

METHOD VALIDATION FOR SPECTROPHOTOMETRIC DETERMINATION OF LISINOPRIL IN PHARMACEUTICALS USING COPPER SULPHATE

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Abstract

This paper presents the statistical validation for the spectrophotometric method of lisinopril determination in bulk and pharmaceutical formulations. The method presented herein underpinned by the reaction between lisinopril and copper sulphate. The maximal absorption for the blue complexation reaction product occurs at a 740 nm wavelength. Accordingly, the calibration curve is described by a second order polynomial regression equation, within the range of 0.1 to 0.7 mg/ml: $A = -0.3171C^2 + 0.9653C + 0.0028$, where A represents the measured absorbance at $\lambda = 740$ nm using cells of 5 cm thickness, and C stands for the concentration expressed in mg/ml, hence rendering $R^2 = 0.9999$ as correlation coefficient. The limits of detection (LOD) and quantification (LOQ), calculated according to ICH guidelines, are 0.026 and 0.079 mg/ml, respectively. Intra-day and inter-day accuracy expressed as relative error are better than 2.5%, while the precision quantified by the relative standard deviation is less than 3.2%. The method was successfully applied for the analysis of two tablet brands containing lisinopril: Lisigamma and Lisinopril Antibiotice Iași 10 mg.

Keywords: lisinopril, copper sulphate, spectrophotometry, pharmaceutical formulation.

VALIDAREA METODEI SPECTROFOTOMETRICE DE DETERMINARE A LISINOPRILULUI DIN FORMELE FARMACEUTICE UTILIZÂND SULFAT DE CUPRU

Rezumat

Lucrarea prezintă validarea statistică a metodei spectrofotometrice de determinare a lisinoprilului substanță pură și din formă farmaceutică, având la bază reacția acestuia cu sulfatul de cupru. Produsul obținut în urma reacției de complexare, colorat albastru, prezintă maxim de absorbție la lungimea de undă 740 nm. În intervalul de concentrații 0.1-0.7 mg/ml curba de calibrare prezintă, pentru $\lambda = 740$ nm și grosimea cuvei 5 cm, expresia unei ecuații polinomiale de gradul doi: $A = -0.3171C^2 + 0.9653C + 0.0028$, astfel rezultând coeficientul de corelare $R^2 = 0.9999$. Limita de detecție (LOD) și de cuantificare (LOQ) calculate în conformitate cu prevederile ICH sunt 0.026, respectiv 0.079 mg/ml. Acuratețea metodei (în aceeași zi și în zile diferite) exprimată prin valoarea erorii relative, a fost mai mare de 2.5%, iar precizia cuantificată prin valoarea deviației standard relative a fost mai mică de 3.2%. Metoda a fost aplicată cu succes pe comprimatele de Lisigamma și Lisinopril Antibiotice Iași, conținând 10 mg lisinopril.

Cuvinte cheie: lisinopril, sulfat de cupru, spectrofotometrie, formă farmaceutică.

INTRODUCTION

Lisinopril dihydrate (LIS, Figure 1), (2*S*)-1-[(2*S*)-6-amino-2[[[(2*S*)-1-hydroxy-1-oxo-4-phenylbutan-2-yl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate is an angiotensin converting enzyme (ACE) inhibitor, the lysine analog of enalaprilat, which is the active metabolite of enalapril. It is used for the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post-myocardial infarction [1,2]. The official methods for the determination of LIS in pure and tablet forms are potentiometric acid-base titration [3] and HPLC using octylsilyl silica gel column at 50 °C, along with phosphate solution-acetonitrile (96:4, v/v) as mobile phase [4].

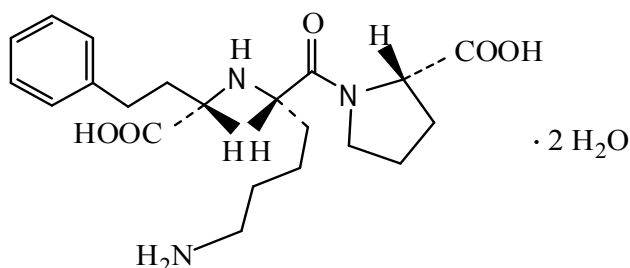


Figure 1. Lisinopril dihydrate: chemical structure.

