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Development and validation of a stability-indicating HPLC method for the simultaneous determination of Losartan potassium, hydrochlorothiazide, and their degradation products

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Abstract

Losartan potassium was the first in a new class of potent angiotensin II receptor antagonists which are well-tolerated in the treatment of hypertension. Losartan potassium is the active ingredient in tablets COZAAR® and is combined with diuretic co-active hydrochlorothiazide (HCTZ) in tablets HYZAAR® for increased efficacy. Losartan potassium has one main impurity and two primary degradates. HCTZ has one major degradate as well as two common process impurities. Historically, separate methods have been used for the analysis of each active and their respective impurities and degradates. The ultimate goal of this work was to develop and validate a single high-performance liquid chromatography method selective for the eight main components of tablets HYZAAR®. A single method was developed to afford simultaneous quantitation of actives and degradates for each of the two existing formulations. Each method is presented herein and demonstrated to be suitable for quantitation to 0.1% levels of all relevant degradates, as well as 100% levels of respective drug substances.

Keywords: HPLC; Losartan potassium; Hydrochlorothizaide; Simultaneous determination; Degradate quantitation; Method development; Method validation

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1. Introduction

Losartan potassium (DuP 753, MK-954, hereafter referred to as Losartan) was the first of a new class of orally active, non-peptide angiotensin II (type AT_1) receptor antagonists for the treatment of hypertension. Losartan has been demonstrated to be superior to previous peptide receptor antagonists and angiotensin converting enzyme (ACE) inhibitors because of its enhanced specificity, selectivity, and tolerability [1–4]. Studies have shown an increase in Losartan efficacy when a low dose of hydrochlorothiazide (HCTZ) is administered concomitantly due to stimulation of the renin–angiotensin system [1,5–7]. In this combination therapy, the effects of both drugs appear to be additive and synergistic for patients whose elevated blood pressure is not well controlled with either substance alone [1,7,8].

The active ingredient Losartan potassium has

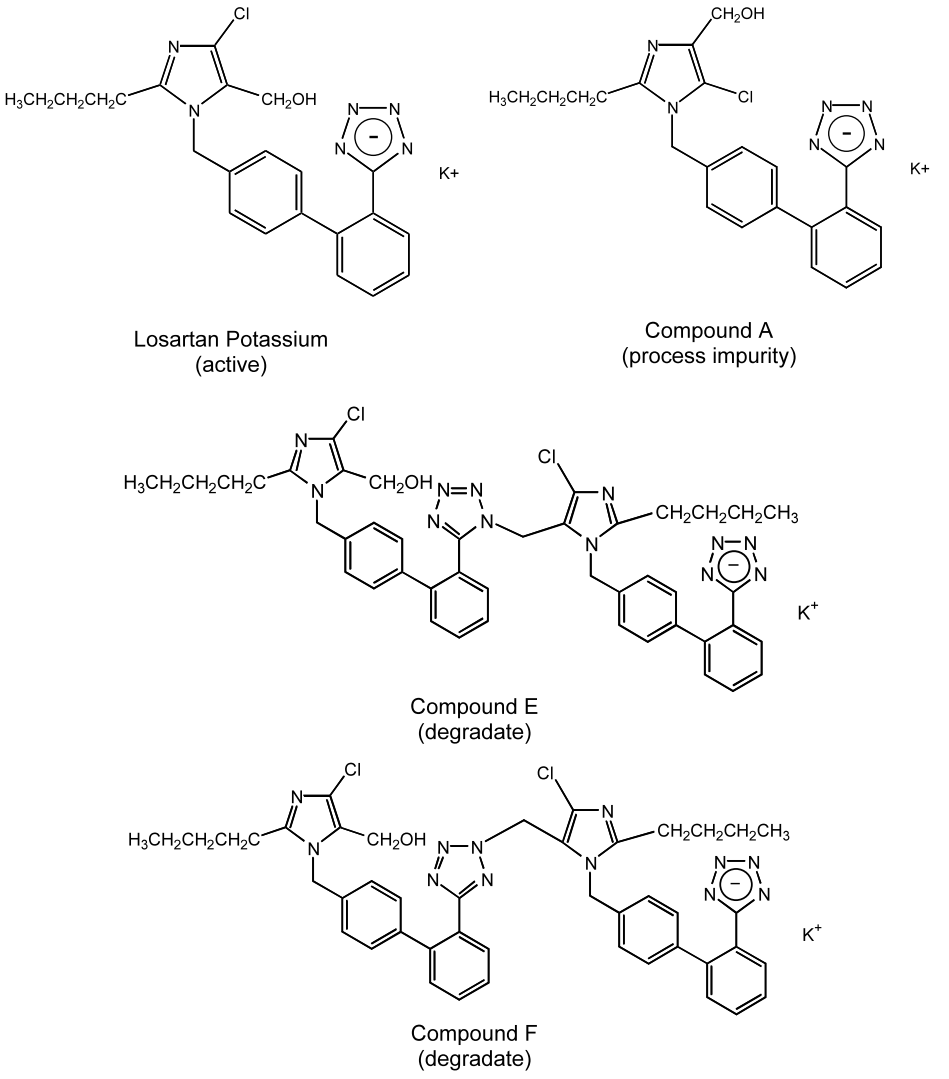

Fig. 1. Structures of Losartan potassium and its related degradates (compounds E and F) and process impurity (compound A). Structures of HCTZ and its related degradate (DSA) and process impurities (CTZ, 5-chloro-HCTZ, and HCTZ–CH_2–HCTZ impurity).

