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Review

HPLC-Based Analysis of Caffeine in Different Matrices: An Appraisal of the Literature Published in 2025

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Abstract: Caffeine, also known as methyltheobromine, is a methylxanthine alkaloid, found in cocoa, coffee, tea, and a few other plants, e.g., *Cola acuminata* Schott & Endl., *Ilex guayusa* Loes, *I. paraguariensis* A. St.-Hil., *I. vomitoria* Sol. ex Aiton, *Paullinia cupana* Kunth. and *P. yoco* R. E. Schult. & Killip. Caffeine is widely used in various energy drinks, cosmetics, and pharmaceutical products. High-performance liquid chromatography (HPLC), inclusive of ultra-performance liquid chromatography (UPLC) or ultra-high-performance liquid chromatography (UHPLC), is the most widely used technique for caffeine analysis. This review article critically appraises the literature published in 2025 on caffeine analysis from different matrices using HPLC, revealing the popularity and usefulness of this chromatographic technique in the analysis and quantification of caffeine in different matrices. While an increase in the use of UPLC (or UHPLC) was observed, conventional HPLC using standard 5 μm columns of 150 or 250 mm length remained the instrument of choice because of its lower price than a UPLC (or UHPLC) system. Only a handful of studies reported in 2025 used an MS detector, but many of the studies used a UV or diode array detector (DAD, also known as photodiode array detector, PDA). Most of the studies published last year involved caffeine analysis in various cocoa, coffee, and tea samples.

Keywords: Caffeine; *Coffea arabica*; *Camelia sinensis*; coffee; tea; cocoa; HPLC

1. Introduction

Caffeine (mol. Formula: $C_8H_{10}N_4O_2$; mol. wt. 194; also known as methyltheobromine; IUPAC name: 1,3,7-trimethyl-3,4,5,7-tetrahydro-1H-purine-2,6-dione) is a methylxanthine alkaloid (Figure 1). It is the most popular central nervous system stimulant, found in cocoa, coffee, tea, and a few other plants, e.g., *Cola acuminata* Schott & Endl., *Ilex guayusa* Loes, *I. paraguariensis* A. St.-Hil., *I. vomitoria* Sol. ex Aiton, *Paullinia cupana* Kunth. and *P. yoco* R. E. Schult. & Killip. Caffeine is extensively used in various energy drinks, cosmetics, and pharmaceutical products [1,2]. Caffeine is an odourless white powder or white glistening needles. It has a bitter taste and is highly soluble in water. Caffeine was first isolated from coffee beans in 1819 by German chemist Friedlieb Ferdinand Runge, during his study of stimulating effects of coffee beans [3]. Later, in 1821, Pierre Jean Robiquet independently isolated it.

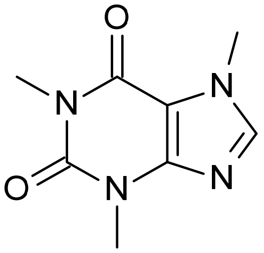

Figure 1. Structure of caffeine.

Caffeine increases alertness, enhances physical performance, improves focus, and boosts metabolism by blocking adenosine receptors [4]. It can be noted that adenosine is a chemical in the brain that produces feelings of tiredness and calm. Some studies suggest that caffeine may mitigate the risk of Parkinson's and liver diseases. This xanthine alkaloid slightly increases blood pressure, stimulates the heart, and acts as a mild diuretic. Excessive consumption of caffeine may lead to anxiety, tremors, insomnia, rapid heart rate, and digestive problems. The recommended safe intake of caffeine per day for healthy adults is 400 mg. Medicinal uses of caffeine include its application in treating apnea of prematurity in newborns and in pain-killer medications in combination with other analgesic drugs, e.g., paracetamol. Caffeine is also recommended for combating drowsiness. Caffeine solution is used externally to reduce under-eye puffiness and dark circles.

Analysis of caffeine in different matrices can be achieved by various chemical, spectroscopic, and chromatographic means, but high-performance liquid chromatography (HPLC), inclusive of ultra-performance liquid chromatography (UPLC) or ultra-high-performance liquid chromatography (UHPLC) [5,6], has emerged as the most widely used technique. For caffeine analysis, HPLC is often coupled with a UV-Vis or photodiode array detector (PDA) [or simply diode array detector (DAD)] as caffeine is UV active (λ_{max} 273 nm), but sometimes, a mass spectrometric (MS) detector is also hyphenated to an HPLC system. This review article critically appraises the literature published in 2025 on caffeine analysis from different matrices using HPLC, obtained from a detailed search using databases including Web of Science, Science Direct, PubMed, and Google Scholar; caffeine, HPLC, and analysis were used as the search terms. Only the information from journal articles, books, and book chapters published in 2025 has been included, and any PhD theses or conference abstracts have been excluded.

2. HPLC Analysis of Caffeine in Various Matrices

In 2025, over 60 publications described HPLC-based methods for the analysis of caffeine in various matrices [7–70] (Table 1), demonstrating the popularity, utility, and versatility of HPLC as an advanced analytical tool for the detection and quantification of caffeine. The matrices analysed for caffeine included cocoa [7–10], coffee [11–28], tea [29–45], plants like *Cucurbita pepo* L., *Desarmillaria tabescens* (Scop.) Emel and *Ilex guayusa* Loes [46–48], various caffeine-containing products [49–65], water and wastewater [66–68], and biological samples, e.g., rat plasma [69] and human urine [70]. Starting from simple HPLC-UV instrumentation (Figure 2) to the most modern UPLC or UHPLC systems, which were used for caffeine analysis, and the most dominant manufacturers for these systems were found to be Agilent, Shimadzu, and Waters. However, the use of Thermo Fisher (Dionex) LC systems has also been noted. C_{18} HPLC columns from Waters and Phenomenex were the most used columns for caffeine analysis. Mobile phases used for caffeine analysis were mostly ACN-water or MeOH-water, often containing a trace amount (0.1%) of acetic acid, HCOOH, phosphoric acid (or *ortho*-phosphoric acid), or trifluoroacetic acid (TFA). It can be noted that acids are added to HPLC mobile phases primarily to control pH, which suppresses the ionization of acidic compounds, improves peak shape, and increases retention in reverse-phase (RP) chromatography. Acids also reduce tailing of basic compounds, e.g., caffeine, by keeping them protonated and preventing unwanted interactions with residual silanol groups (Si-O-) on the silica column, which can cause peak broadening. A clear trend in the increasing use of shorter columns (50–100 mm) with sub-2-micron particle size (for UPLC or UHPLC) was observed. The use of mathematical or computational models, e.g., response surface methodology (RSM), in optimizing the extraction, separation, and quantification of caffeine has also been noted. HPLC analyses of caffeine in various matrices are discussed below.

Table 1. HPLC analysis of caffeine from different matrices.

