resolution good (the peak symmetry and a reduction in the tailing factor).

Several substances (lisinopril, amlodipine, tianeptine, nortryptiline, maprotilin, desipramine, sertraline and tryptamine) were tested as internal standards. Among these, tryptamine met all the typical requirements of a compound to be used as IS—it was stable during the analysis, it was easily available, its elution time was shorter than that of Flu, and its peak did not interfere with the matrix of tablets samples. The extraction recovery value for the IS (tryptamine) was 97.5 ± 1.12% (n = 10). Flu and the internal standard tryptamine were well separated within 5 min using isocratic elution with acetonitrile: water (80:20, v/v) at a room temperature of 23 ± 1 °C and a flow rate of 1 mL min^{-1}. On the other hand, the mobile phase in the proposed method acetonitrile-water instead of buffered systems used in previousyl reported HPLC methods [14–16]. Therefore flushing of the column after analysis is not required. Flu and the IS were well resolved with good symmetry with respective retention times of 4.807 and 4.079 min (Figs. 2, 3). The method uses a simple mobile phase composition, easy to prepare with little or no variation. Besides, according to the other methods the retention time is quite short [11–14, 18]. It is a highly specific and precise analytical procedure and its chromatographic run time of 6 min allows the analysis of a large number of samples in a short period of time.

Linearity, Limit of Detection and Limit of Quantification

The calibration curves for Flu were linear for HPLC method over the concentration range of 45–145 ng mL^{-1}. The results showed an excellent linearity between peak area ratios (Flu/internal standard) and concentration. The equations of the calibration curves were obtained by the least-squares linear regression analysis and calculated as: $A = 0.2158C + 7.90 \times 10^{-3}$. The limit of detection and the limit of quantification for HPLC analysis for Flu were 15 and 50 ng mL^{-1}, respectively. Standard deviations of the slope and intercept for the calibration curves generated on six replicates were 0.005 and 0.029, respectively. The correlation coefficient (r) of all the calibration curves were consistently greater than 0.999. The method exhibited good selectivity and sensitivity. In this study the purpose of the derivatization reaction is the raise of sensitivity thus the possibility of working in low concentrations (LOD and LOQ are quite low) has been occurred. When this developed method is compared with the methods in literature, the concentration interval of LOD and LOQ was seen [5–7] very low. The calibration curve had a much lower value than those obtained by other methods [5–7].

Accuracy and Precision

Intra- and inter-days precision (repeatability) and accuracy were determined from the standard solutions by replicate analyses of three concentration levels of Flu (45, 85 and 145 ng mL^{-1}). The intra-day precision (percentage of RSD, n = 7), and inter-day precision (days 1 and 4, n = 7) are shown in Table 1. The intra-assay RSD at 45, 85 and 145 ng mL^{-1} of Flu were 1.88, 1.07 and 0.66% (n = 7), indicating good intra-day precision, respectively. The inter-assay RSD at the above concentrations were %2.04, %1.14 and %0.68 (n = 7), respectively.

Recovery

The mean relative recoveries for Flu at 45, 65, 85 and 145 ng mL^{-1} were 97.8 ± 0.65%, 98.51 ± 0.74, 98.51 ± 0.91 and 98.84 ± 0.95 (n = 7), respectively. The mean relative recovery for IS at 45 ng was 97.5 ± 1.12% (n = 10). In this study the recovery rate from the Flu tablets is high [5–7], the processes of derivatization and extraction do not cost much time.

Specificity

The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there was not another peak in the retention time of Flu.

System Suitability

The system suitability studies were carried out as specified in ICH [25] and [26]. These parameters include column efficiency (N), resolution time (R_t), tailing factor (T) and capacity factor (k') like retention time (t_r) tailing or symmetrical factor (T), area, height, plates, resolution and HETP (mm). Number of theoretical plates for C_{18} (9,373–9,829) was higher

than 2,000, which is the minimum value to consider the method acceptable [25, 26] (Table 2).

Stability

Stock solutions of Flu and IS were stable for at least 15 days when stored at 4 °C. FLu and IS–NQ derivatives solutions were stable for 12 h at room temperature and 5 days at 4 °C.

Robustness

At the recommended flow rate of 1.0 mL min^{-1}, the retention times for Flu was 4.807 min. At 1.1 mL min^{-1}, the retention times was 4.75• and at 0.90 mL min^{-1}, 5.2 min. At 10% higher and lower flow rate, no significant change in peak areas were observed. A resolution factor of > 2 were observed with either a 10% increase or decrease in flow rate. The robustness as a measure of method capacity to remain unaffected by small, but deliberate, variations in method parameters was studied testing influences of small changes in mobile phase composition. It was investigated, that mobile phase composition had very strong influence on peak retention time and separation. The standard deviation of peak areas was calculated for each parameter and RSD was found to be less than 0.67%. All critical separations were achieved with the indicated minimum baseline resolution (Table 3).

