International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

R E S E A R C H   A R T I C L E

Development and Validation of Micellar Electrokinetic Chromatographic Assay Method for Simultaneous Estimation of Antihypertensive Drugs in Pharmaceutical Formulations

A. Ramyasree[1*], Dr. R. Sekar[2]

[1]NRI college of Pharmacy, Vijayawada, Andhra Pradesh
[2]Indian Institute of Chemical Technology (IICT), Hyderabad, Telangana

A B S T R A C T

A micellar electrokinetic capillary chromatographic (MEKC) method has been developed for the simultaneous determination of anti-hypertensive drugs amlodipine (AM) and lisinopril (LS) in pharmaceutical formulations. Analysis was performed in a 75 µm ID uncoated fused-silica capillary with 50 cm length using a buffer consisting of 12.5 mM sodium dodecyl sulphate (SDS) and 10 mM sodium tetraborate (STB), with pH 9.7. All analytes were separated within 11 min with the applied voltage of +25 kV (current ~ 50 µA). Samples were injected hydrodynamically by applying 25mbar pressure for 12 s. The developed method was validated in terms of linearity, accuracy, precision, limit of quantitation (LOQ), and limit of detection (LOD). The intra- and inter-day precision for amlodipine and lisinopril were 1.02, 1.81, 1.51, and 1.68 respectively. The linearity of method was tested over the range of 5-100 µg/ml (r2 = 0.998) for AM, (r2 = 0.999) for LS. Recovery of standard mixtures was found to be 99.56% with the relative standard deviation (RSD) 1.30%. The LOQ were 2.5 µg/ml, (RSD. 1.10%, n=5), 2.0 µg/ml, (RSD. 0.98%, n=5), LOD were found to be 1.0 and 0.5 µg/ml for AM and LS respectively. The method was applied for the analysis of both drugs in combined dosage form. The average content of the both drugs in pharmaceutical formulations were ranging from 98.6 to 101.8% respectively. The present method is also suitable for the determination of counter ion besylate (benzene sulphonate) in pharmaceutical formulations.

Keywords: Micellar electrokinetic capillary chromatography, amlodipine, lisinopril, pharmaceutical formulations

A R T I C L E   I N F O

Corresponding Author
A. Ramyasree
NRI college of Pharmacy,
Vijayawada, Andhra Pradesh
Email: ramya.attaluri@gmail.com
MS-ID: IJCPS3648

PAPER-QRCODE

A R T I C L E   H I S T O R Y: Received 25 July 2018, **Accepted** 19 August 2018, **Available Online**10 October 2018

Copyright© 2018 A. Ramyasree. *Production and hosting by Pharma Research Library. All rights reserved.*

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Citation: *A. Ramyasree.* Development and Validation of Micellar Electrokinetic Chromatographic Assay Method for Simultaneous Estimation of Antihypertensive Drugs in Pharmaceutical Formulations. *Int. J. Med. Pharm. Res., 2018, 6(5): 197-202.*

C O N T E N T S

1. Introduction. 198
2. Materials and Method. 198

3. Results and Discussion. 199
4. Conclusion. 202
5. References. 202

1. Introduction

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 operating and consumable costs, short analysis time and easy to optimize the analytical conditions.. 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[1-3].

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, 5-dicarboxylate benzene sulphonate 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.

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. The present study is primarily aimed at developing a simple, accurate, precise and sensitive MEKC method for the separation and determination of anionic, cationic and neutral species in combined pharmaceutical formulations of AM and LS and mainly involve in the determination of counter ion besylate in pharmaceutical formulations. The final method is validated as per ICH guidelines.

