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# Determination of losartan and hydrochlorothiazide in tablets by CE and CEC

M.G. Quaglia [a,*], E. Donati [a], G. Carlucci [b], P. Mazzeo [b], S. Fanali [c]

[a] *Dipartimento Studi Farmaceutici, Università degli Studi di Roma, 'La Sapienza', Piazzale Aldo Moro 5, 00185 Rome, Italy*
[b] *Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università dell'Aquila, Via Vetoio, 67010 Coppito, L'Aquila, Italy*
[c] *Istituto di Cromatografia del Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy*

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**Abstract**

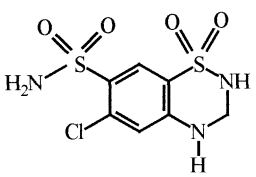
Capillary Electrophoresis (CE) and Capillary Electrochromatography (CEC) have been used to determine losartan and hydrochlorothiazide. The CE separation was carried out in an uncoated capillary filled with a 100 mM sodium borate pH 9 solution containing trimethyl-β-cyclodextrins. CEC was performed using a capillary packed with a RP-18 stationary phase. The mobile phase was a mixture of 50 mM ammonium acetate pH 7, water, acetonitrile (1/1.5/7.5). By CE and CEC suitable methods to determine simultaneously losartan and hydrochlorothiazide in working standard mixture or pharmaceutical form were obtained. The proposed methods are very simple and both gave accurate and precise results. 

*Keywords:* High performance capillary electrophoresis; Micellar chromatography; External and internal standardization

## 1. Introduction

Losartan and hydrochlorothiazide are the two active compounds of an oral pharmaceutical formulation, widely used in antihypertensive therapies. Losartan, 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol, is a selective non-peptide angiotensin II receptor antagonist which has an oral bioavailability of about 33% and a half life of about 2 h. Hydrochlorothiazide, 6-chloro-3,4dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1dioxide, is a benzothiadiazinic diuretic drug widely used in the antihypertensive formulations, alone or in combination with other drugs as in this case [1–4].

**HYDROCHLOROTHIAZIDE**



6-chloro-3,4-dihydro-2 *H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide

* Corresponding author
*E-mail address:* mariagiovanna.quaglia@uniroma1.it (M.G. Quaglia).

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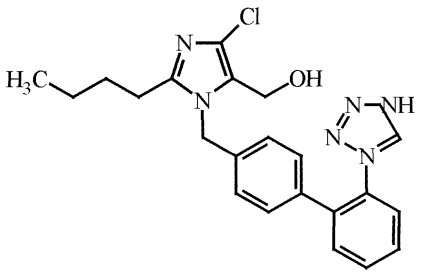
**LOSARTAN**



2-butyl-4-chloro-1-[[2'-(1 *H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl methyl]-1 *H*-imidazole-5-methanol

Losartan alone or together with its active metabolite, the particularly potent 5-carboxylic acid [5–8] was determined by HPLC and high performance capillary electrophoresis (HPCE) [9,10]. Further electrochemical and spectrophotometric procedures were used to analyze hydrochlorothiazide, alone or with other active substances [11–16]. The contemporary analysis of the two drugs in the same pharmaceutical form by HPLC was described by us in a previous paper [17].

The good quality of a drug raw material and finished product must be assured, therefore also the related impurities could be included in an analytical investigation. In the monographs of Pharmacopeia the impurities are generally mentioned, but this investigation is not always possible. Actually it is difficult to find the impurities standard on the market. In the Italian Pharmacopeia [18] only the monograph of hydrochlorothiazide is reported, including three impurities which can remain in the raw material. These impurities are:

- Chlorothiazide;
- 4-amino-6-chlorobenzene-1,3-disulphonamide;
- 4-chloro-6-[6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiazidine-7-yl-(1,1-dioxide)sulphonyl]amino] methyl]-amino]benzen-1,3-disulphonamide.

As above mentioned, we did not find the standards of these impurities, therefore we observed the purity of two active compounds.

