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## Simultaneous estimation of S(-) Amlodipine Besylate Hemipentahydrate and Losartan Potassium in Combined Dosage Form by Using UV-Spectroscopy

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### ABSTRACT

Three simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of losartan potassium and s(-)amlodipine besylate hemipentahydrate in tablet dosage form have been developed. First method employs formation and solving of simultaneous equation using 247 nm and 354 nm as two analytical wavelengths for both drugs in methanol. The second method is Q value analysis based on measurement of absorptivity at 253 nm (as an iso-absorptive point) and 354 nm. Losartan potassium and s(-)amlodipine besylate hemipentahydrate at their respective  $\lambda_{max}$  247 nm and 354 nm and at isoabsorptive point 253 nm shows linearity in a concentration range of 2-20  $\mu\text{g/mL}$ . Third method is first order derivative spectrophotometric analysis based on measurement of absorptivity at 249 nm for s(-)amlodipine besylate hemipentahydrate and 220 nm for losartan potassium with cross point. Recovery studies range from 99.99% for losartan potassium and 99.98% for s(-)amlodipine besylate hemipentahydrate in case of simultaneous equation method and 99.97% for losartan potassium and 100.02% for s(-) amlodipine besylate hemipentahydrate in case of Q - analysis method and 99.98% for Losartan Potassium and 101.01% for s(-)amlodipine besylate hemipentahydrate in case of derivative spectrophotometric method, confirming the accuracy of the proposed method. The proposed methods are recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

**Key words:** Method development; Validation; Derivative Spectroscopy.

**Abbreviations:** AMLO, S (-)Amlodipine Besylate Hemipentahydrate; LOP, Losartan Potassium.

### INTRODUCTION

Losartan potassium (I, 2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-yl)phenyl] benzyl]-imidazole 5-methanol monopotassium salt) (Fig.1) is a highly selective, orally active, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension. It has a more potent active metabolite (II, 2-n-butyl-4-chloro-1-[2-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl] imidazole-5-carboxyl acid) [1]. The determination of Losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography [2,3] in urine by gas chromatography-mass spectrometry [4] and, simultaneously with its active metabolite in biological fluids and normal, by HPLC and HPTLC [5-11]. s(-) Amlodipine Besylate Hemipentahydrate, chemically, s2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester (Fig.1), is an antihypertensive and an antianginal agent in the form of the besylate salt, s(-) Amlodipine Besylate Hemipentahydrate. It is not official in any Pharmacopoeia. Various analytical methods have been reported for the assay of Amlodipine besylate [12,13] in pure form as well as in pharmaceutical formulations. They include

high performance liquid chromatography[14-19] reversed phase high performance liquid chromatography,[20-23] high performance thin layer chromatography [24-27] gas chromatography [28] gas chromatography–mass spectrometry [29] liquid chromatography with tandem mass spectrometry [30] and fluorimetry [31] derivative spectroscopy [32-34] simultaneous multicomponent mode of analysis and difference spectrophotometry [35-38]. By these two methods no UV spectrophotometric study on losartan potassium and amlodipine besylate in tablet dosage form in pharmaceutical preparations has been found in literature survey. There was only one method has been reported [39] for estimation of losartan potassium and amlodipin besylate in tablet by absorption correction method, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of losartan potassium and s(-) amlodipine besylate hemipentahydrate in tablet dosage form. This communication forms the first report of three simple, sensitive and reproducible methods for the simultaneous estimation of losartan potassium and s(-) amlodipine besylate hemipentahydrate from combined dosage form.

## MATERIALS AND METHODS

### Materials:

Spectral runs were made on a Lab-India UV-Visible spectrophotometer, model- 3200 (India) was employed with spectral bandwidth of 1 nm and wavelength accuracy of  $\pm 0.3$  nm with automatic wavelength corrections with a pair of 10 mm quartz cells. Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. s(-) amlodipine besylate hemipentahydrate and losartan potassium reference standard was kindly provided by Emcure Pharmaceuticals Ltd. Pune (M.H.). The pharmaceutical preparations of combination of losartan potassium and s(-)amlodipine besylate that is Esam LT tablet (Torrent Pharma, Ahmedabad, Gujrat). Methanol of analytical reagent grade was purchased by Loba Chemie Pvt. Ltd. (India). All the solutions were protected for light and were analyzed on the day of preparations.

### Selection of common solvent:

Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

### Preparation of Standard Drug Solution:

Standard stock solutions containing Losartan Potassium (LOP) and s(-)Amlodipine besylate (AMLO) were prepared individually by dissolving 2.5 mg of LOP and quantity of AMLO equivalent to Amlodipine base 2.5 mg separately in 20 ml of methanol. It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 50 ml with methanol to get stock solutions containing 50  $\mu\text{g}/\text{mL}$  each of LOP and AMLO in two different 50 ml volumetric flasks.

