VERSITA

Cent. Eur. J. Chem. • 9(3) • 2011 • 446-452
DOI: 10.2478/s11532-011-0022-x

Central European Journal of **Chemistry**

Development and validation of an ultra-fast liquid chromatographic method for the quality control of famotidine formulations using a short monolithic stationary phase

Research Article

Paraskevas D. Tzanavaras

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki,GR-54124 Thessaloniki, Greece

Received 5 November 2010; Accepted 31 January 2011

Abstract: An HPLC method for the quality control of famotidine (FMT) containing formulations has been developed and validated. The combination of a short monolithic column (Chromolith® RP-18e 50× 4.6 mm i.d.) and an elevated flow rate (3.0 mL min^{-1}) enabled the proposal of a high-throughput analytical scheme capable of reliable operation in a demanding industrial environment. Detection was carried out at 265 nm. Thorough validation of the method included linearity (5–150%), limits of detection (0.13%) and quantification (0.41%), selectivity, precision (within- and day-to-day), accuracy and ruggedness. The new method was applied successfully to the analysis of samples (assay, dissolution, dosage & blending uniformity) during the production of four validation batches of FMT-containing tablets.

Keywords: *Famotidine • High Performance Liquid Chromatography • Monolithic column • Quality Control • Pharmaceutical formulations*

1. Introduction

Famotidine (FMT, Fig. 1) is a histamine H2-receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcers and gastro-esophageal reflux. Compared to its "cousins" cimetidine and ranitidine, famotidine is 32 times stronger in its ability to inhibit stomach acid than cimetidine and is 9 times stronger than ranitidine [1,2]. Unlike cimetidine, that was the first H2 antagonist, famotidine has no effect on the cytochrome P450 enzyme system, and does not appear to interact with other drugs [3]. Regarding side-effects, there have been some reports of exacerbating heart rhythm problems with patients who already have such problems. Other less common side effects may include anxiety, nausea, sleepiness, fatigue and muscle and bone pain.

The development of an ultra-fast / high-throughput analytical scheme is of paramount importance in modern pharmaceuticals manufacturing. Compliance to international regulations and guidelines requires the exhaustive analysis of starting materials, intermediate products and final formulations. This demand is even more compulsory during process-validation for the development of new dosage forms [4]. High Performance Liquid Chromatography (HPLC) is the analytical technique that dominates the pharmaceutical quality control in the industrial environment. On the basis of the above-mentioned comments / demands, there is a continuously growing trend focussing on the speeding-up of HPLC-based methods [5]. A breakthrough was made with the introduction of the Ultra-High Pressure LC (UHPLC) technique based on the use of sub-2 μm particles [6,7]. The primary bottleneck with this approach for small-scale manufacturers is the cost for replacing the conventional HPLC instruments with those capable of operating in the range of 400-1000 bar. A viable alternative could be the use of monolithic-based stationary phases [8]. Monolithic columns allow efficient separations at higher flow rates and lower back-pressures than conventional particulate-based columns. Additionally, the unique structural characteristics of monoliths (mesopores and nanopores) offer fast mass transfer kinetics. Such systems have already proven to have useful features for a number of applications in the pharmaceutical industry [9].

* *E-mail: ptzanava@chem.auth.gr*

There are various methods in the literature reporting the analysis of FMT-containing formulations. The majority of the published methods appear to be based on a chemical transformation of FMT through reactions with suitable reagents (complexation, oxidation *etc*.) followed typically by spectrophotometric or spectrofluorimetric detection [10-17]. Although such methods might provide some interesting data on the behavior of the drugs, it is obvious that they are "academic" approaches, unattractive or even unsuitable for real-world analysis in a demanding industrial environment. Capillary electrophoresis (CE)-based methods offer some advantages for quality control (QC) purposes in terms of low operational cost and waste production [18,19], but CE continues to struggle to find its position in the international pharmacopoeias and the pharmaceutical industry. On the other hand, all reported HPLC methods for the determination of FMT in pharmaceuticals utilize particulate columns with retention times of FMT in the range of 4-8 min [20-22]. Additionally, due to the basic character of the drug these methods employ mobile-phase additives such as pentane sulfonic acid or triethylamine.

