ANALYTICAL TECHNIQUES FOR ESTIMATION OF EPROSARTAN MESYLATE: AN OVERVIEW

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ABSTRACT
The Aim of the present review to study analytical and detection techniques for Estimation of Eprosartan Mesylate. Eprosartan Mesylate is a potent, long-lasting, non peptide antagonist of the angiotensin II type-1 (AT₁) receptor that is indicated for the treatment of essential hypertension. It selectively inhibits stimulation of the AT₁ receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. There has been significant research on broad range of analytical and detection techniques that could be useful in its estimation in formulations and biological matrices. Practical requirements for high-sensitivity analysis create challenges for routine analysis. There are a number of methods used, but to our knowledge, high performance liquid chromatography with UV detection is more likely to be popular. In this review discusses methods such as Ultraviolet (UV) spectrophotometry, High Performance Liquid Chromatography (HPLC), High Pressure Thin Layer Chromatography (HPTLC), Liquid Chromatography- Mass spectrophotometry (LC-MS) and Capillary Zone Electrophoresis (CE).

KEYWORDS: Eprosartan Mesylate, Angiotensin, Estimation Methods, Bulk formulation.

INTRODUCTION
Angiotensin-II-receptor antagonists (ARA-IIs) are safe and effective agents for the treatment of hypertension and heart failure, either alone, or in conjunction with diuretics. They have been proposed as an alternative to the more traditional angiotensin-converting enzyme (ACE) inhibitors, because they selectively block the angiotensin type 1 (AT₁) receptor, which is responsible for vasoconstriction, and for salt and water retention. The Angiotensin type 2
(AT₂) receptor, which is thought to have cardioprotective and inhibitory effects on growth, is left unaffected by ARA-IIs [1–4]. There are six ARA-IIs available on the market: Candesartan (C), Eprosartan mesylate (E), Irbesartan (I), Losartan potassium, (L) Telmisartan (T), and Valsartan (V). The structure of Eprosartan differs from that of the other ARA-II compounds. Eprosartan Mesylate, an angiotensin II receptor antagonist (ARB), is used alone or with other antihypertensive agents to treat hypertension. Eprosartan Mesylate competes with angiotensin II for binding at the AT1 receptor subtype. As with other angiotensin II receptor antagonists, Eprosartan Mesylate is generally better tolerated than enalapril (an ACE inhibitor), especially among the elderly [5].

Eprosartan Mesylate (EPM) is chemically monomethane sulfonate of (E)-2-butyl-1-(p-carboxybenzyl)-a-2-thienylmethylimidazole-5-acrylicacid (Fig. 1) is a new antihypertensive agent as an angiotension II receptor antagonist that is highly selective to elicit a higher reduction in systolic blood pressure than other antihypertensive drugs [6–8]. The drug acts on the rennin angiotension system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotension II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic nor epinephrine production further reducing blood pressure [9–10].

**Eprosartan Mesylate:** [11–15]
- **Molecular Formula:** C₂₃H₂₄N₂O₇S₂
- **Molecular weight:** 520.63 g/mol
- **Drug Category:** Class of Angiotensin-II Receptor Antagonist.
- **Chemical Name:** 4-[[2-buty-5-(2-carboxy-3-thiophen-2-yl-prop-1-enyl)-imidazol-1-yl]methyl] Benzoic acid. Mesylate.

![Fig.1: Structure of Eprosartan Mesylate](image)
Description:

- **Appearance**: Crystalline powder
- **Odour**: Odourless
- **Colour**: White off white crystalline powder
- **Melting Point**: 248-250°C
- **Solubility**: Freely Soluble in ethanol, practically insoluble in water.
- **Stability & Storage**: Protect from warm temperatures (Below 25°C) & Stored at 40 ± 1°C and RH 75 ±5% conditions.

**Mechanism of action**: Eprosartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT found in many tissues (e.g., vascular smooth muscle, adrenal gland). There is also an AT receptor found in many tissues but it is not known to be associated with cardiovascular homeostasis. Eprosartan does not exhibit any partial agonist activity at the AT receptor. Its affinity for the AT receptor is 1,000 times greater than for the AT receptor. In vitro binding studies indicate that eprosartan is a reversible, competitive inhibitor of the AT receptor. Eprosartan has also been shown to bind to AT receptors both presynaptically and synthetically. Its action on presynaptic AT receptors results in the inhibition of sympathetically stimulated noradrenaline release. Unlike ACE inhibitors, eprosartan and other ARBs do not interfere with response to bradykinins and substance P, which allows for the absence of adverse effects that are present in ACE inhibitors (eg. dry cough).

