



New Fluorimetric Method of Determination for Lisinopril Dosage Forms

CM Jamakhandi^{1*}, Chandrashekar Javali¹, Satish Kumar², Santosh Kumar¹,
Sanjay Kumar DS¹

¹Pharmaceutical Chemistry Department, Government College of Pharmacy, Bangalore-560027, India

²Pharmacology Department, Government College of Pharmacy, Bangalore-560027, India

ABSTRACT

New fluorimetric analytical method which is simple, accurate, precise, specific is developed for determination of Lisinopril. The fluorimetric determination of Lisinopril is based on the formation of complex between Lisinopril and Fluorescein, measured at excitation wavelength of 366 nm and emission wavelength of 475 nm. Linearity was observed in the range of 0.03 – 0.15 $\mu\text{g ml}^{-1}$. The fluorimetric method shows regression coefficient of 0.99971, and Relative Standard Deviation 0.527. Tablet dosage forms were estimated were complied with percentage recovery studies of 99-100 %. The method was validated for linearity, precision, accuracy, specificity and statistically expressed.

Keywords: Lisinopril, Fluorescein, Coupling reaction, Fluorimetry.

INTRODUCTION

Lisinopril is ACE inhibitor which is used as antihypertensive and in the treatment of cataract. [1] The official analytical methods for Lisinopril described are potentiometric titration and HPLC [2-4], various spectrophotometric methods [5-18], chromatographic methods of analysis such as micellar electrokinetic chromatography and gas liquid chromatography [19-20], Capillary electrophoresis, fluoroimmunoassay, radioimmunoassay and fluoroenzymatic assay have also been reported. [21-22] The estimation with ninhydrin reported in sodium hydroxide and sodium carbonate associated with interference of concentrated blank solution in absorption and is time consuming. The available methods are associated with drawbacks such as low reliability due to isomerisation, less sensitive, measurement at lower wavelength, pH dependent, inaccessibility and requirement of expertise.

So there is need to develop new simple, accurate, precise, specific analytical method which sensitive and is easily accessible. The present study aimed to develop fluorimetric method to determine the Lisinopril in pure form or in formulation. Fluorimetric method is based upon the condensation reaction between primary amino group of Lisinopril and Fluorescein to form fluorescent derivative (LSFN) in methanol at 60°C for 5 min (Fig. 4).

The formation of fluorescent derivative was confirmed by the UV (λ_{max} 227 nm), NMR, Mass and IR spectra (Fig. 5-10). Relative fluorescent intensity was measured with excitation filter of 366 nm and emission filter of 475 nm setting the fluorometer to 100% intensity with concentration of 0.1 $\mu\text{g/ml}$ standard solution using methanol as blank. The reagent fluorescein shows 0.00009 $\mu\text{g ml}^{-1}$ as maximum of limit of measurable concentration whereas fluorescent derivative (LSFN) limit is beyond the range of reagent (0.03 - 0.19 $\mu\text{g ml}^{-1}$). Under the limit of measurement, fluorescent intensity is proportional to the concentration of analyte. The stability of reaction mixture was determined. The developed method was validated for parameters as per the ICH guidelines.

MATERIALS AND METHODS

Elico Fluorimeter, model CL-53 was used for fluorimetric determination. The fluorescence intensity of test and reference solutions was recorded in 3 ml borosilicate cells. The Relative Intensity was measured with filters of excitation wavelength of 366 nm and emission wavelength of 475 nm.

Standard drug, marketed formulations and reagents used

The experimentation Lisinopril dihydrate standard drug was procured from Unimark Pharmaceuticals Ltd, Vapi, Gujarat, India, and certified to contain 99.3%. All the chemicals, solvents and reagents used in the study were of analytical grade. Lisinopril 5 mg manufactured by Torrent Pharmaceuticals Ltd, Lipril 10 mg manufactured by Lupin Ltd and Lisinopril 5 mg manufactured by Ipca Laboratories Ltd were three commercial tablets of Lisinopril used for sample estimation.

***Corresponding author: Mr. C. M. Jamakhandi,**
Pharmaceutical Chemistry Department, Government College of Pharmacy, Bangalore-560027, India;
E-mail: cmjamakhandi@gmail.com

Fluorimetric Method

Lisinopril of 100 mg was transferred to 100 ml volumetric flask. Add 50 ml of methanol, shake well to dissolve, and then Fluorescein of 0.07526 mg was added. The mixture was shaken and heated at 60°C for 5 min, cooled, and then final volume was made with methanol. Various volumes were transferred to 50 ml volumetric flasks so as to produce the different concentrations in the range of 0.03 µg-0.15 µg. Standard Lisinopril solution of 0.1 µg/ml was used to set 100% intensity using methanol as blank.

