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Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar® tablets

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Abstract

Reversed phase liquid chromatography, micellar electrokinetic chromatography, and packed column supercritical fluid chromatography were used to measure the amount of Losartan Potassium drug substance in pharmaceutical tablets. Tablet extract samples were analyzed and results were used to compare accuracy, precision, and linearity of calibration of the instrumental techniques. All three techniques have satisfactory precision (less than 1% RSD) and accuracy to be used for the analysis of the drug substance in tablets and the advantages of each technique are discussed.

Keywords: Cozaar®; Micellar electrokinetic chromatography; Losartan Potassium; Reversed phase liquid chromatography; Supercritical fluid chromatography

1. Introduction

High performance liquid chromatography (LC) has been used for many years in the pharmaceutical industry as the primary analytical technique for measuring purity and stability of drug substances and drug products. Recently two other separation techniques, supercritical fluid chromatography (SFC) and capillary electrophoresis (CE), have been developed and applied to the analysis of pharmaceutical compounds [1–4]. SFC and CE both have unique features which may be advantageous in the separation and analysis of certain types of pharmaceutical compounds [5,6]. Both are considered complementary to LC as separation methods in the pharmaceutical industry and both are being evaluated as alternative analytical techniques.

SFC is done either in packed columns or in open tubular capillaries. Packed column SFC resembles normal phase LC in many aspects: LC columns, UV detectors, injection valves, column ovens, and mobile phase pumps are used as equip-

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ment [1]. This similarity in equipment and sample handling make packed column SFC attractive to the pharmaceutical chemist who is familiar with LC. However, SFC differs from LC in that a supercritical fluid, usually CO_2, is used as the mobile phase. A major advantage of SFC is that the CO_2 mobile phase can be released to the atmosphere so that little waste solvent is generated. Although SFC is best applied to separation of nonpolar compounds, polar modifiers can also be added to the mobile phase so that polar pharmaceutical compounds can be separated and analyzed [7]. Packed column SFC has been applied to the separation and analysis of pharmaceutical compounds [8,9] although detailed data on precision and accuracy are limited.

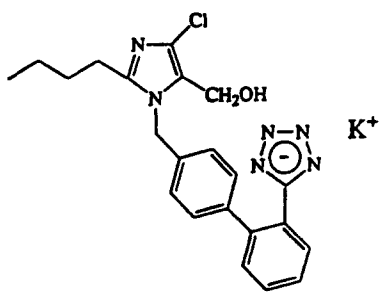
Capillary electrophoresis (CE) was first developed as a means of separating high molecular weight biomolecules but in recent years it has been applied to the analysis of inorganic ions and low molecular weight organic compounds. CE is best applied to separation of polar water-soluble compounds but it can also be used for less polar compounds by including surfactants [10,11] in the run buffer (a technique called micellar electrokinetic capillary chromatography or MEKC). The CE technique has the advantage of using only very small amounts of sample and run buffer but it has also suffered from a reputation of poor quantitative reproducibility and low detector sensitivity [3]. A variety of pharmaceutical compounds have been separated by MEKC methods [3,4,6,13] and recent studies have shown that it can be used to assay drug products with satisfactory accuracy [12,13]. Interlaboratory studies have also shown that a properly developed and validated MEKC method can be satisfactorily transferred between laboratories that use different types of CE equipment [13]. Precision of measurements in these laboratories ranged between 0.5 and 2.0% RSD.

The purpose of this study was to use a typical pharmaceutical application to compare the accuracy, precision and linearity of packed column SFC, MEKC and reversed phase LC; to the authors' knowledge the three techniques have not been directly compared before in this way in the literature. The analysis of Losartan Potassium (losartan) drug substance in Cozaar® tablets was chosen for this study; the drug substance is a potent Angiotensin II receptor antagonist and Cozaar® is a new pharmaceutical drug product used in the treatment of hypertension. Losartan Potassium (Fig. 1) has a molecular weight of 461, a pK_a value of 4.9, and an aqueous solubility of 3.3 mg ml^{-1} at pH 7.8. Although reversed phase LC is presently used for the quality control testing of losartan in Cozaar®, packed column SFC and MEKC can also be used for this analysis. The MEKC technique has already been investigated as a method to separate and determine losartan in different drug products which contain both losartan and hydrochlorothiazide [14]; the precision of MEKC measurements in that study was in the range of 1.5–2.5% RSD. However, for this study, extract samples of Cozaar® tablets were analyzed by all three techniques and the results were used to directly compare the linearity, precision, and accuracy of packed column SFC and MEKC to that of reversed phase LC.

