DRUG FORMULATIONS AND CLINICAL METHODS

Simultaneous Determination of Potassium Clavulanate and Cefixime in Synthetic Mixtures by High-Performance Liquid Chromatography

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A simple, precise, and sensitive high-performance liquid chromatographic method was developed and validated for the simultaneous determination of potassium clavulanate and cefixime in synthetic mixture form. The analytes were separated on a C18 column by using 0.03 M disodium hydrogen phosphate buffer (pH 6.5)–methanol (84 + 16, v/v) as the mobile phase with detection at 220 nm. The method exhibited high sensitivity and good linearity in the concentration ranges of 12.5–62.5 and 20–100 μg/mL for potassium clavulanate and cefixime, respectively. The total run time for the 2 components was <8 min, and the average recovery was >101.5% with a relative standard deviation of <1.0%. The proposed method was validated according to guidelines of the International Conference on Harmonization by evaluation of linearity, recovery, selectivity, robustness, limits of detection and quantitation, and within- and between-day precision. The results obtained for the synthetic mixture show that the method is highly precise and accurate for the simultaneous determination of potassium clavulanate and cefixime.

Cefixime (Figure 1) is a semisynthetic cephalosporin antibiotic for oral administration with the chemical name (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7^2-(*Z*)-[*O*-(carboxymethyl)oxime] trihydrate. It is used to treat different types of bacterial infections such as bronchitis, tonsillitis, ear and skin infections, gonorrhea, and urinary tract infections (1). Potassium clavulanate (Figure 2) is a white-to-off-white powder produced by fermentation of *Streptomyces clavuligerus* and is chemically designated (2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate. Although clavulanic acid has weak antibacterial activity, it acts as a potent irreversible β-lactamase inhibitor. It forms stable inactive complexes with β-lactamases and thus protects against antibiotic degradation (2). It is usually supplied mixed with Avicel (microcrystalline cellulose) and Syloid 244 (colloidal silicon dioxide; 3). The combination of cefixime and potassium clavulanate has recently been approved by Central Drugs Standard Control Organization India (4). Because of the additive effects of both drugs, some pharmaceutical companies are preparing to launch a combined form of potassium clavulanate and cefixime.

Both potassium clavulanate and cefixime are β-lactam antibiotics. A large number of analytical methods have already been published for both drugs, either alone or in combination with other drugs. These methods include the use of high-performance liquid chromatography (HPLC; 5–10), high-performance thin-layer chromatography (HPTLC; 11), and fast inverse Laplace transform (12) for cefixime and HPLC (13–24) for potassium clavulanate. According to our information, no method has yet been reported for the simultaneous determination of cefixime and potassium clavulanate. Therefore, the work described in this paper focuses on the optimum chromatographic conditions for the simultaneous determination of cefixime and potassium clavulanate in a synthetic mixture.

We describe here a simple, sensitive, and validated HPLC method with isocratic elution for the simultaneous determination of cefixime and potassium clavulanate. The developed method can be used successfully for quality control and for other analytical purposes.

Experimental

Chemicals and Reagents

Reference potassium clavulanate and cefixime with purity claims of 99.55 and 99.68%, respectively, were obtained from Ideal Pharmaceutical & Pharmagen Ltd. (Lahore, Pakistan). The disodium hydrogen phosphate (Merck, Rahway, NJ) and methanol (Fisher Scientific, Pittsburgh, PA) used were HPLC grade. All excipients used were pharmaceutical grade. Starch was purchased from Rafhan (Faisalabad, Pakistan), lactose was from Borculo Domo (Borculo, The Netherlands), magnesium stearate was from Coin Chemical (Taiwan, China), and Avicel was from JRS Pharma (Rosenberg, Germany). Water for injection was used throughout the experiment. The mobile phase was degassed by Sonicator PSO 13000 A and filtered by using 0.45 μm nylon filters from

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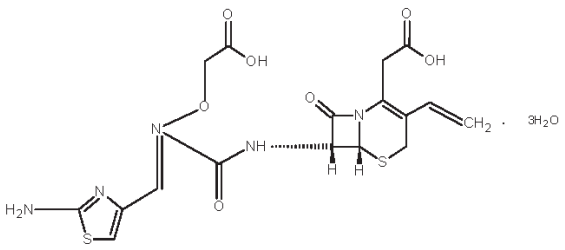

Figure 1. Chemical structure of cefixime.

