Microchemical Journal 218 (2025) 115616

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Review Article

Evaluation of green and practical analytical techniques for the determination of the antihypertensive drugs amlodipine and valsartan

Shaikh Manirul Haque [a,*], Abuzar Kabir [b], Mohd. Rafatullah [c], Zakariya Sadique [a], Adrián Fuente-Ballesteros [d,*]

[a] Department of Chemical Engineering, Jubail Industrial College, Jubail Industrial City 31961, Saudi Arabia
[b] Global Forensic and Justice Center, Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th Street, Miami, FL 33196, USA
[c] University Center for Research and Development, Chandigarh University, Mohali, Punjab 140413, India
[d] Analytical Chemistry Group (TESEA), I. U. CINQUIMA, Faculty of Sciences, University of Valladolid, Valladolid 47011, Spain

ARTICLE INFO

Keywords:
Amlodipine
Valsartan
Green analytical chemistry
Analytical techniques
Analytical eco-scale
AGREE
BAGI

ABSTRACT

The development of sustainable pharmaceutical analysis is essential to ensure drug safety, efficacy, and environmental responsibility. Amlodipine (AML) and valsartan (VAL) are widely prescribed antihypertensive agents often used in combination due to their synergistic effects on cardiovascular and renal health. This review presents a comparison of recent analytical methodologies developed for the quantification of AML and VAL in active pharmaceutical ingredients, dosage forms, and biological fluids. The techniques evaluated include ultraviolet-visible (UV–Vis) spectrophotometry, spectrofluorimetry, high-performance liquid chromatography (HPLC), micellar electrokinetic chromatography (MEKC), and ultra-high-performance liquid chromatography (UHPLC). Each method is critically assessed in terms of analytical performance and environmental sustainability, following the twelve principles of green analytical chemistry (GAC). To evaluate the environmental impact and practical applicability of the methods, three complementary assessment tools were applied: the analytical Eco-Scale, the analytical GREEnness metric (AGREE), and the blue applicability grade index (BAGI). The results indicate that while all reviewed techniques are capable of accurately quantifying AML and VAL, simpler methods such as UV–Vis and MEKC show higher green scores, whereas spectrofluorimetry and UHPLC offer greater sensitivity and speed. This work aims to guide researchers in selecting or developing analytical methods that balance performance, sustainability, and practical implementation in pharmaceutical analysis.

1. Introduction

Hypertension is considered one of the most prevalent and dangerous risk factors for cardiovascular diseases globally. It significantly increases the probability of developing ischemic stroke, primarily due to reduced blood flow to the brain. In addition, haemorrhagic strokes caused by blood vessel rupture and internal bleeding in the brain are also frequently associated with uncontrolled blood pressure [1]. Beyond cerebrovascular complications, hypertension is strongly linked to other chronic conditions, such as kidney disease, congestive heart failure, chest pain, visual impairments, and erectile dysfunction. Addressing this health challenge requires a comprehensive strategy, including lifestyle modifications, such as improved dietary habits, increased physical activity, and reduction of tobacco and alcohol consumption, as well as pharmacological intervention. Despite preventive measures, a large proportion of patients require drug therapy to manage their blood

Abbreviations: AGREE, analytical GREEnness metric; AML, amlodipine; APIs, active pharmaceutical ingredients; BAGI, blue applicability grade index; C_{18}, octadecylsilane; C_8, octylsilane; CC, column chromatography; CEC, capillary electrochromatography; ComplexMoGAPI, complex modified GAPI; CZE, capillary zone electrophoresis; GAC, green analytical chemistry; GC, gas chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MEKC, micellar electrokinetic chromatography; PPs, penalty points; PLS, partial least squares; RAPI, red analytical performance index; SDS, sodium dodecyl sulfate; STABLE, stability toolkit for the appraisal of bio/pharmaceuticals' level of endurance; TLC, thin-layer chromatography; UHPLC, ultra-high-performance liquid chromatography; UV–Vis, ultraviolet-visible spectrophotometry; VAL, valsartan; VIGI, violet innovation grade index; WAC, white analytical chemistry..

* Corresponding authors.
E-mail addresses: haque_m@rcjy.edu.sa (S.M. Haque), adrian.fuente.ballesteros@uva.es (A. Fuente-Ballesteros).

https://doi.org/10.1016/j.microc.2025.115616
Received 2 August 2025; Received in revised form 29 September 2025; Accepted 1 October 2025
Available online 8 October 2025

pressure effectively. In many cases, a single therapeutic agent is insufficient to achieve desired blood pressure targets, prompting the use of fixed-dose combinations that offer complementary mechanisms of action. Among the most prescribed combinations are amlodipine besylate (AML) and valsartan (VAL), which are widely used due to their synergistic antihypertensive effects and ability to reduce adverse events when compared to monotherapies [2]. This co-administration strategy has shown considerable effectiveness in improving therapeutic outcomes in patients with moderate to severe hypertension [3].

AML, chemically described as benzenesulfonic acid; 3-O-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, is a dihydropyridine calcium channel blocker approved by the Food and Drug Administration in 1987. Its primary mode of action is the inhibition of calcium influx into vascular smooth muscle cells and cardiac muscle fibers, leading to vasodilation, reduced peripheral resistance, and improved blood flow. AML also exhibits antioxidant properties, enhances nitric oxide production, and is considered vasoactive, contributing to its efficacy in blood pressure control [4]. VAL, on the other hand, is an angiotensin II receptor blocker with the chemical name 3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid. It acts by inhibiting the binding of angiotensin II to the AT1 receptor, leading to vasodilation and reduced secretion of vasopressin and aldosterone. This mechanism contributes to decreased blood pressure and improved renal and cardiovascular outcomes [5]. The combination of AML and VAL, therefore, represents a rational therapeutic approach to managing hypertension and related cardiovascular complications.

The development of pharmaceutical products is a complex and multistage process requiring strict compliance with quality standards and regulatory guidelines. Ensuring the chemical and physical stability of active pharmaceutical ingredients (APIs), as well as the quality of excipients and final dosage forms, is essential to guarantee efficacy and safety. Impurities generated during manufacturing, whether from residual solvents, degradation products, or other process-related contaminants, must be identified and kept within regulatory limits to prevent adverse effects [6–11]. As such, robust and reliable analytical techniques are critical throughout the drug development and quality control processes.

Due to the clinical relevance of AML and VAL, a wide range of analytical methods have been developed to quantify these drugs in bulk substances, pharmaceutical formulations, and biological matrices. As illustrated in Fig. 1, there has been a notable increase in publications focusing on AML and VAL quantification over the past decade. These methods address not only the drugs themselves but also their combinations, related impurities, degradation products, and metabolites, as well as their presence in different pharmaceutical forms and biological fluids such as plasma and urine [12–14]. Furthermore, some of these analytical approaches are specifically designed to detect genotoxic impurities, which are critical for ensuring drug safety and compliance with international regulatory guidelines. Fig. 2 summarizes the most frequently used analytical techniques employed to quantify AML and VAL from 2015 to 2024. The data highlight the wide variety of methodologies, including spectrophotometric and chromatographic techniques, which are currently applied in pharmaceutical quality control and research laboratories.

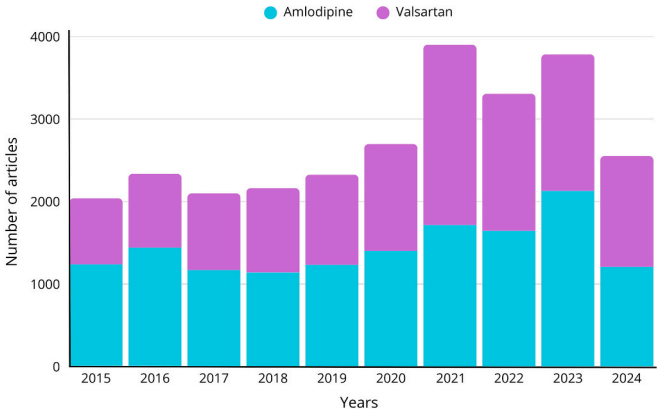


Fig. 1. The number of publications per year (2015–2024) to quantify AML and VAL.

In recent years, there has been a growing awareness of the environmental impact of chemical and analytical practices. The field of green chemistry, introduced in the 1990s, advocates for the design of chemical processes that minimize the use and generation of hazardous substances, while maintaining or enhancing scientific performance [15]. Green analytical chemistry (GAC) [16], as a sub-discipline, focuses on developing analytical procedures that are safer, more sustainable, and more energy-efficient. The implementation of GAC principles is especially important in pharmaceutical analysis, which traditionally relies on large volumes of organic solvents, energy-intensive instruments, and multistep sample preparation protocols. The push for sustainability in pharmaceutical research has encouraged the development of novel methods that minimize environmental impact. Researchers are increasingly adopting greener solvents, biodegradable reagents, reduced sample sizes, and more energy-efficient instrumentation [17–19]. Additionally, tools like analytical quality by design, Taguchi models, and response surface methodology are now commonly applied to optimize analytical procedures within the framework of white analytical chemistry (WAC); a concept that balances analytical performance, greenness, and practicality [20]. To evaluate the environmental performance of analytical methods, several assessment tools have been developed [21]. Some of these include the analytical Eco-Scale [22], the analytical GREEnness metric (AGREE) [23], and complex modified GAPI (ComplexMoGAPI) [24]. Other complementary tools for evaluating different parameters, such as the blue applicability grade index (BAGI) [25], red analytical performance index (RAPI) [26], violet innovation grade index (VIGI) [27], and the stability toolkit for the appraisal of bio/pharmaceuticals' level of endurance (STABLE) [28], have also been introduced. Recently, a multi-color assessment platform (MA tool) for WAC was proposed [29]. Moreover, emerging approaches such as biosensor-based detection platforms [30] and artificial intelligence-based works with chemometrics [31–35] are gaining attention and may further strengthen the future readiness of pharmaceutical analysis.

