SPECTROPHOTOMETRIC METHOD FOR LISINOPRIL DETERMINATION USING NINHYDRIN

LAURA SBÂRCEA[1*], LUCREŢIA UDRESCU[2], LIANA DRĂGAN[1], CRISTINA TRANDAFIRESCU[1], ZOLTÁN SZABADAI[2], MARIUS BOJIŢĂ[3]

"Victor Babeş" University of Medicine and Pharmacy, Faculty of Pharmacy, 2 Eftimie Murgu Square, 300041, Timişoara, Romania
[1] Departament of Pharmaceutical Chemistry
[2] Departament of Drug Analysis
[3] "Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Departament of Drug Analysis, 6 Louis Pasteur St., 400015, Cluj-Napoca, Romania
[*]corresponding author: laura_sbarcea@yahoo.com

Abstract

This paper presents a sensitive, accurate and rapid method for quantitative determination of lisinopril dihidrate (LIS) in bulk and pharmaceutical formulations based on the reaction with ninhydrin in the presence of potassium hydroxide. The reaction quantitatively proceeds at a temperature of 95 ± 2ºC, in 10 min and the end product, purple coloured, exhibits maximum absorption at 567 nm. The Lambert-Beer law is obayed within the range of 10 – 30 μg/mL, while the calibration curve is described by the regression equation $A = 0.0312\ C$ (μg/mL) – 0.2262, having a correlation coefficient of $R^2 = 0.9981$ (n = 8). The influence of temperature, heating time and reactive concentrations on the end product was also analyzed, so as to render the optimal conditions for reaction. In order to validate the method, the linearity, the limit of detection (LOD), limit of quantification (LOQ), sensitivity, selectivity, precision, accuracy and recovery were determined according to ICH guidelines. The LOD and LOQ values were calculated to be 1.16 μg/mL and 3.52 μg/mL respectively. Intra-day and inter-day accuracy expressed as relative error was less than 1.12% with precision quantified as relative standard deviation ranging from 1.14 to 1.98%. The percent recovery ranged from 98.12 to 102.57%. No interferences from the common excipients were detected and therefore the method was considered specific. The proposed method was successfully applied for the quantitative determination of lisinopril from three pharmaceutical products, namely Lisigamma®, Lisinopril® Sandoz and Ranolip®.

Rezumat

Lucrarea prezintă o metodă spectrofotometrică sensibilă, precisă şi rapidă de determinare cantitativă a lisinoprilului dihidrat (LIS) în substanţa pură şi din forma farmaceutică pe baza reacţiei cu ninhidrina în prezenţa hidroxidului de potasiu. Reacţia decurge cantitativ la temperatura de 95 ± 2ºC în 10 min, iar produsul obţinut, colorat violet, prezintă maxim de absorbţie la 567 nm. Legea Lambert-Beer se respectă în intervalul de concentraţii 10 – 30 μg/mL, iar dreapta de calibrare este descrisă de ecuaţia de regresie $A = 0{,}0312C$ (μg/mL) – 0.2262, cu coeficientul de corelare $R^2 = 0{,}9981$ ($n = 8$). S-a studiat

influenţa temperaturii, a timpului de încălzire şi a concentraţiilor reactivilor asupra produsului final şi s-au stabilit condiţiile optime de reacţie. În vederea validării metodei, s-au evaluat conform ghidurilor ICH linearitatea metodei, limita de detecţie (LOD) şi limita de cuantificare (LOQ), sensibilitatea, selectivitatea, precizia, acurateţea şi procentul de regăsire ale metodei. Valorile calculate corespunzătoare pentru LOD şi LOQ sunt 1,16 µg/mL şi respectiv 3,52 µg/mL. Acurateţea metodei, exprimată ca valoare a erorii relative, a fost mai mică de 1,12% cu o precizie exprimată ca valoare a deviaţiei standard relative variind între 1,14 şi 1,98%. Procentul regăsirii prin metoda propusă s-a situat între 98,12 şi 102,57%. Nu s-au raportat interferenţe cu excipienţii utilizaţi în mod frecvent, fapt ce conduce la concluzia că metoda este specifică. Metoda propusă a fost aplicată cu succes la determinarea cantitativă a lisinoprilului din trei specialităţi farmaceutice: Lisigamma®, Lisinopril® Sandoz şi Ranolip® comprimate.