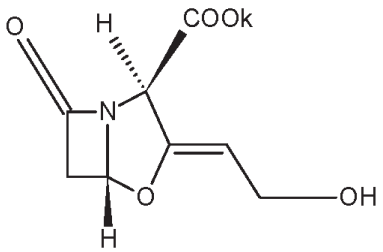

Figure 2. Chemical structure of potassium clavulanate.

Millipore (Billerica, MA); Whatman No. 41 filter paper (purchased from the local market) was used in the preparation of the sample solution.

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Thermo Separation Products (Piscataway, NJ) P-100 liquid chromatograph, Version 4.05, equipped with detector UV 150, Version 3.05, and a Rheodyne (Cotati, CA) injection valve with a 20 μL loop. Software CSW 1.7 was used for recording the chromatograms and calculating the chromatographic parameters. Isocratic separation of both the components was achieved by using a Hypersil C18 column (Gloucestershire, UK), 250 × 4.6 mm, 5 μm, at a flow rate of 1.0 mL/min. The UV detector was set at 220 nm. All the analyses were performed at room temperature (25 ± 2°C).

Preparation of Mobile Phase

The mobile phase was prepared by mixing 0.03 M disodium hydrogen phosphate buffer (pH 6.5) and methanol in the ratio of 84 + 16 (v/v). After adjusting the pH of the buffer to 6.5 with 10% phosphoric acid, the solution was filtered through 0.45 μm nylon filters and degassed before use.

Preparation of Standard Solution

The standard stock solution of potassium clavulanate and cefixime (0.125 and 0.2 mg/mL, respectively) was prepared by dissolving 25 mg potassium clavulanate and 20 mg cefixime in a small amount of mobile phase in a 100 mL volumetric flask and then diluting to volume with mobile phase. The working standard solution containing potassium clavulanate at 37.5 μg/mL and cefixime at 60 μg/mL was prepared by diluting the stock solution with mobile phase.

Linearity

The linearity of the developed method was evaluated by analyzing 5 solutions in the range of 12.5–62.5 μg/mL for potassium clavulanate and 20–100 μg/mL for cefixime. Each concentration was analyzed in triplicate.

Accuracy

To check the accuracy of the developed method, known amounts of potassium clavulanate and cefixime were added to the previously prepared standard solution. The experimental and theoretical concentrations were then compared. From the standard solutions of potassium clavulanate and cefixime, 5 mL aliquots were transferred to separate 50 mL volumetric flasks containing 2.5, 6.25, 10, 13.75, and 17.5 mL potassium clavulanate and cefixime standard solutions (0.125 and 0.2 mg/mL, respectively). The contents of the flask were then diluted to volume with mobile phase to obtain concentrations of 18.75, 28.13, 37.5, 46.87, and 56.25 μg/mL for potassium clavulanate and 30, 45, 60, 75, and 90 μg/mL for cefixime. These concentrations correspond to 50, 75, 100, 125, and 150% of the nominal analytical concentrations, which are 37.5 μg/mL for potassium clavulanate and 60 μg/mL for cefixime.

Preparation of the Synthetic Mixture and Its Analysis

The selectivity of the proposed method was checked by preparing a synthetic mixture of both analytes with commonly occurring excipients that are found in most of the tablet formulations and then measuring the percent recovery of each component in the presence of excipients. For this purpose, 25 mg potassium clavulanate, 20 mg cefixime, and 30 mg each of starch, lactose, magnesium stearate, and Avicel were accurately weighed and transferred to a 100 mL volumetric flask. The mixture was dissolved well by shaking. After dilution to volume with mobile phase, the solution was filtered through Whatman No. 41 filter paper. A quantity equal to 8 mL of this filtrate was diluted to 50 mL with mobile phase to obtain final concentrations of 20 μg/mL for potassium clavulanate and 32 μg/mL for cefixime.

