www.sphinxsai.com

PharmTech
International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 556-562, Jan-Mar 2010

Simultaneous estimation of Amlodipine Besylate and Lisinopril Dihydrate as A.P.I. and in tablet dosage forms by modified form of simultaneous equation method using derivative UV- Spectrophotometry

PERMENDER RATHEE*[1], SUSHILA RATHEE[1], SHYAMA THAKUR[2] & VIKASH KUMAR[1]

[1]PDM College of Pharmacy, Bahadurgarh,India.

[2]School of Pharmacy, Shoolini Institute of Life Sciences & Business Management, Solan,India.

Corres.author: coolpr79@rediffmail.com
Phone No. 09466593965

ABSTRACT: A new second derivative UV-Spectrophotometric method has been described for the simultaneous assay of Amlodipine Besylate and Lisinopril Dihydrate in bulk drug and in tablet dosage forms using double distilled water as the solvent. The method is based on simultaneous equation or Vierodt's method. The λ_{max} values for Amlodipine Besylate and Lisinopril Dihydrate in the solvent medium were found to be 256 nm and 216 nm respectively. The systems obey Beer's law in the range of 10.0 to 70.0 µg/ml and 4.0 to 40.0 µg/ml with correlation coefficient of 0.9994 and 0.9996 for Amlodipine Besylate and Lisinopril Dihydrate respectively. Repeatability, Interday and intraday precision were found to be 0.134, 0.280, 0.349 and 0.205, 0.530, 0.569 respectively. No interference was observed from common tablet adjuvants. t –test and F-test have been applied for the recovery studies of the method. The method was successfully applied to the assay of Amlodipine Besylate and Lisinopril Dihydrate in tablet formulations.
KEYWORDS: Amlodipine Besylate; Lisinopril Dihydrate; Derivative Spectroscopy; UV Spectrophotometry; Vierodt's method

1. INTRODUCTION

Amlodipine Besylate [1] (AML) chemically 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate is a long-acting calcium channel blocker used for hypertension and angina pectoris [2-4]. Amlodipine Besylate block the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature relaxing the smooth muscle and dilating arterioles thereby decreasing peripheral resistance. Hence improving blood pressure; in angina it improves blood flow to the myocardium.

Lisinopril Dihydrate [5] (LID) chemically (2S)-1-[(2S)-6-amino-2-[{(1S)-1-carboxy-3-phenylpropyl} amino] hexanoyl] pyrrole-2-carboxylic acid is an ACE inhibitor which acts by directly blocking the formation of AT-II & at the same time by increasing bradykinin level.

The official method for estimation of AML includes Non-aqueous titration [6] & HPLC [7] and for LID is Potentiometry [5]. The methods reported for simultaneous estimation of either drug in combination are RP-HPLC [8], HPTLC [9], Derivative Spectrophotometry [10], UV- Spectrophotometric [11-14], HPLC [15], Spectrofluorimetric [16].

In the present method double distilled water is used as solvent for simultaneous estimation of both the drugs by simultaneous equation or Vierodt's method using UV-Spectrophotometry. The present method is relying on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that

achieved by sophisticated and expensive technique like HPLC & HPTLC.

2.1 MATERIAL & METHODS

2.1.1 Instrument

ELICO SL 160 Double beam UV-VIS Spectrophotometer with spectral band width of 1.8 nm, wavelength accuracy of ± 2 nm and matched quartz cells of 10 mm optical path length was used for all spectral and absorbance measurements.

2.1.2 Reagents and materials

All chemicals used were of analytical reagent grade and double distilled water was used to prepare the solvent medium. Pharmaceutical grade AML and LID procured from Torrent labs, Hyderabad, India were used as received. A standard solution of AML and LID was prepared by dissolving 10.0 mg of pure drugs in double distilled water and diluting to 100 ml with double distilled water. These stock solutions (100.0 µg/ml) were diluted with double distilled water to get working concentrations of 10.0 to 70.0 µg/ml for AML and 4.0 to 40.0 µg/ml for LID

2.1.3 Selection of Wavelength

The λ_{max} values for Amlodipine Besylate and Lisinopril Dihydrate respectively in the solvent medium were found to be 256 nm and 216 nm in second derivative spectroscopy using zero crossing point method in double distilled water and the spectrum was derivatized and smoothed by Savitsky – Golay method (Fig. 1).