Generally the quality control of pharmaceuticals is performed by HPLC, but now the presence more and more numerous of the CE instruments in the industrial pharmaceutical laboratories shows the increasing interest in this separative technique. Actually the technological development of the new instrumentation allows the use of the CE and recently of the CEC in the quality control of pharmaceuticals. CEC is a powerful electrophoretic technique useful for the separation of numerous substances belonging to different classes of compounds [20]. The capillaries used in CEC are generally packed with same phases of the chromatographic columns. The separation principle of the CEC are weither chromatographic or electrophoretic, achieving high efficiency and peak separation.

In this paper we propose the quality control of losartan and hydrochlorothiazide by CE and CEC. The CE analyses were made in an uncoated capillary filled with a borate buffer at pH 9, added or not with trimethyl-β-cyclodextrins or sodium dodecylsulphate (SDS).

The CEC were carried out in a capillary packed with a RP-18 stationary phase. The determination of both active compounds was performed using a working standard mixture and a pharmaceutical formulation (tablets). The suitability of CE and CEC in the determination of losartan and hydrochlorothiazide was compared with them of HPLC.

## 2. Experimental

### 2.1. Reagents and chemicals

Losartan was kindly supplied by the Dipartimento di Medicina Interna of the University of L'Aquila. Hydrochlorothiazide and trimethyl-β-cyclodextrins were purchased from Sigma-Aldrich (Milan, Italy).

SDS and Lichrosphere 100 RP-18 (5 μm), used to fill CEC capillaries, were obtained from Merck-Eurolab (Milan, Italy).

All other chemicals used were provided by Merck-Eurolab (Milan, Italy) and were all of analytical or HPLC grade, water included.

### 2.2. Apparatus

Standards and samples were analyzed by HPCE or CEC using a Hewlett-Packard$^{3D}$ CE Instrument (Waldbronne, Germany) equipped with a linear UV–visible diode array detector and an autosampler. The instrument was controlled and the data were evaluated by a ChemStation and a computer HP KAYAK XM 600 Pentium 3. The uncoated fused silica capillaries (Hewlett-Packard CE capillaries, Germany) have been used for the analyses by HPCE. In the CEC experiments the fused silica capillaries 100 μm I.D. (375 μm O.D.), purchased from composite Metal Services (Hallow, Wores, UK), were packed with Lichrosphere 100 RP-18 (5 μm) (Merck-Eurolab, Milan, Italy).

### 2.3. High Performance capillary electrophoresis

The standards and samples analyses were carried out in an uncoated capillary (48.5 cm total length, 40 cm effective length, 50 μ I.D.) As running buffer a 100 mM sodium borate pH 9 has been used. This running buffer has been directly used or alternatively added with 6 mM trimethyl-β-cyclodextrins or with 10 mM SDS as micelle generator. The HPCE analytical conditions were studied using a mixture of losartan and hydrochlorothiazide as working standard mixture.

### 2.4. Electrochromatography

The fused-silica capillary was prepared in the laboratory of one of us using the conditions previous described [19]. This capillary (total length 33 cm) was completely packed with a RP-18 stationary phase. The effective length of the capillary was 24.5 cm.

A mixture of 50 mM ammonium acetate pH 7, water, acetonitrile (1/1.5/7.5) was used as mobile phase. The capillary conditioning was carried out with the mobile phase, applying 12 bar pressure at the inlet end of the capillary. The pressure (10 bar) and voltage (20 kV) were applied till when a stable current and a good baseline signal were monitored (about 15 min).

CEC experiments were performed applying − 20 kV and 10 bar pressure at both ends of the capillary. Injection was done at the anodic end of the capillary by high pressure application (12 bar for 12 s). The capillary temperature was maintained at 25 °C.

### 2.5. Analytical procedures

#### 2.5.1. Standard solutions

Methanolic solutions of losartan potassium (0.53 mg $ml^{-1}$) and hydrochlorothiazide (0.488 mg $ml^{-1}$) were separately prepared by dissolving each individual drug, exactly weighed, in a 50 ml volumetric flask. By dilution with methanol a series of losartan (concentration range between 0.106 and 0.48 mg $ml^{-1}$) and hydrochlorothiazide (concentration range between 0.0244 and 0.108 mg $ml^{-1}$) solutions were obtained. These solutions were used to control the linear correlation between absorbance values and concentrations.

