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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING CE ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND LISINOPRIL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, accurate, precise and sensitive capillary electrophoresis method has been optimized for the separation and stability indicating assay of antihypertensive drugs, amlodipine and lisinopril in the commercial tablet formulations. The separation of both drugs and their degradation products were tested and evaluated under different electrophoretic conditions and best results were obtained using a fused silica capillary with 25mM phosphoric acid buffer (pH 2.45). The detection was performed at a UV wavelength of 214 nm. In this optimized method, 4-Aminopyridine has been used as an internal standard and is stable over stressed conditions used in this experiment. The drugs were subjected to oxidation, hydrolysis, photolysis and heat to apply stress conditions. The proposed CE method is also superior over the reported TLC method in terms of simplicity, cost and resolving the number of degradation products. The method was also validated. This is the first report on an ideal stability indicating CE method that quantifies selectively amlodipine and lisinopril in the presence of degradation products.

Keywords: Capillary zone electrophoresis, specific stability indicating assay, amlodipine, lisinopril, pharmaceutical formulations

## INTRODUCTION

Lisinopril (LS) chemically (2S)-1-[(2S)-6-amino-2-[{(1S)-1-carboxy-3-phenylpropyl} Amino] hexanoyl] pyrrole-2-carboxylic acid, an angiotensin converting enzyme (ACE) inhibitor is used in the management of hypertension. Similarly, amlodipine (AM) chemically 3-Ethyl-5-methyl (4RS)-

2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5dicarboxylate benzene sulphonate (2) a long-acting calcium channel blocker used in the management of hypertension, chronic stable angina pectoris and coronary artery disease. Combined dosage form of both drugs has a marked additional effect on blood pressure as compared with their mono-therapy(1,3).

A literature survey reveals that a few analytical methods have been reported for separate determination of AM and LS of single dosage form, as well as combined dosage forms of these drugs with combination of other drugs. Methods reported include UV – visible spectrophotometry, HPLC methods. To our knowledge no stability indicating CE method has yet been reported for separation and determination of AM and LS in pharmaceutical formulations.

Capillary electrophoresis (CE) is an advanced technique in the field of separation science, as it displays an enormous efficiency and possesses inherent advantages over conventional separation techniques due to its advantages such as small sample volume, high separation efficiency, low operationg and consumable costs, short analysis time and easy to optimize the analytical conditions(4-6). Currently, it has been increasingly used as an alternative separation method capable of faster analysis and higher efficiency than HPLC or complementary technique to HPLC in drug and pharmaceutical analysis(7-8).

Now, we are reporting a stability indicating CE method for the determination of AM and LS in bulk drug and combined pharmaceutical formulations. An ideal stability indicating assay method is one that quantifies selectively in the presence of degradation products. Therefore, an aim of present work is to develop an accurate, specific, reproducible and cost-effective stability indicating CE method for determination of AM and LS in pharmaceutical formulations and validated as per ICH guidelines.

## EXPERIMENTAL

### Instrumentation

All the CE experiments were performed using a CE system (Prince Technologies, model no.460, The Netherlands) equipped with a Lambda 1010 UV-vis detector and an auto sampler. An uncoated fused silica capillary of 75 µm i.d. (Polymicro, Phoenix, AZ, USA) with a total length of 50 cm (effective length 43 cm) was used for the separation. A new capillary was conditioned by rinsing with 1.0 M sodium hydroxide for 30 min, water for 15 min, and finally with the buffer solution for 15 min. Between each run, the capillary was rinsed with water for 3 min, 0.1 M sodium hydroxide for 2 min, water for 3 min, and the buffer solution for 3 min successively. The capillary was thermostated at $25^0$C. A sample was kept in auto sampler and the normal stacking was performed using hydrodynamic injection by applying pressure of 25 mbar for 12 s. Both peak height and corrected peak area (peak area divided by migration time) increased in proportion to the injection time. The 12 s injection was the most suitable in terms of peak shapes. For much longer injection time peaks showed asymmetric shapes. A constant voltage of +20 kV was applied throughout the analysis. Analyte detection was performed at a UV wavelength of 214 nm. Data acquisition and analysis were carried out with the DAX software supplied by Prince Technologies. Chemicals and Reagents

