Capillary Electrophoresis: MEKC assay method for simultaneous determination of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in tablets

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ABSTRACT

Objectives: A simple, fast, accurate and precise micellar electrokinetic chromatographic (MEKC) method was developed and validated for the simultaneous quantification of olmesartan medoxomil (OLM), amlodipine besylate (AMB) and hydrochlorothiazide (HCT) in tablets. **Methodology:** The analytes were separated and quantified on untreated fused silica capillary with the help of background electrolyte comprising of 40 mM phosphate buffer, 20 mM sodium dodecyl sulphate (pH 6.0) and acetonitrile (90%:10% v/v). Losartan was selected as an internal standard and the detector was adjusted at 220 nm. The concentration of buffer and SDS, pH of buffer, the organic modifier, injection time, temperature and the voltage applied were optimized. The developed method was validated according to ICH guidelines. **Results:** The method demonstrated wide linearity ranges of 2-80 μg/ml, 1-40 μg/ml and 5-200 μg/ml with LODs of 1.25 μg/mL, 0.44 μg/mL and 3.81 μg/mL for OLM, AMB and HCT respectively. Furthermore, the intra day and inter day repeatability was well within the acceptable range. From the formulation, the mean recoveries of HCT, OLM, and AMB were found to be 99.8%, 99.1% and 100.11% respectively. **Conclusion:** Finally, the MEKC method was effectively applied for the assay of HCT, OLM, and AMB from their formulation and results were compared with the reported RP-HPLC method. In terms of precision and accuracy, no significant difference was observed in the outcomes of both the methods.

Key words: Amlodipine, Hydrochlorothiazide, MEKC, Olmesartan, Simultaneous determination.

INTRODUCTION

The most important cause for morbidity and mortality is cardiovascular diseases in general and hypertension in particular. The management of hypertension is essential to reduce the risk of CV events; for example: stroke, ischemic attack, congestive heart failure etc.[1,2] Recent studies have demonstrated that combination of antihypertensive drugs with different mechanism of action facilitate in lowering the blood pressure goals and it was found to be five times more effective in reducing systolic blood pressure than doubling the dose of single initial drug or monotherapy.[3] The most commonly used combination includes angiotensin 2 receptor blockers (ARBs) such telmisartan, valsartan, olmesartan, etc. with calcium channel blocker (amlodipine) and/or thiazide diuretics (hydrochlorothiazide) for the management of hypertension.[4,5] These three class of drugs have complementary mechanism of action, which help in rapid control of hypertension Hydrochlorothiazide (HCT, Figure 1a), chemically designated as 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide,[6] a thiazide diuretic, reduces blood volume by increasing the excretion of equal amounts of sodium and chloride along with water. One of the side effects of thiazide diuretics, loss of potassium ions, is reduced when co administred with ARBs.[4,5] Olmesartan medoxomil

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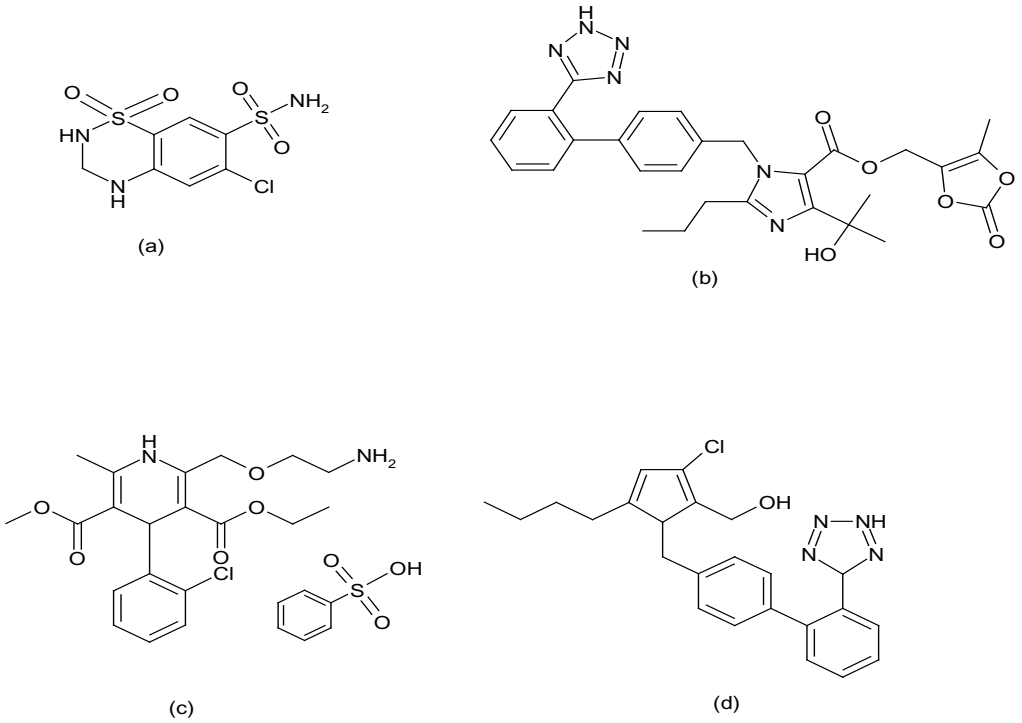

