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Review

Analytical Methods Applied to Auraptene: A Mini Review

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Abstract: Auraptene (7-geranyloxycoumarin), a prenyloxycoumarin primarily found in *Citrus* species, has garnered significant attention due to its diverse bioactivities and presence in natural products. Accurate analytical techniques are crucial for the qualitative and quantitative assessment of auraptene in plant materials and formulations. This review provides an exhaustive summary of the analytical methods applied to auraptene, encompassing classical extraction, chromatographic separation, spectroscopic identification, and advanced hyphenated techniques. Critical evaluations of sample preparation, detection modes, and method validation are presented, alongside a discussion on current challenges and future directions in auraptene analysis.

Keywords: auraptene; HPLC; GC-MS; UV-Vis spectroscopy; extraction methods; method validation

1. Introduction

Auraptene (7-geranyloxycoumarin) (Figure 1) is a naturally occurring prenyloxycoumarin widely found in the peel of *Citrus* fruits such as oranges, mandarins, and grapefruits, and several other plants belonging to the families of Rutaceae, Apiaceae, and a few others. This rare secondary metabolite is characterized by a coumarin nucleus linked via an ether bond to a hydrophobic geranyl side chain, conferring unique chemical and physical properties [1].

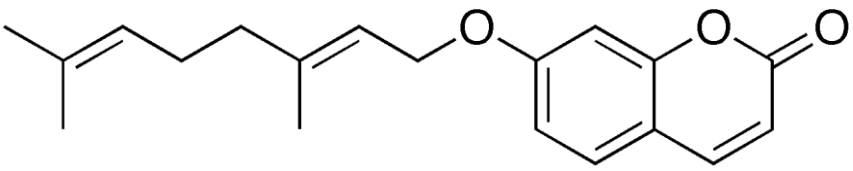

Figure 1. Structure of auraptene.

Due to its bioactivity and presence in *Citrus* essential oils, auraptene has attracted significant interest in natural product research, pharmaceutical, and food sciences. Several recently published review articles explicatively describe the relevance of the title natural product in phytochemical, pharmaceutical, and fodd sciences as well as lists all its occurrence in the plant kingdom [1–4]. Analytical methods for auraptene are essential for its identification, quantification, and quality control in various matrices. These methods include classical solvent extractions, chromatographic separations, and spectroscopic detection techniques. Given its hydrophobic nature and moderate volatility, specific challenges arise in sample preparation and analysis. This review provides an in-depth and updated overview of analytical methodologies applied to auraptene, including their principles, instrumentation, optimization, validation, and applications in natural products research.

2. Chemical and Physical Properties

Chemical name: 7-geranyloxycoumarin
Molecular formula: $C_{19}H_{22}O_3$
Molecular weight: 298.37 g/mol
Chemical structure: Coumarin nucleus with an ether-linked geranyl substituent at position 7
Physical state: Pale yellow oily liquid or crystalline solid depending on purity
Melting point: 66–68 °C
UV absorption maxima: 322 nm (MeOH)
Solubility: Sparingly soluble in H_2O; soluble in MeOH, EtOH, acetone, chloroform
Stability: Light and heat sensitive; prone to oxidation, best stored under inert atmosphere and darkness
LogP: 5.2

3. Extraction and Sample Preparation Techniques

Traditional extraction methods like maceration and Soxhlet extraction have been utilized for extracting auraptene and related coumarins from plant matrices using solvents like petroleum ether, diethyl ether dichloromethane, acetone, ethanol, and methanol [5,6]. While these methods are straightforward and require minimal equipment, they often involve longer extraction times and may yield lower concentrations of the target compound compared to modern techniques. Furthermore, low yields (<0.2%) have been obtained applying such methodologies. For this reason, several powerful and alternative techniques have been set up and validated for the same purposes. The most meaningful and explicative examples are highlighted below.

