International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 1073-1082, 2017

Cost Effective Stabilty Indicating Reverse Phase High Performance Liquid Chromatography Analytical Method Validation for Determination of Related Substance of Cyproterone Acetate

Asheesh Singh[1]*, Parul Singh[2]

[1]Alpa Laboratories Limited, Pharma zone, Pithampur (India)-454775
[2]Department of Pharmacy, Guru Ghasidas University, Bilaspur(India)-495009

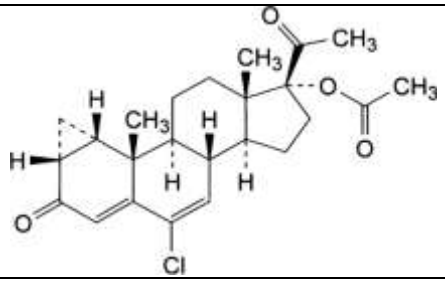
Abstract : Cyproterone acetate (CPA) is a steroidal synthetic progestagen and anti-androgenic compound used as a treatment for metastatic prostate cancer. A simple, sensitive, stability-indicating reversed-phase high-performance liquid chromatographic method was developed for the determination of Cyproterone acetate and related impurities in Cyproterone acetate Active Pharmaceutical ingredient. The chromatographic separation was achieved on a Waters, Spherisorb ODS II, 125 mm x 4.6 mm, 3 µm column, using a mobile phase consisting of Water: Acetonitrile (60: 40) %, v/v, at a flow rate of 1.5 mL/min and temperature of 25°C. Quantification was achieved with photodiode array detection at 254 nm. The described method showed excellent linearity over a range of limits of quantification to 150% of specification limit. The drug product was subjected to the stress conditions of oxidative, acid, base, thermal and photolytic degradation. Cyproterone acetate degradation was observed in acid hydrolysis, base hydrolysis and peroxide stress conditions. Cyproterone acetate was stable in thermal and photolytic degradation conditions. The method is validated for the quantification of impurities and degradation of Cyproterone acetate in Cyproterone acetate Active Pharmaceutical ingredient. This method was validated for Specificity, accuracy, precision, linearity and Robustness as per ICH guidelines.
Keywords : Cyproterone acetate, progestagen, Analytical Method, Validation, High performance Liquid Chromatography.

Introduction:

Cyproterone acetate (CPA) is a steroidal synthetic progestagen and anti-androgenic compound widely administered in patients with metastatic prostate cancer. The first case of CPA-induced fulminant hepatitis with a fatal outcome was reported in 1989. A variety of hepatotoxic reactions have been documented, including immunoallergic cytotoxic reactions, cholestasis, autoimmune hepatitis, acute hepatitis, and fulminant hepatic failure. Despite its low incidence, the prognosis of hepatic failure induced by CPA is fatal. Only 1 of 14 reported patients has survived.[1–3] CPA has been widely prescribed as an anti-androgen to suppress the progression of metastatic prostate cancer. Considering the high use of CPA by urologists late into the treatment process, more discussion about the complication of this drug is needed. It is well-known that patients with prostate cancer have a relatively good prognosis and even patients with bone metastasis can have extended survival periods. Unfortunately, CPA-induced hepatic failure may encroach upon the considerably favourable survival period among patients with metastatic prostate cancer[4-5]. Cyproterone acetate was stable in thermal and

photolytic degradation conditions. The method is validated for the quantification of impurities and degradation of Cyproterone acetate in Cyproterone acetate Active Pharmaceutical ingredient.This method was validated for Specificity, accuracy, precision, linearity and Robustness as per ICH guidelines[6-7].

Table No. 1: Drug Substance Information

Name of Drug Substance	**:**	**Cyproterone Acetate API**
Description of Drug Substance	**:**	White or almost white crystalline powder
Chemical Name	**:**	6-chloro–3,20-dioxo-1β,2β-dihydro-3'H-clopropa [1,2]pregna-1,4,6-trien-17-yl acetate.
Structure	**:**	
Molecular Formula	**:**	$C_{24}H_{29}ClO_4$
Molecular Weight	**:**	416.94
CAS No.	**:**	427-51-0

Experimental Section:

Instruments, Reagents and Materials

Following instruments were used for the validation studies.