2.1.4 Procedure

Aliquots of pure AML and LID solutions (1.0-7.0 ml) and (0.4- 4.0 ml) were transferred into a series of 10 ml calibrated flasks and the total volume was adjusted upto the mark with double distilled water. The absorbance's of the resulting solutions were then measured at 256 nm and 216 nm respectively in triplicate against double distilled water as blank and calibration curves were plotted between absorbance v/s concentrations (Fig. 2 & Fig. 3).

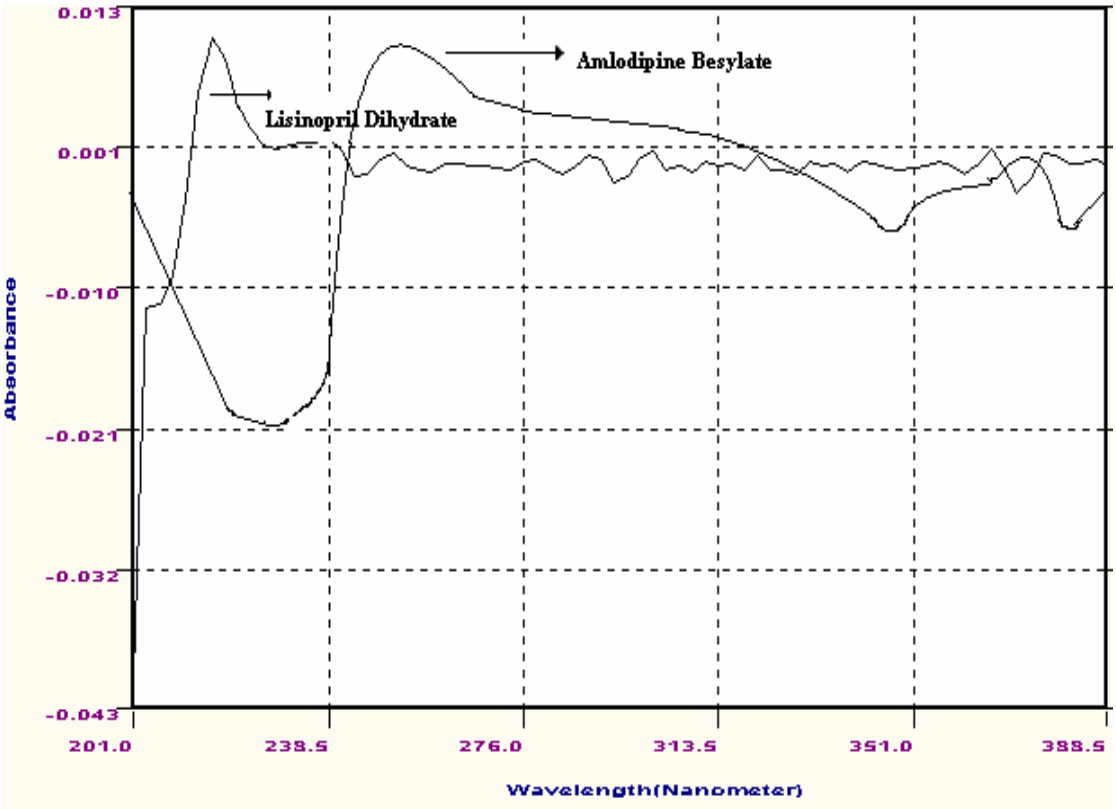

Figure 1: Overlain spectra of Amlodipine Besylate and Lisinopril Dihydrate in second derivative mode

