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Development and validation of a fully automated method for the chromatographic determination of content uniformity of drug tablets

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Abstract

A fully automated method for the content uniformity analysis of LAS 34475 25 mg tablets has been developed by using an automated procedure. This automated method has been validated within the requirements of ICH guidelines Q2A–Q2B.

Standard and sample solutions are processed by an automated benchtop system. The operations automated include the phases of disintegration of the dosage form, filtration of the resultant homogenate and injection of the clear sample into the chromatographic system.

Although a manual method validated according to ICH guidelines already existed for this compound, the benefits of applying appropriate automation should provide continuous operation, increased precision, an affordable electronic audit trail and significantly reduced time consumption as well as reducing the exposure of the analyst to the drug substance.

The objective of this work was to adapt the manual method to an automated workstation. Considerable effort went into developing and validating an automated method. The results obtained in the validation of this automated method were equivalent to the manual method in terms of system precision, linearity, accuracy, robustness and sensitivity (limits of detection, LOD and limits of quantification, LOQ), and carry-over.
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1. Introduction

LAS 34475 is a novel, highly selective COX-2 inhibitor which has exhibited analgesic and anti-inflammatory activities in pre-clinical models. This drug is targeted for use in the treatment of rheumatoid arthritis, osteoarthritis, and acute pain.

The first part of this work consisted of the development of an automated method to extract the drug component from the tablet matrix. Several key parameters such as the number of pulses, the probe speed and homogenisation time were also optimised.

The manual determination of the content uniformity of LAS 34475 25 mg tablets is a simple, but rather cumbersome task when a large number of samples are to be analysed. However, the operations involved in the overall procedure can be automated by adequate adaptation of certain already commercialised robotics to the sample's complexities [1].

An automated benchtop system has been used to proceed with the analysis of 25 mg tablets of LAS 34475 [2]. Automated methods based on the analysis of pharmaceutical compounds using this robot have been proposed with several applications [3–5].

The automated system performs quantitative sample preparation of pharmaceutical dosage forms such as, tablets, capsules, powders, granulates, powder blends, dissolutions and suspensions. The system is a benchtop platform that fully automates the quantitative sample preparation of pharmaceutical dosage forms. Typical applications include: composite assay, stability assay, content uniformity, and blend uniformity analysis. The workstation automatically extracts the active ingredient(s) from the sample matrix using a high efficiency dispersion module. Extracts are then filtered and diluted if necessary, with the final solution either being stored in an HPLC vial for subsequent analysis, or introduced in HPLC or UV–vis for on-line analysis.

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Two internal balances automatically track all of the sample preparation steps.

This capacity results in a comprehensive electronic sample audit trail providing defensible documentation to facilitate regulatory compliance or out of specification (OOS) investigations. The system is capable of running up to 100 samples automatically, thereby increasing productivity and lowering the cost/analysis.

The mechanical sample preparation approach eliminates analyst-dependent biases, so improving the quality of the resultant data. The specific system used in this work is 21 CFR Part 11 compliant [6,7].

The optimisation of the sample treatment focused on the critical parameters of the automated system. In the manual procedure the tablets are dispersed in the sample solvent using a magnetic stirrer and an ultrasonic bath, then clarified by centrifugation. The corresponding steps of the automated method are performed using an Ultraturax homogeniser and a filtration station. Parameters relating to dispersion of the sample (probe speed, homogenisation time and number of pulses among others) were in consequence optimised to achieve good recovery. The carry-over effect was also improved by vessel rinsing studies. The method requires a final automatically coupled HPLC analysis of the standards and sample solutions.

The second part of the work was the validation of the developed automated method, within the requirements of ICH Guidelines Q2A–Q2B [8,9]. Parameters such as system precision, limits of detection and limits of quantification, linearity, recovery, robustness and equivalence between the manual and automated methods were successfully determined.

This article describes a robotic method for the automated chromatographic determination of content uniformity of LAS 34475 25 mg tablets, so allowing the development of this routine analysis with minimal human participation.

2. Material and methods

2.1. Apparatus

The chromatographic system used in the analysis of content uniformity of LAS 34475 25 mg tablets consisted of a 510 HPLC Pump by Waters (Waters Corporation, MA, USA) and a 486 UV detector from the same company. Data were collected using Millennium on a PC.