Despite the therapeutic relevance and widespread use of AML and VAL, no comprehensive review has been published to date that jointly examines the analytical methodologies for their quantification, particularly from a green chemistry perspective. This gap is especially relevant given the recent proliferation of small and mid-sized pharmaceutical manufacturers, especially in developing countries, where the risk of producing substandard or non-compliant formulations has become a concern. Some of these products may fail to meet regulatory specifications for content uniformity or impurity levels due to inadequate analytical testing. Consequently, there is a pressing need to identify and promote analytical procedures that are not only accurate and precise, but also environmentally friendly, safe, and practical for routine application. To address this need, the present review provides a critical overview of the most relevant analytical techniques used to determine AML and VAL in APIs, pharmaceutical formulations, and biological samples over the past ten years. In addition to reviewing conventional analytical performance metrics, this work incorporates a comparative assessment of each method's greenness and practical applicability, based on the Analytical Eco-Scale, AGREE, and BAGI. The aim is to offer researchers, analysts, and quality control professionals a consolidated resource that supports the development and selection of analytical methodologies aligned with the principles of WAC.

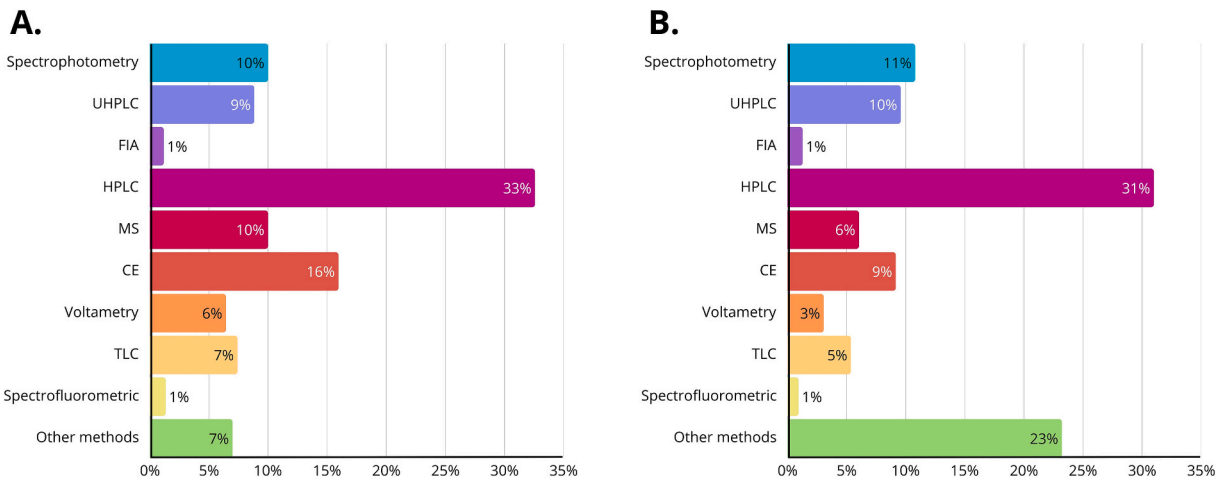


Fig. 2. The contribution of different techniques for determining AML (**A**) and VAL (**B**) across years (2015–2024).

2. Quantification analytical techniques for amlodipine and valsartan

Several analytical techniques with diverse experimental configurations have been developed for the quantitative determination of AML and valsartan VAL, either individually or in combination with other drugs. However, to date, no comprehensive review has been published that compiles and critically discusses these methodologies within a unified framework, an essential task considering the therapeutic relevance of both compounds. The literature search was conducted using Scopus, Web of Science, ScienceDirect, and Google Scholar as primary databases. The following keyword combinations were applied: "amlodipine" OR "valsartan" AND "analytical methods" OR "analytical techniques" OR "green analytical chemistry" AND "sustainability". Only peer-reviewed articles published in English were considered. Each article was carefully screened, and only those directly relevant to the objectives of this review were included. This review focuses on a selection of recent analytical methods developed for the quantification of AML and VAL in APIs, pharmaceutical dosage forms, and biological fluids (see **Tables 1** and **2**). In the following sections, the main detection techniques employed are described and evaluated in terms of their analytical performance and suitability for sustainable pharmaceutical analysis.

2.1. Ultra-violet and visible spectrophotometry

Spectroscopic techniques are widely applied for the quantification of drugs in pharmaceutical dosage forms. Among them, ultraviolet and visible (UV–Vis) spectrophotometry is among the most commonly used methods for quantitative analysis (see Fig. 3). UV spectrophotometry is a well-established and reliable tool for both qualitative and quantitative studies. These methods are typically simple, fast, cost-effective, and require little to no sample pre-treatment prior to analysis [36–43]. Despite these advantages, UV spectrophotometry also has limitations, particularly its inability to accurately quantify multiple compounds with overlapping absorption spectra, especially in the presence of excipients or degradation products. In contrast, visible spectrophotometry is based on chemical reactions such as complex formation, redox reactions, or catalytic processes, which lead to the formation of coloured species. The absorbance of these coloured products is then measured to quantify the analyte. Since most drug substances are colourless, they must first react with appropriate reagents under suitable conditions to generate detectable coloured compounds [44–47]. Partial least squares (PLS) regression offers significant advantages in spectrophotometric analysis, particularly in resolving complex mixtures. It enables the use of the full spectral data for the rapid quantification of target compounds, often eliminating the need for prior separation. A key benefit of PLS is that calibration can be performed by focusing solely on the analyte of interest, without requiring knowledge of the concentrations of interfering components. In addition, derivative spectrophotometry proves highly effective for extracting both qualitative and quantitative information from overlapping spectral bands, making it a valuable tool when analytes cannot be completely resolved using conventional absorbance measurements. As illustrated in Fig. 2, approximately 11 % of the studies on AML and VAL quantification employed spectrophotometric techniques. UV–Vis spectrophotometry, in particular, has been successfully applied to the quantification (**Tables 1** and **2**) of AML [48–82] and VAL [41,54,83–106], offering wide linearity ranges along with high precision, accuracy, and robustness.

2.2. High-performance liquid chromatography

Chromatographic techniques are widely employed in pharmaceutical analysis due to their versatility and capacity for high-resolution separations. Various approaches such as thin-layer chromatography (TLC), column chromatography (CC), gas chromatography (GC), high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), and capillary electrochromatography (CEC) are applied depending on the physicochemical nature of the analyte and the analytical goal. Among these, HPLC remains one of the most frequently used techniques in both pharmaceutical and biomedical fields.

In recent years, HPLC has gained increasing attention for the analysis of active pharmaceutical ingredients (APIs), finished pharmaceutical formulations, and biological fluids such as urine and plasma [107–110]. The technique is favored for its operational simplicity, high repeatability, and excellent reproducibility. Most of the reported methods for the quantification of AML [51,60–71] and VAL [41,60,61,89–94] have employed reversed-phase columns based on octylsilane (C_8) or octadecylsilane (C_{18}), with varying dimensions and particle sizes. Both isocratic and gradient elution modes have been used (see **Tables 1** and **2**), with flow rates and column temperatures optimized according to the specific detection wavelengths. Mobile phases typically consist of single solvents or mixtures of polar solvents, including buffers at various pH levels and organic modifiers such as acetonitrile, methanol, and ethanol. The proportion and composition of the mobile phase are crucial for achieving optimal separation, as reflected in parameters like theoretical plate number and resolution. Detection is generally performed using UV, photodiode array, or fluorescence detectors, depending on the sensitivity and selectivity required. Most HPLC procedures for AML and VAL quantification employ acidic buffer solutions and flow rates in the range of 0.8–1.1 mL/min for AML and 0.8–1.2 mL/min for VAL. The methods

Table 1
Analytical techniques applied to quantify AML in API, pharmaceutical formulations and biological fluids.

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
Tablets	UV spectrophotometric	The double divisor ratio spectra method (DDR) selected two-wavelength at 373.4, 288.8 nm and the ratio first derivative method (RFD) at 353.8 nm in pharmaceutical dosage forms. The UV proves that the software is used with a 1 cm cuvette and 2 nm opening breath for the determination	1–10 1–10	0.151 0.237	0.454 0.782	[48]
API, Pharmaceutical Formulations	UV–Vis spectrophotometric	AML is mixed with the 2,6-dihydroxybenzoic acid solution, followed by a para-dimethylamino-benzaldehyde solution. The absorbance of the yellow-coloured complex was measured at 402 nm against the reagent blank without the presence of the drug	2–40	0.969	3.228	[49]
API, Pharmaceutical Formulations	UV spectrophotometric	AML was detected at 365 nm. Methanol is used as a solvent for pure drugs and formulations	2–10	0.145	0.152	[50]
API and synthetic mixtures	UV–Vis spectrophotometric	Methods including factorized spectrum (Method I) and dual amplitude difference combined absorbance subtraction (Method II) were measured at 360 and 331 nm, respectively	5–35	0.49 0.15	1.66 0.49	[51]
Quality control samples and pharmaceutical formulations	UV spectrophotometric	The determination included the absorption subtraction method (Method I), which selected two wavelengths at maximum (365 nm) and at isoabsorptive point (237 nm) based on the extended ratio subtraction method (Method II) at wavelength 239 nm; Dual wavelength method (Method III) at 246 and 259 nm; Method IV based on second order derivative spectrophotometry at 227 nm	2–25 2–25 2–25 2–25	0.454 0.390 0.382 0.520	1.373 1.182 1.158 1.577	[52]
Bulk and tablets	UV spectrophotometric	All the measurements were performed at a wavelength of 360 nm. Ethanol is used as a solvent	5–40	0.081	0.246	[53]
API, synthetic mixtures and pharmaceutical formulations	UV spectrophotometric	The calibration curve was constructed at wavelength 237.5 nm with the linearity range concentrations	4–40	NR	NR	[54]
Tablet formulations	UV–Vis spectrophotometric	The direct spectrophotometry method was detected at 365 nm	2–25	0.495	1.500	[55]
API and tablet formulation	UV–Vis spectrophotometric	Five different procedures were applied: dual-wavelength ratio, modified absorption factor, successive ratio-derivative, first derivative combined amplitude factor and modified amplitude center method. Methanol is used as a solvent for standard solution preparation	2–100 2–100 2–100 2–100 2–100	0.642 0.634 0.561 0.621 0.495	1.927 1.902 1.684 1.864 1.486	[56]
Pharmaceutical dosage forms	UV–Vis spectrophotometric	Acetonitrile was used as a solvent for the simultaneous quantification at wavelength 357 nm	5–65	1	3	[57]
Synthetic mixture and tablets	Chemometric-assisted spectrophotometric	Artificial neural network and least squares support vector machine were applied for determination	5–25	NR	NR	[58]
API and tablets	UV spectrophotometric	Ethanol-water (50 % *v*/v) was used as a solvent. The measurements were conducted at 371, 369.4 & 297.5 and 390.4 nm, respectively	1–9 1–9 1–9	0.262 0.316 0.270	0.781 0.948 0.810	[59]
API and synthetic mixtures	HPLC	The reversed-phase isocratic elution was performed with the Waters Spherisorb C_{18} (150 × 4.6 mm, 5 μm) column. The mobile phase combined the phosphate buffer (pH–2.5), acetonitrile and methanol with a 30, 65 and 5 (*v*/*v*/v) ratio with a controlled flow rate of 1.5 mL/min and detected a 240 nm	5–40	0.02	0.06	[51]
Rat plasma	HPLC	A gradient elution procedure containing mobile phase as 50 mM phosphate buffer (pH 3) and methanol. The separation was enhanced by the Zorbax Extend-C_{18} (4.6 × 250 mm, 5 μm) column. The mobile phase flow rate was 1 mL/min, maintaining the column temperature at 25 °C. The detection was performed at 210 nm	1–50	0.28	0.85	[60]
Pharmaceutical formulations	HPLC	The separation was performed using Waters X-Bridge Shield RP_{18} (100 × 4.6 mm, 3.5 μm) as a column with a temperature of 40 °C. The mobile phase comprised a mixture of formic acid (0.1 %) and ethanol (40:60, *v*/v). It maintained a flow rate of 1 mL/min, detected at 220 nm	150–450	0.06	0.20	[61]
Tablets	HPLC	The separation was speedily eluted in 5 min. An octylsilane (C_8) column with a dimension of 150 and 4.6 mm included 5 μm as particle size and column temperature of 40 °C used with a mobile phase ethanol: phosphate buffer (37:63, v/v) at a flow rate of 0.8 mL/min. All the measurements were performed at 254 nm	50–150	NR	NR	[62]
Pure and pharmaceutical formulations	HPLC	The isocratic separation was carried out with a (250 × 4.6 mm, 5.0 μm) column, which contains a mixture of phosphate buffer of pH -3 and acetonitrile (65:35, v/v) and controlled a flow rate of 1 mL/min with detection wavelength at 240 nm	1–100	0.094	0.313	[63]
Formulations	HPLC	The separation was enhanced by applying 20 mM phosphate buffer at pH 5.8 and acetonitrile with a ratio of 55 and 45. The elution was performed on a Zorbax C_{18} 150 column with a flow rate of 1.1 mL/min and 230 nm	5–50	0.11	0.33	[64]
API & formulations	HPLC	The mobile phase contains solution A, solution B and C as the mobile phase. Solution A is a phosphate buffer of pH–3, solution B is acetonitrile, and solution C is a mixture of acetonitrile and	1.4–9.7 0.2–4.3	0.19 0.06	0.57 0.17	[65]