2. Experimental

2.1. Equipment

The LC analysis of the Cozaar® tablets was done with a Millipore Waters LC system consisting of two Waters 510 pumps, a Waters Automated Gradient Controller, a Waters Plus 717 autosampler, a Waters column oven, and an ABI Spectroflow 783 variable-wavelength UV detector.



Losartan Potassium

Fig. 1. Structure of losartan potassium.

Samples were analyzed on a 25 cm × 4.6 mm i.d. Lichrosorb 10 RP-8 column from Phenomenex.

A Perkin-Elmer HT 270A capillary electrophoresis system equipped with a variable-wavelength UV detector was used for MEKC analysis of the tablet extracts. Samples were hydrodynamically injected by vacuum (5 in. Hg) applied at the exit end of the capillary. Fused silica capillaries (350 μm o.d. × 75 μm i.d.) were obtained from Polymicro Technologies (Phoenix, AZ) and cut to 72 cm in length. These capillaries were coated with a UV-transparent polymer so that detection windows did not have to be scraped or burned into the capillary. All capillaries were preconditioned before first use by flushing with 1 N NaOH for 30 min followed by a 30 min water wash. When stored for long periods, capillaries were flushed for 10 min with water and then for another 10 min with air.

The packed column SFC analysis was done with a Hewlett Packard model 1205 supercritical chromatography system equipped with a variable-wavelength UV detector, and an automated injection system with a Rheodyne valve containing a fixed 10 μl injection loop. The mobile phase was delivered by two pumps; one pump delivered CO_2 while the second pump metered in the premixed solution of methanol and trifluoracetic acid which was used as the mobile phase modifier (see conditions in Fig. 3). The SFC system was controlled by HP SFC 3D software (#G1855A Revision A.01.02) on a HP Vectra computer. The samples were analyzed on a 25 cm × 4.6 mm i.d. Zorbax SB CN column (5 μm packing) from Rockland Technologies (Wilmington, DE).

The detector output from each system was interfaced to a Fisons Multichrom software program (Version 1.8-3) on a VAX 6000 Series computer for data reduction and generation of chromatograms and electropherograms.

A Zymark Tablet Processing Workstation Plus (Milton, MA) was used for the robotic extraction of the Cozaar® tablets. The work station consisted of a robot arm, a balance for measuring weights, a solvent dispensing system, an automated pipettor, and a homogenizer for disintegrating and extracting tablets.

2.2. Chemicals

The losartan drug substance and the Cozaar® tablets were obtained from the DuPont Merck Pharmaceutical Company in Wilmington, DE. The butylparaben used as the internal standard was from Aldrich Chemical (Milwaukee, WI). The sodium dodecylsulphate used in the CE studies was the Ultra pure grade from Gibco BRL (Gaithersburg, MD). The sodium borate 10-hydrate and the LC-grade trifluroacetic acid (TFA) came from J. T. Baker (Phillipsburg, NJ). The sodium phosphate, monobasic, and the LC grades of acetonitrile and methanol were from EM Science (Gibbstown, NJ).

2.3. Sample preparation

Extracted samples of 50 mg Cozaar® tablets were prepared manually by placing 10 tablets in a 1000 ml volumetric flask, adding a 10 mM NaH_2PO_4 pH 8 solution and stirring for 30 min. The volumetric flasks were filled to the mark with the sample solvent and mixed. The solution was filtered through a 0.45 μm PVDF Whatman syringeless filter (Clifton, NJ) and used for LC and SFC analysis. For MEKC analysis this solution was further diluted 1:25 with a solution of 8% methanol/92% 10 mM NaH_2PO_4 (pH 8) so that sample peaks would stay within the linear range of the CE detector. This solution was centrifuged at 2000 rev min^{-1} for 2 min before MEKC analysis. All manual extract samples were assayed for losartan content by the three techniques using external standards for calibration.

