Journal of Chromatographic Science, 2016, 1–6
doi: 10.1093/chromsci/bmw068
Article

Article

Comparison of Stability-Indicating LC Methods Using Light Scattering and Photodiode Array Detection with Monolithic Column for Determination of Quinapril and Hydrochlorothiazide

Marta de Diego*, Ricardo Godoy, Sigrid Mennickent, Carola Vergara, Henry Charnock, and Camilo Hernández

Faculty of Pharmacy, University of Concepción, PO Box 237, Concepción, Chile

*Author to whom correspondence should be addressed. Email: mdediego@udec.cl

Received 13 January 2016; Revised 3 March 2016

Abstract

Rapid stability-indicating LC methods for simultaneous analysis of quinapril and hydrochlorothiazide were developed, validated and compared using evaporative light scattering detection (ELSD) and diode array detection (DAD). For the separation of quinapril, hydrochlorothiazide and its major degradation products, a monolithic column was used and the analytes were eluted within 7 min, applying gradient mobile phase in both methods. Quinapril was subjected to hydrolytic, oxidative, thermal, humidity and photolytic stress conditions. Degradation products were well resolved from main peaks and from each other, proving the stability-indicating power of the methods. The response with DAD was linear and the response with ELSD was fitted to a power function, for quinapril and hydrochlorothiazide concentrations of 20–160 and 12.5–100 μg mL^{-1}, respectively. DAD method achieved better precision than ELSD method, the LOQ of DAD was lower and the accuracy of the methods was similar. Quinapril degrade by hydrolysis and thermal stress, showing the formation of quinaprilat and quinapril diketopiperazine as degradants, which were identified by MS–MS. The methods were successfully applied to quantify quinapril and hydrochlorothiazide in commercial tablets. LC–DAD and LC–ELSD methods are suitable to assess the stability and routine analysis of quinapril and hydrochlorothiazide in pharmaceutical industry.

Introduction

The stability of a pharmaceutical product is closely related to its potency; therefore, whether the compounds are degraded, a decrease of the therapeutic effect or changes in their toxicological properties would be produced, affecting their efficacy and safety. Thus, it is mandatory to maintain a stable pharmaceutical product and also to have analytical tools to demonstrate stability (1). Therefore, stability-indicating methods are required to quality control of pharmaceuticals (2).

Quinapril hydrochloride (QUIN) and hydrochlorothiazide (HCTZ) (Figure 1) are drugs used as single therapy or in association in the treatment of hypertension. HCTZ is a thiazide diuretic and QUIN is an angiotensin-converting enzyme inhibitor, which is a prodrug with little pharmacological activity until hydrolyzed to quinaprilat (3). Both drugs have chemical structures susceptible to degradation, so it is important to have suitable methods in order to evaluate their stability. QUIN undergoes hydrolysis and cyclization intramolecular, forming two degradation products: quinaprilat and quinapril diketopiperazine (DKP), respectively (4–8), and HCTZ also undergoes hydrolysis forming 4-amino-6-chloro-1,3-benzendisulfonamine (DSA) and formaldehyde by hydrolysis (9–13). Stability-indicating LC methods for the

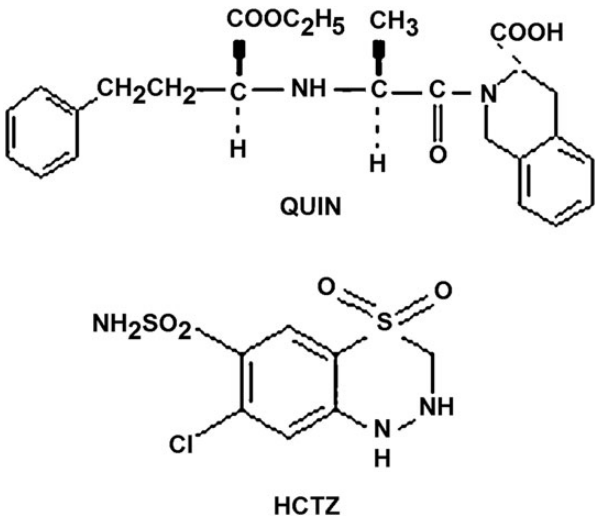

Figure 1. Chemical structures of QUIN and HCTZ.