Matrices/Sources	Type of Analysis	HPLC Methods	References
Cocoa			
Cocoa bean shells	Integrated extraction and isolation	A pressurised liquid extraction (PLE) and solid-phase extraction (SPE) coupled with HPLC-PDA was used. An Acquity Waters UPLC H-Class system, a Waters Acquity C_{18} column (2.1 mm × 5.0 mm, 1.7 µm) at 40 °C, with a flow rate of 0.5 mL/ min and an injection volume of 3 µL were used. The mobile phases comprised water (A) and ACN (B), both containing 0.1% acetic acid in the following gradient: 0–0.5 min: 5% of B; 0.5–5.5 min: 5–30% B; 5.5–6 min: 30–100% B; 6–7 min: 100% B. The column equilibration time = 2.5 min; monitoring wavelength = 210 and 400 nm; quantification wavelength = 272 nm. 30% MeOH in water was the mobile phase.	[7]
	Quantification for extraction optimization	A Shimadzu HPLC model LC20AD coupled with a DAD and a Phenomenex RP-C_{18} column (150 mm × 4.6 mm, 5.0 µm) with a guard column (12.5 mm × 4.6 mm) was used. The column oven temperature = 45 °C; flow rate = 1 mL/min; monitoring wavelength = 200–400 nm. The mobile phase comprised water–acetic acid (99:1) and ACN. An isocratic elution with 80% water–acetic acid in ACN.	[8]
Cocoa hulls (*Theobroma cacao* L.)	Quantification	An RP-HPLC-UV was used with a gradient elution, set at a flow rate of 1 mL/min, using the mobile phases A = HCOOH/water (0.5%) and B = HCOOH/ACN (0.5%). The gradient programme was as follows: 0–30 min: 90–75% A; 30–35 min: 75–0% A; 35–39 min: 0% A isocratic; 39–40 min: 0–90% A; 40–50 min: 90% isocratic A. The UV detector was set at 280 nm, and the column was kept at room temperature (25 °C). Caffeine retention time = 13.8 min.	[9]
Colombian Criollo cacao liquors	Composition analysis	An Agilent Infinity 1260 HPLC-PDA, together with a Zorbax Eclipse XBD-C_{18} column (250 mm × 4.6 mm, 5 µm), coupled to an Eclipse XBD-C_{18} precolumn (12.5 mm × 4.6 mm, 5 µm), was used. Injection volume = 0.3 mL; flow rate = 1 mL/min; run time = 33 min; detection wavelength = 280 nm. A MeOH-water gradient was used.	[10]
Coffee			
Cascara (coffee pulp) beverage prepared by cold brewing	Quantification	A Shimadzu RP-HPLC-PDA was used. Separation was performed on the YMC-Triart C_{18} column (150 mm × 3 mm, 3 µm) with the mobile phase comprising solvent A = water 0.3% HCOOH in water and solvent B = MeOH. The optimal gradient programme was as follows: 0 min at 10% B, 6 min at 33% B, 9 min at 33% B, 13 min at 55% B, 18 min at 90% B, and 20 min at 10% B. The mobile phase flow rate, injection volume, and column temperature were 0.4 mL/min, 2.0 µL, and 30 °C, respectively. Detection wavelength was 273 nm.	[11]
Coffee (*Coffea arabica* L.)	Quantification	An Agilent 1260 Infinity II with a C_{18} column (250 mm × 4.6 mm, 5 µm) was used with a mobile phase consisting of acetic acid:ACN (8:2) at a flow rate of 0.7 mL/min; isocratic elution. Injection volume = 10 µL; detection wavelength = 272 nm.	[12]
	Simultaneous detection and quantification	A Hitachi LaChrom HPLC system coupled with a PDA and a Supelcosil C_{18} column (300 mm × 4 mm, 5 µm) was used. Flow rate = 1 mL/min; detection wavelength = 273 nm. The isocratic mobile phase consisted of 40% MeOH in water.	[13]
Coffee beverages	Quantification	A Dionex Ultimate 3000 HPLC (Thermo Fisher) and a BDS C_{18} Hypersil reversed-phase column (250 mm × 4.6 mm, 5 µm) were used. Flow rate = 1 mL/min; injection volume = 10 µL; wavelength = 272 nm; column temperature = 25 °C; isocratic run time = 8 min.	[14]
Coffea canephora cv. Conilon (seeds, husks, and leaves)	Identification and chemical profiling	A Shimadzu RP-HPLC-DAD system and a Phenomenex Luna C_{18} column (250 mm × 4.6 mm, 5 µm) were used. Isocratic elution with 40% MeOH in water at a flow rate of 1 mL/min was used, and the detection wavelength was 272 nm.	[15]
Coffea canephora (flowers and infusions)	Identification and chemical profiling	Identification was performed with an LC–MS, and the quantification was achieved with an HPLC–DAD.	[16]
Coffee containing javamide I/II	Quantification	An Agilent 1260 RP-HPLC was used. A Nova-Pak C_{18} (150 mm × 2.1 mm, 4 µm) and a gradient condition were utilized; 0–5 min: buffer A (20 mM NaH_2PO_4, pH 4.3), 5–18 min: a first gradient from buffer A to buffer B (40% ACN), 18–25 min: a second gradient from buffer B to buffer C (60% ACN) and 5 min: buffer C at the flow rate of 1 mL/min.	[17]
Coffee pulp	Quantification	A Shimadzu 1100 HPLC-DAD was used. A Zorbax ODS column (250 mm × 4.6 mm, 5 µm) coupled with a precolumn (Zorbax SB C_{18}, 4.6 mm × 12.5 mm, 4 mm), and an isocratic mobile phase comprising 30% MeOH in water were employed.	[18]
Coffee (*Coffea arabica* var. *Catimor*) pulp	Quantification	An Agilent 1260 RP-HPLC-DAD was used. The gradient started with 80% phase A (water acidified with 1% acetic acid) and 20% phase B (MeOH), held for 1 min; adjusted to 75% A in B until 3.5 min; then transitioned to 100% B from 4 to 8 min; and returned to 80% A in B, stabilized until 11 min. Injection volume = 10 µL; flow rate = 1 mL/min; column temperature = 30 °C; detection at 272 nm.	[19]
Coffee waste	Multisolvent metabolic profiling	A Vanquish UHPLC system coupled with a Q Exactive^{TM} HF-X Quadrupole-Orbitrap MS and a Hypersil GOLD^{TM} C_{18} column (100 mm × 2.1 mm, 1.9 µm, 40 °C) was used. The mobile phase was 0.1% HCOOH in water (A) and 0.1% HCOOH in ACN (B), with gradient elution over 25 min; flow rate = 0.4 mL/min. Mass spectrometry was performed in +ve and −ve ion ESI modes, with MS1 resolution at 120,000 and data-dependent MS2 (dd-MS2) across a 100–1500 *m/z* range.	[20]

Table 2. *Cont.*

Matrices/Sources	Type of Analysis	HPLC Methods	References
		Coffee	
Conventional, Civet, and Elephant coffees	Identification and quantification	An RP-HPLC-UV using an ACE 100A Excel 2 µm C_{18}-amide column (150 mm × 4.6 mm) was used. A gradient elution was performed using mobile phases A (water with 0.5% acetic acid) and B (ACN with 0.5% acetic acid) as follows: 4% B in A at 0 min, 15% B in A at 10 min, 21% B in A at 14 min, 22% B in A at 25 min, 41% B in A at 34 min, 100% B at 38 min and continued until 48 min, 4% B in A at 51 min, which was used for equilibration for the next 10 min. Injection volume = 10 µL; flow rate = 1 mL/min; column temperature = 33 °C. Detection of analytes was performed at 194–500 nm. Quantification was performed at 260 and 300 nm.	[21]
Dark and green coffee	Quantification	A Waters Alliance 2690 HPLC coupled with a Waters 996 PDA detector was used. A Luna-Cyano column (250 mm × 4.6 mm, 5 µm) was used with gradient mobile phases comprising solvents A (1% TFA in water) and B (ACN). The gradient programme consisted of two linear transitions: the first from A-B (95:5) to A-B (92:8) over 0 to 4 min and the second from A-B (92:8) to A-B (0:100) between 4 and 5 min. This was followed by an isocratic elution with 100% solvent B from 5 to 7 min. A third linear transition was performed from A-B (0:100) to A-B (95:5) from 7 to 8 min, and finally, an isocratic elution of A and B (95:5) from 8 to 11 min. Flow rate = 1.5 mL/min; detection wavelength = 254 nm; column temperature = 22.	[22]
Green and roasted coffee extracts	Simultaneous quantification	An Agilent 1260 HPLC-DAD was used. A Luna Omega Polar C_{18} column (150 mm × 4.6 mm, 5 µm) connected to an Eclipse XDB-C_{18} precolumn (12.5 mm × 4.6 mm, 5 µm) was employed. A gradient elution using the mobile phase consisting of water with 1% acetic acid (solvent A) and ACN (solvent B), and the following programme: 15% solvent B in solvent A for 5 min, and column cleaning and 25% B in A for another 10 min, with the detector at 272 nm. Column temperature = 40 °C; injection volume = 10 µL; flow rate = 1 mL/min.	[23]
Roasted coffee beans	Simultaneous quantification	A Waters RP-HPLC-PDA was used. An XBridge C_{18} column (100 mm × 4.6 mm, 3.5 µm) was eluted with a mobile phase consisting of aqueous ACN (50%) [A] and water acidified with orthophosphoric acid to pH 2.7 [B]. A gradient mode was applied. The concentration of [A] increased to 50% within 50 min; then, returned to 1% over the next 10 min. Flow rate = 1 mL/min; detection wavelength = 320 nm.	[24]
Spent coffee grounds	Identification and quantification	An Agilent 1100 HPLC coupled with a DAD was used. The injection volume was 3 µL. A Gemini C_{18} (250 mm × 3.0 mm, 5 µm) analytical column coupled with a Security Guard C_{18} (4 cm × 3 mm, 5 µm) precolumn was used. The column temperature was set at 40 °C. A gradient elution was conducted using water (A) and MeOH (B), both acidified with 0.1% HCOOH. The gradient programme was as follows: starting with 20% B; 0–10 min: 35% B; 10–15 min: 55% B; 15–20 min: 85% B; subsequent conditioning was then performed for 10 min at the initial 20% B. Flow rate = 0.5 mL/min; detection wavelength = 270 nm.	[25]
	Identification and quantification	A Shimadzu analytical HPLC-DAD was used. A Waters C_{18} Spherisorb ODS2 column (250 mm × 4.6 mm; 5 µm) was used, with the mobile phase comprising 5% HCOOH (A) and MeOH (B), starting with 5% B. Injection volume = 20 µL; a gradient programme was applied with a solvent flow rate of 1.2 mL/min; detection wavelengths = 260, 272, and 320 nm.	[26]
Thai single-origin green coffee beans	Quantification	A Waters Alliance 2695 HPLC equipped with a 2489 UV/Visible detector and a Zorbax Eclipse XDB C_{18} column (150 mm × 4.6 mm, 5 µm) was used. Detection was conducted at 275 nm. The mobile phase consisted of solvent A (0.1% HCOOH in water) and solvent B (0.1% HCOOH in MeOH). A gradient elution was applied as follows: 0–8 min, 25–50% B; 8–9 min, 50% B constant; 9–11 min, 50–25% B.	[27]
Yunnan Arabica coffee	Quantification	HPLC analysis was performed using a Thermo Scientific-VF coupled with a PDA. Injection volume = 10 µL; flow rate = 1 mL/min; column temperature = 35 °C. A GL Sciences InrtSustain-C_{18} column (100 mm × 4.6 mm, 3.5 µm) was used. A gradient elution was performed with the mobile phase comprising water (A) and MeOH (B), both containing 0.1% phosphoric. The composition of the mobile phase varied as follows: 0–10 min, isocratic condition, 10% B; 10–15 min, increased to 60% B; 15–20 min, increased to 90% B; 20 min, isocratic condition, 90% B, then decreased to 10% B in 5 min.	[28]
		Tea	
Assam tea	Chemical profiling	A Waters RP-HPLC-PDA and a SunFire C_{18} RP-HPLC column (250 mm × 4.6 mm, 5 µm), eluted isocratically with 0.1% orthophosphoric acid in water (phase A) and MeOH (phase B), at a 75:25 (A:B) ratio, were used. Flow rate = 0.5 mL/min; run time = 30 min; injection volume = 2 µL; column temperature = 35 °C.	[29]
Black tea	Quantification	The mobile phase comprised solvent A (0.1% *ortho*-phosphoric acid in water) and solvent B (0.1% *ortho*-phosphoric acid in ACN). The HPLC elution gradient was as follows: starting at 10% solvent B, increasing to 11% B over 5 min, then rising to 20% B over the next 5 min. This was followed by a 6-min hold at 20% B, an increase to 30% B over 8 min, and a further rise to 45% B over 8 min. The gradient then came back to 90% B over 2 min, held at 90% B for 2 min, and concluded with a 4-min re-equilibration to the initial 10% B condition. Run time = 40 min; flow rate = 0.4 mL/min; column temperature = 25 °C; injection volume = 10 µL.	[30]
Black tea subjected to blue and red-light withering	Quantification	An Agilent HPLC and a COSMOSIL 5C_{18} AR-II column (250 mm × 4.6 mm, 5 µm) were used. The mobile phase comprised ACN/0.1% HCOOH (95:5) in an isocratic elution programme at a flow rate of 1.0 mL/min.	[31]