### Determination of Absorption Maxima:

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10  $\mu\text{g ml}^{-1}$  of LOP and 10  $\mu\text{g ml}^{-1}$  of AMLO were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. LOP and AMLO showed absorbance maxima at 247 nm ( $\lambda_1$ ) and 354 nm ( $\lambda_2$ ) respectively (Fig: 2&3). The overlain spectra showed  $\lambda$  max of both drugs and also isoabsorptive points at 253 nm (Fig. 4).

The derivative spectra show maximum absorption for both drug in first order derivatize is 249 nm for s(-)amlodipine besylate (Fig. 5) and 220 nm for losartan potassium (Fig. 6) and the cross point for both drugs (Fig.7).

### Method I (Simultaneous equation method):

Two wavelengths selected for the method are 247 nm and 354 nm that are absorption maximas of LOP and AMLO respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 2-20  $\mu\text{g}/\text{ml}$  concentrations. The absorbances were measured at the selected wavelengths and absorptivities ( $A$  1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2}) \quad \dots\dots\dots\text{Eq. (i)}$$

$$C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2}) \quad \dots\dots\dots\text{Eq. (ii)}$$

Where,  $A_1$  and  $A_2$  are absorbances of mixture at 247 nm and 354 nm respectively,  $a_{x1}$  and  $a_{x2}$  are absorptivities of LOP at  $\lambda_1$  and  $\lambda_2$  respectively and  $a_{y1}$  and  $a_{y2}$  are absorptivities of AMLO at  $\lambda_1$  and  $\lambda_2$  respectively.  $C_x$  and  $C_y$  are concentrations of LOP and AMLO respectively. (Table. 1)

**Method II (Absorbance ratio or Q-analysis method):**

From the overlain spectrum of LOP and AMLO, two wavelengths were selected one at 253 nm which is the isoabsorptive point for both the drugs and the other at 354 nm which is  $\lambda_{\text{max}}$  of AMLO. The absorbances of the sample solutions prepared in a similar manner as in the previous method, were measured and the absorptivity values for both drugs at the selected wavelengths were also calculated. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula,

For LOP

$$C1 = Q0 - Q2 / Q1 - Q2 \times a1 / A \quad \dots\dots\dots \text{Eq. (iii)}$$

For AMLO

$$C2 = Q0 - Q1 / Q2 - Q1 \times a2 / A \quad \dots\dots\dots \text{Eq. (iv)}$$

Where,

Absorbance of sample at 354 nm

Q0 =

Absorbance of sample at 253 nm

Absorptivity of LP at 354 nm

Q1 =

Absorptivity of LP at 253nm

Absorptivity of AB at 354 nm

Q2 = Absorptivity of AB at 253nm

A = Absorbance of sample at isoabsorptive point, a1 and a2 = Absorptivities of LOP and AMLO respectively at isoabsorptive point. (Table. 1)

**Method III (Derivative spectroscopic method):**

From the derivative spectrum of LOP and AMLO, in this method first order derivative spectra form by derivatized the normal spectra and select cross point for both drug and two wavelengths were selected for measuring of absorptivity one at 220 nm which is for LOP and 249 nm for AMLO. The absorbance's of the sample solutions prepared in a similar manner as in the previous method were measured and the absorptivity values for both drugs at the selected wavelengths were also calculated. The method employs derivative spectroscopic values and the concentrations of drugs in sample solution were determined by using the following formula, (Table. 1)

$$dA / d\lambda = (dA/dt) / (d\lambda / dt) = (dA/dt)(1/C) \quad \dots\dots\dots \text{Eq. (v)}$$

**Application of the proposed method for the determination of LP and AB in tablets:**

Twenty tablets of marketed formulation Esam LT (Torrent Pharma, Ahemadabad) containing LOP 50 mg and AMLO equivalent to Amlodipine base 2.5 mg were weighted, and finely powdered. For analysis of drug, a standard addition method was used. An accurately weighted 2.5 mg of pure AMLO was added to finely powdered samples to bring the concentration of AMLO in linearity range. With this addition, the ratio of LOP and AMLO in the samples was brought to 1:1. Quantity of powder equivalent to 2.5 mg of LOP and 5 mg of S(-)Amlodipine base was weighed and dissolved in 40 mL of methanol and sonicated for 10 minutes. Then the solution was filtered through whatman filter paper no. 41 and then final volume of the solution was made up to 50 ml with methanol to get a stock solution containing 100  $\mu\text{g ml}^{-1}$  of LOP and 100  $\mu\text{g ml}^{-1}$  AMLO. Appropriate aliquots of LOP and AMLO within the Beer's law limit were taken. In Method I, the concentration of both LOP and AMLO were determined by measuring the absorbance of the sample at 247 nm and 354 nm. Values were substituted in the respective formula to obtain concentrations.