individual determination of QUIN in an extemporaneous formulation (6) and as a drug substance have been reported (7). Some stability-indicating LC methods to quantify HCTZ in combination with other drugs, such as losartan (10, 11), irbesartan (14), ramipril (15), enalapril (9), spironolactone (16), aliskiren (17), losartan and atenolol (18) and valsartan with amlodipine, were reported (19). A stability-indicating LC method for the simultaneous determination of QUIN and HCTZ was developed by the authors (4), in which a conventional RP-18 column and UV detector were used, as well as a monograph employing a LC method for organic impurities reported in USP (20). In the present study, we present two methods, one with DAD and the other with evaporative light scattering detection (ELSD) that are faster by using a monolithic column, and enable peak purity determination with DAD and detection of possible degradation products undetected by UV detector, with ELSD.

ELSD measures the intensity of light scattered by solids that remain after the solvent has been evaporated, and its response is independent of the chemical structures of the compounds (21, 22). ELSD has been principally used for the detection of compounds lacking chromophores in the pharmaceutical field, such as gabapentin, spectinomycin and tobramycin (21, 23, 24). Some comparative studies have been developed to assess the performance of methods using ELSD versus UV (22); however, to the best of our knowledge, there is no detailed comparison between both detectors in stability-indicating methods.

Monolithic columns are made from a single piece of highly porous monolithic rods of silica with a bimodal pore structure of macropores and mesopores. Macropores allow a rapid flow of the mobile phase at low pressure and the mesopores provide high surface area for good efficiency. Therefore, monolithic columns are used as an alternative to microparticle columns (25).

The aim of this work was to develop, validate and compared two rapid stability-indicating LC methods using monolithic stationary phase with DAD and ELSD for the simultaneous analysis of QUIN and HCTZ in pharmaceutical samples. Both methods were applied to the stability study and analysis of real commercial tablets of QUIN and HCTZ. Moreover, a characterization of degradation products formed under stress testing was performed by direct infusion into the mass spectrometer in order to confirm the identity of the compounds.

Experimental

Instrumentation and reagents

Chemicals and reagents

Standards of QUIN, quinaprilat, DKP and DSA (>99.0% purity) were obtained from USP (Rockville, MD, USA). Standard of HCTZ (>99.0% purity) was obtained from Sigma (St. Louis, MO, USA). QUIN and HCTZ drug substance were obtained from Diprolab (Santiago, Chile). Acetonitrile and methanol LC grade, KH_2PO_4, sodium hydroxide, triethylamine, acetic acid, hydrochloric acid and hydrogen peroxide p.a. grade were purchased from Merck (Darmstadt, Germany). Milli-Q grade water was used for the preparation of mobile phase. The commercial drug tablets containing 20 mg of QUIN and 12.5 mg of HCTZ were purchased from a Chilean pharmacy.

Instrumentation

The LC/DAD analysis was performed on a Series Flexar HPLC system (Perkin Elmer, Norwalk, CT, USA). The system consists of a binary LC pump, a DAD and a column oven, equipped with a manual injector and a 20-µL loop. Chromera software was used for the data collection. The LC/ELSD analysis was performed on a YL9100 HPLC system (Young Lin Instrument, Anyang, Korea). The system consists of a YL9110 quaternary pump, a YL9101 vacuum degasser, a YL9130 column compartment and a Sedex model 85 LT-ELSD detector (low temperature evaporative light scattering detector) (Sedere S.A., Alfortiville Cedex, France), equipped with a manual injector and a 20-µL loop. YL-Clarity Software version 3.0.4.444 was used for the data collection. The MS analyses were carried out with a 3200 QTrap LC–MS-MS Applied Biosystems® (MDS Sciex, California, USA). Instrument control and data collection system were carried out using a CLASS-VP DAD Shimadzu Chromatography Data System and Analyst software (version 1.5.2) for MS^2 analysis.