3.1. Ethanol-Based Ultrasound-Assisted Extraction (UAE)

A notable study focused on pomegranate (*Punica granatum* L.) seed extracts employed absolute EtOH as the extraction solvent, combined with ultrasound-assisted maceration [7]. This method significantly enhanced the extraction efficiency of auraptene. Specifically, a 1-min ultrasound-assisted maceration yielded auraptene concentrations of 1.99 ± 0.08 μg/g of dry extract. The use of absolute EtOH was chosen due to its effectiveness in extracting *O*-prenylated coumarins and its compliance with food and medicinal plant extraction standards. Advantages of this methods are rapid extraction process (1-min duration), high extraction efficiency for auraptene, good extractive yields (>0.5%), and the utilization of food-grade solvent. The only putative limitation relies on the potential degradation of thermolabile auraptene if ultrasound parameters are not optimized.

3.2. Supercritical Fluid Extraction (SFE)

SFE has been explored for the extraction of coumarins from plant matrices [8]. In a comparative study on *Citrus maxima* peels, SFE using carbon dioxide at 50 °C and 27.6 MPa, with EtOH as a modifier, yielded 1.28 mg/g of auraptene. This suggested that SFE could be a viable method for its extraction, offering benefits such as reduced solvent residues and preservation of thermolabile compounds. Advantages of this method are selective extraction with minimal solvent residues and the preservation of chemical stability due to moderate temperatures and good to very good extractive yields (>0.7%). Limitations consist of high operational costs and equipment requirements.

3.3. Solid-Phase Extraction (SPE)

SPE has been applied to the selective extraction of oxyprenylated phenylpropanoids, including auraptene, from complex matrices such as Tea tree oil [9]. In this method, the oil was adsorbed onto Al_2O_3 (Brockmann activity II), washed with MeOH, and then treated with dichloromethane. This approach effectively isolated auraptene in the final extracts. Advantages of this method are the enhanced selectivity for the target phytochemical and the reduction of matrix interferences in subsequent analyses and good to very good extractive yields (>0.7%).

The extraction of auraptene from plant matrices has been advanced through various methodologies, each with its own set of advantages and limitations. EtOH-based UAE stands out for its efficiency and compliance with food-grade standards. SFE presents a promising alternative, though further studies focusing on auraptene are needed. SPE offers enhanced selectivity but requires careful optimization to prevent analyte loss due to tight retention onto the solid sorbent and thus difficulties in desorption. Future research is largely open to optimize these extraction methods, focusing on scalability, environmental impact, and integration with advanced analytical techniques to facilitate comprehensive studies on auraptene's potential applications.

4. Chromatographic Techniques

Chromatography is central to the separation and quantification of auraptene. Key techniques include: thin layer chromatography (TLC), high performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC), and gas chromatography (GC). All these methodologies are often coupled to spectroscopic techniques such as UV and/or mass detection. The combination of a separation technique like chromatography with a detection technique like spectroscopy provides the so called "hyphenated techniques".

