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Validation of Stability-Indicating Reverse Phase HPLC Method for the Determination of Related Substances in Fosaprepitant Dimeglumine Drug Substance

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ABSTRACT

A gradient reversed phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the determination of related substances of Fosaprepitant dimeglumine drug substance. The well chromatographic separation of Fosaprepitant dimeglumine from its process and degradation related substances was achieved on Unison UK-Phenyl, 3µm (250mm × 4.6mm) column *i.e* Phenyl silane chemically bonded to porous silica particles of 3 µm diameter maintained column oven temperature at 25°C. Phosphoric acid buffer as mobile phase A and acetonitrile as mobile phase B. Wavelength for UV detection: 210nm, flow rate: 1.0 ml/min and Injection volume: 20µl. The method suitability was checked and validated according to the ICH guidelines for specificity, linearity, accuracy, precision, limit of quantification, limit of detection robustness and ruggedness and also Fosaprepitant dimeglumine was subjected to stress conditions of thermal, hydrolysis, humidity, peroxide and photolytic to observe the degradation products. Limit of detection of each impurity is less than 0.011% w/w indicating that the developed method is highly sensitive. The experiment results are given in detail in this research article.

1.0 INTRODUCTION

Fosaprepitant dimeglumine (FPD) is chemically known as 1-Deoxy-1-(Methylamino)-D-Glucitol [3-[[(2*R*,3*S*)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4 morpholinyl]methyl]-2,5-dihydro-5-oxo-1*H*-1,2,4-triazol-1-yl]phosphonate (2:1), molecular formula is $C_{23}H_{22}F_7N_4O_6P.2(C_7H_{17}NO_5)$ and molecular weight is 1004.83. FPD belongs to antiemetic therapeutic category, an intravenous neurokinin-1 antagonist for the prevention of chemotherapy induced nausea and vomiting [1]. It is a phosphorylated prodrug that is rapidly converted to aprepitant, an oral selective neurokinnin-I receptor antagonist approved [2-4]. Fosaprepitant is an intravenous prodrug of aprepitant that offers a new alternative to patients with chemotherapy induced nausea and vomiting (CINV). Currently, FPD can substitute oral aprepitant in day 1 of a 3-day regimen. Recent studies show that a single day FP regimen is also bioequivalent to the 3 day aprepitant regimen; this could significantly simplify the care for CINV patients in the future [5]. Fosaprepitant (FP) 115 mg was generally well tolerated at a final drug concentration of 1 mg/ml, and FP 115 mg was AUC bioequivalent to aprepitant 125 mg. FP in the dose of 115 mg has been approved by the US FDA, the EU and the Australian authorities. FP may be a useful parenteral alternative to oral aprepitant [6]. FPD is marketed under trade name EMEND IV [7]. FPD is lyophilized powder in single dose vial for reconstitution and each vial contains FPD equivalent to 150 mg of FP which corresponds to 130.5 mg of aprepitant. After reconstitution and dilution 1 ml of solution contains 1 mg of FP (1 mg/ml). The chemical structure of FPD is shown in Figure 1.

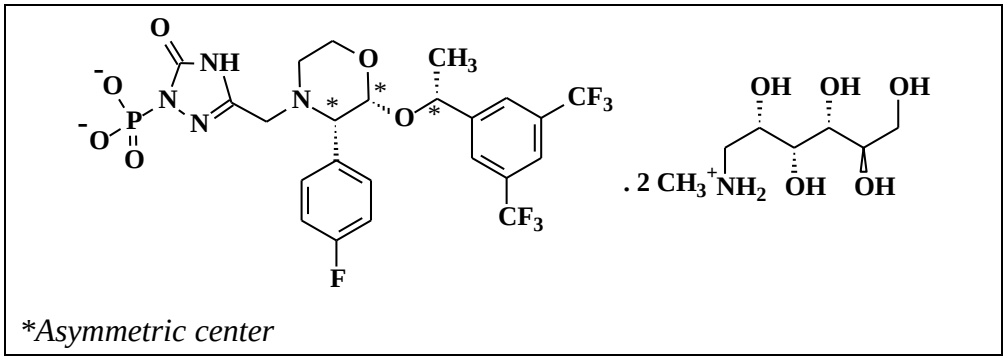


Fig. 1: Chemical structure of Fosaprepitant dimeglumine

There is no single pharmacopeial monograph available for this drug substance or drug product and no HPLC method is available in literature for quantification of FPD related substances. However, few of methods have been reported in literature for the determination of FP in formulated products and plasma. Determination of FP in FP injection by HPLC was