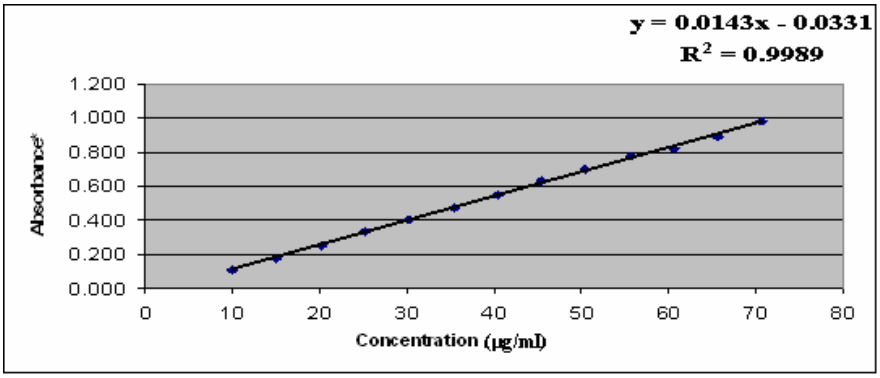

Figure 2: Calibration curve of Amlodipine Besylate at 256 nm

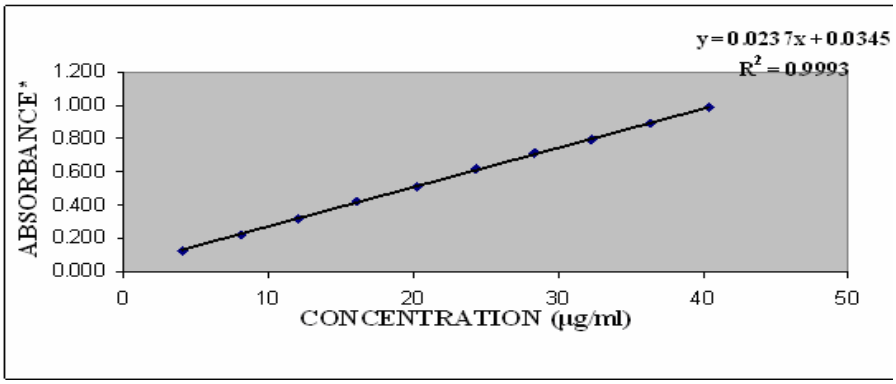

Figure 3: Calibration curve of Lisinopril Dihydrate at 216 nm

2.1.5 Assay of formulations

Twenty Tablets (of same respective batch number) of two pharmaceutical companies were accurately weighed and powdered. A quantity of powder (143.5 mg for Amlodac-L and 124.4 mg for Amlopress-L) equivalent to 5.0 mg of Amlodipine Besylate/5.0 mg of Lisinopril Dihydrate was transferred into 100 ml volumetric flask and dissolved in 25 ml of double distilled water. The solution was sonicated for 20 minutes and was filtered through Whatmann No. 40 filter paper. The residue was washed with double distilled water and the washings were added to the filtrate. The volume was made upto the mark with double distilled water so as to get a concentration of 50.0 µg/ml of Amlodipine Besylate and 50.0 µg/ml of Lisinopril Dihydrate. From this solution, (1.6 ml) was pipetted out into 10 ml volumetric flask and diluted upto the mark with double distilled water so as to get a concentration of 8.0 µg/ml of Amlodipine Besylate and 8.0 µg/ml of Lisinopril Dihydrate. The absorbances of these solutions were measured in triplicate at 238.4 nm and 216 nm using double distilled water as blank.

3. RESULTS AND DISCUSSION:

3.1 Analytical data

A linear correlation was found between absorbances at λ_{max} and concentrations of AML and LID. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values are given in Table 1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table 1. The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and x concentration in µg/ml. The limit of detection and quantification calculated according to ICH guidelines [17] and reveals a very high sensitivity of the methods.

3.2 METHOD VALIDATION:

3.2.1 Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within the working limits) were analyzed, each determination being repeated three times. The relative standard deviations (%) were less than 1 and indicate the high accuracy and precision for the methods (Table 2). For intra-day and interday precision the relative standard deviation values were in the range of 0.280 -0.569 % and represent the best appraisal of the methods in routine use.

