J. Iran. Chem. Soc., Vol. 8, No. 1, March 2011, pp. 31-37.

JOURNAL OF THE
Iranian
Chemical Society

Development and Validation of RP-HPLC Method for Simultaneous Determination of Glipizide, Rosiglitazone, Pioglitazone, Glibenclamide and Glimepiride in Pharmaceutical Dosage Forms and Human Plasma

K.S. Lakshmi and T. Rajesh*

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur-603203, Tamil Nadu, India

(Received 16 May 2009, Accepted 16 December 2009)

A simple, high performance liquid chromatographic method has been developed for the simultaneous determination of glipizide, rosiglitazone, pioglitazone, glibenclamide and glimepiride in pharmaceutical dosage forms and human plasma. The elution was performed using a mobile phase mixture of 0.05% Triethylamine (pH-3.5, adjusted with *ortho* phosphoric acid), acetonitrile and methanol in the ratio of 55:15:30 at a flow rate of 1 ml min^{-1} on a phenomenex C_{18} column (150 × 4.6 mm, i.d., 5 µm) at ambient temperature. The drugs were monitored at a wavelength of 248 nm and were separated within 20 min. Mixtures of formulations were prepared in suitable dilutions and plasma samples were prepared by extraction with acetonitrile. The method was successful in detecting the drugs at a concentration of less than 0.1 µg ml^{-1} for each drug and %RSD for intra- and inter-day studies was found to be less than 4.34 for all the selected concentrations. Moreover, the method was validated as per ICH guidelines and the results were found to be within the acceptable range. Hence, the proposed method can be used for the routine quality control of the drugs and can also be applied to pharmacokinetic studies.

Keywords: Thiazolidenediones, Sulfonyl ureas, Reverse phase HPLC, Validation, Human plasma

INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders characterized by abnormalities in carbohydrate, protein, and lipid metabolism [1]. For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to maintain effective control [2]. Drugs belonging to classes such as sulfonyl ureas (*e.g.* glipizide, glibenclamide, glimepiride) and thiazolidinedione (TZD) derivatives (pioglitazone, rosiglitazone) (structures shown in Fig. 1), are the commonly prescribed hypoglycemic drugs for the treatment of non-insulin dependent type II diabetes mellitus. Thiazolidinedione class of drugs exert their glucose-lowering effect by binding to Peroxisome Proliferator-Activated Receptors gamma (PPARγ), thus increasing the receptor sensitivity to insulin [3-5]. Sulfonylurea drugs act by increasing the secretion of insulin by the functioning β-cells of the pancreas. Such a combination can be attained by taking each of the drugs separately, or alternatively by fixed formulations. A combination tablet formulation is beneficial in terms of its convenience and patient compliance.

The human dose of the drugs was 2 mg for rosiglitazone and glimepiride, 5 mg for glipizide and glibenclamide, and 15-30 mg for pioglitazone. These drugs were found to be more than 98-99% bound to plasma protein with a half life of about 2-7 h after single oral dose except glibenclamide which was extensively bound to serum proteins and had a half life of 10