Procedure for fluorimetric estimation of commercial tablets

Weigh accurately 20 tablets and crushed to fine powder. The tablet powder equivalent to 100 mg was weighed and added to 40 ml of methanol. The mixture was stirred well filtered; the filtrate was transferred to 100 ml of volumetric flask. Fluorescein of 0.07526 mg was weighed accurately and dissolved in 50 ml of methanol and then transfer this solution to the Lisinopril solution. Mixture was heated at 60°C for 5 min, cooled to room temperature and then final volume was made with methanol. Standard Lisinopril solution of 0.1 µg/ml was used to set 100% intensity using methanol as blank. The relative intensities of other sample dilutions were recorded using methanol as blank solution.

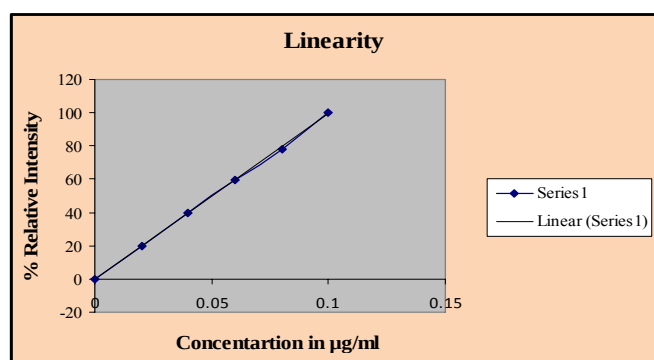


Fig. 1: Linear relationship between % Relative intensities and Concentration

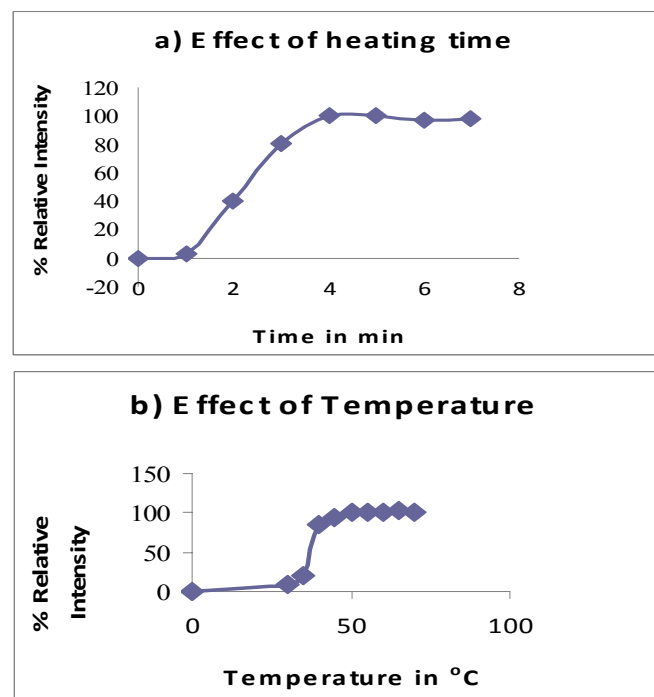


Fig. 2: Graphical representation of effect of (a) heating time and (b) temperature on % Relative Intensity in Fluorimetric determination

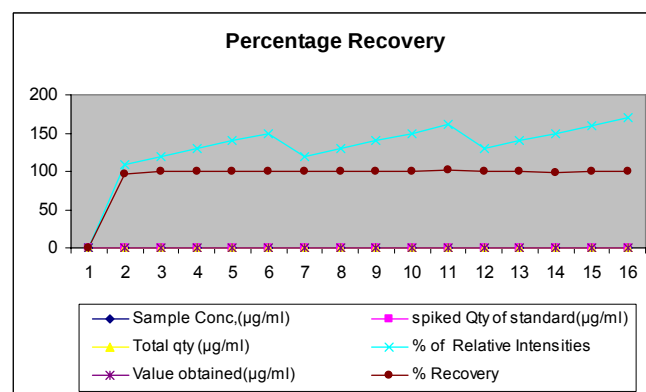


Fig. 3: Fluorimetric determination: Graphical representation of % Recovery studies with constant quantity of sample and spiked quantity of drug

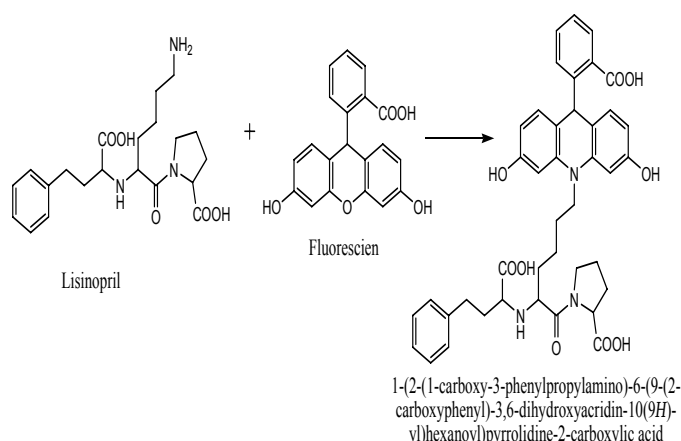


Fig. 4: Proposed reaction mechanism for the formation of fluorescent derivative of Lisinopril with Fluorescein