Chromatographic conditions

HPLC analyses were carried out on a Chromolith® High Resolution RP-18 column (100 × 4.6 mm; Merck). For LC/DAD analyses, the mobile phase consisted of acetonitrile (A) and phosphate buffer (pH 3.0; 0.01 M) (B) in a gradient mode; T_{min}/A %; T_0/15; T_2/15; T_4/65; T_6/65; T_8/15 with 8 min for column re-equilibration. The flow rate was set to 1.5 mL min^{-1} with UV detector wavelength fixed at 215 nm and the column temperature was set at 30°C. For LC/ELSD analyses, the mobile phase consisted of acetonitrile (A) and water with acetic acid (0.086 M) and triethylamine (0.007 M) (pH 3.3) (B) in a gradient mode; T_{min}/A %; T_0/15; T_2/15; T_4/75; T_6/75; T_8/15; with 8 min for column re-equilibration. The flow rate was set to 1.0 mL min^{-1} and the column temperature was set at 35°C. ELSD evaporation temperature was set at 40°C, the gain was 7 and the nebulizer gas pressure was kept at 3 bar. The optimum column temperature, evaporation temperature and gas pressure were obtained using an experimental design procedure by means of the software Statgraphics Centurion XV, version 15.2.05. Preliminary identification of degradation products was made by $ESI\text{-}MS^n$, employing the following parameters: positive ionization mode; drying temperature, 350°C; ion spray voltage, +4000 V; nebulizer gas, 30 psi; auxiliary gas, 20 psi; collision energy, 30; scan range, 100–1200 *m/z*.

Standard solutions and sample solution preparation

The stock solution of HCTZ, DSA, DKP and quinaprilat was prepared in methanol at 1.0 mg mL^{-1}. The stock solution of QUIN was prepared in acetonitrile at 1.0 mg mL^{-1}. The standard solutions were prepared from the stock solutions after adequate dilution with water.

To prepare the sample solution, 20 tablets were weighed and thoroughly homogenized. An amount equivalent to 2.0 mg of QUIN and 1.25 mg of HCTZ was accurately weighed into a 25 mL volumetric flask, and 5 mL of water was added. The sample was vortexed for 15 s and sonicated for 15 min. Then 5 mL of acetonitrile was added, and the solution was vortexed for 15 s and sonicated for 15 min. Then the volume was made up with water and finally the solution was filtered by sample filter (0.45 µm nylon membrane filter). The final concentrations were of 80.0 µg mL^{-1} for QUIN and 50.0 µg mL^{-1} for HCTZ.

Methods

Method validation

The method was validated according to the ICH Q2 (R1) guideline (26). Linearity, precision, accuracy, selectivity, limits of detection and quantification, and robustness were used as the validation parameters. The results of validation parameters obtained from LC/DAD and LC/ELSD methods were statistically compared at the 95% confidence level; *F*-test and *t*-test were used to compare precision and accuracy, respectively.

Linearity

The linearity was investigated for the calibration curves in which the concentrations of the drugs were plotted against the peak areas. Five calibration solutions of 20, 40, 80, 120 and 160 µg mL^{-1} for QUIN and 12.5, 25, 50, 75 and 100 µg $mL^{-1}$for HCTZ were prepared by serial dilution of stock solution, each solution was injected three times.

Precision

The precision of the method was determined by intraday and interday precision. The intraday precision was examined by carrying out three independent assays in three concentrations on the same day under the same experimental conditions, and the interday precision was assessed by carrying out the assays on three different days. The precision was calculated by relative standard deviation.

Accuracy

The accuracy was evaluated by recovery test, applying the method to the analysis of a mixture of common tablet excipients (lactose, starch and magnesium stearate) with known amounts of standard of the drugs, equivalent to 80, 100 and 120% of the nominal levels in the tablets. Three replicates of each level were analyzed. The results were expressed as the percentage of QUIN and HCTZ recovered from the formulation matrix.

Selectivity

The selectivity was evaluated through the stress studies in order to demonstrate the separation between QUIN, HCTZ and their degradation products. It was also evaluated by observing any interference from excipients used in the tablets; therefore, samples of the commercial products were analyzed. The peak purity test was evaluated using DAD to confirm that there was no co-eluting analytes.

LOD and LOQ

The LOD and LOQ were calculated from the slope (*S*) and the standard deviation of the intercept (σ) of the mean of three calibration curves, using the equations: LOD = 3.3 σ/S and LOQ = 10 σ/S.

The LOQ was validated by triplicate analysis of samples prepared at a concentration near to that experimentally obtained.

Robustness

The robustness of the method was tested by changing the flow rate and pH of aqueous phase of mobile phase in ±0.2 units. Replicate injections ($n = 3$) of test solutions containing 80 µg mL^{-1} of QUIN and 50 µg mL^{-1} of HCTZ were performed. The theoretical plate number and peak tailing factor were calculated as comparison parameters.