(*continued on next page*)

Table 1 (*continued*)

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
		methanol with a ratio of 50:50, v/v. The first method applied solutions A and B, whereas the second solution was A and C. The 1st method used a Thermo hypersil column (250 × 4.6 mm, 5 μm) with a 1 mL/min flow rate and column temperature of 30 °C. However, the 2nd procedure included an ACE C_8 (150 × 4.6 mm, 5 μm) column. It maintained a column temperature of 40 °C with a 1.5 mL/min flow rate. Both methods applied detection wavelength at 237 nm				
Pharmaceutical drugs	HPLC	The column is selected as Shim-Pack GIST C18 with a dimension of 150 × 4.6 mm and a particle size of 5 μm. The mobile phase was a mixture of phosphate buffer (30 %) and acetonitrile (70 %) with a flow rate of 0.8 mL/min with a controlled column temperature of 40 °C and detection at 254 nm	0.01–0.5	0.002	0.006	[66]
Pharmaceutical dosage forms	HPLC	Eclipse plus C_{18} (250 × 4.6 mm, 5 μm) was used as the stationary phase to produce a higher resolution. The mobile phase A (ethanol) and B (phosphate buffer of pH–3.5) with gradient elution were used with a PDA detector at 233 nm for the detection with a controlled column temperature of 35 °C	1–6	0.12	0.37	[67]
API	HPLC	HPLC with core-shell C_{18} column dimensions of 100 × 4.6 mm and particle size of 2.6 μm. The gradient procedure enhanced higher separation within 15 min. The mobile phase included ammonium hydroxide in water (0.4 %) and methanol. It was detected at 237 nm with a 1 mL/min flow rate	0.1–2	NR	NR	[68]
Tablet	HPLC	The chromatographic separation was assessed with C_{18} (250 × 4.6 mm, 5 μm). Four variables were selected: flow rate, column temperature, methanol ratio, and pH. The wavelength was 215 nm and a 5 μL volume was injected throughout the analysis	0.8–2.4	0.0631	0.19	[69]
Rat plasma	HPLC	The chromatographic performance was conducted on Eclipse plus C_{18} (250 × 5 mm, 4.6 μm), including L1 packing. The mobile phase comprises methanol and acetate buffer (pH 4.5) with a ratio of 70:30 % v/v). The flow rate was maintained at 1 mL/min and was detected at 228 nm	0.06–0.42	NR	0.18	[70]
Tablets	HPLC–Fluorescence	The analytes were separated with a C_{18} reverse phase column with a dimension of 250 × 4.6 mm and 5 μm particle size. The mobile phase contained a mixture of 50 mM phosphate buffer of pH–5.5 and acetonitrile (40:60, v/v) by controlled column temperature at 40 °C with a 1 mL/min flow rate. The excitation and emission wavelengths were selected as 360 and 446 nm	0.05–2	0.017	0.05	[71]
Rat plasma	MEKC	The background electrolyte (BGE) comprises borate buffer (50 mM, pH–9), including sodium lauryl sulphate (50 mM) and acetonitrile as an organic modifier (10 %). The analyte was separated on a fused silica capillary with a length of 41.5 cm and an internal diameter of 50 μm. The wavelength used was 220 nm with a run time of 7 min	5–50	1.49	4.50	[60]
Human urine	CE	The chiral selector was used as a carboxymethyl-β-cyclodextrin in triethylamine (125 mmol/L, pH 6)	0.3–2.1	NR	0.3	[72]
API and pharmaceutical formulations	MEKC	The background electrolyte was prepared by adding tetraborate buffer (10 mM, pH–10.5), including sodium dodecyl sulphate (25 mM) and n-propanol (11 %). The detection was carried out at 200 nm, entered a bandwidth of 10 nm, and the capillary temperature of 25 °C	1–30	0.32	1	[73]
Bulk, dosage forms, human plasma and urine	Spectroflurometric	Adding basic drugs (AML) with xanthene-based dyes (Eosin Y) produced quenched fluorescence that was relied upon to develop a study at a wavelength of 415 nm. The analysis was performed by applying water as a solvent	0.03–0.9	0.009	0.028	[74]
Pharmaceutical formulations	Spectroflurometric	The 1st method performed at 434 nm after excitation at 358 nm. But for the 2nd, $\Delta\lambda = 70$ nm, measured at 363 nm. Methanol was used as a solvent	0.25–7 0.25–7	0.06 0.08	0.18 0.23	[75]
Raw materials, pharmaceutical formulations and spiked human plasma	Spectroflurometric	The proposed method is based on developing binary complexes by adding eosin dye and drug in an acidic medium (acetate buffer of pH 4.4), which excited at 425 nm to get its fluorescence intensity at 544 nm	0.3–3	0.07	0.22	[76]
Bulk powder and dosage forms	Spectroflurometric	The intensities were measured at 443 nm after being excited at 338 nm	0.05–0.75	0.013	0.042	[77]
Bulk and pharmaceutical dosage forms	UPLC	The separation was carried out using phosphate buffer (0.02 M, pH–3.2) and methanol with a volume ratio of 70 and 30 as a mobile phase with a flow rate of 0.3 mL/min. The detection was performed with Cosmosil C_{18} (100 × 2.1 mm, 1.7 μm) column at a fixed wavelength of 224 nm with a run time of 2.5 min	5–25	0.12	0.37	[78]
Bulk and pharmaceutical dosage forms	UPLC	The performance was achieved with the Acquity UHPLC BEH C_{18} column with a dimension of 50 × 2.1 mm and particle size of 1.8 μm. The formic acid and ACN exhibit different ratios as mobile phases with gradient elution developing a flow rate of 0.2 mL/min for separation at a wavelength of 237 nm	2–50	0.012	0.038	[79]

(*continued on next page*)

Table 1 (*continued*)

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
Pure and Tablets	UPLC	The analysis was performed with a BEH C_{18} column with a dimension of 50 × 2.1 mm with a particle size of 1.7 μm. The mobile phase contains 1 N HCl and methanol with a ratio of 1:1 and a constant flow rate of 1 mL/min at room temperature with a wavelength of 272 nm	10–50	10.06	30.32	[80]
Pharmaceutical formulation	TLC	The silica gel 60 plates with dimensions of 20 × 10 cm were utilized with 250 mm thickness. The developing system comprises a mobile phase of 1–propanol, diethylamine and ethyl acetate (0.2:1:9, v/v). The intensity of the radiated light was determined after excitation at 264 nm	TLC-Abs 50–600 ng/band	14 ng/band	45.2 ng/band	[81]
			TLC-FL 15–150 ng/band	4 ng/band	12.2 ng/band	
Human plasma	LCMS/MS	The chromatographic separation was achieved with the Intersil ODS gum C_{18} (50 × 4.6 mm, 3.5 μm) column. The mobile phase contained water, formic acid, and acetonitrile with gradient elution carried 0.5 mL/min as the flow rate, which utilized the mass spectrometer's ESI mode. The analysis time was 8.5 min	0.001–0.13	NR	0.00101	[82]

typically achieve low limits of detection (LOD) and quantification (LOQ), making them particularly suitable for trace-level analysis.

The main advantages of HPLC for the determination of AML and VAL include: (a) high resolution and rapid analysis, (b) large surface area of interaction between analyte and stationary phase, (c) precise flow control under high pressure with a broad selection of stationary phases, (d) accurate peak identification using minimal sample volumes, and (e) high sensitivity and robustness of detection systems.

