

Simultaneous Quantitation of Multiple Antihypertensive Drugs in Plasma: A Comprehensive Review with Focus on Herb–Drug Interactions

Shifa Mammed Kutty K M *, Wafa T, Saranya Mohan, Najah P A, Meena P V
Department of Pharmaceutical Analysis, Al Shifa College of Pharmacy (Kerala University of Health Sciences),
Perinthalmanna, Malappuram

Corresponding Author: Shifa Mammed Kutty K M

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ABSTRACT

The common practice of using herbal medicines in combination with prescribed antihypertensive treatments highlights the urgent requirement for precise and effective concurrent determination of multiple cardiovascular drugs in plasma. Herb–drug interactions (HDIs) can profoundly impact pharmacokinetic factors i.e., C_{max} and AUC via mechanisms such as CYP450 modulation and transporter regulation, with severe implications varying from failure of therapy to toxicity. Versatile analytical methods using mostly LC–MS/MS support high-throughput and sensitive quantification of numerous antihypertensive drugs from plasma matrices. This paper integrates present methodologies to carryout simultaneous drug quantitation, summarizes mechanistic findings and evidence from HDI research, and discusses future directions such as AI-based decision-support tools and the necessity for clinical validation frameworks.

KEY WORDS: Herb-drug interactions, Antihypertensive drugs, Pharmacokinetics, LC-MS/MS, CYP450 modulation, Simultaneous drug quantitation, Clinical validation

I. INTRODUCTION

Hypertension is still one of the most common chronic diseases globally, accounting for a high percentage of morbidity and mortality. (1)Hypertension management is highly dependent on drug treatment, and to control blood pressure, several drugs are often combined. The most frequently co-prescribed classes include angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), calcium channel blockers (CCBs), β -blockers, and diuretics.(2)

Herb-drug interactions (HDIs) are a serious clinical dilemma, particularly in hypertensive patients who often self-treat with

herbal supplements alongside prescribed medications. (3)Various studies indicate that more than 50% of hypertensive patients use traditional pharmacotherapy in combination with botanicals for perceived additive effects. These interactions can produce interaction effects through two main mechanisms:

(1) pharmacokinetic modulation—through the action of CYP enzymes such as CYP3A4 and drug transporters such as P-glycoprotein
(2) pharmacodynamic effects such as additive hypotension or bleeding risk. (4,5)Detection and quantitation of such interactions necessitate strong, concurrent analytical platforms capable of precisely measuring several antihypertensive drugs in limited plasma volumes. The advent of LC-MS/MS and UHPLC-based methodologies has therefore become pivotal to HDI research.(6,7)

Analytical Techniques for Simultaneous Quantitation

Current LC–MS systems allow for accurate, multi-analyte determination with high throughput:

- A validated LC-MS/MS assay for hydrochlorothiazide (HCT) and metoprolol (MET) in human plasma was linear over 3–1,000 ng/mL, with intra and inter-day precision below 6.2% and accuracies at $\pm 3.8\%$ (HCT) and $\pm 2.6\%$ (MET).(8,9)
- An enhanced UPLC-MS/MS assay determined candesartan (CN) and HCT in human plasma, with a low quantitation limit of 1 ng/mL, fast run time (2 min), and suitability for bioequivalence trials.(9)
- A sensitive LC-MS/MS assay simultaneously quantified MET, amlodipine (AML), canrenone (CAN), and HCT in patients with resistant hypertension. With the use of isotope-labelled internal standards, the assay had detection limits of 0.05–8.54 $\mu\text{g/L}$, precision in the range of 1.7–14%, and short 2.5-minute

run time. For olmesartan (OLM) and HCT, a high-throughput LC-MS/MS strategy based on liquid-liquid extraction was validated according to USFDA guidelines and offered a 3-minute chromatographic cycle, increased stability in hemolytic or lipemic samples, well-suited for PK and bioequivalence studies.(8–10)

- Concomitant candesartan and HCT analysis with negative ion mode, with low LLOQ and high matrix factor reproducibility.(9)
- Pioglitazone and telmisartan analysis in rat plasma for PK use amlodipine, valsartan, and HCT measurement in rat plasma simultaneously for pharmacokinetic modeling.(8–10)

These methodologies reflect a trend: migrating toward multiplexed, sensitive, rapid-turn around assays with robust validation metrics—essential to enabling reproducible detection of PK changes in HDI research.

