Few researchers have published their research work on mentioned drug (i.e. Lercanidipine and Enalapril). Available literature (not limited to below list) was reviewed and will be used to develop better analytical approach for R & S Lercanidipine and Enalapril (and active metabolite) considering clinical biological matrices.

As mentioned earlier; The lercanidipine molecule has got one asymmetric carbon atom. While the S-enantiomer is more effective than the R-enantiomer, marketed formulations contain a 1:1 mixture of both (i.e., the racemate). Only one researchers have published their work without attaining enantiomeric separation of R and S enantiomer, while many have quantified both as merged single peak. In my research my focus will be to attain a bioanalytical strategy to separately quantify two enantiomer in single analytical run.

Few available bio-analytical research publications for lercanidipine quantiation:

- In a latest publication; **Darshan V. Chaudhary** and his colleagues (2016) have shared an ultra-performance liquid chromatography/tandem mass spectrometry (UPLC–MS/MS) using solid phase extraction technique. Research work published in J. of Phar. Analysis, mentions linear range of 0.010–20.0 ng/mL in plasma. Assay was used to support a bioequivalence study performed with 36 healthy subjects after oral administration of 10 mg of lercanidipine and the assay reproducibility was evaluated by reanalysis of 133 incurred samples.

- **M. Sunitha Reddy et.al. (2015)** published their research work in World journal of Pharmaceutical Research (Vol. 4; Issue. 10). Researchers published their work on UV Spectrophometric assay for lercanidipine at 239 nm in bulk formulation samples. Method does not include any chromatographic separation and had linearity from 5 to 25 ppm.

- **Kareem M. Younesa and Ehab F. El Kadyb (2013)**; published their research work on bioanalytical assay for lercanidipine assay from plasma. Their research was titled as ‘A new validated bio-analytical liquid chromatographic -tandem mass spectrometric method for the quantification of Lercanidipine in human plasma’ in Int. J. of Analytical and Bioanalytical chemistry (2013). Mentioned assay involved liquid-liquid extraction followed by LC-MS/MS detection. Assay was linear for a range of 0.10 to 10.0 ng/mL.

- **H. O. Kaila et.al. (2011)** published isocratic reversed phase stability indicating HPLC method for the simultaneous determination of atenolol and lercanidipine hydrochloride in
commercial tablets. The chromatographic separation was achieved on phenomenex Gemini C18 (250×4.6 mm, 5 μm) column using a mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate pH 3.5) in the ratio of (55:45, v/v) at a flow rate of 1.0 ml/min and UV detection at 235 nm. The linearity of the proposed method was investigated in the range of 40-160 μg/ml for atenolol and 8-32 μg/ml for lercanidipine. Injection volume for mentioned method was 20 µL and run time was 10 minutes (with lercanidipine peak eluting at approx. 6 minutes).

- **Nilesh jain et.al. (2011)** published their research work titled: Simultaneous Spectrophotometric Estimation of Lercanidipine Hydrochloride and Atenolol in Tablet Dosage Form in Eurasian Journal of Analytical Chemistry. Method included absorbance measurement at λmax (242 nm) for lercanidipine with mean recovery of 98.60±0.36%. Method was validated as per ICH guidelines and applied for simultaneous determination of Lercanidipine hydrochloride and Atenolol for routine industrial samples.


- In one of review article by **G.L.Erny and A. Cifuentes (2006)** Titled: Liquid separation techniques coupled with mass spectrometry for chiral analysis of pharmaceutical compounds and their metabolites in biological fluids; Table-1; the LOQ for this method was mentioned as 25 ng/mL against the summary refence of 0.025 ng/mL. Verification and correlation of these facts is beyond scope of my research.

The limitations of proposed method were its long run time and issues of compatibility between normal phase chromatographic solvents and mass spectrometers.


- **A.B. Baranda et.al. (2005)** shared Development of a liquid–liquid extraction procedure for five 1,4-dihydropyridines calcium channel antagonists from human plasma using experimental design.

- **I.I. Salem et.al. (2004)** Selective and rapid liquid chromatography mass spectrometry method for the determination of lercanidipine in human plasma
V. A. Jabor et.al. (2004); Further same researchers group extended their research by publishing next article (Title: Enantioselective pharmacokinetics of lercanidipine in healthy volunteers; Journal of Chromatography B) in 2004; covering enantioselective kinetic disposition of lercanidipine, in six healthy male volunteers following a single 20mg racemic oral dose.

Proposed method used hexane: ethanol: diethylamine (95:5:0.1; v/v) composition mobile phase on Chiralpak AD column. Liquid-liquid extraction was used as extraction method and ionization source in LC-MS/MS was ESI.