#### 2.5.2. Internal standardization

A methanolic solution of indapamide was used as internal standard. To verify the linearity of calibration curve, indapamide standard solution was prepared by transferring 128.3 mg, exactly weighed, in 50 ml volumetric flask and diluting up to mark with methanol. The calibration curve was plotted in a concentration range between 0.025 and 0.44 mg $ml^{-1}$.

The quantitative determination has been carried out by adding 1 ml of indapamide solution (0.26 mg $ml^{-1}$) in each sample solution examined.

#### 2.5.3. Working standard solution

One milliliter of indapamide (2.57 mg), 1 ml hydrochlorothiazide (0.48 mg) and 3 ml of losartan (1.6 mg) solutions were transferred in 10 ml volumetric flask and diluted up to mark with methanol. This solution was used to point out the analytical conditions.

#### 2.5.4. Analysis of tablets

A tablet of samples analyzed, containing 12.5 mg of hydrochlorothiazide and 45.8 mg of losar-

tan, had a middle weight of 256.6 mg. Five of these tablets were accurately crushed and about 110 mg of powdered material were weighed. This weighed powder has been transferred into a 100 ml beaker, partially filled with about 40 ml of methanol. The mixture was put in an ultrasonic bath for 30 min. Afterward 10 ml of the internal standard solution was added in the supernatant and transferred, after filtration by a Whatman anotop (25 mm plus 0.2 μm), in a 100 ml volumetric flask. The extraction was repeated using 20 ml of methanol then added in the volumetric flask. The solution has been filled up to mark with methanol and used weither for the analyses by HPCE or by CEC.

*2.5.5. Samples injection*

The samples were introduced in the instrument by hydrodynamic mode applying:

50 mbar for 3 s. in CE

12 bar for 12 s in CEC

## 3. Results and discussion

At the beginning of our experiments regarding the quality control of losartan and hydrochlorothiazide, raw materials and pharmaceutical formulations, we used a capillary zone electrophoretic method (CZE). The analyses have been performed using an uncoated capillary, filled with 100 mM borate buffer at pH 9. In few minutes a good separation as anions of two drugs has been obtained (Fig. 1). However, we noted that, repeating the analyses (six repeated analyses intraday), the losartan migration time remained unchanged, while the migration time of hydrochlorothiazide slowly increased. Hydrochlorothiazide seemed to interact with internal capillary wall.

To avoid this interaction we tried two other methods by adding to B.G.E. trimethyl-β-cyclodextrins, to form an inclusion complex (Fig. 2a), or SDS, as micelles generator to perform the micellar electrokinetic chromatography (MEKC) (Fig. 2b). Also in these conditions the two drugs were negatively charged.

The performances of two methods were similar both allowing a good resolution of two drugs and a good repeatability of their migration times.

For the determination of hydrochlorothiazide and losartan we preferred to use the trimethyl-β-cyclodextrins, added in the running buffer.

It is generally known that in CE the sample volume injected is measured not by the injection volume, as in HPLC, but by the injection time. The more used sample introduction method is the hydrodynamic mode. This method is based on pressure differences, applied for few seconds between the inlet and outlet ends of the capillary. In a capillary with a fixed length and diameter, the hydrodynamic flow is proportional to the pressure difference and inversed proportional to the viscosity of the running buffer. To significantly reduce the injection related imprecision [20,21] and to

