ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Novel RP-HPLC Method for Simultaneous Estimation of Silodosin and Dutasteride in Multiunit Solid Dosage Form.

Hardik P Shah[1*],Amit Khandar[2] , Shirish Deshpande[1] , and Shashikant Bagade[1]

[1]Department of Pharmaceutical Chemistry, SVKM's NMIMS, School of Pharmacy & Technology Management, Mumbai-Agra Highway, Shirpur, Dist. Dhule, MS, India, 425405.
[2]ZydusCadila Healthcare Pvt. Ltd. Ahmedabad , Gujarat.

ABSTRACT

Silodosin and Dutasteride both are approved drugs by USFDA (Food & Drug Administration). On literature survey, it was found that no methods have been reported for simultaneous estimation of Silodosin and Dutasteride. Therefore, it was thought of interest to develop a simple, accurate, precise, sensitive and economic analytical method and to validate as per ICH guidelines. So RP-HPLC method was developed and validated for simultaneous estimation of Silodosin and Dutasteride in multiunit system. Separation was achieved on Agilent technology HPLC; Zorbax SB C8 Column (250 mm × 4.6 mm , 5 µm) at 40°C temperature by using a mobile phase containing Buffer (Dipotassium hydrogen phosphate , pH 3) & Organic mixture (methanol : acetonitrile in 50:50 ratio) in the ratio of 20:80. Analysis was done at the flow rate of 1.0 mL/min and UV detection was carried out by wavelength gradient at 270 nm (0 to 5 min.) and 210 nm (5 min to 10 min) . The retention time of Silodosin and Dutasteride was found to be 2.7 min & 6.7 min respectively. The specificity of the method was determined by assessing interference from placebo. Samples were subjected to forced degradation study by using 5N HCl, 5N NaOH, 30% hydrogen Peroxide , Humidity , thermal and photo degradation. Degradation was found at 30% peroxide condition for Silodosin and mild degradation was found for Dutasteride.The method was validated in terms of linearity, precision, accuracy, specificity, robustness, ruggedness, solution stability. The linearity were found to be in the range of 39.56 -118.68 µg/mL& 2.43-7.29 µg/mL for Silodosin and Dutasteride with correlation coefficient of 0.997 for Silodosin and 0.995 for Dutasteride. %RSD of method precision was found to be less than 2%, this indicates that the method is precise.
Keywords: Forced degradation; Method development; RP-HPLC ; Validation.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Silodosin [1], a novel indoline 7-carboxamide derivative used in the treatment of Benign Prostatic Hyperplasia and Urinary Tract Infections. Silodosin is designated chemically as 1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2, 2, 2-trifluoroethoxy) phenoxy] ethyl} amino) propyl] indoline-7-carboxamide. Structure of silodosin is shown in figure 1. Silodosin is α1A adrenergic receptor antagonist[2-3]that selectively affects the prostate and urinary bladder as a therapeutic agent for the treatment of the signs and symptoms of the benign prostatic hyperplasia. It causes smooth muscle relaxation by antagonizing the α1A adrenergic receptor in the lower urinary tract.

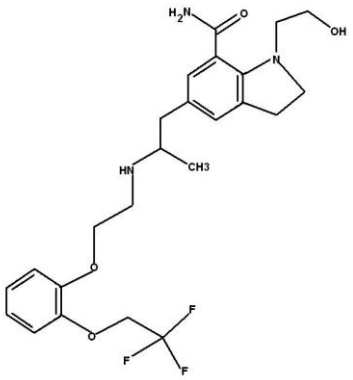

Figure 1: Chemical structure of Silodosin

Dutasteride, chemically known as (5α, 17β)-N-{2,5bis(trifluoromethyl)phenyl}-3-oxo-4- azaandrost-l-ene-17-carboxamide, is a synthetic 4-azasteroid compound with antiandrogenic activity. Structure of Dutasteride is shown in figure 2. Dutasteride is used to treat benign prostatic hyperplasia in men having an enlarged prostate gland and in the treatment of male pattern baldness. It belongs to a class of drugs called 5α-reductase inhibitors, which competitively and specifically inhibits type 1 (active in the sebaceous glands of most regions of skin and liver) and type 2 (primarily active in the reproductive tissues like prostate, seminal vesicles, epididymides, hair follicles and liver) isoforms of 5α-reductase, an intracellular enzyme that converts testosterone to 5α- dihydrotestosterone. The decrease in dihydrotestosterone levels may mitigate or prevent enlargement of the prostate gland [4-6].

