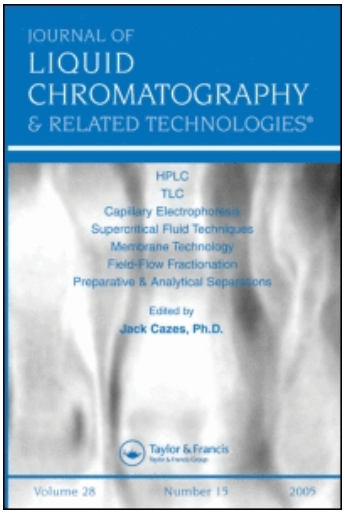

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 30 September 2001

To cite this Article Özkan, Sibel A.(2001) 'SIMULTANEOUS DETERMINATION OF LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE FROM TABLETS AND HUMAN SERUM BY RP-HPLC', Journal of Liquid Chromatography & Related Technologies, 24: 15, 2337 — 2346
To link to this Article: DOI: 10.1081/JLC-100105145
URL: http://dx.doi.org/10.1081/JLC-100105145

J. LIQ. CHROM. & REL. TECHNOL., 24(15), 2337–2346 (2001)

SIMULTANEOUS DETERMINATION OF LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE FROM TABLETS AND HUMAN SERUM BY RP-HPLC

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ABSTRACT

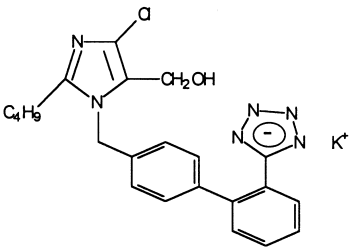
A new, simple, precise, rapid, and accurate RP-HPLC method has been developed for the simultaneous determination of losartan potassium and hydrochlorothiazide from tablets and human serum. Chromatography was carried out on a C_{18} reversed-phase column using a mixture of 0.01 M KH_2PO_4: acetonitrile (65:35; v/v) adjusted to pH 3.1 with H_3PO_4 at a flow rate 1.0 mL/min.

Detection was realised at 232 nm using a UV detector. Linearity was obtained in the concentration range of 25–10000 ng/mL and 50–10000 ng/mL for losartan potassium and hydrochlorothiazide, respectively. The limit of detection and the limit of quantification of the procedure were found to be 1.02 ng/mL and 3.39 ng/mL for losartan potassium; 4.49 ng/mL and 14.96 ng/mL for hydrochlorothiazide, respectively.

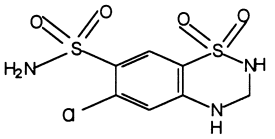
This method was succesfully applied without any interferences to the simultaneous analysis of losartan potassium and hydrochlorothiazide in human serum and pharmaceutical dosage forms in the presence of each other.

INTRODUCTION

Losartan potassium (LOS) is the first created AT_2 receptor antagonist . The importance of angiotensin II in regulating cardiovascular function has led to the development of nonpeptide receptor antagonists of the angiotensin II receptor for clinical use. The use of this compound is mainly for hypertension therapy. Hydrochlorothiazide (HCT) is currently one of the most commonly used diuretic agents. The active ingredients of the drug are a combination of LOS and HCT, having concentrations of 50 mg and 12.5 mg, respectively(1,2).



Losartan potassium



Hydrochlorothiazide

Various analytical techniques for the determination of LOS individually or in its metabolite, the pharmaceuticals or bulk form, are described. These include HPLC,(3-7) capillary electrophoresis,(8,9) supercritical fluid chromatography,(10) liquid chromatography-mass spectrometry(11).

There have been several reports(12-20) on the determination of HCT individually or in its combination with other drugs, including the use of liquid chromatography(12-15), capillary zone electrophoresis(16), spectrophotometry(17-20).

However, to the best of our knowledge, no information about the simultaneous determination of LOS and HCT in bulk form, pharmaceutical formulations, and human serum have appeared in the literature and pharmacopoeias.

This work constitutes the first stage of a reseach schedule focused on the proposal of a new HPLC procedures for the simultaneous determination of LOS and HCT. This paper describes a reliable, simple, time and money saving reversed-phase HPLC method to the simultaneous determination of LOS and HCT in bulk material, pharmaceutical dosage forms, and human serum.

EXPERIMENTAL

Apparatus

The HPLC system used consists of a Waters Isocratic LC pump (Waters Model 510; Waters Assoc., Milford M.A., USA), an autosampler (Model 717 plus), UV detector (Waters, Model 481), a LC_{18} column (150x4.6 mm; 5 μm particle size; Waters Assoc.).