2. Materials and Methods

Instrumentation and electrophoresis procedure

All the CE experiments were performed using a CE system (Prince Technologies, model no.760, 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 73.6 cm (effective length 62 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 250C. 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 +25 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

All chemicals used in the analysis were of analytical reagent grade. Standard drugs amlodipine (AM) and lisinopril (LS) were gift samples from Mass Spectrometry Division, IICT. Combination tablets of different brands were purchased from local pharmacies. Sodium tetra borate (STB), sodium dodecyl sulfate (SDS) were purchased from Qualigens Fine Chemicals (Mumbai, India). Sodium hydroxide was purchased from S.D.Fine Chem. (Mumbai, India). Deionized water was obtained by using a Milli-Q (Millipore, Molsheim, France) water purification system.

Preparation of Running Buffer and Drug Standard Solutions

The running buffer solutions were prepared by mixing an appropriate amount of sodium tetraborate decahydrate (10-40 mM) and SDS (25-100 mM) in deionized water. The required pH of running buffer solutions was prepared prior to analysis. All the solutions were filtered using 0.45 µm micro filters. Stock and working standard solutions of drugs and internal standard were prepared as follows: Stock solutions of each standard (AM and LS) and PABA (IS) was prepared in methanol at an individual concentration of 1.0 mg/ml. A required concentration of working standard of individual drugs and mixtures were prepared by diluting the stock standard solutions with deionized water prior to CE injection.

Preparation of pharmaceutical samples

Twenty tablets were weighed, crushed into fine powder in a mortar and homogenized. 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 and shaken mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and makes volume up to the mark with methanol. The solution was filtered with a Whatmann filter paper no.1. For all quantitative determinations, a constant amount of PABA (IS) 50 µg/ml was added to the drug solution. Before the analysis, both standard and sample solutions were filtered using a Millipore filter (0.45 µm).

Stability of Sample Solution

Sample and standard solutions were stored in a refrigerator at 40C. Under these storage conditions they were stable for

the period of one month. Samples were filtered through 0.45 µm micro syringe filters prior to use.

3. Results and Discussion

Micellar electrokinetic capillary chromatography (MEKC) requires the addition of charged surfactants, forming micelles, to the background electrolyte at a level greater than its critical micelle concentration [12-17]. The micelles formed have their own electrophoretic mobility that is different from the surrounding aqueous phase. Analytes may differentially partition themselves between the micellar and the aqueous phases depending on their polarity thus promoting selectivity. Hence, the migration order can be a way to predict the polarity of the compounds. The hydrophobic core of the micelles provides sites of interaction that greatly enhance the solubility of insoluble non-polar compounds in aqueous media [13]. This is the case of AM, which are insoluble in aqueous media due to their neutral nature in the pH range, whereas LS has solubility in water.

Method Development

The chemical structures and pKa values of LS and AM are shown in Fig.1 and 2. The primary and secondary amine groups in drugs AM and LS recommended the use of strong acidic buffer solution for the capillary zone electrophoretic (CZE) separation. In MEKC, Sodium dodecyl sulfate (SDS), sodium tetraborate (STB) is used as running buffer, with pH 9.7. Under this electrophoretic condition, it could separate the two drugs AM and LS within 11 min. However, peak broadening and tailing were observed for AM. Micellar electrokinetic capillary chromatography (MEKC) has been used for the separation of neutral and charged molecules. Therefore, MEKC has been optimized for the simultaneous separation of AM and LS and the conditions are reported in Table. 1.

Table 1: Optimized capillary electrophoretic conditions

MEKC METHOD	
Capillary	uncoated fused silica capillary of 75 µm i.d., with a total length of 73.6 cm (effective length 62 cm)
Buffer	12.5 mM SDS, 10 mM STB, pH 9.7
Voltage	+20 kV, 15 min runtime
Temperature	25^0c
Sample injection	Hydrodynamic injection at 25 mbar/12 s
Detection	ultraviolet, 214 nm

Effect of SDS Concentration

SDS is the most widely used micellar medium for the separation of neutral as well as for the charged analytes in MEKC [12]. The effect of SDS concentration on the separation was tested in the range of 12.5-25 mM, keeping a constant amount of 10 mM sodium tetraborate (pH 9.7) solution. On increasing SDS concentration, the migration time of AM was significantly increased over LS. The relatively higher hydrophobic AM was more solubilized in the SDS micelle and thus migration time increased. Such that in increased SDS concentration, baseline shift were produced due to higher current (joule heating) and therefore, further optimizations were carried out with BGE.