For Method II, the concentration of both LOP and AMLO were determined by measuring absorbance of the sample at 253 nm and 354 nm and values were substituted in the respective formula to obtain concentrations. Results of tablet analysis are shown in Table 3.

For Method III, the concentration of both LOP and AMLO were determined by measuring absorbance of the sample at 253 nm and 354 nm and values between cross point and measure the absorptivity. (Table. 2)

**VALIDATION:**

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

**Accuracy:**

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for LOP and AMLO, by all the three methods, was found in the range of 100.46% to 99.99%.

**Linearity:**

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of LOP and AMLO. For simultaneous equation method, Q analysis and derivative method, the Beer- Lambert's concentration range was found to be 2-20 µg/ml for LOP and AMLO.

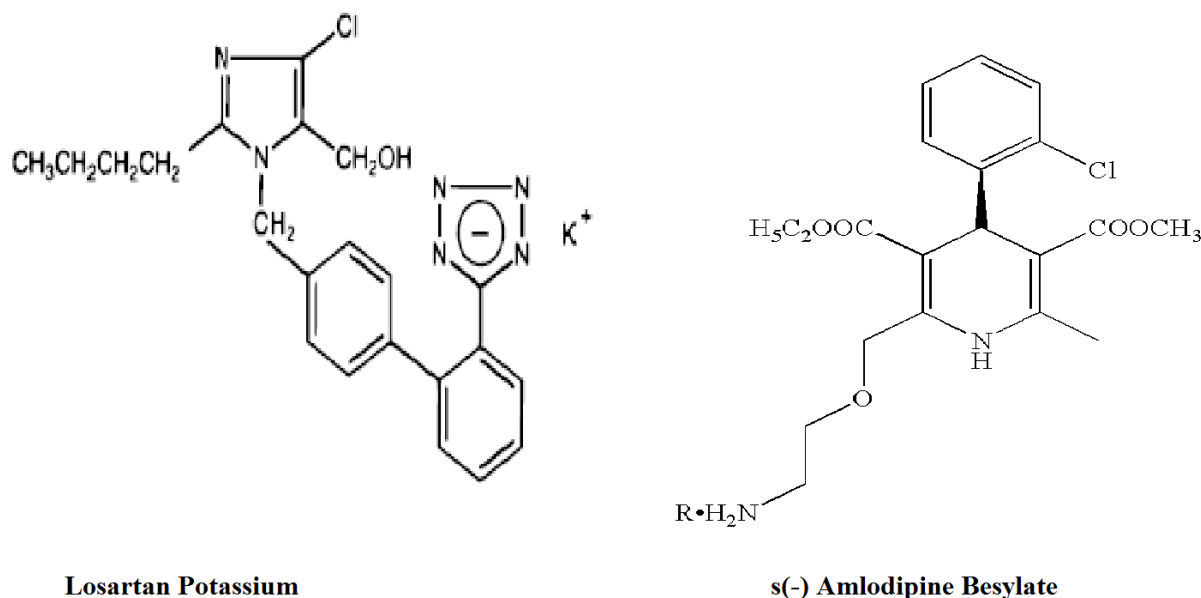
**Precision:**

Precision was studied to find out intra and inter-day variations in the test method of LOP and AMLO. Calibration curves prepared in medium were run in triplicate in same day and for three days. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are tabulated in Table 4.

**RESULTS AND DISCUSSION**

The overlain spectra of LOP and AMLO exhibit  $\lambda_{max}$  of 247 nm and 354 nm for LOP and AMLO respectively which are quite separated from each other. Additionally one isoabsorptive point was observed at 253 nm. This wavelength was selected for simultaneous estimation of LOP and AMLO for Q value analysis and it is assume to be sensitive wavelength. Standard calibration curves for LOP and AMLO were linear with correlation coefficients ( $r$ ) values in the range of 0.9988- 0.9997 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.03 – 0.82. The calibration curves were repeated three times in a day and the average % RSD was found to be 0.592 for LOP and 1.12 for AMLO, similarly the method was repeated for three different days and average % RSD was found to be 1.91 for LOP and 1.33 for AMLO. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of standard additions; recovery in the range of 95 – 110% justifies the accuracy of method.