Table 1: Optical and regression parameters of AML and LID in double distilled water

Parameters	**AML**		**LID**
λ max (nm)	**256 nm**	**216 nm**	**216 nm**
Beer's law limit (μg/ml)	10.0-70.0	4.0-28.0	4.0-40.0
Molar absorptivity (l mole^{-1}cm^{-1})	7.43 x 10^3	17.01x 10^3	11.48 x 10^3
Sandell's sensitivity (mg/cm^2/.001absorbance unit)	0.1345	0.0333	0.08704
Correlation coefficient (r) **(R^2)**	0.9994 0.9989	0.9997 0.9995	0.9996 0.9993
Regression equation (y= a + bc) **slope (b)** **intercept (a)** **S_a**** = Standard deviation of slope **S_b**** = Standard deviation of intercept	 0.0143 0.0331 0.002 0.011	 0.0402 0.1122 0.001 0.002	 0.0237 0.0345 0.002 0.014

(Y)* = a + bX where Y is the absorbance and x concentration in μg/ml

Table 2: Summary of validation parameters for AML & LID

S. No.	**Parameters**	**AML**	**LID**
1.	**Specificity:** -% interference -% agreement	 ≤ 0.5% 100.14-100.37	 ≤ 0.5% 100.16-100.52
2.	**Range (μg/ml):** -Working range -Linearity range -Target range -Test conc. (100%)	 0.87-70.70 0.87-70.07 38.38- 57.57 47.98	 4.67-40.4 16.16-40.40 22.62- 28.28 28.28
3.	**Precision: (RSD)** -Repeatability (n =7) -Intraday (n=3) -Interday (3 days)	 0.134 0.280 0.349	 0.205 0.530 0.569
4.	**Accuracy %**	98.23-99.21	98.02-99.43
5.	**Limit of detection, μg mL^{-1}**	0.229	0.248
6.	**Limit of quantification, μg mL^{-1}**	0.694	0.751

3.2.2 Interference study

To investigate the effect of tablet fillers on the measurements involved in the method 1.0, 1.6 and 2.0 ml of Amlodipine Besylate and 1.0, 1.6 and 2.0 ml of Lisinopril Dihydrate were mixed in three 10 ml different volumetric flasks and the volume was made upto mark with double distilled water. The absorbances of the final resulting solutions were measured at 256 nm and 216 nm. Then a solution containing excipients was added to the above preanalysed solution and filtered the solution using whatmann no. 40 filter paper. The ratio of drug: excipients were 1: 14. The residues were washed with double distilled water and washings were mixed the filtrates. The absorbance's of the resulting solutions were then measured in triplicate and compared to the results of the preanalysed solutions (Fig. 4 & Table 3).