4.1. TLC

TLC is a widely used, simple, and cost-effective chromatographic technique for preliminary qualitative and semi-quantitative analysis of natural products [10], including coumarins like auraptene [11,12]. This technique remains valuable for rapid screening, fraction identification, and purity checks of auraptene in crude plant extracts before more advanced chromatographic analysis. Commonly used stationary phase are silica gel 60 F_{254} precoated aluminium or glass plates (layer thickness 0.2–0.25 mm) TLC plates. Auraptene's moderate lipophilicity requires carefully chosen solvent systems to achieve good resolution and R_f values. Reported mobile phases in the literature include: toluene/ethyl acetate (93:7 *v*/*v*) (R_f = 0.63), hexane/ethyl acetate (8:2 *v*/*v*) (R_f = 0.61), petroleum ether/ethyl acetate (8:2 *v*/*v*) (R_f = 0.62), and chloroform/methanol (9:1 *v*/*v*) (R_f = 0.75). With such eluents as the mobile phase, typical R_f values for auraptene range between 0.50 and 0.65 depending on the solvent system and plate type. Detection is performed using UV light 254 nm and 365 nm. Furthermore, auraptene exhibits characteristic fluorescence under long-wave UV (365 nm) due to its coumarin chromophore. Alternatively chemical sprays such as anisaldehyde + conc. H_2SO_4 sulfuric acid reagent (it produces yellow to orange coloured spots upon heating), diphenylboric acid aminoethyl ester, Naturstoff reagent, and Charring reagent can be used for TLC visualization. Quantification can also be achieved by densitometric scanning at 325 nm on TLC plates. Calibration with auraptene pure standards spotted in increasing amounts (e.g., 10–100 ng/spot), and linearity, LOD, and LOQ can be determined using TLC densitometry systems. Table 1 summarizes the main examples of TLC analysis of auraptene reported in the literature.

Table 1. Literature examples of TLC analysis of auraptene.

Source	Mobile Phase	R_f	Detection	Ref.
Citrus sinensis (peel)	Toluene/Ethyl acetate 93:7	0.58	UV 365 nm fluorescence	[11]
Citrus limon (peel)	Hexane/Ethyl acetate 8:2	0.52	Anisaldehyde spray	[11]
Citrus aurantium (leaves)	Petroleum ether/Ethyl acetate 8:2	0.55	UV and densitometry	[11]
Other Rutaceae species	Chloroform/Methanol 9:1	0.60	UV 365 nm	[12]

Strengths of TLC for auraptene include low cost and minimal solvent use, rapid qualitative screening of extracts, suitable for preparative TLC to isolate auraptene fractions, visualization via UV fluorescence is direct and sensitive, and compatibility with densitometric quantification. On the other hand limitations are represented by lower resolution and sensitivity compared to other techniques (e.g., HPLC/UHPLC), limited quantification precision, matrix complexity that can cause overlapping spots, and the need for a careful optimization of solvent systems. In summary, TLC remains a valuable and practical tool for initial screening, qualitative identification, and semi-quantitative analysis of auraptene in plant matrices. Its advantages in cost and speed make it a first-choice method before employing more sophisticated chromatographic techniques.

4.2. HPLC

Currently, HPLC is the predominant analytical method applied for the quantification of auraptene in plant matrices due to its high resolution, reproducibility, and ability to separate the title compound from closely related coumarins and other phytochemicals [6,13–15]. To run such analyses, reverse-phase C18 silica columns (150 × 4.6 mm; 5 μm particle size) are the gold standard. Waters Symmetry C18, Agilent Zorbax Eclipse Plus, Phenomenex Luna C18 have been most frequently used to this purpose. Filtration through 0.22 μm or 0.45 μm PTFE filters before injection and possibly SPE cleanup can be applied to reduce matrix effects in complex samples. Mobile phases typically include H_2O (with 0.1% formic or acetic acid) and CH_3CN or MeOH. Acid modifiers are an undoubtedly advantageous as they improve peak shape by suppressing ionization of acidic groups. A gradient elution has been frequently employed, especially in the case of complex matrices, with a flow rate of 0.8–1.2 mL/min and column temperature set in the range 25–35 °C. Detection methods include a) UV-Visible spectra,

based on the fact that auraptene exhibits a strong UV absorbance peak around 322–330 nm due to its conjugated coumarin chromophore (most studies detect auraptene using diode array detectors (DAD) at these wavelengths), b) fluorescence, for which coumarins fluoresce naturally with excitation and emission wavelengths of 320 nm 400 nm, respectively, and c) mass spectrometry (LC-MS/MS) adopting an ESI positive mode and having the precursor ion *m/z* 299 $[M + H]^+$ for auraptene as the reference. Table 2 summarizes the most relevant analytical parameters and the related values for the HPLC method validation according to ICH guidelines [16].