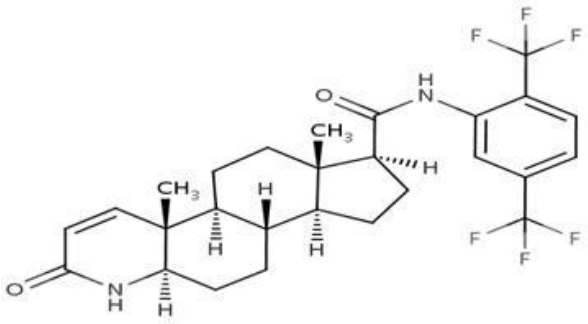

Figure 2 Chemical structure of Dutasteride

The recent literature survey showed that a rapid, sensitive LC/MS [7-8]methods were developed for the determination of silodosin in human plasma. There are several method available for estimation of silodosinby UV Spectrophotometric methods [9-10], by RP-HPLC[11-13]and HPTLC method[14]were reported. A limited number of analytical method have been reported for the quantitative determination of Dutasteride in pharmaceutical preparations and human plasma when present alone or in combination with other drugs (Alfuzosin and Tamsulosin)[15-17]. These techniques are LC–MS[18-19], HPTLC[20], Enzyme-linked immunosorbent assay[21], HPLC and stability-indicating RP-HPLC[22-23] , UV spectrophotometric method[24]، UPLC method[25].

None of the reported methods, however, addressed the analysis multiunit system ofSilodosin and Dutasteride. The objective of this study was therefore to develop a simple, sensitive, and precise HPLC method for the simultaneous analysis of Silodosin and Dutasteride .

MATERIALS AND METHODS

Chemicals and reagents

Silodosin: Working standard grade was supplied by ZydusCadila Healthcare Limited (Ahmedabad , India) , its claimed purity was 98.99%. Dutasteride: Working standard grade was supplied by ZydusCadila Healthcare Limited (Ahmedabad , India) , its claimed purity was 97.20% Combination drug products of Silodosin and Dutasteride (Lable claim of 8 mg & 0.5 mg respectively) and Placebo were manufactured and supplied by ZydusCadila Healthcare Limited (Ahmedabad , India). acetonitrile, methanol used were of HPLC grade and were purchased from Spectrochem Private Limited, India.

Instrumentation and chromatographic conditions

Optimization of Method

Various method development trials were done and according to that trials, the method was optimized. The HPLC was of Agilent technology 1200 series with UV Visible detector. Column used was ZorbaxSB C8, 5 µm (250 mm × 4.6 mm). The system was run at a flow rate of 1.0 mL/min, 5 µL of sample was injected in the chromatographic system and a UV Visible detector was used for simultaneous determination of Silodosin and Dutasteride. Mobile phase comprising of 10.5mMdipotassium hydrogen phosphate buffer: Organic mixture: (20:80) adjust pH 3.0 with 5% o-phosphoric acid. Organic mixture comprising of methanol:acetonitrile (50:50). Column temperature was maintained at 40°C and UV detection at 270 nm for 0 to 5 min and 210 nm for 5 to 10 min.

Preparation of standard solutions

50 mg of working standard of Dutasteride was dissolved in methanol in 100 mL volumetric flask ,sonicate it for 2 min and volume was made up to the mark with methanol(Stock A). 40 mg of Silodosin was dissolved in 20 mL methanol in 50 mL volumetric flask ,then add 5 mL of stock A solution and sonicate it for 2 min and volume was made up

ISSN: 0975-8585

by methanol(B). 5 mL aliquot from B solution was transferred in 50 mL volumetric flask and volume was made up to the mark with mobile phase. The final concentration of Silodosin and Dutasteride was 80 ppm and 5 ppm for Silodosin and Dutasteride respectively.