Effect of BGE Concentration

It is well known that the BGE concentration has a significant effect on separation of analytes, as it not only influences the zeta potential, but also the critical micellar concentration (CMC) of the surfactant used [12]. The effect of BGE concentration on the separation behaviour of the analytes was studied by varying the concentration of STB from 10-20 mM, containing 12.5 mM SDS at pH 9.7. The results showed that higher STB concentration produce longer migration time of analytes. The reason for the longer migration time of analytes is due to the decreasing EOF velocity on increasing the BGE concentration. Further, the CMC decreases with increasing BGE concentration, this because the interaction of charged hydrophilic head groups are weakened, thus favoring the formation of micelles and hence the effective mobility of neutral solute in MEKC is proportional to the mobility of the micellar phase. At higher BGE concentration AM and LS are separated at large migration time. In order to get better separation and minimize the above mentioned effects, 10 mM were selected as the optimum BGE concentration and was used throughout the rest of the experiments.

Internal Standard (IS)

The use of internal standard is crucial in order to compensate the injection errors, evaporation loss of solvents, and migration time fluctuations. The following compounds were screened to identify the suitable internal standard: p-amino benzoic acid (PABA), 4- Amino pyridine (4-AP), nicotinic acid, and p-aminophenol. Among them, PABA was found to be suitable in terms of relatively shorter migration time and better UV response at 214 nm whereas 4-AP migrated after long time.

Method Validation

The developed MEKC method was validated according to the International Conference for Harmonization (ICH) guidelines[18-21] under the optimized experimental conditions: 12.5 mM sodium tetraborate (STB) (pH9.7) containing 10 mM sodium dodecyl sulfate (SDS), hydrodynamic injection, 50 mbar for 12 s, applied voltage, 25 kV, detection wavelength 214 nm, capillary temperature 250C.

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. 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.

Linearity:

Under the optimized conditions, linearity was assessed for the two drug standards in mixtures with the concentration ranging of 5-100 µg/ml for AM and LS respectively. In all above cases 100 µg/ml of p-amino benzoic acid (PABA) were added as IS. Calibration curves were plotted from the standard drug concentration versus peak area ratios of AM

and LS to the IS (Fig. 1 and 2). The statistical data of regression equation are shown in Table.2.

Accuracy & Precision:

Accuracy of the proposed method was assessed by recovery studies. This was performed by adding 25, 50, and 100% of nominal test concentration of the drug standard solutions of AM and LS with IS to the analytical placebo solution. All the samples were injected in six replicates for each concentration. The recovery percentage was calculated against the concentration added. The analysis results were summarized in Table.2. The recoveries of AM and LS were found to be 100.08 % respectively which indicates that the additives and excipients did not interfere in the determination of the analytes.

Repeatability (intra-day) of the analytical method was tested by analyzing all the three analytes in a mixture of a solution. In order to determine the repeatability of this method, replicate injections (n=3) of a standard solution containing lower, middle and higher linearity range were carried out in the optimum condition as described previously. The precision was calculated as percentage of relative standard deviation (% RSD) of relative migration time and the peak area ratio. From Table.4, the repeatability of relative migration time (RMT 0.24 and 0.215 %RSD) and peak area ratio (PAR 1.39 and 1.33 % RSD) for AM and LS can been seen respectively.

The intermediate precision was also evaluated over 7days by performing six successive injections each day. The results are summarized in Table. 4. This performance suggests that the proposed CE method presents acceptable reproducibility.

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. Robustness testing was performed by the selected parameters like buffer concentration, buffer pH, SDS concentration, voltage, and temperature, which are varied around (higher and lower level) the standard conditions in the method to reflect changes likely to rise in different test environments. Only one parameter was changed at a time and each injection carried out in triplicate for all experiments. The mean and % RSD of the RMT and peak area ratio (PAR) of the drugs AM and LS with respect to IS were listed in Table .5 [A] and [B], showing the acceptable robustness of the method.