The organic solvents used for sample preparation and separation were of analytical reagent grade. Deionized water was produced by a Millipore water purifying system (Millipore, Molsheim, France). Orthophosphoric acid was purchased from Qualigens Fine Chemicals (Mumbai, India). Sodium hydroxide, hydrochloric acid, hydrogen peroxide, 4- aminpyridine were purchased from S.D.Fine Chem. (Mumbai, India). Amlodipine (AM) working standard and lisinopril (LS) working standard were provided by Mass Spectrometry Division, IICT. Combination tablets of different brands were purchased from a local pharmacy shop.

Preparation of running buffer and standards

Required concentration phosphate buffer was prepared using ortho-phosphoric acid and the pH (2.0- 4.0) was adjusted with 1.0 M sodium hydroxide. A standard stock solution 1.0 mg/ ml of each AM and LS was prepared separately by dissolving in methanol: water (20:80). The required working standards were prepared by diluting the stock solution with 10 mM phosphoric acid prior to the analysis. The stock solution was protected from light and stored in refrigerator.

Preparation of Commercial sample

Twenty tablets (combined dosage of LS and AM) were accurately weighed and finely powdered. A quantity of powder equivalent to 5.0 mg of LS and 5.0 mg of AM was weighed and transferred to a 5.0 ml flask. The drug is dissolved in methanol:water (20:80) and shaken mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and makes the volume up to the mark with10 mM phosphoric acid. The solution was filtered with a whatmann filter paper no.1.

Before the analysis, both standard and sample solutions were filtered using a Millipore filter (0.45 µm).

Stress degradation studies

All degradation studies were carried out at 1 mg/mL concentration of AM and LS in 0.1 N HCl and 0.1 N NaOH solutions (1000 ng/µl) separately and these mixtures were refluxed for 8 hr at 80 ºC. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. Oxidative degradation was carried out in 3% h2o2 at room temperature for 6 and 24h. For thermal degradation, the drugs were placed in hot air oven at 800c for 2h. Photolytic degradation were carried out in direct sunlight for 48 hours during the daytime for six days, respectively. UV degradation was carried by drugs which are evenly spread in thin layer in a covered petridish and were kept at uv short (254 nm) and uv long (366 nm) light for 12 hr. The buffer and sample solutions were filtered using 0.45µm micro syringe filters prior to the CE analysis.

Drug solutions stability was evaluated under the refrigerated ($4^0$c) storage conditions for a period of 1 month. The concentrations of freshly made solutions and those tested after a period of 1 month did not vary much (<1%).

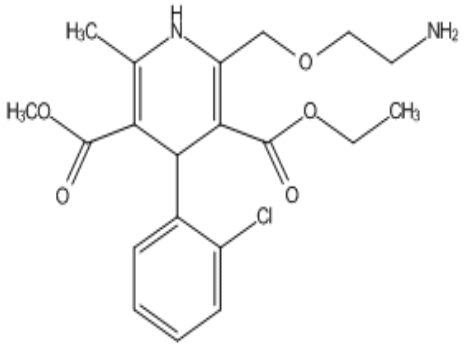
RESULTS AND DISCUSSIONS

Method development

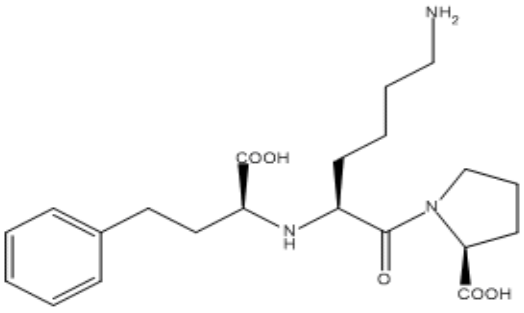
LS is characterized by the presence of an ionizable carboxylic and amine groups. It possesses four pKa values 2.5 and 4.0 for the two carboxylic groups; 6.7 and 10.1 for the primary and secondary amine groups. Similarly AM has pKa value of about 8.6. Both drugs are expected to be protonated