Figure 1: Chemical structures of hydrochlorothiazide (a), Olmesartan medoxomil (b), amlodipine besylate (c), and losartan (d)

(OLM, Figure 1b) is chemically named as 5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl) – 2 – propyl – 1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate[6], reduces BP by specifically blocking angiotensin II type 1 receptor,where by avoid the binding of angiotensin II, which results in vasodilation and blockade of aldosterone secreting effects of angiotensin II. Amlodipine besylate (AMB, Figure 1c) chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxy[6] is dihydropyridine calcium channel blocker, which blocks the transfer of calcium ions across the vascular and cardiac smooth muscles. This causes peripheral arteries vasodilation and reduce vascular resistance for blood flow thus causing the reduction in blood pressure.

On literature survey it was found that methods such as UV-Vis spectrophotometry and High performance liquid chromatography(HPLC), have been described for the determination of olmesartan alone and also in combination with other drugs in formulations and biological fluids.[7-12] Numerous methods were documented for the estimation of hydrochlorothiazide single or along with other drugs.[13-18] The determination of amlodipine had been reported in the literature using UV Spectrophotometry,[19] RP-HPLC,[20-22] liquid chromatography tandem mass spectrometry.[23-25] However, few methods[26-30] were reported for the concurrent separation and quantification of OLM, AMB and HCT. In one of the reported methods, four different spectrophotometric techniques, such as second derivative of the ratio spectra, first derivative of the double devisor ratio spectra, successive spectrophotometric resolution technique and mean centering of the ratio spectra were applied for the determination of these three analytes.[26] Another method reported was stability indicating RP-HPLC, using gradient elution method consisting of different proportion of triethyl amine and acetonitrile.[28] In another RP-HPLC method, these three drugs were separated on hypersil ODS C 18 column using methanol, phosphate buffer and triethyl amine as mobile phase. Darwish *et. al.*[30] reported three multivariate spectroscopic methods for the simultaneous determination of title drugs.

Capillary electrophoresis is an alternative analytical method with numerous advantages, such as,the use of lesser amount of samples, reagents and buffers with higher resolution of analytical separation in a short time.[31] A micellar elctrokinetic capillary chromatography (MEKC) method is a type of capillary electrophoresis method, where separation of analytes takes place by partition between solvent and micelles. Further, MEKC method increases the resolution of neutral and water insoluble compounds.[32] Several capillary electrophoresis methods[33-38] have been described in the literature for the estimation of OLM, AMB and HCT alone and in combination with other medications. However, until date, no CE method has been described for the assay of OLM, AMB and HCT in their combined formula-

