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Article

Fast and Environmentally Friendly Method for Simultaneous Determination of Hydrochlorothiazide, Losartan and Potassium by Capillary Electrophoresis with Capacitively Coupled Contactless Conductivity Detection

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Losartan potassium and hydrochlorothiazide are often combined in pharmaceutical formulations for the treatment of hypertension. Therefore, the determination of these compounds in a single run is highly desirable for rapid quality control applications. The present study describes an ultra-fast (ca. 85 injections h^{-1}) and environmentally friendly method based on capillary electrophoresis (CE) with capacitively coupled contactless conductivity detection for simultaneous quantification of potassium, losartan and hydrochlorothiazide. Cation (potassium) and anions (losartan and hydrochlorothiazide) were analyzed in a single run using a background electrolyte composed by 10.0 mmol L^{-1} boric acid (pH = 9.0, adjusted with sodium hydroxide). The limits of detection were 4.0, 3.0 and 10.0 µmol L^{-1} for potassium, losartan and hydrochlorothiazide, respectively. The proposed method is simple, fast, with minimal waste generation, and accurate (recovery values between 98 and 102%). The results obtained with the CE method were statistically similar (95% confidence level) to those obtained by high-performance liquid chromatography (losartan and hydrochlorothiazide) and flame photometry (potassium).

Keywords: minimal waste, pharmaceutical analysis, stoichiometry ratio, sub minute analysis, tablets

Introduction

Losartan potassium (KLOS salt) is one of the most prescribed active ingredients for the treatment of arterial hypertension due to its greater specificity, selectivity, and tolerability.[1] The administration of KLOS salt results in a decrease in total peripheral resistance and in cardiac venous return.[2] Hydrochlorothiazide (HCT) is a thiazide diuretic drug that increases renal excretion of water and electrolytes and is used in the treatment of edema associated with congestive heart failure, liver cirrhosis and corticosteroid therapy.[3] KLOS salt and HCT are used separately or combined in a pharmaceutical formulation for the treatment of hypertension and cardiovascular disease. In the treatment of hypertension, the combination of both active ingredients was considered more effective in cases of patients whose blood pressure is not adequately controlled by a single active ingredient. This is due to the synergistic and additive effect that stimulates the renin-angiotensin-aldosterone system.[4]

The possibility of simultaneous determination of losartan (LOS) and HCT has already been demonstrated in the literature using different techniques, such as UV-Vis spectrophotometry,[5,6] thin-layer chromatography,[7] high performance liquid chromatography,[1-3,8] capillary electrophoresis,[9-11] and electrochemistry (differential-pulse voltammetry).[4] All previously developed methods have advantages and disadvantages; however, neither of these have the ability for simultaneous determination of LOS, HCT, and potassium (K).

In the pharmaceutical industry, around 50% of molecules used as active ingredients are administered in the form of salts in order to improve their biological and physicochemical properties (stability, solubility and bioavailability).[10] In this context, it would be very helpful if quality control methods had the ability to detect both cations and anions (active ingredient and respective

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counter-ion) in a single run to obtain additional and useful information about the composition of the pharmaceutical sample.[12] The accurate determination of the stoichiometry of pharmaceutical active ingredients may be helpful in detecting the presence of impurities and/or the existence of degradation/hydrolysis processes of the active organic ingredient.[12,13]

Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) is a widely used technique for the separation and detection of inorganic and organic compounds.[14,15] All analytes that have different electrophoretic mobilities than the used background electrolyte (BGE) can be detected by C^4D and, therefore, it can be considered as a universal detection system. This detector is particularly useful when the sample under examination contains species with very different physicochemical characteristics, such as salts of active pharmaceutical ingredients (acid drugs with Na^+, K^+, Ca^{2+}, Mg^{2+} or NH_4^+ as counter-ions or basic drugs with hydrochloride, bromide, tartrate, sulfate or phosphate as counter-ions).[16]

In the present work, we describe a sub-minute method for simultaneous determination of K, LOS and HCT in pharmaceutical formulations using CE-C^4D. The results obtained with the single-run electrophoretic method were statistically similar ($p < 0.05$) to those obtained by flame photometry (K) and by liquid chromatography (LOS and HCT).