For the manual sample analysis, a Waters WISP 917 autoinjector was used. For the robotic analysis, a Benchmate Tablet Processing Workstation (TPW II) (Zymark, Corp., Hopkinton, MA) replaced the autoinjector. The TPW II is a single robot arm consisting of a unique sample dispersion vessel, a 3-decimal electronic balance and a 4-decimal electronic balance, an automatic vortex mixer, automatic filtration using standard 0.45 μm nylon syringe filters, an integrated HPLC injector (Rheodyne model 7010 injector valve, 20 μl loop volume) and a powder transfer station. The 3- and 4-decimal balances determine sample weight and gravimetrically determine solvent volume for dilution purposes. At the back of the instrument there is a bank of solvent and sample's dissolution delivery. All operations are controlled by a PC that also generates a spreadsheet during every run, tracking all pertinent weights, volumes and speed as well as any errors that may have occurred during the run.

2.2. Chemicals and reagents

LAS 34475 tablets were prepared following internal protocols by Almirall Prodesfarma, S.A. The major ingredient of the tablets was lactose (DMV International, Netherlands). *Ortho*-phosphoric acid was supplied by Scharlau Chemie S.A. (Barcelona, Spain). Sodium hydroxide was supplied by Merck (Darmstadt, Germany). Qualitative standard 2,4-difluorophenol used for the system suitability test was purchased from Aldrich Chemie (Steinheim, Germany). HPLC grade acetonitrile and methanol solvents were from Scharlau Chemie S.A. (Barcelona, Spain). HPLC grade water was provided by a Milli-Q system gradient A10 (Millipore Corporation, Bedford, USA). Samples were prepared at a concentration of 0.1 mg/ml of LAS 34475 in methanol as solvent. Substances related to LAS 34475 were supplied by the Process Development Department of Almirall Prodesfarma, S.A.

2.3. Procedure for analytical optimisation of the automated method

The type and solvent volume were already optimised during the previous development of the manual method. 250 ml of methanol was the volume of the solvent of choice in the optimised manual method. We checked the critical steps of the automated sample preparation, focusing on extraction and rinsing.

2.4. Preparation of sample and standard solutions

2.4.1. Standard solutions preparation

2.4.1.1. Qualitative standard solution. A methanol solution of approximately 0.1 mg/ml of LAS 34475 and 2,4-difluorophenol, respectively, was prepared for qualitative purposes (system suitability test). In the system suitability test the resolution between the peaks corresponding to LAS 34475 and 2,4-difluorophenol must be $\geq$2. The solution could be stored in a refrigerator for 1 month.

2.4.1.2. Quantitative standard solution. Three standard solutions of respectively, 0.12 mg/ml (standard A), 0.10 mg/ml (standard B) and 0.075 mg/ml, (standard C) of LAS 34475 in methanol were prepared to quantify the

content of LAS 34475 in the tablets by means of external calibration. The stability over 24 h of this solution had been determined in the validation of manual method.

2.4.2. Samples

2.4.2.1. Manual sample solution preparation. The samples were prepared at the concentration of 0.1 mg/ml of LAS 34475. All samples were dissolved in methanol. One tablet was ground in a mortar and then transferred to a 250 ml volumetric flask. Two hundred milliliter of methanol were added and an ultrasound bath was used to assure total disintegration of the tablet (10 min approximately). The solution was then stirred for 1 h. Once room temperature is reached the solution is made up with methanol, and finally centrifuged at 3000 rpm for 20 min. The resultant clear solution was injected into the chromatographic system. Sample solutions must be prepared immediately after grinding the tablets and protected from light using amber glass flasks.

2.4.2.2. Automated sample solution preparation. The sample was placed in the corresponding tube of the workstation and the next phases were automatically carried out according the following scheme:

In step 1 the automated system dispenses into the homogeniser vessel 250 ml of methanol. The solvent is dispensed gravimetrically based on previously recorded solvent densities. In step 2, the tablet of the sample tube is transferred into the homogenisation vessel with the aid of the robot arm and a tipper assembly. In steps 3 and 5 of the method the homogenisation vessel disperses the tablet by performing a particular number of pulses at the established probe speed (Kilorevolutions per minute (K rpm)). In step 3 the tablet is dispersed using 4 pulses of 15 s at 8 Krpm, and in step 5 the tablet is dispersed using 60 pulses of 15 s at 5 Krpm. In steps 4 and 6 the solution is allowed to soak/settle for 60 s. In step 7 the robot arm first places a new filter in the filter holder. A 5.0 ml sample aliquot of homogenate is then aspirated from the vessel and passed through the filter to eliminate particles. In step 8 a filtered sample aliquot of 0.5 ml is passed through the injector, and 20 μl of this aliquot are injected into the HPLC system. This step is carried out in duplicate. In step 9 a clean-up procedure is performed to prepare for the next sample. The homogenisation vessel is emptied. Finally, it is rinsed twice with 200 ml of methanol. The filter path is also rinsed with methanol.