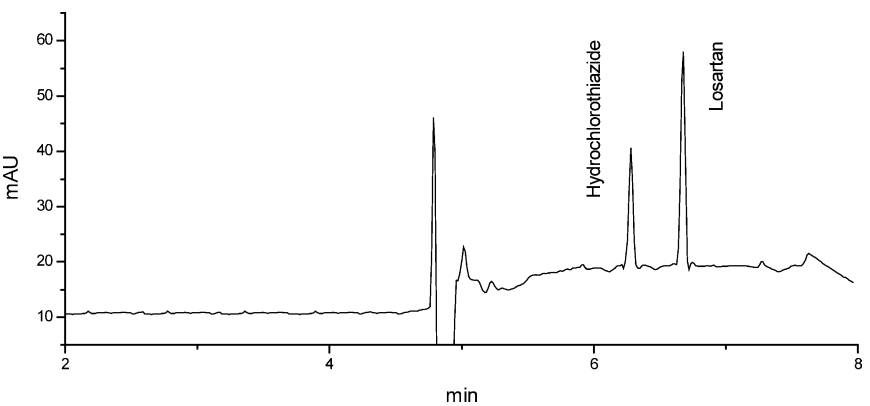



Fig. 1. Standard mixture of hydrochlorothiazide and losartan. B.G.E.: 100 mM borate buffer pH 9, applied voltage 15 kV, temperature 25 °C, injection 50 m bar × 3 s. Capillary: 50 μm I.D., 48.5 cm total length (40 cm effective length).

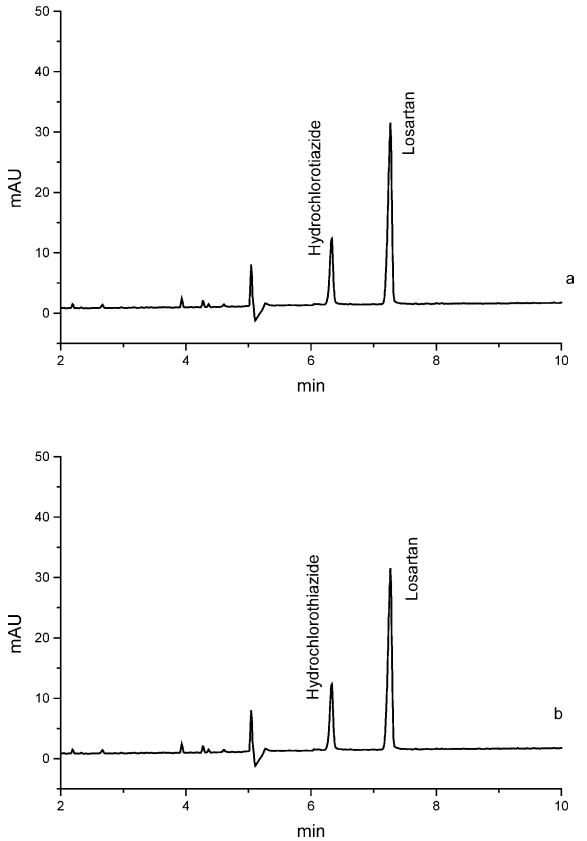



Fig. 2. Standard mixture of hydrochlorothiazide and losartan. B.G.E.: 100 mM borate buffer pH 9 added with 6 mM trimethyl-β-cyclodextrins (a) or 10 mM SDS (b). Applied voltage 15 kV, temperature 25 °C, injection 50 m bar × 3 s. Capillary: 50 μm I.D., 48.5 cm total length (40 cm effective length).

assure a better reproducibility and greater control over the sample amount injected, the use of an internal standard in the quantitative analysis is generally preferred. In this case we chose indapamide as internal standard because it was well resolved from the two other drugs (Fig. 3).

The concentrations of the two drugs in the tablets are very different (12.5 mg of hydrochlorothiazide and 45.8 mg of losartan). Therefore, to increase the hydrochlorothiazide sensitivity the analyses have been performed at its maximum absorbance value (226 nm) even if the maximum absorbance value of losartan is 200 nm (Fig. 4).

A standard solution containing the same amount of indapamide, hydrochlorothiazide and losartan has been used to compare the peak areas. The areas have been then normalized (peak area/migration time). The percentage ratio between internal standard (I.S) and losartan (or hydrochlorothiazide) area was measured, obtaining a correction factor.

The stability of standard solution was observed. These solutions, stored in the night at 4 °C for over 1 month showed no evidence of decomposition.

