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Recent Advances in Analytical Method Development and Validation for Simultaneous Estimation of Amlodipine and Fimasartan: A Comprehensive Review

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Abstract: ***Hypertension is one of the main causes of heart-related health problems and deaths worldwide. Fixed-dose combinations of Fimasartan, which is an angiotensin II receptor blocker, and Amlodipine, a calcium channel blocker, have become important in clinical practice due to their better effectiveness in lowering blood pressure and improving patient adherence. Reliable analytical methods are crucial for quality control, stability testing, and meeting regulatory standards for these drug formulations. This review outlines the analytical methods used for estimating Fimasartan and Amlodipine together, including UV spectrophotometry, RP-HPLC, UPLC, HPTLC, and LC-MS/MS. The focus is on developing chromatographic methods, optimizing mobile phases, and validating procedures according to ICH guidelines. It also covers stability-indicating analytical methods. New concepts like Analytical Quality by Design, green analytical chemistry, and tools for assessing environmental impact are discussed as well. The review highlights current gaps in research and future chances to create eco-friendly, quick, and effective analytical methods for this blood pressure treatment combination.***

Keywords: Fimasartan, Amlodipine, RP-HPLC, Method Development, Method Validation, Stability-Indicating Method, Green Analytical Chemistry, AQbD

1. Introduction

Hypertension is one of the most prevalent chronic non-communicable diseases worldwide and remains a major public health challenge. It is a significant risk factor for cardiovascular diseases, including stroke, myocardial infarction, heart failure, and chronic kidney disease. The increasing prevalence of hypertension is associated with population aging, sedentary lifestyle, obesity, unhealthy dietary habits, excessive salt intake, smoking, alcohol consumption, and genetic predisposition. Despite the availability of several antihypertensive drugs, blood pressure remains inadequately controlled in many patients, increasing the risk of cardiovascular morbidity and mortality.[1] [2] (World Health Organization, 2023; Whelton et al., 2018)

Combination therapy has become the preferred treatment strategy for patients with moderate-to-severe hypertension because it provides superior blood pressure reduction compared with monotherapy while minimizing dose-related adverse effects. Fixed-dose combinations improve patient compliance, reduce pill burden, and enhance long-term treatment outcomes. The combination of Amlodipine and Fimasartan has demonstrated significant clinical efficacy due to the complementary mechanisms of action of the two drugs.[3] [4] (Williams et al., 2018; Lee et al., 2015)

Amlodipine is a third-generation dihydropyridine calcium channel blocker that inhibits the influx of calcium ions through L-type calcium channels in vascular smooth muscle, resulting in peripheral vasodilation and sustained reduction of blood pressure. Because of its long elimination half-life, once-daily dosing, and excellent safety profile, amlodipine is widely prescribed for hypertension, chronic stable angina, and vasospastic angina.[5] [6] (Meredith & Elliott, 2004; Burnier, 2019)

Fimasartan is a newer angiotensin II receptor blocker (ARB) developed for the treatment of essential hypertension. It selectively blocks the angiotensin II type-1 (AT_1) receptor, thereby inhibiting vasoconstriction, aldosterone secretion, sodium retention, and cardiovascular remodeling mediated through the renin–angiotensin–aldosterone system. Clinical studies have demonstrated that fimasartan possesses potent antihypertensive activity, favorable pharmacokinetics, and excellent tolerability when used either alone or in combination with amlodipine.[7] [8] (Lee and Oh, 2016; Kim et al., 2015)

The increasing commercialization of fixed-dose combination formulations has created a demand for reliable analytical methods capable of simultaneously estimating amlodipine and fimasartan in bulk drugs and pharmaceutical dosage forms. RP-HPLC is the most widely employed analytical technique because of its excellent sensitivity, specificity, precision, and reproducibility. Recent advances in analytical chemistry have also focused on stability-indicating methods, green analytical chemistry, and Quality-by-Design approaches to improve method robustness while reducing environmental impact.[9] [10] (ICH Q2(R2), 2023; Snyder et al., 2010)

Clinical Importance of the Combination

Amlodipine and fimasartan work through two different but complementary pathways. Amlodipine relaxes blood vessels by blocking calcium entry, while fimasartan works by suppressing the renin–angiotensin–aldosterone system. When used together, these mechanisms provide stronger and more stable blood pressure control than either drug alone. This combination is also beneficial in reducing side effects, such as swelling in the legs caused by amlodipine, since fimasartan helps balance fluid pressure within blood vessels. Because of these advantages, the combination is widely considered effective for long-term improve patient adherence.