at acidic pH. Therefore, preliminary studies were carried out with 25 mM phosphoric acid (pH 2.5). The electrophoresis buffer is of key importance in CE because its composition fundamentally determines the migration behaviour of the analytes. The ionic strength or concentration of buffer has significant efforts on drug mobilities and separation efficiency. Varied the buffer concentrations ranging from 10-75 mM and separation efficiency are studied at fixed pH of 2.5.

The degree of ionization of species present in the electrolyte system depends on pH of the solution. Differences in the degree of ionization give rise to differences in electrophoretic and electroosmotic mobilities which exhibits good separation efficiency and flow velocities. Owing to the amphoteric character of LS and AM a pH value of 2.5 – 4.0 is expected to be ideal for separation of both analytes. Therefore, pH of the buffer was varied from 2.0 to 4.0 (Fig.2.2). Below pH 2.0, LS possess good peak symmetry but it co-eluted with AM. The separations of the both drugs were almost similar over a pH range 2.5- 4.0. At lower pH the reduction of the electro osmotic flow (EOF) increased the analysis time and resulted in a loss of efficiency. It can be seen that both drugs and the degradation products are well separated at 25 mM phosphate buffer at pH 2.5 was chosen for further studies.




Amlodipine (pka 8.6)




Lisinopril (pka 6.7 and 10.1)

Stress Degradation Behaviour

The degradation studies indicate that the active drug ingredients AM and LS under goes hydrolysis in acidic and basic as evident from the additional peak appeared (migration time 11.905 (AM), 13.368, 13.812 (LS) min, with a proportionate difference in migration time of the main drug at 11.645 (AM) and 13.288 (LS) and almost. The drugs was degraded in hydrogen peroxide (3%) in dark at room temperature and shows degradation product. The drugs also showed little amount of degradation under photo light and under ultraviolet light. The active ingredients also undergo degradation under dry heat.

Comparison of CE and TLC methods

The comparison of CE and TLC methods were performed by analyzing different stress degradation samples under the optimized CE and TLC (mobile phase: n-butanol: acetic acid: water; 6:2:2 v/v/v; at wavelength 560nm) conditions. Under different stressed conditions the number of degradation products observed in CE is higher than that of TLC method. The reason may be due to the superior resolving power of CE, the use of lower wavelength 214 nm and lower solvent consumption (14).

Method Validation

Validation steps were carried out in accordance with ICH guideline Q2 (R1) [15-18]. The validated CE method was then adapted to the assessment of marketed AM and LS combination tablets (913).

Selectivity/Specificity

According with ICH, the term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method which provides responses for a number of chemical entities that may be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective.

This test was performed by recording electropherograms of placebo blank solution (Fig.2.6) and the drug mixture spiked in placebo solution (Fig.2.9).Under the optimized conditions,

the drugs from combined formulation have been well separated without interference of excipients and degradation products. Therefore, the present method is selective and specific.