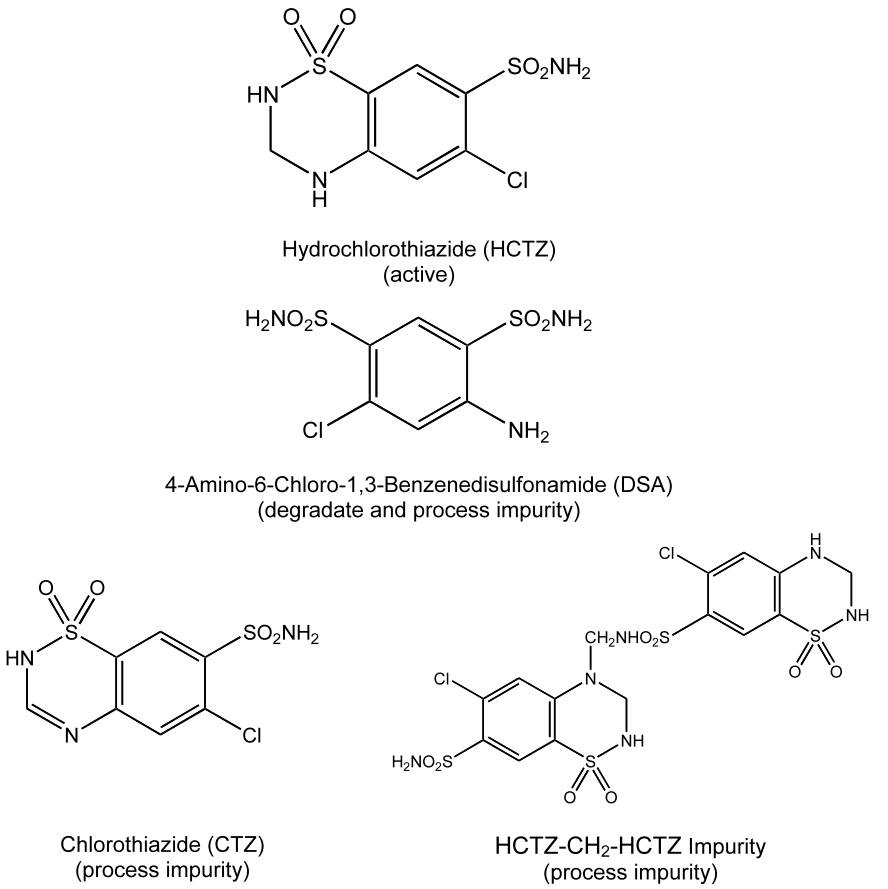

Fig. 1. (*Continued*)

one isomeric process impurity (compound A) and two primary degradates previously identified as the acid dimers, compounds E and F, which form thermally under acidic conditions [9,10] (Fig. 1). A literature survey revealed few analytical reports for the analysis of Losartan in tablet formulations. Furtek [11] and Lee [12] describe HPLC methods for Losartan determination in plasma and Williams [13] compared liquid chromatography, capillary electrophoresis, and super-critical fluid chromatography for the determination of Losartan in tablets. However, only one literature assay by McCarthy [14] using high-performance thin-layer chromatography was shown to be selective and sensitive enough to monitor the less-polar Losartan degradates.

In contrast to this relatively new drug, the diuretic agent HCTZ is one of the oldest and most widely used active ingredients. HCTZ formulations are listed in both the US [15] and European [16] Pharmacopoeias as a monotherapy and as co-therapies with other numerous drugs. HCTZ has one primary degradate pathway which yields 4-amino-6-chloro-1,3-benzendisulfonamine (DSA) (Fig. 1) and formaldehyde by hydrolysis [17]. Chlorothiazide (CTZ) and the recently characterized HCTZ–CH_2–HCTZ impurity are two main process impurities present in HCTZ (Fig. 1B) [16,18]. HPLC techniques are most commonly used for analysis of tablets containing HCTZ and a second active ingredient such as enalapril maleate [19,20], methyldopa [21,22], or amiloride

HCl [23]. Recently, Carlucci [24] described a rapid HPLC method and Erk [25] described a similar HPLC method as well as two spectrophotometric methods for the simultaneous determination of HCTZ and Losartan in tablets. However, a method that is capable of quantitating both actives at the 100% level and related substances down to 0.1% levels using a single sample preparation has not been documented to date for either formulation.

The focus of the work described herein was to develop selective and sensitive HPLC methods for each tablet formulation for which time and efficiency are optimized for use in routine testing. Adequate retention of the parent compound, Losartan, while maintaining a reasonable elution time for the significantly less-polar degradates, compounds E and F, was the main analytical challenge and was not feasible with an isocratic separation with a run time under 1 h. Therefore, a new shortened gradient method was successfully developed and validated for Losartan (COZAAR®) tablets. A longer, steeper gradient separation was required to provide resolution of the significantly more polar HCTZ-related compounds in the presence of Losartan and the very hydrophobic dimer degradates in Losartan/HCTZ (HYZAAR®) tablets. Several analytical challenges common to steep profile gradient methods were encountered and addressed in achieving the final methods. The newly developed methods for Losartan and Losartan/HCTZ tablets were validated for the quantitation of actives at the 100% level and for degradates down to 0.1% levels. Robustness studies were completed to ensure continued performance of the methods in diverse analytical environments. Both methods have been successfully transferred to numerous laboratories throughout the world and are currently in use for routine release and stability testing for assay and degradates for COZAAR® and HYZAAR® tablets.

2. Experimental

2.1. Chemicals and reagents

Losartan potassium tablets and Losartan potassium/HCTZ tablets were manufactured by DuPont Pharmaceuticals Company (Wilmington, DE, USA). Placebo mixtures were prepared in the laboratory using US Pharmacopoeia (USP) grade excipients. Losartan, HCTZ, DSA, and CTZ reference standards were obtained internally at Merck. Reagent grade sodium dihydrogen phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), potassium dihydrogen phosphate monobasic (KH_2PO_4) anhydrous, sodium hydrogen phosphate dibasic (Na_2HPO_4) anhydrous, phosphoric acid 85% solution, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and were used as received. Deionized water (>18 MΩ), purified using a Barnstead Nanopure Bioresearch water system (Dubuque, IA, USA), was used to prepare the mobile phase and the sample and standard solutions.