Experimental

Reagents and samples

All reagents used were of analytical grade (purity ≥ 98%) and were used without further purification. Boric acid, histidine (HIS) and methanol were obtained from Vetec (Duque de Caxias, RJ, Brazil), 2-(*N*-cyclohexylamino) ethanesulfonic acid (CHES) and KLOS salt from Sigma-Aldrich (St. Louis, United States); HCT from Attivos Magistral (São Paulo, SP, Brazil), and sodium hydroxide (NaOH) from Panreac (Castellar del Vallès, Spain). All solutions were prepared with deionized water (resistivity ≥ 18 MΩ cm) obtained from a purification system called Direct-Q-System (Millipore, Bedford, MA, USA). Boric acid solution (10.0 mmol L^{-1}) with pH adjusted to 9.0 with the addition of NaOH was used as background electrolyte (BGE). Pharmaceutical samples containing HCT and KLOS salt were obtained from local drug stories.

Ten tablets from each sample were accurately weighed and then ground to a fine and homogeneous powder. All standard and sample solutions were prepared using methanol as solvent and then kept under ultrasound (Ultracleaner 1400A UNIQUE) for 10 min for complete solubilization. Prior to injection into the CE-C^4D system, sample solutions were filtered through membranes with 0.45 µm pore sizes and properly diluted in deionized water to a concentration within the linear range of the respective calibration curve.

Instrumentation

CE analyses were performed using a CE-C^4D equipment built in the research group of Prof Claudimir L. do Lago (Institute of Chemistry, University of São Paulo). The CE-C^4D system is equipped with two compact detectors fixedly positioned at 10 cm from both ends of the separation capillary (total capillary length = 50 cm). That is, the effective capillary lengths are fixed for the first (10 cm) and second (40 cm) detectors, respectively. Both compact C^4D detectors operate at a fixed frequency (1.1 MHz) which has been optimized for use with capillaries with internal diameters ranging from 20 to 100 µm.[14] A software in LabView 8 version[17] was used for controlling the equipment and acquiring data. The used fused silica capillary was 50 µm of internal diameter, 375 µm of external diameter, and 50 cm of total length (Agilent, Folsom, CA, USA).

Before the analyses, the capillary was conditioned by the following rinse cycles: 0.1 mol L^{-1} NaOH for 15 min, deionized water for 10 min and, finally, with BGE to be used in the experiments for 10 min. Standard and sample solutions were hydrodynamically injected (with constant pressure of 25 kPa) through the right side of the equipment (anode). All experiments (except for optimization) were performed with separation voltage of +20 kV (inlet side) and using normal electroosmotic flow (EOF) condition.

Comparison analyses were performed by high-performance liquid chromatography (HPLC) (LOS and HCT) and by flame photometry (K). In HPLC analysis, a Shimadzu LC-10 VP equipment with the following configuration was used: UV-Vis detector (SPD-10AV), C18 column (Macherey-Naguel 10 cm, 5 µm), manual injector (20 µL) and pump (LC-10AD-VP). The mobile phase was composed by acetonitrile/phosphate buffer (35:65, v/v; pH 4.0; 0.1 mol L^{-1}). The selected wavelength and the flow rate were 230 nm and 1.0 mL min^{-1}, respectively. These conditions were adapted from a previously published work.[3] The photometric determination of K (adapted from Okumura *et al.*[18]) a 910M equipment (Analyzer Instrumentação Analítica, São Paulo, Brazil) located at the undergraduate laboratory of the Institute of Chemistry of UFU was used.