Table 2. HPLC method validation and performance parameters [16].

	Reported Values
Linearity range	0.1–50 μg/mL; $r^2 > 0.998$
Limit of Detection (LOD)	10–50 ng/mL
Limit of Quantification (LOQ)	30–100 ng/mL
Precision (RSD)	<3% intra-day, <5% inter-day
Recovery (%)	90–105%
Accuracy	±5% relative error

HPLC remains the most robust, precise, and reproducible method for auraptene analysis in complex plant matrices. Advances in extraction methods (UAE, MAE), detection (LC-MS/MS, fluorescence), and instrumentation have dramatically improved throughput, sensitivity, and accuracy. Integrating metabolomics and chemometrics with HPLC-MS, in order to explore auraptene biosynthesis and bioactivity relationships, will lead to further significant enhancements.

4.3. UHPLC

UHPLC provides a superior analytical platform for the rapid and precise quantification of auraptene in complex plant extracts [7,9,17–19]. Typically, to run such analyses RP C18 columns packed with sub-2 μm particles (e.g., 50 × 2.1 mm, 1.7 μm particle size) are preferred. Commercially available columns from Waters, Agilent, Phenomenex have been most frequently used. The mobile phase consists of an acidified H_2O (0.1% formic or acetic acid) and CH_3CN or MeOH mixture with the adoption of a gradient elution at a flow rate of 0.3–0.5 mL/min for narrow-bore UHPLC columns. Column temperature is usually set in the range 25–30 °C. Sample preparation remains similar to conventional HPLC. Injection volumes are in the range 1.0–5.0 μL. Detection modes are practically the same as the ones described in the case of HPLC analysis. Table 3 summarizes the most relevant analytical parameters and the related values for the UHPLC method validation according to ICH guidelines [20].

Table 3. UHPLC method validation and performance parameters [20].

	Reported Values
Linearity range	0.01–50 μg/mL $r^2 > 0.999$
Limit of Detection (LOD)	5–10 ng/mL
Limit of Quantification (LOQ)	5–10 ng/mL
Precision (RSD)	<3% intra-day, <3% inter-day
Recovery (%)	92–105%
Accuracy	±1.5% relative error

For what concerns auraptene analysis, UHPLC shows improved retention time stability due to controlled temperature and system pressure. However, until now UHPLC has found limited usage for the qualitative and quantitative determination of this coumarin in complex plant matrices. Considering its undoubted advantages, it is desirable that such a technique could be used in many more cases for the detection of auraptene, but also for other structurally similar secondary metabolites. Indeed, UHPLC can find a large and convenient application in quality control of *Citrus*-derived nutraceuticals and herbal products enriched in auraptene. To this aim, Table 4 reports a comparison between HPLC and UHPLC when applied to the analysis of auraptene in different matrices. Samples of both HPLC and UHPLC chromatograms of auraptene from plant extracts can be easily found in the literature [5–7].

Table 4. Advantages of UHPLC over conventional HPLC for auraptene analysis [16,20].

	UHPLC	Conventional HPLC
Particle Size	<2 μm	3–5 μm
Operating Pressure	Up to 15,000 psi (1034 bar)	Typically ≤ 6000 psi (400 bar)
Analysis Time	3–10 min	15–30 min
Resolution	Higher resolution and peak capacity	Lower compared to UHPLC
Solvent Consumption	Reduced by 30–80%	Higher solvent usage
Sensitivity	Improved peak shapes, better S/N (LOD 5–10 ng/mL)	Lower sensitivity (LOD 10–50 ng/mL)
Sample Throughput	Higher (up to 10×)	Lower