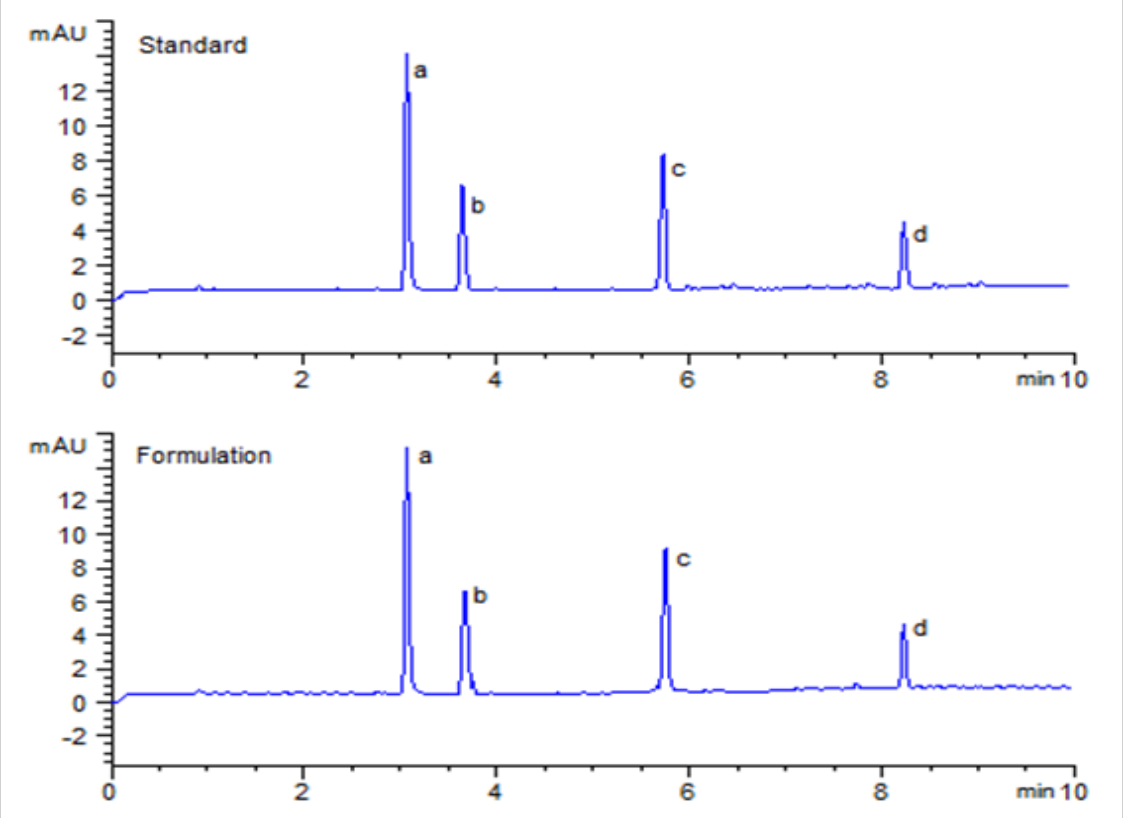

Figure 2: The electropherograms of standard and formulation solutions. Electrophoretic conditions: 40 mM phosphate buffer, 20 mM sodium dodecyl sulphate (pH 6.0) and acetonitrile (90%:10% v/v)., fused silica capillary column (55 cm length, 50 μm i.d.) hydrodynamic injection time of 4 s at pressure of 50 mbar, separation voltage of 25 kV and column temperature of 30°C, at 220 nm. a; HCT, b; AMB, c; OLM and d; IS

tion. Therefore, an effort has been made in this study to develop a rapid, precise and accurate MEKC assay process for separation and quantification of OLM, AMB and HCT in a solid dosage form.

EXPERIMENTAL

Agilent CE instrument (Agilent technologies, Germany) equipped with auto sampler, diode array detector, and a thermostat chamber was used for performing all the experiments. Moreover, Agilent chemstation software (version B.04.03) was used for data acquisition and analysis. A 55 cm long untreated fused silica column (47 cm from injection point to detector) with 50 μm i.d. was adopted for separation of analytes. Samples were loaded using hydrodynamic injection system at a pressure of 50 mbar for 4 seconds. The new capillary was irrigated with 0.1 M sodium hydroxide for 20 min, deionized water for 15 min and with background buffer for 15 min, sequentially and to attain good reproducibility in between the run, capillary was flushed with 0.1 M sodium hydroxide for 3 min, water for 2 min and background buffer for 3 min. To measure the pH of the solutions Omega PHH 222 (Stamford, USA) pH meter was used.

Chemicals and reagents

Millipore water was consumed for the preparation of buffer and other aqueous solutions. OLM, AMB, and losartan (Figure 1d) were purchased from Sigma Aldrich, (Germany). A pure sample of HCT was provided as a gift sample by Micro Labs Ltd, India. Sodium phosphate mono basic and sodium phosphate dibasic were procured from Scharlau (Spain). Analytical grade acetonitrile, orthophosphoric acid and sodium hydroxide were supplied by Loba Chemie (India). Analytical standard sodium dodecyl sulphate (SDS) was delivered by Sigma (Spain). Solid dosage form consisting of OLM (20 mg), AMB (5 mg) and HCT (12.5 mg) per tablet were acquired from pharmacy.

