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Bio-analytical method development and validation of a sensitive RP-HPLC method for simultaneous determination of eprosartan mesylate and hydrochlorothiazide in human plasma

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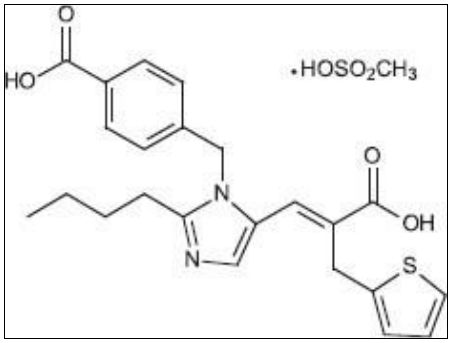
Abstract
Eprosartan mesylate and hydrochlorothiazide are antihypertensive agents used in combination therapy. The objective of the present study was to develop and validate a simple, rapid and sensitive reversed-phase high-performance liquid chromatographic (RP-HPLC) method for simultaneous determination of eprosartan mesylate and hydrochlorothiazide in human plasma. Methods: Chromatographic separation was achieved on an Agilent C18 column (150 × 4.6 mm, 5 µm) using 0.1% orthophosphoric acid (pH 2.2) and acetonitrile (60:40, v/v) as the mobile phase under isocratic conditions. The flow rate was 1.0 mL/min, the injection volume was 20 µL, the column temperature was 30 °C and detection was performed at 240 nm. Valsartan was used as the internal standard. Plasma samples were processed by liquid-liquid extraction with acetonitrile. Results: Eprosartan and hydrochlorothiazide were well resolved from the internal standard, with reported retention times of approximately 2.318, 2.713 and 3.698 min, respectively. The method was linear over 80–3200 ng/mL for eprosartan and 8.5–340 ng/mL for hydrochlorothiazide, with a reported coefficient of determination (r^2) of 0.999. The reported overall mean recoveries were 98.858% for eprosartan and 98.67% for hydrochlorothiazide. Selectivity, precision, accuracy and stability results were reported to meet the stated acceptance requirements. Conclusion: The developed RP-HPLC method provides a practical analytical approach for simultaneous determination of eprosartan mesylate and hydrochlorothiazide in human plasma.

Keywords: Eprosartan mesylate, hydrochlorothiazide, rp-hplc, human plasma., bioanalytical method validation, valsartan

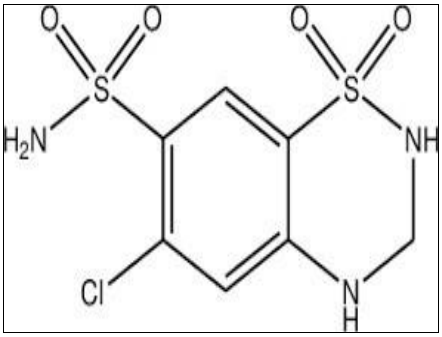
Introduction

Hypertension is a major cardiovascular risk factor and frequently requires combination pharmacotherapy to achieve adequate blood-pressure control. Eprosartan mesylate is an angiotensin II receptor type 1 antagonist, whereas hydrochlorothiazide is a thiazide diuretic. Their complementary mechanisms provide a pharmacological rationale for combined antihypertensive therapy.

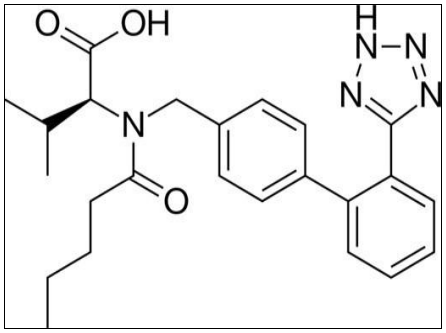
Analytical methods capable of measuring both drugs in biological matrices are important for pharmacokinetic, bioavailability and related bioanalytical studies. The present work therefore focused on development and validation of an RP-HPLC method for simultaneous determination of eprosartan mesylate and hydrochlorothiazide in human plasma.