Chemicals and Reagents

Losartan potassium and its pharmaceutical dosage forms (Hyzaar®) were kindly provided by Merck Sharp & Dohme Pharmaceuticals, Inc. (Istanbul, Turkey). Hydrochlorothiazide and internal standard (furosemide) were kindly supplied by Nobel Pharmaceuticals Inc. (Istanbul, Turkey) and Fako Pharmaceuticals Inc. (Istanbul,Turkey), respectively. Acetonitrile was of HPLC grade, purchased from Merck (Darmstadt, Germany). All other chemicals were commercial analytical grade. Doubly distilled water was used for preparing solutions.

Chromatographic Conditions

The proposed method was conducted using a reversed phase technique, UV monitoring at 232 nm, and furosemide as an internal standard. A mixture of 0.01 M KH_2PO_4: acetonitrile (65:35; v/v), adjusted to pH 3.1 with H_3PO_4 was used as a mobile phase. The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 1.0 mL/min. 50 μL of each solution was injected and chromatograms were recorded.

Standard Stock Solutions

Accurately weighed 10 mg of standard LOS and HCT were taken separately in a 10 mL volumetric flask. 10 mL methanol was added and kept in an ultrasonic bath for 5 min. Standard solutions for HPLC were prepared with mobile phase by varying the concentration of LOS in the range of 25-10000 ng/mL, and HCT in the range of 50–10000 ng/mL, maintaining the concentration of furosemide (internal standard) at a constant level of 1 μg/mL. A calibration curve for HPLC analysis was obtained by plotting the peak area ratio of the drug to internal standard against the drug concentration.

Analysis of Tablets

Not less than ten tablets were weighed. The average weight per tablet was calculated from the weight of 10 tablets. Ten tablets were reduced to a fine powder. A quantity of composite equivalent to 50 mg of LOS (12.5 mg of HCT) was weighed and transferred into a 10 mL flask, diluted with methanol, sonicated for 5 minutes, and then completed to the volume with the same solvent. After filtration, an appropriate volume of the filtered solution was taken in a 10 mL flask. An appropriate amount of internal standard was added and diluted up to the mark with the mobile phase. The amount of LOS and HCT per tablet was calculated from the related linear regression equations.

Recovery Studies

In order to establish the reliability, suitability, accuracy, reproducibility, and to check the interference from excipients used in the formulation, of the above method, recovery experiments were carried out. The known amounts of the pure sample solutions were added to the preanalysed formulations of each drug, including a constant level of the internal standard, and the mixtures were analysed by the proposed method. From the total amount of drug found, the percentage recovery was calculated. After five repeated experiments, the recoveries were calculated.

Recovery Studies in Human Serum

Serum sample, obtained from healthy individuals (after obtaining their written consent), were stored frozen until assay. After gentle, thawing, 2 mL aliquots of serum were spiked with 1000 μg/mL of LOS and 1000 μg/mL of HCT (dissolved in methanol), 500 μL acetonitrile (for participitation of proteins). The tubes were vortexed for 5 min and then centrifuged for 10 min at 4000 g. The supernatant was taken carefully. An appropriate amount of supernatant including LOS and HCT was transferred in a 2 mL volumetric tube, and maintaining the concentration of internal standard was added at a constant level and then diluted to the volume with mobile phase. Serum samples including various concentration of LOS and HCT and constant amount of internal standard were injected into the HPLC column.

RESULTS AND DISCUSSION

Various mobile phase systems and their various proportions were prepared and used for chromatographic separation, but the proposed mobile phase com-

Table 1. Statistical Analysis Results of the Calibration Plots of LOS and HCT by RP-HPLC

Sample	Linearity Range* (ng/mL)	Slope	Intercept	S.E. of Slope	S.E. of Intercept	Correl. Coeff.	Detection Limit (ng/mL)	Quantitation Limit (ng/mL)
LOS	20-10000	4.52×10^{-4}	−0.024	$4.04\text{x}10^{-6}$	0.015	0.9999	1.02	3.39
HCT	50-10000	3.34×10^{-4}	0.016	$2.73\text{x}10^{-6}$	0.011	0.9999	4.49	14.96

* Data represents 5 replicate injections of standard solutions.

prising 0.01 M KH_2PO_4: acetonitrile (65:15; v/v), adjusted to pH 3.1 with H_3PO_4 (10%), gave a better resolution and sensitivity of LOS and HCT. Under the described conditions the analyte peaks were well defined, resolved, and free from tailing. The elution order was HCT (t_r: 2.93 min), furosemide (t_r: 6.50 min), and LOS (t_r: 8.68 min) at a flow rate of 1.0 mL/min. A wavelength of 232 nm was selected for the detection purpose to match the sensitivities of these two drugs.

