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Research Article

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Rapid and Sensitive HPLC Method for Simultaneous Estimation of Atorvastatin, Hydrochlorothiazide and Losartan and Quantitative Application to Polypill Based Synthetic Ternary Mixture

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ABSTRACT

A rapid and sensitive isocractic RP-HPLC method has been developed for the purpose of analysis of losartan (LOS), hydrochlorothiazide (HCZ) and atorvastatin (ATR) drugs simultaneously in cardiovascular polypill. Analysis was performed on BDS Hypersil C18 (250 × 4.6mm id, 5-µm particle size) column with acetonitrile-0.2M phosphate buffer (pH 3) 50:50 (v/v) as mobile phase, a flow rate of 1.5 ml min^{-1} and column temperature 40 ^{o}C. UV detection was performed at 230 nm. The run time under these chromatographic conditions was less than 10 min. The method was linear in the range of 10-100 µg ml^{-1} for LOS, 10-90 µg ml^{-1} for HCZ and 10-150 µg ml^{-1} for ATR. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. The sensitivity of the method, as the limits of detection (LOD) and quantification (LOQ) for each active ingredient was also determined. The validated method was successfully applied for the quantification of losartan, hydrochlorothiazide and atorvastatin simultaneously in laboratory prepared pharmaceutical tablets and the average % amount ranges from 99.08 % to 99.60 % with relative standard deviation less than 1.5 % indicating satisfactory accuracy of the method.

Keywords: Losartan, Hydrochlorothiazide, Atorvastatin, RP-HPLC, Polypill.

INTRODUCTION

Blood pressure and LDL cholesterol are causal risk factors for cardiovascular diseases (CVD) and their combined effects make this disease common. A pill containing different active ingredients (polypill) to overcome these factors is more beneficial than the common pills with only one, in terms of cost and patient compliance. Losartan, hydrochlorothiazide and atorvastatin are commonly prescribed active ingredients for CVD (1). Losartan (LOS) chemically (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl) biphenyl-4-yl]methyl}-1*H*-imidazol-5-yl)methanol (Fig 1a) is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). It was the first angiotensin II receptor antagonist to be marketed [1]. Hydrochlorothiazide (HCZ) chemically is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Fig 1b), it is a first-line diuretic drug of the thiazide class[1]. Atorvastatin (ATR) chemically is (3*R*,5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenyl-carbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoate (Fig 1c). It is a member of the drug class known as statins, used for lowering blood cholesterol [1].

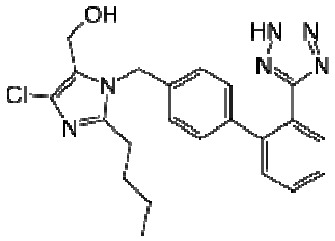

Fig 1a: Losartan

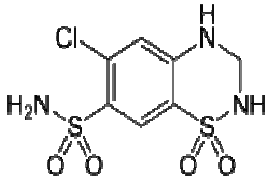

Fig 1b: Hydrochlorothiazide

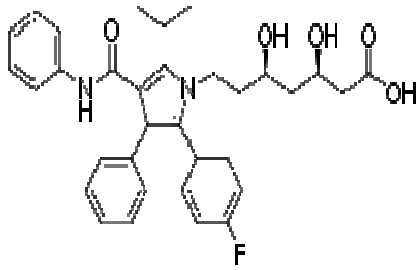

Fig 1c: Atorvastatin

Numerous analytical methods have been reported for estimation of losartan and hydrochlorothiazide individually or in combined mixtures. They include HPLC [2-5], HPTLC [6], capillary electrophoresis [7, 8] and spectrophotometric methods [9-11]. Analysis of atorvastatin in pharmaceutical formulations was achieved using chromatographic [12-14] and electrophoretic [15,16] methods and spectrophotometric techniques [17-19]. A thorough literature survey revealed that no analytical methods were reported for the simultaneous determination of LOS, HCZ and ATR in ternary mixtures. The present work describes a simple LC method, which is rapid, sensitive, selective, precise and accurate isocratic reverse phase HPLC method for simultaneous estimation of losartan, hydrochlorothiazide and atorvastatin in synthetic polypill.

EXPERIMENTAL SECTION

2.1. Materials and Reagents

Pure LOS and HCZ analytical standards and their tablets combination (Kanzar-H, labeled to contain 100 mg LOS and 25 mg HCZ) were kindly provided by Hikma company, Cairo- Egypt. Pure analytical standard of ATR was kindly provided by Eipico company, Cairo- Egypt. Potassium dihydrogen phosphate (KH_2PO_4) and 85% ortho-phosphoric acid (H_3PO_4) of analytical reagent grade were purchased from Sigma-Aldrich company. HPLC grade methanol and acetonitrile (ACN) were purchased from same supplier. Water was deionized and purified by a Milli-Q water purification system.