Comparative Drug Profile of Amlodipine and Fimasartan

Parameter	Amlodipine	Fimasartan
Drug Class	Dihydropyridine calcium channel blocker	Angiotensin II receptor blocker (ARB)
Mechanism of Action	Blocks L-type calcium channels in vascular smooth muscle, leading to vasodilation and reduced peripheral resistance	Selectively blocks AT_1 receptors of angiotensin II, preventing vasoconstriction, aldosterone release, and sodium retention
Primary Effect	Peripheral vasodilation and reduction in blood pressure	Inhibition of renin–angiotensin–aldosterone system (RAAS) leading to vasodilation and fluid balance regulation
Onset of Action	Gradual onset	Relatively faster onset
Peak Plasma Time (Tmax)	6–12 hours	0.5–3 hours
Bioavailability	~60–80%	Low to moderate (due to first-pass metabolism)
Protein Binding	~93–98%	High (~95%)
Metabolism	Hepatic via CYP3A4	Hepatic metabolism (limited CYP involvement)
Elimination Route	Urine (as inactive metabolites)	Urine and bile
Half-Life	30–50 hours	9–16 hours
Dosing Frequency	Once daily	Once daily
Primary Uses	Hypertension, chronic stable angina, vasospastic angina	Essential hypertension
Major Advantage	Long duration of action with stable 24-hour BP control	Strong RAAS inhibition with good tolerability
Common Adverse Effects	Peripheral edema, headache, flushing	Dizziness, hypotension (less common cough vs ACE inhibitors)
Special Benefit	Smooth BP reduction with minimal reflex tachycardia	No bradykinin-related cough, good vascular protection
Role in Combination Therapy	Provides potent vasodilation	Enhances BP control and reduces CCB-induced edema

Analytical Method Development

Method development for simultaneous estimation begins with understanding drug properties such as solubility, pKa, and UV absorption. RP-HPLC is most widely used.

- UV–Visible Spectrophotometry: Simple, economical, and suitable for routine analysis, although limited by overlapping absorption spectra.
- Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC): The most widely employed technique because it offers high sensitivity, excellent selectivity, and reproducible separation.
- High-Performance Thin-Layer Chromatography (HPTLC): Useful for rapid screening and simultaneous analysis of multiple samples.
- Ultra-High-Performance Liquid Chromatography (UHPLC): Provides shorter run times, improved efficiency, and reduced solvent consumption.
- Liquid Chromatography–Mass Spectrometry (LC–MS/MS): Primarily used for bioanalytical applications requiring very high sensitivity and specificity.

Among these techniques, RP-HPLC remains the preferred method for simultaneous estimation of amlodipine and fimasartan in pharmaceutical dosage forms due to its robustness, precision, and widespread regulatory acceptance.

Selection of Chromatographic Column

The stationary phase is one of the most important factors affecting chromatographic separation.

Commonly used columns include:

- C18 (octadecylsilane)
- C8
- Phenyl columns

Mobile Phase Optimization

Mobile phase composition plays a crucial role in achieving satisfactory chromatographic performance.

Frequently used components include:

- Acetonitrile
- Methanol
- Phosphate buffer
- Ammonium acetate buffer

Buffer Selection and pH Optimization

Buffer systems help maintain consistent pH during chromatographic analysis, thereby improving peak shape and reproducibility.

Parameters to optimize include:

- Buffer type
- Buffer concentration
- Mobile phase pH

Detection Wavelength Selection

The selected wavelength should:

- Maximize detector response.
- Minimize baseline noise.
- Permit simultaneous quantification.
- Ensure acceptable sensitivity.

Flow Rate Optimization.

- Retention time
- Resolution
- Peak width
- System pressure
- Total analysis time

Sample Preparation

- Accurate weighing of standards and samples.
- Dissolution in a suitable solvent.
- Sonication to ensure complete extraction.
- Filtration through an appropriate membrane filter.
- Dilution to the required concentration.

System Suitability Testing:

- Retention time
- Resolution

- Tailing factor
- Number of theoretical plates
- Repeatability of peak area

Method validation:
Analytical method validation is an essential part of pharmaceutical analysis, as it confirms that a developed method is reliable, consistent, and suitable for its intended application. For the simultaneous estimation of amlodipine and fimasartan, validation ensures that both drugs can be accurately measured in bulk materials and pharmaceutical formulations without compromising data quality. The validation process is carried out according to the recommendations of the International Council for Harmonisation (ICH Q2(R2), 2023), which defines the key parameters required to demonstrate method performance.

Specificity
Specificity describes the ability of an analytical method to accurately measure the target drugs without interference from excipients, impurities, degradation products, or other components present in the formulation. In RP-HPLC analysis, clear separation of amlodipine and fimasartan with well-defined peaks confirms that the method is specific. This characteristic is particularly important for stability studies, where degradation products may be formed during storage or stress testing.