Table No.2: List of Instrument Used

Sr No	Instrument	Make	Software	Detector/Model No
1	HPLC	Waters	Empower Software	2489 dual wavelength
2	HPLC	Waters	Empower Software	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

Table No.3: List of Column used

Sr. No.	Make	Dimension
1	Spherisorb ODS2	125mm x 4.6mm, 3µm
2	Spherisorb ODS2	125 mm x 4.6mm, 3µm

Table no. 4: Working standard/Sample Details:

Name	Batch Number	Potency (%)
Cyproterone Acetate WS	ADWSF009	99.6
Cyproterone Acetate API	NT1914M	NA

Methodology:

Reagents and Solvents:

Purified Water (HPLC grade or equivalent)

Acetonitrile (HPLC grade)

Chromatographic Conditions:

Column	:	Waters, Spherisorb ODS II , 125 mm x 4.6 mm, 3 µm
Mobile Phase	:	Water :Acetonitrile (60 : 40) %, v/v
Column Temperature	:	Ambient
Detector	:	UV Detector
Detector Wavelength	:	254 nm
Injection Volume	:	20 µL
Flow	:	1.5 ml/min
Retention time	:	22 minutes
Run Time	:	50 minutes
Diluent	:	Acetonitrile (HPLC grade)

Preparation of Standard Stock Solution: Weigh and transfer accurately about 10 mg of Cyproterone acetate Working Standard and transfer it into 10 ml volumetric flask. Add about 5 ml of Acetonitrile and sonicate to dissolve. Allow it to come to room temperature and dilute up to the mark with diluent and mix. (Concentration of Cyproterone Acetate: about 1000 ppm)

Preparation of Reference Solution (a): Transfer 1.0 ml of Standard Stock Solution into 100 ml volumetric flask and dilute up to the mark with diluent and mix. (Concentration of Cyproterone acetate: about 10 ppm)

Preparation of Reference Solution (b): Dissolve the contents of a vial of Cyproterone impurity mixture CRS (impurities F and I) in 1.0 ml of the test solution.

Preparation of Reference Solution (c): Dissolve the contents of a vial of Cyproterone acetate for peak identification CRS (containing impurities B, C, E and G) in 1.0 ml of Acetonitrile.

Preparation of Sample Solution: Weigh and transfer accurately about 10 mg of sample and transfer it into 10 ml volumetric flask. Add about 5 ml of Acetonitrile and sonicate to dissolve. Allow it to come to room temperature and dilute up to the mark with diluent and mix. (Concentration of Cyproterone Acetate: about 1000 ppm)

Evaluation of System suitability:

i) Resolution between Cyproterone acetate and impurity I peaks obtained in System Reference Solution (b) should be minimum 1.5

ii) The relative standard deviation of peak area counts of Cyproterone acetate from six replicate injections of Reference Solution (b) should be not more than 5%.

Relative Retention Time, Relative Response Factor (RRT, RRF) for impurities as the order of elution are given in tabular form below table no. 5:

Table no. 5: order of elution for impurities

Sr. No.	Name of the impurities	RRT	RRF
1.	Impurity E	about 0.27	0.7
2.	Impurity G	about 0.30	1.0
3.	Impurity F	about 0.50	1.0
4.	Impurity B	about 0.70	1.0
5.	Impurity I	about 0.90	1.0
6.	Impurity C	about 1.50	1.8

Specificity:

The specificity of a method is its suitability for the analysis of a substance in the presence of potential impurities. Stress testing of a drug substance can help to identify likely degradation products, which can establish degradation pathways and the intrinsic stability of the molecule. The peak purity of the Cyproterone Acetate was found to be satisfactory. The specificity of the LC method for Cyproterone Acetate was determined in the presence of their known impurities. The retention time of the peak of Cyproterone Acetate in sample solution and diluted standard solution were found to be comparable. Hence the method is specific with respect to retention time and relative retention time of analyte peaks and their known impurities. % degradation and peak purity reported intable no. 6 and system suitability and sample chromatograms reported in figure no 2 &3.