Comparison with Official Method

Commercially available tablets of Fluvoxamine malete (Faverin® 50 mg) were subjected to analysis by the proposed method.

On comparison of the results obtained by the proposed methods with the official method [27] using the *t* test for the accuracy and *F* test for the precision assessment, the calculated values did not exceed the corresponding theoretical values (tabulated value of *t* test and *F* test is under confidence level 95% = 6.388 and 2.78 for $n = 5$ degrees of freedom and $n\text{-}1 = 4$, respectively) indicating insignificant differences the results. Results of analysis for the two methods are summarized in Table 4.

Table 3. Influence of changes in experimental parameters of the HPLC method

Parameter	Modification	Flu (percentage of recovery)
Mobile phase ratio (*v*/*v*) acetonitrile–water	80:20 (Optimum)	99.12
	75:25	98.24
	85:15	98.87
	60:40	97.45
Flow rate (mL min^{-1})	1.0 (Optimum)	99.08
	1.1	98.31
	0.9	97.79
Wavelength (λ_{max}, nm)	450 (Optimum)	99.16
	451 nm	97.05
	449 nm	97.12

Table 4. Comparison of the results obtained by proposed method and official methods ($n = 5$) Faverin tablet (50 mg)

Statistical value	Proposed method	Official method [27]
Mean	49.59	48.24
Recovery (%)	99.18	96.48
RSD (%)	0.56	0.84
t test of significance	0.96	
F test of significance	2.25	

$P = 0.05$, $t = 2.78$, $F = 6.388$

Conclusions

A simple and quick, new analytical method has been developed to be applied in the analysis of commercially available dosage forms. The method uses a simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 6 min and the relatively low flow rate (1 mL min^{-1}) allows the analysis of large number of samples with less mobile phase that proves to be cost-effective. All statistical values (percentage recoveries, RSD, confidence limits of the slope and intercept, LOD and LOQ) were within the acceptable limits. Hence, this HPLC/UV visible method can be used for the routine drug analysis.

References

1. The Merck Index (2001) 748:Fluvoxamine
2. Wilde MI, Plosker GL, Benfield P (1993) Drugs 46:896
3. Berzas Nevado JJ, Villaseñor Lierena MJ, Contento Salcedo AM, Aguas Nuevo E (2005) J Pharm Biomed Anal 38:52–594
4. Berzas JJ, Guiberteau C, Villaseñor MJ, Rodriguez V (2004) Anal Chim Acta 519:219–230\
5. Skibinski R, Misztal G (2004) J Planar Chromatogr Mod TLC 17:224–228
6. Skibinski R, Misztal G (2001) Acta Poloniae Pharmaceutica Drug Res 58:97–100
7. Starczewska B, Mielech K (2000) J Pharm Biomed Anal 23:243–247
8. Berzas Nevado JJ, Contento Salcedo AM, Villaseñor Llerena MJ, Aguas Nuevo E (2000) Anal Chim Acta 417:169–176
9. Elmali F, Alpdogan G, Sungur S, Aycan S (2000) Turk J Chem 24:299–302
10. Misztal G, Skibinski R (1999) Acta Poloniae Pharm Drug Res 56:95–100
11. Foda NH (1995) J Liq Chromatogr 18:1591–1601
12. Atmaca S, Tatar S (1995) Acta Pharm Turc 37:33–3713
13. Atmaca S, Tatar S (1994) Pharmazie 49:458–459
14. Yasui-Furukori Inoue N, Kaneko YS, Otani K (2005) J Pharm Biomed Anal 37:121–125
15. Titier K, Castaing N, Scotto-Gomez E, Pehourcq F, Moore N, Molimard M (2003) Ther Drug Monit 25:581–587
16. Duverneuil C, de la Grandmaison GL, Mazancourt P, Alvarez JC (2003) Ther Drug Monit 25:565–573
17. Wong SH, Kranzler HR, Della Fera S, Fernandes R (1994) Biomed Chromatogr 8:278–282
18. Hartter S, Wetzel H, Hiemke C (1992) Clin Chem 38:2082–2086
19. Van Der Meersch-Mougeot V, Diquet B (1991) J Chromatogr 567:441–449
20. Foglia JP, Birder LA, Perel JM (1989) J Chromatogr B 495:295–302
21. Lhermitte M (1989) Biomed Chromatogr 3:177–179
22. Pullen RH, Fatmi AA (1992) J Chromatogr 574:101–107
23. Schweitzer C, Spahn H, Mutschler E (1986) J Chromatogr B 382:412–414
24. British Pharmacopoeia, Her Majesty's Stationery Office, London (1998) 799–800
25. Validation of analytical procedures: Methodology ICH Harmonised Tripartite Guideline, Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on, 6 November (1996)
26. Shabir GA (2003) J Chromatogr A 987:57–66
27. British Pharmacopoeia (2001) 765