2.2. Chromatographic equipment and conditions

The development and validation work was performed on a ThermoSeparations HPLC system (Riviera Beach, FL, USA) consisting of a P4000 pump, an AS3000 injector, a SpectraFOCUS variable-wavelength UV detector, and a SN4000 controller with PC1000 Software on an IBM computer. Ruggedness studies were performed on similar ThermoSeparations HPLC systems and Dionex HPLC systems (Marlton, NJ, USA). The analytical columns used to achieve chromatographic separation were Symmetry C8 columns (150×3.9 mm I.D., 5 μm particle size) purchased from Waters Corporation (Milford, MA, USA).

The mobile phase for both the Losartan tablets and the Losartan/HCTZ tablets methods was made by first preparing a phosphate buffer solution of KH_2PO_4 and Na_2HPO_4 (pH 7.0, 0.02 M). This buffer solution was then mixed with acetonitrile in ratios of 85:15 (v/v) and 93:7 (v/v) buffer–acetonitrile to yield Mobile Phase A for Losartan tablets and Losartan/HCTZ tablets, respectively. Mobile Phase B is 100% acetonitrile for both methods. Mobile phases for pH robustness studies involved pH adjustment of the 0.02 M phosphate buffer with NaOH or H_3PO_4 prior to mixing with acetonitrile.

For Losartan tablets, the profile is a linear gradient from Mobile Phase A–Mobile Phase B

(80:20, v/v) to Mobile Phase A–Mobile Phase B (40:60, v/v) over 10 min. The mobile phase composition is ramped back to Mobile Phase A–Mobile Phase B (80:20, v/v) for equilibration prior to the next injection for a total run time of 15 min. The mobile phase flow rate is 1.0 ml/min with ambient column temperature, 10 μl injection volume, and UV detection at 250 nm. The 250 nm wavelength was chosen to minimize gradient contributions to the signal while still maintaining sufficient signal from the Losartan related substances. For Losartan/HCTZ tablets, the two-step gradient profile begins with a 12 min linear gradient from 100% Mobile Phase A to Mobile Phase A–Mobile Phase B (92:8, v/v). This is followed by a linear ramp over the next 16 min to Mobile Phase A–Mobile Phase B (38:62, v/v). Finally, the composition is returned to 100% Mobile Phase A over 2 min followed by a 5 min equilibration for a total run time of 35 min. The UV detection wavelength for Losartan/HCTZ tablets was changed to 280 nm to achieve similar signal strengths for the two actives due to the absorption minimum in the UV spectrum for HCTZ at approximately 250 nm (Fig. 2) and the 4:1 ratio of Losartan to HCTZ in the tablets. The mobile phase flow rate is 1.0 ml/min, the column temperature is controlled at 35 °C, and the injection volume is 20 μl.

2.3. Standard and sample solution preparations

2.3.1. Losartan tablets

To prepare the 100% standard for Losartan tablets, Losartan potassium reference standard was dissolved in Mobile Phase A to a concentration of 0.25 mg/ml. The 100% standard was further diluted with Mobile Phase A to prepare the 1% degradate level standard and the 0.1% sensitivity solution. All standards and samples were filtered through the Gelman 0.45 μm Acrodisc CR PTFE-membrane filter from Fisher Scientific (Fair Lawn). Degradate reference standards were not readily available for routine use; therefore, an in situ system suitability solution containing approximately 0.2–0.5% of the Losartan degradates, compounds E and F, was produced with acid stressing under accelerated heating conditions of 105 °C for 1–2 h. Sample Losartan tablets were dissolved in Mobile Phase A with sonication and

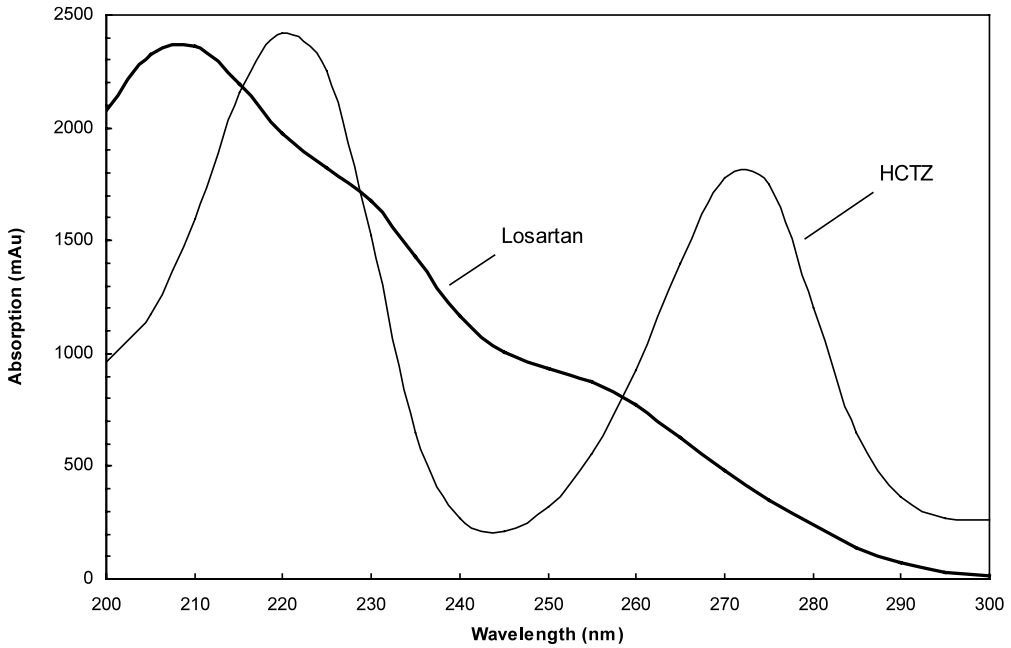

Fig. 2. UV/Vis spectra of HCTZ and Losartan potassium.

intermittent shaking. Samples were then diluted and filtered prior to injection to yield a final Losartan concentration of 0.25 mg/ml.