Table 1 : Statistical calculation of validation parameters

S. No	Parameter	Statistical Value
1.	Standard Deviation	0.03162
2.	Relative Standard Deviation	0.52704628
3.	Coefficient of Variation	52.7046283
4.	ANOVA	36.30868
5.	Correlation Coefficient	0.99971
6.	Lower limit of Detection	0.0001049 µg/ml
7.	Lower limit of Quantification	0.000318 µg/ml
8.	Slope	994.06536
9.	Intercept	0.083576
10.	% Recovery	99-100 %

a. Equation of linearity is Absorbance or Relative Intensity = Slope × Concentration + Intercept.

RESULTS AND DISCUSSION

The developed fluorimetric method makes use of simple reagent, shows linearity range of 0.03 µg to 0.15 µg (Fig. 1), producing the data at appreciable sensitivity even in low concentration. Fluorimetric determination is optimized for the factors affecting the coupling reaction (Fig. 2). Statistical analysis of experiment shows the accuracy of 99-100 % (Fig. 3), relative standard deviation was 0.527 and coefficient of correlation was 0.99971. Samples of marketed formulation was estimated. The method was validated for analytical parameters like accuracy, Linearity, precision, specificity, Limit of Detection and Limit of quantification and statistically expressed were found to be within the standard specification (Table 1). Hence developed method simple, accurate, specific and precise which can applied in quality control for the estimation of lisinopril.