4.4. GC

Auraptene has a moderate volatility (allowing vaporization without decomposition) and thermal stability and thus can be analyzed by GC, often coupled with sensitive detectors like Flame Ionization Detector (FID) or MS [8]. In fact auraptene is sufficiently stable under typical GC injector and column temperatures (up to ~280 °C) and does not require any derivatization. GC is particularly suited for essential oil or volatile extract analysis where auraptene is a minor but important bioactive constituent. Unlike HPLC and UHPLC, hydrodistillation or steam distillation, along with conventional extraction methodologies, can be used as sample preparation techniques as auraptene can be present in essential oils and volatile fractions from *Citrus* peels and Rutaceae plants in general [21]. Typical settings for GC analysis of auraptene in different matrices include the use of a 30 m × 0.25 mm i.d., 0.25 μm film thickness capillary column (e.g., DB-5MS or HP-5MS), an injection volume of 1–2 μL, Helium at a flow rate of 1.0 mL/min as the carrier gas, a split or splitless (split ratio 20:1 or optimized) injection mode and the injector temperature in the range 250–280 °C. The oven temperature program can be summarized as the following: initial 60 °C (hold 1–3 min.), ramp up 5–10 °C/min. to 280 °C, and finally hold at 280 °C for 5–10 min. FID (280 °C) or MS (electron impact, 70 eV) can be used as the detector systems. In the first case, external standards of auraptene for calibration is required, while in the latter structural confirmation can be achieved by comparing mass spectra to reference libraries (NIST, Wiley) or authentic auraptene standard MS spectra. Reported Kovats indices are 1540 for polar and 2634 for polar and apolar columns respectively. With respect to method validation parameters, a greater variability than HPLC and UHPLC is observed for GC, of course depending on how the process set up is recorded. Linear ranges are typically from low ng to μg per injection and the LOD is in the low ng-level depending on the type of detector. The advantages of GC for auraptene analysis include high resolution and good separation, sensitive and selective detection especially with MS, rapid analysis (typically <30 min), and a great usefulness for fingerprinting and chemotaxonomic studies. In summary, GC, especially coupled with MS detection, is an excellent technique for analyzing auraptene in volatile plant extracts, essential oils, and headspace samples. The technique requires optimized sample preparation and chromatographic conditions to ensure accurate identification and quantification.

5. Spectroscopic and Hyphenated Techniques

5.1. Ultraviolet-Visible (UV/Vis) Spectroscopy

Auraptene, due to its conjugated π-electron system, exhibits characteristic absorbance in the UV region. The coumarin moiety strongly absorbs in the 310–330 nm region, while the geranyl side chain contributes little to UV absorption. The λ_{max} of the title phytochemical typically falls between 323–328 nm upon spectrum recording in MeOH. Molar absorptivity (ε) is comprised in the range 15.000–20.000 $L \cdot mol^{-1} \cdot cm^{-1}$. Minor bathochromic shifts have been observed in polar solvents [22]. Samples of UV spectra of auraptene can be easily ound in literature [23]. UV/Vis spectroscopy has mainly been employed for the qualitative identification of auraptene in *Citrus* extracts and for its preliminary quantification, often in conjunction with TLC or HPLC [14]. However, this technique is featured by some limitations like the low specificity due to spectral overlap with other coumarins and the ineffectiveness in complex matrices without prior chromatographic separation.

5.2. Infrared (IR) and Fourier-Transform IR (FTIR) Spectroscopy

FTIR analysis reveals the key absorption bands of auraptene as indicated in Table 5.

Table 5. Key IR absorption bands of auraptene.

Functional Group	Wavenumber (cm^{-1})	Description
C=O (lactone)	~1730	Strong sharp stretch
C=C (aromatic)	~1610, 1580	Medium, conjugated ring
C–O–C stretch	~1260–1280	Ether linkage (geranyl-oxygen)
C–H (alkene)	~3010	=C–H stretch
Isoprenoid CH_3	~880	Out-of-plane bend

While IR and FT-IR are both of limited interest for structure elucidation, but have found applications for the confirmation of typical bands as depicted in Table 5 and for fingerprinting purposes [24,25].