Results and Discussion

According to the data obtained in the literature,[19] the pK_a values of the target analytes are the following: 3.9 and 5.9 for LOS, and 9.1, 9.8 and 11.3 for HCT (Figure 1). These pK_a values indicate that the two species can exist in their ionic forms in aqueous solutions and, therefore, can be separated by capillary zone electrophoresis (CZE). LOS is an amphoteric molecule (pI = 4.9) and can exist in both cationic (pH << 4.9) and anionic (pH >> 4.9) forms. HCT is a weak acid and can exist in anionic form in aqueous solutions with pH > 7.1. Moreover, in pharmaceutical formulations, LOS is added as a potassium salt (KLOS)[20] and, therefore, if K can also be detected, the stoichiometric determination of the KLOS salt can also be performed. In order to achieve the determination of the three analytes (K, HCT and LOS) simultaneously, a condition that enabled the simultaneous determination of cations and anions by CZE was evaluated.

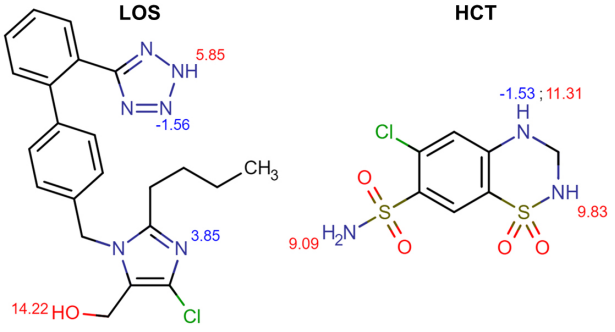

Figure 1. Chemical structures of LOS and HCT and the respective pK_a values (adapted from reference 19).

The strategy adopted in this work for the simultaneous determination of the three compounds (K, HCT and LOS) by CZE was the use of normal polarity and the use of a BGE with pH value > 7.5 (condition where HCT is in anionic form). In this condition, cationic species migrate in the direction of the detector in co-EOF mode (EOF mobility + electrophoretic mobility) and anionic species with low mobility in counter-EOF mode (EOF mobility >> electrophoretic mobility). The configuration of the CE-C^{4}D system is shown in Figure S1 (Supplementary Information (SI) section). Initially, the performance of different BGEs with buffering capacity at pH close to 9.0 (boric acid, CHES, and HIS; 10.0 mmol L^{-1} of each with pH adjusted to 9.0 with NaOH) were evaluated. Better results regarding separation efficiency, peak shape and detectability (Figure 2) were obtained with a BGE composed by boric acid 10.0 mmol L^{-1} (pH = 9.0, pH adjusted with NaOH). Probably, the BGE composed by CHES/NaOH could also be used, however, the separation efficiency and detectability were slightly worse. Therefore, the BGE composed by boric acid/NaOH was selected for subsequent studies. In all studies, analyte concentrations used in the studies were similar to those found in pharmaceutical formulations.

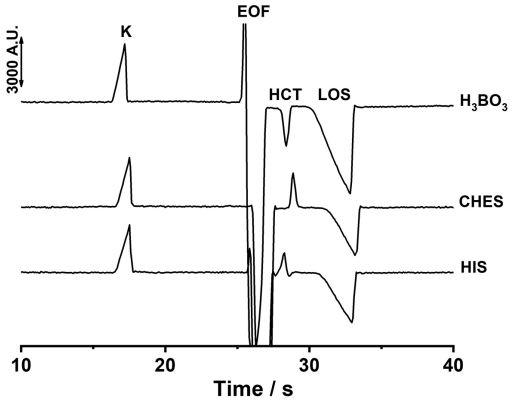

Figure 2. Electropherograms obtained for a standard solution containing KLOS salt (650.0 µmol L^{-1}) and HCT (250.0 µmol L^{-1}) using BGEs with different compositions: 10 mmol L^{-1} of boric acid (H_3BO_3), CHES or HIS with pH adjusted to 9.0 using NaOH. Hydrodynamic injection: 25 kPa for 1.0 s; separation voltage: +20 kV (injection side); total and effective capillary length: 50 and 10 cm, respectively; EOF: normal.