2.3. Micellar electrokinetic chromatography

Micellar electrokinetic chromatography (MEKC) is a hybrid separation technique that combines principles of capillary electrophoresis (CE) and chromatography. It is particularly suited for the separation and quantification of neutral analytes, which are typically challenging to analyze using conventional electrophoretic methods. In MEKC, a surfactant is added to the buffer at concentrations above its critical micelle concentration, resulting in the formation of micelles that act as a pseudo-stationary phase. Analytes are partitioned between the aqueous phase and the micellar phase based on their hydrophobicity, allowing for effective separation without relying solely on electrophoretic mobility. The most commonly used surfactant in MEKC is sodium dodecyl sulfate (SDS), an anionic compound. Both SDS micelles and monomers exhibit electrophoretic mobility opposite to the direction of electroosmotic flow (EOF), further enhancing separation dynamics. This method is especially advantageous for pharmaceutical compounds, which are often neutral in charge. Unlike classical capillary zone electrophoresis (CZE), which is limited to ionic analytes and separates based on electrophoretic mobility alone, MEKC can efficiently resolve both neutral and ionic compounds. Importantly, MEKC and CZE share the same instrumentation (see Fig. 4); the key difference lies in the use of micellar solutions instead of simple buffers, which significantly increases the versatility of MEKC. The technique has demonstrated excellent separation efficiency and is capable of detecting these analytes at very low concentrations, making it a valuable tool for trace analysis in complex matrices [60,72,73,95].

2.4. Spectrofluorimetry

Spectrofluorimetry is a highly sensitive analytical technique that involves the absorption of ultraviolet light followed by the emission of visible light to measure the fluorescence intensity of a sample (see Fig. 5). Fluorescence arises when a molecule absorbs energy and transitions from the ground state to an excited singlet state. Only certain molecules are capable of existing in this excited state and subsequently returning to a lower energy level by emitting photons as fluorescent light. The emission occurs as the molecule relaxes to its lowest vibrational energy level, making it possible to detect trace levels of analytes based on their characteristic emission spectra. This technique is particularly valued in pharmaceutical and biological analysis due to its simplicity, low cost, high sensitivity, and non-destructive nature [111]. It is widely used for the detection and characterization of fluorescent organic and inorganic compounds. Spectrofluorimetry offers advantages over other techniques in terms of detection limits and rapid response. Synchronous fluorescence spectroscopy, a more advanced variant, improves upon conventional fluorescence by simultaneously scanning excitation and emission wavelengths. This approach significantly enhances selectivity and sensitivity when analyzing multi-component samples. For AML, the reported linearity, LOD, and LOQ values range from 0.03 to 7 μg/mL, 0.009–0.08 μg/mL, and 0.028–0.23 μg/mL, respectively [74–77]. For VAL, the corresponding ranges are 0.01–600 μg/mL (linearity), 0.0027–14.39 μg/mL (LOD), and 0.00821–47.96 μg/mL (LOQ) [96–100]. These methods have been successfully applied in the analysis of bulk materials, pharmaceutical formulations, and biological fluids. In terms of environmental impact, the use of water as a solvent enhances the green character of spectrofluorimetric methods. However, the occasional use of organic solvents such as methanol or acetonitrile diminishes their overall sustainability and should be considered when evaluating method greenness.

2.5. Ultra-high performance liquid chromatography

Ultra–high performance liquid chromatography (UHPLC) offers several advantages over conventional HPLC, particularly in terms of reduced analysis time, lower solvent consumption, and improved resolution. These improvements are largely attributed to the use of shorter columns packed with sub-2 μm particles, which enhance chromatographic efficiency while maintaining high separation power. Consequently, UHPLC is increasingly favored in pharmaceutical analysis for its compatibility with the principles of green analytical chemistry.

However, UHPLC also presents certain challenges. Compared to HPLC, it requires tighter control over method parameters and the use of specialized instrumentation capable of withstanding higher system pressures. The method development and transfer can be more complex due to these stringent operational requirements. Nevertheless, each technique presents a unique balance of advantages and limitations, and it is the responsibility of the analyst to select and optimize the method according to the intended application. UHPLC has been successfully applied for the quantification of AML [78–80] and VAL [79,101–103]. Most studies utilized reversed-phase C_8 or C_{18} columns with lengths of 50–100 mm, internal diameters ranging from 2.1 to 4.6 mm, and particle sizes of 1.7–1.8 μm. These configurations enabled rapid elution of target analytes either as single components or in combination within less than

Table 2
Analytical techniques applied to VAL in API, pharmaceutical formulations and biological fluids.

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
Pharmaceutical formulations, spiked wastewater and biological fluids	UV–Vis spectrophotometric	The wavelength is 352 nm, including the initial rate, fixed–time, and equilibrium methods for quantification	2–24 2–24 2–24	1.85 0.77 0.91	5.63 2.33 2.81	[41]
Dosage forms	UV spectrophotometric	The study included sodium acetate (1 %, *w/v*) solution as a solvent and quantified at a wavelength of 247.2 nm	4–48	0.125	0.05	[83]
Biological fluids	UV spectrophotometric	The molecularly imprinted polymer was used for the dispersive magnetic micro solid phase extraction (DM-μ-SPE) as a nanosorbent. The absorbance was measured at 254 nm against the reagent blank	0.01–0.1	0.00056	0.00186	[84]
Pharmaceutical formulations	UV spectrophotometric	Four UV spectrophotometry procedures involving constant value, concentration value, absorbance subtraction and amplitude modulation approximation were applied to the pharmaceutical formulation	1–30	0.35	1.06	[85]
Pure and formulations	UV spectrophotometric	The multivariate analysis was partial least square (PLS) and principal component regression (PCR), whereas for univariate style ratio difference (RD), derivative ratio (DD1), constant center spectrum subtraction (CC-SS), constant value coupled with amplitude difference (CV-AD), advanced concentration value (ACV), constant value coupled with amplitude difference (CV-AD), ratio difference (RD) and amplitude difference (AD) and modified difference amplitude modulation method (MD-AM)	5–25 5–25 2–20 2–25 1–20 2–25 2–25 1–25	NR NR 0.67 0.67 0.33 0.67 0.67 0.33	NR NR 2 2 1 2 2 1	[86]
API, synthetic mixtures and pharmaceutical formulations	UV spectrophotometric	The detection was carried out at a wavelength of 250 nm. Ethanol was used as a solvent	4–44	NR	NR	[54]
Pharmaceutical dosage forms	UV spectrophotometric	Methanol was used as a solvent, and 290 nm as a detection wavelength	2–50	0.154	0.521	[87]
Bulk, synthetic mixture and pharmaceutical formulations	UV spectrophotometric	Different spectrophotometric procedures, such as area under curve (246–256 nm), first derivative of ratio spectra (253.4 nm) and ratio difference (ΔP = 239.2–301.5 nm) method and multivariate methods (272–282 nm), were simultaneously applied to estimate VAL in different resources. Methanol was used as a solvent for the analysis	4–80 4–80 4–80	0.956 1.305 1.183	3.155 3.976 3.903	[88]
Rat plasma	HPLC	The gradient system enhanced the separation efficiency by containing 50 mM phosphate buffer (pH–3) and methanol as a mobile phase with a column of Zorbax extend-C_{18} (250 × 4.6 mm, 5 μm). The separation was performed at 210 nm with a 1 mL/min flow rate	2–200	0.57	1.72	[66]
Pharmaceutical formulations	HPLC	The column used Waters X-Bridge Shield RP_{18} with dimensions of 100 × 4.6 mm and particle size of 3.5 μm with 40 °C as column temperature. Ethanol and 0.1 % formic acid were used as mobile phases with ratios of 60 and 30 (*v/v*) with a 1 mL/min flow rate, detected at 220 nm	150–450	0.14	0.46	[67]
Pharmaceutical formulations, spiked wastewater and biological fluids	HPLC	Applying BDS Hypersil C_{18} column dimension of 150 × 4.6 mm and particle size of 5 μm, comprises phosphate buffer with a range of pH and methanol as per the design assigned as mobile phase. The detection was carried out at a wavelength of 254 nm with a flow rate of 0.84 mL/min	0.25–11.25	0.09	0.27	[41]
Active substances and pharmaceutical formulations	HPLC	The separation involves mobile phase A and phase B with a ratio of 35:65 (v/v). Mobile phase A contained 200 mL trifluoroacetic acid (0.1 % in water) and 800 mL trifluoroacetic acid (0.1 % in acetonitrile), whereas mobile phase B included 800 mL trifluoroacetic acid (0.1 % in water) and 200 mL trifluoroacetic acid (0.1 % in acetonitrile). The detection was executed at a wavelength of 254 nm with Waters Spherisorb (60 × 4 mm, 3 μm) column and a controlled temperature of 40 °C	20–155	0.17	0.58	[89]

(*continued on next page*)

Table 2 (*continued*)

