Simultaneous determination of olmesartan medoxomil and irbesartan and hydrochlorothiazide in pharmaceutical formulations and human serum using high performance liquid chromatography

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Abstract A simple selective sensitive precise simultaneous high performance liquid chromatographic analysis of serum samples and commercial tablet formulation containing hydrochlorothiazide olmesartan medoxomil and irbesartan are reported. Good chromatographic separation was achieved using a µ-Bondapak C18 column 15 cm x 4.6 mm i.d 5 µm and a mobile phase consisting of acetonitrile-0.2% acetic acid aqueous solution 50:50 v/v at a flow rate of 1.0 mL/min. The ultraviolet detector was set at a wavelength of 260 nm. Hydrochlorothiazide olmesartan medoxomil and irbesartan were eluted at 1.2 3.8 and 4.4 min respectively. No extraneous materials were found to interfere. The method uses protein precipitation with acetonitrile for the preparation of serum sample. The linear ranges for hydrochlorothiazide olmesartan medoxomil and irbesartan were 6.25 – 18.75 20 – 60 and 75 – 225 ng/mL respectively. The recoveries of hydrochlorothiazide olmesartan medoxomil and irbesartan in spiked samples were all greater than 98% and their relative standard deviations were less than 2.0%. The limits of detection were 1 2 and 2 ng/mL for hydrochlorothiazide olmesartan medoxomil and irbesartan respectively and the limits of quantification were 3 ng/mL which allow their determination at the expected serum concentration levels.

Key words high performance liquid chromatography HPLC hydrochlorothiazide olmesartan medoxomil irbesartan pharmaceutical formulations human serum

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The absolute risk of cardiovascular events is mainly determined by high blood pressure although there are some other important contributors such as age race and presence of other cardiovascular risk factors. Hence antihypertensive therapy enables to reduce considerably the risk of developing cardiovascular complications that cause a high mortality rate in the industrialized countries. International guidelines for the treatment of resistant hypertension recommend that the treatment includes at least three antihypertensive drugs in adequate dosages including a diuretic. The usual combinations associate to the diuretic first either an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker and second either a beta-blocker or a calcium channel blocker. In the present study we have selected hydrochlorothiazide and two angiotensin receptor blockers olmesartan medoxomil and irbesartan both are equally effective with combination to hydrochlorothiazide.

Hydrochlorothiazide 6-chloro-3[4-dihydro-2H-1]2-[4-benzothiadiazine-7-sulfonamide-1][1-dioxide is a thiazide diuretic of the class of benzothiadiazines an almost white and odorless crystalline powder slightly soluble in water and sparingly soluble in methanol. It reduces the reabsorption of electrolytes from the renal tubules which results in the increase of the excretion of sodium and chloride ions and consequently of water. The ex-
creatinine of other ions such as magnesium and potassium while the loss of calcium is reduced.

Olmesartan medoxomil is 5-methyl-2-oxo-1β-dioxolen-4-yl[meta-]methoxy-[4]-1-hydroxy-1-methylethyl[2]-2-propyl-1-[4]-2 tetrazol-5-yl-[phenyl] phe- nyln methylimidazol-5-carboxylate cyclic 2β-carbonate is a potent and selective angiotensin

AT1 receptor blocker. Irbesartan 2-butyl-3β-[2'-1H-tetrazol-5-yl]-biphenyl-4-yl[methyl]-1β-dia- zaspirid 4-[H]-non-1-ene-4-one is the first member of a new chemical class of a nonpeptide angiotensin II receptor antagonist. The first approved indication for irbesartan is for hypertension. Structural formulas of hydrochlorothiazide olmesartan medoxomil and irbesartan are shown in Fig. 1.

![Chemical structures](image)

Fig. 1 Chemical structures of a hydrochlorothiazide b olmesartan medoxomil and c irbesartan

A combination of hydrochlorothiazide with olmesartan medoxomil and hydrochlorothiazide with irbesartan is indicated in the treatment and management of edema and hypertension. Up to date olmesartan medoxomil has been determined individually in plasma and other biological fluids using high performance liquid chromatography HPLC coupled to fluorescent detection and tandem mass spectrometry. To our knowledge no analytical method has been reported for the simultaneous determination of hydrochlorothiazide olmesartan medoxomil and irbesartan in biological fluids and dosage forms.

There are a number of methods with different analytical techniques like HPLC [6-15] spectrophotometry [16-24] voltammetry [25] and capillary zone electrophoresis [26-29] which have been reported for the determination of hydrochlorothiazide individually or in combination with other drugs in pharmaceutical formulations and biological fluids. Analytical methods developed for the determination of irbesartan are HPLC [30-37] ultraviolet UV derivative spectrophotometry [38] liquid chromatography-mass spectrometry [39] liquid chromatography with fluorimetric detection [40] and capillary electrophoresis [41]. Almost all reported methods have some drawbacks and lack in some validation parameters especially all reported HPLC methods use a high strength ionic buffered mobile phase which is hazardous for column efficiency and need prolonged time for column saturation and washing.