LOQ and LOD:

The LOQ is defined as the lowest concentration that can be measured with acceptable precision and accuracy (S/N = 10) were 2.5 µg/ml, (RSD 1.10%, n=5), 2.0 µg/ml, (RSD 0.98%, n=5), for AM and LS. The LOD defined as the concentration where the signal-to-noise ratio of 3:1, were found to be 1.0 and 0.5 µg/ml for AM and LS. The electropherogram is represented in Fig.3. 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 and LS in commercially available pharmaceutical formulations obtained from different sources as shown in Table. 6. Fig. 4 and 5 shows the electropherogram of standard mixtures containing 100 µg/ml concentration of each drug and combination tablet solution of the two drugs with a constant amount of (100µg/ml) IS. The present method includes the determination of counter ion besylate (benzene sulphonate) in pharmaceutical formulations. Each pharmaceutical formulation was analyzed with six independent determinations and each series was injected three times. The percentage recoveries (Table. 6) of the drugs were between 99.00 and 100.4 % indicating the good agreement with the label claims. The recover ranges observed with better precision (<1.18% RSD) and good selectivity in real tablet sample.

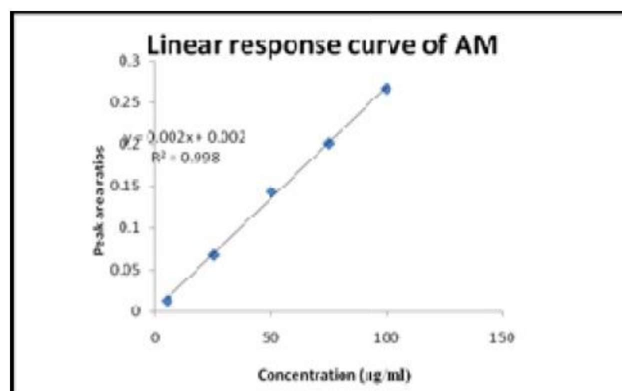

Fig 1: Linearity graph of AM representing peak area ratios (PAR) vs concentration (µg/ml)

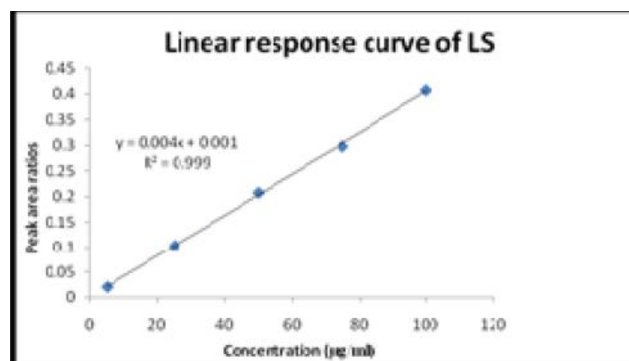

Fig 2: Linearity graph of LS representing peak area ratios (PAR) vs concentration (µg/ml)

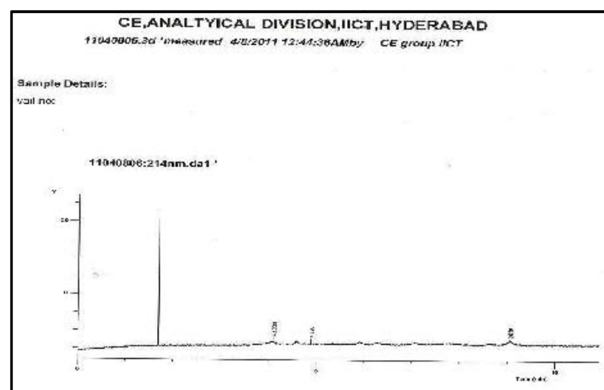

Fig 3: Electropherogram of standard mixtures of AM and LS, with a constant amount of IS, representing LOD