The individual calibration curves for indapamide, hydrochlorothiazide and losartan were set up on standard solutions in the suitable concentration range (losartan 0.106–0.48 mg $ml^{-1}$; hydrochlorothiazide 0.0244–0.108 mg $ml^{-1}$; indapamide 0.025–0.49 mg $ml^{-1}$). In the considered concentration ranges good linearity was always found. The RSD values of the slope were 1.5% for losartan and 1.9% for hydrochlorothiazide.

The LOD and LOQ of two drugs were, respectively 0.032 and 0.096 ng for losartan while 0.04 and 0.12 ng were the values of hydrochlothiazide.

The calibration curves, better described in Section 2, were obtained by plotting the peak area ratio of each drug to indapamide versus its concentration or by plotting peak area ratio versus

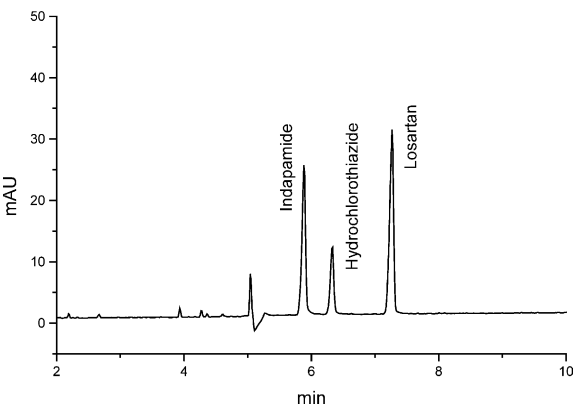



Fig. 3. Standard mixture of hydrochlorothiazide and losartan added with indapamide as internal standard. B.G.E.: 100 mM borate buffer pH 9 added with 6 mM trimethyl-β-cyclodextrins. Applied voltage 15 kV, temperature 25 °C, injection 50 mbar × 3 s. Capillary: 50 μm I.D., 48.5 cm total length (40 cm effective length).

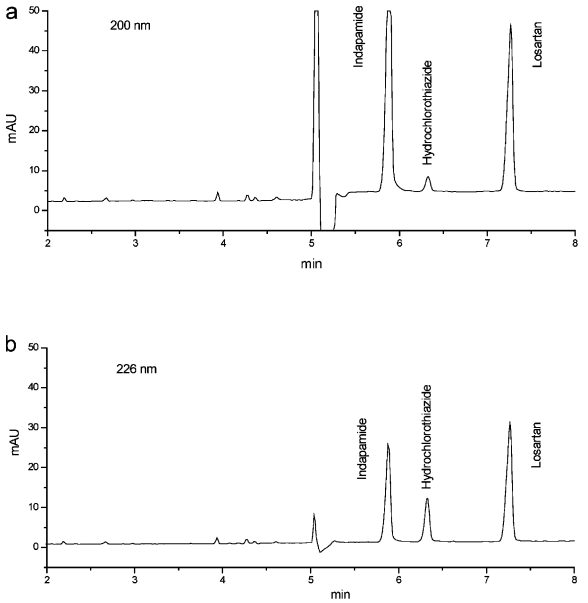



Fig. 4. Standard mixture of hydrochlorothiazide and losartan added with indapamide as internal standard. B.G.E.: 100 mM borate buffer pH 9 added with 6 mM trimethyl-β-cyclodextrins. Applied voltage 15 kV, temperature 25 °C, injection 50 mbar × 3 s. Detection wavelength 200 nm (a) or 226 nm (b). Capillary: 50 μm I.D., 48.5 cm total length (40 cm effective length).

concentration, respectively of hydrochlorothiazide and losartan. The RSD values of the slope were 1.5% for losartan and 2.2% for hydrochlorothiazide.

The quantitative analyses were performed by a working standard mixture, containing known amounts of losartan, hydrochlorothiazide and I.S., or pharmaceutical form. The recovery from the tablets extraction was 97.6% for hydrochlorothiazide and of 97% for losartan.