Stress testing

The stress study was carried out according to the ICH stability testing guidance Q1A(R2) (27). The forced degradation studies of HCTZ were evaluated previously by the authors using LC with UV detection (9) and a method using LC/DAD and LC/ELSD is under consideration at this time, for simultaneous determination of HCTZ and candesartan, finding that HCTZ degrade via hydrolysis with formation of one degradation product and was stable under photolytic, thermal and oxidative conditions; therefore, only stress testing of QUIN is described. As mentioned in Introduction, a stability study of QUIN was developed by the authors in which a conventional RP-18 column and UV detector were used (4). In the present work, stress testing of QUIN was performed using LC/DAD and LC/ELSD with a monolithic stationary phase. QUIN was stressed under different conditions until to produce ~5–20% degradation, in order to obtain a relevant degradation product (primary degradation product) (28). After degradation, the samples were diluted with water to 120 µg mL^{-1}. Acid, alkaline and neutral degradation were carried out on a hot plate at 60°C, with sample solutions of 400 µg mL^{-1} prepared in 0.1 N HCl, 0.1 N NaOH and water for different periods of time. Acid and alkaline solution were neutralized before analysis. The oxidative degradation was induced by storing sample solutions of 400 µg mL^{-1} in 6% H_2O_2 at room temperature (25 ± 2°C) for 7 days in the dark. For thermal and humidity stress, solid drugs were spread in a thin layer on a petri-plate and subjected to dry heat at 60°C in an oven for 20 days and at 75% RH at room temperature (25 ± 2°C) over a saturated NaCl solution, for 24 h. Photodegradation was carried out according to option 2 of the ICH Q1B guidelines (29). Samples of 400 µg mL^{-1} and solid drug in 1 mm layer in a petri-plate were exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 watt hours/m^2.

The peak purity in the degraded samples was checked using a DAD.

Results

Method development and optimization

The sample for optimization of the chromatographic conditions was a mixture of standards of QUIN at 120 µg mL^{-1}, HCTZ at 75 µg mL^{-1} and quinaprilat, DKP and DSA at 40 µg mL^{-1}.

For LC/ELSD method, three different factors were evaluated in order to determine their influence on the chromatographic detection of both compounds. Column temperature (35–45°C), evaporation temperature (40–60°C) and gas pressure (3–4 bar) in a two-level full factorial design (2^3) with a central point and two replicates to assess their impact on the ELSD response. The measured response was peak height and was plotted in relation to each modified factor. A schematic representation of the analysis was obtained in order to easily highlight the significance of each effect on the response.

Optimal chromatographic conditions, with good peak shapes and appropriate resolution in a short separation time, were obtained as described in Chromatographic conditions section. System suitability parameters are within the suitable range: Rs > 2.0 between all peaks,

peak tailing factor between 1.2 and 1.4 for QUIN and HCTZ and theoretical plates ~80,000 for QUIN and 8,000 for HCTZ.

As shown in Figure 2, the LC/DAD and LC/ELSD methods enabled separation between QUIN, HCTZ and their degradation products; therefore, they proved to be stability-indicating.

Method validation

Linearity

The linearity data showed that the DAD response is linear; the equations of the calibration curves were $y = 38.148x + 133.4$; $r^2 = 0.9983$ for QUIN, and $y = 73.661x + 95.68$; $r^2 = 0.9995$ for HCTZ. According to statistical analyses by analysis of variance, both calibration curves were linear ($P < 0.005$). For ELSD, a nonlinear response was observed, and its response can be fitted to a power function; the equations of the calibration curves were $y = 0.156x^{1.871}$; $r^2 = 0.996$ for QUIN, and $y = 0.077x^{1.829}$; $r^2 = 0.995$ for HCTZ. As the response is nonlinear, a log–log transformation was applied producing linear curves with $r^2 = 0.996$ for QUIN and $r^2 = 0.995$ for HCTZ.