As a crucial part of the drug development process a rapid sensitive selective assay is required to measure drug concentrations in human serum samples from clinical pharmacokinetic studies. Here we report an accurate and a sensitive validated HPLC method with UV detection for the simultaneous determination of hydrochlorothiazide irbesartan and olmesartan medoxomil in human serum and their respective dosage forms.

1 Experimental

1.1 Apparatus

A chromatographic system consisted of a Shimadzu LC20A series pump with an autosampler equipped with an SPD20A variable wavelength UV/Vis detector. The detector was set at 260 nm 0.01 Aufs and peak areas were integrated automatically by computer using the CSW-32 software program.

1.2 Chemicals and reagents

Hydrochlorothiazide was purchased from Sigma-Aldrich. Olmesartan medoxomil and irbesar- tan were kindly supplied by Cipla Chemical Ltd. India. HPLC grade acetic acid and acetonitrile
were purchased from Merck® Germany®.

1.3 HPLC procedure

1.3.1 Chromatographic conditions

The analytical column was a μ-Bondapak® C18
15 cm × 4.6 mm [5 μm]®. The mobile phase consisted of acetonitrile and 0.2% acetic acid aqueous solution® 50:50® v/v®. All analyses were done under isocratic conditions at a flow rate of 1.0 mL/min and at room temperature. Solutions and mobile phase were freshly prepared at the time of use.

1.3.2 Standard solution preparation

Stock solutions of hydrochlorothiazide® olmesartan medoxomil® and irbesartan were prepared daily by dissolving the appropriate amount of drug standards in mobile phase to yield a final drug concentration of 0.125® 0.40® and 1.50 mg/mL® respectively. Separate stock solutions were prepared for the calibration standards and quality control samples. Further® solutions were obtained by serial dilutions of stock solutions with mobile phase.

1.3.3 Preparation of spiked serum sample

An aliquot of serum® 200 μL® and standard solution® 200 μL® was pipetted out into a 10 mL tapered bottom centrifuge tube® and the volume was made up by acetonitrile. The mixture was vortex mixed briefly® and after standing for 5 min at room temperature® the mixture was centrifuged at 4,000 r/min for 20 min. The final concentration of the spiked serum sample containing 12.5 mg/mL of hydrochlorothiazide® 40 mg/mL of olmesartan medoxomil® and 150 ng/mL of irbesartan was obtained by further dilution with mobile phase.

1.3.4 Preparation of pharmaceutical dosage form sample

Pharmaceutical formulations of the two different brands® containing hydrochlorothiazide with irbesartan® Avide tablets® and hydrochlorothiazide with olmesartan medoxomil® Benicar hydrochlorothiazide tablets® commercially available in many countries were evaluated. In each case® groups of ten tablets were individually weighed® mixed® and finely powdered in a mortar. Portions of the powder equivalent to about 12.5 mg of hydrochlorothiazide® 40 mg of olmesartan medoxomil® and 150 mg of irbesartan were accurately weighed and diluted with mobile phase to get the final concentration of 12.5® 40® and 150 ng/mL® respectively.

1.4 Calibration and linearity

Calibration curves were constructed in the ranges of 6.25 – 18.75® 20 – 60® and 75 – 225 mg/mL for hydrochlorothiazide® olmesartan medoxomil® and irbesartan® respectively® to encompass the expected concentrations in the measured samples. Curves were obtained by plotting the peak area against concentrations of these drugs. Linear calibration curves were generated by weighted

1/y² linear regression analysis and obtained over the respective standard concentrations ranges. The suitability of the calibration models was confirmed by back-calculating the concentrations of the calibration standards.

1.5 Accuracy

Absolute recoveries of three different concentrations of hydrochlorothiazide® 6.25® 12.5® and 18.75 ng/mL® olmesartan medoxomil® 20® 40® and 60 ng/mL® and irbesartan® 75® 150® and 225 ng/mL® in serum and in placebo of respective dosage forms were determined by assaying the samples as described above and comparing the peak areas of the sample solutions with respective standards. Mean recoveries and the relative standard deviations® RSDs® were calculated by standard method®.

1.6 Precision

The precision of the assay method was considered on two levels® repeatability and intermediate precision. These levels were ascertained based on the analysis of spiked serum samples and spiked placebo sample of respective dosage forms. The concentrations for hydrochlorothiazide® olmesartan medoxomil® and irbesartan were 12.5® 40® and 150 ng/mL® respectively. Six replicates at 100% test concentration for each drug were analyzed for repeatability® and six replicates samples were analyzed on a second day by a different analyst on a different instrument to ascertain intermediate precision. Overall mean® RSD® and confidence interval for both levels were calculated by
standard methods\textsuperscript{[39]}.