C.A. Mueller et.al. (2004); Screening for dihydropyridine calcium channel blockers in plasma by automated solid-phase extraction and liquid chromatography/tandem mass spectrometry.

Previous year; V. A. Jabor et.al. (2003) published approach for enantioselective analysis of the calcium antagonist lercanidipine in human plasma by high performance liquid chromatography (HPLC) employing tandem mass spectrometric (MS) detection. Routine determination of lercanidipine enantiomers in human plasma in the working range of 0.025-50.0 ng/ml plasma for each enantiomer with an accuracy and precision less than 15% was achieved. Application of the method to a stereospecific study of the pharmacokinetics showed that plasma levels after an oral dose of rac-lercanidipine administered to a healthy volunteer were found to be higher for the (S)-enantiomer.

In conclusion, none of the researchers {except Jabor’s (2003) research work} is able to develop an enantioselective analysis of the calcium antagonist lercanidipine in human plasma by high performance liquid chromatography (HPLC) employing tandem mass spectrometric (MS) detection. Limitations of Jabor’s research methodology have also been mentioned earlier (i.e. normal phase chromatography and longer chromatographic run time)

On other hand several analytical methods have been published for extraction of Enalapril and its active metabolite (i.e. Enalaprilat). Following are most relevant research articles published till date on mentioned compounds:

UV-detection. Linearity range was established for 1 to 200 µg/mL. Limit of detection for enalapril was 0.125 µg/mL and 0.5 µg/mL for enalaprilat. Method reported a separation for peaks of enalaprilat and enalapril maleate.

- **Jaeick Lee et al. (2003)** published their research work titled ‘Simultaneous quantitation of enalapril and enalaprilat in human plasma by 96-well solid-phase extraction and liquid chromatography/tandem mass spectrometry’ in Rapid Comm. in Mass Spec. journal. Concentration linearity ranges were 0.2–200 and 1.0–100 ng/mL for enalapril and enalaprilat, respectively. Samples were extracted by solid phase extraction followed by LC-MS/MS detection. Article lacks some critical details e.g. recovery of analytes from biological matrix.

- **Kyung-Hwan Yoon et al. (2004)** shared a protein precipitation, reverse phase chromatography and selected ion monitoring (SIM) mode approach for quantitation of enalapril in human plasma. Lower limit of quantitation for proposed method was 1 ng/mL. Method was used to determine pharmacokinetic profile for 24 subjects over 9 hours time period.

- **Gu Q et al. (2004)** published a research article in Journal of Chromatography B.; entitled as Simultaneous determination of enalapril and enalaprilat in human plasma by liquid chromatography-tandem mass spectrometry. Paper mentions extraction from plasma samples by liquid-liquid extraction, separated on a Zorbax Extend-C18 column, and detected by LC-MS/MS. The chromatographic run time was approximately 3.5 min. The calibration curves for both enalapril and enalaprilat was in the concentration ranges of 0.10-100.0 ng/mL in human plasma. A post validation experiment, the method was applied to evaluation of the pharmacokinetics of enalapril and enalaprilat in 20 volunteers after an oral dose of 10 mg enalapril maleate. One major observation in mentioned was varying recoveries for two analytes. Recovery for enalapril was approx. 65%, while recovery for metabolite (i.e. enalaprilat) was around 24%. This difference is primarily attributed to difference in their hydrophobicity.

- **Shan Lu et al. (2009)** published their research work titled ‘Simultaneous quantification of enalapril and enalaprilat in human plasma by high-performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study’ in Journal of pharmaceutical and biomedical analysis. Article describes high-performance liquid chromatography–tandem mass spectrometry method developed to simultaneously determine enalapril and enalaprilat in human plasma. Benazepril was used as internal standard. Sample pretreatment involved protein precipitation with methanol of 0.2 mL plasma sample aliquots.
Analysis was performed on an Ultimate™ XB-C18 column (50 mm × 2.1 mm, i.d., 3 μm) with mobile phase consisting of methanol–water–formic acid (62:38:0.2, v/v/v). The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction-monitoring (MRM) mode via electrospray ionization (ESI) source. Each plasma sample was chromatographed within 2.5 min. The linear calibration curves for enalapril and enalaprilat were both obtained in the concentration range of 0.638–255 ng/mL. Validated method was applied to determine pharmacokinetic study of enalapril maleate capsules in 20 healthy male volunteers after oral administration.