Keywords: lisinopril, ninhydrin, spectrophotometry, pharmaceutical formulation

Introduction

Lisinopril dihydrate (Figure 1), *(2S)-1-[(2S)-6-amino-2[[(2S)-1-hydroxy-1-oxo-4-phenylbutan-2-yl]hexanoyl]pyrrolidine-2-carboxilic acid dihydrate* (LIS) is an angiotensin converting enzyme (ACE) inhibitor, widely used for the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post myocardial infarction [5,14]. The official methods for LIS determination in both bulk and tablets are potentiometric acid-base titration [44], and HPLC using octylsilyl silica gel column at 50ºC with phosphate solution-acetonitrile (96:4, v/v) as mobile phase [46].

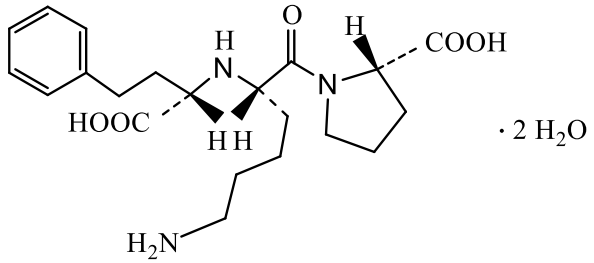


Figure 1
Lisinopril dihydrate chemical structure

Analytical methods such as HPLC [4,9,18,24,35,40], LC/MS [17,26,29,35], gas chromatography with mass detection [20,21], densitometric HPTLC [10], polarography [1,8,33], capillary electrophoresis [15,16], spectrofluorimetry [9,11,13,43], radioimmunoassay [41], fluoroimmunoassay [42] have been developed for the determination of lisinopril in biological fluids and pharmaceutical formulations, either alone

or in combination with hydrochlorotiazide. Most of these methods require the use of expensive equipment and reagents. Spectrophotometric methods still have practical and economical advantages over the other methods, and thus are widely used for the assay of pharmaceuticals in bulk and dosage forms.

In the available literature several different spectrophotometric methods have been reported for LIS quantification in pharmaceutical dosage forms: using different reagents [3,6,7,9,19,27,30,32,34,37,43] and derivative UV spectrophotometric methods [10,12,25,28,39].

Among the reagents used for the assay of LIS in pharmaceutical formulations, ninhydrin has been employed in a few papers. Thus, the method reported by Rahman et al. [32] used ninhydrin in N,N'-dimethylformamide (DMF) medium at room tempetaure for the determination of LIS by initial-rate, rate-constant and fixed time (10 min) procedures, the last one having a molar absorbtivity value of 4.70×10^3 $Lmol^{-1}cm^{-1}$. The reagent in DMF medium but involving elevated temperature (80 ± 5 ºC), when a greenish blue product which absorbs maximally at 600 nm was formed, have been used by Raza et al. [34] for the assay of LIS; the molar absorbtivity value was 4.083×10^3 $Lmol^{-1}cm^{-1}$. The common feature of these two methods [32,34] is the use of DMF medium, an organic solvent which does not lack toxicity. The method reported by Basavaiah *et al*. [3] is based on the reaction of LIS with ninhydrin in aqueous medium in the presence of bicarbonate producing a yellow color product which exhibits maximum absorption at 420 nm; the molar absorbtivity value was 2.02×10^3 $Lmol^{-1}cm^{-1}$.

This paper presents a sensitive and precise spectrophotometric method for the assay of LIS in bulk and pharmaceutical formulation based on the reaction of LIS with ninhydrin in ethanol medium in the presence of potassium hydroxide. The calculated molar absorbtivity value is 7.98×10^3 $Lmol^{-1}cm^{-1}$. From the ninhydrin using methods, the proposed method has the greatest molar absorptivity value, and therefore the highest sensitivity. The method was also validated.

Materials and Methods

Apparatus

The spectrophotometric measurements were performed using a Spectronic Unicam - UV 300 UV-Visible Spectrometer with 1 cm matched glass cells.

Materials and reagents

All the chemicals used were of analytical reagent grade.

0.5% Ninhydrin. 500 mg of chemical (Merck) were dissolved in ethanol and brought to 100 mL with ethanol.

0.1 M Potassium hydroxide. The solution was prepared by dissolving 561 mg of chemical in ethanol and diluting to 100 mL in a calibrated flask.