Table 2. *Cont.*

Matrices/Sources	Type of Analysis	HPLC Methods	References
		Tea	
Dancong tea	Quantification	A Waters Alliance 2695 coupled with a UV-Vis detector was used. An XSelect HSS C$_{18}$ SB column (250 mm × 4.6 mm, 5 µm) at a flow rate of 0.9 mL/min and a column temperature of 35 °C was employed. Injection volume = 10 µL; detection wavelength = 280 nm. The mobile phase consisted of 100% MeOH (A) and 100% water (B) with 30% A/70% B isocratic elution.	[32]
Dark tea extract	Detection and quantification	A Shimadzu HPLC-UV-Vis and an Agilent TCC$_{18}$ column (250 mm × 4.6 mm, 5 µm) were used. Injection volume = 10 µL; detection wavelength = 280 nm; flow rate = 1 mL/min. The mobile phases consisted of ACN, acetic acid, and water. Mobile phase A was composed of 2% ACN in water (mobile phase A), and 3% acetic acid in ACN (mobile phase B). The gradient elution was initiated with 20% B in A, transitioning to 40% B in A over 32 min. The mobile phase composition returned to 20% B in A for the final 3 min.	[33]
Fenggang green tea	Quality components analysis	Chromatographic analysis was conducted on an Eclassical 3200 HPLC system coupled with a UV detector, and using a Supersil ODS2 column (250 mm × 4.6 mm, 5 µm) at 40 °C. The UV detection wavelength was 278 nm. The mobile phase consisted of A = 0.5% acetic acid, B = ACN, and C = MeOH at a flow rate of 1 mL/min. Gradient elution was performed using the following programme: 0–16 min, 85% A, 10% B, 5% C; 16–20 min, 70% A; 20% B, 10% C; 20–25 min, 72% A, 20% B, 8% C; 25–26 min, 85% A, 10% B, 5% C. The injection volume was 1 µL.	[34]
Flavoured black tea	Chemical profiling	An Agilent 1100 HPLC-DAD system and an Agilent C$_{18}$ column (250 mm × 4.6 mm, 5 µm) at 25 °C were used. The system was run in isocratic mode (water:MeOH:*ortho*-phosphoric acid 75:24.5:0.5) at a flow rate of 0.5 mL/min. Injection volume = 20 µL; detection wavelength = 330 nm.	[35]
Green tea extract	Quantification	An Agilent Infinity RP-HPLC 1260 system equipped with a DAD and a Zorbax Rx-SIL C$_{18}$ column was used. A gradient elution was employed with a mobile phase consisting of 0.1% *ortho*-phosphoric acid in water (solvent A) and 0.1% *ortho*-phosphoric acid in MeOH (solvent B) at a flow rate of 1 mL/min. The gradient programme was as follows: 20% B for 0−5 min, followed by linear increases of 20−24% B from 5−7 min, 24% B from 7−10 min, 24−40% B from 10−20 min, and 40−50% B from 20−25 min. The column temperature was maintained at 25 °C.	[36]
Green tea residues	Quantification	The HPLC-PDA analysis was performed using a Shimadzu P-series system. A C$_{18}$ YMC column (250 mm × 4.6 mm, 5 µm) was used. Column oven temperature = 40 °C; injection volume = 10 µL; flow rate = 1.2 mL/min; run time = 60 min; detection wavelengths = 280 and 320 nm. The mobile phase comprised solvent A (water, pH adjusted to 2.6 with glacial acetic acid and ACN in a ratio of 80:20), and solvent B (100% ACN), and a gradient elution was performed.	[37]
Huangjin green tea	Evaluation of key taste components	An Agilent 1200 HPLC-UV was used. A Diamonsil C$_{18}$ column (250 mm × 4.6 mm, 5 µm) was employed. Flow rate = 1 mL/min; column temperature = 35 °C; injection volume = 10 µL; detector wavelength = 231 nm. The mobile phases contained solution A (100% ACN) and B (0.1% HCOOH), and the gradient elution was as follows: 4–6% solvent A from 0 to 15 min, 6–12% solvent A from 15 to 30 min, 12–18% solvent A from 30 to 58 min.	[38]
Huizhou rock tea and Wuyi rock tea	Quality component analysis	A Waters Alliance 2695 HPLC-UV was used. An XSelect HSS C$_{18}$ column (250 mm × 4.6 mm, 5 mm) maintained at 35 °C was employed. Injection volume = 10 µL; flow rate = 0.9 mL/min; detection wavelength = 280 nm. An isocratic elution with equal volumes of MeOH and water.	[39]
Light-fermented sour tea obtained from the Yunnan large-leaf variety of sun-dried green tea	Quantification	The HPLC analyses were performed on a Waters 2489 HPLC system using a Waters XBridge® C$_{18}$ column (250 mm × 4.6 mm, 5 µm). The mobile phase was 0.4% phosphoric acid in water (A) and MeOH (B) with an isocratic elution with 25% B in A. Detection wavelength = 280 nm; flow rate = 0.8 mL/min; the column temperature = 30 °C; injection volume = 10 µL.	[40]
Longjing tea infusion	Quantification	A Dionex Ultimate 3000 UHPLC system (coupled with an MS) with an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 µm) at a temperature of 35 °C and a mobile phase consisting of 0.1% HCOOH in water (solvent A), and ACN with 0.1% HCOOH (solvent B) was used. High-resolution full-scan data (FWHM = 70,000) were acquired in both ESI (+) and ESI (−) modes, covering a mass range of 100–1000.	[41]
Pu-erh tea	Quality assessment	UHPLC-Orbitrap-MS/MS analysis was conducted using a Nexera X2 Shimadzu UHPLC system coupled with an AB Sciex TripleTOF 5600 mass spectrometer, a Waters HSS T3 column, and a mobile phase consisting of water (A) and MeOH (B). The gradient programme was as follows: 0–5 min: 2% B; 5–7 min: 2–13% B; 7–21 min: 13–21% B; 21–23 min: 21–30% B; 23–28 min: 30–100% B; 28–30 min: 100% B. ESI in +ve and −ve ion modes, a mass range of 100–1200 *m/z* for TOF-MS scan, and 50–1200 *m/z* for TOF-MS/ MS scan were used. Metabolite identification utilized databases such as The Human Metabolome Database (HMDB), Lipid Maps database, Metlin database, and a self-built database.	[42]