Extract samples of 12.5 mg Cozaar® tablets were prepared robotically with a Zymark Tablet Processing Workstation Plus. Ten tablets were placed in a 300 ml flask with 200 ml of a solution containing 75% methanol/25% NaH_2PO_4 (10 mM, pH 8) and butylparben at a concentration of 1.25 mg ml^{-1} as an internal standard. The sample was then homogenized for 10 min to extract the losartan from the tablets; the internal standard was necessary to compensate for any evaporation of methanol during this process. This solution was filtered through a 0.45 μm PDVF syringeless Whatman filter and then used for LC and SFC

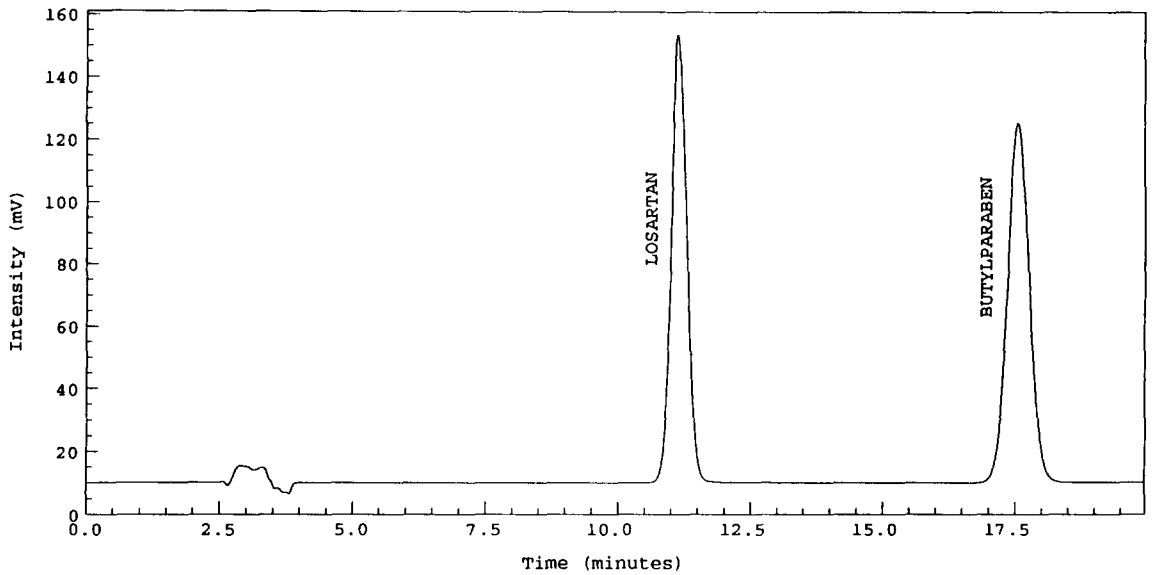


Fig. 2. HPLC chromatogram of robotic extract of Cozaar® tablet. Separation conditions: C8 column; 40% ACN–60% aqueous NaH_2PO_4 (10 mM, pH 2.5); 1.0 ml min^{-1}; 35°C; UV at 230 nm; 4 μl extract solution.

analysis. This solution was further prepared for CE analysis by diluting 1:25 with 8% methanol/92% NaH_2PO_4 (10 mM, pH 8) as described above; the 8% methanol was necessary in order to keep the butylparaben from precipitating from the CE sample solution onto the sides of the plastic sample vials used in the CE system. These samples were also centrifuged at 2000 rev min^{-1} for 2 min before MEKC analysis. All robotic extract samples were assayed for losartan content by the three techniques using butylparaben as an internal standard for calibration.

3. Results and discussion

3.1. LC analysis

The Cozaar® tablet extracts were analyzed for losartan content on a Phenomenex C-8 column (see conditions and chromatogram in Fig. 2). All degradation products were strongly retained and separated from losartan and the butylparaben under these conditions. The manual extracts were analyzed using external standards for quantitation; the robotic extracts were quantitated using butylparaben as an internal standard. All samples were detected at 230 nm in the UV which is a maximum in the losartan absorption spectrum.

3.2. SFC analysis

The sample extracts were analyzed on a 25 cm × 4.6 mm i.d. LC column with a cyanopropyl stationary phase (see chromatograms and conditions in Fig. 3). The detection was at 280 nm in the UV since the TFA in the SFC modifier absorbed at the 230 nm wavelength used for LC analysis. All degradents are separated from the losartan and internal standard peaks under the conditions shown in Fig. 3. Several variables were considered for optimizing the SFC separation including composition of modifier, pressure, flow temperature, etc. Better peak symmetry was obtained with higher CO_2 pressure in the mobile phase. Methanol was used as mobile phase modifier since the extract sample solvent was methanol/water. The TFA was also a necessary component of the modifier for maintaining the peak shape of the acidic losartan.