*Corresponding author. E-mail: rajeshtirumala@hotmail.com

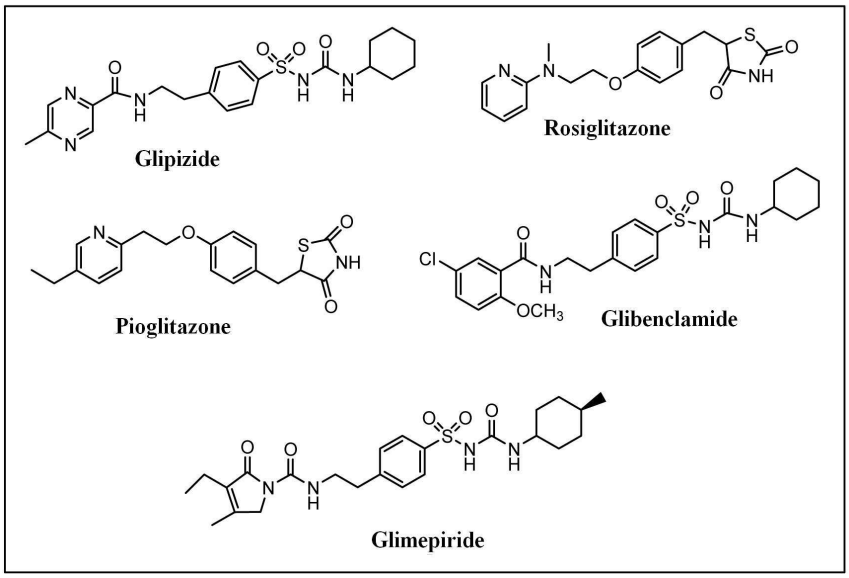

Fig. 1. Structures of anti-diabetic drugs.

h. They undergo hepatic biotransformation; the major metabolites of glipizide are products of aromatic hydroxylation and have no hypoglycemic activity. Rosiglitazone is extensively metabolized in the liver to inactive metabolites *via* N-demethylation, hydroxylation, and conjugation with sulfate and glucuronic acid, whereas pioglitazone is extensively metabolized by hydroxylation and oxidation. Glibenclamide has a major metabolite 4-trans-hydroxy derivative. A second metabolite, the 3-cis-hydroxy derivative, also occurs. But these contribute no significant hypoglycemic action in humans as they are only weakly active. Glimepiride is completely metabolized by oxidative biotransformation to a major metabolite, cyclohexyl hydroxymethyl derivative which is further metabolized to the carboxyl derivative.

To our knowledge, based on the literature survey, LC-MS/MS method for 10 anti-diabetic drugs [6], HPLC gradient method in human plasma [7], isocratic method for pharmaceutical formulations [8] for 6 anti-diabetic drugs and an HPLC method in human plasma for metformin with three sulfonylurea drugs, *i.e.* glibenclamide, glipizide and gliclazide [9] are reported along with few others: LC-MS, HPLC, MEKC and UV methods are confined to single [10-19] or two drugs in combination [20-23].

The present paper describes a simple simultaneous method for the determination of glipizide, rosiglitazone, pioglitazone, glibenclamide, glimepiride by reverse phase HPLC in isocratic mode in pharmaceutical dosage forms, and in human plasma, which could be applied especially to the determination of drugs in clinical data monitoring, and in pharmacokinetic investigations, since the combination of thiazolidinediones and sulfonyl ureas was found to be successful in the treatment of type II Diabetes. The method would help the assay of drugs in a single run which reduces the time of analysis and does not require separate methods for each drug. The method was also validated for the parameters as per ICH guidelines [24].

EXPERIMENTAL

Chemicals and Reagents

Pioglitazone (PGL) and glipizide (GPZ) were obtained from Macleoids Pharmaceutical Ltd., Mumbai, India. Glibenclamide (GBM) was obtained from Medley Pharmaceuticals, Diu and Daman, India. Rosiglitazone (RGL) and glimepiride (GMP) were obtained from Orchid Chemicals and Pharmaceuticals, Chennai, India. Acetonitrile (Rankem, New Delhi, India) and Methanol (Qualigens, Mumbai, India) HPLC grade were used. All the other reagents used were of

analytical grade. MilliQ water (Millipore Q Gard) was used through out the analysis and pharmaceutical formulations were purchased from the local pharmacy.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Vis detector (Shimadzu, Kyoto, Japan) with manual mode of injection. The data acquisition was made by Spincotech® software 1.7 version (Spinco Biotech Ltd., Chennai, India). The analysis was carried out at 248 nm with a Gemini C_{18} reversed phase column (Phenomenex, Torrance, USA) 150 × 4.6 mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted of 0.05% Triethylamine (pH-3.5, adjusted with ortho phosphoric acid), acetonitrile and methanol in the ratio of 55:15:30 v/v and was set at a flow rate of 1 ml min^{-1}.

Preparation of Standard Stock and Sample Solutions

Stock solution of GPZ, RGL, PGL, GBM and GMP was prepared by dissolving appropriate amounts of compounds in methanol to get a final concentration of 1000 µg ml^{-1}. The linearity range was tested in the range of 0.1, 1, 5, 10, 20, 50, 100 µg ml^{-1} using methanol.