Table 2. *Cont.*

Matrices/Sources	Type of Analysis	HPLC Methods	References
		Tea	
Tea leaf extract	Chemical profiling using a green analytical method	A Kromasil Eternity C_{18} column (50 mm × 2.1 mm, 2.5 µm) and a Kinetex C_{18} column (150 mm × 4.6 mm, 5 µm) were used. Analyses were performed on a Shimadzu Nexera UHPLC system coupled with a DAD, and a Shimadzu Prominence HPLC system hyphenated with a DAD. Separation was conducted using a binary mobile phase of dimethyl carbonate/water (2:98) with a flow rate of 0.25 mL/min. The column thermostat was set at 30 °C, and the autosampler temperature was set at 5 °C. Injection volume = 10 µL; detection wavelength = 273 nm.	[43]
Tea infusion	Quantification	An RP-HPLC-UV system and an SVEA C_{18} Core Shell column (150 mm × 3.0 mm, 2.6 µm) were used. A water–MeOH (60:40) mobile phase, 275 nm detection, 1 mL/min flow rate, 40 °C column temperature, an injection volume of 100 µL, and a total run time of 5 min were applied.	[44]
Wild tea *Camellia yungkiangensis* var. *yuanbaoshanica* Dung bis & Hakoda	Characterization	UPLC/ESI-Q-TOF/MS analysis. An Agilent 1290 Infinity UPLC coupled with a hybrid ESI-QTOF Premier mass spectrometer was used. An ACQUITY UHPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm) column and a mobile phase comprising A (0.1% HCOOH/water) and eluent B (ACN), and a linear gradient of 95% A over 5 min, 95 to 75% A over 16 min, 75 to 0% A over 7 min, and 0% A over 2 min were used. Flow rate = 0.3 mL/min; injection volume = 2 µL; column temperature = 45 °C. Detection of the eluent was performed in −ve ESI mode over a *m/z* ratio range of 50 to 1200. The mass spectrometer was operated under the following parameters: desolvation temperature at 375 °C; desolvation nitrogen gas flow at 850 L/h; capillary voltage at 4 kV; cone voltage at 65 eV; and collision energy at 135 V. Nebulization was achieved using nitrogen gas at a pressure of 40 psi. The MS system was calibrated using sodium formate, and raffinose was used as the lockmass. Metabolites were identified based on accurate masses and retention times relative to authentic standards.	[45]
		Other plant sources	
Cucurbita pepo L. seeds	Identification and quantification	An analytical HPLC-DAD and an ODS Hypersil (250 mm × 4.6 mm, 5 µm) column coupled with a LiChrospher 100 RP-18, 5 µm (4 mm × 4 mm) guard column were used. The gradient mobile phase was composed of MeOH–acetic acid–water (5:1:44) as solvent A and EtOH–acetic acid–water (45:1:4) as solvent B.	[46]
Ilex guayusa Loes leaves	Quantification	A Waters HPLC-UV with a Symmetry C_{18} column (250 mm × 4.6 mm, 5 µm) was used. The mobile phase consisted of 0.1% HCOOH in water (solvent A) and ACN (solvent B). The gradient elution programme was: 0−8 min 0−15% B; 8−15 min 15− 90% B, and 15−23 min 90−7.5% B. Flow rate = 1.0 mL/min; injection volume = 10 µL; detection at 273 nm; run time = 23 min. For HPLC-MS analysis, a Waters Acquity I-Class UPLC coupled to tandem mass spectrometry (UPLC-MS/MS) was used with a Waters Acquity UPLC HSS T3 C_{18} column (100 mm × 2.1 mm, 1.8 µm) and a mobile phase comprising 0.1% HCOOH in water (solvent A) and ACN 100% (solvent B). Flow rate = 0.5 mL/min; injection volume = 10 µL. The gradient programme was: 0.0−1.0 min, 1−99% B; 1.0−12.0 min, 99−100% B; 12.0−14.0 min, 100% B; 14.0−14.5 min, 100−1% B; and 14.5− 16.5 1% B. Electrospray ionization (ESI) was performed in +ve ion mode for MS data acquisitions.	[47]
		Caffeine-containing products	
Analgesic tablets	Simultaneous quantification	An RP-HPLC-UV was used. Three different columns, XBridge® BEH C_{18} 2.5 µm (150 mm × 3.0 mm), XBridge® BEH Amide 2.5 µm (150 mm × 3.0 mm), and Kinetex 2.5 µm phenyl–hexyl 100 Å (100 mm × 3.0 mm) were used. The mobile phase consisted of water and MeOH. Isocratic as well as gradient elution was applied. The run time was 30–40 min.	[48]
Beverages, pharmaceuticals, and environmental water samples	Separation and trace determination	A high-temperature Agilent 1200 HPLC, equipped with a multiple wavelength detector (MWD), was used. A Zir-Chrom-PBD column (150 mm × 4.6 mm, 5 µm) eluting with an ACN-water gradient was employed. Flow rate = 1 mL/min; injection volume = 10 µL; detection wavelength = 220 nm. The column temperatures were: for the 100% water mobile phase: 100, 110, 120, 130, and 140 °C; for the 20% ACN mobile phase: 40, 50, 60, 70, 80, 90, 100, and 110 °C; and for the 30% ACN mobile phase: 30, 40, and 50 °C.	[49]
Caffeine-supplemented fungal growth medium	Degradation [by *Desarmillaria tabescens* (Scop.) Emel] study by transcriptomics	An Agilent HPLC and a Yuexu Ulimate® Plus C_{18} column (100 mm × 2.1 mm, 1.8 µm) were used. The mobile phase comprised solvent A (water with 0.1% phosphoric acid) and solvent B (ACN) with a gradient elution: 0–15 min (95% A → 90% A), 15–40 min (90% A → 80% A), 40–50 min (80% A → 65% A), 50–55 min (65% A → 15% A), 55–70 min (15% A → 5% A). Flow rate = 1 mL/min; the column = 34 °C; injection volume = 15 µL; detection wavelength = 280 nm.	[50]
Electronic cigarette oil	Quantitative determination	An HPLC-MS using a mixed-functional column, FMD Comixsil ACRP column (100 mm × 2.1 mm, 3.0 µm) at 30 °C was used. The mobile phase comprised ACN and a 0.1% HCOOH solution containing 10 mmol/L ammonium formate and a gradient elution was used. Flow rate = 0.2 mL/min; injection volume = 10 µL. Mass spectrometric detection was conducted with ESI in +ve ion with multiple reaction monitoring (MRM).	[51]

Table 2. *Cont.*

Matrices/Sources	Type of Analysis	HPLC Methods	References
		Caffeine-containing products	
Energy drinks	Detection and quantification	An Agilent 1290 Infinity II UHPLC with an Agilent 6545 Q-TOF HRMS detector was used. Agilent Jet Stream (AJS) ESI mode was used, and the source parameters were gas (N_2) temperature 320 °C; gas flow 8 L/min; nebulizer pressure 35 psi; sheath gas (N_2) temperature 350 °C; sheath gas flow 11 L/min; VCap +3.5 kV; nozzle voltage 1000 V; fragmentor voltage 175 V. The *m/z* range was set to 100–3000 with a scan rate of 1 spectra/sec. An autosampler with 2 µL injections was used, and detection was in positive ion mode. Caffeine quantitation was based on 8 standard calibration curves ($R^2 > 0.997$). A Chromolith® HighResolution® HPLC monolithic column (C_{18}, 100 mm × 4.6 mm) was used. A gradient elution was performed with water-ACN.	[52]
	Quantification	A Waters Acquity H-Class UPLC system hyphenated with a PDA was used. A Waters UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 µm), eluting with a mobile phase of MeOH and 0.1 M phosphate buffer (pH 2.15) at a 40.7:59.3 ratio with 0.4 mL/L triethylamine, was employed. Flow rate = 0.24 mL/ min; column temperature = 58.9 °C; injection volume = 1.0 µL; detection wavelength = 272.4 nm.	[53]
Energy and cola drinks	Identification and quantification	An Agilent 1260 UHPLC system hyphenated with a DAD was used. A high-resolution silica-based C_{18} monolithic column (250 mm × 4.6 mm), eluting with a gradient elution. The initial conditions were 10% ACN in water (both containing 0.1% HCOOH), followed by a gradient running to 60% ACN (containing 0.1% HCOOH) at 0.47 min. Before repeat injections, the column was re-equilibrated with the initial mobile phase. Flow rate = 4 mL/min; injection volume = 5 µL; detection wavelength = 254 nm.	[54]
Energy and sports drinks	Detection and quantification	An Agilent 1260 Infinity Rapid Resolution Liquid Chromatography system coupled with a DAD was used. A Poroshell 120 EC-C_{18} (50 mm × 3.0 mm, 2.7 µm) analytical column was employed. Column oven temperature = 25 °C; flow rate = 1 mL/min; injection volume = 2.5 µL. The mobile phase was composed of phosphate buffer (pH = 3.8) and ACN (85/15) for the isocratic elution. The detector was set at 230, 260, and 275 nm.	[55]
Fixed-dose combination tablet	Simultaneous determination	An Agilent 1260 Infinity Series LC with a 1260 Quaternary Pump coupled with a variable wavelength detector was used. The separation was conducted at room temperature using a Zorbax SD-C_8 column (150 mm ×4.6 mm, 5 µm). The mobile phase, ACN: phosphate buffer (35: 65), pH 5, was filtered through a 0.45 µm membrane filter and degassed ultrasonically for 15 min. Flow rate = 1 mL/min; detection wavelength = 220 nm; injection volume = 10 µL.	[56]
Green tea drinks	Elution behaviour study	A Shimadzu Prominence RP-UHPLC-DAD with a Shim-pack Velox C_{18} RP-HPLC column (150 mm × 4.6 mm, 2.7 µm) maintained at 40 °C was used. Flow rate = 1 mL/min; detection wavelength = 232 nm; injection volume = 10 µL. The mobile phase comprised ACN and water.	[57]
Intravenous fluids	Stability testing	An Agilent 1220 Infinity HPLC coupled with a UV detector at 273 nm was used. A Zorbax SB-C_8 analytical column (150 mm × 4.6 mm, 5 µm) was used with a mobile phase consisting of phosphate buffer at pH 6.8 with a total phosphate concentration of 10 mM, prepared from KH_2PO_4 and Na_2 HPO_4, mixed with ACN in a ratio of 82:18. Injection volume = 20 µL; flow rate = 1 mL/min.	[58]
Lemon-flavoured beverages enriched with black or green tea extracts	Greenness assessment	A Waters Alliance 2695 HPLC-DAD was used. An isocratic mobile phase of 15% EtOH in water at 1 mL/min was employed. A core–shell Kinetex® C_{18} column (150 mm × 3 mm, 2.6 µm) at 25 °C was used. Injection volume = 3 µL; detection wavelength = 273 nm; run time = 20 min.	[59]
Nanoparticle formulation	Simultaneous Estimation	A Waters 1525 Binary HPLC Pump, coupled with a manual injector and a Waters 489 UV/visible detector, and a Varian C_{18} column (250 mm × 4.6 mm, 5 µm) was used. The initial mobile phase comprised ACN, purified water, and 1.25×10^{-4} M *ortho*-phosphoric acid (pH 3.78) in the ratio of 55:45:0.05. The flow rate was 0.9 mL/min, and the injection volume was 20 µL.	[60]
Pharmaceutical dosage forms	Separation and quantification	An RP-HPLC-DAD using a C_{18} RP HPLC column (250 mm × 4.6 mm, 5 mm).	[61]
Pharmaceutical tablets	Simultaneous quantification	A Waters e2695 system equipped with a UV detector and a Shimadzu LC-10 series system for method verification was used. A C_{18} column (150 mm × 4.6 mm, 5 µm) and a mobile phase consisting of 30% MeOH in phosphate buffer (pH 4.0) were employed. Flow rate = 1 mL/min; detection wavelength = 215 nm; injection volume = 20 µL.	[62]
Tea and carbonated beverages	Isolation and quantification	An RP-HPLC using a C_{18} RP-ODS column (250 mm × 4.6 mm, 5 µm) and a mobile phase containing 30:70 MeOH and water. Column temperature = 40 °C; flow rate = 1 mL/min; injection volume = 20 µL; detection at 278 nm.	[63]
TrichoConcept line	Degradation study	An RP-HPLC-UV analysis using a MeOH-water gradient.	[64]
Weight-loss teas	Estimation	A Shimadzu LC-2030 Plus Prominence-iRP-HPLC with a UV detector set at 275 nm was used. Flow rate = 0.3 mL/min; injection volume = 5 µL; column temperature = 40 °C; run time = 8 min. A Shimadzu Nexcol C_{18} 5 µm 50 mm × 3.0 mm column was used with a column guard. The mobile phase was composed of 20% MeOH in water with 0.1% HCOOH and 5 mM ammonium formate, and an isocratic run was performed.	[65]