Standard solutions preparation

Stock solutions of analytes were arranged by dissolving precisely weighed amount of drugs in 50% acetonitrile separately (1 mg/ml). All these solutions were stored at 4°C and before use; the solutions were brought to 25°C. The stock solutions were diluted with 40 mM phosphate buffer pH 6.0, consisting of 20 mM SDS to give the required concentrations of analytes.

Preparation of Pharmaceutical samples

Accurate weight of twenty tablets was recorded and then the tablets were powdered. The tablet powder equivalent to 20 mg OLM, 5 mg AMB and 12.5 mg HCT was taken and dissolved in 50 ml of 50% acetonitrile. This solution was filtered through Whatman filter paper (0.45 micron) in to 100 ml graduated flask and the volume was made up to the mark with 50%acetonitrile. Then the sample solution was diluted with 40 mM phosphate buffer pH 6.0, consisting of 20 mM SDS to get analyte concentrations in the range of calibration curve.

RESULTS AND DISCUSSION

Newly proposed MEKC procedure is able to separate and quantify all three analytes in their combined pharmaceutical formulation with good resolution under optimized capillary electrophoretic conditions. Figure 2, represents the typical electropherogram of standard laboratory mixed analytes and the migration times for the HCT, AMB, OLM, and IS was found to be 3.09, 3.61, 5.75 and 8.24 min respectively.

Optimization of MEKC condition

Previous reports suggested that OLM was not stable in methanol and in aqueous solution particularly at basic pH. In addition, OLM was not soluble in water at pH 2-6. Further, Pka of AMB was 8.6; hence, amlodipine had positive charge under acidic condition. However, in basic condition above pH 6, the active mobility decreased due to deprotonation of AMB. To overcome these problems, MEKC method had been developed in acidic media. Also with MEKC method, a good separation of HCT from sartans could be achieved in acidic media. The MEKC method required addition of surfactants, such as SDS, which formd micelles. These micelles had different electrophoretic mobility from the surrounding buffer solution. Analytes partition between micelles and buffer solution in different concentration depending upon their polarity. The hydrophobic nature of micelles provided sites for interaction that greatly increase the solubility of OLM in aqueous solution.

Table 1: System suitability and validation parameters of HCT, AMB and OLM and results obtained by MEKC method

Analytical Parameters	HCT	AMB	OLM
Resolution (min)	--	3.6	12.36
Peak symmetry	1.05	0.99	1.00
Number of Theoretical plates	6658	6400	15596
Linearity range (µg/mL)	5-200	1-40	2-80
Intercept	0.1396	0.1058	0.0233
Slope	0.4233	0.9317	0.0969
Correlation coefficient (r)	0.9998	0.9999	0.9995
LOD (µg/mL)	1.22	0.13	0.32
LOQ (µg/mL)	3.81	0.44	1.25

Standard running buffers such as phosphate, acetate and borate were investigated for the separation of HCT, AMB and OLM. Good results were obtained using phosphate buffer in terms of migration time, resolution, peak shape and electric current produced.

Initially, to select detection wave length; solutions of HCT, AMB and OLM were scanned in the UV range (200-400 nm). The maximum detection sensitivities of all the three analytes were observed at 220 nm. Internal standards were suggested for better quantitative analysis and to avoid minor errors. Losartan was used as internal standard.

The concentration of running buffer also played a very important role in separation of analytes and the current generated in the capillary. The role of phosphate buffer concertation was examined in the range of 10 to 50 mM. It was clear from the results that, the increase in the concentration increased the migration time and resolution. A 40 mM was used throughout the experiment due to optimum migration time and resolution.

The influence of the SDS concentration was studied on migration time and resolution. With increase in the SDS concentration, OLM and ADM peaks overlapping was observed due to increase of the electroosmotic flow. Therefore, 20 mM was considered as optimum for the good resolution.

The use of organic modifier to the running buffer was beneficial in terms of reducing noise level and alter partitioning of analytes and viscosity of EOF. As OLM was not stable in methanol, acetonitrile in three concentrations (5, 10 and 15%) was investigated using an initial buffer comprised of 40 mM phosphate buffer, pH 6 and 20 mM SDS. With the increase in the amount of acetonitrile, migration time was increased. As it was evident from the result, that 10% acetonitrile was adequate for the separation of the three drugs with good resolution.