2.3.2. Losartan/HCTZ tablets

The standard and sample diluent is a 0.02M NaH_2PO_4 solution with the pH adjusted to 2.5 with phosphoric acid. The 100% working standard solution for Losartan/HCTZ tablets was prepared by dissolving appropriate amounts of reference standards in diluent and acetonitrile to yield concentrations of 0.4 mg/ml Losartan, 0.1 mg/ml HCTZ, and 0.001 mg/ml CTZ and DSA in a final solution composition of diluent-acetonitrile (70:30 v/v). A 1% degradate level standard (used for degradate quantitation) and a 0.1% sensitivity standard containing HCTZ, Losartan, DSA, and CTZ were prepared by successive dilution of the 100% working standard solution. The System Suitability Solution contains HCTZ, DSA, CTZ, Losartan as well as Losartan degradate products, compounds E and F, and is prepared by combining the 100% standard with the stressed Losartan solution described above.

A single sample solution used to quantitate all the active ingredients and degradates is prepared by dissolving the tablets in a 60:40 (v/v) mixture of acetonitrile and diluent with mechanical shaking for 30 min. Upon disintegration of the tablets, diluent was added to the sample solutions to yield a final acetonitrile composition of 30% by volume and Losartan/HCTZ concentrations of 0.4 and 0.1 mg/ml, respectively. Samples were filtered as above prior to injection.

3. Results and discussion

3.1. Methods development and optimization

3.1.1. Losartan tablets

The method described herein was developed for the simultaneous determination of the active ingredient at the 100% level and degradation products at 0.1% levels in Losartan tablets. The method has replaced drug development methods that determine Losartan utilizing a 15 min isocratic assay and compounds E and F utilizing a 50 min gradient assay. Due to detection of extraneous peaks in the 'blank' run which interfered with the quantitation of compounds E and F, the degradate method often required more than 16 h to pass system suitability. The main analytical challenge during development of a new method was obtaining adequate retention of the polar parent compound, Losartan, while maintaining a reasonable elution time for the less-polar degradates, compounds E and F in order to reduce testing time for the finished product and improve chromatographic performance.

To achieve this end, Phenomenex C18, Nucleosil C18, Symmetry C18, Zorbax C8, Inertsil Phenyl, and Supelco C4 columns were investigated. The Waters Symmetry C8 (150 × 3.9 mm, 5 μm particle size) column afforded the best peak resolution and shortest retention times for the active and degradate compounds. An ion-pairing reagent and more concentrated phosphate buffer were also examined in unsuccessful attempts to further decrease the disparity between the active and degradate retention times in Losartan tablets. Higher ratios of acetonitrile mobile phase content and a methanol organic phase were investigated in an effort to elute the active and degradate compounds isocratically. However, an isocratic method for all three components with run times less than 1 h was not realized.

Therefore, the 15 min gradient method described herein was developed and successfully validated for the quantitation of Losartan and its degradates. This method is also selective for numerous known Losartan process impurities of which only compound A is typically observed in the product. Overall, the original, in-house gradient degradate method had three main limitations: the interference of acetonitrile at the detection wavelength (230 nm), sensitivity to impurities in the phosphate salts, and the combination of a highly aqueous (95%) initial mobile phase condition with a very hydrophobic (C18) column. These three issues have been circumvented and diluent peaks have been minimized by using a less hydrophobic (Waters Symmetry C8) column, 20% acetonitrile starting gradient conditions, a detection wavelength of 250 nm, and fresh phosphate salts.

3.1.2. Losartan/HCTZ tablets

The method described for Losartan tablets was then taken a step further in development for simultaneous quantitation of all actives and degradates in Losartan/HCTZ tablets including HCTZ and Losartan potassium at the 100% level and the degradates (DSA, compound E, and F) at 0.1% of the method concentrations. Additional method selectivity was also required for the Losartan process impurity (compound A), the HCTZ process impurities CTZ and the HCTZ–CH_2–HCTZ impurity, and excipients to ensure accurate quantitation of degradate compounds. Previously, three separate methods were utilized for analysis of Losartan/HCTZ tablets including the two original methods described above for Losartan tablets and a separate 15 min isocratic assay for determination of HCTZ and DSA. The goal was also to considerably reduce testing time and improve chromatographic performance.

To achieve these goals for Losartan/HCTZ tablets, the mobile phase (phosphate buffer) pH was changed from 2.5 to 7.0 to optimize the Losartan retention time, while still allowing separation of CTZ, DSA, and HCTZ from the solvent front and each other. HCTZ related compounds exhibited a strong temperature dependence, in contrast to the Losartan compounds, which required column temperature control at 35 °C. Normal fluctuations in ambient laboratory temperatures affected the resolution of the HCTZ–CH_2–HCTZ impurity and Losartan. Biological growth in the mobile phase phosphate buffer (pH 7) was inhibited by premixing the buffer with acetonitrile at the initial gradient conditions. The sample preparation was changed to 30% acetonitrile to ensure complete recovery of both actives and 70% phosphate buffer (pH 2.5) to ensure HCTZ stability.

Losartan is present at four times the concentration of HCTZ in the tablet formulations; therefore, a higher wavelength of 280 nm was chosen to achieve similar absorbance values for the two active ingredient peaks (Fig. 2). Also, the concentration of sample and standard solutions were decreased to retain Losartan stability at pH 2.5 through 72 h and retain linearity of HCTZ from 0.1 to 150% of the target concentrations (0.4 mg/ml Losartan and 0.1 mg/ml HCTZ). The 35 min, two-step gradient described in the Section 2 allows for simultaneous quantitation of HCTZ, Losartan, DSA, compound E, and compound F and is selective for CTZ, the HCTZ–CH_2–HCTZ impurity, and excipients.

3.2. Method validation

3.2.1. Losartan tablets

3.2.1.1. Selectivity. The diluent chromatogram in Fig. 3 shows that the tablet diluent has negligible contribution after the void volume at the method detection wavelength of 250 nm. Related species relevant to quality control for Losartan tablets were determined through stress studies involving acid, base, peroxide, and heat as well as analysis of samples stored under long term ICH stability conditions (i.e. 40 °C and 75% relative humidity for 6 months). The worst-case placebo mixture, containing excipients from all Losartan tablet formulations, shows one peak eluting just after the void volume (Fig. 3) which can be attributed to a UV absorbing compound in the film coating of some tablet formulations. Sample, standard, and system suitability solution chromatograms are also shown in Fig. 3 to demonstrate method selectivity.