For the analysis of pharmaceutical formulations, ten tablets of each drug were weighed and powdered individually. The mixture of formulations was prepared by weighing amount equivalent to labeled claim from the powdered formulations. To this, a suitable amount of methanol was added. The mixture was subjected to sonication for 30 min for a complete extraction of the drugs, and then filtered and diluted with mobile phase at a suitable concentration range and injected into HPLC system for the analysis.

Extraction of Anti-Diabetic Drugs from Human Plasma

The analytes at a concentration of 10 µg ml^{-1} were spiked into 1 ml of human plasma and to this was added 1 ml of acetonitrile [4] and vortex mixed. The supernatant was then collected and the organic layer evaporated. The residue left was reconstituted using mobile phase and 20 µl of this was injected into HPLC system for analysis.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The drugs were soluble in organic solvents like methanol and acetonitrile. During the development phase, the mobile phase containing methanol-water and methanol-buffer solutions, resulted in peaks with poor resolution and the acetonitrile-water resulted in asymmetric peaks with a greater tailing factor (>2) and high run time. The successful use of both acetonitrile and methanol, along with 0.05% TEA in water, pH adjusted to 3.5 (based on the pKa values of the drugs 6-7) reduced tailing and resulted in good peak symmetry and resolution. The optimized mobile phase contained 0.05% TEA in water, acetonitrile, and methanol in the ratio of 55:15:30 at a flow rate of 1 ml min^{-1}. The analytes were monitored at 248 nm and the retention times were found to be 4.51, 9.56, 11.3, 14.76 and 17.78 min for GPZ, RGL, PGL, GBM and GMP, respectively (Fig. 2a).

Validation of the Developed Method

The proposed method was validated as per the guidelines in ICH for its linearity, accuracy, precision, specificity and selectivity, robustness and stability *etc*.

Linearity. The linearity was tested for the concentration range of 0.1, 1, 5, 10, 20, 50, 100 µg ml^{-1} and the calibration curve was constructed and evaluated by its correlation coefficient. The correlation coefficient (r^2) for all the calibration curves was consistently greater than 0.9990 ± 0.0004.

Accuracy and Precision. The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percent difference (%bias) between the measured mean concentrations and the corresponding nominal concentrations. The accuracy of the proposed method was tested by recovery experiments by adding known amounts of each anti-diabetic drug corresponding to 80, 100 and 120% of the label claim from the respective standard solution. The accuracy was then calculated as the percentage of each anti-diabetic drug recovered by the assay (Table 1). The precision of the proposed method was assayed by replicate injections of anti-diabetic drugs mixture