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
Formulations	HPLC	The mobile phase contains glacial acetic acid, water and acetonitrile with a ratio of 0.1, 50 and 50 (v/v/v) and is detected at 230 nm. The column (250 × 4.6 mm, 10 μm) was separated with a 1 mL/min flow rate and diluent as acetonitrile and water with a 50: 50 (v/v) ratio	50–150	0.284	0.852	[90]
Pure and pharmaceutical formulations	HPLC	The column was used as L1, 150 mm with a controlled temperature of 40 °C. The mobile phase consists of acetonitrile and methanol with a ratio of 50:50 (v/v) and maintaining pH–2.4 with triethyl amine, governing a flow rate of 1 mL/min and detected at 239 nm	40–120	8.45	25.36	[91]
Pharmaceutical formulations	HPLC	The mobile phase system for the 1st mixture was ethanol and phosphate buffer of pH–3 (79:21, v/v). In contrast, for the 2nd mixture, there were similar solvent combinations, but the ratios were different, as 65 and 35 (v/v). The flow rates were 1.2 and 0.7 mL/min with total run times of 3 and 6 min, respectively. Both methods' column temperature and detection wavelengths were 40 °C and 254 nm, respectively	5–100 2.5–50	NR	NR	[92]
Pharmaceutical dosage forms	HPLC	The chromatographic separation included a reversed–phase octadecyl (C_{18}) symmetry (75 × 4.6 mm, 3.5 μm) column with a 0.8 mL/min flow rate. The approach included a gradient system carrying a mobile phase A as acetate buffer (0.02 M. pH–7.2) and solvent B as ethanol. The detection was performed at 230 nm with a maintained column temperature of 40 °C	16–112	1.4	4.5	[93]
Dosage forms	HPLC	The greener HPLC methodology was achieved with the Discovery C_{18} column with a dimension of 150 × 4.6 mm, including 5 μm particle size. The mobile phase is composed of acetonitrile (20 %), 0.16 % of ammonium acetate (80 %) and 1.5 M tetramethylammonium hydroxide (0.2 %) with flow rate and column temperature of 1 mL/min and 30 °C, respectively. The detection was performed at 225 and 237 nm	160–960	200	900	[94]
Rat plasma	MEKC	The BGE used as applied 50 mM borate buffer (pH–9), which involved acetonitrile and sodium lauryl sulphate. The drug samples utilized fused silica capillary 41.5 cm × 50 μm with a wavelength of 210 nm	20–200	4.74	14.36	[60]
API and pharmaceutical formulations	MEKC	The analysis was achieved within 12 min using the background electrolyte as 10 nM tetraborate buffer (pH–10.5), including SDS and n-propanol. The performance was accomplished with fused-silica capillary contributed a total length of 488 mm, with an effective length of 405 mm with a temperature of 25 °C and applied a separation voltage of +30 KV	0.5–20	0.16	0.5	[73]
Pharmaceutical formulations	MEKC	The BGE was used as phosphate buffer (10 mM, pH–10) containing SDS (25 mM) with capillary fused silica with a length of 400 mm and internal diameter of about 50 μm id). Methanol was used as a solvent to prepare the required solutions. The separation was executed at a wavelength of 250 nm	10–300	3.12	9.45	[95]
Tablets	Spectroflurometric	Two spectrofluorimetric approaches were used: first-order level spectral derivatization measured at 230 nm and dual-wavelength mathematical style at (226–241) nm. Methanol is used as a diluent	60–200 80–600	7.03 14.39	23.44 47.96	[96]
Pharmaceutical formulations and spiked plasma samples	Spectroflurometric	VAL was dissolved in methanol and made up the required concentration with citric acid (0.1 M). The fluorescence intensity was measured at 260 and 410 nm for excitation and emission	0.03–1	0.0134	0.0406	[97]
Pharmaceutical dosage form, human plasma and urine	Spectroflurometric	Based on a first-order derivative with zero crossing point at 262.8 nm. The drug sample was dissolved in methanol and diluted with acetic acid (0.1 M) solution before measurements as needed	0.01–0.1	0.0027	0.00821	[98]
Tablets	Spectroflurometric	The derivative ratio method was used to measure intensity at 258–295 nm without any	0.06–0.2	0.01258	0.04193	[99]

(*continued on next page*)

Table 2 (*continued*)

Samples	Methods	Conditions	Linearity (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Ref.
		separation steps, and 600 Volts was used as the detector operating voltage				
Pure and pharmaceutical formulations	Spectroflurometric	Quantification was based upon excitation at 240 nm and emission at 615 nm. The first derivative process is employed to eliminate the interference. Acetonitrile was used as a solvent	0.01–1	0.02393	0.07250	[100]
Bulk and pharmaceutical dosage forms	UPLC	The separation was eluted with an Acquity UHPLC BEH C_{18} column (50 × 2.1 mm, 1.8 μm). The chromatographic procedure included a gradient system with acetonitrile and formic acid. However, the optimized conditions showed formic acid (0.172 %) higher separation efficiency with a column temperature of 27.86 °C. The detection was executed at 237 nm with a 0.2 mL/min flow rate	2–50	0.033	0.101	[79]
Bulk, dosage forms and spiked human plasma	UPLC	The separation was performed with C_{18} Kinetex (50 × 2.1 mm, 1.7 μm) column at room temperature, maintaining a gradient elution with solvent A as phosphate buffer (0.05 M, pH–5) and solvent B as 0.10 M sodium dodecyl sulfate (0.1 M) and 15–25 % isopropanol. It maintained a flow rate of 0.2 mL/min and detection wavelength at 230 nm, including metformin as an internal standard	0.5–25	0.1	0.33	[101]
Formulations	UPLC	The column was used as BEH C_{18} with a dimension of 50 × 2.1 mm with a particle size of 1.7 μm and maintained a temperature of 45 °C. The isocratic elution system was applied with ammonium formate, acetonitrile and methanol with a ratio of 40:15:45 (v/v/v) with a flow rate of 0.35 mL/min and detection wavelength of 236 nm	1–50	1.56	4.73	[102]
Bulk and finished products	UPLC	Accucore XL C_8 column with a dimension of 4.6 × 100 mm and a particle size of 3 μm at 30 °C with gradient system with solvent A as perchloric acid (0.1 %) and tetrahydrofuran (92:8, v/v) and solvent B as acetonitrile, tetrahydrofuran, water (80:5:15, v/v/v) with a flow rate of 0.6 mL/min and PDA detector	0.25–2.25	0.03	0.25	[103]
API and pharmaceutical formulations	UFLC–MS/MS	Phenomenex biphenyl column having dimensions of 150 × 4.6 mm and particle size of 3 μm with a temperature of about 55 °C. The gradient elution enhanced the separation procedure with formic acid (0.1 % in water) as solvent A and 0.1 % formic acid (in methanol) as solvent B mobile phase	0.00006–0.05	NDMA–0.15 NDEA–0.06 NMBA–0.06 NDPA–0.06 NDIPA–0.06 NDBA–0.06 NEIPA–0.06 NMPA–0.06	NDMA–0.25 NDEA–0.15 NMBA–0.15 NDPA–0.15 NDIPA–0.15 NDBA–0.15 NEIPA–0.15 NMPA–0.15	[104]
Human plasma	LC-MS/MS	Separation with Lichrocart RP Select (125 × 4 mm, 5 μm) column with a temperature of 35. The mobile phase involved acetate buffer (10 mM) and acetonitrile (5:95, v/v) with a 0.5 mL/min flow rate	0.0502–6.0186	NR	0.1455	[105]
API & finished products	LC-APCI-MS/MS	Poroshell HPH C_{18} column with dimensions of 150 × 4.6 mm, 2.7 μm involved gradient system employing formic acid (0.2 % in water) as mobile phase A and methanol as mobile phase B. Sample injection volume about 20 μL with a total run time of 17 min. The column temperature was maintained at 20 °C with an autosampler of 8 °C	0.005–0.05	NDBA–0.002 NDEA–0.004 DIPNA–0.002 NDMA–0.007 EIPNA–0.002 NMBA–0.008 NMIPA–0.001 NMEA–0.005 NMPhA–0.007 NPIP–0.003 NpyR–0.002	NDBA–0.008 NDEA–0.013 DIPNA–0.008 NDMA–0.022 EIPNA–0.008 NMBA–0.025 NMIPA–0.050 NMEA–0.018 NMPhA–0.023 NPIP–0.010 NpyR–0.008	[106]

3 min. The methods demonstrated excellent separation efficiency, reproducibility, and analytical performance for both detection and quantification purposes.

2.6. Comparison of the analytical techniques

As discussed above, a variety of analytical techniques have been employed for the quantification of AML and valsartan VAL, each offering distinct advantages and limitations in terms of analytical performance, matrix compatibility, and environmental sustainability (see Table 3). UV–Vis spectrophotometry stands out for its operational simplicity, low cost, and minimal use of reagents and solvents, making it one of the most environmentally friendly options. However, its application is limited when dealing with complex matrices or multi-component systems, due to the potential for spectral overlap between analytes and excipients. Spectrofluorimetry offers higher sensitivity and lower limits of detection compared to UV–Vis, making it suitable for trace-level quantification in biological and pharmaceutical samples. Its green profile is enhanced

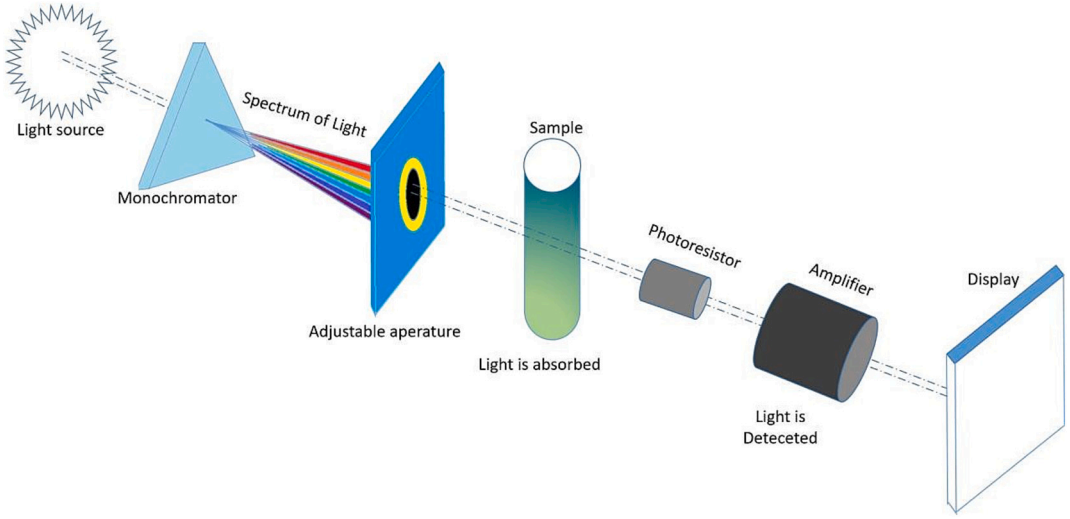


Fig. 3. Schematic diagram of general working principles of UV–Vis spectrophotometer.