Table No.6: Stress Testing (Forced Degradation) Data

Stress Condition	Cyproterone Acetate % degradation	Purity Angle	Purity Threshold
Control Sample	NA		
Acid Sample 0 Hrs	29.2	0.556	0.869
Base Sample 0 Hrs	7.2	0.785	1.820
Base Sample 24 Hrs	15.2	0.982	1.102
Peroxide Sample 0 Hrs	5.1	0.752	1.212
Peroxide Sample 24 Hrs	14.2	0.743	1.252
Photo Sample	2.8	1.124	2.143
Thermal Sample	1.3	1.415	2.546
Humidity Sample	Stable	0.623	1.675

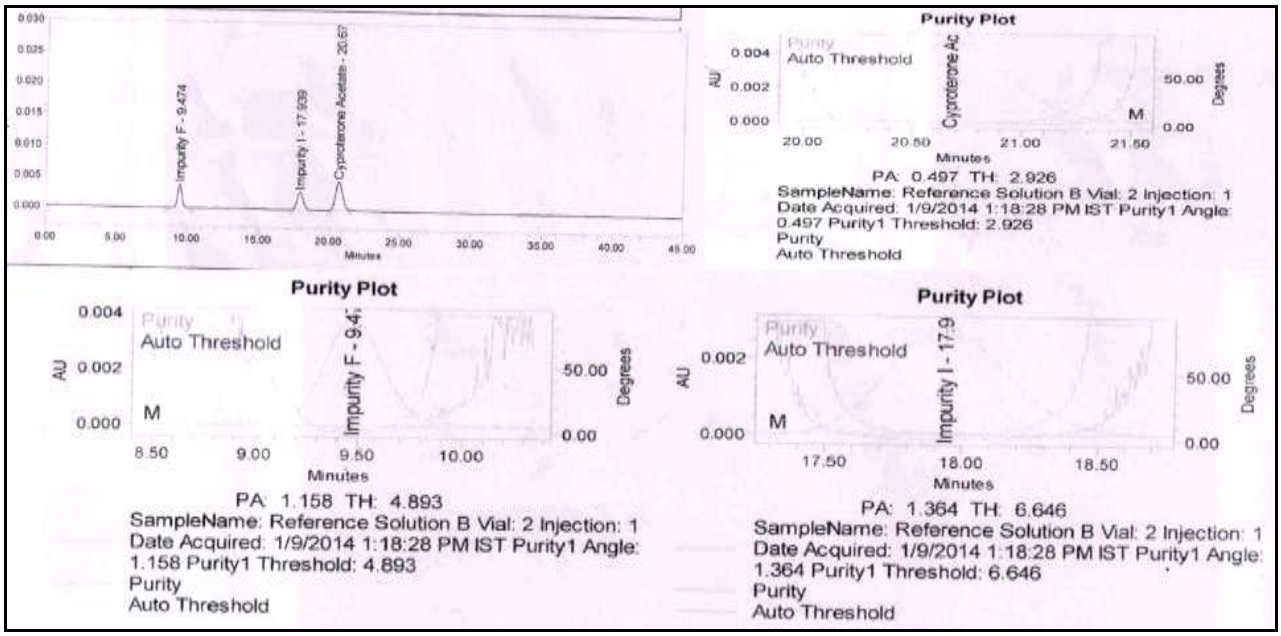
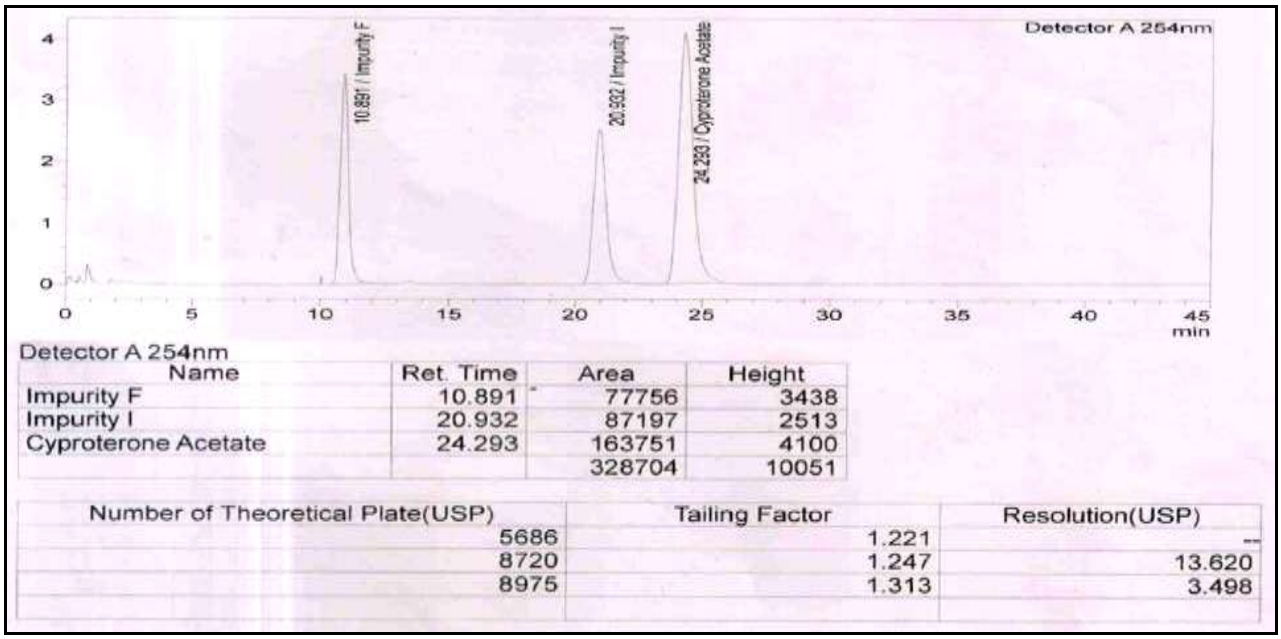