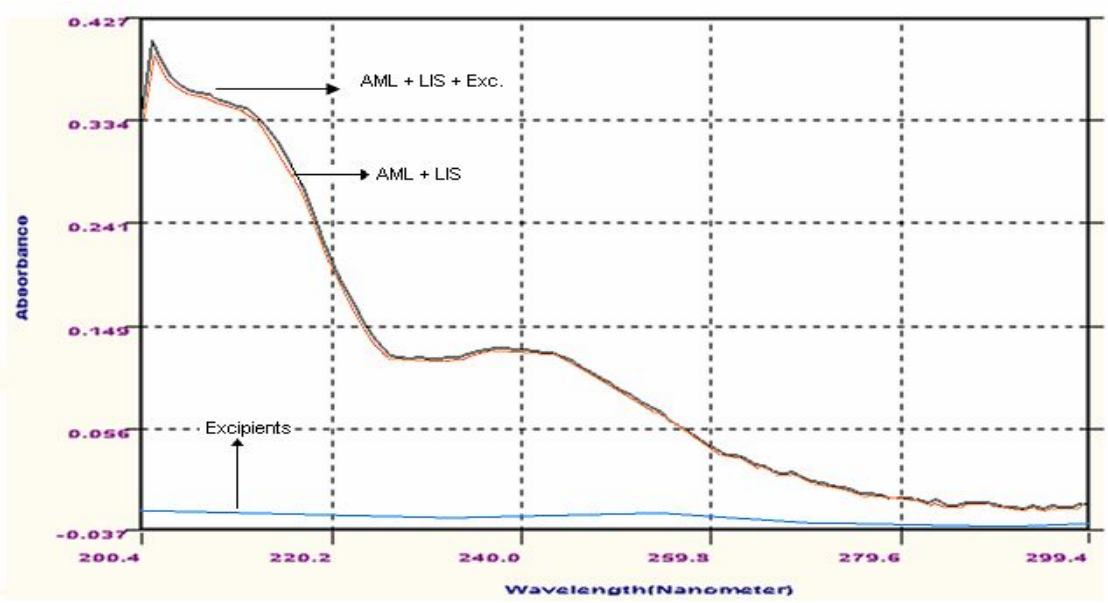

Figure 4: Overlain spectra of AML + LIS, AML + LIS + excipients and excipients at 256 and 216 nm

Table 3: Result of specificity study for the synthetic mixture of AML and LID

S. No.	In absence of excipients		In presence of excipients		% Interference $\frac{(Cp - Ca)*100}{Ca}$	% Agreement Cp/ Ca*100
	Abs.*	Conc. (µg/ml) Ca	Abs.*	Conc. (µg/ml) Cp		
1.	0.106	9.97	0.108	10.01	0.384	100.40
	0.265	9.94	0.267	9.99	0.526	100.50
2.	0.175	16.08	0.178	16.11	0.214	100.19
	0.408	16.01	0.411	16.08	0.422	100.44
3.	0.252	19.98	0.254	20.03	0.233	100.25
	0.525	20.05	0.527	20.10	0.226	100.25

3.2.3 Application to analysis of commercial samples

In order to check the validity of the proposed method, AML and LID were determined in some commercial formulations. Table 4 present the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labeled claim.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powders were spiked with pure AML and LID standard solutions at three different levels and the concentration of the sum total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug solution added was quantitative (99.85-99.88 % and 99.80-99.92 % respectively) and revealed that co-formulated substances did not interfere in the determination.

Table 4: Results of simultaneous estimation of AML and LID in tablet dosage form of different brands

Brand	Labeled amount (mg/tab)	Conc. selected (μg/ml)	Amount found (mg/tab)	% Recovery	% Mean recovery ± SD
Amlodac-L	5 +5	20 + 20	19.98 + 19.95	99.90 + 99.75	99.85 ± 0.132
			19.99 + 19.92	99. 95 + 99.60	99.80 ± 0.229
			19.94 + 20.01	99.70 + 100.05	
Amlopores-L	5 +5	20 + 20	19.99 + 20.04	99.95 + 100.20	99.88 ± 0.076
			19.96 + 19.98	99.80 + 99.90	99.92 ± 0.275
			19.98 + 19.93	99.90 + 99.65	

Data represents mean ± SD; n = 3.

CONCLUSION

The methods for the determination of Amlodipine Besylate and Lisinopril Dihydrate have been developed and validated. These are applicable over a range of 10.0-70.0 μg/ml for AML and 4.0-40.0 μg/ml for LID and molar absorptivity of 7.43 x 10^3 L $mole^{-1}$ cm^{-1} for AML and 11.48 x 10^3 L $mole^{-1}$ cm^{-1} for LID. The recoveries of AML and LID from two brands have been compared by using t- test and F-test for both the methods and results are shown in Table 5. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC, HPTLC. Thus these can be used as alternatives for rapid and routine determination of bulk sample and tablets.

Here, t_{crit} and F_{crit} is greater than t_{stat} and F_{stat}; hence significant difference between the recoveries of Amlodipine Besylate and Lisinopril Dihydrate by using the two brands does not exist.

ACKNOWLEDGEMENT: The authors gratefully acknowledge the receipt of pure Amlodipine Besylate and Lisinopril Dihydrate from Torrent labs, Ahmedabad.

MATHEMATICAL CALCULATIONS:

The API mixture in the ratio of 1:1 (n=4) was transferred and diluted upto the mark with double distilled water. The absorbances of these solutions were measured at 256 nm and 216 nm.

Amounts of Amlodipine Besylate and Lisinopril Dihydrate were determined by solving the simultaneous equations**.** Two equations were formed using absorptivity coefficient values.