The scope of the present study was to develop and validate an ultra-fast HPLC method for the determination of FMT in various pharmaceutical samples (assay, dissolution, dosage & blending uniformity). Using a short monolithic column, the separation/detection cycle was completed in 60 s at a flow rate of 3.0 mL min^{-1}, offering a high-throughput analytical scheme. To the best of our knowledge there is only one previous article reporting the determination of FMT in human plasma using a monolithic column [23]. However, in this article the authors did not explore, nor took advantage of the ability of these columns to operate at elevated flow rates, since their main goal was selectivity and not rapidity. The proposed method was fully validated in terms of critical performance parameters and tested exhaustively during the production of four validation batches of an FMT-containing formulation in a pharmaceutical industry (Cosmopharm Ltd, Greece).

2. Experimental Procedure

2.1. Reagents and solutions

All chemicals were provided by Merck (Germany), unless stated otherwise. Purified water (κ < 4.3 μs cm^{-1}) was used for preparing solutions, while HPLC grade water and acetonitrile (ACN) were used for the preparation of the HPLC mobile phase.

The FMT working standard (Lot no: F279 / QC 2005/14 / assay = 99.20%) was provided by Ercros (division of Fyse, Spain). Standard solutions were prepared daily by dissolving an appropriate amount of the solid working standard in 0.1% v/v of CH_3COOH.

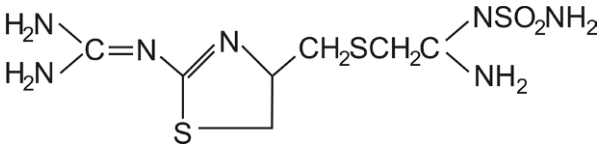

Figure 1. Structure of famotidine.

The mobile phase consisted of a (20:80 v/v) mixture of ACN and phosphate buffer (20 mmol L^{-1} NaH_2PO_4, pH adjusted to 6.0 with 1.0 mol L^{-1} NaOH). Before use, the mobile phase was filtered under vacuum (0.45 μm nylon membrane filters, Whatman) and degassed ultrasonically for 30 min.

The pharmaceutical excipients used in the selectivity and accuracy studies (magnesium stearate, maize starch and microcrystalline cellulose) were provided by domestic suppliers. The placebo mixture (all excipients except for the active ingredient) contained per gram: 581.0 mg microcrystalline cellulose, 412.4 mg maize starch and 6.7 mg magnesium stearate.

2.2. Instrumentation

The HPLC equipment used was an HP 1100 system (Agilent Technologies, USA), comprising a quaternary pump (G1311A), a vacuum degasser (G1322A), a column thermostat (G1316A), an autosampler (G1313A) and a DAD spectrophotometric detector (G1315A). Chromatographic parameters such as peak areas, retention times, theoretical plates *etc*. were calculated using the Chem Station® software.

A Chromolith® RP-18e monolithic column (50×4.6 mm i.d., Merck) was used throughout this study, while the mobile phase was filtered using a Schleicher and Schuell (Germany) vacuum filtration system.

Dissolution experiments were carried out using a Distek Premiere 5100 system equipped with a programmable auto-sampler.

2.3. HPLC analysis

20 μL of the samples/standards were injected into the monolithic column via the autosampler. The flow rate of the mobile phase was set at 3.0 mL min^{-1} and the column was thermostated at 25°C. Detection was carried out spectrophotometrically at 265 nm. Peak area evaluation was used in all cases, and each sample or standard was injected in triplicate. Under the above-mentioned conditions, separation/detection was completed in 60 s, offering a practical sampling rate of 20 h^{-1} (taking into account the duration of the cycle injection time of the autosampler).

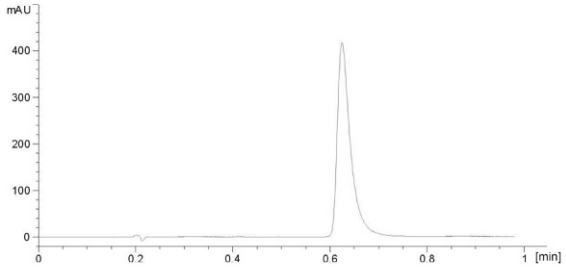

Figure 2. Representative chromatogram of famotidine from dissolution samples.

2.4. System suitability

Inject six replicates of FMT test solution at the 100% level (40 mg L^{-1}).

(i)The relative standard deviation (R.S.D.) of the peak areas should not be greater than 2.0%.

(ii) The number of theoretical plates per column meter should not be less than 5000.

2.5. Processing of pharmaceutical samples

Assay analysis. 20 tablets of each batch were manually ground to a fine powder. Appropriate, accurately weighed amounts were dissolved in 0.1% v/v CH_3COOH, sonicated for 20 min, filtered through 0.45 μm disposable syringe filters (Whatman) and injected into the monolithic column. Three sub-samples were prepared independently for statistical evaluation.