published in 2015 by Zhao and *et al* [8]. Simultaneous determination of Aprepitant and FP in plasma by HPLC method was reported in 2016 by Xu, Meijuan and *et al* [9]. In this research paper, development of a stability indicating HPLC method for the simultaneous detection and quantitative determination of the four impurities in FPD drug substance has been reported. The chemical structures of these four related substances [RS –I to IV] are given in Figure 2. Forced degradation studies were carried out to establish stability indicating nature of the method according to ICH stability guidelines [10]. Limit of detection (LOD), limit of quantification (LOQ) and linearity were established as per ICH guidelines. The limit of RS - I, II and IV have been considered as 0.15% in accordance with ICH guideline based on maximum daily dose [11]. Further, limit for RS - III has been considered as 0.5%, where related substance- III (aprepitant) is allowed for higher limit, as this related substance is a metabolite and FP is a prodrug of aprepitant. The developed chromatographic method can resolve four related substances with acceptable resolution to achieve good chromatography and the optimized methodology have been validated to accomplish ICH guidelines on validation [12].

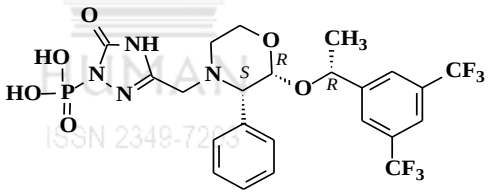
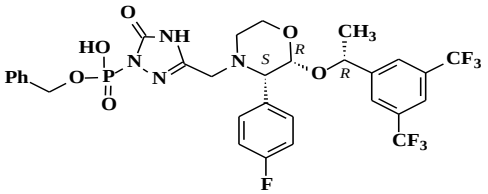
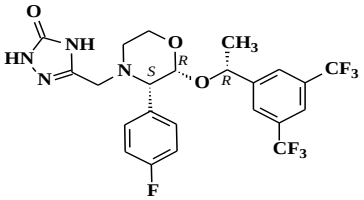
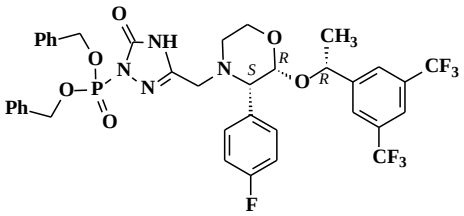
Impurity	Chemical Structure
RS-I	
RS-II	
RS-III	
RS-IV	

Fig. 2: Chemical structures of Fosaprepitant dimeglumine related substances

2.0 MATERIALS AND METHODS

2.1 Chemicals, reagents, standards and samples

The investigated samples of FPD drug substance and reference sample, its related substances and FPD for system suitability (FPD enriched with RS-I) were gifted from APL Research Centre-II Laboratories (A division of Aurobindo Pharma Ltd., Hyderabad). AR grade of potassium dihydrogen phosphate, disodium hydrogen phosphate anhydrous, acetonitrile, and orthophosphoric acid (~88%), hydrochloric acid, sodium hydroxide and hydrogen peroxide were procured from Merck, India and pure milli-Q water was used with the help of Millipore purification system (Millipore®, Milford, MA, USA).