Standard drug solution. The lisinopril dihydrate was kindly provided by Medochemie, Limassol (Cyprus). A stock standard solution of 1000 µg/mL LIS was prepared by dissolving 100 mg of pure drug in distilled water and diluting to 100 mL in a calibrated flask with water. The standard LIS solution (500 µg/mL) was prepared from the stock solution, by appropriate water dilution.

Proposed procedure

Different aliquots of 500 µg/mL LIS solution (10-30 µg/mL) were accurately measured and transferred in heating tubes. 1 mL of 0.5% ninhydrin and 0.1 mL of 0.1 M potassium hydroxide were added to each tube. The mixture was kept in a water bath at 95 ± 2 ºC for 10 minutes, then cooled to room temperature and transferred into a 25 mL volumetric flask. The volume was made up to the mark by adding water. The absorbance was measured at 567 nm against the reagent blank, which was similarly prepared by omitting the drug. The calibration curve was performed by plotting the measured absorbance values *versus* concentration.

Procedure for pharmaceutical formulation

Twenty tablets were accurately weighed and powdered. A quantity of powder containing 50 mg of lisinopril was transferred into a 100 mL volumetric flask with 60 mL water. The mixture was shaken for 15 min, diluted to volume with water and then filtered using 0.2 µm Nylon filter membrane. The filtrate was subsequently subjected to analysis using the above described procedure.

Results and Discussion

Ninhydrin reagent is used for the determination of aliphatic primary amines, amino acids, peptides and their related compounds [2,22,23,31,38].

LIS interacts with ninhydrin in the presence of potassium hydroxide in ethanol medium *via* oxidative deamination of the primary aliphatic amino group of the lisyne rest contained in the lisinopril molecule followed by the

condensation of the reduced ninhydrin to form the purple colored reaction complex with λ_{max} at 567 nm (Figure 2 and Scheme I).

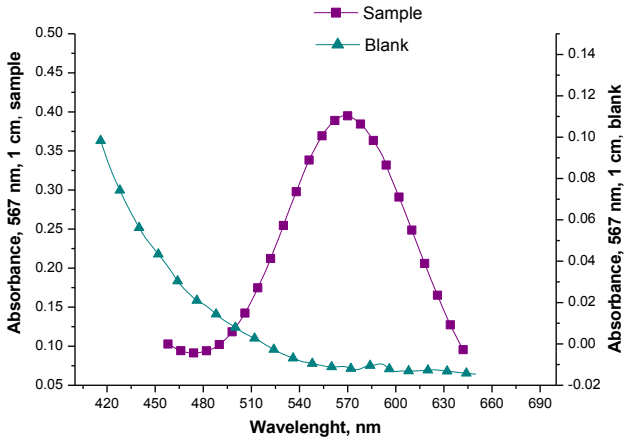
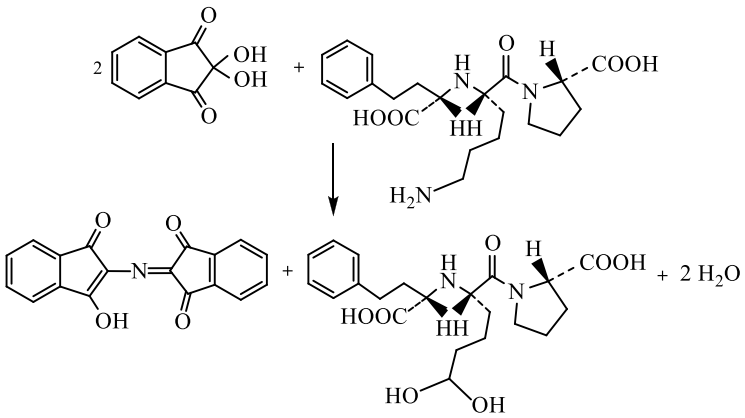


Figure 2
The absorbtion spectra for the purple end product and blank



Scheme I
Sugessted reaction pathway between LIS and ninhydrin

Optimization of reaction conditions

Different parameters such as the temperature, heating time, reagents concentration have been analyzed, in order to render the optimal conditions for reaction. It has been noted that the complete color development was attained at 95 ± 2 ºC. Optimum reaction time has been determined by heating the reaction mixture on a water bath at 95 ± 2 ºC. A heating time of 10 minutes was found as optimal for the development of the purple color product (Figure 3).

In order to investigate the effect of ninhydrin concentration on the reaction product color, the change in absorbance generated by varying the concentration of ninhydrin on fixed concentration of LIS (20 µg/mL) has been measured against reagent blank. The optimum value was found to be 1 mL of 0.5% ninhydrin (Figure 4).