Content uniformity analysis. The QC test requires an independent treatment of each tablet. On this basis, 20 tablets of each batch were manually ground to a fine powder (independently). The HPLC analysis was performed after treatment as mentioned above.

Blending uniformity analysis. Twelve samples were collected from the final blend of the product during production and before tabletting. Sampling was carried out from four positions × three depths (Top/Middle/Bottom). The HPLC analysis was performed after treatment as mentioned above.

Dissolution testing. 12 tablets were weighed and introduced to the dissolution apparatus in batches of six. The dissolution profile was recorded *via* automated sampling at 5, 10, 15, 20, 30, 45 and 60 min (n = 7). Phosphate buffer (0.1 mol L^{-1}, pH = 4.5) was used as dissolution medium. The dissolution buffer was degassed under vacuum and kept at 35°C until use. The temperature was kept constant at 37.0 ± 0.5°C and the volume of the dissolution medium was 900 mL in all cases [24]. The paddle dissolution approach (Type II) was used throughout the experiments (at 50 r.p.m.), while the withdrawn aliquots were filtered in-line using 45 μm PTFE disc filters. The results from the analysis of the dissolution samples were compared to an in-house- validated HPLC assay (based on the Drug Master File of the manufacturer of the active ingredient). A Hypersil BDS C_{18} column (250×4 mm i.d. / 5 μm) was used throughout the experiments. The retention time of the analyte was *ca*. 6 min.

3. Results and Discussion

3.1. Method development - HPLC conditions

The chromatographic behavior of FMT was investigated in terms of mobile phase composition (pH, ionic strength of buffer, organic modifier) and flow rate. All experiments were carried out with the SpeedRod® Chromolith monolithic column (50×4.6 mm i.d.) at a temperature of 25°C and UV detection at 265 nm.

FMT is a basic compound with reported pK_a values in the range of 6.5 – 7.1 [25]. A lower pH is therefore necessary in order to suppress the ionization of the analyte and improve its retention on the reversed phase monolithic stationary phase. A slight increase in the retention of FMT was observed in the pH range of 3.0 – 6.0 without any important changes in peak symmetry. The latter value was therefore selected for further experiments. In terms of the ionic strength of the phosphate buffer, our findings were in agreement with Wanwimolruk who observed irreproducible retention times at low ionic strength buffers at an ODS Hypersil particulate stationary phase [26]. An amount concentration of 20 mmol L^{-1} phosphate buffer was therefore adopted throughout the study.

The manufacturer of the monolithic columns recommends the use of ACN as organic modifier in terms of column performance [27]. However, in the case of FMT – besides the expected higher eluotropic strength of ACN – no significant differences were observed with peak symmetry compared to MeOH. The latter, at a volume fraction of 20%, provided a retention factor of FMT of >2.0 and was selected for further investigations.

Since the most unique characteristic of monolithic materials is the ability to operate at elevated flow rates with low backpressures, the effect of the flow rate of the mobile phase was examined in the range of 1.0 – 5.0 mL min^{-1}. The goal of this series of experiments was to reduce the duration of the analytical cycle and develop an ultra-fast assay lasting less than 60 s. This objective was achieved at a flow rate of 3.0 mL min^{-1} without sacrificing peak symmetry and retention factor. Higher flow rates do not offer any additional advantages since the sampling rate is dictated by the operation cycle-time of the autosampler and there is unnecessary consumption of the mobile phase.

3.2. Method validation

The proposed HPLC method was validated in terms of linearity, precision (within-day and day-to-day), limits of detection and quantification, selectivity, accuracy (within-day and day-to-day) and ruggedness. Based on the strength of the formulation (40 mg FMT per tablet) and the expected theoretical concentration of the drug in the samples after the dissolution experiments (ca. 40 mg FMT in 900 mL of dissolution medium), a mass concentration of 40 mg L^{-1} FMT was set as the 100% level.

Linearity. Linearity was evaluated between 5–150% FMT (2 – 60 mg L^{-1}, n = 8 in all cases) validating different ranges depending on the QC test. For example, for dissolution experiments a range between 5–125% is necessary to ensure effective monitoring of a dissolution profile. In a similar experiment, 80–120% (assay) and 50–150% (dosage and blending uniformity) were examined. The detailed experimental results can be found in Table 1. The acceptance criteria for these parameters were a correlation coefficient (r) of higher than 0.99 and percent residuals in the range of ±3%. As can be seen from the values of Table 1, the acceptance criteria were met in all cases.