3.2.1.2. Accuracy. The accuracy of the Losartan tablets method was evaluated by the determination of the recovery of Losartan in duplicate on each of 2 days at five levels from 50 to 150% of the method concentration (0.25 mg/ml). Worst-case placebo solutions that contained excipients for all formulations were utilized in this experiment. Individual recoveries of Losartan ranged from 98.6 to 100.9%. The mean recoveries for each component at each level and the respective R.S.D. are shown in Table 1. The accuracy was also measured at the degradate level in a similar manner at six levels from 0.1 to 1.5% of the method concentration (0.25 mg/ml). The structures of the dimer degradation products, compounds E and F, are very similar to the structure of Losartan, and therefore, the chromophores of the three compounds are nearly identical. This

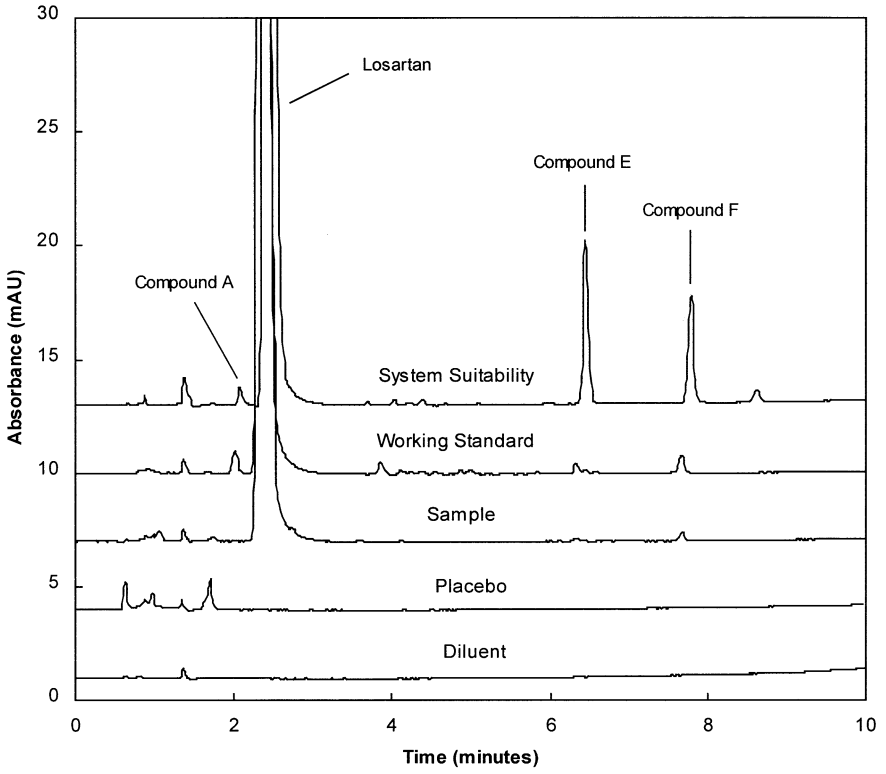

Fig. 3. HPLC chromatograms of Losartan tablet solutions. Separation conditions: waters symmetry C8 column; 1.0 ml/min flow rate; ambient temperature; 10 μl injection volume; gradient of 20 mM phosphate buffer (pH 7) and acetonitrile; UV detection at 250 nm.

Table 1
Summary of accuracy, recovery, and linearity precision data-active level

Approximate level	COZAAR		HYZAAR			
	Losartan		HCTZ		Losartan	
	Mean	R.S.D.	Mean	R.S.D.	Mean	R.S.D.
50	100.8	0.3	100.6	0.8	101.5	0.2
75	100.6	0.2	100.1	1.0	100.5	0.1
100	99.7	0.2	99.4	0.2	99.6	0.2
125	99.4	0.4	98.9	0.5	98.6	0.3
150	98.9	0.2	97.7	0.8	98.2	0.2
Slope	0.979		0.965		0.964	
y-Intercept	1.741		0.026		0.028	
Correlation coefficient	0.9999		0.9998		0.9999	

permits area percent calculations based on Losartan for the determination of compounds E and F. Individual recoveries of Losartan at the degradate level ranged from 97.2 to 104.5%. The mean recoveries for each component at each level and the respective R.S.D. are shown in Table 2.

3.2.1.3. Precision. The measurement (injection) precision was evaluated by performing seven replicate injections of Losartan standards at 1 and 100% levels of the method concentration (0.25 mg/ml). Peak area measurements were performed and excellent measurement precision was achieved at both levels with R.S.D. of 0.2 and 0.3% ($n = 7$) for the 1 and 100% levels, respectively. Standard preparation precision was evaluated for six independent preparations of the 1 and 100% level of Losartan standards. Individual response factors (measured area/standard weight) yielded excellent method precision with R.S.D. of 0.4 and 0.2% ($n = 6$) for the 1 and 100% levels, respectively.

3.2.1.4. Linearity and sensitivity. The linearity of the detector response for this method was determined at both the parent level (50–150%) and degradate level (0.1–1.5%). The data acquired for accuracy measurements was plotted as percent measured versus percent added to yield correlation coefficients, y-intercepts, and slopes for parent and degradate levels, respectively (see Tables 1 and 2). In all cases the slopes were near one and the correlation coefficients were greater than 0.999. The ability to detect Losartan in four independent preparations at the 0.1% level with an R.S.D. of 3.2% was established. Typical signal/noise (S/N) ratios between 20 and 45 are obtained for the Losartan peak in 0.1% sensitivity solutions.

3.2.1.5. Ruggedness. The method ruggedness was evaluated by a second analyst using a different instrument, different reagents, and freshly made solutions, mobile phase, standards, and samples. A different analytical column from the same vendor was also used. The retention times for all components reported by the ruggedness chemist were within 0.5 min from those observed by the developing chemist. When duplicate preparations ($n = 2$) of each sample lot were performed by each analyst, the results agreed within 2.1% for Losartan and all degradate values were below the limit of quantitation (0.1%). These results demonstrated acceptable method ruggedness for product quality control on release and stability.