Table 2. *Cont.*

Matrices/Sources	Type of Analysis	HPLC Methods	References
Water and wastewater			
Groundwater	Detection and quantification	An 844 UV/VIS compact Ion Chromatography equipped with a Hichrom-Altima C_{18} HPLC column (250 mm × 4.6 mm, 5 µm) was used. Flow rate = 1 mL/min; injection volume = 250 µL; detection wavelength = 270 nm. The mobile phase comprised ACN and water (pH = 3.0) at 1:1, and it was run isocratically.	[66]
Lake water (Karst Lake Punta Laguna of the Yucatán peninsula)	Quantification	An Agilent 1290 Infinity HPLC-DAD was used with a Zorbax C_{18} column (150 mm × 4.6 mm, 5 µm). An isocratic run was conducted with 35% MeOH in water at a flow rate of 1 mL/min and detection at 295 mm.	[67]
Water and wastewater	Trace pharmaceutical monitoring	A UHPLC system coupled with quadrupole mass spectrometry with a HESI (heated electrospray ionization) ion source was used. The analysis utilized a Chromolith® Performance RP-18e column (100 mm × 4.6 mm) at 20 °C. An isocratic flow rate of 0.5 mL/min was applied using a mobile phase consisting of eluent A (0.1% HCOOH in water) and eluent B (ACN), mixed in a 1:1 ratio. Injection volume = 20 µL.	[68]
Biological samples			
Rat plasma	Plasma concentration	An Agilent 1100 Series HPLC system coupled with a DAD was used. A Chromasil C_{18} analytical column (250 mm × 4.6 mm, 5 µm), linked to a Chromasil C_{18} guard column (20 mm × 4.6 mm, 10 µm), was employed at a column temperature of 30 °C. A mobile phase consisting of (A) 0.1% HCOOH in water and (B) ACN containing 0.1% HCOOH was used with a flow rate of 1.0 mL/min. The injection volume was 20 µL and the gradient elution was as follows: 10% B from 0 to 5 min, increased to 15% B from 5 to 7 min, held at 15% B from 7 to 10 min, ramped to 25% B from 10 to 20 min, increased to 40% B from 20 to 25 min and maintained at 40% B until 30 min. The gradient was then returned to the initial composition (10% B) within 1 min and re-equilibrated for 5 min before the next injection. A wavelength of 280 nm was used to monitor the effluent.	[69]
Urine sample	Drug metabolism study	An HPLC–ESI–MS/MS and internal standards labelled with stable isotopes were used to measure urinary caffeine and caffeine metabolites.	[70]

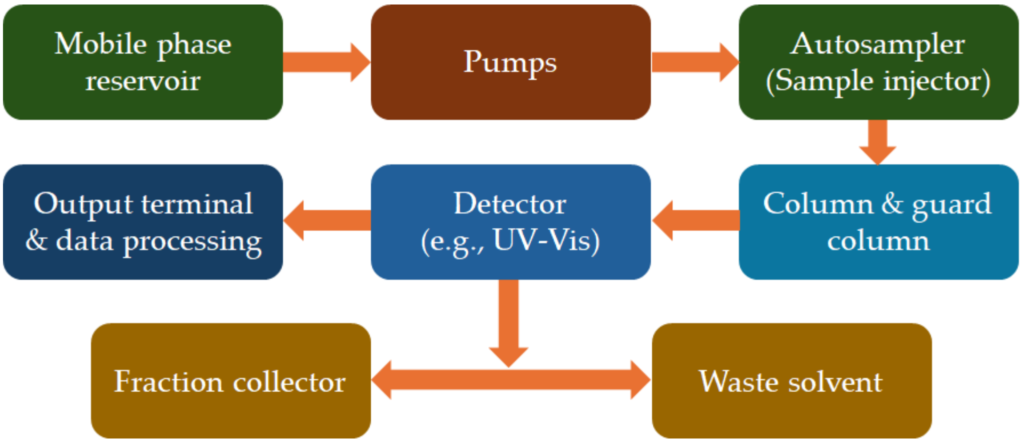

Figure 2. Schematic presentation of components of a conventional HPLC system.

2.1. Cocoa

Cocoa is a powder produced from fermented, dried, and roasted beans of the *Theobroma cacao* L. tree [7–10,71]. It contains caffeine among various other bioactive compounds. Cocoa, predominantly produced in Africa and South America, has been widely used in the food industry, primarily through chocolate and as an additive to several food products, e.g., cookies, ice creams, and cakes [7]. An integrated caffeine extraction and isolation protocol for cocoa bean shells was reported [7], which was based on pressurised liquid extraction using an ethanol (EtOH)-water gradient and solid-phase extraction (SPE) utilizing Phenomenex Sepra$^{\text{TM}}$ C_{18}-E (50 µm, 65 Å; Phenomenex) and Waters PoraPak Rxn RP Bulk (80 µm) coupled with a Waters UPLC-PDA analysis using a UPLC column and ACN-water mobile phase at a slightly elevated temperature at 40 °C (Table 1). Process optimization was accomplished with COSMO-RS-based computational analysis. This protocol aligns with the principles of green chemistry, green analytical chemistry, and green sample preparation by promoting process automation, enabling real-time analysis, reducing the number of steps, minimizing solvent consumption, and decreasing waste generation. Similarly, a much simpler HPLC-PDA method using a conventional HPLC column (150 mm × 4.6 mm, 5.0 µm) at 45 °C and an ACN-water mobile phase was reported for the extraction optimization and quantification of caffeine in cocoa bean shells [8]. Cocoa hulls are also a good source of caffeine, which was quantified by a simple RP-HPLC method at room temperature (Table 1) [9]. As part of an RP-HPLC-based composition analysis using an Agilent Infinity 1260 HPLC-PDA, together with a Zorbax Eclipse XBD-C_{18} (250 mm × 4.6 mm, 5 μm) and a MeOH-water gradient, caffeine was quantified in Colombian Criollo cacao liquors (Montana, Olivo, and Trinitario varieties) [10].

2.2. Coffee

One of the major sources of caffeine is obviously coffee, which is a globally consumed beverage popular for its sensory attributes and bioactive compounds, including caffeine, chlorogenic acid, and trigonelline, and a good number of publications were published about the HPLC-based detection and quantification of caffeine in coffee and coffee-based consumer products last year (Table 1) [11–28]. Cascara (Spanish for "husk") is a tea-like infusion made from the dried skins and pulp of coffee cherries, which are usually discarded as a by-product of coffee production. Muriqi et al. [11] quantified caffeine in cascara beverage (prepared by cold brewing) using a Shimadzu RP-HPLC-PDA system and a YMC-Triart C_{18} column (150 mm × 3 mm, 3 μm) with a gradient mobile phase comprising water acidified with 0.3% HCOOH and MeOH. RP-HPLC, using a UV or a PDA detector, was used for the detection and quantification of caffeine in coffee from *Coffea arabica* L. [12–14], *C. canephora* cv. Conilon (seeds, husks, and leaves) [15] and *C. canephora* (flowers and infusions); the mobile phase was either isocratic or gradient using ACN-water or MeOH-water [16] (Table 1). Effects of roasting degree and grinding size on caffeine content and sensorial quality of Ethiopian Arabica coffee (*C. arabica* L.) were assessed by HPLC [12] using RSM-based optimisation [72]. It was found that the higher caffeine content ranged from 1.30 to 1.53 g/100 g, with finer powder and lighter roasts, which was due to reduced thermal degradation and increased extraction efficiency. Similar HPLC methods were reported for the simultaneous detection and quantification of major bioactive components in coffee and coffee beverages [13,14], where a long column (250–300 mm) with a diameter of 4.0–4.6 mm and 5 μm particle size at room temperature was used with an isocratic mobile phase comprising MeOH-water (flow rate = 1 mL/min; detection wavelength = 272 or 273 nm).