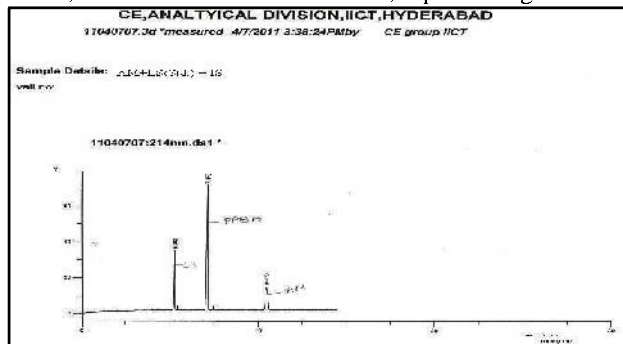

Fig 4: Electropherogram of standard mixtures of AM and LS, with a constant amount of IS

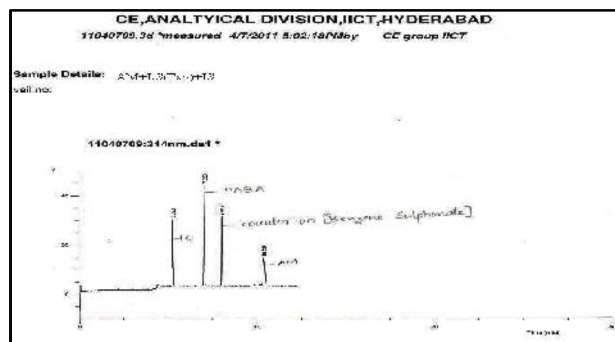

Fig 5: Electropherogram of combined tablet formulation of AM and LS with a constant amount of IS

Table 2: Statistical data of regression equations for AM and LS by developed MEKC method

Drug	AM	LS
Correlation coefficient (R2)	0.998	0.999
Slope	0.002	0.004
Std. error of slope	0.0001	0.0001
Intercept	0.002	0.001
Std. error of intercept	0.001	0.001

Table 3: Accuracy data for the assay of AM and LS

Drug	Conc. Added (µg/ml)	Found (mg/ml)	Recovery (%)[a]	% RSD (n=6)
AM	25	24.92	99.68	1.2
	50	49.78	99.56	1.29
	100	100.01	100.01	1.3
LS	25	25.02	100.08	1.18
	50	49.87	99.74	1.26
	100	99.69	99.69	1.29

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

Table 4: Repeatability and immediate precision data for the drugs AM and LS

Drug	Conc. Added (µg/ml)	Intraday		Inter day	
		RMT	PAR	RMT	PAR
		% RSD	% RSD	% RSD	% RSD
AM	25	0.124	1.24	1.231	2.51
	50	0.164	1.17	0.881	2.83
	100	0.248	1.39	1.49	3.58
LS	25	0.1405	1.1	1.286	2.98
	50	0.214	1.28	1.412	3.6
	100	0.1096	1.33	0.956	2.41

Table 5(A): Robustness data results for AM, LS and IS (50 µg/ml each) (n=3) representing Relative migration time (RMT)

		AM		LS	
		Mean	% RSD	Mean	% RSD
Standard conditions*		1.458	0.38	0.736	0.87
Buffer pH	9.7	1.457	0.38	0.739	0.88
	9.5	1.501	0.4	0.896	0.91
Buffer conc. (mM)	11	1.458	0.37	0.736	0.86
	12	1.583	0.38	0.637	0.87
SDS Conc. (mM)	12	1.46	0.39	0.738	0.88
	13	1.579	0.41	0.66	0.92
Applied voltage(kV)	24	1.564	1.36	0.676	1.18

	26	1.509	1.29	0.798	1.01

Table 5(B): Robustness data results for AM, LS and IS (50 µg/ml each) (n=3) representing Peak area ratios (PAR)