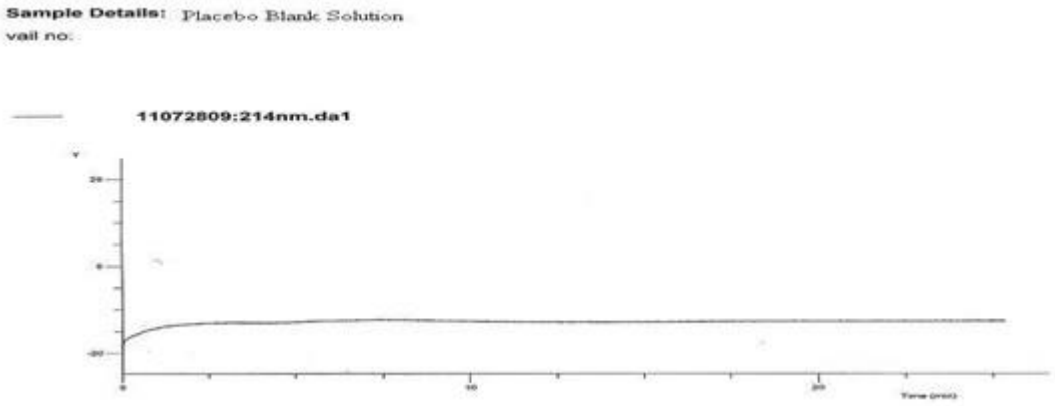



Fig. Electropherogram of placebo blank solution

Linearity

According to ICH guidelines, the linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes in samples within a given range. Linearity is evaluated graphically.

In present study, the linearity range was calculated under optimized conditions with the concentration range of 5.0 – 100.0 µg/ml. The concentrations of drugs vs. peak area ratio of drugs with internals standard were plotted (Fig.2.7 and 2.8) and the validation parameters obtained was summarized in the Table.2.2.

Table. validation parameters for the determination of standard solutions of AM and LS by proposed method

| Parameters | Amlodipine (AM) | Lisinopril (LS) |
|---|---|---|
| Conc. Range (µg/ml) | 5-100 | 5-100 |
| Intercept(a) | -0.001 | -0.003 |
| Slope(b) | 0.003 | 0.002 |
| Correlation coefficient (R2) | 0.998 | 0.998 |

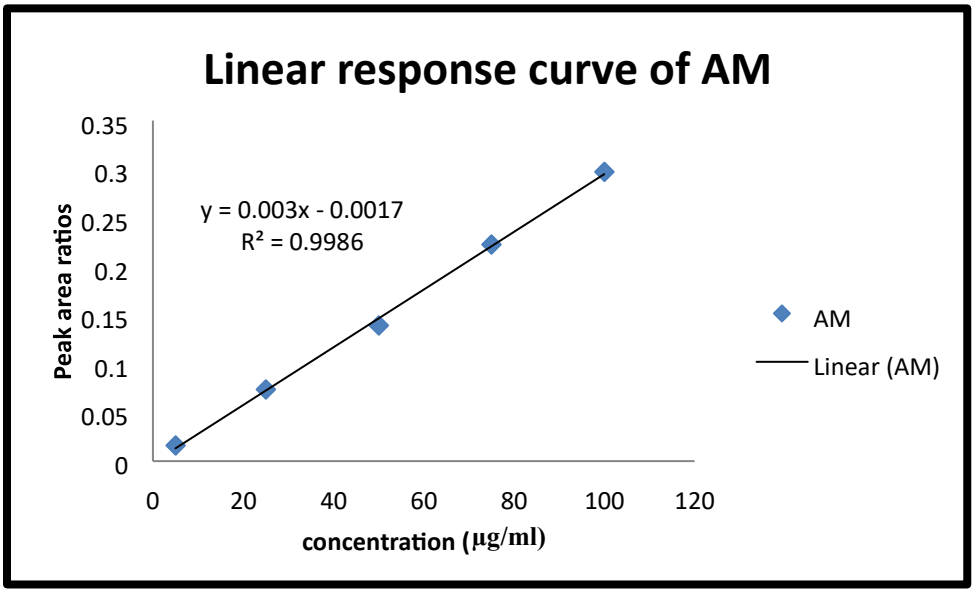



Fig.2.7 Linear response curve of AM, determined by peak area ratios (PAR) vs concentration (µg/ml)