Limit of detection (LOD) & quantification (LOQ). The LOD and LOQ of the proposed method were evaluated by the signal-to-noise ratio criterion. The estimated values were LOD (S/N=3) = 0.13% (0.05 mg L^{-1}) and LOQ (S/N=10) = 0.41% (0.17 mg L^{-1}). Precision experiments at the 1% level of FMT (close to the LOQ) confirmed the validity of the estimated values since the R.S.D. of the peak areas was in the range of 4 – 6% (n = 12).

Precision. The within-day precision was validated by eight repetitive injections of FMT standards at the 10, 50, 100 and 150% levels. The acceptance criterion was set at a relative standard deviation of not more than 2.0% (n = 8). The experiments confirmed the repeatability of the method since the values of the R.S.D. were in the range of 0.2 – 0.9%.

The day-to-day precision of the procedure was evaluated within a time-frame of eight non-consecutive days by constructing respective calibration curves in the range of 5–150%. The acceptance criterion was a R.S.D. of the slopes of not more than 5%. The experiments met the pre-established criterion since the R.S.D. of the slopes was calculated to be 3.1% (n = 8).

Selectivity. The selectivity of the HPLC method was evaluated by the placebo approach. For the assay and uniformity tests 50 mg of placebo (see section 2.1) were dispersed in 100 mL of 0.1% acetic acid, sonicated for 15 min, centrifuged (15 min at 5000 r.p.m.) and finally filtered through disposable syringe filters. For the dissolution tests 500 mg of the placebo were dispersed in 900 mL of dissolution medium and subjected to the test for 30 min as described in section 2.5. In both cases, the HPLC analysis of the resulting samples revealed no interfering peaks. It should be noted that the selected concentration of the placebo is 2-fold higher than the theoretically expected in the real samples (the average weight of the tablets is 258 mg).

Accuracy. The accuracy for the assay and uniformity tests was evaluated by analyzing synthetic mixtures at 50, 80, 100, 120 and 150% FMT containing 500 mg L^{-1} placebo. For the dissolution test synthetic samples (10, 25, 50, 100, 120% FMT) were subjected to dissolution experiments for 30 min (section 2.5). In all cases the evaluation of the accuracy was carried out by two independent analysts and on two consecutive days. The acceptance criteria were set at recoveries in the range of 97.5–102.5% for the assay / uniformity tests and 95.0–105.0% for the dissolution tests. Since quantification by the use of a single standard is typical in international pharmacopoeias, the accuracy was evaluated by this approach as well as using an aqueous standard at the 100% level. Representative results are tabulated in Table 2. The acceptance criteria were met in all cases with the percent recoveries ranging between 98.21–101.64% (assay / uniformity) and 97.01–102.35% (dissolution).

Ruggedness. The ruggedness of the assay was validated by evaluating its ability to maintain its accuracy within certain limits, after small deliberate changes in critical instrumental and chemical parameters of the method. The varied parameters were: (i) the volume fraction of ACN (±3%); (ii) the sample injection volume (±2%); (iii) the flow rate of the mobile phase (±3%); (iv) the pH of the mobile phase (±3%); (v) the temperature

Table 1. Linearity of the proposed HPLC method.

Quality control test	Range (%) [a]	Range (mg L^{-1})	r	Residuals (%)
Dissolution	5 – 125	2 – 50	0.9997	- 1.6 / + 0.9
Assay	80 – 120	32 – 48	0.9999	- 0.3 / + 0.4
Dosage/blending uniformity	50 – 150	20 – 60	0.9999	- 0.6 / + 0.5

[a] *In all cases, n = 8.*

Table 2. Within-day accuracy of the HPLC method [a]

Sample	FMT level (%)	Theoretical concentration (mg L^{-1})	Recovery (%)	
			Calibration curve	Single standard
1	50	20.0	101.56	101.16
2	50	20.0	101.40	99.06
3	80	32.0	98.84	100.88
4	80	32.0	100.01	100.29
5	100	40.0	100.30	100.50
6	100	40.0	100.18	100.37
7	120	48.0	99.63	99.45
8	120	48.0	99.80	101.23
9	150	60.0	99.71	99.47
10	150	60.0	100.85	98.94
Average recovery (%)			100.22	100.13
Standard deviation			0.83	0.72

[a] *For experimental details see text.*

Table 3. Ruggedness of the HPLC method.