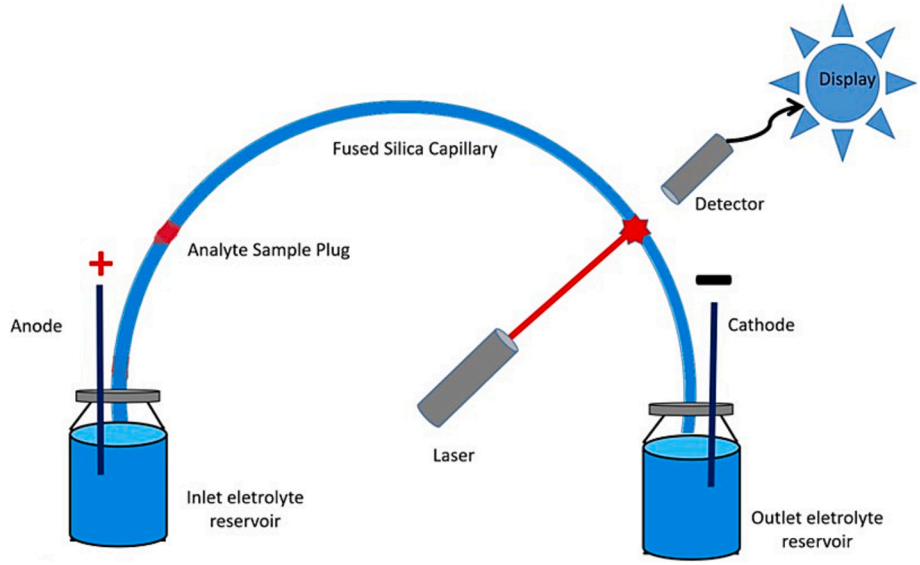


Fig. 4. Schematic diagram of the capillary electrophoresis system.

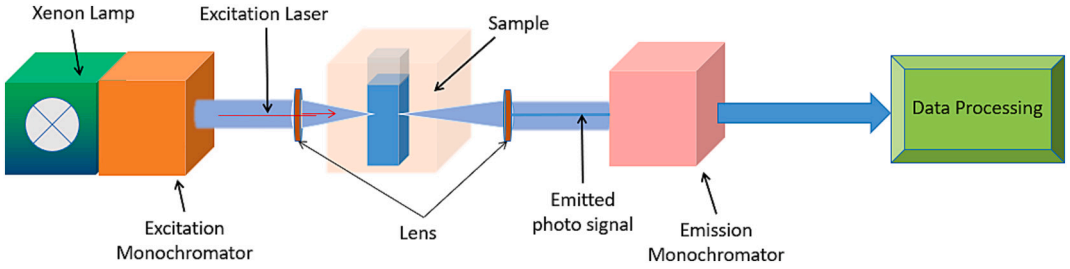


Fig. 5. Schematic diagram of general working principles of spectrofluorimetric system.

when water is used as the solvent; however, the use of organic solvents such as methanol or acetonitrile, which is common in certain derivatization reactions, reduces its overall sustainability. HPLC remains the most widely used technique for AML and VAL due to its robustness, versatility, and high resolution. It is particularly effective in multi-analyte determinations and stability studies. Nevertheless, HPLC often involves larger volumes of organic solvents and longer analysis times. To mitigate its environmental impact, efforts can be made to reduce sample preparation steps, use greener solvents (e.g., ethanol), and optimize column and mobile phase conditions. MEKC provides a more sustainable alternative by replacing organic mobile phases with aqueous buffers containing surfactants. This allows for effective separation of both neutral and ionic species, especially relevant for AML, a molecule with amphiphilic character. MEKC also offers excellent resolution in complex

Table 3
Comparative overview of analytical techniques for AML and VAL determination, including advantages, disadvantages, and GAC-related aspects.

Technique	Advantages	Disadvantages	GAC aspects
UV–Vis spectrophotometry	Extremely low operational cost; portable instrumentation for on-site testing; rapid screening in developing countries	Limited selectivity in multi-component samples; relatively low sensitivity	Minimal solvent use; low energy demand; excellent greenness for routine assays
Spectrofluorimetry	High sensitivity at trace levels; strong signal-to-noise ratio; suitable for biological samples	Fluorescence quenching by excipients; requires careful calibration	Greenness enhanced with water as solvent, but decreases if derivatization with organic solvents is required
High-performance liquid chromatography	Widely standardized in pharmacopeias; adaptable with detectors (DAD, MS)	High solvent consumption and disposal costs; relatively long analysis times	Greenness depends on solvent choice; greener alternatives include ethanol or reduced sample volumes
Micellar electrokinetic chromatography	Simultaneous analysis of neutral and ionic species; lower reagent costs than LC	Less common in regulatory validation; requires optimization of surfactant systems	Largely aqueous buffers replace organic solvents, reducing environmental impact
Ultra-high performance liquid chromatography	High throughput; shorter run times; reduced solvent use per sample	Expensive equipment and columns; method transfer from HPLC not always straightforward	Improved greenness vs. HPLC due to lower solvent consumption and faster separations

formulations and biological fluids, with significantly lower solvent consumption and waste generation. UHPLC represents a more advanced chromatographic approach that enables faster separations and reduced solvent use through the use of sub-2 μm particles and shorter columns. While the method requires specialized, high-pressure instrumentation and precise control over variables, its overall efficiency and reduced environmental footprint make it a promising tool for high-throughput analysis of AML and VAL, particularly when rapid quantification is needed in quality control environments.

When comparing these techniques by a GAC approach, UV–Vis and MEKC generally achieve higher greenness scores due to their lower energy demands and minimal solvent usage. Nevertheless, chromatographic methods such as HPLC and UHPLC, though traditionally less green, can approach comparable levels of sustainability when properly optimized and aligned with the 12 principles of green analytical chemistry. In particular, selecting appropriate column chemistries, reducing analysis times, and implementing miniaturized or automated workflows can significantly reduce their environmental impact. Finally, the choice of analytical technique for AML and VAL should consider not only greenness but also method sensitivity, specificity, matrix compatibility, and regulatory requirements. For example, in formulations combining both drugs or in biological matrices such as plasma, more selective and sensitive techniques like UHPLC or spectrofluorimetry may be necessary. In contrast, for routine assay of single compounds in bulk APIs or tablets, simpler techniques such as UV–Vis or MEKC may be sufficient and more sustainable.

It is also important to highlight that the real-world application of these methods shows differences depending on the sample type. For biological matrices such as plasma or urine, techniques with higher sensitivity and selectivity, including spectrofluorimetry, UHPLC, and HPLC coupled with MS detectors, have been most widely employed to reliably quantify trace levels of AML and VAL in the presence of endogenous interferences. In contrast, simpler approaches such as UV–Vis or MEKC have found broader use in pharmaceutical formulations, where matrix complexity is lower and regulatory compliance often prioritizes cost-effectiveness, robustness, and throughput. In this context, the choice of technique is not only determined by analytical performance and greenness, but also by the practical requirements of the target matrix and the quality control context in which the methods are implemented.

3. Greenness and blueness assessment applied to the determination of amlodipine and valsartan

GAC is closely associated with key sustainability principles, including environmental protection, waste minimization, and the development of safer, cleaner methodologies. In the context of pharmaceutical analysis, especially during quality control and routine testing, there is a growing imperative to incorporate GAC principles into method development and validation. This involves critically assessing whether analytical methods align with the 12 principles of GAC, which include the use of safer solvents, reduction in energy consumption, minimization of sample and reagent volumes, and preference for in situ measurements, among others [16]. Given the high therapeutic relevance and widespread use of AML and VAL, ensuring that their analytical determination is not only effective but also environmentally responsible is a priority. Researchers have increasingly aimed to develop methods that maintain analytical performance while improving sustainability. As a result, several green and blue assessment tools have been created and applied to evaluate the “greenness” and “blueness” of analytical methods (see Tables 4–6). The application of the selected metrics was carried out according to the published guidelines and softwares available for each of them.

3.1. Analytical eco–scale

The Analytical Eco-Scale, first proposed by Galuszka et al. [22], is a semi-quantitative tool that assesses the environmental friendliness of an analytical procedure through a system of penalty points (PPs). These points are assigned to specific parameters that deviate from the principles of GAC, such as the use of toxic reagents, hazardous solvents, excessive energy consumption, and waste generation (see Table 4). The Eco-Scale provides a pragmatic approach by quantifying the greenness of a method on a scale where 100 represents an ideal green analysis, with no penalty points deducted. The primary goal of any analytical procedure evaluated by this metric is to favor safer reagents and solvents, ideally derived from renewable and biodegradable sources, and to minimize the use of hazardous substances. In addition, preference is given to non-invasive, in situ analytical methods that do not require derivatization, as well as miniaturized techniques that reduce solvent and sample volumes, thereby decreasing waste generation and energy consumption [112]. A method that scores above 75 is considered excellent in terms of greenness, while scores between 50 and 75 are deemed acceptable. The simplicity and accessibility of the Eco-Scale make it particularly suitable for research laboratories and academic environments, where it serves as a valuable guide for both established and novel methodologies aiming to enhance sustainability.

3.2. Analytical GREEnness metric

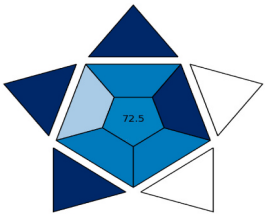
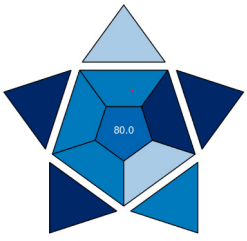
AGREE software, introduced by Pena-Pereira et al. [23], is a comprehensive, flexible, and user-friendly tool designed to assess the greenness of analytical procedures. It evaluates analytical methods based on the twelve principles of GAC, providing an integrated score on a scale from 0 to 1. This metric is visually represented by a circular pictogram divided into twelve segments, each corresponding to one GAC

Table 4
Greenness assessment of AML and VAL according to analytical Eco-Scale applied to different analytical techniques.