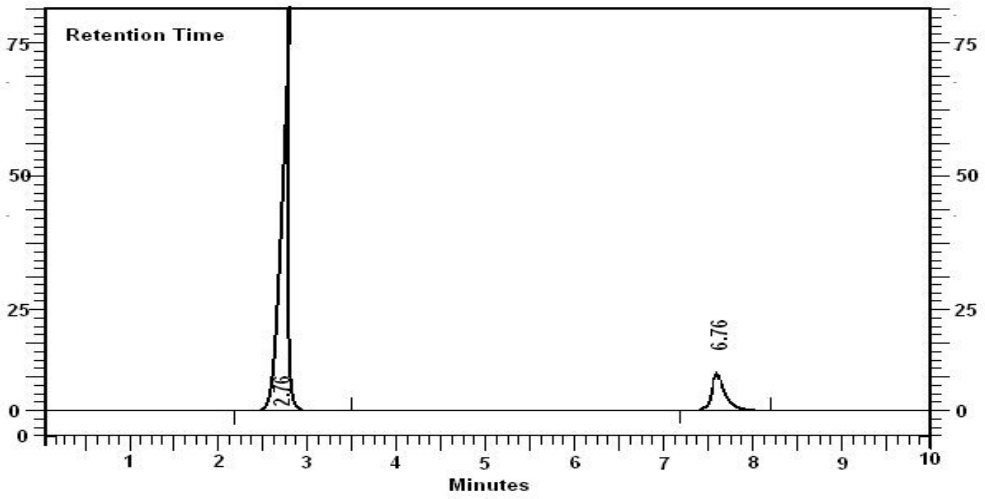

Figure 3 Chromatograph of standard solution of silodosin and dutasteride at optimized chromatographic condition.

Preparation of sample solution

5 capsules were weighed and all contents of 5 capsules were taken in 500 mL volumetric flask. Add 100 mL methanol and sonicate it for 10 min , after that Volume was made up with mobile phase. The solution filtered through 0.45µ PVDC filter. The final concentration of Silodosin and Dutasteride was 80 ppm and 5 ppm respectively.

Validation of analytical method [26-27]

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The linearity for Silodosin and Dutasteride was found to be 39.56 -118.68 µg/mL & 2.43-7.29 µg/mL respectively

Procedure for linearity

The linearity was determined at 5 levels over the range of 50% to 150% of standard concentration .Standard stock solution of Silodosin and Dutasteride was prepared (B solution). From that, aliquots of 2.5, 4 , 5 , 6 , 7.5 mL was taken in to 50 mL volumetric flask. Volume was made up to the mark with Diluent. A graph of mean area versus concentration was plotted and the correlation co-efficient, y intercept, slope of regression were calculated.

ISSN: 0975-8585

Table: 1 Calibration data for Silodosin & Dutasteride:

Parameters	Silodosin	Dutasteride
Correlation co-efficient	0.9974	0.9951
Standard deviation of response	280328.05924	52355.58529
Slope (m)	96540.00	235159.00
Intercept (b)	257801.00	146229.00

Table 2: Linearity data for Silodosin and Dutasteride:

Linearity level	Silodosin		Dutasteride	
	Conc. in µg/ml	Peak area	Conc. in µg/ml	Peak area
50%	39.56	4108796	2.43	745744
80%	63.29	6261319	3.88	1052774
100%	79.12	8092726	4.86	1254373
120%	94.94	9237852	5.83	1495262
150%	118.68	11778595	7.29	1895010