**Therapeutic category**: Eprosartan is an angiotensin II receptor antagonist (angiotensin receptor blocker, ARB) used in the management of hypertension.

**Indication**: Eprosartan is indicated in the treatment of essential hypertension.

**Contraindication**: Eprosartan is contraindicated during pregnancy. Like other drugs affecting the renin angiotensin system (RAS), Eprosartan can cause birth defects, stillbirths and neonatal deaths. It should not be taken by breastfeeding women since it is not known whether the drug passes into the breast milk.

**Adverse effect**: Side effects are similar to other angiotensin II receptor antagonists and include tachycardia and bradycardia (fast or slow heartbeat), hypotension (low blood
pressure), edema (swelling of arms, legs, lips, tongue, or throat, the latter leading to breathing problems), and allergic reactions.

- **Administration:** Initial dose: 600 mg once daily assuming adequate intravascular volume & Maintenance dose: total daily doses of 400 to 800 mg administered once or twice daily
- **Bioavailability:** 13%
- **Protein Binding:** approximately 98%
- **Half-life:** 5-9 hour

**ANALYTICAL TECHNIQUES FOR ESTIMATION OF EPROSARTAN MESYLATE:** Various analytical methods are available for estimation of eprosartan Mesylate in different matrices from either single drug, combined dosage form or from biological matrices. There are several spectrophotometric and chromatographic methods reported in the literature that are capable of analyzing multiple cardiotropic drugs using one protocol. One advantage of these methods is that multiple sample preparation and chromatographic runs are not required for therapeutic drug monitoring purpose in patients receiving multiple drugs.

1. FOR ESTIMATION IN BULK AND DOSAGE FORMS:
   A. Chromatographic Methods:
   I. **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.**
      - **Ravichandrababu Rupakula et.al.**\(^{[16]}\) reported that develop a novel, sensitive and selective HPLC method for the determination of process impurities of Eprosartan Mesylate Drug substance (EPM) and characterization of impurities using Mass Spectrometry and NMR. EPM and its impurities were determined by Agilent 1200 series HPLC with PDA detector. A phenomeneX GeminiC18 (250 mm _ 4.6 mm _ 5.0 mm) column was employed for the separation of impurities from EPM. The mobile phase consists of 10 Mm Ammonium acetate buffer (pH to 3.0) with acetic acid as solvent A and Acetonitrile as solvent B in gradient programme. All the impurities were well resolved from one another and EPM peak indicating the specificity of the proposed method to quantify EPM and its four impurities. Precision, method and intermediate precision for EPM was checked at specification level and the % RSD were found to be 0.36, 0.29 and 0.52. The developed HPLC method was found to be simple, sensitive, and selective. Detection limit for impurities was found to be as low as
0.01% and was found to have excellent resolution for four impurities indicating high sensitivity and selectivity of the validated method.

- **Devika G S et.al.**[^17] reported that A simple, rapid, sensitive and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Eprosartan Mesylate and hydrochlorothiazide in combination. Chromatographic separation of the two drugs was performed on a Purospher BDS C18 column (150 mm× 4.6 mm id, 5μm particle size). The mobile phase comprising of acetonitrile: methanol: 0.01M KH₂PO₄ buffer (40:40:10) was delivered at a flow rate of 1.0mL/min. The pH of the mobile phase is adjusted to 4 with ortho phosphoric acid. Detection was performed at 270nm. The total run time is 5 min and the retention time of Eprosartan mesylate is 3.56 min and hydrochlorothiazide is 4.62 min respectively. The described method is linear for the assay of Eprosartan mesylate and hydrochlorothiazide over a concentration range of 216-576μ g/mL and 9-24μg/mL respectively. Results of the analysis have been validated and by recovery studies. The excipients present in the formulations do not interfere with the assay procedure. The developed method was successfully applied to determine Eprosartan Mesylate and hydrochlorothiazide in pharmaceutical formulations.