		AM		**LS**	
		Mean	**% RSD**	**Mean**	**% RSD**
Standard conditions*		0.387	1.28	0.297	0.99
Buffer pH	9.7	0.387	1.29	0.298	1
	9.5	0.402	1.2	0.31	1.1
Buffer conc. (mM)	11	0.389	1.28	0.295	0.97
	12	0.398	1.32	0.279	1.12
SDS Conc. (mM)	12	0.386	1.27	0.296	0.98
	13	0.406	1.39	0.28	1.2
Applied voltage(kV)	24	0.384	1.36	0.276	1.08
	26	0.39	1.29	0.298	1.01

Table 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	4.95	99	1.1
	LS	5	5.02	100.4	0.96
Amlopres - L	AM	5	5.01	100.2	1.12
	LS	5	4.98	99.6	0.99
Amlosafe – LS	AM	5	4.93	98.6	1.18
	LS	5	4.95	99	1.13
Amlokind	AM	5	4.98	99.6	0.97
Lipril	LS	5	4.96	99.2	0.98

4. Conclusion

The present micellar electrokinetic chromatography (MEKC) method is simple, selective and suitable for the simultaneous determination of AM and LS in pharmaceutical formulations. The good separation of both the drugs was achieved within 15 min, using 12.5 mM Sodium dodecyl sulfate (SDS), 10 mM sodium tetraborate (STB), with pH 9.7. The proposed MEKC method shows recover ranges (99.00 and 100.4 %) with better precision (<1.18% 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.

5. References

[1] Beasley, C. A., Shaw, J., Zhao, Z., Reed, R. A. J Pharma Biomed Anal. 2005, 37, 559-567.
[2] Jain, H. K., Agrawal, R. K. Indian Drugs, 2000, 37: 196-199.
[3] Fakhari, A. R., Nojavan S, Haghgoo S. Mohammadi A . Electrophoresis. 2008, 29: 4583-4592.
[4] Jankovics, P., Nem, T., Nem-Pals, J., Koszegi-Szalai, H. Chromatographia. 2008, 68: S43-S48
[5] Martinez, V., Lopez, J. A., Alonso, R. M., Jimenez, R. M. J Chromatogr. 1999, 836:189-199.
[6] Hillaert, S., de Grauwe, K., van den Bossche, W J Chromatogr. 2001, 924:439-449.
[7] Hillaert, S., van den Bossche, W. J Pharma Biomed Anal.2001, 25: 775-783.
[8] Wätzig H, Dette C. Pharmazie .1994, 49:83–96.
[9] Malesuik, M. D., Cardoso, S. G, Bajerski, L., Lanzanova, A. J of AOAC Int .1994, 89:359-364
[10] Panzade, P. D., Mahadik, L. R. Indian Drugs.1999, 36: 321-323.
[11] Qin, X. Z., Nguyen, D.-S. T., Ip, D. P. Jl of Liq Chromatogr.1993, 16: 3713-3734
[12] S.F.Y.Li, Capillary electrophoresis, J. Chromatogr Library, 52, Elsevier, Amsterdam, 1992
[13] Silva M. Electrophoresis.2007, 28: 174-92
[14] G.H. Cocolas, in: J.N. Delgado, W.A. Remers (Eds.), Text- book of Organic Medicinal and Pharmaceutical Chemistry, 10th ed., Lippincott-Raven, Philadelphia, New York, 1998, p.603
[15] Syed A Ali Rizvi, Duc Phuc Do, Ayman Md Saleh, Eur J Chem (2011), vol 2
[16] Sami El Deeb, Md Abu Iriban, Ronald Gust. Electrophoresis.2011, 32: 166–183.
[17] ICH Q1A (R2) (2003) Stability testing of new drug substances and products. International conference on Harmonization, Geneva, February 2003.
[18] Naguib, Abdelkawy. Eur. J Medicinal Chem 45 (2010), 3719-3725.
[19] International Conference on Harmonization (2003) IFPMA, Geneva.
[20] Bakshi M, Singh S. J Pharm Biomed Anal. 2002, 28,:1011-1040.