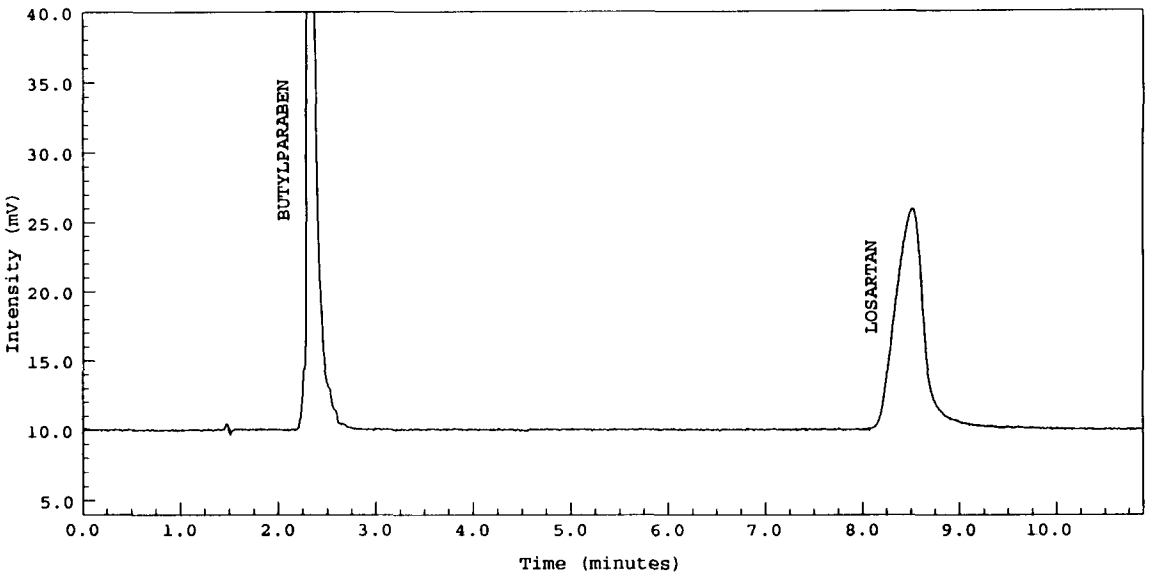


Fig. 3. Packed column SFC chromatogram of robotic extract of Cozaar® tablet. Separation conditions: cyanopropyl column; 90% CO_2–10% MeOH: TFA (98:2 v/v); 2.0 ml min^{-1}; 300 atm; 45°C; UV at 280 nm; 10 μl of extract solution.

3.3. CE analysis

The tablet extracts were separated by MEKC which is a CE technique in which 50 mM SDS, a micellar agent, was added to the 10 mM Na_2BO_4 run buffer at concentrations large enough to form a lipophilic secondary phase of organic micelles (the critical micelle concentration of SDS in water is approximately 12 mM). The separation mechanism is similar to reversed phase LC but with a different selectivity. Electropherograms and conditions are found in Fig. 4; all degradation products were strongly retained and separated from the losartan and internal standard peaks under these conditions. Several variables were considered for optimizing the separation including run buffer concentration and pH, run buffer replenishment, run voltage, sample volume, sample concentration, capillary diameter and capillary washes.

A fresh vial (4 ml volume) of run buffer had to be used after analyzing every 10 samples in order to maintain constant migration time and to prevent distortion of the butylparaben peak; the CE system was programmed to automatically change to fresh run buffer vials. Run buffers were filtered through a 0.45 μm filter before use and all samples were centrifuged at 2000 rev min^{-1} for 2 min before analysis. Capillaries of 75 μm i.d. were used for analysis because they were less prone to pluggage from particulate matter than the 50 μm i.d. capillaries. A 10 mM concentration of the NaH_2PO_4 was sufficient to control migration time without producing an excessive amount of heat in the 75 μm i.d. capillary. A short wash of the capillary with water followed by a 4 min wash with run buffer before each sample was found to be effective for control of migration time. All sample extracts had to be diluted either 1:10 or 1:25 in order to stay within the linear range of the CE detector.