2 Results and discussion

2.1 Chromatograms of samples

The aim of this research was to develop a new\[ simple\] more accurate\[ reproducible\] sensitive HPLC method for the simultaneous determination of hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan in human serum and pharmaceutical dosage form. A satisfactory separation of each drug from biological endogenous components and pharmaceutical excipients was obtained. To optimize the appropriate HPLC conditions for separation of the examined drugs\[ various reversed-phase columns\] isocratic\[ and gradient mobile phase systems were tried. The optimum wave-length for detection was 260 nm at which much better detector responses for the three drugs were obtained. The mobile phase was found to be suitable to improve the sharpness and thinness of the hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan peaks. Fig. 2-a\[ b\] and c shows the chromatograms obtained with drug-free serum mixed with placebo of respective dosage forms\[ spiked serum sample\] and spiked placebo sample\[ respectively. The retention times for the investigated drugs were found to be 1.25 min \[ hydrochlorothiazide\] 3.8 min \[ olmesartan medoxomil\] and 4.4 min \[ irbesartan\]. No endogenous serum components and pharmaceutical excipients eluted at the retention times of the peaks of interest.

![HPLC chromatograms](image)

Fig. 2 HPLC chromatograms of a\[ a drug-free serum sample mixed with placebo\] b\[ a serum sample spiked with 12.5 ng/mL of hydrochlorothiazide\] 40 ng/mL of olmesartan medoxomil\] and 150 ng/mL of irbesartan\] and c\[ a placebo sample spiked with 12.5 ng/mL of hydrochlorothiazide\] 40 ng/mL of olmesartan medoxomil\] and 150 ng/mL of irbesartan.

1. hydrochlorothiazide\[ 2. olmesartan medoxomil\] 3. irbesartan.

2.2 Method validated

The method was validated with regard to specificity\[ linearity\] limit of detection\[ LOD\] limit of quantification\[ LOQ\] precision\[ accuracy\] and robustness.

Peak areas of hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan of calibration standards were proportional to the concentration in serum and dosage forms over the ranges tested 6.25 – 18.75 \[ 20 – 60\] and 75 – 225 ng/mL respectively. Each concentration was tested in triplicate. The slope values for hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan were calculated as 0.819 840\[ 0.552 830\] and 0.343 045\[ respectively\] with intercept values of −0.097 4\[ −0.529\] and −0.632 4\[ respectively. The standard deviations on slope were calculated as 0.002 527\[ 0.004 999\] and 0.000 651\[ respectively\] and similarly standard deviations on intercept were calculated as 0.033 507\[ 0.173 907\] and 0.103 625 respectively for hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan. The calibration curves were fitted by linear least-square regression and showed correlation coefficients greater than 0.999 9 with standard error of estimate of 0.024 97\[ 0.129 62\] and 0.077 24\[ respectively for hydrochlorothiazide\[ olmesartan medoxomil\] and irbesartan.

The LODs and LOQs of irbesartan\[ olmesartan medoxomil and hydrochlorothiazide were calculated on the peak area using the following equations: LOD = 3 × N/B\] LOQ = 10 × N/B where N\]
the noise estimate is the standard deviation of the peak areas of three injections of the drugs and $B$ is the slope of the corresponding calibration curve. The LODs of hydrochlorothiazide, olmesartan medoxomil and irbesartan were found to be 12 ± 2 and 2 ng/mL and the LOQ was 3 ng/mL for each drug.

The absolute recovery was calculated by comparing the peak areas obtained from the standard working solutions with the peak areas obtained from spiked serum and placebo samples. The recoveries of hydrochlorothiazide, olmesartan medoxomil and irbesartan were found to be 99%–101%. The stability of standard and sample solutions was determined for the storage condition at +5°C, −17°C and ambient temperature. The standard and sample solutions were checked after three successive days of storage and the data were compared with freshly prepared samples. In each case, the RSD values of the assay were found to be below 2.0%.

### 3 Conclusions

The chromatographic method described is adequate for quantitation of hydrochlorothiazide, olmesartan medoxomil and irbesartan in human serum and pharmaceutical dosage forms at different concentration levels. It is a simple, accurate and effective and provided no interference peaks for endogenous components and pharmaceutical excipients. In spite of the complex matrix analyzed, acceptable values of precision and accuracy have been obtained at all levels by this method regarding the guidelines for assay validation. The separation of three drugs takes 6 min in one chromatogram so a large number of samples can be analyzed in a short period of time. The method uses simple mobile phase and is very beneficial for column life. In summary, the method can be successfully applied to samples of pharmaceutical dosage form and clinical and pharmacokinetic studies.

### References

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