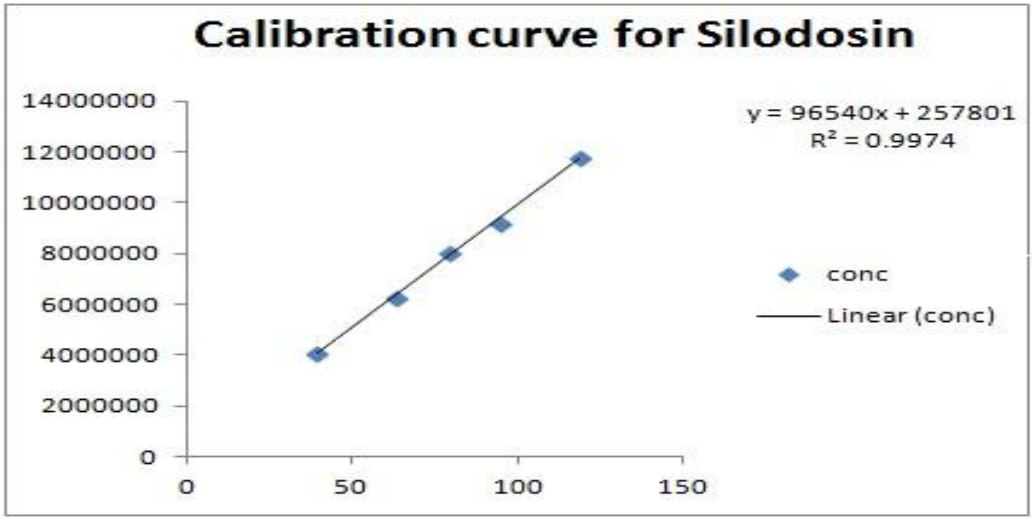

Figure 4 Calibration curve for Silodosin

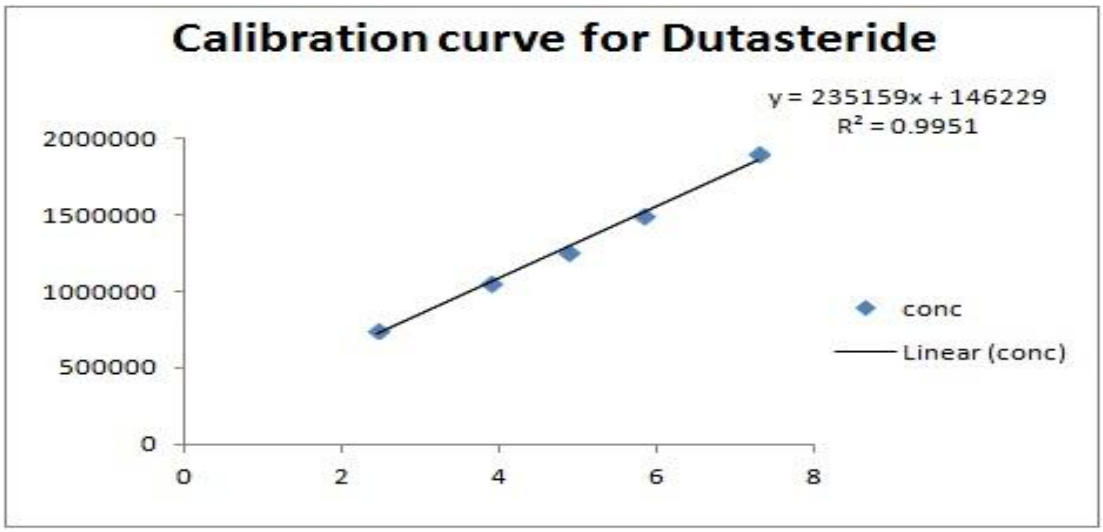

Figure 5 Calibration curve for Dutasteride

Method Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It was Performed by preparing 6 samples as per the test method representing a single batch.

Intermediate Precision (Ruggedness)

Intermediate Precision was determined by analyzing in combined solution their respective calibration range by changing the analyst , equipement and column etc.

Accuracy

Accuracy may often be expressed as percentage recovery. It was determined by calculating the recovery of Silodosin and Dutasteride by application of the analytical method to mixtures of the drug product contents to which known amount of analyte have been added within the range of the method. It was determined at 3 levels of 50% to 150 % , each in triplicate.

Limit of detection (LOD) & Limit of quantification (LOQ)

LOD & LOQ was calculated by Calibration curve.

$$LOD = 3.3 \times \sigma/s$$
$$LOQ = 10 \times \sigma/s$$

Here σ = Standard deviation of Response
s = Slope of the calibration curve

Specificity

It is the ability of the analytical method to measure specifically the analyte of interest without interferences from blank and placebo.

Check for interference from blank and placebo

Diluent was used as blank. Standard and sample were prepared as per test procedure. Diluent , standard preparation , placebo preparation , placebo spiked with standard solution , sample solution were prepared and injected.