- **Harsha U. Patel, at.al.**[^18] reported that a simple, precise and accurate isocratic reversed phase column HPLC method has been developed for simultaneous analysis of eprosartan and HCT in tablet formulation. Isocratic RP-HPLC separation was achieved on phenomenex C18 column (250x4.6 mm i.d., 5μm particle size) using mobile phase composed of 0.5% formic acid-methanol-acetonitrile at flow rate of 1.0ml/min. The retention time for EPR and HCT was 7.69±0.10 and 4.24±0.09 minutes, respectively. The detection was performed at 272nm. The method was linear in the concentration range of 60-600 μg/ml for EPR and 2.5-25μg/ml for HCT with a correlation coefficient of 0.9992 and 0.9997, respectively. The method was validated and successfully used for determination of the drugs in tablets.

- **C. Sun et.al.**[^19] reported that simple and sensitive liquid chromatography tandem multi-stage mass spectrometry (HPLC/MSn) method suitable for eprosartan analysis was developed, by which an unknown impurity in bulk drug eprosartan was detected. The fragmentation behavior of eprosartan and the impurity in negative mode was investigated. Two molecules of CO₂ lost from eprosartan precursor ion were observed, while four
molecules of CO₂ were extruded from the deprotonated molecular ion to the MS³ product ions of the impurity. Furthermore, a characteristic fragmentation ion at m/z 335 was observed in both eprosartan and the impurity indicated that the impurity might have two eprosartan units. The unknown impurity was initially proposed to be eprosartan dimer connected via methylene unit at the thiophene moiety on the basis of the multi-stage mass spectrometric and exact mass evidences, and it was finally elucidated as 4,4-(5,5-(1E,1E)-3,3-(4,4-methylenebis(thiophene-4,2-diyl))bis(2-carboxyprop-1-ene-3,1-diyl))bis(2-butyl-1H-imidazole-5,1diyl))bis(methylene)dibenzoic acid by NMR experiments including 1D (¹H NMR, [¹³C NMR, DEPT135⁰) and 2D (1H-1HCOSY, HMQC and HMBC) data.

- V. Kiran Kumar et al. [20], reported that simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Eprosartan Mesylate in tablet dosage form. An Xterra KP18 150x4.6 mm, 5 μm particle sizes, with mobile phase consisting of acetonitrile and 0.03 M potassium dihydrogen phosphate (pH adjusted to 3.0±0.05 with ortho phosphoric acid) in the ratio of 35:65 v/v was used. The flow rate was 1 ml/min and the effluents were monitored at 215 nm. The retention time was 5.549 min. The detector response was linear in the concentration of 1-25 mcg/ml. The respective linear regression equation being Y= 6669.355x+892.3405. The limit of detection and limit of quantification was 0.1 and 0.5 mcg/ml respectively. The percentage assay of Eprosartan mesylate was 99.77 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RPHPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Eprosartan mesylate in bulk drug and in its pharmaceutical dosage form.

- K. R. Venugopala Reddy et al. [21] reported that a simple precise, accurate Reverse phase High performance liquid chromatographic method has been developed for the estimation of Eprosartan Mesylate in bulk. In this method a C18, 25 cm 5µ 4.6 mm ID (Oyster) column with mobile phase consisting of Sodium acetate buffer pH 3.0: Acetonitrile (70:30) was used. The detection wavelength is 235 nm and the flow rate is 1.0 ml/min. The linearity of Eprosartan shows regration coefficient of 0.9999. The proposed method is sufficiently selective to distinguish the parent drugs and the degradation products after hydrolysis photolysis or chemical oxidation.
- A.B.N. Nageswara Rao et al. [22] reported that an accurate, highly sensitive, precise and reproducible isocratic RP-HPLC method was developed and subsequent validated for the simultaneous analysis of Eprosartan and Hydrochlorothiazide in bulk and tablet dosage forms. Method development was carried out on Agilent Eclipse XBD-C18 (5μm, 150mm × 4.6mm I.D.) column. The mobile phase was a mixture of buffer (20mM KH2PO4) and methanol in the ratio of 80:20 v/v. The flow rate was set at 1.0 ml/min and UV detection at 225nm. The retention time of Hydrochlorothiazide and Eprosartan were found to be 3.34 min and 4.75 min respectively. Validation parameters such as linearity, accuracy, precision, and robustness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. In the linearity study, the regression equations Hydrochlorothiazide and Eprosartan were found to be y=0.0123x+ 0.0019 and y=0.0034x - 0.0163. Correlation coefficient was 0.9984 and 0.9989 for Hydrochlorothiazide and Eprosartan respectively. The proposed method was successfully applied for the quantification of bulk and active pharmaceutical present in tablet dosage form.