As a part of an investigation of the health effects of coffee in rats fed with a high-fat diet, caffeine was quantified from the coffee containing javamide I/II using a simple RP-HPLC method (Table 1) [17]. In this method, a buffer (20 mM $NaH_2\ PO_4$, pH 4.3) was used in the gradient mobile phase along with ACN, and a Nova-Pak C_{18} column with a shorter diameter (150 mm × 2.1 mm, 4 μm) was employed. Caffeine (12 mg), javamide I (0.048 mg), javamide II (0.36 mg), and chlorogenic acids (10 mg) were quantified in 30 mL of coffee containing javamide I/II. Additionally, an LC-MS method using an Agilent Eclipse Plus RP C_{18} UHPLC column (150 mm × 2.1 mm, 1.8 μm). The chemical composition of coffee (ca. 700 compounds including caffeine) was determined using a two-tier ID approach; in tier 1, peak pairs were matched against an in-house standards metabolite library based on triple parameters (accurate molecular masses, retention times, and MSM/MS spectra), while in tier 2, the remaining peak pairs were identified using a linked identity library which includes well over 7000 pathway related metabolites. The quantification of caffeine in coffee pulps from two varieties of *C. arabica* was accomplished with RP-HPLC methods using a standard RP C_{18} column and acidified MeOH-water mobile phase [18,19]. A UHPLC-HRMS/MS protocol, along with a molecular networking approach, was described for multisolvent metabolite profiling of coffee waste [20]. It can be noted that coffee processing wastes include defective green beans and spent coffee grounds, which are underutilized by-products, yet rich in bioactive compounds with potential uses in nutraceuticals and cosmetics. In this study, metabolites from Arabica and Robusta wastes were extracted using five solvents of varying polarity: EtOH, ethyl acetate (EtOAc), toluene, xylene, and *n*-hexane. Quantification of chlorogenic acid and caffeine was performed using HPLC-DAD, while comprehensive metabolite profiling was conducted via UHPLC-HRMS/MS and GC-MS. Molecular networking visualized structurally related metabolite clusters and illustrated solvent- and material-associated distributions [20]. This study demonstrated that coffee waste could be a rich source of caffeine and other bioactive molecules, and EtOH emerged as the most suitable solvent for extracting caffeine from coffee waste.

The use of a standard RP-HPLC system, employing an acidified ACN-water mobile phase, to determine caffeine in various coffee varieties, e.g., conventional, Civet, and elephant coffee [21], dark and green coffee [22], roasted coffee beans [23,24] are well documented in the literature (Table 1). In addition to a standard RP C_{18} column, a C_{18}-amide [21] or a cyano column [22] could also be used in these analyses.

Spent coffee grounds are considered a rich source of caffeine and a high-value-added biomass [73,74]. Solid waste spent coffee grounds are obtained in the process of producing coffee products. Spent coffee grounds have been widely applied in food, pharmaceutical, cosmetic, energy, and materials sectors. Pompei et al. [25] reported an RP-HPLC-DAD method for the identification and quantification of caffeine in spent coffee grounds, where a Gemini C_{18} (250 mm × 3.0 mm, 5 μm) analytical column coupled with a Security Guard C_{18} (4 cm × 3 mm, 5 μm) precolumn, maintained at 40 °C, was used. A gradient elution was conducted using water (A) and MeOH (B), both acidified with 0.1% HCOOH. The gradient programme was as follows: starting with 20% B, between 0 and 10 min it increased to 35% B; between 10 and 15 min it increased to 55% B; between 15 and 20 min it increased to 85% B; subsequent conditioning was then performed for 10 min at the initial 20% B. The flow rate was 0.5 mL/min. The wavelength used was 270 nm. In this study, the extraction of caffeine and other bioactive components from spent coffee grounds was accomplished by ultrasound-assisted extraction using water-based binary deep eutectic solvents. Earlier, a similar HPLC method was also reported by Fernandes et al. [26].

Coffee from different geographical origins, e.g., Thai single-origin green coffee beans [27] and Yunnan Arabica coffee [28], was studied for its caffeine content using HPLC methods. The columns used were 100–150 mm in length, 4.6 mm in diameter, and 3.5–5 µm in particle size, and the mobile phase was based on an acidified MeOH-water mixture; ether 0.1% HCOOH or 0.1 phosphoric acid was used for acidification of the mobile phase. The study with Yunnan Arabica coffee [28] aimed at identifying key aroma compounds formation during the roasting process using molecular sensory science approaches, in addition to HPLC. It is worth noting that Yunnan Province is the major coffee cultivation region in China, and coffee beans produced in Yunnan account for approximately 99% of the total national coffee production [75].

2.3. Tea

Tea [*Camelia sinensis* (L.) Kuntze], the most popular beverage in the world because of its attractive aroma, exceptional taste, health-promoting effects, and pharmaceutical potential, is another major source of caffeine [76,77]. *Camellia sinensis* is a species of evergreen shrub or small tree in the flowering plant family Theaceae and is widely distributed in the Far East and Southeast Asia, with China being the country where the consumption of tea began during the Shang dynasty more than 4000 years ago. Besides, China, Bangladesh, India, and Sri Lanka are three major tea-growing countries. Its leaves, twigs, and stems are used to produce tea. HPLC-based methods have been used routinely for the identification and quantification of caffeine in different tea samples, and many of these methods were published last year (Table 1) [29–45].

Assam tea is a type of black tea produced from *C. sinensis* var *assamica* in Assam, India, the world's largest tea-growing region by production. The tropical climate in Assam contributes to a unique malty taste of Assam tea, a feature for which this tea is well known. A Waters RP-HPLC-PDA and a SunFire C_{18} RP-HPLC column (250 mm × 4.6 mm, 5 µm), eluted isocratically with 0.1% *ortho*-phosphoric acid in water (phase A) and MeOH (phase B), at a 75:25 (A:B) ratio, were used in the chemical profiling of Assam tea, and to quantify caffeine in it [29]. The flow rate was 0.5 mL/min, the run time was 30 min, the injection volume was 2 µL and the column temperature was maintained at 35 °C. A similar RP-HPLC method, but using an acidified ACN-water as the mobile phase, a longer run time (40 min), and the column temperature of 25 °C was used for quantifying caffeine in black tea samples [30]. Black tea subjected to blue and red-light withering was analysed, and the quantification of caffeine was accomplished using an Agilent RP-HPLC and a COSMOSIL $5C_{18}$ AR-II column (250 mm × 4.6 mm, 5 µm), eluted isocratically with ACN/0.1% HCOOH (95:5) [31]. This study demonstrated that red-light withering could increase the aroma content and diversity, and blue-light withering could increase the fresh sweetness of black tea. The reduction of caffeine, catechins, and flavonoids by blue light could be due to the downregulation of key genes (chalcone synthase and chalcone isomerase) in the catechin synthesis pathway by blue light.

Dancong tea is a type of Oolong tea from Fenghuang Shan (Phoenix mountains, 350–1560 m above sea level) in Guangdong province, China [32]. Niaoji Shan (1560 m) is the highest mountain, while Wudong Shan (1391 m) is the main Dancong production site. Dancong tea comes with different flavours, e.g., Magnolia flavour, Pomelo blossom flavour, or Honey-Orchid flavour. The flavour components and aroma compounds of fresh leaves from four Dancong ancient tea gardens, Baixiang, Da′an, Xialiao, and Zimao, at different altitudes, were determined by HPLC (Table 1) and GC–MS [32]. Low altitude tea gardens, Zimao had a higher caffeine content (5.21%) and an astringency index of 0.82 compared to other high-altitude tea gardens, which had a more bitter taste and astringent sensation. Yu et al. [33] reported an RP-HPLC protocol using a Shimadzu HPLC-UV-Vis and an Agilent TCC_{18} column (250 mm × 4.6 mm, 5 µm) for the analysis of caffeine in dark tea extracts [33]. In this HPLC analysis, the injection volume was 10 µL, the detection wavelength was set at 280 nm, and the flow rate was 1 mL/min. Mobile phase A was composed of ACN and water (2:98), and mobile phase B of ACN and acetic acid (84:3). The gradient elution was initiated with 80% A and 20% B, transitioning to 60% A and 40% B over 32 min. The mobile phase composition returned to 80% A and 20% B for the final 3 min. This study demonstrated that phytochemicals, including caffeine, from a fractionated dark tea water extract enhanced digestive enzyme inhibition, antioxidant capacities, and glucose-lipid balance. Caffeine was substantially enriched in the 50% aqueous EtOH fraction, with a concentration of 17.13%, representing a 3.6-fold increase compared to the crude water extract. The water extract and 95% EtOH fraction had lower concentrations of caffeine.

Fenggang green tea is known to be rich in zinc, selenium, and other trace elements, in addition to organic bioactive molecules like caffeine. For the key quality components analysis of three Fenggang green tea samples from Fenggang County, namely Liumingxing green tea (high-zinc green tea) with elevated zinc content, Cuidianxiang green tea (medium-zinc green tea) containing moderate zinc levels, Linxiankang green tea (low-zinc green tea) characterized by reduced zinc concentration, were used [34]. The contents of amino acids, caffeine, and catechins were determined by RP-HPLC, conducted on an Eclassical 3200 HPLC system coupled with a UV detector, and using a Supersil ODS2 column (250 mm × 4.6 mm, 5 µm) at 40 °C. The UV detection wavelength was 278 nm. The mobile phase consisted of 0.5% acetic acid (A), ACN (B), and MeOH (C) at a flow rate of 1 mL/min. Gradient elution was performed using the following programme: 0–16 min, 85% A, 10% B, 5% C; 16–20 min, 70% A; 20% B, 10% C; 20–25 min, 72% A, 20% B, 8% C; 25–26 min, 85% A, 10% B, 5% C. The injection volume was 1 µL. Similarly, chemical profiling and quantification of caffeine were conducted with flavoured black tea [35], green tea extract [36], and green tea residues [37] using RP-HPLC-PDA methods employing various RP columns and MeOH-water or ACN-water mobile phases (Table 1).