- **K. Makwana et.al. (2013)** published their research work in Paripex-Indian journal of Research on bioanalytical method validation for Enalapril in Human Serum by LC-MS/MS Detection. Methodology included liquid-liquid extraction (n-Hexane: Ethyl acetate combination added to basified matrix sample), followed by chromatographic separation of analyte (Enalapril) and internal standard (Fluoxetine) on ODS column. Mass spectrometric detection was performed in negative mode for selected ion monitoring (SIM) m/z 375.07 for Enalapril and 310.2 for internal standard. Linearity range for proposed method was established form 0.25 to 50 ng/mL. Method reported a recovery of approx. 35% for analyte.

- **Bjoern B. Burckhardt and Stephanie Laeer (2015)**; published a detailed research work in International Journal of Analytical Chemistry, entitled: ‘Sample Preparation and Extraction in Small Sample Volumes Suitable for Pediatric Clinical Studies: Challenges, Advances, and Experiences of a Bioanalytical HPLC-MS/MS Method Validation Using Enalapril and Enalaprilat’. Several countries (US, EU) have posed regulations on child-appropriate medications. Medical companies need to generate higher extent of pharmacokinetic data for prescription drugs before suggesting it for pediatric use. Need from bioanalytical scientists are associated with low sample volumes and higher throughput assays. Broadly used HPLC-MS/MS, being able to cope with small volumes, is susceptible to matrix effects. The matrix effect restrains the accurate and precise drug quantification through signal suppression/ enhancements. Extensive optimization was performed to select best ion-exchange SPE protocol. A scale-up from vacuum manifold to positive pressure manifold was conducted to meet the demands of high-throughput within a clinical setting. Enalapril, enalaprilat, and benazepril were selected as test drugs. The applied sample preparation and extraction successfully reduced the absolute and relative matrix effect to comply with international
guidelines. Extensive optimization of extraction protocol was performed using ion-exchange sorbent chemistries during solid phase extraction optimization. Recoveries ranged from 77 to 104% for enalapril and from 93 to 118% for enalaprilat. The bioanalytical method comprising sample extraction by solid-phase extraction was fully validated according to FDA and EMA bioanalytical guidelines and was used in a Phase I study in 24 volunteers. Calibration curves ranged between 0.2 – 200 ng/mL (for Enalapril) and 0.18-180 ng/mL (for Enalaprilat).

- **Hossein Danafar and Mehrdad Hamidi (2015)** published a research article in Journal of pharmaceutical and Biomedical research on simultaneous quantitation of enalapril and enalaprilat in human plasma. Sample preparation involved single step protein precipitation (using per-chloric acid). Chromatography separation was achieved on ODS column using mobile phase composite of methanol and diluted aqueous formic acid solution. Chromatographic run was 1.25 minutes and linearity range was 0.1 to 20 ng/mL. Same group again published their methodology in Iranian Journal of Pharmaceutical Sc. (2016) with added data on pharmacokinetic study for 10 mg enalapril maleate on 12 healthy male volunteers.

To cover voltametric detection strategies; main references include:

- **Y. Altun et.al. (2010)** had shared a study on Electroanalytical characterization of lercanidipine and its voltammetric determination in pharmaceuticals and human serum on boron-doped diamond electrode.

- **F. Ozturk et.al. (2011)** shared a study with a new voltammetric method for the determination of lercanidipine in biological samples.

On side of pharmacology references; main references include:

- **R. Fogari et.al. (2003)** had shared a study on Differential effects of lercanidipine and nifedipine GITS on plasma norepinephrine in chronic treatment of hypertension.

- **Later L.M. Bang et.al. (2003)** had mentioned some critical effects in a review of Lercanidipine and its efficacy in the management of hypertension.

- **Earlier in (2005) C. Borghi** had mentioned pharmacology of Lercanidipine in hypertension.
• **P.I. Hair et al. (2007)**; first time discussed on Fixed-dose combination lercanidipine/enalapril Drugs.

• **M.T. Pruijm et al. (2008)**; on Patient adherence and the choice of antihypertensive drugs focusing lercanidipine.

• **Arnaout S (2015)** shared results of an observational study in Efficacy and safety of lercanidipine/enalapril fixed combination in Lebanon.

• **Grassi G (2016)** shared a research publication on Lercanidipine/enalapril combination in the management of obesity-related hypertension.

In recent times several clinical studies have been completed, advocating benefits of Fixed-dose combinations (FDC) for hypertensive drugs. One example is research study reported by João Maldonado and his colleagues (2014). Clinical observational study collected data for 315 patients with hypertension treated by 46 specialists at clinics across Portugal with lercanidipine/enalapril (10/20 mg). Treatment with the fixed-dose combination lercanidipine/enalapril was associated with significant reductions in systolic and diastolic blood pressure (BP) and a significant increase in the BP control rate. Till date no pooled analytical research work has been conducted on these drugs, to establish a comprehensive analytical strategy for evaluation of mentioned FDC regimen.