Design of the Forced Degradation Study

Accelerated degradation studies were performed to evaluate the specificity of the method. For acid degradation, 1 mL 0.1 M HCl was added to 5 mL standard stock solution, and the solution was allowed to stand for 40 min at 25°C. The solution was then diluted to 25 mL with mobile phase. For basic degradation, 3 mL 0.05 M NaOH was added to 5 mL standard stock solution, and the solution was allowed to stand for 30 min at 25°C. The solution was then diluted to 25 mL with mobile phase. For thermal degradation, 5 mL standard stock solution was heated to 80°C for 70 min and then diluted to 25 mL with mobile phase. For oxidative degradation,

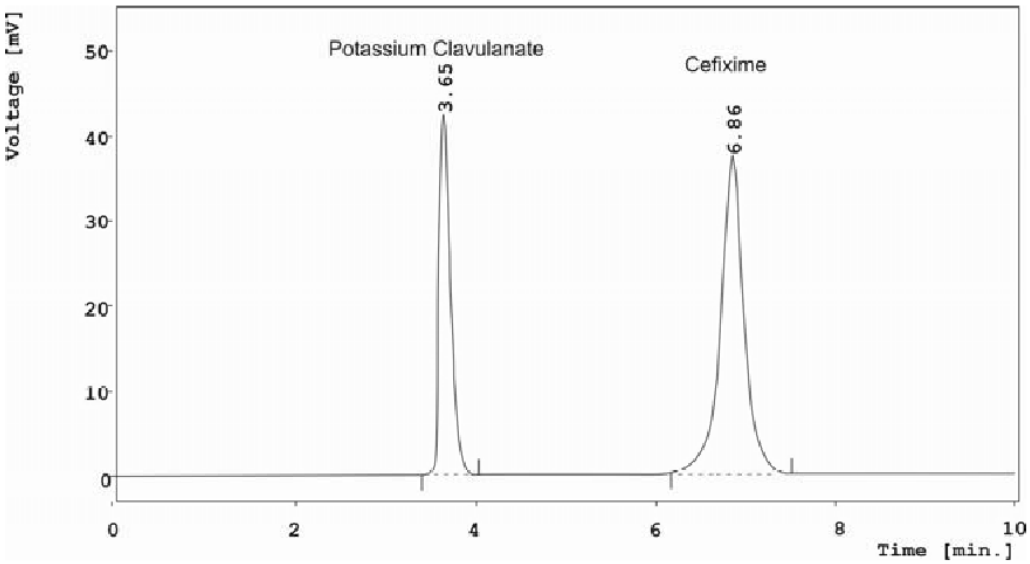

Figure 3. Chromatogram obtained for potassium clavulanate and cefixime reference standards.

0.5 mL 1.25% H_2O_2 was added to 5 mL stock solution, and the solution was allowed to stand for 20 min at 25°C and then diluted to 25 mL.

Robustness

The robustness of the method was evaluated by deliberately changing the chromatographic conditions such as composition of the mobile phase, flow rate, and pH of the buffer solution. The percent recoveries of each analyte along with chromatographic parameters like retention time, tailing factor, resolution, and number of theoretical plates were measured for each condition tested.

Results and Discussion

Not only are both potassium clavulanate and cefixime official drugs in the *United States Pharmacopeia*, but they are also found in individual monograms. The aim of the present research was to develop an HPLC method for the simultaneous determination of potassium clavulanate and cefixime in synthetic formulations.