- T.Srinivasua et.al. [23] Reported that validated stability-indicating high-performance liquid chromatographic method has been developed for the simultaneous determination of Eprosartan and hydrochlorothiazide in tablet dosage forms. Chromatographic separation was performed on HPLC system of waters Model 2997 using X Bridge Shield RP18 (150 x 3.0 mm i.d., 3.5 μm particle size) column with a mixture of 0.1% formic acid and acetonitrile as mobile phase with a flow rate of 0.8 mL/min (gradient mode) with UV detection at 235 nm. The combination of drugs was subjected to stress conditions such as acidic, alkaline, oxidation photolytic and thermal degradations and the method was validated as per ICH guidelines.

- Shah et al. [24] Reported that the degradation of Eprosartan was evaluated under ICH/WHO prescribed stress conditions. The drug degraded to only one product under photo alkali condition, whereas it was stable to conditions of hydrolysis, oxidation and thermal stress. The drug and its co-eluting product were well separated on RP-HPLC in a gradient mode. Subsequently, LC-MS/TOF and on-line H/D exchange studies were performed on both of them. The two showed same molecular mass, similar fragment ions and even the same number of labile hydrogens indicating the product to be an isomer of the drug. To confirm the same, 1H and COSY LC-NMR studies were carried out by using an enriched sample.
Distinguishing behaviour of chemical shifts proved the product to be (Z)-4-((2-butyl-5-(2-carboxy-3-(thiophen-2-yl) prop-1-enyl)-1H-imidazol-1-yl)Methyl)benzoic acid. The structure was further supported by differential LC-MS ion intensities of the drug and the product.

• **Venu et al.** [25] reported that a simple, sensitive and specific RP-HPLC method was developed for the determination of Eprosartan in pure and tablet forms. The method showed a linear response for concentrations in the range of 20-120μg/mL using Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium di-hydrogen orthophosphate in water. Adjust pH of solution to 6.85 with orthophosphoric acid) in the ratio (45:35:20) as the mobile phase with detection at 232 nm using photodiode array (PDA) detector and a flow rate of 1 mL/min and retention time 7.1 min. The value of correlation coefficient, slope and intercept were, 0.9998, 1661.8 and 114.82, respectively. The method was validated as per ICH guidelines for precision, recovery, ruggedness and robustness. The specificity of the method was investigated under different stress conditions including acidic, basic, photochemical and thermal as recommended by ICH guidelines. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

• **SYEDA KULSUM et.al.** [26] reported that a simple, specific, accurate, precise and sensitive Reverse phase high performance liquid chromatographic method has been developed for the quantitation of Eprosartan mesylate in both pure and pharmaceutical dosage forms. An Phenomenex Luna 5 μ C-18(2) 100A column having 250 x 4.6 mm internal diameter in isocratic mode with mobile phase containing Acetonitrile : 1% Diethyl amine : 1% Glacial acetic Acid (13 : 3 : 4 v/v/v). The flow rate was 0.6 mL/min and the effluents were monitored at 242 nm. The retention time was 4.757 min. The linearity was in the range of 5-20 μg/mL. This method was validated for linearity, precision, limit of detection, limit of quantitation and accuracy. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug.

II. **HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY:** The focal attributes of modern TLC are the use of fine particle layers for fast and efficient separations; sorbents
with a wide range of sorption properties to optimize selectivity; the use of instrumentation for convenient (automated) sample application, development and detection; and the accurate and precise in situ recording and quantitation of chromatograms. Its uses have flourished because of its simple instrumentation and low operation costs\[27\].

- **Patel H.U. et.al.**\[28\] reported that developed a high-performance thin-layer chromatographic (HPTLC) method has been established for simultaneous analysis of Eprosartan Mesylate and hydrochlorothiazide in tablet formulations. Standard and sample solutions of Eprosartan Mesylate and hydrochlorothiazide were applied to precoated silica gel G 60 F254 HPTLC plates by means of Desaga AS30Win sample applicator equipped with 100-μL applicator syringe and were developed with benzene–methanol–formic acid 7:3:0.1 (v/v/v) as mobile phase. Detection and evaluation of chromatograms was performed densitometrically at 272 nm. The linear range was 4.8–43.2 μg/spot for EPR and 0.15–1.35 μg/spot for HCT.