Next, the effect of pH was carried out in a pH range close to the pK_a value (9.2) of boric acid. The electropherograms obtained in this study are shown in Figure 3.

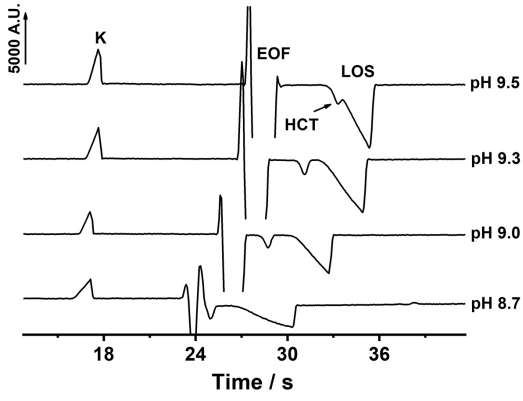

Figure 3. Electropherograms obtained for a standard solution containing KLOS salt (650.0 µmol L^{-1}) and HCT (250.0 µmol L^{-1}) using BGEs with different pH values (8.7, 9.0, 9.3, and 9.5). BGE: 10 mmol L^{-1} boric acid with pH adjusted with NaOH; hydrodynamic injection: 25 kPa for 1.0 s; separation voltage: +20 kV (injection side); total and effective capillary length: 50 and 10 cm, respectively; EOF: normal.

As can be seen in Figure 3, the separation of the target species with acceptable peak resolution (> 1.6) was possible in the pH range between 9.0 and 9.3. At pH 9.5, the co-migration of HCT and LOS was observed, however, with the decrease in the pH of the BGE, the separation was possible because the electrophoretic mobility of HCT (pK_a = 9.1) decreased while LOS mobility (pI = 4.9) remained constant (100% in anionic form). The effect of

the concentration (ionic strength and conductivity of the BGE) of boric acid (5 to 50 mmol L^{-1}) with pH adjusted to 9.0 with NaOH was also evaluated (Figure S2, SI section). Better results (analysis time, peak symmetry, and peak resolution) were obtained with 10 mmol L^{-1} boric acid, which was used in subsequent studies.

Next, the influence of electrophoretic parameters, such as injection time (0.5 to 2.0 s; 25 kPa; Figure S3, SI section), separation voltage (+15 to +25 kV; Figure S4, SI section) and effective capillary length (10 or 40 cm; Figure S5, SI section) was evaluated. Adequate peak separation ($r > 1.5$) and peak shapes were observed for injection times between 0.5 and 1.2 s (Figure S3) and the time of 1.0 s was used in subsequent studies. The separation voltage selected for subsequent analysis was +20 kV (Figure S4). At +25 kV, the peak for HCT was too close to the EOF signal ($r < 1.5$) and at +15 kV, only an increase in analysis time was observed. The effective capillary length was evaluated considering two conditions, 10 or 40 cm (Figure S5). The length of 10 cm was selected because the peak resolution was > 1.5 and the analysis time was three times faster (85 and 28 injections *per* hour, respectively).

Using the optimized conditions, the linearity of the method was determined by injection of standard solutions containing increase concentrations of the three target analytes (Figure S6a, SI section; KLOS salt: 52.0 to 1300.0 μmol L^{-1}; HCT: 20.0 to 500.0 μmol L^{-1}). As can be observed in Figure S6b, wide linear ranges (correlation coefficient > 0.995) were achieved for the three target analytes. The intra-day repeatability (instrumental precision) of the proposed method was evaluated by ten successive injections of a standard solution containing KLOS salt (650.0 μmol L^{-1}) and HCT (250.0 μmol L^{-1}). The first, fifth and tenth electropherograms of this study are shown in Figure 4. These results were obtained without cleaning procedures (flushing with BGE) between replicates.