Several analytical methods have been described for the determination of lisinopril in biological fluids or pharmaceutical formulation, whether alone or in combination with hydrochlorothiazide: HPLC [5-10], gas chromatography with mass detection [11, 12], LC/MS [13-15], densitometric HPTLC [16], capillary electrophoresis [17, 18], spectrofluorimetry [6, 19, 20], polarography [21-23]. Most of the above mentioned techniques are sensitive but cumbersome and expensive. Within this paper, the spectrophotometric methods represent the technique of choice due to their inherent simplicity and economical advantages; nevertheless, they are already used for the assay of a wide variety of pharmaceuticals, in bulk and dosage form. Moreover, several literature references are reporting spectrophotometric methods as valid tools for the assay of LIS in pharmaceutical formulations, which are based either on the reaction with different reagents [6,24-30] or on derivative UV-spectrophotometry [31-33]. Most of these methods involve organic solvents as reaction medium (often undesirable because of their toxicity), require longer heating time, and use expensive reagents.

This paper tries to deal with the above stated drawbacks, by presenting a simple, fast, economical and environmentally-friendly spectrophotometric method, for the assay of lisinopril in pure and pharmaceutical formulations. The method is based on the reaction between lisinopril and copper sulphate, in water medium, at room

temperature. No organic solvent and no heating time were required.

MATERIALS AND METHODS

Apparatus

All spectrophotometric measurements were performed using a SPECTRONIC UNICAM – UV 300 UV-VISIBLE SPECTROMETER, with 5 cm matched glass cells.

Materials and reagents

All chemicals used within this paper's experiments are of analytical reagent grade.

10 mg/ml Copper sulphate 5-hydrate. 2000 mg of chemical (Reactivul București) was dissolved in distilled water and made up to 200 ml with the solvent.

Standard drug solution. Lisinopril dihydrate was kindly provided by Medochemie, Limassol, Cyprus and it was used as received. A standard stock solution of 5 mg/ml LIS was prepared by dissolving 500 mg pure drug in distilled water and then diluting it to 100 ml in a calibrated flask with water. The standard LIS solution (2.5 mg/ml) was prepared from the stock solution, by appropriate water dilution.

Proposed procedure

Aliquots of 2.5 mg/ml LIS solution (0.1–0.7 mg/ml) were accurately measured and transferred into a series of 25 ml standard volumetric flasks. 10 ml of 10 mg/ml copper sulphate 5-hydrate were added to each flask. The volume was made up to the mark with distilled water. The absorbance was measured using the 5 cm cells at 740 nm, against reagent blank (i.e. similarly prepared, but omitting the drug). The calibration graph was generated by plotting the measured absorbance values, according to concentration variation.

Pharmaceutical formulation

Twenty tablets were accurately weighed and powdered. A quantity of powder, containing

100 mg of lisinopril, was transferred into a 50 ml volumetric flask with 30 ml water. The mixture was shaken for 15 min, diluted to volume with water, and then filtered. The filtrate was subjected to analysis, using the above described procedure.

RESULTS AND DISCUSSION

As presented in [34], the reaction between LIS and copper sulphate is a reversible reaction. For shifting this equilibrium towards the final product, an excess of copper ions is required. For the given reaction conditions, the rank of the matrix of the absorbance values proves that only one final product is to be obtained [34]. The complexation product has a maximum absorbance at 740 nm (see Figure 2).

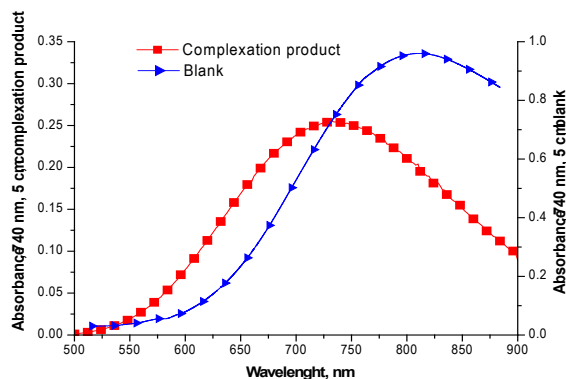


Figure 2. Absorption spectra for the complexation products and blank.