- **Emad M Hussien et.al.**\[29\] reported that developed a method based on TLC separation of Eprosartan Mesylate and hydrochlorothiazide followed by densitometric measurements of their spots at 290 and 270 nm for EPR and HCT, respectively. The separation was carried out on silica gel 60 F254 using n-butyl acetate: ethanol: water: 33% ammonia (40:40:10:1, v/v/v/v) as mobile phase. The calibration curves were linear in the range 2.0–20.0 μg/spot for EPR and 2.0–9.0 μg/spot for HCT. The suggested method was successfully applied for the analysis of pharmaceutical preparations.

III. CAPILLARY ZONE ELECTROPHORESIS:

The individual detection of closely related angiotensin-II-receptor antagonists (ARA-IIs) requires sophisticated separation technique in addition to high sensitivity. The effective and fast separation of components by capillary electrophoresis is based upon charge and mass dependent migration in an electrical field.

- **Hillaert S. et.al.**\[30\] illustrated the potential of the capillary zone electrophoretic method and micellar electrokinetic capillary chromatographic method to simultaneously separate candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, valsartan and hydrochlorothiazide. Separation was achieved on a Crystal Thermo Capillary Electrophoresis system wherein a fused-silica capillary (85cm x 50μm ID) was applied with a constant voltage of 25kV. UV absorbance was detected at 214 nm. In Capillary Zone Electrophoresis, 60 mM sodium phosphate buffer (pH 2.5) was used as mobile phase. With micellar
electrokinetic capillary chromatography, separation is achieved by 55 mM sodium phosphate buffer solution containing 15 mM sodium dodecyl sulfate. These methods are suitable for the qualitative as well as quantitative determination of combined dosage forms of ARA-Is and Hydrochlorothiazide.

### B. Spectrophotometric Method

- **Anandakumar K, et.al** [31] reported that A simple efficient, precise and accurate Simultaneous equation method have been developed for the Simultaneous estimation of Eprosartan Mesylate and Hydrochlorothiazide in pure and in fixed dose combinations. In this method, UV Spectra of Eprosartan Mesylate and Hydrochlorothiazide were overlained. The linearity ranges for Eprosartan Mesylate and Hydrochlorothiazide were 6-36μg/ml and 1-10μg/ml, respectively. The proposed procedures were successfully applied for the simultaneous determination of both drugs in the laboratory prepared mixtures and in commercial tablet preparations. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 99.36 ± 0.701 and 98.9 ± 0.728 for Eprosartan Mesylate and Hydrochlorothiazide, respectively. The proposed procedure is rapid, simple, require no preliminary separation steps and can be used for routine analysis of both drugs in quality control laboratories. The results of analysis have been validated statistically and by recovery studies confirmed the accuracy of the proposed method.

- **Jain R, et.al.** [32] reported that Two simple, accurate, novel, safe and precise methods were developed for the simultaneous estimation of poorly water-soluble drugs Eprosartan Mesylate and Hydrochlorothiazide in tablet dosage form using 2M Sodium acetate and 8M Urea solution (50:50% W/V) as a mixed hydrotropic solution. Eprosartan Mesylate and Hydrochlorothiazide show maximum absorbances at 267.5 and 271.5 nm respectively. Sodium acetate and Urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs was seen. Eprosartan Mesylate and Hydrochlorothiazide follows the Beer’s law in the concentration range of 15-75 and 5-25 μg/ml ($r^2= 0.9994$ and 0.9996). Method-A employs a simultaneous equation method using 267.5 and 271.5 nm as two analytical wavelengths, Method-B, an absorption ratio method, uses 271.5 and 277 nm as two analytical wavelengths for estimation of Eprosartan Mesylate and Hydrochlorothiazide. The optimized methods showed good reproducibility and recovery with ranging from 95.08±0.086 to 99.82±0.097 EPS and HCZ respectively. The developed methods were
validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values therefore the both methods can be used for routine monitoring of EPS and HCZ in industry in the assay of bulk drug and tablets.

- **Kamila M. et.al.**[^33] reported that a simple, sensitive and accurate UV spectrophotometric method has been developed for the determination of Eprosartan Mesylate in raw material and experimental tablets. Beer’s law was obeyed in the concentration range 230 µg mL¹ for the drug (= 232nm) with an apparent molar absorptivity and Sandell sensitivity of 2.8 x 10⁴ L.mol⁻¹cm⁻¹ and 0.01854 µg cm⁻²/0.001A, respectively. The limits of detection and quantitation were calculated to be 0.3623 and 1.098 µg mL¹, respectively. Results were validated statistically according to ICH guidelines. Validation of the method yielded good results in the concerning range (2-30 µg mL¹), linearity (r² = 0.9998), precision and accuracy. The excipients present in the experimental tablets did not interfere with the method.