2.2. Instrumentation

The LC system, used for method development and validation was Agilent 1200 series liquid chromatographic RRHT (Rapid Resolution High Throughput) system comprising of binary pumps, column oven, autosampler and UV detector. Analysis was performed on a BDS Hypersil C18 of 250 × 4.6 mm id, 5-µm particle size; the mobile phase consists of a mixture of 0.02 mM sodium dihydrogen orthophosphate (pH adjusted to 3 using orthophosphoric acid) and acetonitrile in 50:50 ratio. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.5 ml/min for 20 min. The column temperature was maintained at 40 °C. The eluate was monitored at 230 nm using UV detector and the injection volume was 20 µl. A Shimadzu 1601 spectrophotometer with quartz cells of 1-cm optical path length and a Hanna Microprocessor HI 9321 with a combined glass-saturated calomel electrode were used.

2.3. Standard solutions and calibration curves

Stock standards (1.0 mg ml^{-1} concentration) of LOS, HCZ and ATR were prepared separately in water for losartan and in methanol for the other two drugs. Working standard solutions of individuals and mixtures were prepared by appropriate dilution of the stock standard solutions (1.0 mg ml^{-1}) with mobile phase over a concentration range of 10-100 µg ml^{-1} for LOS, 10-90 µg ml^{-1} for HCZ and 10-150µg ml^{-1} for ATR.

2.4. Preparation of synthetic Sample Solutions

Twenty tablets of kanzar- H (marketed losartan and hydrochlorothiazide combination) were weighed finely powdered and mixed thoroughly. To the weight of tablet triturates equivalent to 100 mg LOS and 25 mg HCZ, 20 mg of ATR standard powder was added. The mixture was transferred into 100 ml volumetric flasks containing 10ml methanol and 50 ml mobile phase, sonicated for few minutes, filtered and the filtrate was then completed to 100 ml with mobile phase. 1ml of the resulting solution was completed to 10 ml with the mobile phase to obtain 100 µg ml^{-1} LOS, 25 µg ml^{-1} HCZ and 20 µg ml^{-1} ATR. This solution was injected and compared to standard mixture solution of the three drugs which was prepared similarly but using 100 mg LOS and 25 mg HCZ standard powder instead of tablet triturates (filtration is not necessary) .

RESULTS AND DISCUSSION

In the present study, a simple isocractic LC method was developed and validated for simultaneous determination of the possible components of a polypill: Losartan, hydrochlorothiazide and atorvastatin**.**

3.1. Optimization of Chromatographic Conditions

UV overlain spectra of the three drugs solutions showed that losartan, hydrochlorothiazide and atorvastatin standard solutions absorbed appreciably at 230 nm. So, detection at this wavelength was selected for method development. A number of eluting systems containing mixtures of acetonitrile and phosphate buffer of different pH (2.5–4.5) at different proportions and flow rates of 0.5-2.0 ml/min, were examined. The column temperature was changed from 25-40 ^{o}C. Separation between losartan and hydrochlorothiazide was best achieved at pH 3 (at pH 4 and more the two drugs were not well separated). A flow rate of 1.5 ml/min and column temperature of 40^{o}C were choiced to decrease retention time especially for atorvastatin. Good resolution, selectivity, sensitivity and rapidity was thus obtained using mobile phase consisting of 0.02 mM NaH_2PO_4 buffer (pH_3) and acetonitrile (50:50 v/v), at a flow rate of 1.5 ml/min and BDS Hypersil C18 of 250 × 4.6 mm id, 5-µm particle size analytical column with temperature maintained at 40 ^{o}C.

3.2. Validation Procedure

Validation of the developed and optimized HPLC method was carried out in the light of ICH Guidelines [20] with respect to validation parameters: linearity, intra and inter-day precision, ruggedness, robustness, accuracy, detection (LOD) and quantification (LOQ) limits and specificity.

3.2.1. Calibration Curve

Calibration plots were constructed by plotting the peak area (y) versus analyte concentration (x). Linearity was established by least squares linear regression analysis of the calibration curve. Peak areas of losartan, hydrochlorothiazide and atorvastatin were plotted against their respective concentrations. The constructed calibration curves were linear over the concentration range of 10-100 µg ml^{-1} for LOS, 10-90 µg ml^{-1} for HCZ and 10- 150µg ml^{-1} for ATR. Correlation coefficient (*n*=3) was found to be 0.9991, 0.9987 and 0.9993 for LOS, HCZ and ATR, respectively. Regression line equation was found to be:

Y= 60.68 + 56.40 X, Y= 72.10+ 65.40 X and Y=35.20+30.15 X for LOS, HCZ and ATR, respectively.