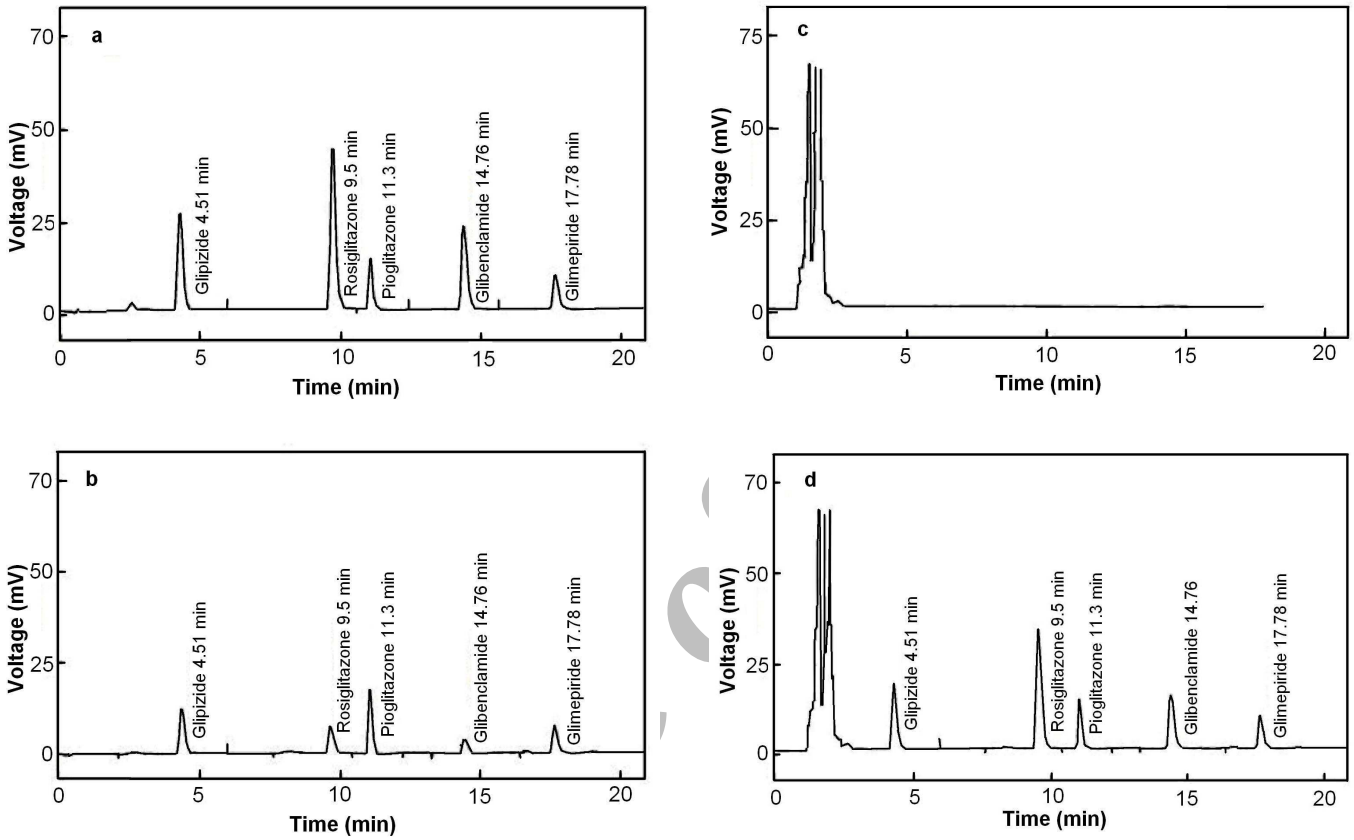

Fig. 2. Chromatograms showing the retention times of Glipizide, Rosiglitazone, Pioglitazone, Glibenclamide and Glimepiride at a concentration of 20 µg ml^{-1} in Pure (a), formulation mixture (b) blank plasma (c) and Human Plasma (d).

of three different concentrations (5, 10 and 15 µg ml^{-1}), three times on three different days. The obtained intra-day and inter-day precision results are depicted in Table 2. The results indicated sufficient accuracy and precision of the developed HPLC method.

Specificity and selectivity. From Fig. 2, it can be seen that the method was sufficiently specific to the analytes. The resolution factor for the drug peaks was found to be more than 1.5 from the nearest resolving peak for all the peaks and no interferences were found in the retention of drugs extracted from plasma (Figs. 2c and 2d).

Robustness. The robustness of the proposed method was found after altering the parameters deliberately: the mobile phase ratio variants: acetonitrile 13% and 17%, flow rate variants: 0.9 and 1.1 ml min^{-1}, the pH of buffer variants: 3.40 ± 0.05 and 3.60 ± 0.05, concentration of TEA variants: 0.04% and 0.06%. The retention time of the compound was evaluated, and the resolution had no significant changes when the parameters were changed. However, there was a change in the retention times with a change in flow rate, but this did not affect the peak symmetry. Each mean value was compared with the mean value obtained by the optimum conditions. A solution of 50 µg ml^{-1} of all the drugs extracted from the plasma was used for the study. The relative standard deviation (%RSD) was found to be less than 2 (Table 3).

Stability. The stability of the drugs extracted from the

Table 1. Accuracy Data Obtained by Recovery Studies of 80%, 100% and 120% of the Drugs

Compound	Labeled (mg)	Amount added			Recovery (%)
		80%	100%	120%	
Glipizide	5	4	5	6	98.76
Rosiglitazone	2	1.6	2	2.4	100.09
Pioglitazone	15	12	15	18	99.07
Glibenclamide	5	4	5	6	98.34
Glimepiride	2	1.6	2	2.4	99.12

n = 3.