Parameter	Peak area	Retention time (min)	Recovery (%)
Optimal conditions	1239.70	0.642	—
φ(ACN) = 20.6 %	1241.78	0.618	100.15
φ(ACN) = 19.4 %	1243.17	0.672	100.28
***V* = 25.5 μL**	1264.31	0.641	101.98
***V* = 24.5 μL**	1217.37	0.640	98.20
Q_v = 3.09 mL min^{-1}	1207.61	0.615	97.41
Q_v = 2.91 mL min^{-1}	1280.36	0.684	103.28
pH = 7.2	1243.70	0.640	100.33
pH = 6.8	1241.47	0.640	100.14
***T* = 30°C**	1242.47	0.624	100.23
***T* = 20°C**	1241.84	0.656	100.18

of the monolithic column (±20%). All experiments were carried out at the 100% level of FMT and acceptable recoveries were in the range of 95.0 – 105.0%. The results can be found in Table 3.

3.3. Method applications

The developed and validated HPLC method was applied to the quality control (assay, dosage / blending uniformity and dissolution) of four validation batches of a famotidine-containing formulation (40 mg FMT per tab / lots: VB1-VB4).

The recoveries from the assay tests were in the range of 98.8–100.3% for all validation batches, while the results from the dosage / blending uniformity tests are shown in Table 4. For the dissolution tests, all batches met the criteria set by the US Pharmacopoeia (not less than 75% in 30 min). A representative chromatogram and dissolution profile of FMT tablets is depicted in Figs. 2 and 3 respectively. The validity of the dissolution profiles was further investigated by comparative analysis of the

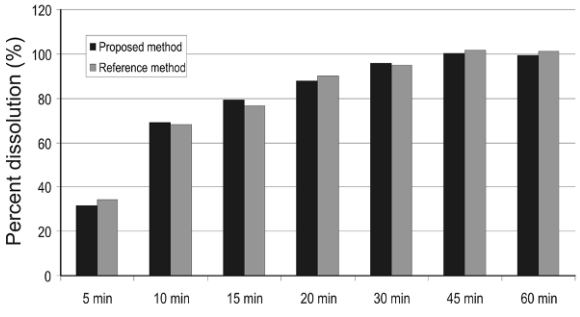

Figure 3. Dissolution profiles of famotidine tablets from validation batches using the proposed (■) and a reference (■) HPLC method.

dissolution samples with a reference HPLC method (see section 2.5). The obtained profiles were compared statistically using the widely established *similarity factor* (f_2) [28]. In all cases, the values of f_2 were in the range of 73 – 84, verifying the statistical validity of the dissolution profiles created by the proposed HPLC method.

Table 4. Dosage and blending uniformity of the FMT tablets.

Sample	Recovery (%)				Sample	Recovery (%)			
	VB1	VB2	VB3	VB4		VB1	VB2	VB3	VB4
Dosage Uniformity									
DU1	97.6	104.6	101.8	96.4	**DU11**	103.2	98.7	92.0	98.1
DU2	98.9	102.7	107.3	97.6	**DU12**	96.5	99.2	101.6	100.7
DU3	96.5	94.8	98.5	103.5	**DU13**	97.6	103.7	105.0	101.9
DU4	102.6	98.6	99.0	103.8	**DU14**	102.8	104.9	95.8	100.2
DU5	98.0	101.3	102.5	97.4	**DU15**	95.4	102.7	96.2	99.4
DU6	99.3	105.1	105.9	104.3	**DU16**	106.1	96.2	104.7	108.2
DU7	103.8	100.8	93.8	95.8	**DU17**	105.0	97.8	103.0	106.7
DU8	102.6	97.0	95.4	99.7	**DU18**	101.9	99.4	99.1	104.5
DU9	98.7	96.7	100.8	101.6	**DU19**	96.5	101.8	98.7	93.1
DU10	95.8	102.9	101.4	93.9	**DU20**	94.8	98.4	99.0	95.0
Blending Uniformity									
TOP1	98.9	99.6	99.0	98.4	**TOP3**	98.8	98.6	102.5	100.5
MID1	98.5	99.1	99.4	97.5	**MID3**	99.7	99.8	101.6	97.6
BOT1	102.1	101.2	101.7	103.0	**BOT3**	101.2	100.5	97.6	101.6
TOP2	99.3	101.6	100.9	101.6	**TOP4**	101.6	99.9	99.0	99.5
MID2	100.8	98.8	100.7	99.5	**MID4**	100.3	101.5	99.6	98.2
BOT2	98.3	99.0	99.5	98.1	**BOT4**	100.7	102.9	102.0	98.9

4. Conclusions

The developed HPLC method for the determination of FMT in various samples from a pharmaceutical quality control offers the critical advantage of a complete separation cycle of 60 s. This is significantly lower than previous reported HPLC and CE methods where FMT is typically eluted between 4-8 min. The analytical procedure is simple, robust and adequately validated and tested in real industrial conditions. It has proven a valuable tool for the rapid testing of hundreds of samples during the validation of a manufacturing process for FMT-containing tablets.