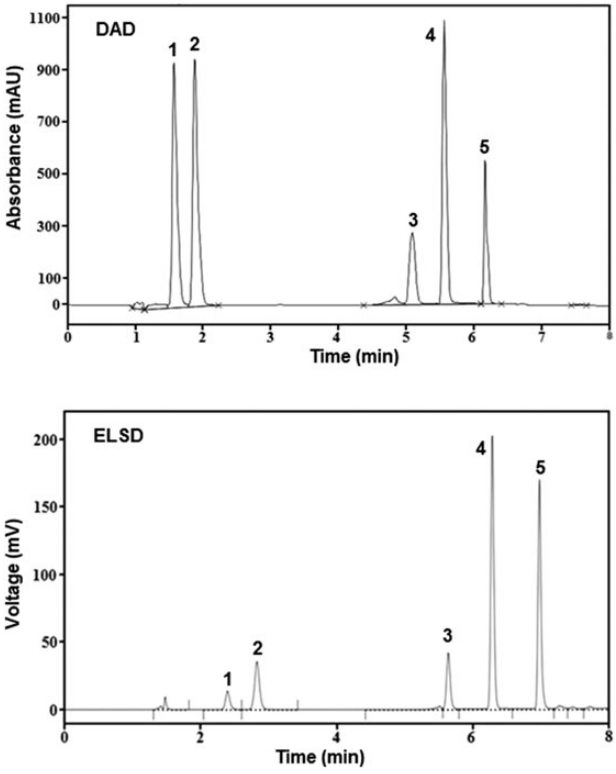

Figure 2. Chromatogram with DAD and ELSD: (**1**) DSA, (**2**) HCTZ, (**3**) quinaprilat, (**4**) QUIN and (**5**) DKP.

Precision

The results of precision study are shown in Table I. The obtained values with DAD and ELSD were compared using *F*-test; the calculated *F*-test values showed that there were significant differences between precision of both methods for QUIN and HCTZ with $P < 0.05$, showing that the LC/DAD method is more precise; anyway, the obtained values show a suitable precision for both methods. These results are in accordance with previous research, which show that UV detection is more precise than ELSD for determination of antidiabetic drugs (22).

Accuracy

The results of accuracy study are shown in Table I. According to *t*-test ($n = 9$, $\alpha = 0.05$), the recovery obtained with DAD and ELSD did not differ from the real value and there were no significant differences between both methods.

Selectivity

Results from the stress studies indicated that the methods were able to resolve all the peaks as shown in Figure 2; besides, no interference from formulation excipients was found. The studies with the DAD showed that all peaks were pure, with values of peak purity index higher than 0.999 for QUIN and HCTZ, proving that the proposed methods are selective.

Limits of detection and quantification

The LOD values were 0.39 and 1.94 μg mL^{-1} for QUIN and 0.30 and 1.78 μg mL^{-1} for HCTZ with DAD and ELSD, respectively, and the LOQ values were 1.20 and 5.88 μg mL^{-1} for QUIN and 0.91 and 5.39 μg mL^{-1} for HCTZ with DAD and ELSD, respectively. According to these results, ELSD was found to have higher LOQ and LOD values; anyway, the obtained values are suitable for pharmaceutical analysis. These results are in accordance with previous research, which show LOD values with UV detection lower than with ELSD for determination of antidiabetic drugs (22). For validation of LOQ,

Table I. Precision and Accuracy Study with DAD and ELSD

Sample level (%)	DAD			ELSD		
	RSD (%)		Recovery (%)	RSD (%)		Recovery (%)
	Intraday[a]	Interday[b]		Intraday[a]	Interday[b]	
QUIN						
80	0.72	0.86	101.5	2.59	1.92	102.9
100	0.58	0.96	100.6	2.09	1.25	98.8
120	0.11	0.84	101.2	3.11	1.14	98.6
HCTZ						
80	0.76	1.25	100.5	0.06	2.63	98.9
100	0.58	1.08	101.4	4.27	1.77	97.8
120	0.68	0.37	99.2	1.95	1.55	103.1

[a]Analyzed on the same day ($n = 3$).
[b]Analyzed on three different days ($n = 9$).

the recovery of QUIN and HCTZ was between 96.8 and 109.4% with RSD lower than 5%.

Robustness

After slight variations of flow rate and pH, the theoretical plate number and peak tailing factor were practically not affected, except at flow rate 1.2 mL min^{-1} for HCTZ with ELSD in which the peak tailing factor increases significantly ($T = 1.8$), probably due to inefficient evaporation of the solvent when mobile phase flow is increased at the predefined temperature of work. As a result, an uneven distribution of dry particulate size is obtained, favoring more than one type of light scattering and consequently obtaining asymmetric peaks.