Linearity and Range
Linearity indicates how well the analytical response changes with increasing drug concentration. It is assessed by preparing a series of standard solutions and plotting the detector response against concentration. A method is considered linear when the calibration curve shows a strong correlation over the selected concentration range. The working range should be broad enough to cover the concentrations commonly encountered during routine quality control.

Accuracy
Accuracy reflects how closely the measured value matches the actual amount of drug present in the sample. It is usually evaluated by recovery studies, in which known quantities of amlodipine and fimasartan are added to pre-analyzed samples. Recovery values within the acceptable limits indicate that the method can accurately determine the drug content without significant error.

Precision
Precision measures the consistency of analytical results when the same sample is analyzed repeatedly. It includes repeatability, which evaluates results under identical conditions, and intermediate precision, which examines the effect of different analysts, instruments, or days of analysis. The results are generally expressed as the percentage relative standard deviation (%RSD), where lower values indicate better precision and method reproducibility.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The limit of detection (LOD) is the smallest amount of analyte that can be detected by the method, while the limit of quantification (LOQ) is the lowest concentration that can be measured accurately and precisely. These parameters reflect the sensitivity of the analytical method and are especially useful during stability studies and trace-level analysis.

Robustness
Robustness evaluates the ability of the method to produce consistent results even when small, intentional changes are made to analytical conditions. Minor variations in factors such as mobile phase composition, flow rate, pH, column temperature, or detection wavelength should not significantly affect the performance of a well-developed method. A robust analytical procedure ensures dependable results during routine laboratory operation.

Ruggedness
Ruggedness refers to the reproducibility of the analytical method under normal laboratory conditions. It is assessed by performing the analysis using different analysts, instruments, or laboratories. Consistent results under these varying conditions demonstrate that the method is reliable and suitable for routine use.

System Suitability
System suitability testing is performed before sample analysis to confirm that the chromatographic system is functioning properly. Parameters such as retention time, peak symmetry, theoretical plates, resolution, and repeatability are evaluated to ensure that the system is capable of generating accurate and reliable chromatographic data.

Stability-Indicating Capability
A stability-indicating method is designed to accurately quantify the active drugs even when degradation products are present. This is verified through forced degradation studies carried out under acidic, alkaline, oxidative, thermal, and photolytic conditions. Successful separation of degradation products from the drug peaks confirms that the method is suitable for stability testing and routine quality assessment.

2. Green Analytical Chemistry

1) Introduction
Green Analytical Chemistry (GAC) is an approach that aims to make analytical methods more environmentally friendly. It mainly focuses on reducing the use of harmful chemicals, cutting down waste, and saving energy, while still ensuring that the method remains accurate and reliable. In pharmaceutical analysis, GAC helps in developing safer and more sustainable methods without affecting the quality of results.

2) Principles of Green Analytical Chemistry
The basic idea of Green Analytical Chemistry is to make laboratory work safer and more sustainable. This is achieved by using smaller amounts of solvents and reagents, choosing less toxic chemicals, reducing waste production, simplifying sample preparation, shortening analysis time, and improving overall energy efficiency.

3) Green Analytical Procedure Index (GAPI)
The Green Analytical Procedure Index (GAPI) is a simple visual tool used to check how eco-friendly an analytical method is. It evaluates the whole analytical process and represents it using a colour-coded diagram. Green shows low

environmental impact, yellow indicates moderate impact, and red highlights areas that need improvement.

4) Analytical GREENNES Metric (AGREE)

AGREE is a modern and user-friendly tool that measures how green an analytical method is based on the twelve principles of Green Analytical Chemistry. It gives a score between 0 and 1, where values closer to 1 indicate a more environmentally friendly method. The results are shown in an easy-to-understand circular diagram.

Analytical Eco-Scale

The Analytical Eco-Scale is another tool used to evaluate the greenness of a method. It works by giving penalty points for factors like toxic chemicals, high energy use, and waste generation. The final score is out of 100, and a higher score means the method is more environmentally friendly.

Comparison of Green Assessment Tools

Tool	Assessment	Output	Main Advantage
GAPI	Entire analytical process	Colour pictogram	Easy visual understanding of method greenness
AGREE	Twelve GAC principles	Score (0–1)	Detailed and quantitative evaluation
Analytical Eco-Scale	Penalty-based system	Score out of 100	Simple and quick comparison of methods

Significance in Pharmaceutical Analysis

Green analytical tools are now widely used in method development to reduce environmental impact while maintaining good analytical performance. In RP-HPLC methods for the simultaneous estimation of amlodipine and fimasartan, these tools help in designing more sustainable, efficient, and regulatory-friendly analytical procedures.