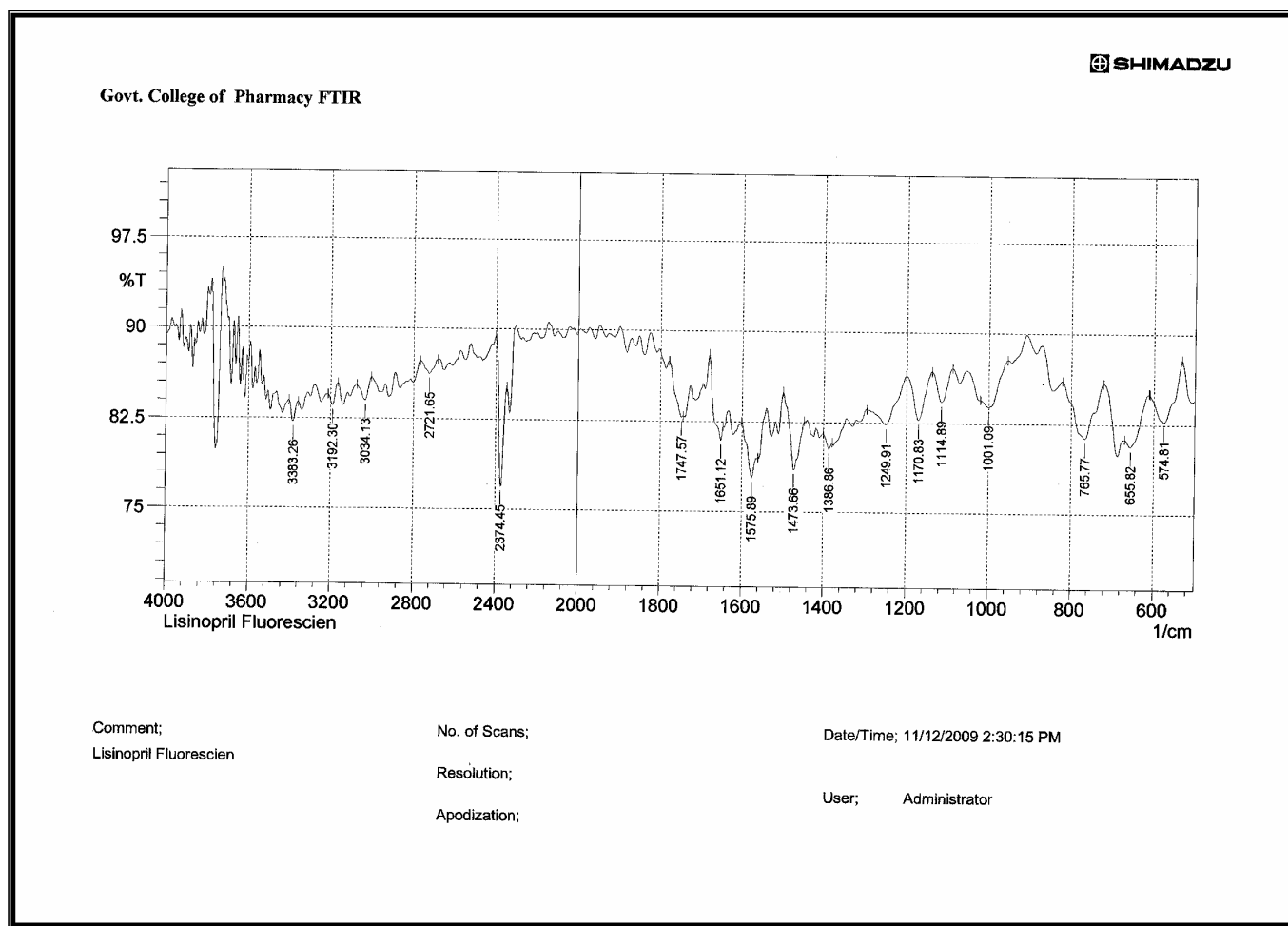


Fig. 5: IR Spectrum of fluorescent derivative

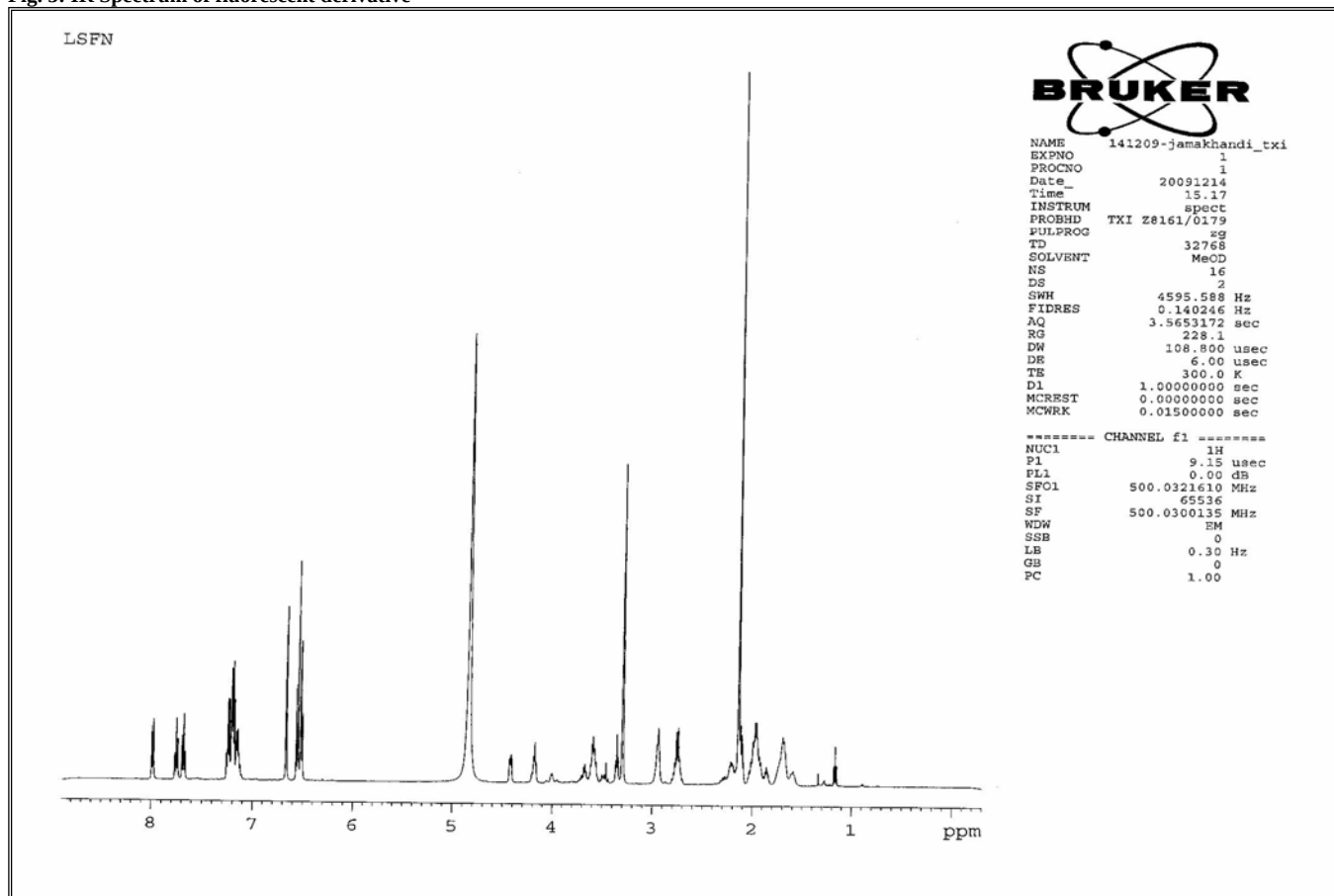


Fig. 6: NMR Spectrum of fluorescent derivative

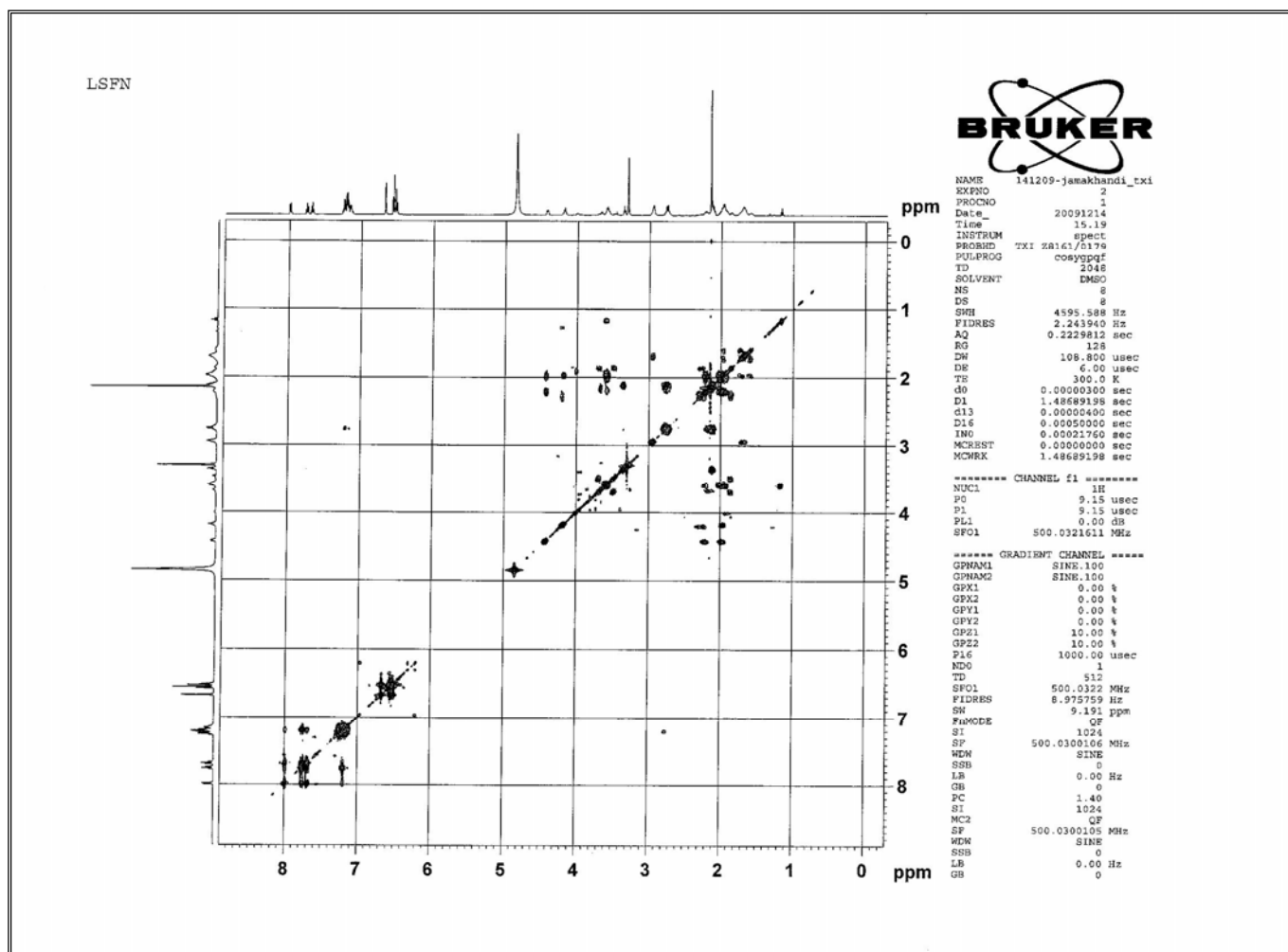