Stress testing

The stability-indicating property of the methods was confirmed by analysis of the degraded samples; all the detected degradation products were satisfactory separated from QUIN, HCTZ and each other. Peak purity results from the DAD confirmed that the QUIN, HCTZ and degradation product peaks obtained from all the stress samples were pure.

QUIN was found to degrade via hydrolysis and thermal stress, and was stable under humidity, photolytic and oxidative conditions; the results of degradation percentage using both detectors are shown in Table II. According to these results, DAD and ELSD display similar degradation with detection of two degradations products. QUIN degraded rapidly and significantly to quinaprilat by alkaline hydrolysis, and to DKP by acid, neutral and thermal conditions; these results are in agreement with previous reports (4–6). Exposure to acid and aqueous conditions yield the apparition of DKP likely because of a cyclization process, which was confirmed for isolation and fragmentation of ion $m/z = 421.2$. In alkaline hydrolysis studies, QUIN molecular ion $[M-H]^+$ at $m/z = 439.3$ is quickly degraded to compound of $m/z = 393.1$ probably as a consequence of both cyclization and hydrolysis process. These findings were in accordance with previous studies (30), demonstrating that in alkaline conditions, QUIN suffers rapid hydrolysis with initial fragmentation to quinaprilat ($m/z = 411.3$) followed by further degradation to ion $m/z = 393.1$.

Table II. Degradation of Quinapril with DAD and ELSD

	DAD				ELSD			
	Degradation (%)				Degradation (%)			
	NaOH	HCl	H_2O	Thermal	NaOH	HCl	H_2O	Thermal
Time	5 min	3 h	3 h	20 days	5 min	3 h	3 h	20 days
	54.9	14.1	4.9	26.5	50.1	15.1	5.3	23.8

Table III. Analysis of Commercial Drugs by DAD and ELSD

Batch	DAD		ELSD	
	Found content (%)		Found content (%)	
	QUIN	HCTZ	QUIN	HCTZ
1	101.5 ± 0.8	100.2 ± 0.6	98.0 ± 1.3	96.1 ± 0.4
2	101.7 ± 2.2	101.8 ± 0.9	96.3 ± 1.9	101.8 ± 1.8
3	104.3 ± 1.3	102.5 ± 1.0	104.1 ± 0.6	100.0 ± 2.1
	Found amount (mg)		Found amount (mg)	
1	20.3	12.5	19.6	12.0
2	20.3	12.7	19.3	12.7
3	20.9	12.8	20.8	12.5

Application to the analysis of commercial formulation

The proposed methods were applied to the determination of QUIN and HCTZ in three different lots of the commercially available tablets. As shown in Table III, both detectors display similar results for all products. According to USP, the amounts found are within the specified limits of 95–105%.

Discussion

According to a previous research (4), the compounds were initially analyzed with gradient elution using acetonitrile as organic modifier of the mobile phase. Two monolithic stationary phases were tested: a Chromolith® HighResolution RP-18 column (100 × 4.6 mm) and a Chromolith® Performance RP-18 column (100 × 3 mm). According to the manufacturer, the difference between both columns is efficiency; HighResolution column is more efficient than Performance column; this was experimentally displayed by analyzing the sample solution with both columns, obtaining theoretical plates of 6,050 and 7,500 for HCTZ, and 38,700 and 80,550 for QUIN, with Performance and HighResolution columns, respectively. The retention time was lower with Performance column, showing a low resolution between HCTZ and DSA with t_R <1 min for both compounds. Therefore, HighResolution column was selected. For LC/DAD different buffer pHs of the aqueous phase of mobile phase were evaluated; at pH >3.0, the quinaprilat peak efficiency decreases considerably (peak tailing factor 1.2 at pH 3.0 and 2.2 at pH 4.6). For LC/ELSD, acetic acid and triethylamine were added into the aqueous phase of mobile phase to enhance sensitivity and peak shapes. Different concentrations of these compounds were tested in order to optimize the mobile phase (data not shown). The factorial design results showed that the ELSD response was decreased by increasing the column temperature, evaporation temperature and gas pressure; the effects of the factors were statistically significant ($\alpha = 0.05$); therefore, the lowest values of these parameters were selected.

These methods represent an alternative to the previously reported (4, 20), which use a conventional RP-18 column and UV detector; with the advantages of shorter analysis (all analytes were eluted within 7 min). On the other hand, the use of DAD allows peak purity determination and ELSD allows detection of possible degradation products that are undetected by UV detector.