The key taste components in Huangjin green tea, which is a premium variety of Chinese oolong tea traditionally from Anxi in Fujian province, were evaluated by RP-HPLC, using an Agilent 1200 HPLC-UV and a Diamonsil C_{18} column (250 mm × 4.6 mm, 5 µm) at a slightly elevated temperature of 35 °C [38]. In this study, the mobile phases contained solution A (100% ACN) and B (0.1% HCOOH), and the gradient elution was as follows: 4–6% solvent A from 0 to 15 min, 6–12% solvent A from 15 to 30 min, 12–18% solvent A from 30 to 58 min with a flow rate of 1 mL/min, an injection volume of 10 µL and detector wavelength set at 231 nm. In another study [39], quality components, including caffeine content, were analysed by a Waters Alliance 2695 HPLC-UV using a conventional 250 mm long RP-HPLC column, eluted with a MeOH-water mobile phase at a flow rate of 0.9 mL/min (Table 1). Caffeine was quantified in light-fermented sour tea obtained from the Yunnan large-leaf variety of sun-dried green tea using RP-HPLC [40], while UPLC or UHPLC was used for the analysis of caffeine in Longjing tea infusion [41] and Pu-erh tea [42] (Table 1). UHPLC-Orbitrap-MS/MS was used for the analysis of Pu-erh tea [42], which is a fermented, aged tea originating from China's Yunnan province, celebrated for its deep, earthy flavour and potential health benefits like improved digestion and weight management [78]. Pu-erh tea is available in raw (Sheng) or ripened (Shou) forms, and it is often compressed into cakes or sold loose, developing more complex, mellow notes over time, like fine wine.

Chemical profiling of tea leaf extract using a green analytical method involving a Shimadzu Nexera UHPLC system coupled with a DAD, and a Shimadzu Prominence HPLC system hyphenated with a DAD, and shorter columns, e.g., a Kromasil Eternity C_{18} column (50 mm × 2.1 mm, 2.5 µm) and a Kinetex C_{18} column (150 mm × 4.6 mm, 5 µm) was reported [43], while samples of tea infusion were analysed for quantifying caffeine by an RP-HPLC-UV system and an SVEA C_{18} Core Shell column (150 mm × 3.0 mm, 2.6 µm) [44].

Camelia sinensis is not the only source of tea, but other *Camelia* species or varieties are also in use as tea, e.g., wild tea *Camellia yungkiangensis* Dung bis & Hakoda var. *yuanbaoshanica,* which is endemic to China and widely distributed in It is distributed in Guizhou, Sichuan, and Yunnan provinces [45]. However, the content of caffeine is generally much lower in these species than it is in *C. sinensis*. In *C. yungkiangensis*, low-caffeine and high-theobromine content was determined by an LC-MS analysis [45]. An Agilent 1290 Infinity UPLC coupled with a hybrid ESI-QTOF Premier mass spectrometer was used. An Acquity UHPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm) column and the mobile phase comprising A (0.1% HCOOH/water) and eluent B (ACN), and a linear gradient of 95% A over 5 min, 95 to 75% A over 16 min, 75 to 0% A over 7 min, and 0% A over 2 min were employed. The flow rate was 0.3 mL/min, the injection volume was 2 µL, and the column temperature was maintained at 45 °C. Detection of the eluent was performed in negative ESI mode over a *m/z* ratio range of 50 to 1200. The mass spectrometer was operated under the following parameters: desolvation temperature at 375 °C; desolvation nitrogen gas flow at 850 L/h; capillary voltage at 4 kV; cone voltage at 65 eV; and collision energy at 135 V. Nebulization was achieved using nitrogen gas at a pressure of 40 psi. The MS system was calibrated using sodium formate, and raffinose was used as lockmass. Metabolites were identified based on accurate masses and retention times relative to authentic standards.

2.4. Other Plant Sources

Caffeine is not only present in cocoa, coffee, and tea, but several other plants also biosynthesize caffeine, e.g., *Cucurbita pepo* L., and *Ilex guayusa* Loes. [46,47]. A conventional RP-HPLC and an ODS Hypersil column were used with a gradient mobile phase consisting of acidified MeOH-water for the analysis of caffeine in *C. pepo* [46], while a UPLC system was used for detecting and quantifying caffeine in *Ilex guayusa* Loes. [47] (Table 1); the latter used UPLC-MS/MS.

2.5. Caffeine-Containing Products

Several products, e.g., energy drinks, cosmetic products, and pharmaceutical preparations, contain caffeine (Table 1). HPLC-based methods are routinely used for the quantification of caffeine and in quality control protocols. During 2025, at least 21 publications described different HPLC methods for the analysis of caffeine in various caffeine-containing products [48–68].

Some analgesic tablets, e.g., Panadol Extra Advance, Anadin Extra, and Syndol, contain caffeine together with other analgesic compounds, e.g., aspirin, ibuprofen, and paracetamol [48,79]. Lener et al. [48] reported a simple RP-HPLC-UV-based method for the simultaneous determination and quantification of caffeine in analgesic tablets; they used three different columns, XBridge® BEH C_{18} 2.5 µm (150 mm × 3.0 mm), XBridge® BEH Amide 2.5 µm (150 mm × 3.0 mm), and Kinetex 2.5 µm phenyl–hexyl 100 Å (100 mm × 3.0 mm) and a mobile phase consisting of acidified water and MeOH. The method was also supported by tandem mass spectrometry (MS). In this study, twelve active pharmaceutical ingredients, including acetylsalicylic acid, bromisoval, caffeine, codeine, narcotine, phenacetine, phenobarbital, salicylic acid, and four distinct barbiturates, were investigated. This study revealed that aminophenazone, barbiturates, caffeine, and phenacetine retained high chemical stability over several decades, with contents within ca. 10% of the declared values. Beverages, pharmaceuticals, and environmental water samples were analysed for their caffeine contents using a high-temperature Agilent 1200 HPLC, equipped with a multiple wavelength detector [49]. In this study, the retention behaviour of caffeine on the thermally stable Zir-Chrom-PBD column (150 mm × 4.6 mm, 5 µm) was investigated thermodynamically at various elevated temperatures utilizing high-temperature liquid chromatography. It was demonstrated that elevated temperatures could be applied to minimize processing time, improve device selectivity, and reduce mobile phase viscosity, allowing for the use of columns packed with smaller particles. Extending the length of the column at high temperatures permitted many theoretical plates to be achieved, thus increasing the efficiency of high-temperature liquid chromatography. A similar HPLC-based method was reported for the simultaneous determination and quantification of caffeine in a fixed combination tablet containing caffeine, ibuprofen, and tramadol [56] (Table 1). The HPLC method used in this study first eluted caffeine (t_R = 2.05 min), then tramadol (t_R = 3.84 min and finally, ibuprofen (t_R = 7.41 min). This reported method achieved fast, robust, and selective chromatographic separation using an environmentally considerate mobile phase, with analysis completed in under 8 min. Jan et al. [62] reported an RP-HPLC-based method for the estimation of caffeine, chlorpheniramine maleate, and paracetamol in pharmaceutical tablets. This method used a Waters e2695 system equipped with a UV detector and a Shimadzu LC-10 series system for method verification. A C_{18} column (150 mm × 4.6 mm, 5 µm) and a mobile phase consisting of 30% MeOH in phosphate buffer (pH 4.0) were employed. The flow rate was 1 mL/min with detection at 215 nm and an injection volume of 20 µL.

The UHPLC study was performed with the caffeine-containing fungal growth medium to study caffeine degradation pathways driven by the fungal strain *Desarmillaria tabescens* (Scop.) Emel, using an Agilent UHPLC linked to a Yuexu UlimateR Plus C_{18} column (100 mm × 2.1 mm, 1.8 µm) [50]. A gradient elution with acidified ACN-water at a 1 mL/min flow rate was used (Table 1). This study determined *D. tabescens* as a potent caffeine-degrading fungus, achieving efficient degradation under optimized conditions (malt extract medium, 900 mg/L caffeine, 28 °C and pH 8). It was further established that a branched catabolic pathway involving *N*-demethylation and C8 oxidation was involved in the formation of key intermediates such as theobromine and 3-methylxanthine. The degradation pathway was as follows: from caffeine to theobromine to 7-methylxanthine, and finally to xanthine.

An HPLC-MS/MS method using a mixed-functional column, FMD Comixsil ACRP column (100 mm × 2.1 mm, 3.0 µm) at 30 °C, a gradient mobile phase comprising ACN and a 0.1% HCOOH solution containing 10 mmol/L ammonium formate at a flow rate of 0.2 mL/min and an injection volume of 1 mL was employed for the determination of caffeine in electronic cigarette oil [51]. Mass spectrometric detection was conducted with ESI in +ve ion with multiple reaction monitoring. Using this simple optimized method, both caffeine and taurine were well retained on the chromatographic column, exhibiting excellent, tailing-free peak shapes. The method showed that the limits of detection of caffeine and taurine were 0.100 and 1.00 mg/kg, and the limits of quantification were 0.250 and 2.50 mg/kg, respectively, making this method ideal for quantifying caffeine and taurine in real electronic cigarette oil samples.