3.4. Linearity

Five standard samples of losartan were prepared in 10 mM Na_2HPO_4 (pH 8) in the range of 50–150% of the label claim values (0.25–0.75 mg ml^{-1}) with butylparaben as internal standard and injected onto both the LC and SFC systems in order to establish linearity. These same standards were diluted 1:10 with 8% MeOH/92% Na_2HPO_4 (10 mM, pH 8) and injected onto the CE system. The correlation coefficients and slopes of the calibration curves for the three methods are shown in

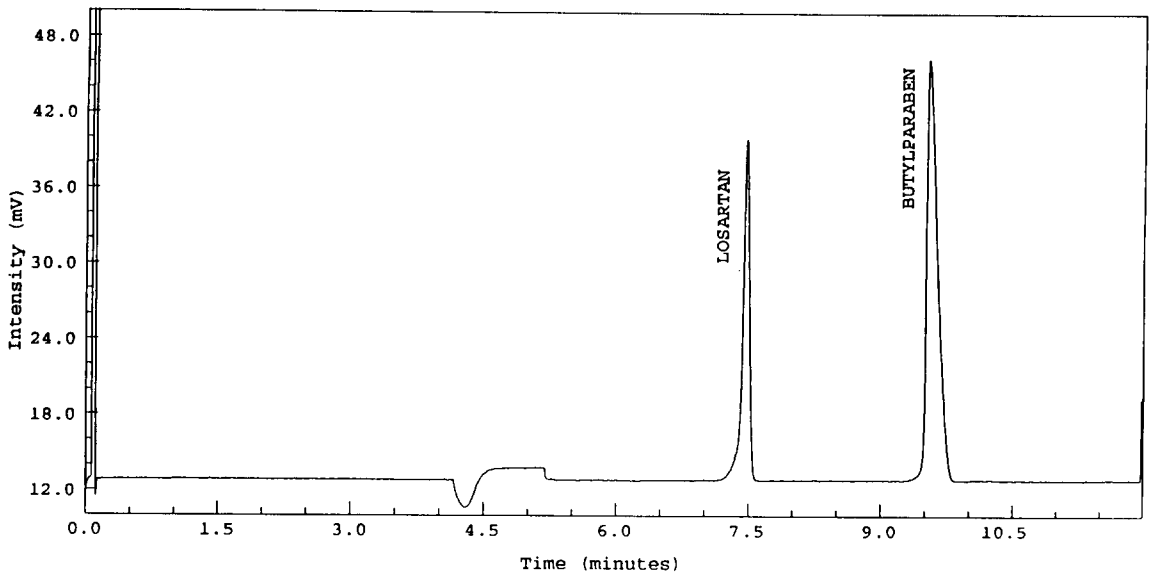


Fig. 4. CE electropherogram of robotic extract of Cozaar® tablet. Separation conditions: 72 cm × 75 μm fused silica capillary; SDS (50 mM)–$Na_2B_4O_7$ (10 mM, pH 9); 15 kV; 30°C; UV at 230 nm, 40 nl extract solution.

Table 1. The Y intercepts are not significantly different from zero for the three techniques. The data in Table 1 show that the three techniques are equivalent in linearity provided the peaks in the CE samples had an absorbance of less than 0.25 AU. The narrow pathlength of the 75 μm i.d. capillary caused the UV detection to be nonlinear above 0.25 AU and all extract samples had to be diluted in order to stay within the linear range of the CE detector.

3.5. Precision

The precision of the HPLC, SFC, and MEKC systems was measured by injecting a standard sample ten times sequentially into each instrument. Retention or migration times and peak areas and area ratios were measured and the RSD values are listed in Table 2. The HPLC technique gave the best precision of retention and peak area repeatability (less than 0.2% RSD) although both SFC and CE also gave satisfactory performance (less than 1.0% RSD) for this application. The precision of quantitation using either internal or external standards was satisfactory for all three techniques.