Validation parameters are appropriate showing that the methods are suitable for its intended use, although the DAD method is more accurate and may determine lower concentrations than ELSD.

Stress study shows that QUIN is considerably degraded via hydrolysis and thermal stress, especially under alkaline conditions, with formation of quinaprilat and DKP as were identified by mass analysis. Therefore, caution should be taken in the production process and on storage of this product in order to prevent degradation. When using it as a drug product, due to compound degradation, it could lead to a decrease in the therapeutic effect or changes in its toxicological properties.

Conclusion

Two new rapid stability-indicating LC methods using a monolithic stationary phase with DAD and ELSD have been developed and validated for simultaneous determination of QUIN and HCTZ. The

performance of both detectors was compared, obtaining that DAD is more precise and has a lower detection limit than ELSD, and the accuracy is similar. Moreover, another advantage of DAD is that it shows a linear relationship, whereas for ELSD shows a nonlinear response. Anyway, the LC–DAD and LC–ELSD methods are suitable to assess the stability and rapid routine analysis of quinapril and HCTZ in pharmaceutical samples. ELSD represents an alternative to DAD in pharmaceutical analysis, especially when non-UV detectable compounds should be analyzed. The stability study of QUIN shows that it is unstable under hydrolysis and thermal stress, showing the formation of quinaprilat and quinapril diketopiperazine as degradants, which were identified by mass spectrometry analysis.

Funding

The authors thank FONDECYT grant 1130447 (CONICYT, Chile).