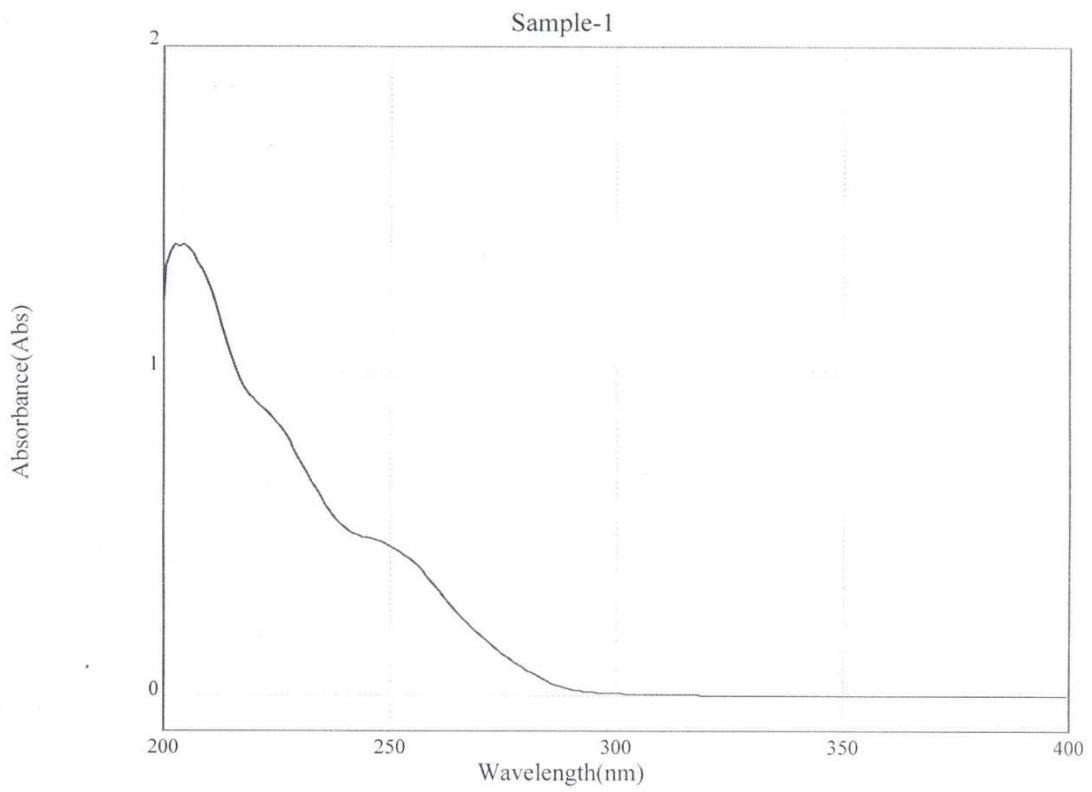
**Fig. 1: Structure of Losartan Potassium and S(-)Amlodipine Besylate.**



**Table 1: Linear regression analysis of calibration curves with their respective absorptivity values**

| Parameter                               | Method I |          | Method II |          | Method III |          |
|---|----------|----------|-----------|----------|------------|----------|
|   | LOP      | AMLO     | LOP       | AMLO     | LOP        | AMLO     |
| Beer's law limit (µg ml-1)              | 2-20     | 2-20     | 2-20      | 2-20     | 2-20       | 2-20     |
| Correlation coefficient (r)             | 0.9988   | 0.9991   | 0.9993    | 0.9970   | 0.9989     | 0.9991   |
| Molar absorptivity (lit/mole/cm)        | 41944.37 | 19635.21 | 21296.41  | 19694.31 | 28299.41   | 17694.30 |
| Sandell's sensitivity (mcg/Sq.cm/0.001) | 0.0107   | 0.0210   | 0.021546  | 0.021872 | 0.01246    | 0.02123  |
| Slope                                   | 0.0955   | 0.0468   | 0.04602   | 0.04325  | 0.04925    | 0.03221  |
| Intercept                               | -0.0063  | -0.0158  | -0.01753  | -0.02353 | -0.0046    | -0.0134  |