OLM was not stable in basic pH for more than 4 hours. Therefore, the acidic pH in the range of 3-6 had been investigated for the separation of analytes. All the analytes were separated in this pH, however, short migration time with good resolution were observed at pH 6 best peak shape.

Temperature and the voltage supplied to the capillary tube influenced the migration of drugs. Hence, three voltage levels (20,25, and 30 kV) and three temperature values (25, 30, and 35°C) were investigated for their effect on migration of drugs. The good peak shape and short migration time was observed at 25 kV applied

Table 2: Precision and accuracy results

Compound		Inter-day			Intra-day		
	Added (µg /mL)	Found[a] (µg /mL)	RSD (%)	RE(%)	Found[b] (µg /mL)	RSD (%)	RE (%)
HCT	50	49.84 ± 0.26	0.52	-0.321	49.52 ± 0.37	0.75	-0.97
	100	99.23 ± 1.39	1.40	-0.776	101.11 ± 1.48	1.46	1.10
	150	150.81 ± 2.01	1.33	0.537	149.23 ± 2.52	1.69	-0.52
OLM	20	19.82 ± 0.22	1.11	-0.908	19.79 ± 0.34	1.72	-1.06
	40	39.73 ± 0.45	1.13	-0.680	40.53 ± 0.59	1.46	1.31
	60	60.72 ± 1.02	1.68	1.186	59.82 ± 1.07	1.79	-0.30
AMB	10	10.05 ± 0.14	1.39	0.498	9.92 ± 0.18	1.81	-0.81
	20	20.04 ± 0.19	0.95	0.200	19.96 ± 0.32	1.60	-0.20
	30	29.89 ± 0.37	1.24	-0.368	29.69 ± 0.42	1.41	-1.04

a: Mean (n=3) ± SD. b: Mean (n=9) ± SD, RE: Relative error, RSD: Relative standard Error

Table 3: Assay results for the determination of the drugs in their formulations by MEKC and reference method

Compound	MEKC method			Reference method (28) % Found
% Found	Amount taken (µg/mL)	Amount found (µg/mL)	% Found	
HCT	3.125	3.16	101.12	98.92
(µg/mL)	6.25	6.23	99.68	98.59
(µg/mL)	12.5	12.31	98.48	101.4
Mean ± S.D			99.76 ± 1.32	99.64 ± 1.53
% RSD	-	-	1.32	-
% Error	-	-	0.20	-
t	-	-	0.35 (2.63)[a]	-
OLM	5	4.91	98.2	100.89
% Error	10	9.91	99.1	98.09
t	20	19.76	98.8	98.61
OLM	5	4.91	98.2	100.89
	10	9.91	99.1	98.09
	20	19.76	98.8	98.61
Mean ± S.D	-	-	98.7 ± 0.45	99.20 ± 1.48
% RSD	-	-	0.46	-
% Error	-	-	0.57	-
t			0.28 (2.49)[a]	-
AMB	1.25	1.24	99.20	98.53
	2.5	2.51	100.4	99.15
	5	4.91	98.2	101.14
Mean ± S.D	-	-	99.30 ± 1.10	99.61 ± 1.36
% RSD	-	-	1.10	-
% Error	-	-	0.40	-
t	-	-	0.72 (2.41)[a]	-

a: t values at P=0.05.

voltage at 30ºC temperature. Therefore, these values were used throughout the experiments.

Injection time affectd the peak area and peak height. Generally, hydrodynamic injection was used for better precision and accuracy. Solutions were injected with 50 mbar for different periods between 1.0 to 5.0 sec. The peak shape of AMB and OLM was deformed after 4 s. Hence, 4 s was designated as optimal injection time.

VALIDATION OF THE MEKC METHOD

The present proposed MEKC method was validated according to the ICH guidelines for linearity, range, limit of detection (LOD), limit of quantification (LOQ) specificity, precision, accuracy, and stability studies.

System suitability tests were performed to know the variations in selectivity, retention time, resolution, peak symmetry and the number of theoretical plates (HETP). The results of freshly prepared solutions showed that the variation in selectivity, retention time, resolution, and peak symmetry were well within in acceptable ranges for all three analytes. The number of theoretical plates (HETP) were well within the acceptable range (Table 1).