2.2 Instrumentation and methodology

The HPLC system used for method development, method validations as well as forced degradation studies were Waters Alliance e2695 separation module equipped with 2998 photodiode array detector and with Empower data handling system i.e Empower 3 software, 2487 UV detector [Waters Corporation, MILFORD, MA 01757, USA] was used. HPLC column: A stainless steel column 250mm long, 4.6 mm internal diameter filled with Phenyl silane chemically bonded to porous silica particles of 3µm diameter. [Unison UK-Phenyl, 3µm (250mm × 4.6mm) (Make: Imtakt)], column oven temperature: 25°C. Mobile phase A: 1.0 ml of Orthophosphoric acid in 1000 ml of water, Mobile phase B: Acetonitrile. Diluent: Degassed mixture of buffer and acetonitrile in the ratio of 50:50 v/v. (Buffer: dissolve 1.15 g of Disodium hydrogen phosphate anhydrous and 0.88 g of potassium dihydrogen phosphate in 1000 ml of water, filter this solution through 0.45 µ or finer porosity membrane filter) Flow rate: 1.0 ml/min, injection volume: 20µl, data acquisition time: 40 min and UV detection: 210 nm. Retention time of FP: about 12 minutes. The pump is in gradient mode and the program is as follows: Time (min)/ A (v/v): B (v/v); $T_{0.01}$/65:35, T_5/65:35, T_{20}/50:50, T_{40}/05:95, T_{42}/65:35, T_{50}/65:35.

2.3 Preparation of solutions

2.3.1 System suitability solution

1 mg/ml concentration of FPD for system suitability (FPD enriched with Impurity-I) in diluent.

2.3.2 Standard solution

0.0015 mg/ml concentration of solution using FPD standard in diluent.

2.3.3 Sample solution

1.0 mg/ml concentration of solution using FPD sample in diluent.

2.3.4 System suitability evaluation:

USP Plate count of FP: Not less than 5000; USP Tailing NMT 1.5 from standard solution.

USP Resolution: RS-I and FP peaks is not less than 1.5 from the system suitability solution.

3.0 RESULTS AND DISCUSSION

3.1 Method validation

The developed method was established through the validation experiments as per the ICH guidelines individually in terms of specificity or selectivity, forced degradation studies, LOD/LOQ, linearity, precision, accuracy, robustness and stability of solutions.

3.1.1 Specificity

Specificity is the ability to assessing unequivocally of analytic in the presence of components which may be expected to be present. For determination of specificity, blank, all individual related substances solutions were prepared and injected to confirm the individual retention times. The solutions of FPD drug substance (Control Sample) and FPD spiked with known related substances at specification level (Spiked Sample) were prepared and injected into HPLC. Peak purity was established by using Empower Software. A typical representative HPLC chromatogram of FPD drug substance spiked with all related substances is shown in Figure 3. The specificity results are tabulated in Table 1.

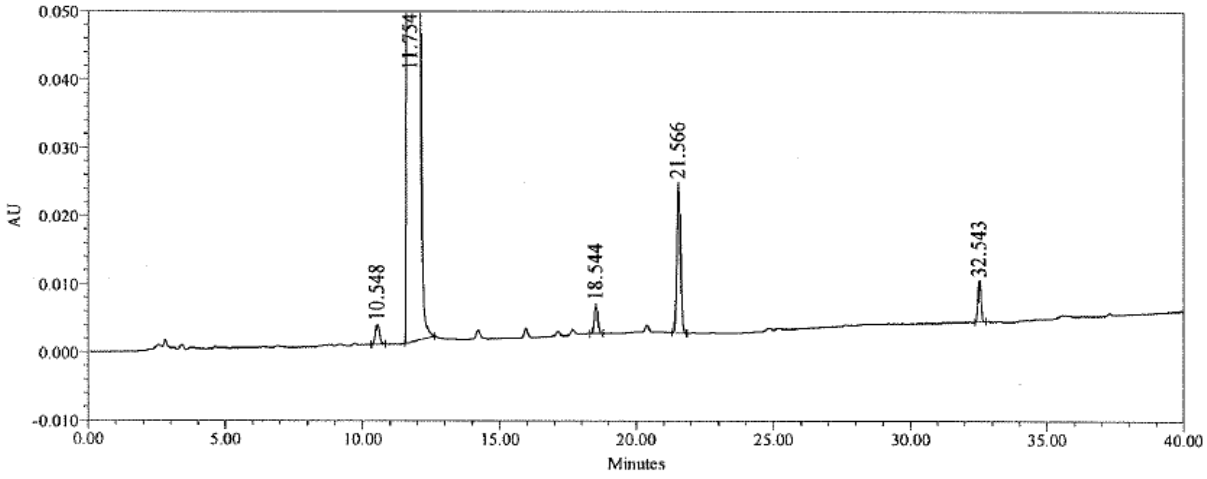


Fig. 3: A typical representative HPLC chromatogram of FPD drug substance spiked with related substances