Figure No.1: Reference Solution B (System Suitability Solution)



Detector A 254nm

Name	Ret. Time	Area	Height
Impurity F	10.891	77756	3438
Impurity I	20.932	87197	2513
Cyproterone Acetate	24.293	163751	4100
		328704	10051

Number of Theoretical Plate(USP)	Tailing Factor	Resolution(USP)
5686	1.221	--
8720	1.247	13.620
8975	1.313	3.498

Figure No.2: Sample Solution

Precision

System Precision:

Six replicate injections of the diluted standard solution were injected into the chromatograph. Peak area counts of Cyproterone Acetate from six replicate injections of diluted standard solution were calculated. System Precision reported in Table No.7.

Table No. 7: System Precision

Injection	Cyproterone Acetate
1	155615
2	154722
3	154856
4	154298
5	154744
6	155120
Mean	**154893**
SD	**442.6**
% RSD	**0.29**

Method Precision & Ruggedness:

Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under the prescribed conditions. Quantification of individual impurities and Cyproterone Acetate was performed for each of the preparations and the percent relative standard deviation (RSD) was determined for the content of the impurities. To evaluate the intermediate precision, the same experiment was repeated with a different lot of column and a different instrument in the same laboratory. % RSD (n=6) for % of unknown Degradation product in six sample solutions and overall % RSD (n=12) for % of unknown Degradation product from Method Precisionand Intermediate Precision results was found within acceptance criteria. Precision data reported in table no.8.

Table No. 8: Over all %RSD Comparison for Impurities in Precision and Ruggedness study

Sr. No.	% Impurity F	% Single Maximum	% Total Impurity
Precision-1	0.05	0.018	0.05
Precision-2	0.05	0.015	0.05
Precision-3	0.05	0.017	0.05
Precision-4	0.05	0.018	0.05
Precision-5	0.05	0.020	0.05
Precision-6	0.05	0.018	0.05
Ruggedness-1	0.05	0.018	0.05
Ruggedness-2	0.05	0.018	0.05
Ruggedness-3	0.05	0.019	0.05
Ruggedness-4	0.05	0.018	0.05
Ruggedness-5	0.05	0.018	0.05
Ruggedness-6	0.05	0.018	0.05
Mean	**0.05**	**0.018**	**0.05**
SD	**0.000**	**0.001**	**0.000**
% RSD	**0.00**	**6.50**	**0.00**

Limit of Dectection (LOD) & Quantitation (LOQ):

Limits of detection and quantification The LOD and LOQ for the Known impurities and Cyproterone Acetate were estimated as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. The precision was also determined at the LOQ level by the analysis of six individual preparations of the impurities and calculating the relative standard deviation (RSD; %) of the peak area for each impurity. Limit of Quantitation (LOQ) was established by Reference solution (a) i.e (0.05%). Limit of Detection (LOD) was established by quantitatively diluting 3.3 ml of LOQ solution into 10ml. Limit of Detection and Limit of Quantitation data are reported in table no. 9 & 10.