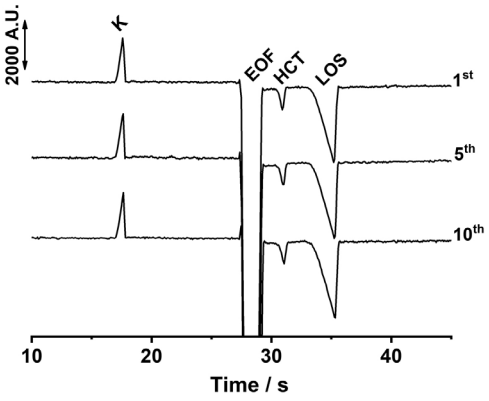

Figure 4. Electropherograms (1st, 5th, and 10th) obtained for successive injections of a standard solution composed of KLOS salt (650.0 μmol L^{-1}) and HCT (250.0 μmol L^{-1}). BGE: 10.0 mmol L^{-1} boric acid with pH adjusted to 9.0 with NaOH; hydrodynamic injection: 25 kPa for 1.0 s; separation potential: +20 kV (injection side); total and effective capillary length: 50 and 10 cm, respectively; EOF: normal.

Intra-day (Figure 4; n = 10) and inter-day (n = 3) relative standard deviation (RSD) values were obtained considering peak area and migration time values. Intra-day RSD values lower than 4.6 and 0.6% and inter-day RSD values lower than 12.9 and 2.1% were obtained for peak area and migration time, respectively. These results indicate that daily calibrations will generate more precise results. All analytical characteristics calculated from previous experiments are summarized in Table 1. Limit of detection (LOD) values were obtained experimentally by injection of standard solutions with peak height three times greater than the mean of the background signal.

Figure 5 shows typical electropherograms obtained with both standard and pharmaceutical sample solutions. As can be seen, both electropherograms presented very similar shapes and without additional peaks from interfering species (e.g., pharmaceutical excipients) of the sample solution. Among the pharmaceutical excipients (microcrystalline cellulose, lactose monohydrate, starch,

Table 1. Analytical characteristics of the proposed CE method (value ± SD)

Characteristic	K	HCT	LOS
Linear range / (μmol L^{-1})	52.0 to 1300.0	20.0 to 500.0	52.0 to 1300.0
r	0.998	0.995	0.997
Migration time / s	17.6 ± 0.1	30.9 ± 0.1	35.2 ± 0.1
Intra-day RSD / %	2.8	3.3	0.5
Inter-day RSD / %	4.5	6.1	2.1
LOD / (μmol L^{-1})	4.0	10.0	3.0
LOD / (mg L^{-1})	0.2	3.0	1.3
Injections *per* hour	85	85	85
Resolution[a]	6.9 ± 0.2	1.7 ± 0.1	2.4 ± 0.1

[a]Resolution between K/EOF, EOF/HCT and, HCT/LOS peaks. EOF: electroosmotic flow; HCT: hydrochlorothiazide; LOS: losartan; r: correlation coefficient; RSD: relative standard deviation; LOD: limit of detection; LOQ: limit of quantification.

magnesium stearate, quinoline yellow aluminum lake, hypromellose, titanium dioxide, macrogol) informed by the manufacturing companies, magnesium could be detected, however, magnesium (53.0 $cm^2\ \Omega^{-1}\ mol^{-1}$) was not detected because its ionic conductivity[21] is similar to sodium (50.1 $cm^2\ \Omega^{-1}\ mol^{-1}$) that is present in the composition of the BGE solution.