3.2.2. Precision, ruggedness and robustness

The precision of the proposed method was assessed as repeatability and intermediate precision by preparing six replicates of three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated over a 3-day period to evaluate day-to-day variability (intermediate precision). The % RSD values for intra- and inter-day assays of the drug performed in the same laboratory by two analysts (Table 1) was found less than 2%, thus indicating the ruggedness of the method.

Small deliberate variations of the experimental conditions were applied in order to determine the effect on retention time, resolution and peak area. Changes in mobile-phase composition (±2%), flow rate (±0.1ml/min) or buffer pH (± 0.2 pH unit) did not affect significantly the chromatographic method illustrating the robustness of the method.

Table 1. Intra-day and inter-day precision of the proposed HPLC method

Intra-day and inter-day precision			
Conc added(µg/ml)	**Intra-day precision** Conc found± SD RSD %		
	LOS	HCZ	AT
30	29.92± 0.25 0.83	30.12 ± 0.38 1.26	30.05±0.27 0.90
50	49.91±0.37 0.74	50.16±0.53 1.05	50.03±0.46 0.92
80	79.99±0.66 0.82	79.87±0.82 1.02	80.01±0.63 0.78
Conc added(µg/ml)	**Inter-day precision** Conc found± SD RSD %		
	LOS	HCZ	AT
30	29.89±0.28 0.93	30.31 ±0.33 1.09	30.14±0.34 1.12
50	49.86±0.41 0.82	50.27±0.68 1.35	50.08±0.42 0.83
80	79.89±0.68 0.85	79.75±0.79 0.99	80.13±0.77 0.96

3.2.3. Accuracy

The previously mentioned method was repeated five times for different concentrations of pure samples. A definite concentration of standard drug (within the range of linearity for each drug) was added to the preanalysed mixture sample solution (100 µg ml^{-1} LOS, 25 µg ml^{-1} HCZ and 20 µg ml^{-1} ATR). The concentrations were calculated each time from the corresponding regression equation, the mean recovery percentage (Table 2) were found to be 99.95, 99. 56 and 99.72 for LOS, HCZ and ATR, respectively; with mean % RSD less than 2. The results indicate the method enables highly accurate simultaneous determination of all the three drugs in their combination.

3.2.4. Detection and quantification limits

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantization (LOQ), which were calculated according to ICH guidelines [20] using following formula: LOD= 3.3(SD)/S and LOQ= 10(SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve. LOD values for losartan, hydrochlorothiazide and atorvastatin were found 1.97 µg/ml, 1.94 and 0.72 µg/ml , respectively; and limits of quantification were found 5.96 µg/ml, 5.87 and 2.18 µg/ml, respectively. Analytical data for the calibration graphs were summarized in Table 2.

Table 2. Analytical data for the calibration graphs of LOS, HCZ and ATR.

Parameters	LOS	HCZ	ATR.
Concentration range(µg ml^{-1})	10-100	10-90	10-150
Slope a	56.40	65.40	30.15
Intercept b	60.68	72.10	35.20
Correlation coefficient (r)	0.9991	0.9987	0.9993
LOD (µg ml^{-1})	1.97	1.94	0.72
LOQ(µg ml^{-1})	5.96	5.87	2.18
Accuracy*(n=5)	99.95±1.05	99. 56±1.27	99.72±1.17

**Mean ± RSD (%).*

3.3. Method Application and specificity

The validated HPLC method was successfully applied for simultaneous determination of LOS, HCZ and ATR in their proposed combined dosage form. Five replicate determinations were made. The obtained mean assay results were in good agreement with the corresponding labeled amount [Table 3]. The average % amount ranges from 99.08 % to 99.60 % with relative standard deviation less than 1.5 % indicating satisfactory accuracy of the method. The chromatograms were checked for the appearance of any extra peaks. It was observed that single peaks for LOS, HCZ and ATR were obtained under optimized conditions (Fig 2a and Fig 2b), showing no interference from common tablets excipients and impurities. These results demonstrate the specificity of the method.