The linearity range was determined by analyzing six sets of all the analytes in the range of 1 to 200 μg/ml. Losartan (10 μg/ml) was used as internal standard for analysis of the drugs in the formulation. The linier calibration curves were created by plotting peak area ratios and drug concentrations and from the curve the least squares regression equations were generated. The good correlation coefficient (r value, >0.999) was obtained in the range of 2-80 μg/ml, 1-40 μg/ml and 5-200 μg/ml for OLM, AMB and HCT respectively . The LOQs of the assay were evaluated on the S/N=10 criterion and were found to be 0.32 μg/mL and 0.13 μg/mL and 1.22 μg/ml for OLM, AMB and HCT respectively. The LODs based on the S/N=3 criterion were found to be 1.25 μg/mL, 0.44 and 3.81 g/mL for OLM, AMB and HCT respectively.

The precision and accuracy were assessed by spiking the formulation sample with three different concentrations (low, medium, and high) of OLM (20, 40, and 60 μg/mL), AMB (10, 20 and 30 μg/mL) and HCT (50, 100, and 150 μg/mL), followed by IS (10 μg/mL), and then the results were tabulated in Table 2. Both intra-day assay and inter-day assay percentage mean standard deviations ranged from 0.52% to 1.69% for HCT, 1.1 % to 1.72% for OLM and 0.95 % to 1.81 % for AMB. Whereas, the percentage relative error values ranged from -0.97% to 1.10% for HT, -1.06% to 1.18% for OLM and -1.04% to 0.49% for AMB. It is evident that the present MEKC procedure is precise and accurate based on all of the results.

The mean recoveries of HCT, OLM, and AMB from formulation were found to be 99.8% and 99.1% and 100.11% respectively, at three different concentrations covering the entire calibration range (data not shown). These results indicate that the reproducibility with this method was high and comparatively constant over the calibration range.

Specificity of the MEKC technique was established by matching the electropherograms acquired from the solutions of standard and formulation. Figure 2 represents the electropherogram of pharmaceutical formulation. No extra peaks were witnessed from the additives of the preparation, indicating the high specificity of the newly developed MEKC method.

Application of MEKC method for analysis

The present MEKC process was effectively utilized for the estimation of HCT, OLM, and AMB in their fixed dose combination formulations. To confirm the accuracy and precision of the method, the same samples were also analyzed using the previously reported validated HPLC method (28). The results were represented in Table 3 and were in good agreement with the reported HPLC method. The comparison of the statistical results obtained using students t test showed that no significant difference was observed in the outcomes of both methods in terms of precision and accuracy.

CONCLUSION

A simple, fast, and accurate MEKC analytical procedure was established and validated for the simultaneous estimation of HCT, AMB and OLM in solid dosage form. The calibration curves were linear over a wide detection range with a low LOQ. Furthermore, the assay method showed high specificity and short analysis time (<9 min), along with excellent precision, recovery, and reproducibility. The method also demonstrated the use of simple and safe electrolytes. Hence, the newly developed MEKC process can be utilized for the assay of HCT, AMB and OLM for quality control purpose by pharmaceutical industries and analytical laboratories.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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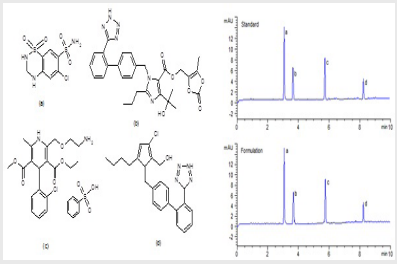
PICTORIAL ABSTRACT



ABBREVIATIONS USED

RP-HLPC: Reverse Phase High Performance Chromatography; **MEKC:** Micellar Electrokinetic Chromatography; **ICH:** International Conference on Harmonisation; **CE:** Capillary Electrophoresis; **CV:** Cardio Vascular; **ARB:** Angiotensin 2 Receptor Blocker; **EOF:** Electroosmotic Flow.

SUMMARY

- A simple, fast, and accurate MEKC analytical procedure was established and validated for the simultaneous estimation of HCT, AMB and OLM in solid dosage form.
- The proposed MEKC method was effectively applied for the assay of HCT, OLM, and AMB from their formulation.
- The MEKC method results were compared with the reported RP-HPLC method.
- In terms of precision and accuracy, no significant difference was observed in the outcomes of both the methods.

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