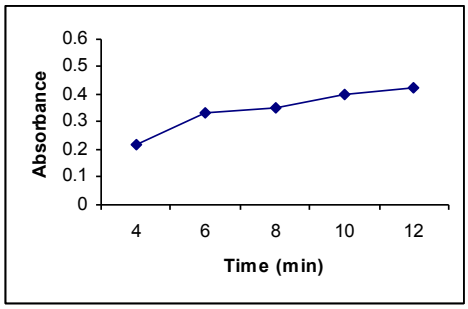


Figure 3. The effect of heating time over the color intensity

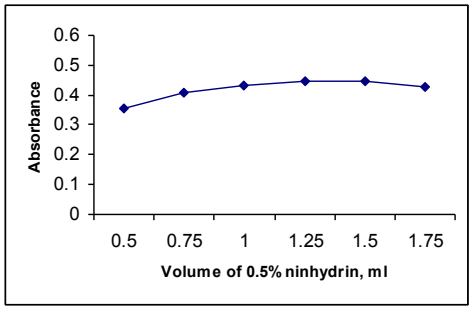


Figure 4. The effect of ninhydrin over the color intensity

It has been observed that a volume over 0.1 mL of 0.1 M potassium hydroxide produces a yellow-brownish reaction product color. Therefore, 0.1 mL of 0.1 M potassium hydroxide was found as being optimum for the development of the purple reaction product. All measurements were performed at 567 nm, against the blank.

Method validation

The proposed method has been validated according to the present ICH guidelines [45].

Linearity and sensitivity

Under optimum conditions, a linear correlation between the absorbance and the concentration of LIS was obtained in the range of 10-30 µg/mL LIS. The calibration curve is described by the equation:

$$Y = a + bC$$

where Y = absorbance, a = intercept, b = slope and C = concentration in µg/mL, which were obtained by the least squares method. The intercept, slope and correlation coefficient are presented in Table I. The sensitivity parameters such as molar absorptivity and Sandell sensitivity are also presented in Table I.

Limits 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 of the response and the slope has been applied, so that 3.3 and 10 times the standard deviation values of y-intercept of regresion line and the regression

equation were used to calculate the LOD and LOQ. The computed values are presented in Table I.

Table I
Regression parameters and sensitivity values

Parameter	Value
λ_{max} / nm	567
Liniarity range, µg/mL	10-30
Molar absorptivity (ε), L/(mol•cm)	7.98×10^3
Sandell sensitivity, µg/cm^2/0.001 abs unit	0.0640
Slope	0.0312
Intercept	-0.2262
Limit of detection (LOD) µg/mL	1.16
Limit of quantification (LOQ) µg/mL	3.52
Standard deviation of slope (S_b)	±0.0011
Standard deviation of intercept (S_a)	± 0.0111
Correlation coefficient (r)	0.9991

Selectivity

The proposed method was tested in order to assess its selectivity using the artificial mixture analysis. It has been confirmed that the measured absorbance was only produced by the analyte. A synthetic mixture was prepared, containing lisinopril (50 mg) as lisinopril dihydrate (54.44 mg), talc (100 mg), starch (150 mg), lactose (180 mg), magnesium stearate (50 mg) and calcium diphosphate (75 mg). The extract was yielded according to the procedure that was described for tablets and subsequently analyzed using the procedure previously described. The replicate analysis (n = 5) for a concentration level of 20 µg/mL LIS has yielded the % LIS recovery at 100.70 ± 1.79, and thus reveled that the inactive ingredients did not interfere with LIS determination.

Precision and accuracy

Intra-day and inter-day precision values have been calculated 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 measurements have indicated a good precision. (Table II)

Accuracy, defined as the closeness between the reference and the found values, has been evaluated, on the other hand, as percentage relative error between the measured and theoretical concentration of LIS. The results are presented in Table II, and show good accuracy for this method.

Table II

Evaluation of intra-day and inter-day accuracy and precision

LIS taken, µg/mL	Intra-day accuracy and precision			Inter-day accuracy and precision		
	LIS found, µg/mL	RE, %	RSD, %	LIS found, µg/mL	RE, %	RSD, %
10	9.94	- 0.60	1.14	9.91	- 0.90	1.18
20	19.87	- 0.65	1.56	19.95	- 0.25	1.90
25	24.72	- 1.12	1.98	24.84	- 0.64	1.95

RE - Relative error; RSD - Relative standard deviation.