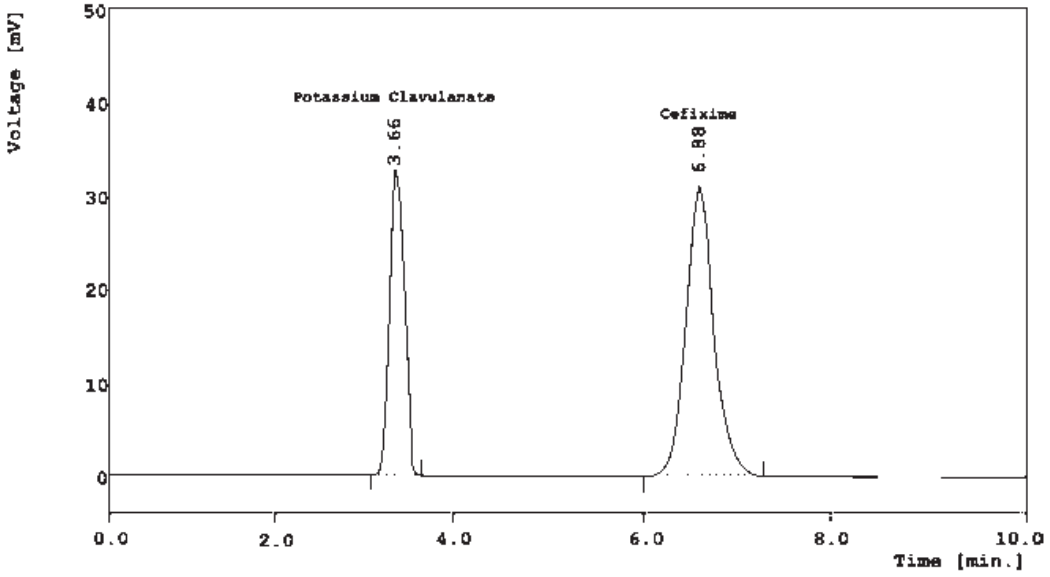

Figure 4. Chromatogram obtained for potassium clavulanate and cefixime in a synthetic mixture.

Table 1. Accuracy of the proposed HPLC method

Compound	% of nominal concn	Added, μg/mL	Found, μg/mL	Avg. recovery, %[a]	RSD, %
Potassium clavulanate	50	18.75	18.80	100.27	0.73
	75	28.13	28.56	101.53	0.35
	100	37.50	37.41	99.76	0.59
	125	46.87	47.21	100.72	0.79
	150	56.25	55.22	98.17	0.58
Cefixime	50	30.0	30.28	100.93	0.71
	75	45.0	45.56	101.24	0.80
	100	60.0	58.90	98.17	0.95
	125	75.0	73.80	98.40	0.25
	150	90.0	91.28	101.42	0.58

[a] $n = 5$.

Table 2. Within-day and between-day precision of the proposed HPLC method

		Within-day precision		Between-day precision	
Compound	Concn, μg/mL	Mean[a]	RSD, %	Mean[a]	RSD, %
Potassium clavulanate	25.0	24.82	0.88	25.15	0.81
	37.5	37.10	0.67	37.59	0.90
	50.0	50.41	0.78	50.32	0.35
Cefixime	20.0	20.21	0.65	20.21	0.58
	40.0	39.87	0.73	40.32	0.89
	60.0	60.24	0.49	60.39	0.59

[a] $n = 6$.

During development of the method, a number of mobile and stationary phases were tried to elute both components simultaneously. First, acetonitrile and phosphate buffer were used in different proportions and with different pH values of the buffer. Although elution of both analytes occurred with acetonitrile–phosphate buffer, pH 4.0 (10 + 90, v/v), tailing was >1.5. The tailing was reduced considerably by increasing the pH of the buffer from 4.0 to 6.5 and replacing the acetonitrile with methanol. The optimum ratio of methanol to phosphate buffer, pH 6.5, in the mobile phase was found to be 16 + 84, v/v. Upon application of the proposed method, well-separated sharp chromatographic peaks were obtained for both potassium clavulanate and cefixime. Representative chromatograms obtained for potassium clavulanate and cefixime are shown in Figures 3 and 4.

The developed chromatographic method was validated by using guidelines of the International Conference on Harmonization (25). Validation parameters evaluated included linearity, limits of detection and quantitation (LOD and LOQ, respectively), selectivity, robustness, accuracy, and repeatability.

The linearity of the method was evaluated by analyzing 5 solutions containing potassium clavulanate in the range of 12.5–62.5 μg/mL and cefixime in the range of 20–100 μg/mL. Each concentration was prepared and analyzed in triplicate. The chromatographic peak areas obtained for each concentration of the analytes were used to build linear regression equations and to determine the value of the correlation coefficients. Good linearity was observed over the above-mentioned ranges with linear regression equations $Y = 26.382X + 13.620$ for potassium clavulanate and $Y = 33.355X - 33.104$ for cefixime. The correlation coefficients were found to be 0.9999 for potassium clavulanate and 0.9998 for cefixime.

To calculate the LOD and LOQ, a blank solution and a solution spiked with progressively decreasing known concentrations of each analyte were prepared and analyzed by the developed method. The LOD and LOQ were then determined by evaluating the minimum concentration at which each analyte can be detected and quantified with accuracy, respectively (signal-to-noise ratio of 3:1 for the LOD and 10:1 for the LOQ). The LOD values were found to be 0.09 μg/mL for potassium clavulanate and 0.06 μg/mL for cefixime. The LOQ values were 0.27 and 0.18 μg/mL for potassium clavulanate and cefixime, respectively.