Chemical structure of Eprosartan



Chemical structure of Hydrochlorothiazide



Chemical Structure of Valsartan (Internal standard)

Materials and Methods

1. Chemicals and reagents

Eprosartan mesylate and hydrochlorothiazide reference standards were used as analytes. Valsartan was used as the internal standard. Acetonitrile and orthophosphoric acid were used for chromatographic and sample-preparation procedures. Human plasma collected in K2EDTA tubes was used as the biological matrix.

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2. Instrumentation and chromatographic conditions

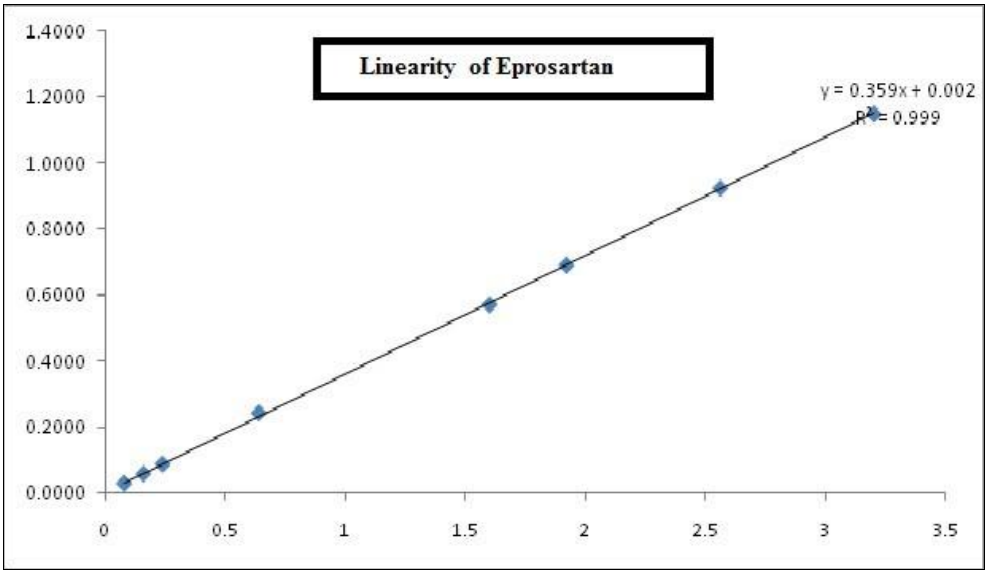
Table 1: Optimized chromatographic conditions.

Parameter	Optimized condition
Column	C18, 150 × 4.6 mm, 5 µm
Mobile phase	0.1% orthophosphoric acid (pH 2.2): acetonitrile (60:40, v/v)
Elution	Isocratic
Flow rate	1.0 mL/min
Injection volume	20 µL
Detection wavelength	240 nm
Column temperature	30 °C
Sample temperature	5 °C
Runtime	10 min
Diluent	0.1% orthophosphoric acid: acetonitrile (50:50, v/v)

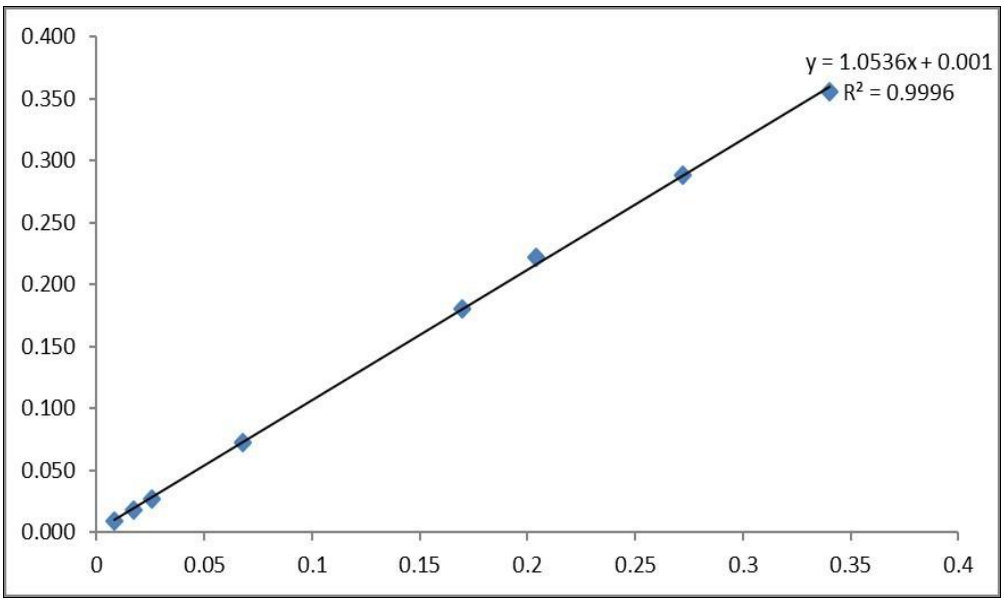
Method development involved evaluation of detection wavelength, stationary phase, mobile-phase composition, pH and flow rate. The final conditions were selected after comparison of alternative chromatographic conditions.