References

1. Lund, W. (ed). *The pharmaceutical codex*, 12th ed. The Pharmaceutical Press, London, (1994), p. 277.
2. *The United States Pharmacopeia*, 35th ed. The United States Pharmacopeial Convention, Inc., Rockville, (2015), pp. 1509–1511.
3. Mc Evoy, G.K. (ed). *AHFS drug information*. American Society of Health–System Pharmacists, Bethesda, (2014), pp. 2047–2050.
4. De Diego, M., Mennickent, S., Muñoz, J., Sanhueza, F., Godoy, R.; A validated stability-indicating LC method for simultaneous determination of quinapril and hydrochlorothiazide in pharmaceutical samples; *Central European Journal of Chemistry*, (2014); 12: 470–475.
5. Stanisz, B., Paszun, S., Strzyzycka, N., Ptaszynski, E.M.; Influence of humidity and hydroxypropyl cellulose, hydroxypropylmethyl cellulose, glyceryl behenate or magnesium stearate on the degradation kinetics of quinapril hydrochloride in solid phase; *Acta Poloniae Pharmaceutica*, (2010); 67: 99–102.
6. Freed, A., Silbering, S., Kolodsick, K., Rossi, D., Mahjour, M., Kingsmill, C.; The development and stability assessment of extemporaneous pediatric formulations of accupril; *International Journal of Pharmaceutics*, (2005); 304: 135–144.
7. Stanisz, B.; The influence of pharmaceutical excipients on quinapril hydrochloride stability; *Acta Poloniae Pharmaceutica*, (2005); 62: 189–193.
8. Stanisz, B.; The stability of quinapril hydrochloride—A mixture of amorphous and crystalline forms (QHCl-AC)—In solid phase; *Acta Poloniae Pharmaceutica*, (2003); 60: 443–450.
9. De Diego, M., Mennickent, S., Godoy, G., Miranda, V.; A validated stability-indicating LC method for simultaneous determination of enalapril and hydrochlorothiazide in pharmaceutical samples; *Current Pharmaceutical Analysis*, (2011); 7: 248–252.
10. Lusina, M., Cindric, T., Tomaic, J., Peko, M., Pozaic, L., Musulin, N.; Stability study of losartan/hydrochlorothiazide tablets; *International Journal of Pharmaceutics*, (2005); 291: 127–137.
11. Hertzog, D., Finnegan, J., Fang, X., Tyrrell, J., Reed, R.; Development and validation of a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 30: 747–760.
12. Deventer, K., Baele, G., Van Eenoo, P., Pozo, O., Delbeke, F.; Stability of selected chlorinated thiazide diuretics; *Journal of Pharmaceutical and Biomedical Analysis*, (2009); 49: 519–524.
13. Fang, X., Todd, R., Mayr, S., Yin, W., Harmon, P., Finnegan, J., *et al.*; Purification and identification of an impurity in bulk hydrochlorothiazide; *Journal of Pharmaceutical Sciences*, (2001); 90: 1800–1809.
14. Rane, V., Patil, K., Sangshetti, J., Yeole, R., Shinde, D.; Stability indicating LC method for simultaneous determination of irbesartan and hydrochlorothiazide in pharmaceutical preparations; *Journal of Chromatographic Science*, (2010); 48: 595–600.
15. Belal, F., Al-Zaagi, I., Gadkariem, E.; A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms; *Journal of Pharmaceutical and Biomedical Analysis*, (2001); 24: 335–342.
16. Hegazy, M., Metwaly, F., Abdelkawy, M., Abdelwahab, N.; Validated chromatographic methods for determination of hydrochlorothiazide and spironolactone in pharmaceutical formulation in presence of impurities and degradants; *Journal of Chromatographic Science*, (2011); 49: 129–135.
17. Karvelis, D., Kalogria, E., Panderi, I.; A stability-indicating HPLC method for the quantification of aliskiren and hydrochlorothiazide in a pharmaceutical formulation; *Journal of AOAC International*, (2014); 97: 1519–1525.
18. Durga, D., Satyanarayana, N., Sait, S., Ramakoti, Y., Mukkanti, K.; Simultaneous determination of losartan potassium atenolol and hydrochlorothiazide in pharmaceutical preparations by stability-indicating UPLC; *Chromatographia*, (2009); 70: 647–651.
19. Vojta, J., Jedlicka, A., Coufal, P., Janecková, L.; A new, rapid, stability-indicating UPLC method for separation and determination of impurities in amlodipine besylate, valsartan and hydrochlorothiazide in their combined tablet dosage form; *Journal of Pharmaceutical and Biomedical Analysis*, (2015); 109: 36–44.
20. *The United States Pharmacopeia*, 35th ed. The United States Pharmacopeial Convention, Inc., Rockville, (2015), pp. 5523–5525.
21. Shaodong, J., Hill Park, J., Lee, J., Won Kwon, S.; Comparison of two aerosol-based detectors for the analysis of gabapentin in pharmaceutical formulations by hydrophilic interaction chromatography; *Talanta*, (2011); 85: 2301–2306.
22. Shaodong, J., Jun Lee, W., Won Ee, J., Hill Park, J., Won Kwon, S., Lee, J.; Comparison of ultraviolet detection, evaporative light scattering detection and charged aerosol detection methods for liquid-chromatographic determination of anti-diabetic drugs; *Journal of Pharmaceutical and Biomedical Analysis*, (2010); 51: 973–978.
23. Zhou, J., Zhang, L., Whang, Y., Yan, C.; HPLC-ELSD analysis of spectinomycin dihydrochloride and its impurities; *Journal of Separation Science*, (2011); 34: 1811–1819.
24. Chopra, S., Vanderheyden, G., Hoogmartens, J., Van Schepdael, A., Adams, E.; Comparative study on the analytical performance of different detectors for the liquid chromatographic analysis of tobramycin; *Journal of Pharmaceutical and Biomedical Analysis*, (2010); 53: 151–157.
25. Al-Othman, Z., Al-Warthan, A., Ali, I.; Advances in enantiomeric resolution on monolithic chiral stationary phases in liquid chromatography and electrochromatography; *Journal of Separation Science*, (2014); 37: 1033–1057.
26. International Conference on Harmonization, ICH guideline Q2 (R1), Validation of Analytical Procedures: Text and Methodology, Step 4 Version, IFMPA, (2005).
27. International Conference on Harmonization, ICH guideline Q1A(R2), Stability testing of new drug substances and products, Step 4 Version, IFMPA, (2003).
28. Baertschi, S.W. (ed). *Pharmaceutical stress testing, predicting drug degradation*. Taylor & Francis Group, Boca Raton, (2005), p. 142.
29. International Conference on Harmonization, ICH guideline Q1B, Photostability Testing of New Drug Substances and Products, Step 4 Version, IFMPA, (1996).
30. Hailu, S., Bogner, R.; Effect of the pH grade of silicates on chemical stability of coground amorphous quinapril hydrochloride and its stabilization using pH-modifiers; *Journal of Pharmaceutical Sciences*, (2009); 98: 3358–3372.