A measure of method precision was made in a separate recovery study (see results in Table 3). Placebo tablets were spiked with known amounts of drug substance, extracted by the robotic method and analyzed by each of the three techniques. The measured amount of drug substance was compared to the amount of weighed drug substance and the results are shown as percent recovery in Table 3. The % RSD of the recovery results is a measure of method precision and was

Table 1
Linearity of calibration curves

	LC	SFC	CE
Correlation	1.0000	0.9999	0.9999
Slope	17.34	1.51	24.72
Intercept	0.00029	−0.0011	0.00089

Table 2
System precision

	LC	SFCCE
Retention (migration) (%)	0.11	0.720.24
Peak area (%)	0.08	0.530.68
Peak area ratio (%)	0.04	0.360.48

Table 3
Recovery study

Tablet value (mg)	% Recovery of drug substance weighed into placebo		
	LC	SFC	CE
10	100.1	99.7	100.5
	100.0	100.2	99.6
	100.4	100.2	99.8
12.5	99.8	100.7	101.9
	99.9	99.9	100.1
	99.5	100.0	101.9
15	99.7	101.2	101.2
	99.7	99.0	100.3
	99.7	100.2	100.5
Mean (%)	99.9	100.1	100.6
%RSD	0.28	0.62	0.84

satisfactory (less than 1.0% RSD) for the analysis of the Cozaar® tablets by all three techniques.

Several variables were considered in the optimization of the MEKC separation and study of the precision of measurement. The size of sample injection and the internal diameter of the capillary were found to be the most important factors for obtaining good precision. Capillaries of 75 μm i.d. gave more reproducible retention and peak area measurements apparently because they were less prone to blockage from particulate matter in extracted samples than were 50 μm i.d. capillaries. The precision of sample injection in the MEKC system was dependent upon injection time (and injection volume). The RSD of peak measurement increased by a factor of three as the injection time was decreased from 3 s (40 nl) to 1 s (13 nl). 3 s injections were used for all studies even though the larger injection volume caused broadening of the losartan peak.

In SFC the most critical variable affecting precision was the reproducibility of the rate of delivery of the mobile phase modifier. The retention of polar compounds is primarily dependent on the concentration of polar modifier in the CO_2 mobile phase [8]. The studies of the effect of methanol/TFA modifier concentration on losartan retention show that small changes in the modifier concentration can produce significant changes in retention time. Accurate control of the modifier concentration is necessary in order to obtain reproducible retention time.

3.6. Accuracy

The accuracy of the three techiques was compared in a recovery study with placebo tablets spiked with known amounts of losartan drug substance. Placebo samples were spiked with drug substance at levels of 10, 12.5, and 15 mg and with the butylparaben internal standards. These samples were analyzed and results are expressed as percent recovery of weighed drug substance (Table 3).

A further study of accuracy was made by analyzing 12.5 mg Cozaar® tablets and blanks that were prepared by robotic methods and 50 mg tablets and blanks that were prepared by the manual method. A total of 23 Cozaar® tablets were extracted manually and analyzed with external standares by the three techniques (see Table 4). A total of 14 Cozaar® tablets were extracted robotically and analyzed with internal standard. The packed column SFC results were also compared with reversed phase LC by regression analysis and the correlation coefficients for the manual and robotic extracts were 0.999 and 0.998 with slopes of 1.001 and 0.988 respectively. The MEKC results were compared with LC results

Table 4
Determination of losartan in tablet extracts

	LC	SFC	CE
Manual extraction of 50 mg tablets (external standards)			
Mean (mg; $n = 23$)	49.5	49.6	49.0
%RSD	1.08	1.60	1.28
Robotic extraction of 12.5 mg tablets (internal standard)			
Mean (mg; $n = 14$)	12.4	12.5	12.5
% RSD	1.92	2.29	2.17

and correlation coefficients for manual and robotic extracts were 0.999 and 0.999 with slopes of 0.988 and 1.001 respectively. All three techiques gave equivalent results for the determination of losartan in Cozaar® tablets.

4. Conclusions

The results of this study show that properly developed reversed phase LC, packed column SFC, or MEKC methods can be used for accurate determination of losartan drug substance in Cozaar® tablets. Reversed phase LC is the most precise technique in terms of both retention and peak measurement (0.5% RSD) and LC equipment is very reliable. Packed column SFC and MEKC showed precision (1% RSD) and accuracy which are sufficient for many pharmaceutical applications. These are relatively new techniques in comparison with LC and improved equipment and better methods are still being developed. Both CE and SFC will find increasing use for suitable applications where they have special advantages or where waste solvents must be minimized. For this application the SFC and CE methods generated less than 10% of the waste mobile phase generated by LC. Both will be viewed as complementary and alternative techniques to LC in the future of pharmaceutical analysis.

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