Energy drinks are known to often contain caffeine [80–82]. It is imperative to measure the caffeine content, as part of the quality control process, to ensure that it is within the allowed limit for such drinks, and HPLC-based methods for this are quite useful and, at the same time, extremely popular. At least four publications reported HPLC-based methods for the analysis of caffeine in energy drinks during the last year [52–55]. Copsey et al. [52] used an Agilent 1290 Infinity II UHPLC with an Agilent 6545 Q-TOF HRMS detector operating in ESI mode, employing a Chromolith® HighResolution® HPLC monolithic column (C_{18}, 100 mm × 4.6 mm) and a gradient elution with water-ACN. Caffeine quantitation was based on eight standard calibration curves ($R^2 > 0.997$). This study tested the performance of HPLC with MS detection by incorporating recently developed radial flow stream (RFS) splitting end fittings to standard HPLC columns. These RFS end fittings enabled the total flow of the mobile phase to be split so that only the central portion was directed to the MS, which enhanced the efficiency in the separation step because of the flow profile in the column. It was observed that the incorporation of the RFS end fittings enabled a threefold increase in analytical throughput compared to standard HPLC columns without any loss in quantitative metrics. The analysis of caffeine in a range of beverages revealed that the concentrations matched the specification, albeit a co-eluting component, trigonelline, could be a problematic impurity under these conditions if using a non-specific detector. UPLC or UHPLC appeared to be the instruments of choice for caffeine analyses in energy drinks, including cola and sports drinks, reported last year [53–55] (Table 1).

Green tea drinks are also commercially available, for example, Coca-Cola Ayataka green tea drink and Master Kong green tea bottle drink. The elution behaviour of eight tea catechins and caffeine in HPLC separation with core-shell type RP columns and its application to the determination of these contents in green tea drinks containing a large amount of the polyphenol catechin was evaluated by Nishi et al. [57]. Lemon-flavoured beverages enriched with black or green tea extracts were analysed for their methylxanthine content, including caffeine, by HPLC-PDA [59]. In this study, a Waters Alliance 2695 HPLC-DAD was used with an isocratic mobile phase of water (85%) and EtOH (15%) at 1 mL/min, and a core–shell Kinetex® C_{18} column (150 mm × 3 mm, 2.6 μm) at 25 °C. The injection volume was 3 μL, the detection wavelength was set at 273 nm, and the run time was 20 min. Uddin et al. [63] described an RP-HPLC-based analytical method for the determination of caffeine in commercially available tea and carbonated beverages. They used a standard C_{18} RP-ODS column (250 mm × 4.6 mm, 5 μm) and a mobile phase containing 30:70 MeOH and water. Column temperature was maintained at 40 °C. The flow rate was 1 mL/min, the injection volume was 20 μL, and the detection wavelength was set at 278 nm. Commercially available weight-loss teas were analysed for caffeine content by HPLC using a short run time of 8 min [65]. In this study, a Shimadzu LC-2030 Plus Prominence-iRP-HPLC with a UV detector set at 275 nm was used. A Shimadzu Nexcol C_{18} 5 μm, 50 mm × 3.0 mm column at 40 °C was used with a column guard. The mobile phase was composed of 20% MeOH in water with 0.1% HCOOH and 5 mM ammonium formate, and an isocratic run was performed at a flow rate of 0.3 mL/min. The injection volume was 5 mL.

Not only pharmaceutical tablets, but also other forms of pharmaceutical formulations may contain caffeine [58,60,61]. An Agilent 1220 Infinity HPLC coupled with a UV detector at 273 nm and a Zorbax SB-C_8 analytical column (150 mm × 4.6 mm, 5 μm) employing a mobile phase consisting of phosphate buffer (pH 6.8) with a total phosphate concentration of 10 mM, prepared from KH_2PO_4—and Na_2 HPO_4, mixed with ACN in a ratio of 41:9 were used for the stability testing of intravenous fluids containing caffeine [58]. The caffeine and misoprostol contents in a pharmaceutical nanoformulation were simultaneously determined using a quality-by-design RP-HPLC-based analytical technique using a standard RP-HPLC column and acidified (with orthophosphoric acid) ACN-water as a mobile phase [60]. Arqarni et al. [61] reported an RP-HPLC-DAD method using a C_{18} RP HPLC column (250 mm × 4.6 mm, 5 μm) for the determination of caffeine in various pharmaceutical dosage forms.

An RP-HPLC-based degradation study involving TrichoConcept line and its caffeine content was reported last year [64]. It can be noted that TrichoConcept™ is a line of vehicles compatible with the active pharmaceutical ingredients and dermaceutical ingredients used to treat alopecia.

2.6. Water and Wastewater

At least three publications that appeared in 2025 were about the analysis of caffeine in groundwater, lake water, and wastewater [66–68]. Evaluation of sources, spatial and temporal distribution, ecological and health risk associated with caffeine and *N,N*-diethyl-*meta*-toluamide contamination in the urban groundwater parts of Vellore city, Tamilnadu, India, using HPLC was reported [66]. In this study, an 844 UV/VIS compact ion chromatography equipped with a Hichrom-Altima C_{18} HPLC column (250 mm × 4.6 mm, 5 mm) was used with a flow rate of 1 mL/min, and an injection volume of 250 μL. The detection wavelength was set at 270 nm. The mobile phase comprised ACN and water (pH = 3.0) at 1:1, and it was run isocratically. It was found that 96.88% of samples containing caffeine posed moderate ecological risks, while 6.25% of *N,N*-diethyl-*meta*-toluamide-containing samples fell into the same category, which highlighted the widespread presence of caffeine and *N,N*-diethyl-*meta*-toluamide in urban groundwater and their associated health and ecological risks. It can be noted that *N,N*-diethyl-*meta*-toluamide is considered toxic to organisms that live in water, and thus the presence of an excessive amounts of this compound in surface water, groundwater, and wastewater has negative ecological consequences. Similarly, the presence of caffeine in high amounts could cause neurotoxicity, stunted growth, and altered reproduction in organisms like clams, fish, and crustaceans that live in water. Additionally, caffeine acts as a stable, high-priority aquatic contaminant that causes the death of seedlings and disrupts ecosystems. Caffeine was quantified in the samples of lake water obtained from the Karst Lake Punta Laguna of the Yucatán peninsula using an Agilent 1290 Infinity HPLC-DAD and a Zorbax C_{18} column (150 mm × 4.6 mm, 5 μm). An isocratic run was conducted with 35% MeOH in water at a flow rate of 1 mL/min and detection at 295 nm [67]. As part of trace pharmaceutical monitoring in water and wastewater, a UHPLC system coupled with quadrupole mass spectrometry with a HESI (heated electrospray ionization) ion source was used [68]. The analysis utilized a Chromolith® Performance RP-18e column (100 mm×4.6 mm) at 20 °C. An isocratic flow rate of 0.5 mL/min was applied using a mobile phase consisting of eluent A (0.1% HCOOH in water) and eluent B (ACN), mixed in a 1:1 ratio. Sample injection volume was 20 μL.

2.7. Biological Samples

HPLC-based quantification of caffeine in the rat plasma using green tea-loaded chitosan-iron oxide nanoparticles, and in human urine samples from diabetic retinopathy patients was reported [69,70]. For the determination of caffeine in rat plasma, an Agilent 1100 Series HPLC system coupled with a DAD was used. A Chromasil C_{18} analytical column (250 mm × 4.6 mm, 5 μm), linked to a Chromasil C_{18} guard column (20 mm × 4.6 mm, 10 μm), was used at a column temperature of 30 °C [69]. A mobile phase consisting of (A) 0.1% HCOOH in water and (B) ACN containing 0.1% HCOOH was used with a flow rate of 1.0 mL/min. The injection volume was 20 μL and the gradient elution was as follows: 10% B from 0 to 5 min, increased to 15% B from 5 to 7 min, held at 15% B from 7 to 10 min, ramped to 25% B from 10 to 20 min, increased to 40% B from 20 to 25 min and maintained at 40% B until 30 min. The gradient was then returned to the initial composition (10% B) within 1 min and re-equilibrated for 5 min before the next injection. A wavelength of 280 nm was used to monitor the effluent. This reported HPLC method showed reliability, sensitivity and precision for the simultaneous quantification of key compounds, caffeine, (+)-catechin, epicatechin, (-)-epigallocatechin gallate, gallic acid, and theaflavin in rat plasma. On the other hand, an HPLC–ESI–MS/MS and internal standards labelled with stable isotopes were used to measure urinary caffeine and caffeine metabolites [70]. The findings from this HPLC study indicated that high urinary levels of various metabolites, including caffeine, were associated with an increased risk of diabetic retinopathy in males with diabetes. Prospective studies are needed to verify these findings.

3. Conclusions

The review of the literature published in 2025 revealed the popularity and usefulness of HPLC (or UPLC/UHPLC)-based methods in the analysis and quantification of caffeine in different matrices. While an increase in the use of UPLC (or UHPLC) was observed, conventional HPLC using standard 5 mm columns of 150 or 250 mm length remained the instrument of choice because of its lower price than that of a UPLC (or UHPLC) system. Only a handful of studies reported in 2025 used an MS detector, but the vast majority of the studies used a UV or diode array detector (DAD, also known as photodiode array detector, PDA). However, the use of LC-MS/MS is expected to grow in the coming years as it offers high specificity and the ability to detect caffeine metabolites or structurally similar compounds in complex matrices, including biological fluids. The use of UHPLC with MS will certainly improve selectivity, especially in pharmacokinetic and environmental studies. Most of the studies published last year involved caffeine analysis in various cocoa, coffee, and tea samples.

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