Parameters	Penalty points	Ref.
Reagents and solvents		
Ethanol	4	
Instrument (Spectrophotometer)		
Energy consumption	0	[54]
Occupational Hazard	0	
Waste	3	
Total PPs	**7**	
Reagents and solvents		
Acetonitrile	8	
Instrument (Spectrophotometer)		
Energy consumption	0	[57]
Occupational Hazard	0	
Waste	8	
Total PPs	**16**	
Reagents and solvents		
Ethanol Amount < 10	1	
Hazardous	2	
Instrument (Spectrophotometer)		[85]
Energy consumption	0	
Occupational Hazard	0	
Waste	1	
Total PPs	**4**	
Reagents and solvents		
Water	0	
Eosin Y	1	
Instrument (Spectrofluorimeter)		
Energy consumption	0	
Occupational Hazard	0	
Waste < 10 mL	3	[74]
Procedure		
Heating: Not required	0	
Cooling: Not required	0	
pH 3.8	0	
Amount of reagent >10 mL	1	
Total PPs	**5**	
Reagents and solvents		
Methanol (1 mL); Hazard–6	3.66	
PDA (0.7 mL); Hazard–6	3.28	
2–ME (0.7 mL); Hazard–8	4.37	
NaOH (0.5 mL); Hazard–1	0.49	[77]
Instrument (Spectrofluorimeter)		
Energy consumption	0	
Occupational Hazard	0	
Waste (10 mL)	3.77	
Total PPs	15.57	
Reagents and solvents		
0.1 M H_2SO_4 amount (<10 mL)	2	
Methanol (<1 mL)	6	
Instrument (Spectrofluorimeter)		
Energy consumption	0	
Occupational Hazard	0	[96]
Waste (10 mL)	6	
Total PPs	**14**	
Reagents and solvents		
Water	0	
HCl (0.2 M)/citric acid (0.1 M)	2	
Instrument (Spectrofluorimeter)		
Energy consumption	0	[97]
Occupational Hazard	0	
Waste		
Production (<1 mL (g) per sample)	1	
Treatment (No treatment involved)	3	
Total PPs	6	
Reagents and solvents		
Methanol	5	
Potassium dihydrogen phosphate	3	
Instrument (HPLC)		[60]
Energy consumption	1	
Occupational Hazard	0	
Waste	3	
Total PPs	**12**	
Reagents and solvents		
Ethanol	9	[62]
SDS aqueous solution	0	
Phosphate buffer solution	0	
Instrument (HPLC)		
Energy consumption	1	
Occupational Hazard	0	
Waste	3	
Total PPs	**13**	
Reagents and solvents		
Acetonitrile	8	
Instrument (HPLC)		
Energy consumption	1	[65]
Occupational Hazard	0	
Waste	8	
Total PPs	**17**	
Reagents and solvents		
Acetonitrile	4	
Trifluoroacetic acid	4	
Methanol	0	
Instrument (HPLC)		[89]
Energy consumption	1	
Sonicator	1	
Waste	3	
Total PPs	**13**	
Reagents and solvents		
Ethanol <10	1	
Hazardous	2	
Methanol	0	
Instrument (HPLC)		
Energy consumption	0	[92]
Waste	6	
Total PPs	**9**	
Reagents and solvents		
Acetonitrile	8	
NaOH	1	
Disodium tetraborate	1	
Instrument (CE)		[60]
Energy consumption	0	
Occupational Hazard	0	
Waste	1	
Total PPs	**11**	
Reagents and solvents		
SDS aqueous solution	0	
NaOH	1	
Disodium tetraborate	1	
Instrument (CE)		[73]
Energy consumption	0	
Occupational Hazard	0	
Waste	1	
Total PPs	**3**	
Reagents and solvents		
Methanol	6	
KH_2PO_4	0	
Isopropanol	4	
SDS	0	
IS	4	[101]
Instrument (UPLC)		
Energy consumption	0	
Occupational Hazard	0	
Waste	3	
Total PPs	**17**	
Reagents and solvents		
Methanol	6	
Instrument (UPLC/MS)		
Energy consumption	2	[104]
Occupational Hazard	0	
Waste Management and Recycling	3	
Total PPs	**11**	

principle. The segments are color-coded from deep red to dark green according to the environmental impact, while the final overall score is displayed at the center of the pictogram. Each criterion is assigned a specific weight, and the combination of these weighted scores produces the final greenness evaluation. The software is freely accessible, which facilitates its application by researchers aiming to incorporate

Table 5
Application of AGREE, BAGI, and analytical Eco-Scale tools to evaluate greenness/blueness analytical procedures to quantify AML.

Method	AGREE	BAGI	Analytical Eco-Scale Score	Ref.
UV–Vis			93	[54]
UV–Vis			84	[57]
Spectrofluorimetry			95	[74]
Spectrofluorimetry			84.43	[77]
HPLC			88	[60]
HPLC			87	[62]

(*continued on next page*)

Table 5 (*continued*)

Method	AGREE	BAGI	Analytical Eco-Scale Score	Ref.
HPLC			83	[65]
CE			89	[60]
CE			97	[73]

sustainability into analytical method development. However, all metrics and tools exhibit also some disadvantages. Particularly, AGREE does not consider the greenness of pre-extraction steps, as factors such as specific reagents, solvents, energy consumption, and waste generation are excluded from its calculation. Furthermore, it does not directly address the environmental impact of sample preparation procedures [113].

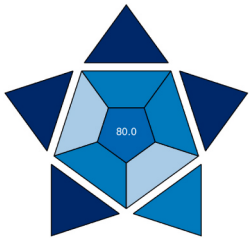
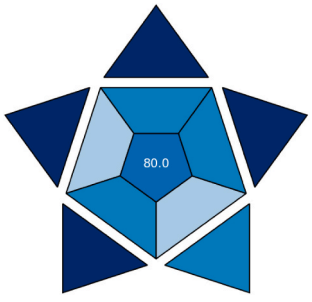
In the present study, most of the evaluated methods for amlodipine and valsartan yielded AGREE scores close to 1, confirming their environmentally friendly nature (see **Tables 5 and 6**) [23]. Regarding Principle 1, which focuses on the sampling procedure, higher greenness levels are associated with direct analysis methods that avoid sample preparation and treatment. Online or in situ analysis with minimal sample mass is preferred. Principle 2 emphasizes reducing the number and size of samples; non-invasive and statistically designed screening methods are considered advantageous in this context. Principle 3 supports direct measurement techniques (off-line, on-line, at-line, or in-line) and encourages the use of portable instruments and real-time analysis. Principle 4 evaluates the number of steps involved in the analytical procedure, such as extraction, sonication, derivatization, and mineralization. Greener methods are characterized by fewer steps, with minimal impact observed when three or fewer are involved. Principle 5 addresses automation and miniaturization; methods that are both automated and miniaturized maintain higher greenness scores, while manual or semi-automated procedures tend to reduce them. Regarding Principle 6, derivatization steps should be avoided when possible. If derivatization is required, the use of eco-friendly reagents is encouraged to minimize environmental impact. Principle 7 concerns the amount of waste generated. Analytical procedures producing less than 0.1 g/mL of waste are considered optimal, while higher volumes negatively affect the greenness score. Principle 8 assesses analytical throughput. A method is favored if a single run includes at least eight samples and if a minimum of eight analyses can be performed per hour. Lower throughput may reduce the overall score. Principle 9 focuses on energy efficiency; procedures consuming less than 0.1 kWh per analysis are considered green, whereas higher energy demands decrease sustainability. Principle 10 promotes the use of bio-based reagents or, when possible, the complete avoidance of reagents. The use of synthetic or non-renewable reagents results in lower greenness scores. Principle 11 recommends avoiding toxic solvents and reagents, as even small quantities can compromise the environmental friendliness of a method. Finally, Principle 12 addresses operator safety. Analytical procedures should avoid hazardous chemicals that are toxic, flammable, corrosive, or harmful to aquatic ecosystems. Ensuring safety for analysts and minimizing risk is essential for achieving high sustainability ratings.

Overall, AGREE provides a valuable framework for systematically evaluating and comparing the environmental sustainability of analytical methods. Its application to the determination of amlodipine and valsartan confirms the growing implementation of green chemistry principles in pharmaceutical analysis.

3.3. Blue applicability grade index

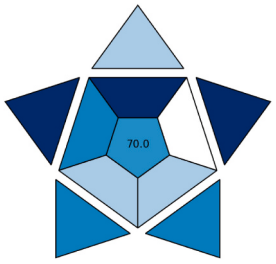
BAGI is a recent metric aligned with the principles of WAC. It was first introduced by Manousi et al. [25] and serves as a complementary tool to establish GAC assessment models. BAGI evaluates the practical applicability of an analytical method through ten predefined criteria, assigning scores from 25 to 100. Each parameter receives a score represented by a color scale, white, light blue, blue, and dark blue, corresponding to 2.5, 5.0, 7.5, and 10.0 points, respectively. The overall score is calculated as the average of all individual scores, with values above 60

Table 6
Application of AGREE, BAGI, and analytical Eco-Scale tools to evaluate greenness/blueness analytical procedures to quantify VAL.

Method	AGREE	BAGI	Analytical Eco-Scale Score	Ref.
UV–Vis			93	[54]
UV–Vis			96	[85]
Spectrofluorimetry			86	[96]
Spectrofluorimetry			94	[97]
HPLC			88	[60]
HPLC			87	[89]

(*continued on next page*)

Table 6 (*continued*)

Method	AGREE	BAGI	Analytical Eco-Scale Score	Ref.
HPLC			91	[92]
CE			89	[60]
CE			97	[73]
UPLC			83	[101]
UPLC-MS			89	[104]

indicating acceptable practical performance. Higher BAGI values reinforce the method's feasibility and utility in routine laboratory practice, providing a quick and effective assessment of its strengths and limitations. However, BAGI does not include the safety, health, and environmental assessment of reagents and waste generation in its evaluation process [113].

This metric was applied to selected case studies for the quantification of AML and VAL, and the results are presented in **Tables 5 and 6**. According to criterion 1, the type of analysis is categorized as qualitative, screening, quantitative, and quantitative-confirmatory, scoring 2.5, 5.0, 7.5, and 10.0 points, respectively. In criterion 2, the number of analytes plays a key role; the analysis of a single compound is considered less applicable (white), whereas simultaneous analysis of more than 15 components is ideal (dark blue, 10 points). Intermediate scenarios, such as analysis of 2–5 similar components or 6–15 compounds from the same or different classes, are scored with light blue or blue depending on complexity. Criterion 3 assesses the instrumentation. Highly sophisticated and less accessible instruments such as GC–MS/MS or LC-MS/MS are marked in white, while standard but still advanced instruments like GC–MS or LC-MS are light blue. Portable and widely available

instruments are preferred and score higher, in blue or dark blue. In criterion 4, the number of samples prepared simultaneously is evaluated; methods that prepare more than 95 samples receive the maximum score (dark blue), while single-sample preparation receives the lowest (white). Preparations involving 13–95 or 2–12 samples fall into blue or light blue categories, respectively. Criterion 5 focuses on the complexity of sample preparation. The most favorable procedures require no preparation or only on-site operations (dark blue), while miniaturized or low-cost techniques are rated as blue or light blue. Multi-step sample preparation significantly reduces the score (white). Criterion 6 considers analytical throughput; methods processing more than 10 samples per hour score highest (dark blue), while those processing only one sample score lowest (white). In criterion 7, the availability and type of reagents are assessed. Procedures using commonly available reagents such as methanol or acetonitrile are favored (dark blue), whereas those requiring reagents synthesized with advanced instrumentation are penalized (white or blue). Criterion 8 relates to pre-concentration. Procedures requiring no pre-concentration step score highest. One-step pre-concentration methods still requiring high sensitivity receive a blue score, while those involving multiple steps, although still complying with regulatory standards, are marked in white. Criterion 9 evaluates the level of automation. Fully automated systems receive the highest score (dark blue), while manual procedures are rated lowest (white). Semi-automated methods using common devices like HPLC autosamplers score in the mid-range (blue or light blue), depending on equipment availability. Finally, criterion 10 addresses the sample amount required. Bioanalytical samples smaller than 100 mg or μL and food/environmental samples below 10 g or mL are ideal (dark blue). Larger quantities, such as 1000 mg or mL or more than 100 g or mL, reduce the method's practicality (white), while intermediate volumes score as light blue or blue.