The accuracy of the method was determined by adding known amounts of potassium clavulanate and cefixime to the previously prepared standard solution. Five concentrations of solutions were prepared for each analyte: 18.75, 28.13, 37.50, 46.87, and 56.25 μg/mL for potassium clavulanate and 30, 45, 60, 75, and 90 μg/mL for cefixime; these values correspond to 50, 75, 100, 125, and 150% of the nominal analytical concentrations. The recovery range for the analytes was found to be 98.17–101.53%, and the relative standard deviation (RSD) ranged from 0.25 to 0.95% (Table 1).

Table 3. Selectivity of the proposed HPLC method

Potassium clavulanate			Cefixime		
Added, μg/mL	Found, μg/mL	Recovery, %	Added, μg/mL	Found, μg/mL	Recovery, %
20.0	20.25	101.25	32.0	32.35	101.09
20.0	20.18	100.90	32.0	31.62	98.81
20.0	19.70	98.50	32.0	32.11	100.34
20.0	20.21	101.05	32.0	32.15	100.47
Mean recovery, %	100.42		100.18		
RSD, %	0.9		0.86		

Table 4. Results from the robustness study of potassium clavulanate

Condition	Assay, %	RT, min[a]	Number of theoretical plates	Tailing
Methanol–buffer (16 + 84)	99.56	3.65	18384	1.35
Methanol–buffer (20 + 80)	100.48	3.36	17001	1.41
Methanol–buffer (12 + 88)	100.25	3.83	18500	1.42
Flow rate, 1.1 mL/min	99.21	3.22	19003	1.33
Flow rate, 0.9 mL/min	99.67	3.84	17900	1.30
Buffer, pH 6.0	100.86	3.60	18970	1.29
Buffer, pH 7.0	99.61	3.61	17884	1.42

[a] RT = Retention time.

To check the precision of the proposed method, 3 different concentrations of each analyte in the mixture were prepared and analyzed. The within-day precision was determined by calculating the RSD for 6 replicate analyses of samples on the same day. The between-day precision was determined by calculating the RSD of the results for the same samples analyzed on 5 consecutive days (Table 2).

The selectivity of the proposed method was checked by preparing a synthetic mixture of both analytes with commonly occurring excipients that are found in most tablet formulations and then calculating the percent recovery of each analyte in the presence of excipients. To test the selectivity of the method, the recovery of each component was determined in the presence of other possible interfering materials such as starch, lactose, magnesium stearate, and Avicel (Table 3).

The specificity of the method was evaluated by accelerated degradation of both analytes in the mixture. For this purpose, the analytes were subjected to acidic, basic, thermal, and oxidative conditions. The samples treated with HCl showed considerable degradation for both analytes, whereas in the case of base, potassium clavulanate degraded to almost 70% with only 4% degradation for cefixime. In the case of thermal degradation, both analytes degraded to almost 12% each, and almost no degradation occurred for both analytes under oxidative conditions. Under all the stress conditions, the chromatographic peaks of the degradation products were well separated from the analyte peaks, and this separation showed the specificity of the method in the presence of the degradation products. A mixture of possible interfering substances (placebo) was also analyzed under the same conditions to evaluate their interference. The absence of chromatographic peaks showed the specificity of the method in the presence of the excipients.

The robustness of the method was evaluated by deliberately changing the chromatographic conditions. The results showed that varying the conditions had no appreciable effect. The results of the robustness study are given in Tables 4 and 5.

In addition, the stability of each analyte in the presence of the other in solution was determined by calculating the percent deviation of the results obtained after 72 h, compared with the data at zero time. The deviation of both analytes was <2% after 72 h.