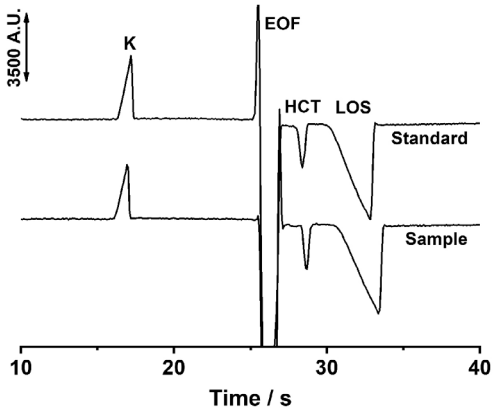

Figure 5. Typical electropherograms obtained for both standard and pharmaceutical sample solutions containing KLOS + HCT (650.0 + 250.0 μmol L^{-1}, respectively). Other conditions see Figure 2.

Next, the accuracy of the proposed CE-C^4D method was evaluated by analyzing two commercial pharmaceutical samples and comparing the results obtained to those obtained by HPLC (LOS and HCT) and flame photometry (K) (Table 2). In this procedure, better results were obtained when narrower linear ranges were used in the calibration curves (Figure S7, SI section).

The results obtained by CE-C^4D (K, HCT and, LOS) were statistically compared with those obtained by HPLC (HCT and LOS) and flame photometry (K) using the paired Student's *t*-test. The calculated *t* values were lower than the tabulated value (4.303; n = 3), which indicates that there were no significant differences between the results (95% confidence level) obtained by CE-C^4D and both HPLC and flame photometry. Furthermore, if the concentrations of K (3.86 g *per* tablet = 10.12 mol *per* tablet) and LOS (42.59 g *per* tablet = 9.93 mol *per* tablet) are converted from mass to molar concentration, information on the stoichiometry of the KLOS salt can also be obtained (10.12:9.93 = 1.00:0.98). This value is close to its theoretical stoichiometric ratio (1:1)[20] and can be used as information about the degradation degree of the active pharmaceutical ingredient (LOS), since K is a stable compound. Finally, recovery studies were also performed by analysis of pharmaceutical samples before and after spiked with known amounts of all target analytes (K, HCT and, LOS). Recovery values (n = 3) close to 100% were obtained for K (98 ± 3%), HCT (102 ± 4%), and LOS (101 ± 2%).

It is worth mentioning that although previously reported methods[1-11] for the simultaneous determination of HCT and LOS have better detectability (< LOD; that is not required to analyze pharmaceutical samples), the developed CE method is much faster (85 injections *per* hour), generate less waste *per* analysis, and is the only one that allows the stoichiometric determination of the KLOS salt (K and LOS).

Conclusions

A fast, eco-friendly, and cost-effective CE method for the simultaneous determination of K, HCT and LOS in pharmaceutical samples was developed. A simple sample preparation step (dissolution and dilution) is only necessary before the measurement procedure. Furthermore, we show here that two analytical techniques can be easily replaced by a single run CE procedure for the quality control of medicines containing KLOS salt and HCT. To the best of our knowledge, this is the first report that shows this ability.

Supplementary Information

Supplementary information (Figures S1-S7) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Table 2. Results obtained in pharmaceutical sample analysis by CE-C^4D (K, LOS, and HCT), HPLC (LOS and HCT) and flame photometry (K)

Sample		Label value / (mg *per* tablet)	CE-C^4D / (mg *per* tablet)	HPLC / (mg *per* tablet)	FP / (mg *per* tablet)
	K	4.20	3.86 ± 0.02		3.95 ± 0.02
A1	HCT	12.50	11.51 ± 0.22	11.84 ± 0.20	
	LOS	45.80	42.59 ± 0.99	42.49 ± 0.20	
	K	4.20	3.97 ± 0.04		4.01 ± 0.03
A2	HCT	12.50	12.96 ± 0.10	13.1 ± 0.30	
	LOS	45.80	44.14 ± 0.58	44.29 ± 0.20	

Analysis time: CE-C^4D (0.60 min), HPLC+FP (7.33 min). CE-C^4D: capillary electrophoresis with capacitively coupled contactless conductivity detection; HPLC: high-performance liquid chromatography; FP: flame photometry; HCT: hydrochlorothiazide; LOS: losartan.

Acknowledgments

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