This process can be summarised in steps as follows:

Step 1: Transfer 250 ml of methanol to the dispersion vessel.
Step 2: Add one tablet to the dispersion vessel.
Step 3: Disperse the tablet using 4 pulses of 15 s, pulses at 8 K rpm.
Step 4: Soak/settle for 60 s.
Step 5: Disperse the tablet using 60 pulses of 15 s, pulses at 5 K rpm.
Step 6: Soak/settle for 60 s.
Step 7: Filter 5.0 ml of dispersion at 0.1 ml/s.
Step 8: Pass through the injector 0.5 ml of sample. Inject in duplicate 20 μl of sample in the HPLC system, with run time of 15 min.
Step 9: End method and clean up for the next sample.

The injector used in the automated system is a Rheodyne model 7010, 20 μl loop volume.

2.5. Optimum chromatographic conditions for manual and automated methods

The column used was a Symmetry C-185 μm, 4.6 × 150 mm. The optimum mobile phase was a mixture of 10 mM phosphoric acid adjusted to pH 3.5 with sodium hydroxide and acetonitrile (64:36 v/v). The flow rate was 1.0 ml/min. The injection volume was 20 μl. The analysis time was 15 min. The detection UV wavelength was 275 nm.

2.6. Method validation

The validation of the developed method was carried out within the requirements of ICH Guidelines Q2A–Q2B. Only the parameters directly related to the automatic preparation of the sample were considered for validation [10–12]. Parameters related to the chromatographic method were previously validated in the manual method.

Selectivity was demonstrated with regard to precursors, synthesis intermediates, synthesis by-products, starting materials and degradation products of LAS 34475 in the manual method development.

The range of linearity was established by injecting solutions of LAS 34475 at concentrations ranging from 40 to 200% of the nominal concentration of LAS 34475 tablets (0.1 mg/ml).

The LOD and LOQ were calculated by using a signal-to-noise ratio of approximately, 3 and 10, respectively.

A system precision was performed to determine intra-day variation in peak areas and retention times. The statistical evaluation was carried out using data from 7 runs on the same day and was determined by injecting LAS 34475 at a concentration of 0.10 mg/ml in the above cited optimal experimental conditions. Precision was calculated as a percentage of relative standard deviation (% R.S.D.) of retention times and peak areas obtained for LAS 34475.

To assess accuracy and method precision using the Benchmate Workstation TPW II, placebo material was spiked with various amounts of LAS 34475 at 75, 100 and 125% of the target concentration (0.1 mg/ml), each level in triplicate.

The rinsing operations were optimised in order to eliminate the carry-over effect. A procedure of alternating sample preparations with blanks was run on the automated system.

The blank solutions were analysed for carry-over with the established chromatographic procedure. The rinsing cycles after homogenisation steps were optimised until no carry-over was observed at the limit of detection in the blank chromatograms.

The robustness test of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The parameters studied to determine the robustness are homogenisation time, solvent volume and probe speed. A variation of ±30% in the initial value of each parameter was made (for the probe speed the variation was ±10%). The results obtained when every parameter was varied must be within the range of values obtained analysing the same batch in the section of comparing the manual and the automatic method.

The final step in the automated method validation was the comparison of content uniformity data generated by the automated system with data generated by the manual procedure. For this determination, ten samples of LAS 34475 25 mg tablets were concomitantly analysed applying the manual and automated methods, and the results obtained were compared.

3. Results and discussion

The purpose of this study was to demonstrate the method development strategies for the automated determination of content uniformity of LAS 34475 25 mg tablets [13]. The proposed automated method was validated taking into account the ICH guidelines (Q2A and Q2B).