Check for interference from forced degradation study

Procedure

In order to establish whether the analytical method for the assay was stability indicating , capsules , pure API of both Silodosin and Dutasteride , Placebo were stressed

under various conditions of acid/base hydrolysis , oxidation , thermal , UV light and humidity , as mentioned in ICH Q1A (R2). Thermal, UV light and humidity degradation of drug substance and drug product was performed in the solid state. For hydrolytic and oxidative degradation , solutions were prepared. All solutions used in forced degradation studies were prepared by dissolving API or drug product in small volume of methanol due to free solubility in methanol and then aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide was added. After the degradation, these solutions were diluted with diluents to yield final concentration.

Acid degradation: Treated with 5 mL 5 N HCl and heated on boiling water bath for 3 hours at 70˚C then cool at room temperature after that add 5 mL 5 N NaOH for neutralize the solution.

Base degradation: treated with 5 mL 5 N NaOH and heated on boiling water bath for 3 hours at 70˚C then cool at room temperature after that add 5 mL 5 N HCl for neutralize the solution.

Peroxide degradation: treated with 5 mL 30% hydrogen peroxide heated on boiling water bath for 3 hours at 70˚C

Thermal degradation: Exposed at 100˚C for 24 hours in oven.

UV light degradation: exposed under UV light in a UV chamber for 24 hours.

Humidity degradation: exposed in humidity chamber at 40 ˚C and 75% RH for 7 days.

Table 3: Results from forced degradation study

Stress Condition	% assay		% degradation	
	Silodosin	Dutasteride	Silodosin	Dutasteride
5.0NHCl / 70˚C/3h	98.00	99.00	2.00	1.00
5.0NNaOH/70˚C/3h	97.00	98 .00	3.00	2.00
30%H_2O_2/70˚C/3 h	56 .00	98 .00	44.00	2.00
Thermal/100˚C/ 24 h	99 .00	99 .05	1.00	0.5
UV/24 h	98 .00	98 .05	2.00	1.05
Humidity/7days	99 .05	98.08	0.5	1.05

Robustness

The following parameters were changed one by one and their effect was observed on system suitability.

- Flow rate of mobile phase: (±10%) to 0.8 mL/min and 1.2mL/min

- Column Oven temperature: (±5˚C) to 35˚C and 45˚C
- Mobile phase ratio: (±2%): 18:78 and 22:82
- pH: (±0.2 absolute) to 2.8 and 3.2

Solution stability

Standard and sample preparation was prepared as per test procedure. The standard and sample preparation were analyzed by HPLC system at regular intervals for 24 hours. The area of the analyte peak for both standard and sample solutions were monitored. The % difference of analyte peak area from initial for both standard and sample solutions were calculated and recorded.

System suitability parameters

System suitability was performed and calculated at the start of study of each validation parameter.

Table 4: The values of system suitability parameters

Sr. No.	Parameters	Silodosine	Dutasteride
1.	**No. of theoretical plates**	4693	8562
2.	**Retention time (min)**	2.7	6.7
3.	**Asymmetry**	1.5%	1.2%

RESULTS AND DISCUSSION

Table 5: Summary of validation parameters:

Parameter	Silodosin	Dutasteride	Remark
Linearity Range	39.56 -118.68 µg/ml	2.43-7.29 µg/ml	Linear
Correlation co-efficient	0.9974	0.9951	Complies
Accuracy	99.7 -101.9 %	99.3-101.4	Complies
Precision	0.2%	0.4%	Complies
LOD	9.58 µg/ml	0.73 µg/ml	
LOQ	95.8 µg/ml	7.30 µg/ml	
Specificity	Peak purity angle is less than purity threshold.	Peak purity angle is less than purity threshold.	Specific
Forced degradation	Degraded at 30% peroxide condition	Mild degradation	Peak purity passes.
Ruggedness	%RSD was less than 2%	%RSD was less than 2%	Rugged
Robustness	%RSD was less than 2%	%RSD was less than 2%	Robust
Solution stability	Stable for 36 h	Stable for 36 h	Stable

The system suitability parameters and system precision are evaluated and found within the limits. A plot is drawn between concentration of the component and the instrumentresponse; It is found to be linear in the concentration range 39.56 -118.68 µg/mL& 2.43-7.29 µg/mL for Silodosin and Dutasteride respectively with good correlation coefficient greater than (r2 0.995). Precision and accuracy of the developed method are

expressed in %RSD and % of recovery of the active pharmaceutical respectively. Low %RSD value and high percent of recovery indicate that the method is highly precise and accurate. All system suitability parameters were found within the standard limit.