Upon completion of validation, this method was transferred from the development laboratory using three lots of Losartan tablets to each of five other laboratories around the world. Agreement between laboratories was within 1.6% for Losartan and degradate values were consistently below the limit of quantitation (0.1%). The laboratories are currently running this method on Hewlett Packard, Waters, and Shimadzu instruments for routine analysis of assay and degradates in Losartan tablets.

3.2.1.6. Robustness. The Losartan tablets method conditions were found to be very robust. Changes in relative retention times were such that small changes in column temperature (ambient to

Table 2
Summary of accuracy, recovery, and linearity precision data-degradate level

Approximate level	COZAAR		HYZAAR			
	Losartan		DSA		Losartan	
	Mean	R.S.D.	Mean	R.S.D.	Mean	R.S.D.
0.10	101.2	3.2	103.2	12.4	122.6	2.2
0.50	99.1	0.5	100.8	4.5	101.7	0.6
0.75	99.9	0.7	99.0	4.4	99.3	1.0
1.00	100.3	0.8	102.0	7.0	100.0	1.8
1.25	99.8	0.2	98.4	3.5	97.1	1.3
1.50	100.2	1.1	97.2	1.3	96.4	1.2
Slope	1.000		0.970		0.950	
y-Intercept	−0.001		0.016		0.035	
Correlation coefficient	0.9998		0.9976		0.9996	

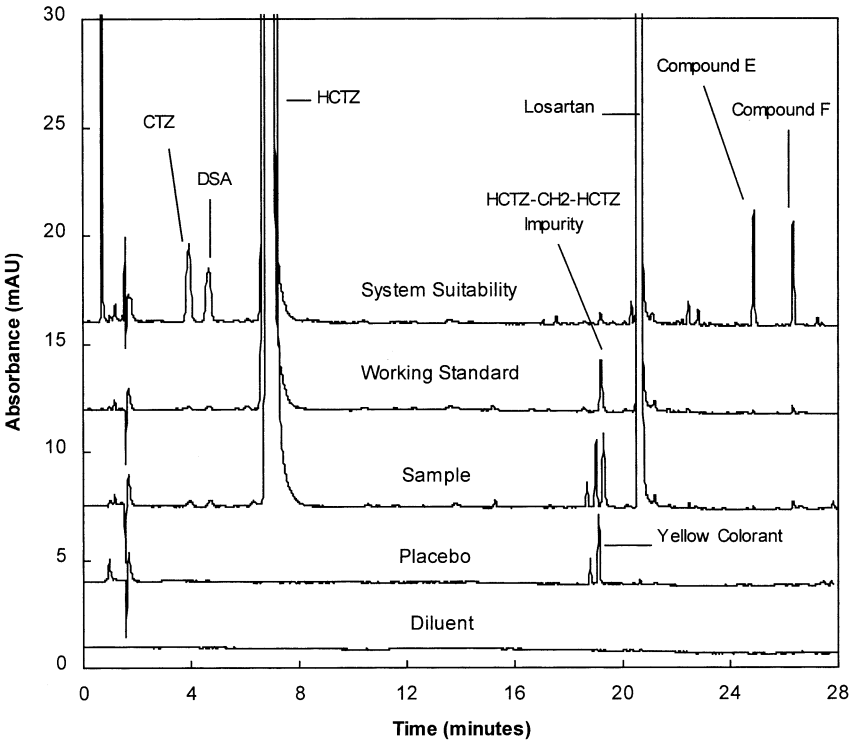

Fig. 4. HPLC chromatograms of Losartan/HCTZ tablet solutions. Separation conditions: waters symmetry C8 column; 1.0 ml/min flow rate; 35 °C; 20 μl injection volume; gradient of 20 mM phosphate buffer (pH 7) and acetonitrile; UV detection at 280 nm.

50 °C), phosphate buffer pH (6.0–8.0), phosphate buffer ionic strength (15–30 mM), mobile phase flow rate (0.75–1.25 ml/min), and percent acetonitrile in mobile phase (10–20%) did not inhibit the separation or quantitation of Losartan, compound A, E, and F.

3.2.2. Losartan/HCTZ tablets

3.2.2.1. Selectivity. A chromatogram of the tablet diluent (Fig. 4) shows negligible contribution at the detection wavelength of 280 nm after the void volume elutes. Related species relevant to quality control for Losartan/HCTZ tablets were determined through stress studies involving acid, base, peroxide, and heat as well as analysis of samples stored under long term ICH stability conditions (i.e. 40 °C and 75% relative humidity for 6 months). The worst-case placebo mixture, containing excipients from all formulation strengths, showed two peaks eluting at approximately 19 min which are seen in the sample chromatogram in Fig. 4. These peaks can be attributed to a UV absorbing excipient in the tablet film coating of some formulations.

3.2.2.2. Accuracy. The accuracy of the Losartan/HCTZ tablets method was evaluated by the determination of the recovery of Losartan and HCTZ in duplicate on each of 2 days at five levels from 50 to 150% of the method concentrations (0.4 mg/ml Losartan and 0.1 mg/ml HCTZ) from worst-case placebo solutions which contained excipients for all formulations. Individual recoveries ranged from 98.1 to 101.7% for Losartan and ranged from 96.6 to 101.4% for HCTZ. The accuracy was also measured at the degradate level for Losartan (compounds E and F) and DSA in a similar manner at six levels from 0.1 to 1.5% of the method concentrations. Individual recoveries at the degradate level ranged from 95.0 to 125.8% for Losartan and ranged from 85.1 to 112.7% for

DSA. The mean recoveries and R.S.D. for each level are shown in Tables 1 and 2 for active and degradate levels, respectively.