3.1. Development and optimisation of the automated method

The development and optimisation of this automated method includes the examination of sample extraction and the carry-over effect in order to create a workable automated procedure.

Sample extraction was evaluated establishing an optimum homogenisation time, probe speed and number of dispersion steps to assure complete disintegration and extraction of the active ingredient from the tablet matrix.

A series of six sample solutions was prepared by varying the number of pulses at the fifth step (10, 30, 60 and 90 pulses). The results are shown in Table 1. There is no difference (at the 95% confidence level) in the mean values for content of LAS 34475 tablets between the manual and the automated method when the number of pulses is 60 and 90 pulses. In the other cases, we obtain significant differences between the mean value of content of LAS 34475 tablets obtained in the manual and in the automated methods. The method using 60 pulses was chosen as improving time efficiency. The results obtained in the manual method for the analysis of content uniformity of LAS 34475 25 mg tablets from the same batch are also shown in Table 1.

Table 1
Results of the mean, standard deviation (S.D.), interval from the lower to higher value (L–H) of the analysis of several samples with the manual method and when the number of pulses of ultraturax is varied from 10 to 90 in the automatic method

Number of pulses	Mean (mg/tablet)	S.D. (mg/tablet)	L–H (mg/tablet)
Manual method			
–	25.31	0.21	25.04–25.76
Automatic method			
10	23.54	0.37	22.94–24.02
30	24.55	0.52	23.98–25.37
60	25.11	0.36	24.34–25.36
90	24.98	0.37	24.52–25.55

The probe speed was subsequently optimised. A series of six sample solutions was prepared by varying the probe speed at the fifth step (20, 8 and 5 K rpm). The results are shown in Table 2 . There is no difference (at the 95% confidence level) in the mean value of content of LAS 34475 tablets between the manual and the automatic methods when the probe speeds are 5 and 8 K rpm. In the case of 20 K rpm we obtain significant differences between the mean value of content of LAS 34475 tablets obtained using the manual and the automatic methods. The differences obtained with this latter probe speed could be due to the scattering of the solvent in the extraction vessel. The probe speed of 5 K rpm was selected because the results were similar and the probe speed was less disturbing.

The carry-over effect was also evaluated by varying the rinsing step after the dispersion step of the 25 mg tablets. A series of six blanks was analysed alternating blanks between six samples of LAS 34475 tablets 25 mg for each rinsing step. The results are shown in Table 3. The signal-to-noise ratio of LAS 34475 chromatographic peak obtained in the last rinsing cycle is lower than limit of detection. The carry-over effect was evaluated establishing a rinsing cycle to assure the thorough cleaning of the extraction vessel. The solvent and volume used was a compromise in the vessel cleaning

Table 2
Results of the mean, standard deviation (S.D.), interval from the lower to higher value (L–H) of the analysis of several samples with the manual method and when the probe speed of Ultraturax is varied from 5 to 20 K rpm in the automatic method

Probe speed (Krpm)	Homogenisation time (s)	Mean (mg/tablet)	S.D. (mg/tablet)	L–H (mg/tablet)
Manual method				
–	–	25.31	0.21	25.04–25.76
Automatic method				
5	1800	25.39	0.24	24.90–25.69
8	1800	25.11	0.36	24.34–25.36
20	1800	26.97	0.58	26.25–27.68

Table 3
Results of carry-over observed with the different rinse cycle indicated as rinse step

Rinse step	Concentration LAS 34475 (μg/ml)
Rinse with water (2 × 200 ml) and with methanol (1 × 100 ml)	1.56
Rinse with water (2 × 200 ml) and with methanol (3 × 100 ml)	0.12
Rinse with methanol (3 × 100 ml)	0.09
Rinse with methanol (4 × 100 ml)	0.09
Rinse with methanol (2 × 200 ml)	<0.03 (LOD)

step that gave minimum carry-over of the drug and maximum efficiency in solvent use. The cleaning step was a rinse with methanol (2 × 200 ml).

Finally, a series of six sample solutions was prepared by varying the time of the pulses of the fifth step (30 and 15 s). The results are shown in Table 4. There is no difference (at the 95% confidence level) in the mean values of content of LAS 34475 25 mg tablets between the manual and the automatic methods when the homogenisation time of the pulses is 15 and 30 s, consequently 15 s time was chosen.