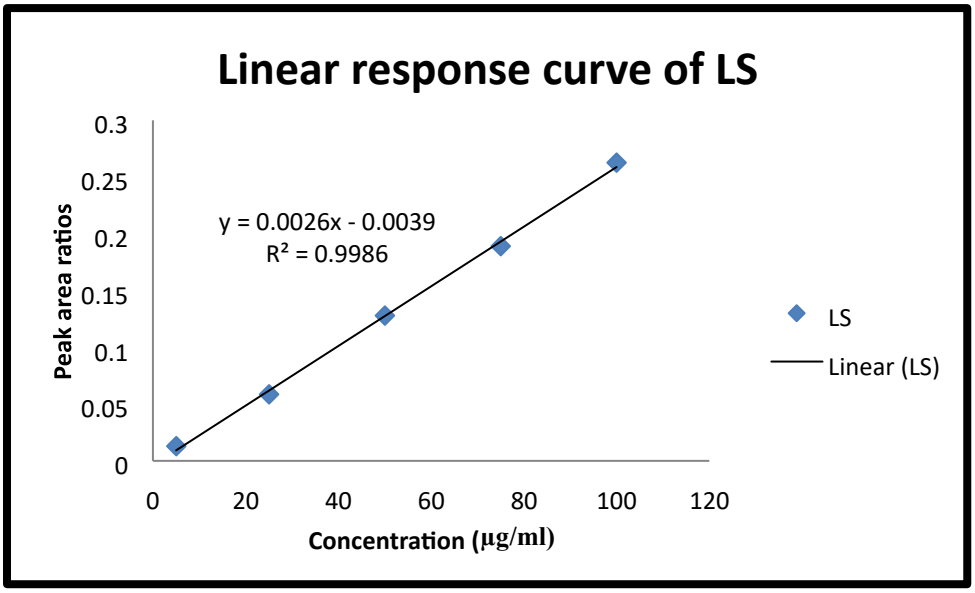



Fig.2.8 Linear response curve of LS, determined by peak area ratios (PAR) vs concentration (µg/ml)

Accuracy & Precision

Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the proposed method was assessed by recover studies. This was performed by comparing the drugs standard solutions with IS. In present work, all the samples were injected in six replicates for each concentration under the optimized conditions. The recovery percentage was calculated against the concentration added. The analysis results are summarized in Table.2.3. The recoveries of AM and LS were found to be ≤100.32 and ≤100.01 respectively.

The precision of this method is evaluated in terms repeatability, intermediate precision and reproducibility. Repeatability (intra-day) of the analytical method was tested by analyzing the analytes in a mixture of a solution. In order to determine the repeatability of this method, replicate injections (n=3) of a standard solutions containing lower, middle and higher linearity range were carried out in the optimum conditions as described previously. The precision was calculated as percentage of relative standard deviation (% RSD) values of relative migration times (RMT) and peak area ratios (PAR) (in relative to the IS) were reported in the following Table.2.4.1 and Table.2.4.2. This performance suggests that the proposed CE method presents acceptable reproducibility.

Table. Accuracy data for the assay of AM and LS

| Drug | Conc. Added (µg/ml) | Found (mg/ml) | Recovery (%)[a] | % RSD (n=6) |
|---|---|---|---|---|
| AM | 25 | 25.08 | 100.32 | 1.01 |
| | 50 | 49.87 | 99.74 | 1.06 |
| | 100 | 99.69 | 99.69 | 0.99 |
| LS | 25 | 24.92 | 99.68 | 1.1 |
| | 50 | 49.78 | 99.56 | 1.07 |
| | 100 | 100.01 | 100.01 | 1.01 |

Recovery (%)[a] = (calculated amount/ theoretical amount) × 100

Table. Repeatability and precision data for AM and LS in tablet formulations [A]
Intraday (n=3)

| Drug | Conc. Added (µg/ml ) | Intraday (n=3) | | Interday | |
|---|---|---|---|---|---|
| | | RMT | PAR | RMT | PAR |
| | | % RSD | % RSD | % RSD | % RSD |
| AM | 25 | 0.765 | 0.968 | 0.798 | 1.108 |
| | 50 | 0.781 | 0.903 | 0.8011 | 1.115 |
| | 100 | 0.802 | 0.9411 | 0.791 | 0.991 |
| LS | 25 | 0.796 | 1.047 | 0.826 | 1.137 |
| | 50 | 0.812 | 1.012 | 0.841 | 1.122 |
| | 100 | 0.803 | 0.993 | 0.836 | 1.13 |

Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In present study, the three parameters were investigated: (1) buffer concentration (24.0 & 26.0 mM), (2) pH (2.4 & 2.6) and (3) the applied voltage (19 & 21 kV).The results are summarized in (Table.2.5 [A] and [B]). The results indicated that there is no significant change in separation and peak efficiencies of the both drugs and internal standard.