Fig. 7: 2D HNMR Spectrum of fluorescent derivative

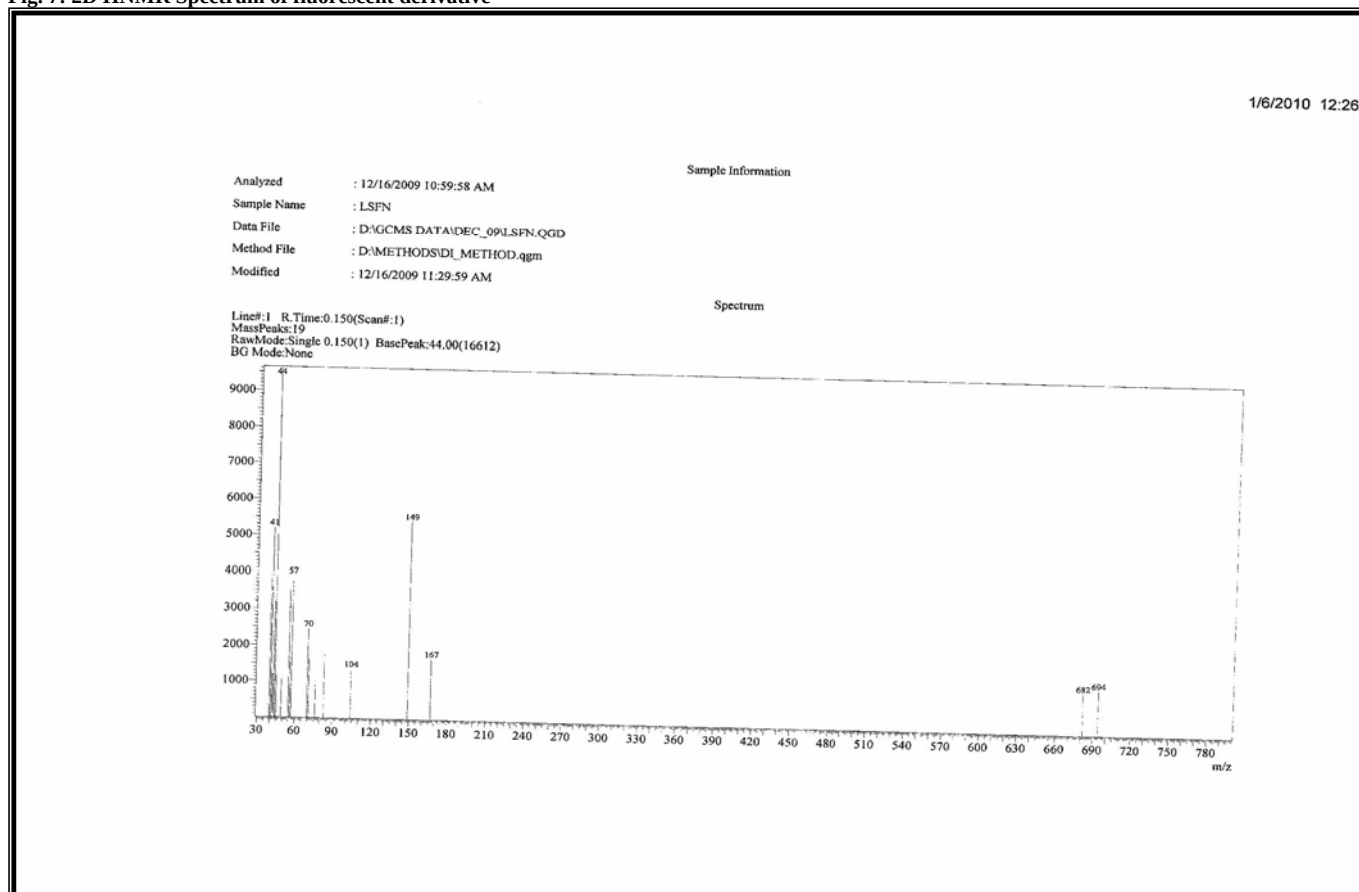


Fig. 8: Mass Spectrum-1of fluorescent derivative

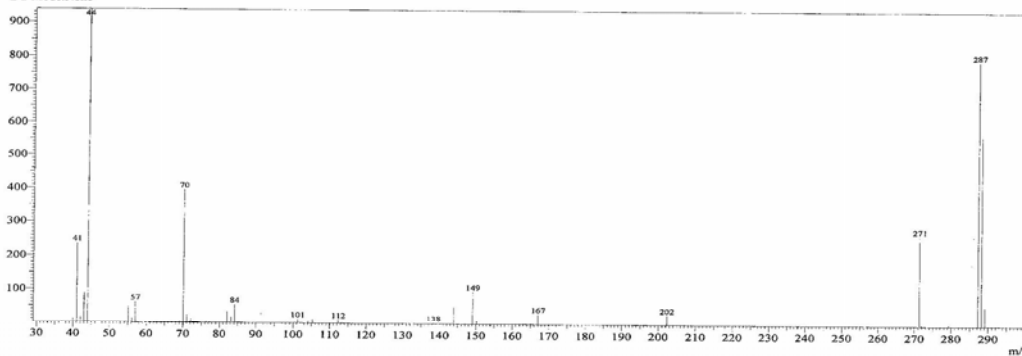
12/17/2009 11:26:43

Sample Information

Analyzed : 12/16/2009 10:59:58 AM
 Sample Name : LSFN
 Data File : D:\GCMS DATA\DEC_09\LSFN.QGD
 Method File : D:\METHODS\DI_METHOD.QGM
 Modified : 12/16/2009 11:29:59 AM