Table 5 Summary of validation parameters

CONCLUSION

All these factors leads to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of Silodosin&Dutasterid in multiunit system without inference and with good selectivity. The proposed validated method was successfully applied to determine Silodosin and Dutasteride in bulk powder and in solid dosage form.

ACKNOWLEDGMENTS

The authors are thankful to ZyduscadilaHealthcare Pvt. Ltd. ,Ahmedabad for the gift sample of Pure Silodosin and Dutasteride.

REFERENCES

[1] Merck & Co. The Merck index: anEnclyclopedia of chemicals, drugs and biologicals. 14th ed. White house station, New Jersey, 2006.
[2] Michel MC, Vrydag W. Br J Pharmacol 2006;147 Suppl:S88–119.
[3] Yoshida M, Homma Y, KawabeK. Expert Opin Investig Drugs 2007;16 (12):1955–65.
[4] Keam SJ, Scott LJ. Drugs 2008; 68(4): 463–485.
[5] Walsh PC.N Engl J Med 2010; 362(13): 1237–8.
[6] Dolder CR. Annals of Pharmacoth 2006; 40: 658–665.
[7] Zhao X, Liu Y, XuJ, et al. J Chromatogr B 2009;877(29):3724-3728.
[8] ShaikJaferVali, Santhikumar Saladi, Shakil S Sait , et al.Am J Pharm Tech Res 2013;1:751-758.
[9] CR Sharma, J Akhtar, NM Jagani, et al. Inventi Rapid: Pharm Analysis & Quality Assurance 2012; 3: 310.
[10] CH. Mounika, N. Umadevi and I. Sudheerbabu. IJRPC 2013; 3(3):595-597.
[11] Harischandran S, Shankar Iyer R, Raju R, et al. IJPRS 2012;1:141-145.
[12] ChinnalalaiahRunja, Ravikumar Pigili .Int J Pharm Sci Rev Res 2012;16(2);52-55.
[13] Aneesh TP and Rajasekaran A.IJBPR 2012; 3(5): 693-696.
[14] Sayana PS, R Shankar Iyer ,Shibi A, et al. The Pharma Innovation 2012; 1(10):60-65.
[15] V Sreelakshmi, V Uma MaheswaraRao, Venkata Praveen M, et al. Inventi Rapid: Pharm Analysis & Quality Assurance 2013;1:1157.
[16] Pande VV, Jadhav JN, Chandorkar JG. JPR 2009;2(3):507-509.
[17] Shivprasad S. Deshmukh, Shweta S Havele, Vaishali V. Musale, et al. Der pharmacia letter 2010; 2(6): 342-349.
[18] Noel A. Gomes, Ashutosh Pudage, Santosh S. Joshi, et al. Chromatographia2008; 69: 9–18.
[19] Sangita Agarwal, K. Veeran Gowda, Amlan Kanti Sarkar, et al. Chromatographia 2008; 67: 893–903.
[20] Kamat SS, Vele VT, Choudhari Swaroop. Asian J of Chem 2008; 20: 5514–5518.

[21] Brun EM, Torres A, Ventura R, et al. Anal ChimActa2010; 671: 70–79.
[22] Dipti B Patel. Indian J Pharm Sci. 2010; 72(1): 113–116.
[23] Rao DVS, Radhakrishnanand P. Chromatographia 2008; 67: 841–845.
[24] Kamila MM, Mondal N, Ghosh LK. Int J of Pharm Tech Res 2010; 2(1):113-117.
[25] Y Koti Reddy, GV Subba Reddy, KN Jaya Veera, et al. Am J Pharma Tech Res. 2012;2(6):702-715.
[26] ICH Q2A, Harmonised tripartite guideline, text on validation of analytical procedures, IFPMA, in: Proceedings of the International Conference on Harmonization. Geneva, 1994, pp. 1–5.
[27] ICH Q2B, Harmonised tripartite guideline, validation of analytical procedure: methodology, IFPMA, in: Proceedings of the International Conference on Harmonization. Geneva, 1996, pp. 1–8.