The effect of the CuSO_4 5-hydrate concentration

In order to study the effect of CuSO_4 5-hydrate concentration on the reaction product color, varying volumes of 10 mg/ml CuSO_4 5-hydrate (2.5–15 ml) were reacted with 6 ml of 2.5 mg/ml LIS into a 25 ml volumetric flask. The absorbance has been measured against reagent blank. 10 ml of 10 mg/ml CuSO_4 5-hydrate has been found as the acceptable tradeoff value (Figure 3).

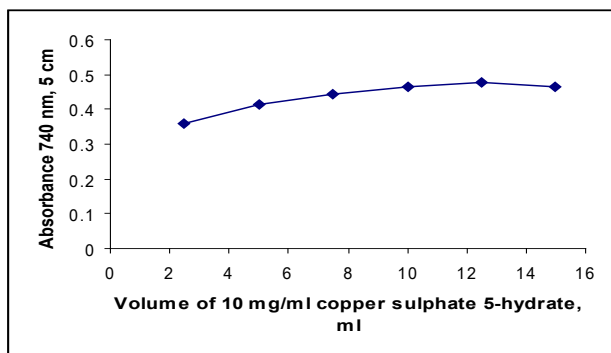


Figure 3. The effect of CuSO_4 5-hydrate concentration.

Method validation

Linearity

The ICH guidelines mention that, for some analytical procedures which do not demonstrate linearity, the analytical response should be described by an appropriate function of concentration for an analyte within a sample [35].

The calibration graph is described by the second order polynomial regression equation:

$A = a + b_1C + b_2C^2$ where A is the absorbance and C is the concentration in mg/ml (Figure 4). Optical characteristics and statistical data, for the regression equation of the proposed method are presented in Table 1.

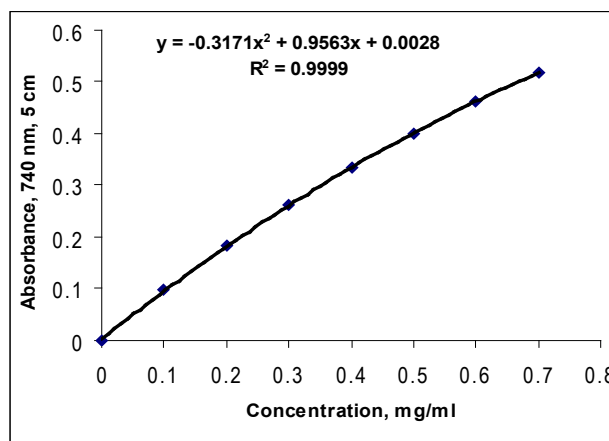


Figure 4. Calibration curve.

Limit of detection and quantification

The ICH guidelines were followed in order to determine the LOD and LOQ. Accordingly, the method based on the standard deviation has been applied, so that three and ten times the standard deviation values for the blank and regression equation were used to calculate the LOD and LOQ. The computed values were found to be 0.026 and 0.079 mg/ml, respectively.

Table I. Regression parameters of the proposed method.

Parameter	Value
$\lambda_{\text{max}} / \text{nm}$	740
Range, mg/ml	0.1 – 0.7
Coefficient 1 (b_1)	0.9563
Coefficient 2 (b_2)	-0.3171
Intercept (a)	0.0028
Limit of detection (LOD) mg/ml	0.026
Limit of quantification (LOQ) mg/ml	0.079
Standard deviation of coefficient b_1 (S_{b_1})	± 0.0280
Standard deviation of coefficient b_2 (S_{b_2})	± 0.0348
Standard deviation of intercept (S_a)	± 0.0015
Correlation coefficient (r)	0.99995

Selectivity test

The proposed method was tested for selectivity by artificial mixture analysis. An artificial mixture containing lisinopril (20 mg) as lisinopril dihydrate (21.78 mg), starch (50 mg), magnesium stearate (20 mg) and calcium diphosphate (10 mg) was prepared. The extract was obtained according to the procedure described for tablets and analyzed using the earlier described procedure. The replicate analysis ($n=5$) of the 0.3 mg/ml LIS concentration level yielded the % recovery of LIS at 99.74 ± 1.32 , therefore revealing that the inactive ingredients did not interfere with LIS determination.