According to USP24, method <621>, system suitability tests are an integral part of a liquid chromatographic method. System suitability tests are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. System suitability tests were carried out on freshly prepared standard stock solutions of LOS and HCT. Resolution and selectivity factors for this system were found to be 5.94 and 4.09, respectively. Tailing factors were obtained as 1.18 for LOS and 1.29 for HCT.

The peak area ratios of LOS and HCT to the internal standard exhibit linear relationship with their concentrations. The characteristics of regression equations and the working concentrations are given in Table 1.

The limit of detection (LOD) and quantification (LOQ) of the procedure was shown in Table 1, which was calculated on the peak area using the following equations:

LOD: 3 s/m LOQ: 10 s/m

Where *s*, the noise estimate, is the standard deviation of the peak areas (five injections) of the drug and *m* is the slope of the corresponding calibration curve.

Intraday precision and accuracy were determined using five samples of two different concentrations at low and medium concentrations, which were prepared and analyzed on the same day (Table 2).

Interday variability was assessed using six samples of two different concentrations (same concentration with intraday experiments) analyzed on three different days over a period of two weeks (Table 2).

Table 2. Intra-Day and Inter-Day Precision of LOS and HCT Standards

Compound	Theoretical Concentration (ng/mL)	Intra-Day Concentration Mean	Measured* (ng/mL) RSD %	Inter-Day Concentration Mean	Measured** (ng/mL) RSD %
LOS	100	99.1	0.95	98.9	1.24
	750	746.3	0.71	756.9	0.83
HCT	100	99.5	0.85	98.6	1.04
	750	748.9	0.41	742.5	1.34

* Mean values represent five different sample standards for each concentration.
**Inter-day reproducibility was determined from five different runs on three different days.

Intra-day and inter-day variabilities were characterized by the relative standard deviation (RSD%) and by the difference between theoretical and measured concentrations. Thus, it was concluded that there was no significant difference for the assay which was tested within-day and between-day.

In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by analysing synthetic mixtures of LOS and HCT which reproduced different composition ratios (Table 3).

Table 3. Resolution of LOS and HCT Laboratory-Made Mixtures by RP-HPLC

Added (ng/mL)		Found (ng/mL)*		Recovery %		RSD %		Mean Recovery (%)	
LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT	LOS	HCT
1000	100	998.8	99.5	100	99.5	0.36	0.41		
1000	250	998.3	249.9	99.85	99.9	0.08	0.45		
1000	750	999.9	748.8	99.9	99.8	0.10	0.08		
1000	1000	997.3	998.8	99.7	99.9	0.21	0.06		
1000	5000	997.7	4992.1	99.8	99.8	0.12	0.11		
								99.9	99.8
100	1000	99.1	998.0	99.1	99.0	0.57	0.18		
250	1000	250.6	1000	100.3	100	0.41	0.10		
750	1000	748.8	998.4	99.8	99.8	0.20	0.12		
1000	1000	997.1	999.1	99.7	99.9	0.22	0.09		
5000	1000	4992.0	999.0	99.9	99.9	0.11	0.08		
								99.8	99.7

*Each result is the mean of three experiments.

Analysis of Formulations

When working on a synthetic mixture, results encourage the use of the methods described for the assay of LOS and HCT in commercial tablet dosage forms. The proposed method can also be used for the simultaneous determination of LOS and HCT in the presence of each other and without prior separation of the excipients. Results obtained from the proposed method of the analysis of LOS and HCT tablets, indicate that the proposed assay can be used for simultaneous quantitation and routine quality control analysis of this binary mixture in pharmaceutical dosage forms. The results are shown in Table 4.

There is no method reported in pharmacopoeias and literatures so far for the simultaneous determination of LOS and HCT in drug dosage forms and biological samples. Moreover, in order to know whether the excipients in the tablet show any interference with the analysis, known amounts of the pure drug were added to the same aliquot portions of the same powdered tablets and mixtures were analysed by the proposed method. The mean percentage recoveries obtained after five repeated experiments were 98.1% and 99.2% with a RSD of 1.58 % and 0.14% for LOS and HCT, respectively. It is concluded, that the proposed method is sufficiently accurate and precise in order to be applied to pharmaceutical dosage forms. High percentage recovery data shows that the method is free from the interferences of the excipients used in the formulations.

Table 4. Assay of LOS and HCT Pharmaceutical Dosage Form

Compound	Labelled Amount (mg per tablet)	Amount Found	Mean*	RSD %
	50.00	49.30		
	50.00	49.97		
LOS	50.00	51.04		
	50.00	49.71		
	50.00	49.27		
			49.88	1.45
	12.50	12.35		
	12.50	12.44		
HCT	12.50	12.48		
	12.50	12.37		
	12.50	12.40		
			12.41	0.42

*Average of five experiments.