Since in the our previous paper we proposed the determination of the same pharmaceutical form by HPLC, we repeated the determination of losartan and hydrochlorothiazide also by CEC, the last born among the separative techniques. The same working standard solution, used in the CE experiments, was introduced in a capillary packed with RP-18 stationary phase. The mobile phase was a mixture of 50 mM sodium acetate/water/acetonitrile (1/1.5/7.5) The electrochromatogram obtained in these conditions showed

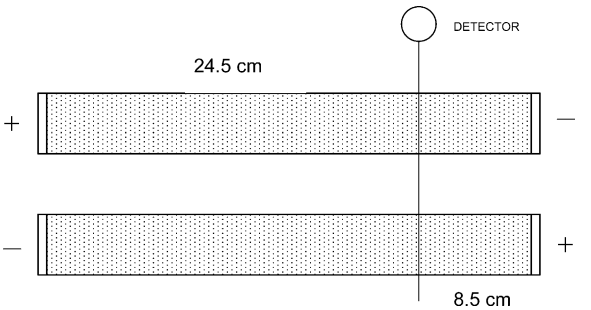



Fig. 5. Long and short effective length of the capillary.

only one peak related to hydrochlorothiazide, but after 60 min losartan was yet retained in the stationary phase. Therefore, we changed the analysis conditions by injecting the sample at the anodic end of the capillary and inverting the polarity (Fig. 5). In this way we reduced the analysis time at about 5 min (Fig. 6). The electrochromatogram is very similar to the chromatogram obtained in our previous paper: the retention times of losartan and hydrochlothiazide have been, respectively 4.6 and 1.7 min by CEC, 6 and 2 min by HPLC. CEC seemed to be more selective than CE. Actually the electrochromatogram showed the same impurity present in the chromatogram. Both impurities showed the same UV spectrum, comparable to that chlorothiazide, one of the possible impurity of hydrochlorothiazide. Increasing the concentration of hydrochlorothiazide injected (about 0.5 mg $ml^{-1}$) two other unidentified impurities appeared.

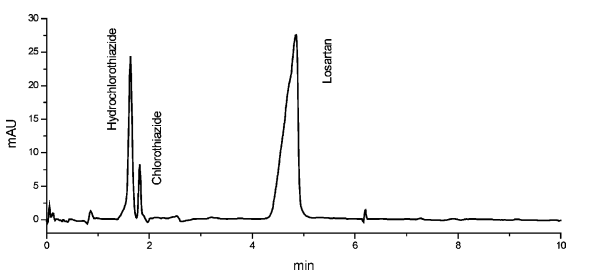



Fig. 6. Electrochromatogram obtained by CEC from the losartan and hydrochlorothiazide analysis. Capillary packed with LiChrospher 100 RP-18 (5 μ). Capillary: 100 μm I.D., 33 cm total length (24.5 cm effective length). Mobile phase: 50 mM sodium acetate buffer pH 7/water/acetonitrile (10/15/75). Applied voltage 20 kV. Applied pressure 10 bar (both sides). Injection: 12 bar × 12 s.

Table 1
Results obtained in the analysis of pharmaceutical form

| | Nominal I (mg) | Found I (mg) | Nominal II (mg) | Found II (mg) |
|---|---|---|---|---|
| CE | 45.8 | 45.27 | 12.5 | 12.23 |
| CEC | 45.8 | 45.30 | 12.5 | 12.34 |
| HPLC | 45.8 | 45.60 | 12.5 | 12.40 |

The linearity of calibration curves of hydrochlorothiazide and losartan obtained by CEC was verified. The RSD values of the slope of losartan and hydrochlorothiazide were 1.5 and 2.2%, respectively. The LOD and LOQ obtained by CEC were 0.025 and 0.075 ng for hydrochlorothiazide and 0.028 and 0.084 ng for losartan.

The quantitative determination of active compounds on tablets, made by CE and CEC, is summarized in Table 1 together with HPLC data.

Our work showed that in the quality control of losartan and hydrochlorothiazide CEC provided the potential selectivity of HPLC and high efficiency of CE.

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