3.2. Method validation

The parameters determined in this study were linearity, detection and quantitation limits, system precision, accuracy and method precision, robustness, and comparison of the manual and automatic methods.

3.2.1. Linearity

Linearity was demonstrated at concentrations from 40 to 200% of the target concentration of drug (0.1 mg/ml). Resulting peak areas were evaluated by linear regression analysis ($r = 0.9998$, $y = 7.1910^7 \times + 3.60 \times 10^4$), where y corresponds to the peak areas and x refers to the LAS 34475 concentration expressed in mg/ml.

3.2.2. Detection and quantitation limits

The LOD calculated using a signal-to-noise ratio of approximately three and the LOQ calculated using a signal-to-noise ratio of approximately, 10 are presented in Table 5. The limit of detection is about 0.03 μg/ml and the limit of quantitation about 0.1 μg/ml based on compliance with those criteria.

Table 4
Results of the mean, standard deviation (S.D.), 95% confidence level (CL), interval from the lower to higher value (L–H) of the analysis of several samples with the manual method and when the time of the pulses is varied from 15 to 30 s in the automatic method

Number of pulses	Time of the pulses(s)	Mean (mg/tablet)	S.D. (mg/tablet)	L–H (mg/tablet)
Manual method				
–	–	25.31	0.21	25.04–25.76
Automatic method				
60	15	25.48	0.23	25.15–25.89
60	30	25.39	0.24	24.90–25.69

Table 5
Limits of detection and quantitation of LAS 34475

	Concentration (μg/ml)	Target[a] (%)	S/N ratio
LOD	0.03	0.03	4
LOQ	0.10	0.1	12

[a] LAS 34475 as a percentage of the nominal concentration.

3.2.3. System precision

A precision test was performed to determine intra-day variation in peak areas and retention times by injecting the same standard solution. The statistical evaluation was carried out with the data from seven runs on the same day and was determined by injecting LAS 34475 at a concentration of 0.1 mg/ml in optimum experimental conditions. System precision calculated as a percentage of relative standard deviation (% R.S.D.) of retention times and peak areas obtained for LAS 34475 is 0.47% and 0.51%, respectively (Table 6).

3.2.4. Accuracy and reproducibility

A recovery study was performed to establish the accuracy of the procedure. Spiked placebos ranging from 75 to 125% (75, 100 and 125%) of the nominal concentration were analysed using the automatic method. The results shown in Table 7 demonstrate excellent accuracy and reproducibility of the method.

3.2.5. Robustness

To demonstrate the robustness of the method, a sample solution was prepared in duplicate under different conditions of sample treatment. Parameters such as homogenisation time, solvent volume and probe speed were varied to evaluate the robustness of the method. In this way, the variation of the homogenisation time and solvent volume was ±30% and the variation of the probe speed was ±10% regarding to initial value. The results obtained are shown in Table 8. In this

Table 6
System precision of LAS 34475

Replicates	Retention time (min)	Area
Automatic method		
	8.39	72,35,212
2	8.39	72,35,907
3	8.40	72,52,891
4	8.42	72,31,010
5	8.43	73,29,338
6	8.43	72,97,872
7	8.50	72,71,367
Mean	8.42	72,64,799
S.D.	0.04	37,184
%R.S.D.	0.47	0.51

Table 7
Recovery of LAS 34475 at three levels injected in triplicate

Level-sample	LAS 34475 spiked (mg)	LAS 34475 recovered (mg)	Recovery (%)
75-1	18.23	17.82	98
75-2	17.83	17.41	98
75-3	18.16	17.65	97
100-1	25.24	24.98	99
100-2	25.09	24.84	99
100-3	24.63	24.36	99
125-1	30.88	30.52	99
125-2	30.74	30.64	100
125-3	30.35	29.98	99
% Mean	–	–	99
% R.S.D.	–	–	0.81

Table 8
Robustness of the automatic method

Variation in homogenisation time (%)		
+30	0 (900 s)	−30
T (mg/tablet)		
25.16	24.96	25.63
Variation in solvent volume (%)		
+30%	0 (250 ml)	−30
T (mg/tablet)		
25.01	24.96	24.72
Variation in probe speed (%)		
+10	0 (8 Krpm)	−10
T (mg/tablet)		
24.97	24.96	24.99

table these results are compared with the results obtained on analysing the same batch without any modification in the previous parameters (0%). The values obtained when the indicated parameters are varied, are within the range of values obtained analysing the same batch in the next section comparing the methods, Table 9 (24.61–25.76 mg/tablet). Consequently, the requirement for robustness was achieved in all cases.