3. Calibration standards and quality-control samples

Calibration standards were prepared by spiking blank plasma to obtain 80, 160, 240, 960, 1600, 1920, 2560 and 3200 ng/mL for eprosartan and 8.5, 17, 25.5, 102, 170, 204, 272 and 340 ng/mL for hydrochlorothiazide. Peak-area ratios of analyte to internal standard were plotted against nominal concentrations.



Calibration plot for Eprosartan (concentration v/s Area ratio)



Calibration plot for Hydrochlorothiazide concentration v/s Area ratio

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4. Plasma sample preparation

A liquid-liquid extraction procedure was used. The source document reports mixing human plasma with internal standard and analyte spiking solutions, followed by addition of acetonitrile, vortex mixing for 2 min and centrifugation for 5 min at 3200 rpm. The supernatant was collected, filtered and 50 µL was injected into the HPLC system. Exact preparation volumes should be checked against the original laboratory worksheet.

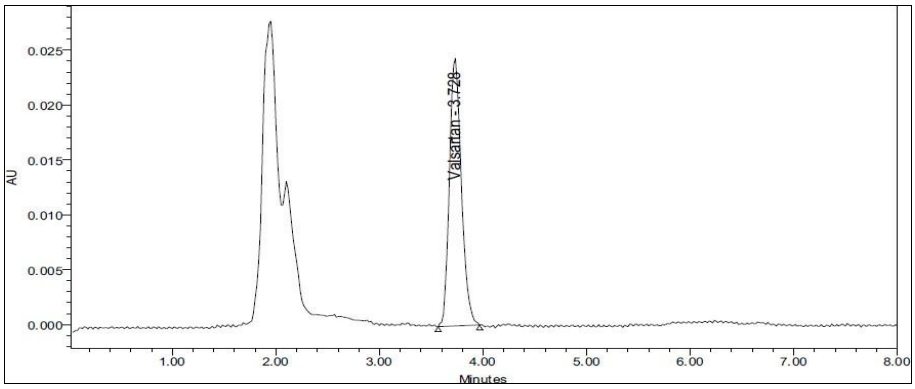
5. Method validation

Validation was performed for selectivity/specificity, system suitability, linearity, sensitivity, precision, accuracy, recovery and stability. Six batches of K2EDTA blank plasma were used for selectivity evaluation.