It can be concluded that our selectivity test has confirmed that the measured absorbance was produced only by the analyte. The study also pointed out that the inactive ingredients, such as yellow iron oxide, red iron oxide and talc do interfere with the active substance, and hence the method cannot be applied for lisinopril quantification in the presence of the above mentioned ingredients.

Precision and accuracy

The precision and accuracy of the method have been evaluated by replicate analysis ($n = 5$) of calibration standard, at three different concentration levels, during the same day, and then during five consecutive days. The RSD (%) values of intra-day and inter-day studies have revealed that good precision was achieved (Table 2). Accuracy has been calculated as relative error (%) between the found concentration and its theoretical counterpart (see Table 2).

Application to pharmaceutical formulation

The proposed method was used for the quantification of LIS in two tablet commercial formulations. The results were compared with those obtained with the reference method [35], by using Student's t-test for accuracy and F-test for precision. The results (presented in Table 3) have failed to reveal any significant difference between the proposed method and the reference method. The values obtained with student's t-test and F-test at a 95 % confidence level are smaller than the theoretical ones, thereby confirming a good agreement with the reference method.

Recovery study

The validity of the proposed method was further demonstrated using recovery studies. Pre-analyzed tablet powder was spiked with pure LIS at three concentration levels (50, 100, and 150 % of that from the tablet powder) and the total has been found by applying the proposed method. The percentage values of LIS added recovery ranged between 97.63 and 101.22, with a standard deviation of 0.92 – 2.57 %; all these results reveal a good degree of recovery (see Table 4).

CONCLUSIONS

The novel spectrophotometric method presented in this paper, which was developed and validated for the quantification of LIS in pharmaceutical formulations is a simple, fast and inexpensive method that involves only one reagent. It has the all the advantages entailed by the fact that requires only simple operations, including the possibility of carrying them out with the common laboratory instruments. Moreover, this method is also environment-friendly since it excludes the use of organic solvents. Further research will focus on a through analysis, regarding the advantages and drawbacks of this method in comparison with the state-of-the-art.

Table II. Evaluation of intra-day and inter-day accuracy and precision.

LIS taken, mg/ml	Intra-day accuracy and precision			Inter-day accuracy and precision		
	LIS found, mg/ml	RE, %	RSD, %	LIS found, mg/ml	RE, %	RSD, %
0.2	0.1988	-0.59	1.29	0.1959	-2.07	3.21
0.4	0.3956	-1.11	0.86	0.3903	-2.42	2.03
0.6	0.5972	-0.47	1.60	0.5870	-2.17	2.31

RE. Relative error; RSD. Relative standard deviation.

Table III. Determination of lisinopril formulation by the proposed and reference method.

Tablet brand name	Label claim, mg/tablet	Found ^a (label claim \pm SD), %	
		Reference method	Proposed method
Lisigamma ^b	10	102.35 \pm 0.63	103.29 \pm 0.72 t = 1.63 F = 1.32
Lisinopril Antibiotice ^c	10	105.68 \pm 0.74	105.30 \pm 0.80 t = 2.36 F = 1.17

^a Mean value of five determinations; ^b Worwag Pharma GmbH & Co, Germany; ^c Antibiotice Iași, Romania;

The tabulated F value at 95% confidence level for four degrees of freedom is 6.39;

The tabulated t value at 95% confidence level for four degrees of freedom is 2.77.

Table IV. Results of recovery study by standard-addition method.

Tablet studied	LIS in tablet, mg/ml	Pure LIS added, mg/ml	Total found, mg/ml	Pure LIS recovered ^a \pm SD, %
Lisigamma	0.207	0.1	0.307	100.87 \pm 2.30
	0.207	0.2	0.404	98.91 \pm 2.57
	0.207	0.3	0.510	101.22 \pm 2.44
Lisinopril Antibiotice	0.211	0.1	0.308	97.63 \pm 1.38
	0.211	0.2	0.412	100.70 \pm 2.05
	0.211	0.3	0.512	100.55 \pm 0.92

^a Mean value of three measurements.