Table. Robustness data results for AM, LS and IS (50 µg/ml each) (n=3) representing

[A] Relative migration time (RMT)

| | AM | | LS | |
|---|---|---|---|---|
| | Mean | % RSD | Mean | % RSD |
| Standard conditions* | 2.264 | 0.88 | 2.736 | 0.73 |
| Buffer pH | | | | |
| 2.4 | 2.2642 | 0.89 | 2.739 | 0.74 |
| 2.6 | 2.2638 | 0.9 | 2.736 | 0.71 |
| Buffer conc. (mM) | | | | |
| 24 | 2.2639 | 0.91 | 2.737 | 0.77 |
| 26 | 2.264 | 0.87 | 2.736 | 0.76 |
| Applied voltage(kV) | | | | |
| 19 | 2.2641 | 0.86 | 2.735 | 0.71 |
| 21 | 2.2642 | 0.88 | 2.737 | 0.75 |

[B] Peak area ratios (PAR)

| | AM | | LS | |
|---|---|---|---|---|
| | Mean | % RSD | Mean | % RSD |
| Standard conditions* | 2.387 | 0.99 | 2.972 | 1.1 |
| Buffer pH | | | | |
| 2.4 | 2.384 | 1.01 | 2.981 | 1.1 |
| 2.6 | 2.387 | 0.99 | 2.976 | 1.11 |
| Buffer conc. (mM) | | | | |

| | | | | |
|---|---|---|---|---|
| 24 | 2.389 | 1.02 | 2.987 | 1.07 |
| 26 | 2.391 | 0.97 | 2.983 | 1.16 |
| Applied voltage(kV) | | | | |
| 19 | 2.388 | 0.98 | 2.965 | 1.21 |
| 21 | 2.39 | 1.08 | 2.969 | 1.07 |

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of quantitation is the minimum injected amount that gives precise measurements. The .LOD and LOQ for AM and LS was determined by using the optimized method. Results obtained were LOD about 0.5 (AM, µg/ml); 1.0 (LS, µg/ml) and LOQ about 2.5(AM, µg/ml); 5.0 (LS, µg/ml). DAx software provided by the instrument manufacturer was used for the calculation of signal-to-noise ratio.

ANALYSIS OF PHARMACEUTICAL FORMULATIONS

The developed and validated method was applied for the simultaneous determination of AM, LS in pharmaceutical formulations obtained from different sources (15,16) and results are shown in Table.2.6. Fig.2.9 and 2.10 shows the electropherograms of standard mixtures of AM and LS, with a constant amount of IS and combined tablet formulation of AM and LS with a constant amount of IS. Each pharmaceutical formulation was analyzed with six independent determinations and each series was injected three times. The percentage recoveries (Table.2.6) of the drugs were between 99.00 and 100.4% indicating the good agreement with the label claims. The recover ranges observed were with better precision (<1.08% RSD) and good selectivity in real tablet sample.

Table.2.6. Results of analysis of combination tablets (AM and LS)

| Commercial tablet | Actual ingredients | Labeled claim (mg) | Amount found (mg) | Recovery (%) | % RSD (n=6) |
|---|---|---|---|---|---|
| Lipril – AM | AM | 5 | 4.98 | 99.6 | 1.08 |

| | | | | | |
|---|---|---|---|---|---|
| | LS | 5 | 5.01 | 100.2 | 0.96 |
| Amlopres – L | AM | 5 | 5.02 | 100.4 | 1.01 |
| | LS | 5 | 5.01 | 100.2 | 0.99 |
| Amlosafe – LS | AM | 5 | 4.96 | 99.2 | 0.98 |
| | LS | 5 | 4.95 | 99 | 1.03 |
| Amlokind | AM | 5 | 5.02 | 100.4 | 0.99 |
| Lipril | LS | 5 | 4.99 | 99.8 | 0.96 |