Application to Biological Samples

In order to check the applicability of the proposed method to biological materials, the recovery studies were performed in human serum. Quantitation was performed by means of the calibration graph method, achieving for each analysis, the specified percent recoveries.

Figure 1(a) shows a typical chromatogram of an extract of fresh blank plasma, and Fig. 1(b) shows a chromatogram obtained when the method was applied to spiked plasma containing 1000 ng/mL of LOS, 1000 ng/mL of HCT, and 1000 ng/mL of the internal standard. HCT, internal standard and LOS gave well-separated, sharp symmetrical peaks, with retention times of 2.87, 6.33, and

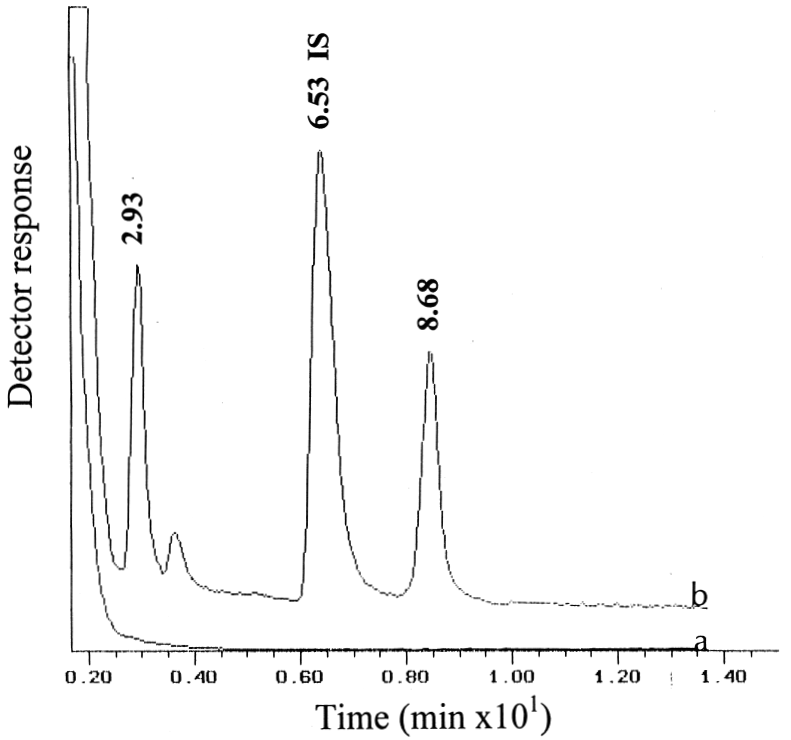


Figure 1. Chromatogram of blank serum (a) and serum spiked with 1000 ng/mL of LOS and 1000 ng/mL of HCT and 1000 ng/mL of Furosemide 1000 ng/mL (IS) (b).

Table 5. Results Obtained for LOS and HCT Analysis from Human Serum

Compound	Added (ng/mL)	Found (ng/mL)	n	RSD (%)	Average Recovery %
LOS	500	5	488.7	1.58	97.7
	1000	5	983.0	0.84	98.3
HCT	500	5	493.7	2.07	98.7
	1000	5	983.5	1.04	98.4

8.48 min, respectively. There are no extraneous peaks in chromatograms obtained for serum samples.

The determination results and recoveries of known amounts of LOS and HCT added to serum samples were given in Table 5. The proposed method gives reproducible results, is easy to perform, and is sensitive enough for the simultaneous determination of LOS and HCT in human serum and in the presence of each other (Table 5).

CONCLUSION

The proposed RP-HPLC method described, herein, provides a simple, rapid, reproducible, and precise simultaneous determination of LOS and HCT in pharmaceutical dosage forms and human serum. LOS and HCT, the active components of Hyzaar tablets, have been succesfully determined, each in admixture with the other, without any interference when applying the proposed method. Therefore, the proposed HPLC method can be used for routine simultaneous analysis of these drugs and can be used as an alternative tool for the drug quality control laboratories. No interferences from endogenous substances were observed in any of the biological samples. The proposed method should be useful for the therapeutic monitoring of levels of LOS and HCT in biological samples, and may have clinical application for patients receiving the drug. In addition, the resulting run time is suitable for processing numerous samples on a daily basis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Merck Sharp and Dohme Pharm. Ind. for supplying Losartan potassium and pharmaceutical dosage form (Hyzaar®). The authors also thank Nobel Drug, Inc. and Fako Drug, Inc. for the supply of hydrochlorothiazide and sulfamethoxazole, respectively.

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Received December 23, 2000 Manuscript 5469
Accepted February 21, 2001