References

1. Parfitt, K. In "Martindale: the Complete Drug Reference", 1999, 32nd eds., p.898, The Pharmaceutical Press, London, United Kingdom
2. Ferrar, R., Guardigli, G., Mele, D., Valgimigli, M., and Ceconi, C., Myocardial ischemia: new evidence for angiotensin-converting enzyme inhibition, *European Heart Journal Supplements*, 2003, 5 (Supplement E), E11 – E17
3. xxx - European Pharmacopoeia, 5th Ed., Council of Europe, Strasbourg, 2004, p.1922-1923
4. The United States Pharmacopoeia, 24th revision, Asian Edition, United States Pharmacopoeial Convention, INC. Twinbrook Parkway, Rockville, MD, USA, 2000, p. 979-980, 2149-2152
5. Wong, Y. C., Charles, B.G., Determination of angiotensin converting enzyme inhibitor lisinopril in urine using solid phase extraction and reverse phase high performance liquid chromatography, *J. Chromatogr. B Biomed. Appl.*, 1995, 673, 306-310
6. El Gindy, A., Ashour, A., Abdel-Fattah, L., Shabana, M.M., Spectrophotometric, Spectrofluorimetric and LC determination of lisinopril, *J. Pharm. Biomed. Anal.*, 2001, 25, 913-922
7. Beasley, C. A., Shaw, J., Zhao, Z., Reed, R. A., Development and validation of a stability indicating HPLC method for determination of lisinopril, lisinopril degradation product and parabens in the lisinopril extemporaneous formulation, *J. Pharm. Biomed. Anal.*, 2005, 37, 559-567
8. Nevin, E., Murat, K., Comparison of high-performance liquid chromatography and absorbance ratio methods for the determination of hydrochlorothiazide and lisinopril in pharmaceutical formulations, *Anal. Lett.*, 1999, 32, 1131-1141
9. Ivanoic, D., Medenica, M., Jancic, B., Knezevic, N., Validation of an analytical procedure for simultaneous determination of hydrochlorothiazide, lisinopril and their impurities, *Acta Chromatogr.*, 2007, 18, 143-156
10. Rudzki, P. J., Bus, K., Ksycinska, H., Kobylinska, K., An overview of chromatographic methods coupled with mass spectrometric detection for determination of angiotensin-converting enzyme inhibitors in biological material, *J. Pharm. Biomed. Anal.*, 2007, 44, 356-367
11. Leis, H. J., Fauler, G., Raspotnig, G. and Windischhofer, W., Quantitative determination of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry, *Rapid Commun. Mass Spectrom.*, 1998, 12, 1591-1594
12. Leis, H. J., Fauler, G., Raspotnig, G. and Windischhofer, W., An improved method for the measurement of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry, *Rapid Commun. Mass Spectrom.*, 1999, 13, 650-653
13. Jinchang Huang, Yu Xu, Fei Liu, Shu Gao and Qingxiang Guo, Development of a liquid chromatography/tandem mass spectrometry assay for quantification of lisinopril in human plasma, *Rapid Commun. Mass Spectrom.*, 2006, 20, 248-252
14. Weiwei Qin, Zunjian Zhang, Yuan Tian, Fengguo Xu, Na Wang and Yun Chen, Rapid quantification of lisinopril in human plasma by liquid chromatography/tandem mass spectrometry, *Biomed. Chromatogr.*, 2007, 21, 415-421
15. Padua, A., Barrientos-Astigarraga, R. E., Rezende, V. M., Mendes G. D., De Nucci, G., Lisinopril quantification in human plasma by liquid chromatography-electrospray tandem mass spectrometry, *J. Chromatogr. B*, 2004, 809, 211-216
16. El Gindy, A., Ashour, A., Abdel-Fattah, L., Shabana, M.M., Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorothiazide in binary mixture, *J. Pharm. Biomed. Anal.*, 2001, 25, 923-931
17. Hillaret, S., Vanden Bopscha, W., Optimization capillary electrophoretic separation of several inhibitors of the angiotensin-converting enzyme, *J. Chromatogr. A*, 2000, 895, 33-42