Spectrum

Line#1 R.Time:0.200(Scan#:7)
 MassPeaks:36
 RawMode:Averaged 0.192-29.142(6-3480) BasePeak:44.00(2995)
 BG Mode:None



1 / 1

Fig. 9: Mass Spectrum-2 of fluorescent derivative

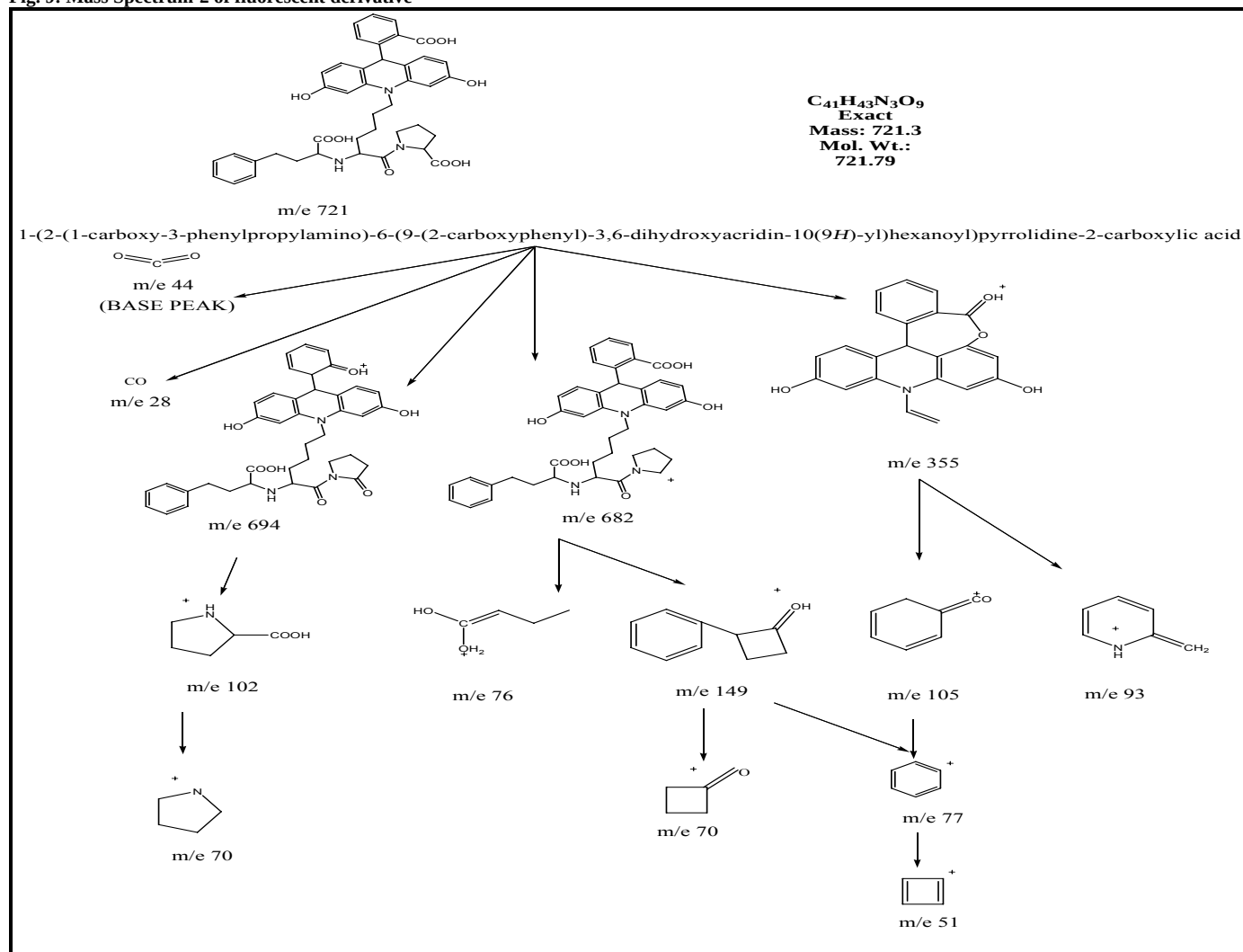


Fig. 10: Fragmentation pattern of LSFN in Mass spectra

ACKNOWLEDGEMENTS

Authors are thankful to Unimark Pharmaceuticals Ltd, Vapi, Gujarat, India for generously providing gift sample of Lisinopril and Indian Institute of Science Bangalore for spectral data. Authors are grateful to Dr. S. Shashidhar, Principal and Professor. M. S. Niranjana HOD, Mr. Chaluvvaraju K Asst Prof of Government College of Pharmacy Bangalore for continuous support and providing laboratory facilities.