**Fig. 2:  $\lambda_{max}$  of Losartan Potassium.**



**Fig. 3:  $\lambda_{max}$  of Amlodipine Besylate.**

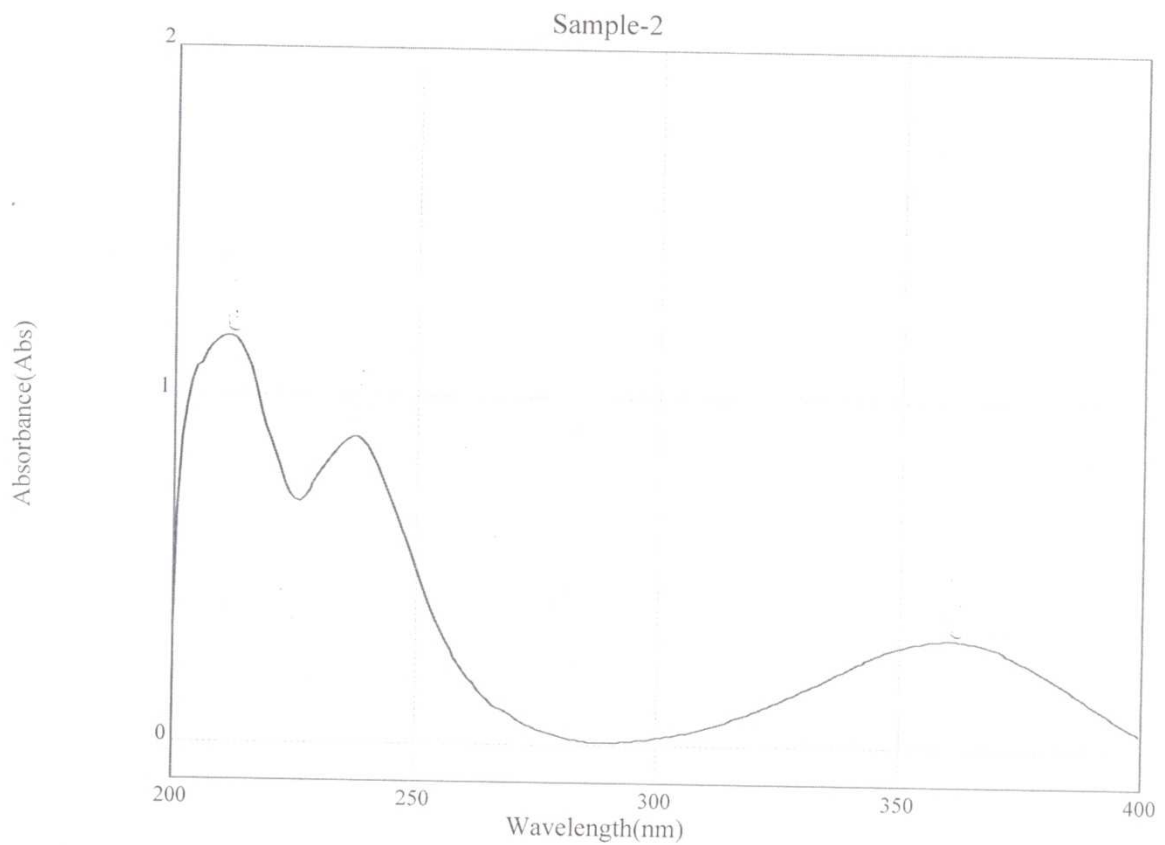


Fig. 4: Overlain spectra for Losartan Potassium and s(-)Amlodipine Besylate.  
Sample-1