3. Challenges and Future Perspectives

3.1 Challenges

Even though many analytical methods have been developed for the simultaneous estimation of amlodipine and fimasartan, there are still a few practical challenges. One of the main difficulties comes from the differences in their chemical nature. Since both drugs behave differently in terms of polarity, solubility, and concentration levels in formulations, it can be tricky to achieve proper separation and sharp, well-resolved peaks in a single run.

Another important challenge is developing methods that can clearly distinguish the drugs from their degradation products. During stress conditions like heat, light, acid, base, or oxidation, both drugs may break down into different compounds. Ensuring that these degradation products do not interfere with the main drug peaks requires careful optimization of chromatographic conditions.

In addition, many conventional RP-HPLC methods still rely on large volumes of organic solvents, which increases cost and raises environmental concerns. Meeting modern regulatory expectations, especially ICH Q2(R2), also requires methods to be not only accurate and precise but also robust enough to perform consistently under small variations in conditions.

3.2 Future Perspectives

In the future, analytical research for this drug combination is expected to move toward simpler, faster, and more environmentally friendly approaches. There is a growing interest in reducing solvent usage, shortening run times, and adopting greener alternatives in routine analysis.

The use of Analytical Quality by Design (AQbD) is also expected to become more common. This approach helps in systematically understanding method variables and developing more reliable and robust analytical procedures from the beginning.

Advanced techniques like UHPLC and LC–MS/MS may further improve speed, sensitivity, and selectivity, especially for complex stability studies. At the same time, digital tools such as artificial intelligence and machine learning are likely to make method development faster and more efficient by helping optimize chromatographic conditions.

Overall, future analytical methods for amlodipine and fimasartan will likely focus on achieving a better balance between performance, sustainability, and regulatory compliance, making them more suitable for modern pharmaceutical quality control needs.

4. Research Gaps

Even though different analytical methods are available for estimating antihypertensive drugs, the amlodipine and fimasartan combination is still not explored in detail compared to many other drug combinations. Only a limited number of studies are available, and most of them focus on routine analysis rather than deeper method development.

Environmental aspects are also not well addressed in many existing methods. The idea of green analytical chemistry is still not widely applied, and many methods continue to use higher amounts of organic solvents and longer run times. Also, tools like AGREE and GAPI, which help measure how eco-friendly a method is, are rarely used in published work for this drug combination.

In addition, there is limited detailed work on stability-indicating methods combined with advanced techniques like LC–MS/MS. Most studies mainly focus on simple quantification, while deeper understanding of degradation behavior and pathways is still missing.

5. Future Analytical Trends

Analytical methods in pharmaceutical science are changing quickly, and the focus is now shifting toward making them faster, cleaner, and more intelligent. For the analysis of amlodipine and fimasartan, future developments are expected to improve both efficiency and environmental safety.

One clear trend is the move toward greener analytical practices. This means using safer solvents, reducing chemical waste, and designing methods that take less time to complete while still giving reliable results.

Advanced techniques like UHPLC and LC–MS/MS are also becoming more common. These systems offer better sensitivity and faster analysis, which is especially useful for detecting small changes, impurities, and degradation products.

At the same time, automation and artificial intelligence (AI) are starting to play a bigger role. These tools can help optimize method conditions more quickly and reduce the need for repeated experiments, saving both time and resources.

Another important shift is toward continuous or lifecycle-based validation, where methods are not just validated once but monitored and improved throughout their use. This approach matches modern regulatory expectations and helps maintain consistent quality over time.

6. Conclusion

The simultaneous estimation of amlodipine and fimasartan is important for maintaining the quality and consistency of combination products used in hypertension treatment. Over time, several analytical methods- especially RP-HPLC-based techniques- have been developed and proven to be reliable for routine analysis as well as stability studies.

In recent years, analytical research has moved toward more advanced and thoughtful approaches. Concepts like and green analytical chemistry have made methods more systematic, efficient, and environmentally friendly. These improvements help in designing analytical procedures that are not only accurate but also more robust and sustainable.

Forced degradation studies also play a key role by showing how well the method performs under stress conditions. They ensure that both drugs can still be properly measured even when degradation products are present. Along with this, tools like GAPI, AGREE, and Eco-Scale are now being used to check how eco-friendly an analytical method is, reflecting the growing importance of sustainability in pharmaceutical analysis.

Overall, analytical methods for amlodipine and fimasartan are steadily evolving toward being faster, greener, and more reliable. Future developments are expected to further improve their efficiency, environmental safety, and scientific robustness, supporting better quality control in pharmaceutical industries.

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