3.2.2.3. Precision. Measurement (injection) precision was evaluated by performing eight replicate injections of the 100 and 1% standard solutions prepared as described above in the Section 2. Excellent measurement precision for all species was obtained with R.S.D. values of 0.1% for HCTZ and Losartan at the 100% level and R.S.D. values of 2.1 and 0.2% for DSA and Losartan, respectively, at the 1% level. Standard method precision was evaluated for eight independent preparations of each the 100 and 1% standards. R.S.D. values for response factors (measured area/standard weight in mg) were 0.5 and 0.2% for HCTZ and Losartan at the 100% level and ranged from 1.2 to 1.6% for DSA, HCTZ, and Losartan at the 1% level. Sample method precision of eight (8) independent sample preparations was also demonstrated with R.S.D. values of 0.6 and 0.4% for actives HCTZ and Losartan and 9.4, 5.0 and 6.0% for degradates DSA, compound E, and F, respectively.

3.2.2.4. Linearity and sensitivity. The linearity of the detector response for this method was determined at the parent level for HCTZ and Losartan and at the degradate level for DSA and Losartan. The data acquired for accuracy measurements was plotted as percent measured versus percent added to yield satisfactory correlation coefficients, y-intercepts, and slopes as shown in Tables 1 and 2. The ability to detect DSA and Losartan in four independent preparations at the 0.1% level with R.S.D. of 12.4 and 2.2%, respectively, was established. Typical signal/noise (S/N) ratios in the

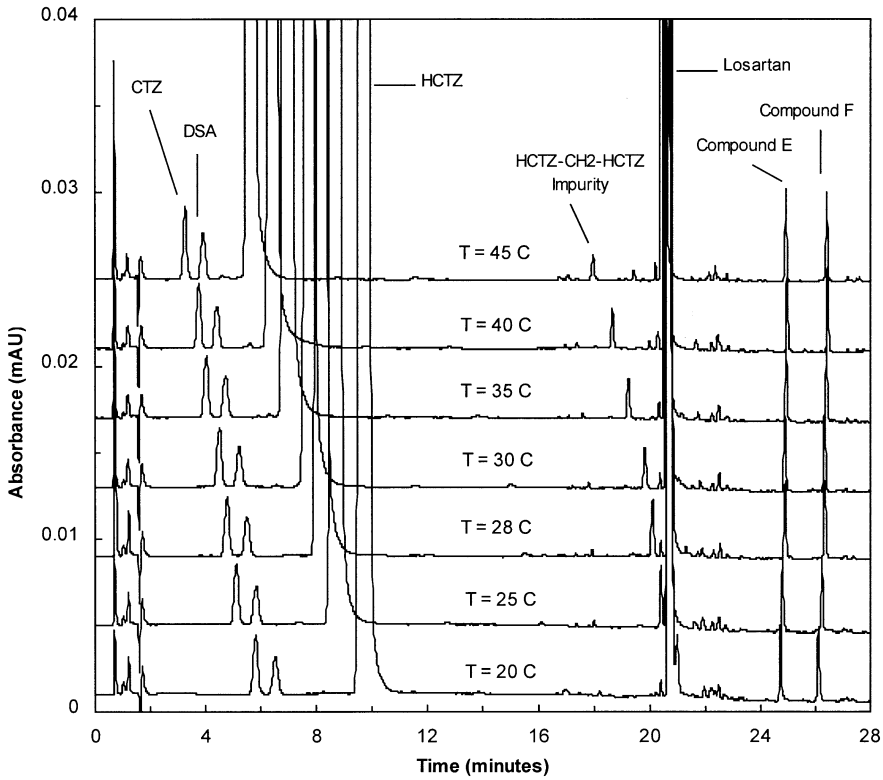

Fig. 5. HPLC chromatograms of system suitability solution for Losartan/HCTZ tablet method—temperature robustness study. Separation conditions: waters symmetry C8 column; 1.0 ml/min flow rate; 20 μl injection volume; gradient of 20 mM phosphate buffer (pH 7) and acetonitrile; UV detection at 280 nm; temperature 20–45 °C.

0.1% sensitivity solutions for DSA have been from 6 to 36 and greater than 40 for Losartan.

3.2.2.5. Ruggedness. Method ruggedness for analysis of the Losartan/HCTZ tablets was evaluated by a second analyst using a different column and instrument, different reagents, and freshly made solutions, mobile phase, standards, and samples. The ruggedness chemist reported retention times for major components detected within 0.2 min from those observed by the developing chemist. When duplicate preparations ($n = 2$) of each sample lot were performed by each analyst, the results agreed within 1.5% for HCTZ, 1.3% for Losartan, and 0.05% for DSA and the HCTZ–CH_2–HCTZ impurity. All other degradate values were below the limit of quantitation (0.1%). These results demonstrated acceptable method ruggedness for product quality control on release and stability.

Upon completion of validation, this method was transferred from the development laboratory using three lots of Losartan/HCTZ tablets to each of six other laboratories around the world. Agreement between laboratories was within 1.9% for HCTZ, 1.8% for Losartan, and 0.1% for DSA. Other degradate values were consistently below the limit of quantitation (0.1%). The laboratories are currently running this method on Hewlett Packard, Waters, and Shimadzu instruments for routine analysis of assay and degradates in Losartan/HCTZ tablets.

3.2.2.6. Robustness. The method robustness for Losartan/HCTZ tablets was also evaluated during method validation to determine how system suitability would be affected by variations in experimental conditions. In this study, the chromatographic parameters monitored were peak retention times, capacity factors, tailing factors, theoretical plates, and resolution. Ionic strength of the mobile phase phosphate buffer was varied by $\pm 20\%$. The chromatography was not visually affected, and the monitored parameters did not exhibit distinguishable trends. Variations of the mobile phase flow rate by $\pm 15\%$ yielded the expected results: peaks were generally shifted earlier with faster flow rates and later with slower flow rates. Relative retention times of CTZ, DSA, and compounds E and F were not affected by this change; and the tailing factors, theoretical plate counts, and peak resolutions of sample components showed only minor fluctuations.