Acknowledgments

All experiments were conducted at the facilities of Cosmopharm Ltd (Korinthos, Greece). Mrs Aspasia Verdoukas (Head of Plant and Production) is kindly acknowledged for her assistance.

References

[1] T. J. Humphries, Scand. J. Gastroenterol. 22, 55 (1987)
[2] B. Simon, P. Muller, H.G. Dammann, Innere Medizin 11, 81 (1984)
[3] T.J. Humphries and G.J. Merritt, Aliment. Pharmacol. Ther. 13, 18 (1999)
[4] P.D. Tzanavaras, D.G. Themelis, Anal. Chim. Acta 588, 1 (2007)
[5] D. Guillarme, D.T.T. Nguyen, S. Rudaz, J-L. Veuthey, Eur. J. Pharm. Biopharm. 68, 430 (2008)
[6] M.E. Swartz, J. Liq. Chromatogr. Relat. Technol. 28, 1253 (2005)
[7] M.W. Dong, LC-GC North America 25, 656 (2007)
[8] K. Cabrera, D. Lubda, H-M. Eggenweiler, H. Minakuchi, K. Nakanishi, J. High Resolut. Chromatogr. 23, 93 (2000)
[9] C.K. Zacharis, J. Chromatogr. Sci. 47, 443 (2009)
[10] R. Isono, H. Kurokawa, N. Suzaki, M. Tomida, S. Kadil, T. Yamaguchi, Y. Fujita, Bunseki Kagaku 58, 715 (2009)
[11] M.I. Walash, A. El-Brashy, N. El-Enany, M.E. Kamel, J. Fruoresc. 19, 333 (2009)
[12] I.A. Darwish, S.A. Hussein, A.M. Mahmoud, A.I. Hassan, Spectrochim. Acta A 69, 33 (2008)
[13] M.I. Walash, M.K. Sharaf-El-Din, M. El-Sayed Metwally, M.R. Shabana, J. Chin. Chem. Soc. (Taipei) 52, 71 (2005)
[14] Z. Korićanac T. Jovanović, J. Petković, D. Minić, J. Serb. Chem. Soc. 69, 485 (2004)
[15] M.S. Elazazy, A. Shalaby, M.N. Elbolkiny, H.M. Khalil, Chin. Pharm. J. 55, 481 (2003)
[16] M.M. Ayad, A. Shalaby, H.E. Abdellatef, M.M. Hosny, Anal. Bioanal. Chem. 376, 710 (2003)
[17] N. Rahman, M. Kashif, Anal. Sci. 19, 907 (2003)

[18] N. Helali, N.T. Tran, L. Monser, M. Taverna, Talanta 74, 694 (2008)
[19] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, E. Bravo, R. Galera, J. Pharm. Biomed. Anal. 30, 1055 (2002)
[20] N. Helali, L. Monser, J. Sep. Sci. 31, 276 (2008)
[21] N. Helali, F. Darghouth, L. Monser, Chromatographia 60, 455 (2004)
[22] C. Ho, H-M. Huang, S-Y. Hsu, C-Y. Shaw, B-L. Chang, Drug Dev. Ind. Pharm. 25, 379 (1999)
[23] A. Zarghi, A. Shafaati, S.M. Foroutan, A. Khoddam, J. Pharm. Biomed. Anal. 39, 677 (2005)
[24] United States Pharmacopoeia, USP30-NF25 2112 (2007)
[25] S.L. Rivera, S. Ghodbane, Int. J. Pharm. 108, 31 (1994)
[26] S. Wanwimolruk, A.R. Zoest, S.Z. Wanwimolruk, C.T. Hung, J. Chromatogr. B 572, 227 (1991)
[27] P.D. Tzanavaras, D.G. Themelis, Anal. Chim. Acta 581, 89 (2007)
[28] Food and Drug Administration: Guidance for Industry. Dissolution Testing of Immediate Release Solid Oral Dosage Forms (US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research, Rockville MD, 1997)