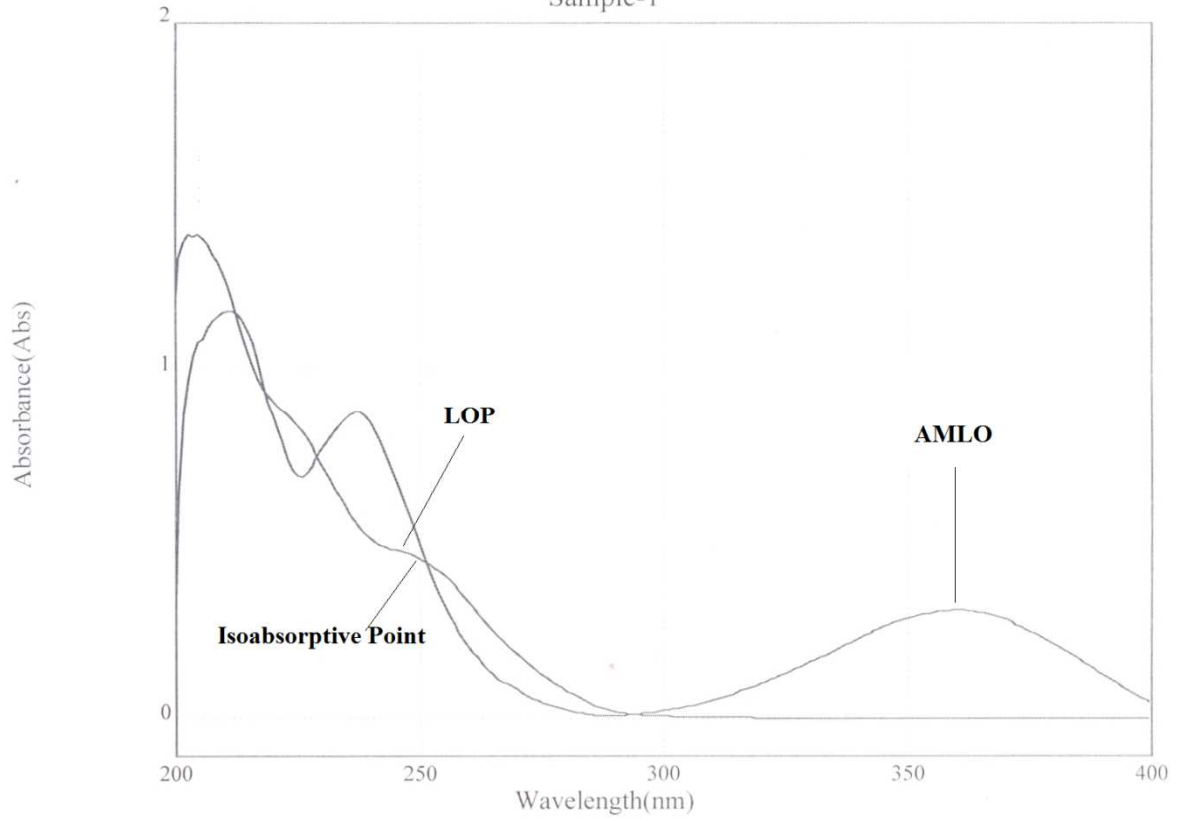
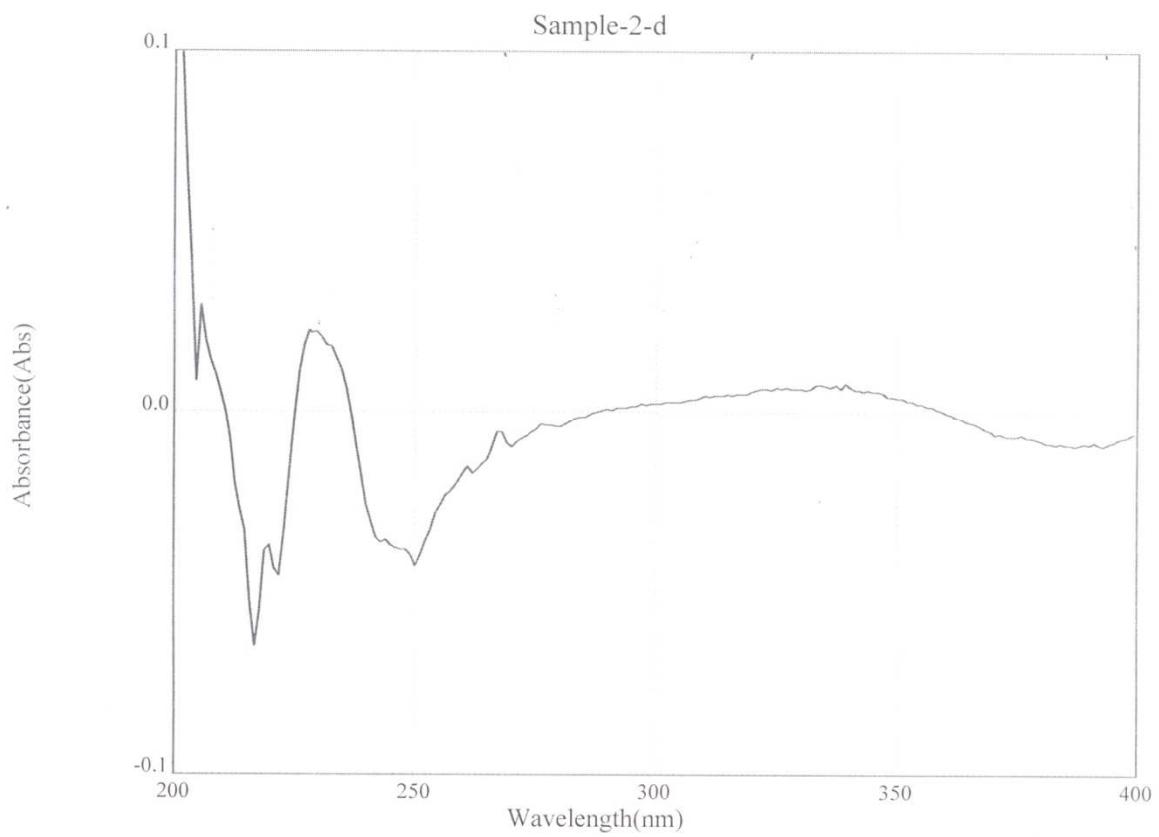
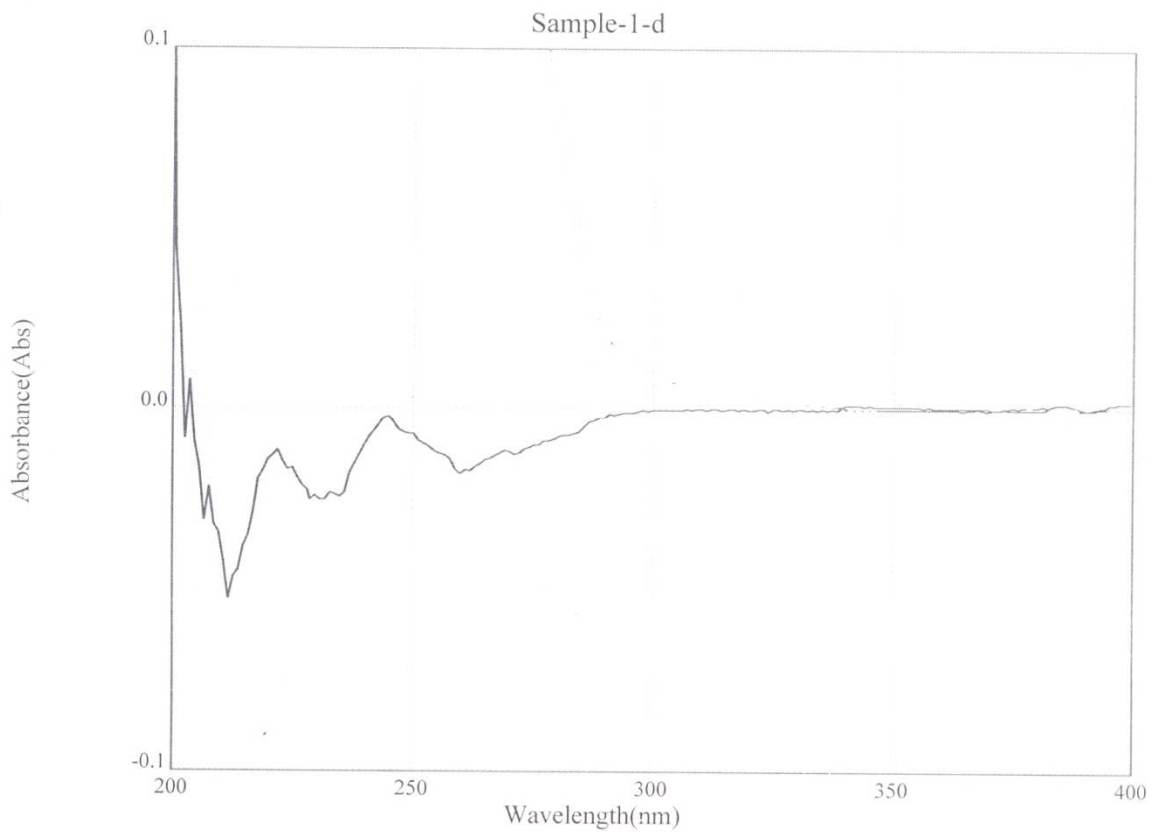