Table No.9: Limit of Detection for Cyproterone Acetate

	Peak Area Counts	**S/N value**
	Cyproterone Acetate	
injection	**Area**	**3.57**
1	668	

Table no.10: Limit of Quantitation for Cyproterone Acetate

	Peak Area Counts	**S/N Value**
	Cyproterone Acetate	**9.79**
Injection	**Area**	
1	1831	
2	1823	
3	1886	
4	1831	
5	1843	
6	1853	
Mean	**1845**	
SD	**22.9**	
%RSD	**1.24**	

ACCURACY:

The studies were carried out at four different levels: LOQ, 50%, 100%, and 150% of limits. The percentage of recoveries of Impurity E, Impurity G, Impurity F, Impurity B, Impurity I and Impurity C were calculated with respect to amount spiked and amount recovered. The percentage recovery at each level was calculated against the Cyproterone Acetate standard. Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels and 85% to 115% for LOQ level. Mean recovery in percentage is reported in Table no. 11.

Table No. 11: Accuracy of Impurity of Cyproterone Acetate

	Mean Recovery (%)			
Name of Impurity	**LOQ**	**Accuracy 50%**	**Accuracy 100%**	**Accuracy 150%**
Impurity E	89.6	95.5	98.2	96.7
Impurity G	92.1	98.2	99.8	99.5
Impurity F	97.4	98.3	101.3	99.3
Impurity B	99.0	98.5	105.8	97.9
Impurity I	90.5	99.2	104.4	98.9
Impurity C	93.5	97.5	100.1	99.5

Filter Study:

Six sample solutions of Cyproterone Acetate were prepared by the specification level and analyzed as per methodology. Prepare 3 sample solutions as per test method. Centrifuge one portion of sample solution and filter the other portion of sample solution through PVDF filter and nylon filters. The Results of Filter Variability are calculated. Samples the m/m difference of maximum single unknown degradation product should not be more than **0.05** and the % m/m total degradation products should not be more than **0.2.** The results are tabulated in Table 12.

Table no. 12: Comparisons of Filter Study of Cyproterone Acetate

Sample Name	Single max Unknown Degradation product	Total Degradation product	Mean (Single maximum)	Mean (Total Degradation product)	Difference for Single maximum	Difference for Total Degradation product
Centrifuge-1	0.034	0.05	**0.034**	**0.05**	0.0000	0.0000
Centrifuge-2	0.034	0.05				
Centrifuge-3	0.033	0.05				
Nylon-1	0.030	0.04	**0.033**	**0.05**	**0.0003**	**0.00**
Nylon-2	0.035	0.05				
(Nylon-3	0.035	0.05				
PVDF-1	0.035	0.04	**0.035**	**0.04**	**0.001**	**0.01**
PVDF-2	0.034	0.04				
PVDF-3	0.035	0.04				

Stability of Analytical Solution:

One sample solution was prepared as per methodology and Injected diluted standard solution, initially and at different time intervals upto 48 hours by storing the sample solutions at Room temperature. Absolute value of (m/m) difference between the area of Cyproterone Acetateinitially (0 hour) and area of Cyproterone Acetateat each time interval with respect to area of Cyproterone Acetateinitially (0 hour) respectively was calculated. Solution stability of Diluted Standard solution, Sample solution and Spike sample Solution are reported in table no. 13 to 15.

For standard the similarity factor for corresponding time interval should be between 0.95 to 1.05. For the sample solution the m/m difference of maximum single unknown degradation product should not be more than 0.05 and for total degradation products should not be more than 0.2.