Table. 2. Precision Data (n = 3)

Compound	Intra-day (SD, %RSD)			Inter-day (SD, %RSD)		
	5	10	15	5	10	15
Glipizide	0.063, 1.26	0.12, 1.24	0.21, 1.36	0.10, 2.06	0.24, 2.37	0.46, 3.11
Rosiglitazone	0.082, 1.71	0.11, 1.19	0.37, 2.79	0.08, 1.67	0.31, 3.14	0.57, 3.85
Pioglitazone	0.090, 1.88	0.08, 0.84	0.31, 2.07	0.13, 2.64	0.42, 4.24	0.64, 4.34
Glibenclamide	0.034, 0.74	0.12, 1.21	0.41, 2.75	0.11, 2.32	0.37, 3.81	0.39, 2.65
Glimepiride	0.101, 2.32	0.36, 3.65	0.76, 1.09	0.08, 1.72	0.41, 4.17	0.38, 2.59

SD and %RSD of concentrations 5, 10, 15 $\mu g\,ml^{-1}$ prepared from the synthetic mixture of formulations of GPZ, RGL, PGL, GBM and GMP.

Table 3. Robustness Data of 50 $\mu g\ ml^{-1}$ Solution of Drugs Mixture Extracted from Plasma (n = 3)

Condition	GPZ	RGL	PGL	GBM	GMP
ACN-13% + MET-32%	1.42	1.62	0.92	1.21	1.07
ACN-17% + MET-28%	1.86	1.79	1.76	1.32	1.31
pH 3.4	1.75	1.82	1.34	1.71	1.81
pH 3.6	1.62	0.93	1.97	1.23	1.09
Conc. of TEA-0.04%	1.36	1.12	0.89	1.68	1.48
Conc. of TEA-0.06%	1.92	1.28	1.91	1.42	1.54
Flow rate 0.9 ml min^{-1}	1.54	0.87	1.21	1.48	1.35
Flow rate 1.1 ml min^{-1}	0.99	1.36	1.19	1.39	1.82

%RSD values.

Table 4. Formulation Assay Results Prepared from the Synthetic Mixtures (n = 3)

Formulation	Labelled amount (mg)	Amount found (mg)	RSD (%)	Assay (%w/w)
Glez (GPZ)	5	4.87	2.09	97.4
Rosicon (RGL)	2	1.98	1.79	99.0
G-Tase G (PGL)	15	14.75	2.76	98.3
(GMP)	2	1.92	1.19	96.0
Glinil (GBM)	5	4.83	2.31	96.0

plasma was subjected to short-term stability by keeping them at room temperature for 24 h, and to long-term stability at -4 °C for 30 days. The study indicated that the samples were stable where the percent ratios were within the acceptable limits of 90-110%.

Assay of Pharmaceutical Formulations

The method developed was sensitive and specific for the quantitative determination of GPZ, RGL, PGL, GBM and GMP and also was subjected to validation for different parameters; hence, it was applied for the estimation of drugs in pharmaceutical formulations. Drug quantity equivalent to the labeled claim was weighed accurately and used for the assay. Each sample was analyzed in triplicate after extracting the drug as was mentioned above in the experimental section (2.3). The amounts of drugs were found to be within the range of 96-102%. None of the tablet excipients were found to interfere with the analyte peak as shown in Fig. 2b. Tthe results are shown in Table 4.

Recovery of Anti-Diabetic Drugs from Human Plasma

The extraction results were obtained from peak area by the comparison of neat standards *vs*. plasma-extracted standards at 10 µg ml^{-1} concentration for all the analytes. The extraction recovery was found to be independent of concentration. The absolute recovery of anti-diabetic drugs from human plasma (with acetonitrile) was found to be more than 90% (Fig. 2c).

CONCLUSIONS

A simple, specific, selective and precise method was developed for the determination of anti-diabetic drugs GPZ, RGL, PGL, GBM and GMP. The mobile phase was easy to prepare with little or no variation and was economical. The analysis time was found to be less than 20 min. The recovery from formulations and human plasma were in good agreement and they suggested no interference in the estimation. Hence, this method can be easily and conveniently used for the routine quality control of the drugs in pharmaceutical dosage forms and can also be applied to clinical studies.