3.2.6. Comparison of the manual and automatic methods

The final step in the validation of the automated method was to compare content uniformity data generated by the automated system with data generated by the manual procedure. Comparison data is provided in Table 9. It was noted that the precision of both methods (shown as % R.S.D.) is similar. The difference between the average of results obtained from both methods is 1.4% and the difference between each mean and the ground mean is 0.7%. Therefore the automated method fulfilled most reasonable acceptance criteria being the above differences less than the accepted variation in content uniformity due to the homogeneity of the batch.

Table 9
Comparison of manual and automated methods: results of the mean, standard deviation (S.D.), 95% confidence level (CL), interval from the lower to higher value (L–H) of the analysis of several samples with the automatic and manual methods

Number of pulses	Homogenisation time (s)	Mean (mg/tablet)	S.D. (mg/tablet)	L–H (mg/tablet)
Manual method				
–	–	25.31	0.21	25.04–25.76
Automatic method				
60	900	24.96	0.99	24.61–25.35

Table 10
Results of HPLC validation with the manual and automatic methods

LOD (μg/ml)	LOQ (μg/ml)	*r*	Precision (area)	Precision (t_R)	Recovery mean (% R.S.D)
Manual method					
0.03	0.10	0.999	0.3	0.2	100 (0.22)
			% Precision (area)	% Precision (t_R)	Recovery mean (% R.S. D.)
Automatic method					
0.03	0.10	0.999	0.5	0.5	99 (0.81)

4. Concluding remarks

The automated method for the sample preparation and analysis of LAS 34475 25 mg tablets has been optimised. The automated procedure includes dissolving, filtration and direct injection of the sample solution into the HPLC chromatographic system.

This automated method has been validated in the context of the pharmaceutical industry requirements and demonstrated excellent accuracy and robustness. This is a simple, precise, reproducible, accurate, sensitive, linear, quick and selective method. The system is capable of running up to 100 samples automatically, thereby increasing productivity and lowering the cost of analysis.

The results obtained in the validation of this automated method were equivalent in terms of system precision, linearity, accuracy, robustness, and sensitivity. Comparison of manual and automatic HPLC validation data is provided in Table 10.

To conclude, the automated system provides a more than suitable tool for the analytical sample preparation of drug tablets and can be used in a general content uniformity analysis of tablets. Furthermore, this approach can be regarded as an excellent starting point for content homogeneity of powder blends as well as for assay determination in stability studies of tablets, when several units are pooled in the same sample solution preparation.

References

[1] J.Y. Zhang, D.M. Fast, A.P. Breau, J. Chromatogr. B 785 (2003) 123–134.

[2] J.A. García Mesa, M.D. Luque de Castro, M. Valcárcel, LRA 529-33.
[3] F. Zick, M. Fisher, B. Gulden, J. Assoc. Lab. Autom. 5 (2000) 40–43.
[4] G.A. Hamlin, J. Autom. Methods Manag. Chem. 22 (2002) 181–186.
[5] I. Mahmud, D. Kim, J. Autom. Methods Manag. Chem. 22 (2002) 199–202.
[6] Center for Drug Evaluation and Research, 21 CFR 211,194.
[7] S.M. Dobro, J. Autom. Methods Manag. Chem. 23 (2003) 183–187.
[8] In: the Proceedings of International Conference on Harmonization Guidance, ICH: Q2A: Guideline for Industry, Text on validation of analytical procedures, arch 1995.
[9] In: the Proceedings of International Conference on Harmonization Guidance, ICH: Q2A: Guideline for Industry, Text on validation of analytical procedures, May 1997.
[10] G.A. ShabiR, J. Chromatogr. A 987 (2003) 57–66.
[11] F. Feng, J. Jiang, H. Dai, J. Wu, J. Chromatogr. Sci. 41 (2003) 17–21.
[12] R. Gonzalo-Lumbreras, R. Izquierdo Hornillos, J. Pharm. Biomed. Anal. 31 (2003) 201–208.
[13] W.F. Shamrock, K. Reilly, D.K. Lloyd, J. Pharm. Biomed. Anal. 21 (2000) 1225–1232.