Mechanistic Insights & Evidence in Herb–Drug Interactions

a. Enzyme and Transporter Modulation:

Herbal ingredients tend to interact with metabolic processes:

- **Grape fruit juice** rich in furanocoumarins, inhibits CYP3A4 in the gut and liver, increasing plasma concentrations of drugs such as calcium-channel blockers, and possibly affecting antihypertensive dosing and safety.(11,12)
- **Ginkgo biloba, green tea extract, sesamin, and piperine inhibit CYP3A4**—risk-ensuring enhanced exposure to CYP3A4-substrate antihypertensives.(8,12,13)
- **St. John's Wort** strongly induces CYP3A4 and P-gp, lowering blood levels of antihypertensive CYP3A4 substrates—e.g., verapamil and others.(12,14)

b. Protein Binding and PK Changes:

- Concurrent–MS/MS experiment evaluated benazepril, gliclazide, and valsartan to determine protein-binding interference by rhein, illustrating the capacity of multiplex techniques to unravel protein-binding-mediated interactions.(15)
- Herbs like Licorice, which are glycyrrhizin-rich, are associated with hypokalemia and arrhythmias when used in combination with diuretics—implying intricate drug

pharmacodynamic interactions with the need for concurrent drug monitoring.(16)

c. Pharmacokinetic Evidence—Preclinical to Clinical:

Lepidium sativum and *Curcuma longa* markedly increased the C_{max} (by 83% and 53%, respectively) and AUC of amlodipine in hypertensive rodents, indicating that enzyme inhibition or changed distribution may be the underlying processes.(10)

Challenges, Limitations & Future Directions

a. Current Challenges

- **Herbal variability:** Herbal product composition varies non-uniformly, making reproducibility and extrapolation difficult(17)
- **Limited clinical verification:** Most studies are still preclinical, which prevents their use in the real world.(18)
- **Analytical requirements:** Balance high sensitivity and multiplexing with throughput and low sample demands.(19)
- **Regulatory and Standardization Gaps:** There are no standard guidelines for herb–drug interaction bioanalytical method validation, as opposed to normal drug–drug interaction studies.(20)
- **Co-elution of Analytes:** Chemically related drugs and metabolites can co-elute in a chromatographic run, causing inaccurate quantitation.(21)
- **Stability Problems:** Some antihypertensive medications and herbal components degrade during sample preparation, storage, or analysis, affecting reliability.(22)
- **Metabolite Interference:** Active or inactive metabolites of antihypertensive drugs can coincide with parent drug peaks, and this interferes with selectivity in concurrent assays.(23)

b. Emerging Directions

- **AI-based decision support systems hold promise:** One architecture uses machine learning to signal potential HDIs, supporting clinicians(24)
- **Knowledge graph models further** hold out promise of predictive insights for the identification of novel HDIs
- **Clinical trials** involving standardized plant extracts and internally validated analytical

techniques are essential to bridge the translational gap.(25)

- **PBPK and Modeling:** Integrate in vitro, in vivo, and analytical information into predictive HDI modeling.(26)
- **HRMS (DIA/PRM) Platforms:** Allow for untargeted screening of drugs and herb constituents in addition to quantitation.(27)
- **Cross-Lab Harmonization:** Validation of ICH-M10 compliant assays across centers for pooling HDI datasets.(28)
- **Automated SPE Workflows:** Enhance throughput and reproducibility for herb-rich matrices.

Application of Simultaneous Quantitation in HDI Research

These validated bioanalytical methods are crucial for HDI studies:

- An approach integrating MET, AML, CAN, and HCT quantitation proved practical application in the assessment of non-adherence and PK deviations in refractory hypertension patients.(8,9)
- Short-term, multiplex assays such as UPLC-MS/MS enhance throughput—suited to clinical PK studies and high-throughput HDI screening(29)
- Preclinical animal models greatly gain from multiplexed assays (e.g., amlodipine/valsartan/HCT), facilitating thorough PK profiling when herbs are co-administered(8,9)

II. CONCLUSION

Herb–drug interactions with antihypertensive drugs present a significant risk through pharmacokinetic and pharmacodynamic changes. Concomitant quantitation methods, particularly LC-MS/MS approaches, are primary tools for explaining such interactions. Although preclinical data highlight the risk of changed drug exposure, more emphasis on standardization, clinical trials, and integrative decision-making support is necessary. Further development toward individualized HDI risk assessment will provide improved and safer cardiovascular patient care.

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