Table 3. Determination of LOS, HCZ and ATR in proposed polypill tablet

synthetic Tablet mixture	Drug	Labeled amount*(µ/ml)	Found amount *(µ/ml) ±SD	*% recovery ±SD RSD%
Kanzar- H +atorvastatin	LOS	100	99.08± 1.01	99.08±1.01 1.01
	HCZ	25	24.82±0.18	99.28±0.72 0.72
	ATR	20	19.92±0.29	99.60±1.47 1.47

**Mean of five determinations*

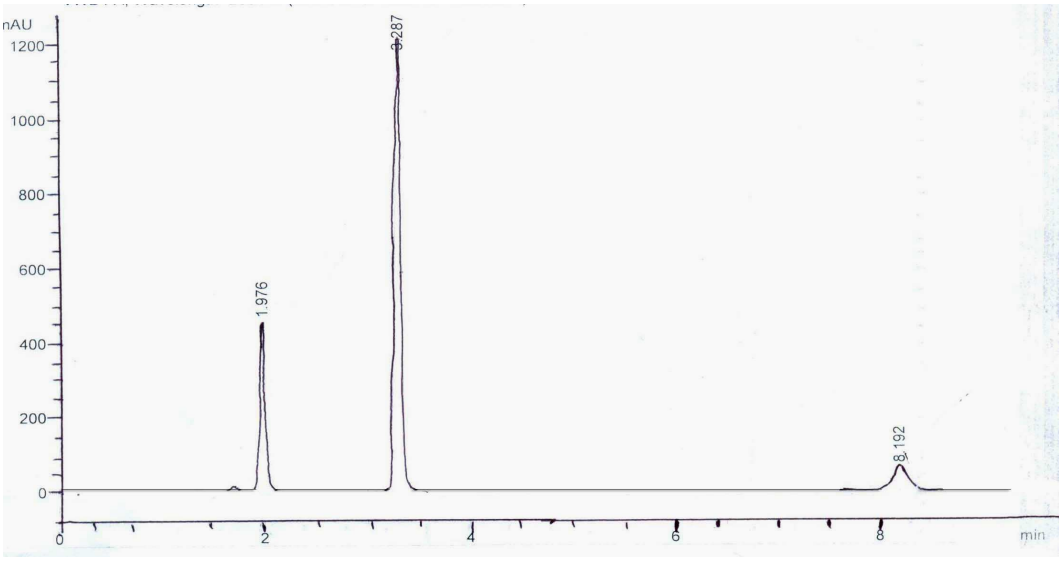

Fig 2a. Chromatogram obtained for standard mixture: 100 µg ml^{-1}LOS, 25 µg ml^{-1} HCZ and 20 µg ml^{-1} ATR (ret time 3.287, 1.976 and 8.192 min, respectively).

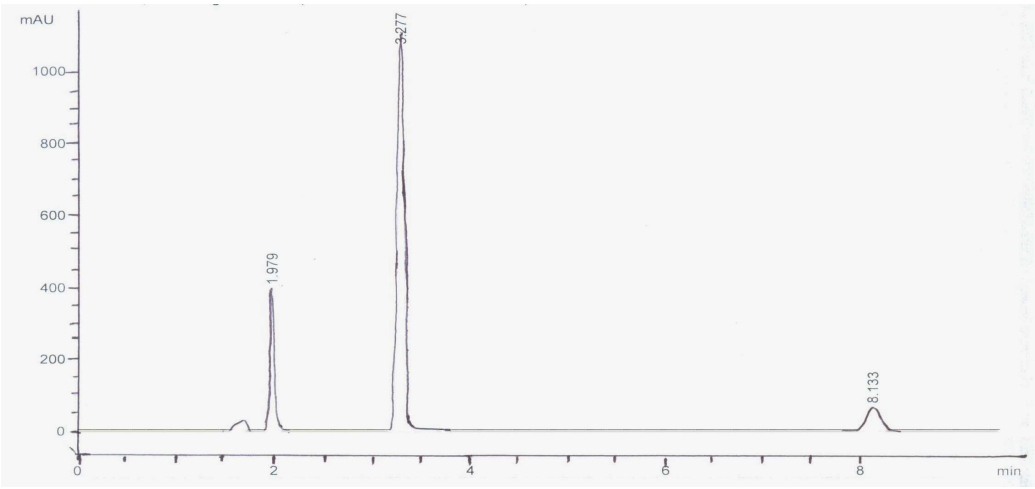

Fig 2b. Chromatogram obtained for prepared tablet : 100 µg ml^{-1}LOS, 25 µg ml^{-1} HCZ and 20 µg ml^{-1} ATR (ret time 3.277, 1.979 and 8.133 min, respectively).

CONCLUSION

The isocractic RP-HPLC method developed is specific and reproducible for the quantitative determination of losartan, hydrochlorothiazide and atorvastatin simultaneously, with good resolution and high sensitivity. The proposed method is simple, relatively rapid, and sufficiently precise and could be applied for routine analysis of the active ingredients LOS, HCZ and ATR in bulk and polypill tablets.

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