Table 1: Specificity experiments results

Name	Retention time (min)	RRT	Peak purity	
			Purity angle	Purity threshold
RS-I	10.548	0.90	0.569	0.956
FP	11.754	1.00	0.034	0.264
RS-II	18.544	1.58	0.460	0.740
RS-III	21.566	1.83	0.090	0.292
RS-IV	32.543	2.77	0.249	0.584
FPD Control sample/diluent	--	--	0.037	0.254
FPD Spiked sample/diluent	--	--	0.034	0.264

3.1.2 Forced degradation

The stability indicating nature of FPD has been studied by performing forced degradation experiments. FPD was subjected to different stress conditions [13] *i.e* acid/base hydrolysis [1M HCl/60°C/20 min & 1M NaOH/60°C/30 min], peroxide degradation under oxidative stress [5% H_2O_2 / 60°C / 10 min], thermal degradation [50°C/120 Hours], humidity degradation study (90% RH/25°C/120 hrs) and photolytic degradation [white Fluorescent light, 1.2million lux hours and UV light, 200 watt hours / m^2] w.r.t ICH option 2 of Q1B [13].

Peak purity of FP peak was established by using PDA detector in these stress samples along with unstressed sample. The forced degradation results are tabulated in Table 2. In all of the above stressed conditions, there was a significant change observed w.r.t known impurity –IV (RS-IV) up to 21.99% in acid degradation condition, 7.08% in thermal condition, 1.59% in photolytic condition, 1.40% in humidity condition, there was no significant change observed w.r.t other known related substances. In peroxide degradation, two unknowns at RRTs 0.24 & 1.07 detected up to 0.40 & 11.79% respectively. In base degradation unknown at RRT, 0.24 was observed up to 7.24%. These results of various stress conditions employed to degrade FPD indicate that drug substance is susceptible to degradation under acidic, basic, oxidative and thermal conditions whereas it is found to be stable in photolytic & humidity stress conditions. Based on the above forced degradation studies, it can be concluded that the origin of Impurity III (RS-III) (Aprepitant) is degradant. Further, the peak purity data of FP peak (refer below Table 2) from every degradation sample showed that it is homogeneous, and there are no co-eluting peaks. Hence, this method is proved as stability indicating. The forced degradation experiments results are shown in Table 2.

Table 2: Forced degradation data of FPD

Degradation mechanism	Degradation condition	Area	Degradation (%)	Peak purity of Fosaprepitant	
				Purity angle	Purity threshold
-	Unstressed sample	9647501	-	0.046	0.254
Acid	1M HCl / 60°C / 20 min	7479319	22.7	0.053	0.246
Base	1M NaOH / 60°C / 30 min	9535870	1.2	0.016	0.252
Peroxide	5% H_2O_2 / 60°C / 10 min	8399089	13.1	0.007	0.247
Thermal	50°C / 120 hours	8972787	7.0	0.037	0.250
Photolytic	White fluorescent light, 1.2million Lux hours and UV light, 200 watt hours / meter square	9565584	0.9	0.016	0.252
Humidity	90% RH / 25°C / 120 hours	9587766	0.6	0.047	0.252

3.1.3 Limit of Detection (LOD) / Limit of Quantification (LOQ)

LOD and LOQ were predicted on the basis of response and slope of the regression equation. These are calculated from the formula 3.3δ/S and 10 δ /S respectively where ‘δ is standard deviation of the y-intercept of the regression line and ‘S’ is slope of the calibration curve

which were predicted from linearity experiment. The precision study was carried out at about predicted LOD and LOQ levels by injecting six replicates and calculating the % RSD of the area of each impurity. LOD and LOQ values are presented in Table 3.

3.1.4 Linearity

A series of solutions were prepared by using FPD and its related substances at concentration levels from LOQ to 150% of specification level and each solution was injected and calculated the statistical values like slope, intercept, STEYX and correlation coefficient from linearity plot drawn for concentration versus area. The statistical values are presented in Table 3.