REFERENCES

1. Parfitt, editor. In "Martindale: The Complete Drug Reference". 32nd ed. The Pharmaceutical Press. London, United Kingdom. ISBN: 0853 69429, 1999, pp. 898.
2. Indian Pharmacopoeia. Ministry of Health and Family Welfare New Delhi, 2007, 2, pp. 1306-08
3. British Pharmacopoeia. Stationery Office Books (TSO) London, United Kingdom, 2005, 2, pp. 1199.
4. United States Pharmacopoeia- USP-24, NF-19, Asian Edition, United States Pharmacopoeial Convention, INC. Twin brook Parkway, Rockville, MD, U.S.A. 2000, pp. 979.
5. Basavaiah K, Tharpa K, Salmara GH, Basavaiah VK. Spectrophotometric Determination of Lisinopril in Pharmaceuticals Using Ninhydrin- a Modified Approach. *J Food Drug Anal.*2009; 17(2):93-99.
6. Devi PA, Mallikarjuna Rao GPV, Krishna Prasad KMM, Sastry C SP. Four simple spectrophotometric determinations of lisinopril in pure state and in tablets. *Indian J Pharm Sci* 2003; 65(3):296-99.
7. Paraskevas JG, Atta Politou, Koupparis M. Spectrophotometric determination of lisinopril in tablets using 1-fluoro-2, 4-dinitrobenzene reagent. *J Pharm Biomed Anal.* 2002; 29(5):865-72.
8. Abdel Razak Belal OSF, Bedair MM, Barakat NS, Haggag RS. Spectrophotometric and polarographic determination of enalapril and lisinopril using 2, 4-dinitrofluorobenzene. *J Pharm Biomed Anal.*2003; 31(4):701-711.
9. Fawzy A, El-Yazbi Heba H, Abdine, Rasha A, Shaalan. Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. *J Pharm Biomed Anal.*1999; 19:819-27.
10. Ali A, El-Emama, Steen Honore, Hansen Mohamed A, Moustafa, Saadia M, El-Ashry Dina T, El-Sherbiny. 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.
11. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana MM. Spectrophotometric, septrofluorimetric and LC determination of lisinopril. *J Pharm Biomed. Anal.*2001; 25:913-22.
12. Asad Raza, Tariq Mahmood Ansaria, Atta-ur-Rehmanb. Spectrophotometric Determination of Lisinopril in Pure and Pharmaceutical Formulations. *J Chine Chem Soci.*2005; 52:1055-59.
13. Jain HK. Agrawal RK. Spectrophotometric method for simultaneous estimation of amlodipine besylate and lisinopril in tablets. *Indian Drugs.* 2000; 37(4):196-99.
14. Shinde V, Trivedi A, Upadhyay PR, Gupta NL, Kanase DG, Chikate R. Identification of a new impurity in lisinopril. *J Pharm Biomed. Anal.* 2007; 43; (1): 381-86.
15. Panzade, PD. K.R. Mahadik. Simultaneous spectrophotometric estimation of Lisinopril and hydrochlorothiazide from combined dosage forms. *Indian Drugs.*1999;36(5):321-23
16. Durisehvar O, Hulya S. Determination of lisinopril from pharmaceutical preparations by derivative UV spectrophotometry. *J Pharm Biomed Anal.*1999; 21:691-95.
17. Ersä SA, Lale E, Olcay S. A new spectrofluorimetric method for the determination of lisinopril in tablets. *IL Farmaco* 2003;58:165-168.
18. El-Yazbi, FA Abdine, HH, Shaalan RA. Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. *J Pharm Biomed Anal.* 1999; 19: 819-827.
19. Sagirli O, Ersoy L. An HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection. *J Chromatogr.* 2004; 809(1): 159-165.
20. Ivanovic D, M Medenica, B. Jancic, N Knezevic, A Malenovic, J Milic.. Validation of an analytical procedure for simultaneous determination of hydrochlorothiazide, lisinopril, and their impurities. *Acta Chromatogr.* 2007; 18.
21. Yuan AS, Gilbert JD. Time-resolved fluoroimmunoassay for the determination of lisinopril and enalaprilat in human serum. *J Pharm Biomed Anal.*1996; 14:773-81.
22. Gotti R, Andrisano V, Cavrini V, Bertucci C, Furlanetto S. Analysis of ACE-inhibitors by CE using alkylsulfonic additives. *J Pharm Biomed Anal.* 2000; 22:423-31.