Conclusions

In this paper, an isocratic HPLC method is presented for the simultaneous determination of potassium clavulanate and cefixime. The developed method is simple, precise, and fast as is evident from the retention times and the results of the recovery study. The commonly found excipients do not interfere with the elution of both analytes. Therefore, the

Table 5. Results from the robustness study of cefixime

Condition	Assay, %	RT, min[a]	Number of theoretical plates	Tailing	Resolution
Methanol–buffer (16 + 84)	100.28	6.86	17155	0.95	10.13
Methanol–buffer (20 + 80)	99.36	5.98	18002	1.15	5.90
Methanol–buffer (12 + 88)	100.58	10.79	18600	1.09	12.30
Flow rate, 1.1 mL/min	99.85	6.52	18100	1.01	9.68
Flow rate, 0.9 mL/min	100.21	6.99	17400	1.11	10.98
Buffer, pH 6.0	99.12	6.79	16586	1.21	9.65
Buffer, pH 7.0	100.69	6.90	18800	1.00	10.05

[a] RT = Retention time.

method can be used for the determination of potassium clavulanate and cefixime both in individual dosage forms and in combined pharmaceutical formulations.

References

(1) http://www.rxlist.com/cgi/generic/cefixime.htm

(2) http://en.wikipedia.org/wiki/Potassium_clavulanate

(3) http://www.drugs.com

(4) http://cdsco.nic.in/index.html

(5) *USP 30-NF 25* (2007) The United States Pharmacopeial Convention, Rockville, MD, pp 1654–1656

(6) Shah, P.B., & Pundarikakshudu, K. (2006) *J. AOAC Int*. **89**, 987–994

(7) Rolando, G., Lauro, N., Laritza, S., Miguel, L., & Joseph, H. (2001) *J. Liq. Chromatogr. Relat. Technol*. **24**, 2315–2324

(8) Liu, G.L., Sha, R.G., Gao, S., Shen,Y.X., & Wang, S.X. (1993) *Yaoxue Xuebao* **28**, 216–221

(9) Falkowski, A.J., Look, Z.M., Noguchi, H., & Silber, B.M. (1987) *J. Chromatogr*. **422**, 145–152

(10) McAteer, J.A., Hiltke, M.F., Silber, B.M., & Faulkner, R.D. (1987) *Clin. Chem*. **33**, 1788–1790

(11) Eric-Jovanovic, S., Agbaba, D., Zivanov-Stakic, D., & Vladimirov, S. (1998) *J. Pharm. Biomed. Anal*. **18**, 893–898

(12) Yamaoka, K., Kanba, M., Toyoda, Y., Yano, Y., & Nakagawa, T. (1990) *J. Pharmacokinet. Biopharm*. **18**, 545–559

(13) Li, C., Geng, Q., Nicolau, D.P., & Nightingale, C.H. (2003) *J. Chromatogr. B* **794**, 227–236

(14) Hoizey, G., Lamiable, D., Frances, C., Trenque, T., Kaltenbach, M., Denis, J., & Millart, H. (2002) *J. Pharm. Biomed. Anal*. **30**, 661–666

(15) Pajchel, G., Pawlowski, K., & Tyski, S. (2002) *J. Pharm. Biomed. Anal.* **29**, 75–81

(16) Tsou, T.L., Wu, J.R., Young, C.D., & Wang, T.M. (1997) *J. Pharm. Biomed. Anal*. **15**, 1197–1205

(17) Jehl, F., Monteil, H., & Brogard, J.M. (1987) *Pathol. Biol. (Paris)* **35**, 702–706

(18) Foulstone, M., & Reading, C. (1982) *Antimicrob. Agents Chemother*. **22**, 753–762

(19) Haginaka, J., Nakagawa, T., Nishino, Y., & Uno, T. (1981) *J. Antibiot. (Tokyo)* **34**, 1189–1194

(20) Reyns, T., De Baere, S., Croubles, S., & De Backer, P. (2006) *J. Mass. Spectrom*. **41**, 1414–1420

(21) Yoon, K.H., Lee, S.Y., Kim, W., Park, J.S., & Kim, H.J. (2004) *J. Chromatogr. B* **813**, 121–127

(22) Aghazadeh, A., & Kazemifard, G. (2001) *J. Pharm. Biomed. Anal.* **25**, 325–329

(23) Haginaka, J., Yasuda, H., Uno, T., & Nahagawa, T. (1986) *J. Chromatogr*. **377**, 269–277

(24) Shah, A.J., Adlard, M.W., & Stride, J.D. (1990) *J. Pharm. Biomed. Anal*. **8**, 437–443

(25) ICH (Q2B) (1996) *Note for guidance on the validation of analytical procedures: methodology,* International Conference on Harmonization, International Federation of Pharmaceutical Manufacturers & Associations, Geneva, Switzerland