18. Gotti, R., Andrisano, V., Cavrini, V., analysis of ACE inhibitors by CE using alkyl sulphonic additives, *J. Pharm. Biomed. Anal.*, 2000, 22, 423-431
19. Esra, S.A., Lale, E., Olcay, S., A new spectrofluorimetric method for the determination of lisinopril in tablets, *Il Farmaco*, 2003, 58, 165-168
20. Zacharis, C., Tzanavaras, P., Themelis, D., Theodoridis, G., Economou, A., Rigas, P., Rapid spectrofluorimetric determination of lisinopril in pharmaceutical tablets using sequential injection analysis, *Anal. Bioanal. Chem.*, 2004, 379, 759-763
21. El-Enany, N., Belal, F., Al-Ghannam, S., Polarographic determination of lisinopril in pharmaceuticals and biological fluids through treatment with nitrous acid, *Microchim. Acta*, 2003, 141, 55-61
22. Rajasekaran, A., Murugesan, S., Polarographic studies of lisinopril, *Asian J. Chem*, 2001, 13, 1245-1246
23. Abdel Razak, O., Belal, S. F., Bedair, M. M., Barakat, N. S., Haggag, R. S., Spectrophotometric and polarographic determination of enalapril and lisinopril using 2,4-dinitrofluorobenzene, *J. Pharm. Biomed. Anal.*, 2003, 31, 701-711
24. Paraskevas, G., Atta-Politou, J., Koupparis, M., Spectrophotometric determination of lisinopril in tablets using 1-fluoro-2,4-dinitrobenzene reagent, *J. Pharm. Biomed. Anal.*, 2002, 29, 865-872
25. El-Emam, A.A., Hansen, S.H., Moustafa, M.A., El-Ashry, S.M., El-Sherbiny, D.T., Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) followed by spectrophotometry or HPLC with fluorimetric detection, *J. Pharm. Biomed. Anal.*, 2004, 34, 35 – 44
26. Rahman, N., Singh, M., Hoda, Md., N., Optimized and validated spectrophotometric methods for the determination of lisinopril in pharmaceutical formulation using ninhydrin and acid ascorbic, *J. Braz. Chem. Soc.*, 2005, 16, 5, 1001-1009
27. Devi, P.A., Mallikarjuna, R.G.P.V., Krishna, P.K.M.N., Sastry, C.S.P., Four simple spectrophotometric determination of lisinopril in pure state and in tablets, *Indian J. Pharm. Sci.*, 2003, 63, 296-299
28. Rahman, N., Anwar, N., Kashif, M., Application of π -acceptors to the spectrophotometric determination of lisinopril in commercial dosage forms, *Il Farmaco*, 2005, 60, 605-611
29. Raza, A., Ansari, T.M., Rehman, A., Spectrophotometric determination of lisinopril in pure and pharmaceutical formulation, *J. Chin. Chem. Soc.*, 2005, 52, 1055-1059
30. Basavaiah, K., Tharpa, K., Hiriyanna, S.G., Vinay, K.B., Spectrophotometric determination of lisinopril in pharmaceuticals using ninhydrin – a modified approach, *Journal of Food and Drug Analysis*, 2009, 17, 93-99
31. Stanisz, B., Estimation of the applicability of differential spectroscopic method for the determination of lisinopril in tablets and for its evaluation of its stability, *Acta Pol. Pharm.*, 2005, 61, 327-334

32. Ozer, D., Senel, H., Determination of lisinopril from pharmaceutical preparations by derivative spectroscopy, J. Pharm. Biomed. Anal., 1999, 21, 691-695
33. Prasad, C.V.N., Saha, R.N., Parimoo, P., simultaneous determination of amlodipine-enalapril maleate and amlodipine-lisinopril in combined tablet preparations by derivative spectrophotometry, Pharm. Pharmacol. Commun., 1999, 5, 383-388
34. Sbârcea, L., Drăgan, L., Szabadai, Z., Udrescu, L., Bojiță, M.,

The determination of lisinopril based on its complexation reaction with Cu^{2+} using spectrophotometric methods, Farmacia, 2007, 2, 165-170

35. ***, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Complementary Guideline on methodology dated 6 November 1996 incorporated in November 2005, London