Calculation of Similarity Factor:

The Similarity Factor for Cyproterone Acetate in standard solution at each time point was calculated with respect to the initial area as follows.

$$\text{Similarity Factor} = \frac{\text{(Area of Std Solution)}}{\text{(Area of initial)}} \times \frac{\text{(Wt of initial)}}{\text{(Wt of STD)}}$$

Table no. 13: Solution stability of Cyproterone Acetate in diluted standard solution

Cyproterone Acetate		
Time Interval	**Area of Cyproterone Acetate**	**Similarity Factor**
Initial	156847	1.00
8 Hrs.	157922	0.99
16 Hrs.	157552	1.00
24 Hrs.	158718	0.99
32 Hrs.	158866	0.99
40 Hrs.	159309	0.99
48 Hrs.	159474	0.99

Table no.14: Solution stability of single maximum unknown Degradation product in sample solution

Time Interval	Cyproterone Acetate	
	% Single Max Degradation product (m/m)	% Difference w.r.t. Initial (m/m)
Initial	0.043	0.000
8 Hrs.	0.036	0.007
16 Hrs.	0.038	0.005
24 Hrs.	0.040	0.003
32 Hrs.	0.035	0.008
40 Hrs.	0.044	0.001
48 Hrs.	0.036	0.007

Table no.15: Solution stability of Total Degradation product in sample solution

Time	Cyproterone Acetate	
	% Total Degradation product (m/m)	% Difference w.r.t. Initial (m/m)
Initial	0.05	0.000
8 Hrs.	0.05	0.003
16 Hrs.	0.05	0.002
24 Hrs.	0.05	0.002
32 Hrs.	0.05	0.003
40 Hrs.	0.05	0.002
48 Hrs.	0.05	0.001

Summary of System Suitability

System suitability was evaluated by injecting Standard solution during different days of validation and monitoring resolution for different parameters .The % relative standard deviation for the peak area counts of cyproterone Acetate from five replicate injections of standard solution was verified at every stage. Results are tabulated in Table no.16.

Table No.16: Table for System Suitability

Sr No	Name of Experiment	Resolution	%RSD
1	System precision, Method Precision,	2.3	0.29
2	LOD & LOQ	4.0	1.00
	Filter study, Solution Stability		0.17
3	Accuracy	2.5	0.26
4	Ruggedness	3.0	0.68
5	Specificity / Force Degradation	2.9	0.39

Summary and Conclusion:

The Validated HPLC method for related substance of Cyproterone Acetate is precise, accurate and specific. The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the Cyproterone Acetate during routine analysis and also for stability studies in view of its capability to separate degradation products.

Acknowledgements:

The authors wish to thank the management of Alpa Laboratories Limited Indore for supporting this work. Authors wish to acknowledge the Analytical research group for providing the necessary facilities for our research and also wish to thanks colleagues in Validation division of analytical research for their co-operation in carrying out this work.

List of Abbreviations:

No.	Number
LOQ	Limit of Quantitation
LOD	Limit of Detection
Imp	Impurity
Unk	Unknown
Max	Maximum
w.r.t	With respect to
Hrs	Hours
HPLC	High performance Liquid Chromatography
RSD	Relative Standard Deviation
RRT	Relative retention time

References:

1. Savidou I, Deutsch M, Soultati AS. Hepatotoxicity induced by cyproterone acetate: a report of three cases. World J Gastroenterol.,2006, 12; 7551–5.
2. Levesque H, Trivalle C, Manchon ND. Fulminant hepatitis due to cyproterone acetate. Lancet.,1989, 1; 215–6.
3. Murphy BJ, Collins BJ. Severe hepatitis and liver failure induced by cyproterone acetate. Aust N Z J Med., 1996, 26; 724-730.
4. Kacar S, Akdogan M, Kosar Y. Estrogen and cyproterone acetate combination induced autoimmune hepatitis. J ClinGastroenterol., 2002, 35; 98–100.
5. Drakos PE, Gez E, Catane R. Hepatitis due to cyproterone acetate. Eur J Cancer., 1992, 28; 1931–2.
6. FDA, Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Guidance for Industry "Bioanalytical Methods Validation for Human Studies". U.S. Department of Health and Human Services; 2001.
7. International Conference on Harmonization Q1A (R2)) Stability Testing of New Drug Substances and Products. 29. International Conference on Harmonization Q3A (R2) Impurities in New Drug Substances.

*****