As stated, BAGI enables a structured and visual evaluation of the analytical method's applicability. When combined with GAC metrics it offers a more holistic view of both the environmental and operational performance of the methods used to quantify AML and VAL.

3.4. Comparison of green and blue tools

Three assessment tools were employed to evaluate and compare the analytical procedures used for the quantification of AML and VAL: the analytical Eco-Scale, AGREE, and BAGI (see **Tables 5** and **6**). Among the wide array of available methodologies, selected techniques were included in this comparative analysis, namely UV–Vis spectrophotometry [54,57,85], spectrofluorimetry [74,77,96,97], HPLC [60,62,65,89,92], CE [60,73], UHPLC [101], and UPLC–MS [104]. These methods were examined for their alignment with the principles of GAC and WAC, as reflected in their respective green and blue assessment scores.

In general, the use of environmentally benign solvents such as ethanol, combined with lower instrument energy consumption and reduced solvent usage, resulted in higher green assessment scores and lower waste generation [54,85]. In contrast, the use of less environmentally favorable solvents such as acetonitrile slightly diminished the final score, though it remains widely employed due to its chromatographic efficiency [57]. Procedures involving multiple derivatization steps [77] are typically discouraged within the GAC framework, as they increase reagent use and operational complexity. On the other hand, the use of water as a primary solvent is strongly preferred for its minimal occupational and ecological impact [74], while alternatives such as methanol may moderately improve the overall score depending on the context and volume used [96,97].

Regarding the mobile phase or background electrolyte composition, several combinations were observed across the methods analyzed, including methanol, ethanol, n-propanol, isopropanol, sodium lauryl sulfate, and acetonitrile [60,62,65,73,89,92]. The environmental burden of these solvents varies, influencing the final greenness scores based on toxicity, volatility, and biodegradability. Despite these differences, the majority of the analytical methods yielded comparable results across the three assessment tools. Scores based on the 12 principles of GAC were generally consistent, supporting the robustness of these frameworks for evaluating the environmental profile of analytical procedures.

Nevertheless, some techniques did not fully comply with green principles due to specific methodological requirements or instrumental limitations. In such cases, the challenge lies in balancing analytical performance with environmental responsibility. To achieve greener and more sustainable practices, future method development should focus on replacing toxic solvents and reagents with safer alternatives, reducing or eliminating derivatization steps, optimizing sample preparation procedures, and promoting multi-analyte, high-throughput capabilities.

The evaluation of the blue dimension using the BAGI metric revealed that methods combining instrumental simplicity, high sample throughput, and minimal sample preparation were rated most favorably. As presented in **Tables 5** and **6**, most BAGI values ranged between 83 and 97, significantly surpassing the minimum recommended threshold of 60. These high scores confirm that the evaluated techniques are not only environmentally friendly but also practical and suitable for implementation in routine laboratory settings. Spectrophotometric and spectrofluorimetric methods consistently achieved the highest BAGI scores due to their straightforward instrumentation, low cost, and high analytical throughput. These techniques also benefit from minimal sample preparation and the use of commonly available, less hazardous solvents. CE also received high BAGI values, attributed to its efficient separations, low reagent consumption, and capability to analyze multiple components without complex preconcentration procedures. Conversely, more sophisticated techniques such as UHPLC and UPLC–MS showed slightly lower BAGI scores, reflecting their greater reliance on advanced equipment and more elaborate operating conditions.

3.5. Alignment with sustainable development goals (SDGs)

Environmental impact assessment (greenness) and practical applicability assessment (blueness) are not sufficient. It is also convenient to provide a snapshot evaluation of the alignment of this review with sustainable development principles such as the SDGs [114,115]. In this context, the analytical methods discussed here demonstrate clear links with several global sustainability objectives. Most evidently, the development of greener and more reliable analytical procedures for AML and VAL contributes directly to SDG 3 (Good Health and Well-Being) by ensuring the quality, safety, and efficacy of essential antihypertensive therapies, thereby supporting cardiovascular health worldwide. The promotion of methodologies that minimize solvent consumption, reduce hazardous waste, and employ energy-efficient instruments aligns with SDG 12 (Responsible Consumption and Production) and SDG 13 (Climate Action), as they lower the environmental footprint of pharmaceutical analysis. Furthermore, by systematizing the application of greenness and blueness assessment tools, this review indirectly supports SDG 4 (Quality Education), offering researchers and students a structured framework to integrate sustainability metrics into their academic and professional training. Also, the collaborative and multidisciplinary approach underlying this review, involving researchers from different institutions and countries, is in agreement with SDG 17 (Partnerships for the Goals) by fostering knowledge exchange and global cooperation in the advancement of sustainable analytical chemistry.

4. Research gaps and future directions

Despite the advances highlighted in this review, several research gaps remain in the field of AML and VAL quantification. One major limitation is the restricted diversity of analytical techniques applied to biological matrices, where most studies rely heavily on HPLC or UHPLC,

often coupled with conventional detectors, while alternative greener approaches such as MEKC or spectrofluorimetry remain underexplored. The integration of green analytical chemistry principles into routine pharmaceutical quality control is still inconsistent, as many methods continue to employ hazardous solvents like acetonitrile and methanol despite the availability of safer alternatives. Additionally, while Eco-Scale, AGREE, and BAGI provide valuable insights, their application has not yet been standardized, leading to variability in how greenness and practicality are reported across studies. Another important gap is the limited evaluation of these methods under real-world conditions, particularly in resource-limited settings or in smaller pharmaceutical industries, where cost and ease of implementation are decisive factors. Furthermore, the role of sample preparation, which often contributes significantly to the environmental burden of analytical processes, is frequently overlooked in green assessments, creating a discrepancy between theoretical greenness and practical application. Moreover, although several systematic reviews exist for analytical techniques in general, there is still a lack of integrative works focused specifically on AML and VAL that combine environmental, practical, and regulatory perspectives.

From a regulatory perspective, it is also important to note that analytical methods for AML and VAL must comply with international guidelines such as those established by the International Council for Harmonisation. These guidelines emphasize key performance attributes such as accuracy, precision, linearity, robustness, and detection limits, which are essential for pharmaceutical quality control. While most of the reported methods fulfill these requirements, the integration of green analytical chemistry principles into validated protocols remains limited. Future efforts should therefore aim to harmonize sustainability considerations with regulatory compliance, ensuring that greener and more practical analytical methodologies can be accepted not only in academic research but also in routine industrial and regulatory environments.

Looking ahead, future research should prioritize the development of miniaturized and automated workflows that reduce solvent and energy consumption while maintaining analytical accuracy and reproducibility. Expanding the application of greener techniques such as MEKC, spectrofluorimetry with water-based systems, or biosensor platforms could provide sustainable alternatives to chromatographic methods, especially for routine analysis of pharmaceutical formulations. In parallel, the incorporation of artificial intelligence and chemometric tools offers promising opportunities for optimizing method design, improving data analysis, and reducing the need for labor-intensive sample preparation steps. Another direction is the integration of comprehensive sustainability assessments into method validation protocols, ensuring that environmental and practical metrics are considered alongside conventional performance parameters. Collaboration between academia, regulatory agencies, and the pharmaceutical industry will also be critical to standardize the use of greenness and applicability metrics, facilitating their acceptance in guidelines and pharmacopeias. In this regard, adopting a holistic vision of analytical methods that integrates greenness, practicality, performance and innovation (e.g., through the application of the MA tool) represents a logical next step. Addressing these future directions will enable the analytical community to move towards methods that are not only accurate and reliable but aligned with global sustainability goals.

5. Conclusions

This review highlights the application of various analytical techniques for the quantification of two widely used antihypertensive drugs, AML and VAL, across different matrices such as bulk drugs, pharmaceutical formulations, and biological samples. Techniques such as UV–Vis spectrophotometry, spectrofluorimetry, MEKC, and UHPLC have been discussed for their analytical performance and environmental sustainability. To evaluate their green and practical character, three complementary assessment tools, analytical Eco-Scale, AGREE, and BAGI, were applied. The results showed that most of the methods reviewed can be considered both environmentally friendly and practical. Eco-Scale scores ranged from 83 to 97, AGREE values varied between 0.59 and 0.90, and BAGI assessments were consistently above the 60-point threshold, supporting the classification of these methods within the concept of WAC. The comparative analysis of these techniques suggests that spectrophotometry and MEKC offer the highest green scores, while UHPLC and HPLC approaches can also align with GAC principles when properly optimized. BAGI further demonstrated that methods combining high throughput, minimal sample preparation, and common reagents are especially suited for routine application. Our findings offer a framework for selecting and designing greener and more practical analytical procedures for the determination of antihypertensive drugs.

Declaration of Use of AI-Assisted Technologies

The authors employed ChatGPT (GPT-3.5) to create the graphical abstract and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Shaikh Manirul Haque: Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **Abuzar Kabir:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Mohd. Rafatullah:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Zakariya Sadique:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Adrián Fuente-Ballesteros:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Adrián Fuente-Ballesteros thanks the University of Valladolid for his PhD contract.

Data availability

No data was used for the research described in the article.

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