- **V Raja Kumar et.al.**[^34] reported that two simple, precise and accurate spectrophotometric methods have been developed for the estimation of Eprosartan in pharmaceutical formulations. Eprosartan exhibits maximum absorbance (λ max) at 233.0 nm (Method A). In Method B (D1) is a first derivative method showing minima at 222.0 nm. The drug obeys the Beer-Lambert’s law in the concentration range of 1-70 µg/ml in these two methods with an apparent molar absorptivity and sandell’s sensitivity of 23.0 x 10⁴and 0.018 in the method A, 0.726 x 10⁴ and 0.588 in the method B. The methods were validated according to the ICH guidelines and can be successfully applied to estimate Eprosartan in pharmaceutical dosage forms. Validation of the method yielded good results in the range (1-70ppm) with linearity (r²=0.999 and 0.999), precision and accuracy.

- **Emad M Hussien et.al.**[^35] reported that developed two spectrophotometric methods for the direct determination of Eprosartan Mesylate and hydrochlorothiazide in bulk powder and combined dosage form, without prior separation. The first method was a first derivative spectrophotometry using a zero crossing technique of measurement wherein the two series solutions were scanned within the range 220-330 nm against methanol as a blank. The absorbance at 240.7 nm (zero crossing of HCT) was measured for the determination of EPR, while the amplitudes at 233.4 nm (zero-crossing of EPR) were recorded for the determination of HCT. The calibration curves were linear in the range 1.0-18.0 for EPR and 1.0-9.0 for

[^33]: Kamila M. et.al. [Link]
[^34]: V Raja Kumar et.al. [Link]
[^35]: Emad M Hussien et.al. [Link]
HCT. The second method is the first derivative of ratio spectrophotometry. The ratio of absorption spectra of EPR and HCT was measured. The first derivative of the ratio spectra was calculated with $\Delta \lambda = 21$ nm and scaling factor (SF) = 20. The absorbencies were measured at 237.0 nm and the values were plotted against the corresponding concentration. The calibration curves were linear in the range 2.0-18.0 μg/ml for EPR and 1.0-9.0 μg/ml for HCT.

2. FOR ESTIMATION FROM BIOLOGICAL MATRICES: Eprosartan Mesylate, in certain clinical situations, may require monitoring of serum or plasma levels to ensure better clinical outcome. Therapeutic drug monitoring involves the proper interpretation of the drug concentration in serum or plasma using pharmacokinetic parameters so that appropriate conclusion can be reached regarding the progress of therapy and dose adjustment. Both HPLC and LC/MS methods perform acceptably and equivalently for clinical measurement within the clinically relevant concentration ranges.

HPLC-UV methods using fast and simple solid phase extraction have been most widely adopted and provide adequate analytical sensitivity.

- *N. Ferreiros et al.* [36] reported that a chemometric approach was applied for the optimization of the extraction and separation of the antihypertensive drug eprosartan from human plasma samples. Multi Simplex program was used to optimize the HPLC-UV method due to the number of experimental and response variables to be studied. The measured responses were the corrected area, the separation of eprosartan chromatographic peak from plasma interferences peaks and the retention time of the analyte. The use of an Atlantis dC18, 100mm×3.9mm i.d. chromatographic column with a 0.026% trifluoroacetic acid (TFA) in the organic phase and 0.031% TFA in the aqueous phase, an initial composition of 80% aqueous phase in the mobile phase, a stepness of acetonitrile of 3% during the gradient elution mode with a flow rate of 1.25 mL/min and a column temperature of 35±0.2 °C allowed the separation of eprosartan and irbesartan used as internal standard from plasma endogenous compounds. In the solid phase extraction procedure, experimental design was used in order to achieve a maximum recovery percentage. Firstly, the significant variables were chosen by way of fractional factorial design; then, a central composite design was run to obtain the more adequate values of the significant variables. Thus, the extraction procedure for spiked human plasma samples was carried out using C8 cartridges, phosphate buffer pH 2 as conditioning...
agent, a drying step of 10 min, a washing step with methanol–phosphate buffer (20:80, v/v) and methanol as eluent liquid. The SPE-HPLC-UV developed method allowed the separation and quantitation of eprosartan from human plasma samples with an adequate resolution and a total analysis time of 1 h.