Application to pharmaceutical formulation

The proposed method was applied for the quantification of LIS, in tablets pertaining to three commercial formulations. The results were compared to those obtained with the reference method [3], using the Student's t-test for accuracy and F-test for precision. The results as presented in Table III reveal no significant differences between the proposed method and the reference method. The Student's t- and the F-values at 95 % confidence level are less than the theoretical one, but nevertheless confirming a good agreement between the results obtained by the proposed method and the reference method.

Table III

Determination of lisinopril formulation by the proposed and the reference methods.

Tablet brand name	Label claim, mg/tablet	Found[a] (label claim ± SD), %	
		Reference method	Proposed method
Lisigamma®[b]	10	101.152 ± 0.853	102.153 ± 1.689 **t** = 1.088 **F** = 3.921
Lisinopril Sandoz®[c]	10	100.367 ± 0.641	100.668 ± 1.362 **t** = 0.557 **F** = 4.510
Ranolip®[d]	10	100.456 ± 0.691	101.118 ± 0.898 **t** = 1.966 **F** = 1.690

[a] Mean value of five determinations; [b] Worwag Pharma GmbH & Co, Germany; [c] Salutas Pharma GmbH, Germany, [d] Terapia-Ranbaxy, 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

Recovery analysis

The validity of the proposed method was further demonstrated by performing recovery studies. Pre-analyzed tablet powder was spiked with pure LIS at three concentration levels (50, 100 and 150 % of that present in tablet powder), and the total has been found using the proposed method. The percentage values for LIS added recovery are ranging between 98.12 and 102.57, with a standard deviation of 0.40 – 1.94%, which reveals a good recovery.

Table IV

Recovery analysis results, obtained with the standard-addition method

Tested tablet	LIS in tablet, µg/mL	Pure LIS added, µg/mL	Total found, µg/mL	Pure LIS recovered[a] ± SD, %
Lisigamma	10.31	5	15.16	98.20 ± 0.93
	10.31	10	20.31	100.01 ± 0.40
	10.31	15	25.27	99.74 ± 1.47
Lisinopril Sandoz	10.07	5	15.27	102.57 ± 1.34
	10.07	10	20.13	100.58 ± 1.94
	10.07	15	24.47	98.12 ± 1.37

[a] Mean value of three measurements

Conclusions

This paper presents a new spectrophotometric method that has been developed and validated for the quantification of lisinopril dihydrate from its pharmaceutical formulations. The proposed method was proven to be selective, sensitive, rapid and inexpensive.

References

1. 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-dinitrofluorobenzen, *J. Pharm. Biomed Anal*., 2003, 31, 701-711
2. Abdellatef, H. E., Khalil, H.M., Colorimetric determination of gabapentin in pharmaceutical formulation, *J. Pharm. Biomed. Anal*., 2003, 31, 209-214
3. 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
4. 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

5. Brown, N. J. and Vaughan, D. E., Angiotensin-Converting Enzyme Inhibitors, *Circulation,* 1998; 97; 1411-1420
6. 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
7. 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 spectrophtometry or HPLC with fluorimetric detection, *J Pharm Biomed Anal.*, 2004, 34, 35 – 44
8. 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
9. 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
10. El Gindy, A., Ashour, A., Abdel-Fattah, L., Shabana, M.M., Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorotiazide in binary mixture, *J. Pharm. Biomed. Anal.*, 2001, 25, 923-931
11. El-Yazbi, F. A., Abdine, H. H., Shaalan, R. A., Spectrophotometric and spectrofluorimetric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms, *J. Pharm. Biomed. Anal*., 1999, 19, 819-827
12. Erk, N., Combined study of the ratio spectra derivative spectrometry derivative spectrometry and Vierord's method applied to the analysis of lisinopril and hydrochlorothiazide in tablets, *Spectrosc. Lett*., 1998, 31, 633-645
13. Esra, S.A., Lale, E., Olcay, S., A new spectrofluorimteric method for the determination of lisinopril in tablets, *Il Farmaco*, 2003, 58, 165-168
14. Aramă C, Varvara A, Monciu CM, Development and validation of a new capillary zone electrophoresis method for the assay of ondansetron, *Farmacia*, 2011, 59(1), 34-43
15. Gotti, R., Andrisano, V., Cavrini, V., Analysis of ACE inhibitors by CE using alkyl sulphonic additives, *J. Pharm. Biomed. Anal*., 2000, 22, 423-431
16. Hillaret, S., Vanden Bopscha, W., Optimization capillary electrophoretic separation of several inhibitors of the angiotensin-converting enzyme, *J. Chromatogr. A*, 2000, 895, 33-42
17. Huang, J., Xu, Y., Liu, F., Gao S. and Guo, Q., Developement of a liquid chromatography/tandem mass spectrometry assay for quantification of lisinopril in human plasma, *Rapid Commun. Mass Spectrom*., 2006, 20, 248-252
18. Ivanovic, 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
19. Jain, H.K., Agrawal, R.K., Spectrophotometric methods for simultaneous determination of amlodipine besylate and lisinopril in tablets, *Indian Drugs*, 2000, 37, 196-199
20. Leis, H. J., Fauler, G., Raspotnig, G. and Windischhofer, W., Quantitative determination of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stabile isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry, *Rapid Commun. Mass Spectrom*., 1998, 12, 1591-1594
21. 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 stabile isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry, *Rapid Commun. Mass Spectrom*., 1999, 13, 650-653
22. McCaldin, D.J., The chemistry of ninhydrin, *Chem. Rev*., 1960, 60, 39-51
23. Meyer, H., The ninhydrin reaction and its analytical applications, *Biochrm. J*., 1957, 67, 333-340