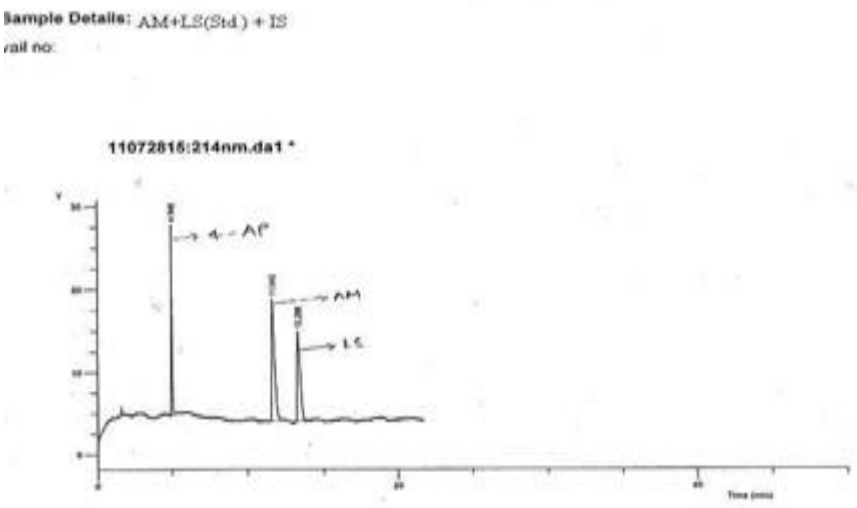



Fig. 2.9 Electropherogram of standard mixtures of AM and LS, with a constant amount of IS

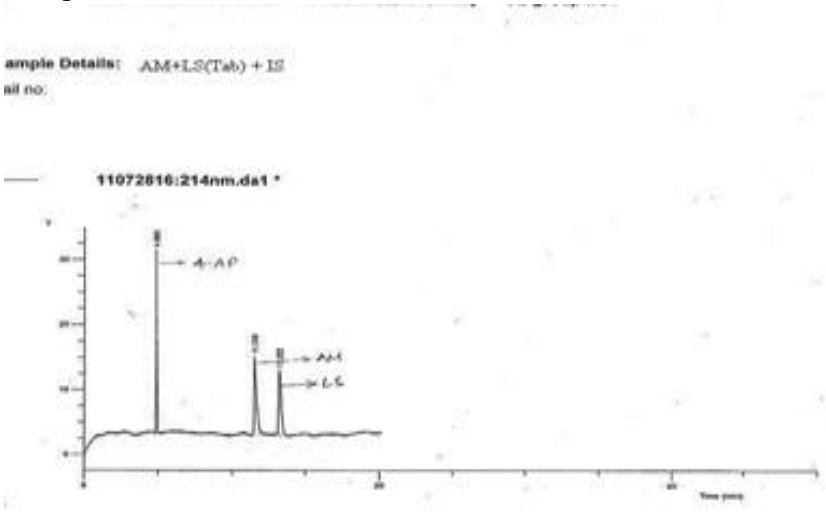


Fig. 2.10 Electropherogram of combined tablet formulation of AM and LS with a constant amount of IS

## CONCLUSION

The present capillary zone electrophoresis (CZE) method is simple, selective and suitable for the stability indicating assay of simultaneous determination of AM and LS in pharmaceutical formulations. The good separation of both the drugs was achieved within 15 min, using 25 mM phosphoric acid (pH 2.5), and acetonitrile (15%) as an organic modifier. The proposed CZE method shows recover ranges (99.00 and 100.4%) with better precision (<1.08% RSD) and good selectivity in real tablet samples. The proposed method is more stable over the existing spectrophotometric method, where the analysis provides better selectivity and accuracy than the zero-crossing derivative spectrophotometric method. Therefore, the developed method is an alternative method for routine analysis of AM and LS in pharmaceutical formulations and there is no interference of degradation products.

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