REFERENCES

[1] C.R. Craig, R.E. Stitzel, Modern Pharmacology with Clinical Applications 6th ed., Lippincott Williams & Wilkins, 2004, p. 767.

[2] P. Martha, M. Arnold, J. Meeker, D. Greene, J. Clin. Pharmacol. 40 (2000) 1494.

[3] J.M. Lehmann, L.B. Moore, T.A. Smith-Oliver, W.O. Wilkison, T.M. Willson, S.A. Kliewer, J. Biol. Chem. 270 (1995) 12953.

[4] T.M. Willson, J.E. Cobb, D.J. Cowan, R.W. Wiethe, I.D. Correa, S.R. Prakash, K.D. Beck, L.B. Moore, S.A. Kliewer, J.M. Lehmann, J. Med. Chem. 39 (1996) 665.

[5] P.W. Young, D.R. Buckle, B.C.C. Cantello, H. Chapman, J.C. Clapham, P.J. Coyle, D. Haigh, R.M. Hindley, J.C. Holder, H. Kallender, A.J. Latter, K.W.M. Lawrie, D. Mossakowska, G.J. Murphy, L. Roxbee Cox, S.A. Smith, J. Pharmacol. Exp. Ther. 284 (1998) 751.

[6] E.N. Ho, K.C. Yiu, T.S. Wan, B.D. Stewart, K.L. Watkins, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 811 (2004) 65.

[7] P. Venkatesh, T. Harisudhan, H. Choudhury, R. Mullangi, N.R. Srinivas, Biomed. Chromatogr. 20 (2006) 1043.
[8] J. Yao, Y.-Q. Shi, Z.-R. Li, S.-H. Jin, J. Chromatogr. B 853 (2007) 254.
[9] S. Aburuz, J. Milership, J. McElnay, J. Chromatogr. B 817 (2005) 277.
[10] B.L. Kolte, B.B. Raut, A.A. Deo, M.A. Bagool, D.B. Shinde, J. Liq. Chromatogr. Rel. Tech. 26 (2003) 1117.
[11] C.-C. Chou, M.-R. Lee, F.-C. Cheng, D.-Y. Yang, J. Chromatogr. A 1097 (2005) 74.
[12] J. He, Y.F. Hu, L.F. Duan, Z.R. Tan, L.S. Wang, D. Wang, W. Zhang, Z. Li, J. Liu, J.H. Tu, Y.M. Yao, H.-H. Zhou, J. Pharm. Biomed. Anal. 43 (2007) 580.
[13] Z.J. Lin, W. Ji, D. Desai-Krieger, L. Shuma, J. Pharm. Biomed. Anal. 33 (2003) 101.
[14] K. Yamashita, H. Murakami, T. Okuda, M. Motohashi, J. Chromatogr. B 677 (1996) 141.
[15] T. Radhakrishna, D. Sreenivas Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 29 (2002) 593.
[16] D.B. Wanjari, N.J. Gaikwad, Ind. J. Pharm. Sci. 67 (2005) 256.
[17] I. Niopas, A.C. Daftsios, J. Pharm. Biomed. Anal. 28 (2002) 653.
[18] C. Gedeon, B. Kapur, K. Aleksa, G. Koren, Clini. Biochem. 41 (2008) 167.
[19] S. Altinoz, D. Tekeli, J. Pharm. Biomed. Anal. 24 (2001) 507.
[20] A. Karthik, G. Subramanian, C.M. Rao, K. Bhat, A. Ranjithkumar, P. Musmade, M. Surulivelrajan, K. Karthikeyan, N. Udupa, Pak. J. Pharm. Sci. 21 (2008) 421.
[21] K.S. Lakshmi, T. Rajesh, S. Sharma, Int. J. Pharmtech. Res. 1 (2009) 496.
[22] R.T. Sane, S.N. Menon, S. Inamdar, M. Mote, G. Gundi, Chromatographia 59 (2004) 451.
[23] C. Yardımcı, N. Őzaltın, Anal. Chim. Acta 549 (2005) 88.
[24] ICH Harmonised Tripartite Guideline, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure: Methodology, 1996.