Table 3: Statistical evaluation of linearity and LOD/LOQ experiments

	RS-I	RS-II	RS-III	RS-IV
Concentration range (µg/mL)	0.203-2.299	0.119-2.260	0.131-7.541	0.107-2.266
Slope	25510	41907	38881	48366
Intercept	192	80	257	-2
STEYX	247	193	738	572
Response factor	1.32	0.80	0.86	0.69
Correlation Coefficient	0.9999	0.9999	0.9999	0.9999
LOD(%w/w)	0.011	0.006	0.007	0.006
%RSD	2.7	3.5	3.6	1.9
LOQ(%w/w)	0.033	0.019	0.021	0.017
%RSD	1.4	1.0	1.9	1.5

3.1.5 Precision

The precision (system precision) was evaluated by injecting six injections of FPD standard solution and calculating the % relative standard deviation. The method precision was checked by injecting six individual preparations of FPD spiked with related substances I, II and IV with 0.15% level, and related substance-III with 0.5% level with respect to sample concentration. The % RSD of content of each related substance was calculated. The intermediate precision of the method was also evaluated using different analyst, different instrument, and different lot of column on different day. The inter day variations were calculated. The precision experiments results are given in Table 4.

Table 4: Precision experiment results

System Precision										
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Mean	SD	% RSD	95% Confidence Interval (±)
FPD Peak area	32125	32627	32286	32302	32451	32196	32331	182	0.6	191

Method Precision										
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Mean (%w/w) *n=6*	SD	% RSD	95% Confidence Interval (±)
RS-I	0.159	0.162	0.164	0.164	0.163	0.159	0.162	0.002	1.2	0.002
RS-II	0.152	0.152	0.154	0.156	0.154	0.153	0.154	0.002	1.3	0.002
RS-III	0.839	0.832	0.850	0.831	0.870	0.839	0.844	0.015	1.8	0.016
RS-IV	0.146	0.146	0.147	0.148	0.146	0.145	0.146	0.001	0.7	0.001

Ruggedness										
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Mean (%w/w) *n=6*	SD	% RSD	95% Confidence Interval (±)
RS-I	0.163	0.165	0.164	0.163	0.162	0.163	0.163	0.001	0.6	0.001
RS-II	0.161	0.161	0.160	0.160	0.159	0.160	0.160	0.001	0.6	0.001
RS-III	0.846	0.846	0.848	0.846	0.847	0.842	0.846	0.002	0.2	0.002
RS-IV	0.139	0.139	0.139	0.139	0.138	0.137	0.139	0.001	0.7	0.001

3.1.6 Accuracy

The accuracy of the method was determined by analyzing FPD (n=3) samples spiked with related substances at different levels (LOQ, 50, 100 and 150% of specification levels). The percentage recovery values for all the impurities are calculated and tabulated in Table.5.

Table 5: Accuracy experiment results

Recovery details (average 3 replicates)		RS-I	RS-II	RS-III	RS-IV
--	% Level				
Added (%w/w)	LOQ	0.0335	0.0197	0.0212	0.0177
	50	0.079	0.078	0.249	0.074
	100	0.158	0.156	0.499	0.148
	150	0.237	0.234	0.748	0.221
Recovered (%w/w)	LOQ	0.0325	0.0183	0.0205	0.0165
	50	0.081	0.075	0.233	0.077
	100	0.170	0.162	0.506	0.151
	150	0.252	0.241	0.741	0.220
Recovery (%)	LOQ	97.0	92.9	96.7	93.2
	50	102.5	96.2	93.6	104.0
	100	107.6	103.8	101.4	102.0
	150	106.3	103.0	99.1	99.5

3.1.7 Robustness

To determine robust of the method, experimental conditions were deliberately changed and evaluate system suitability requirement as per methodology. For this evaluation, system suitability solution and sample solution spiked with impurities at specification level were prepared as per test method and injected into HPLC. To study the effect of flow rate, 10% variation (±0.1 units) of flow rate was changed. The effect of column temperature was studied by keeping 20°C and 30°C instead of 25°C. In the same manner, detection wavelength (±3 nm) and organic in mobile phase (±2% absolute in Gradient Composition) have been verified and the results obtained from these experiments are summarized in Table 6.