C_2 = (a-c)/absorptivity of C_2 at λ_2 ---------- (1)

c = (b/absorptivity of C_1 at λ_1) x absorptivity of C_1 at λ_2

C_1 = b/absorptivity of C_1 at λ_1 ---------- (2)

Where C_1 and C_2 are concentration of Amlodipine Besylate and Lisinopril Dihydrate respectively in gm/liter in the sample solution, a and b are the absorbances of the mixture at 256 nm and 216 nm respectively and the concentrations of Amlodipine Besylate and Lisinopril Dihydrate were calculated using these two equations.

Table 5: Results of t-test and F-test applied for AML & LID for Amlodac-L & Amlopores-L brands

S. No.	Parameters	Amlodac-L	Amlopores-L
1	Mean	98.94	98.74
2	Variance	0.029	0.087
3	t Stat	1.012	
4	P (T<=t) two-tail	0.368	
5	t Critical two-tail	2.78	
6	$F_{(2,2)}$ Statistical	2.95	
7	$F_{(2,2)}$ critical	19.0	

REFERENCES

1. Martindale. The Complete Drug Reference. 34th Ed. Pharmaceutical Press, USA; 2005.
2. Tripathi KD. Essential of Medical Pharmacology. 5th Ed. Jaypee Brothers Medical Publishers (P) Ltd.: New Delhi; 2003.
3. Williams A. Foye's Principles of Medicinal Chemistry. 5th Ed. published by B.I. publications Pvt. Ltd.; 2004.
4. Myeek MJ, Harvey RA. Lippincott's Illustrated Reviews, Pharmacology. 2nd Ed. 2004.
5. British Pharmacopoeia, Vol. III. Published by MHRA: UK; 2004, 2270-2271.
6. Indian Pharmacopoeia. Vol. II. Published by The Controller of Publication Delhi; 1996.
7. British Pharmacopoeia. Vol. III. Published by MHRA: UK; 2004.
8. Naidu KR, Kale UN, Shingare MS Stability indicating RP-HPLC method for simultaneous determination of Amlodipine and Benazepril Hcl from their combination drug product. J Pharm Biomed Anal 2005; 39(1-2): 147-155.
9. Argekar AP, Powar SG Simultaneous determination of Atenolol and Amlodipine in tablets by HPTLC. J Pharm Biomed Anal 2000; 21(6): 1137-1142.
10. Prasad CVN, Saha RN, Parimoo P Simultaneous determination of Amlodipine-Enalapril Maleate and Amlodipine-Lisinopril in combined tablet preparations by derivative Spectrophotometry. Pharmacy and Pharmacology communications 1999; 5(5): 383-388.
11. Topale PR Simultaneous UV Spectrophotometric estimation of Amlodipine and Losartan Potassium in tablet dosage forms. Indian drugs 2003; 40(2): 119-121.
12. Rahman N, Singh M, Md. Hoda N Optimized and Validated Spectrophotometric Methods for the Determination of Lisinopril in Pharmaceutical Formulations Using Ninhydrin and Ascorbic Acid. J. Braz. Chem. Soc. 2005; 16(5): 1001-1009.
13. Stanisz B Estimation of the applicability of differential spectrophotometric method for determination of Lisinipril in tablets and for evaluation of its stability. Acta Pol Pharm. 2004; 61(5): 327-34.
14. Paraskevas G, Atta- Politou, Koupparis M. Spectrophotometric determination of Lisinipril in tablets using 1- fluoro-2, 4-dinitrobenzene. J Pharm Biomed Anal. 2002; 29(5): 865-72.
15. Beasley CA, Shaw J, Zhao Z, Reed R A Development and validation of a stability indicating HPLC method for determination of Lisinipril, Lisinopril degradation products and parabens in Lisinopril extemporaneous formulations. J Pharm Biomed Anal. 2005; 37(3): 559-67.
16. El-Gindy A, Ashour A, Abdul- Fattah L, Shabana M M Spectrophotometric, Spectrofluorimetric and LC determination of Lisinipril. J Pharm Biomed Anal. 2001; 25(5-6): 913-22.
17. I.C.H. Harmonized Tripartite Guidelines; Text on Validation of Analytical Procedures *Q2A & Q2B,* 1994 & 1996.

*****