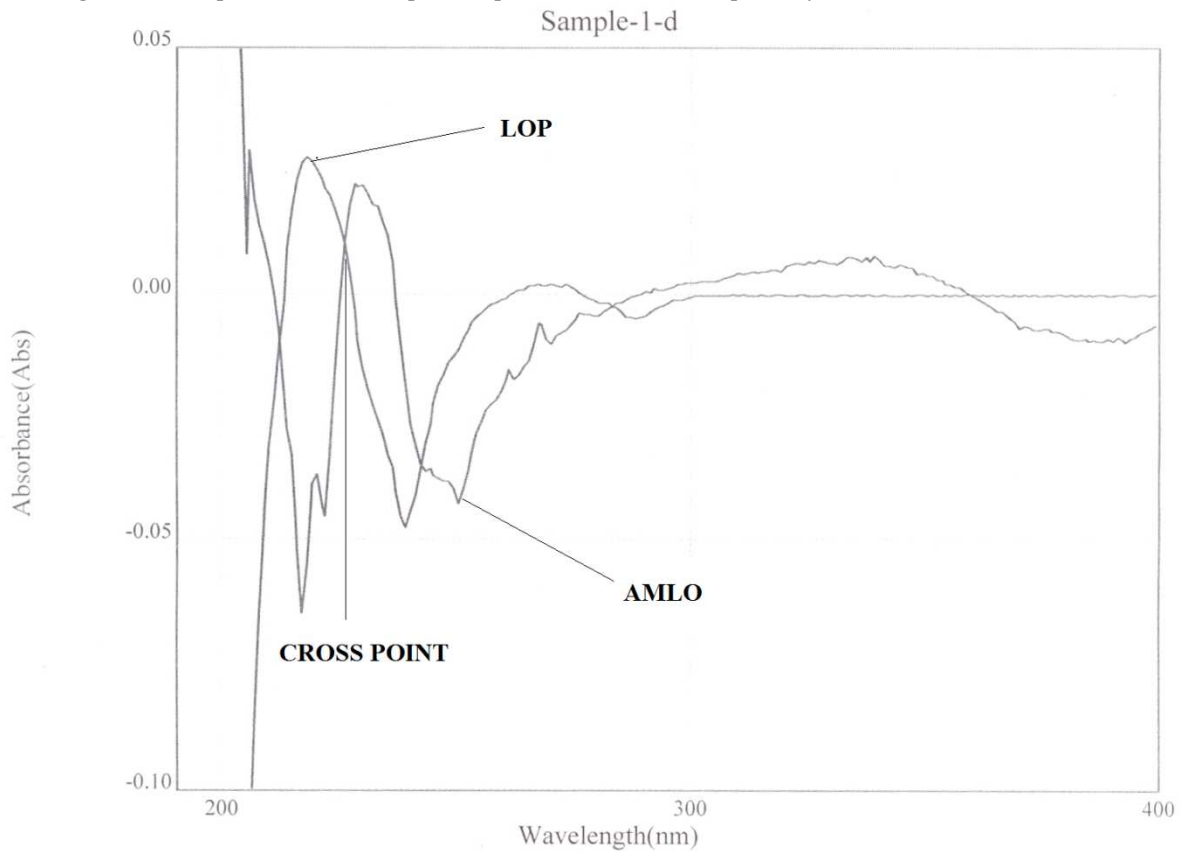
Fig. 5: First order derivative spectra for s(-)Amlodipine Besylate.