Table 6: Robustness experiment results

Condition	Variation	System Suitability			Spiked Sample (RRT)			
		USP Resolution	USP Plate count	USP Tailing	RS-I	RS-II	RS-III	RS-IV
Original Method parameters	-	3.8	49827	1.1	0.91	1.54	1.81	2.64
Flow	-10%	3.7	54422	1.1	0.91	1.52	1.80	2.62
	+10%	3.8	50405	1.1	0.90	1.55	1.83	2.67
Wavelength	-3 nm	3.8	51984	1.1	0.90	1.54	1.80	2.63
	+3 nm	3.8	53203	1.1	0.91	1.54	1.80	2.63
% Organic in gradient variation	-2% absolute	3.4	28833	1.1	0.89	1.67	2.00	3.11
	+2% absolute	4.1	83070	1.1	0.92	1.45	1.67	2.31
Column Oven Temperature	-5°C	4.0	52078	1.1	0.90	1.53	1.80	2.63
	+5°C	3.5	52724	1.1	0.91	1.55	1.81	2.64

3.1.8 Stability of solutions

Standard solution and sample solution spiked with impurities were prepared and analyzed initially and at different time intervals by keeping the solutions at room temperature (~ 25°C) and refrigerator condition (~6°C). Experimental results show that Standard solution is stable up to 24 hours at 25°C+2°C and Sample solution is not stable at 25°C+2°C, but stable up to 24 hours at 5°C±3°C.

4.0 CONCLUSION

A reverse phase stability indicating HPLC method was developed and validated for the quantitative determination of related substances of Fosaprepitant dimeglumine. The present research work will help the manufacturers and suppliers of Fosaprepitant dimeglumine to quantify and qualify the quality in terms of purity based on experimental results. Thus, it can be used for routine analysis, quality control and for determining quality during the stability studies of pharmaceutical analysis.

5.0 ACKNOWLEDGEMENT

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6.0 REFERENCES

[1] Van Belle SJ, Cocquvt V. Fosaprepitant dimeglumine (MK-0517 or L-785,298), an intravenous neurokinin-1 antagonist for the prevention of chemotherapy induced nausea and vomiting. Expert Opin Pharmacother. 2008; 9(18): 3261-3270.

[2] Roila F, Herrstedt J, Aapro M, Gralla RJ and *et al.* Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. Ann Oncol. 2010; 21(5): 232-243.

[3] Feyer P, Jordan K. Update and new trends in antiemetic therapy: the continuing need for novel therapies. Ann Oncol. 2011; 22(1): 30-38.

[4] Celio L, Ricchini F, De Braud F. Safety, efficacy, and patient acceptability of single-dose fosaprepitant regimen for the prevention of chemotherapy-induced nausea and vomiting. Patient Prefer Adherence. 2013; 7: 391-400.

[5] Colon Gonzalez F, Kraft WK. Pharmacokinetic evaluation of fosaprepitant dimeglumine. Expert Opin Drug Metab Toxicol. 2010; 6(10): 1277-1286.

[6] Navari RM. Fosaprepitant: a neurokinin-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. Expert Rev Anticancer Ther. 2008; 8(11): 1733-1742.

[7] USFDA Approval; EMEND (Fosaprepitant dimeglumine) for injection, Merck Sharp & Dohme Corp. a subsidiary of MERCK & CO., INC., 2008.

[8] Jing Z, Cong-cong R, Wei-chong D, Ye J. Determination of Fosaprepitant dimeglumine for injection by HPLC. Huaxi Yaoxue Zazhi. 2015; 30(4): 476-477.

[9] Meijuan X, Jihong C, Ting W, Wenzheng J, Changyin L, Jun Z, Shijia L, Guoliang D. A method for simultaneous detection of fosaprepitant and aprepitant in plasma. Faming Zhuanli shenquing. 2016.

[10] The International Conference on Harmonization, Q1A(R2), Stability Testing of New Drug Substances and Products: Text and Methodology, 2003.

[11] The International Conference on Harmonization, Q3A(R2), Impurities in New Drug Substances: Text and Methodology, 2006.

[12] The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 2005.

[13] The International Conference on Harmonization, Q1 (B), Stability Testing: Photostability Testing of New Drug Substances and Products: Text and Methodology, 2005.