24. 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
25. Ozer, D., Senel, H., Determination of lisinopril from pharmaceutical preparations by derivative spectroscopy, *J. Pharm. Biomed. Anal.*, 1999, 21, 691-695
26. 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
27. 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
28. 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
29. Qin, W., Zhang, Z., Tian, Y., Xu, F., Wang N. and Chen, Y., Rapid quantification of lisinopril in human plasma by liquid chromatography/tandem mass spectrometry, *Biomed. Chromatogr.*, 2007, 21, 415-421
30. 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
31. Rahman, N., Kashif, M., Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations, *Il Farmaco*, 2003, 58, 10, 1045-1050
32. 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
33. Rajasekaran, A., Murugesan, S., Polarographic studies of lisinopril, *Asian J. Chem*, 2001, 13, 1245-1246
34. Raza, A., Ansari, T.M., Rehman, A., Spectrophotometric determination of lisinopril in pure and pharmaceutical formulation, *J. Chin. Chem. Soc*., 2005, 52, 1055-1059
35. Razen J., Senica D., Ustar, M., Drnovsek, P., Kogej, S., Pavko, A., Concentration of lisinopril purified by liquid chromatography-a comparison between reverse osmosis and evaporation, *Acta Chim. Slov.*, 2001, 48, 597-612
36. 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 materia, *J. Pharm. Biomed. Anal*., 2007, 44, 356-367
37. Sbârcea, L., Drăgan, L., Szabadai, Z., Udrescu, L., Bojiţă, M., The determination of lisinopril based on its complexation reaction with Cu^{2+} using spectrophotometric methods, *Farmacia*, 2007, 2, 165-170
38. Siddiqui, F. A., Arayne, M. S., Sultana, N., Quireshi, F., Mirza, A. Z., Zuberi, M. H., Bahadur, S. S., Afridi, N. S., Shamshad H., Rehman N., Spectrophotometric determination of gabapentin in pharmaceutical formulations using ninhydrin and π-acceptors, *European Journal of Medicinal Chemistry*, 2010, 45, 2761-2767
39. 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
40. Wong, Y. C., Charles, B.G., Determination of angiotensin converting enzyme inhibitor lisinopril in urine using solide phase extraction and reverse phase high performance liquid chromatography, *J. Chromatogr. B Biomed. Appl*., 1995, 673, 306-310
41. Worland, P. J., Jarrott, B., Radioimmunoassay for quantification of lisinopril and enalaprilat, *J. Pharm. Sci.*, 1986, 75, 512-516
42. Yuan, A. S., Gilbert, J. D., Time-resolved fluoroimmunoassay for the determination of lisinopril and enalaprilat in human serum, *J. Pharm. Biomed. Anal*., 1996, 14, 773-781

43. 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
44. ***, European Pharmacopoea, 5th Ed., Council of Europe, Strasbourg, 2004, p.1922-1923
45. ***, 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
46. The Unites States Pharmacopoeia, 24th revision, Asian Edition, United States Pharmacopoeial Convention, INC. Twinbrook Parkway, Rockville, MD, USA, 2000, p. 979-980, 2149-2152

Manuscript received: March 6th 2011