**Fig. 6: First order derivative spectra for Losartan Potassium.**



**Fig. 7: Overlain spectra of derivative spectroscopic method for s(-)Amlodipine Besylate and Losartan Potassium.**



**Table 2: Results of analysis of laboratory samples**

| Analyte                             | Method I         |                  | Method II        |                   | Method III       |                  |
|-------------------------------------|------------------|------------------|------------------|-------------------|------------------|------------------|
|                                     | LOP              | AMLO             | LOP              | AMLO              | LOP              | AMLO             |
| % Conc. Estimated (Mean $\pm$ S.D.) | 99.79 $\pm$ 0.13 | 99.74 $\pm$ 0.15 | 99.85 $\pm$ 0.03 | 101.09 $\pm$ 0.82 | 99.99 $\pm$ 0.04 | 99.89 $\pm$ 0.09 |
| Coefficient of variance             | 0.0008           | 0.0002           | 0.0001           | 0.0004            | 0.0002           | 0.0001           |

\*Average of three determinations; R.S.D.; Relative Standard Deviation.

**Table 3: Results of analysis of tablet samples**

| Method | Drug | Label Claim | %Label Claim $\pm$ R. S. D. | Coefficient of variance | %Recovery* (Mean $\pm$ R. S. D) |
|--------|------|-------------|-----------------------------|-------------------------|---------------------------------|
| I      | LOP  | 2.5         | 102.33 $\pm$ 1.124          | 0.0155                  | 99.99 $\pm$ 0.92                |
|        | AMLO | 50          | 101.01 $\pm$ 1.25           | 0.111                   | 99.93 $\pm$ 0.71                |
| II     | LOP  | 2.5         | 100.93 $\pm$ 1.66           | 0.072                   | 99.97 $\pm$ 0.22                |
|        | AMLO | 50          | 102.05 $\pm$ 1.25           | 0.111                   | 100.02 $\pm$ 0.39               |
| III    | LOP  | 2.5         | 102.01 $\pm$ 1.15           | 0.0145                  | 99.98 $\pm$ 0.33                |
|        | AMLO | 50          | 100.23 $\pm$ 1.11           | 0.112                   | 101.01 $\pm$ 0.13               |

\*Average of three determinations; R.S.D.; Relative Standard Deviation

**Table 4: Results of intermediate precisions**

| Day      | Method I                                       |                   | Method II                                      |                  | Method III                                     |                   |
|----------|--|-------------------|--|------------------|--|-------------------|
|          | % Label claim estimated* (Mean $\pm$ % R.S.D.) |                   | % Label claim estimated* (Mean $\pm$ % R.S.D.) |                  | % Label claim estimated* (Mean $\pm$ % R.S.D.) |                   |
|          | LOP  | AMLO              | LOP  | AMLO             | LOP  | AMLO              |
| Intraday | 105.83 $\pm$ 0.73                              | 100.04 $\pm$ 0.43 | 100.87 $\pm$ 0.56                              | 112.5 $\pm$ 2.2  | 100.86 $\pm$ 0.53                              | 105.81 $\pm$ 0.76 |
| Interday | 106.86 $\pm$ 1.27                              | 108.05 $\pm$ 1.71 | 99.25 $\pm$ 1.76                               | 100.6 $\pm$ 0.36 | 105.05 $\pm$ 1.72                              | 100.5 $\pm$ 0.31  |

## CONCLUSION

The most striking feature of this method is its simplicity and rapidity, non- requiring- consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These are new and novel methods and can be employed for routine analysis in quality control analysis. The described methods give accurate and precise results for determination of Losartan potassium and s(-)Amlodipine besylate mixture in marketed formulation.

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