- **Cyronak M. et.al** [37] reported that developed a sensitive, selective and rugged analytical method for the determination of Eprosartan Mesylate in human plasma. The new method employed a simple solid-phase extraction procedure to isolate the drug and its internal standard from plasma samples. The SPE cartridges were conditioned with methanol, washed with ethyl acetate containing 0.1% triethylamine and elution with methanol-0.05 M acetic acid (90:10 v/v). Separation was achieved by BDS Hypersil C18 column (150 x 2 ID mm, 5μm) supported with BDS Hypersil C18 column (20 x 2 ID mm, 5μm). The mobile phase was optimized to 0.05 M citrate buffer (pH 3.5) tetrahydrofuran (34:16, v/v) and the flow rate was set to 0.25 ml/min. The assay was based on analysis by reverse phase high performance liquid chromatography with UV absorbance detection. The dynamic range of the assay was 10 to 5000 ng/ml.

- **Xue-Ning Li et.al.** [38] reported that developed and validated a protein precipitation, liquid chromatography/ tandem mass spectrometry (LC/MS/MS) method for the determination of eprosartan mesylate in human plasma and urine. The solvent system also served as a protein precipitation reagent. The chromatographic separation was achieved on a Capcell Pak C18 column (50 x 2.0 ID mm, 5 μm). A mobile phase was consisted of 0.5% formic acid in water and 0.5% formic acid in acetonitrile (72:28 v/v). Detection was by positive ion electrospray tandem mass spectrometry on a Sciex API3000. The standard curves, which ranged from 5 to 2000 ng/ml in human plasma and from 0.25 to 50 μg/ml in urine, were fitted to a 1/x weighted quadratic regression model. The method proved to be accurate, specific and sensitive enough to be successfully applied to a pharmacokinetic study.

- **Manish TR et.al.** [39] reported that developed a simple, rapid, selective and sensitive HPLC method for the determination of eprosartan from human plasma. The drug was extracted with a mixture of 0.05M sodium hydroxide and ethyl acetate. Eprosartan was measured in plasma using a validated a HPLC method with UV detector at 235nm. Chromatographic peaks were separated on 5μm Intensil, C18 column (4.6 x 250 ID mm, 5μm) using phosphate buffer pH 4 and acetonitrile (60:40 v/v) as mobile phase at a flow rate of 1 ml/min. The method was
linear over the concentration range of 300 to 20,000 ng/ml. This method was successfully applied to pharmacokinetics studies.

- *Vineeta Khanvilkar et al.* [40] reported that a simple high performance thin-layer chromatographic method was developed and validated for estimation of Eprosartan Mesylate in human plasma using losartan as internal standard. Protein precipitation technique was used for extraction of drugs from human plasma. Separation was achieved on precoated silica gel 60 F254 TLC plates using mobile phase ethyl acetate: acetonitrile: glacial acetic acid in the ratio 6:4:0.2 (v/v/v). Detection was performed by densitometric analysis at 238 nm. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 0.7-32 μg/ml. The percent recovery of eprosartan mesylate was found to be 61.50-66.58%. The Rf values for eprosartan mesylate and losartan were 0.3±0.03 and 0.59±0.03, respectively. The method was validated in accordance with the requirements of European Medicines Agency (EMA) guidelines. The proposed method can be applied for quantitative analysis of Eprosartan in clinical samples.

**CONCLUSION**

A wide range of analytical techniques are available for separation and detection of Eprosartan Mesylate for practical analysis. This review highlighted some recent development and new techniques that have been used in the analysis and detection of Eprosartan Mesylate. The presented information would be useful for the researchers especially those involved in the formulation development and quality control of Eprosartan Mesylate. High Performance Liquid Chromatography with UV detector is the most widely employed technique for determination of Eprosartan Mesylate in formulation and biological matrices as well as for evaluation of its pharmacokinetics. Capillary Zone Electrophoresis is the commonly utilized method for simultaneous quantitative estimation of Eprosartan Mesylate along with other angiotensin-II receptor antagonists (ARA-IIs). As shown, although there have been several recent successes in its detection, new methods are still required to achieve higher sensitivity and address other challenges that are posed. The application of MS in conjunction with other tools for decreasing limits of detection has been of increased interest in the recent times. Future trend would be to concentrate on designing of more rapid and sensitive tool for the estimation purpose.
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