While the above observations are typical for many chromatographic methods, the critical separation factors for the Losartan/HCTZ method were determined to be column temperature and the pH of the phosphate buffer used in the mobile phase. The chromatograms shown in Fig. 5 depict the effect of column temperature ranging from 20 to 45 °C. As the column temperature is decreased, the retention times, capacity factors, tailing factors, and theoretical plates for HCTZ-related peaks increased while these parameters for Losartan and its degradates were not significantly affected. This resulted in a loss of resolution between the HCTZ–CH_2–HCTZ impurity and Losartan below 25 °C since the capacity factor of the HCTZ–CH_2–HCTZ impurity approached that of Losartan. Above 40 °C, sufficient retention of CTZ, DSA, and HCTZ was not achieved to establish the required theoretical plate counts and resolution. Therefore, it was concluded that system suitability could be met for all peaks in Losartan/HCTZ tablet samples at temperatures between 28 and 40 °C.

As a drop in temperature caused a loss of resolution of the HCTZ–CH_2–HCTZ impurity and Losartan, a drop in pH yielded a similar affect with CTZ and DSA. The chromatograms in Fig. 6 show the effect of varying the mobile phase phosphate buffer pH by ± 0.5 pH units. As with temperature variations, the chromatographic parameters of Losartan and its degradates show only minor fluctuations with mobile phase pH changes. While the retention times, capacity and tailing factors, and plate counts of HCTZ, DSA, and the HCTZ–CH_2–HCTZ impurity also showed little change with pH, the retention of CTZ was significantly altered with mobile phase pH. In contrast, both the DSA and HCTZ peak retention times were largely unaffected over the same pH range. The known pKa's for HCTZ (8.6–8.8 and 9.9–10.4) [26] and CTZ (6.9 and 9.5) [27] are consistent with the observed retention time shift with pH. This shift is significantly more

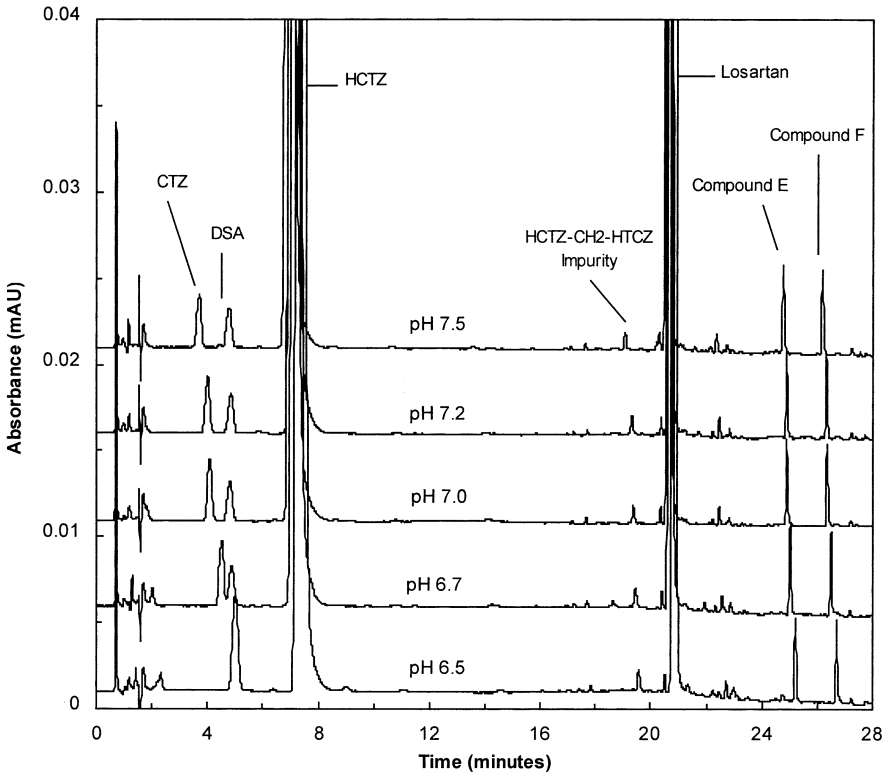

Fig. 6. HPLC chromatograms of system suitability solution for Losartan/HCTZ tablet method—phosphate buffer pH robustness study. Separation conditions: waters symmetry C8 column; 1.0 ml/min flow rate; 35 °C; 20 μl injection volume; gradient of 20 mM phosphate buffer (pH varied) and acetonitrile; UV detection at 280 nm.

pronounced for CTZ since the protonation state of the drug is changing dramatically over the 6.5–7.5 pH range. Furthermore, the pKa's for DSA are expected to be similar to HCTZ, based on the molecular structure (Fig. 1) and are further supported by the lack of retention time change over the examined pH range. At pH 7.5, the resolution of CTZ and DSA was calculated as 3.2 and decreases to 2.3 at pH 7.0. Baseline resolution was lost at pH 6.8 and the two components co-elute at pH 6.5. Therefore, it was concluded that it is necessary to maintain mobile phase buffer pH between 7.0 and 7.5 to achieve acceptable chromatography. It is undesirable to use buffers at pH values higher than this range to preserve long column lifetimes.

4. Conclusions

The goal of this work was achieved by separating and quantitating all related compounds in Tablets COZAAR® and in Tablets HYZAAR® by high performance liquid chromatography. While Losartan tablets presented challenges with the acid dimer degradates, Losartan/HCTZ tablets have four additional components which also must be separated to allow simultaneous quantitation of all actives and degradates in the dosage formulation. Reversed-phase, gradient HPLC methods on Waters Symmetry C8 column have been developed and validated as described herein for the simultaneous determination of active ingredients and degradation products from the 100% level to

the 0.1% level in Losartan tablets and Losartan/HCTZ tablets. The concomitant quantitation and analysis in these methods provides significant time-savings advantages in pharmaceutical laboratory analysis as well as significant decreases in sample preparation, instrument run time, and solvent and drug waste over the original, separate methods for analysis.

Each HPLC method has been extensively validated for the quantitation of necessary components as shown by the accuracy, linearity, recovery, and precision data. Ruggedness of the methods has also been demonstrated both between analysts in the same laboratory as well as between laboratories as these methods are now in use at Merck sites. The robustness data gathered during the method validation shows that the Losartan tablets method is not susceptible small changes in chromatographic conditions. Whereas, for the Losartan/HCTZ tablets method, critical chromatographic conditions such as temperature and pH have been identified.

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