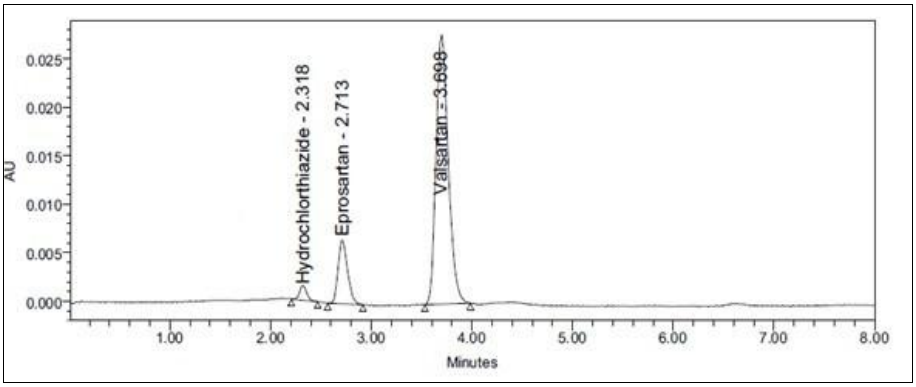
Results and Discussion

1. Chromatographic optimization

The optimized chromatographic conditions produced well-defined and resolved peaks for eprosartan, hydrochlorothiazide and valsartan. The retention times were approximately 2.318 min for eprosartan, 2.713 min for hydrochlorothiazide and 3.698 min for valsartan, with no endogenous interference at the analyte or internal-standard retention times.



Chromatogram of Blank in human plasma spiked with IS



Standard chromatogram of Eprosartan, Hydrochlorothiazide and Valsartan in human plasma

2. System suitability

Table 2: Representative system-suitability results.

Parameter	Eprosartan	Hydrochlorothiazide
Mean analyte retention time (min)	2.839	3.379
%CV of analyte retention time	0.94	0.26
%CV of IS retention time	0.65	0.65
%CV of analyte/IS area ratio	0.6746	0.88

The system-suitability results showed low variability. Analyte and internal-standard retention-time %CV values were below 2%, and analyte-to-IS peak-area-ratio variability was below the stated 5% criterion.

3. Selectivity

Selectivity was evaluated using six batches of K2EDTA blank human plasma. The source data report no significant interfering peaks at the retention times of eprosartan, hydrochlorothiazide or valsartan.

4. Linearity

Table 3: Linearity characteristics.

Analyte	Calibration range	Reported r^2
Eprosartan	80–3200 ng/mL	0.999
Hydrochlorothiazide	8.5–340 ng/mL	0.999

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The method demonstrated excellent linearity over the investigated concentration ranges. The calibration curves consistently showed a minimum r^2 of 0.999.

5. Sensitivity

Table 4: Sensitivity data.

Analyte	LOD	LLOQ	S/N value
Eprosartan	0.25 µg/mL	2.5 µg/mL	22
Hydrochlorothiazide	0.05 µg/mL	0.65 µg/mL	27

6. Accuracy and precision

The data reports low coefficients of variation across evaluated quality-control levels and mean accuracy values close to nominal concentrations. Representative stability/QC results showed % CV values generally below 2%.

7. Recovery

Table 5: Overall recovery

Analyte	Overall mean recovery	Overall SD	Overall %CV
Eprosartan	98.858%	0.3078	0.31
Hydrochlorothiazide	98.67%	o.6833	0.69

Extraction recovery was evaluated by comparison of extracted plasma samples with corresponding unextracted reference responses. The source overall mean recoveries of 98.858% for eprosartan and 98.67% for hydrochlorothiazide.

8. Stability

Table 6: Representative stability results for matrix samples stored at −80 ± 5 °C for 37 days.

Analyte	QC level	Reported accuracy (%)	Reported stability (%)
Eprosartan	HQC	100.84	100.90
Eprosartan	LQC	100.66	100.17
Hydrochlorothiazide	HQC	100.17	100.24
Hydrochlorothiazide	LQC	100.79	101.11

The stability results indicate that the analytes remained stable under the investigated storage condition. The source conclusion states that stability of analytes in K2EDTA human plasma, stock solutions and stock dilutions showed little degradation across the designated conditions and durations.

Conclusion

Based on the study findings, the validated method is suitable for accurately estimating Eprosartan and Hydrochlorothiazide concentrations in human plasma within the ranges of 80–3200ng/ml for Eprosartan and 8.5–340ng/l for Hydrochlorothiazide.

With regard to selectivity, precision, accuracy, linearity, and recovery, the HPLC detection method for determining the presence of eprosartan and hydrochlorothiazide in human plasma satisfied the acceptance requirements.

The results of stability tests conducted in k2EDTA human plasma, stock solutions, and stock dilutions demonstrated little degradation of Eprosartan and Hydrochlorothiazide across the designated storage conditions and durations, meeting the acceptance criteria.

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