5.3. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR remains the most definitive tool for the structural characterization of auraptene [21]. Table 6 summarizes the diagnostic peaks in the ^1H NMR spectrum ($CDCl_3$, 400 MHz).

Table 6. ^1H NMR diagnostic peaks for the structural assignment of auraptene.

δ (ppm)	Multiplicity	Assignment
1.62–1.68	m	CH_3 of geranyl group
2.05	s	Allylic CH_3
4.65	d	OCH_2 linked to coumarin ring
6.20–7.80	m	Aromatic protons of coumarin
5.00–5.20	m	Vinyl protons of geranyl group

In the ^{13}C NMR spectrum (100 MHz, $CDCl_3$) the diagnostic peaks are represented by the lactone carbon (C=O at ~161–163 ppm), prenyl C=C (125–135 ppm), and OCH_2 (66–68 ppm).

5.4. Mass Spectrometry (MS)

Auraptene (MW = 298.37 g/mol) displays consistent ionization behaviour under electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Typical MS and MS/MS data are the following: $[M + H]^+$ ion at *m/z* = 299.3 and fragmentation ions *m/z* = 231.0 related to the loss of C_5H_8 from the prenyl chain, and *m/z* = 163.0 corresponding to the characteristic coumarin core fragment. MS applied to auraptene has found wide use for the definition of its pharmacokinetic and metabolic profile (e.g., detection of phase I/II metabolites) [26], and in non-targeted metabolomics [27]. Due to MS it has been found that auraptene, administered at a dose of 50 mg/Kg to 6–8 week old C57BL/6 male mice (20–25 g), has a half-life in the range 2.5–3.8 h in rats (plasma, liver, and brain tissues), a C_{max} = 1.8–3.2 μg/mL (oral dose), a T_{max} = 1.5–2.0 h, an oral bioavailability of 35–40%, and finally hydroxylated auraptene derivatives (e.g., 8-hydroxyauraptene, *m/z* 315.1) and their conjugation as glucuronates (detected as *m/z* 509) and sulfate (detected as *m/z* 395) have been detected as the main phase I and phase II metabolites, respectively. These are detectable in urine and faeces up to 12–24 h post oral intake.

Table 7 reports a comparison between the efficiency and performance of the above listed spectroscopic and hyphenated techniques. Samples of MS spectra of auraptene can be easily ound in literature [28,29].

Table 7. Method comparison and critical discussion.

Technique	Strengths	Limitations
UV/DAD	Fast, low-cost, moderate sensitivity	Low selectivity
FTIR	Functional group ID	Low resolution for complex mixtures
NMR	Full structural info	Requires pure sample, expensive
LC-MS/MS	High sensitivity & selectivity	Cost, matrix effects

Modern analytical approaches combining high-resolution MS, quantitative LC-MS/MS, and NMR spectroscopy provide comprehensive coverage for the qualitative and quantitative analysis of auraptene. While traditional methods like UV and TLC still remain valuable for screening and preliminary studies, they should be complemented by more robust and sensitive techniques in research requiring precision. Emerging techniques such as UPLC-QTOF-MS, LC-NMR, and DESI-MS imaging represent future frontiers for assessing more details about auraptene localization in tissues, metabolomics, and in vivo biotransformation. Additionally, the development of validated green analytical methods (e.g., miniaturized extractions, solvent-free sample preparation) is an important

step toward sustainable phytochemical analysis. Moreover, the application of more peculiar methodologies like spatial and imaging metabolomics to map auraptene accumulation in plant tissues (e.g., *Citrus* peel vs. pulp) and its distribution in animal organs after oral ingestion, integration with other -*omics*, like transcriptomics (e.g., to assess how CYP expression correlates with auraptene hydroxylation), proteomics (e.g., to determine how auraptene affects detoxification enzymes), and lipidomic (e.g., to assess how auraptene interacts with lipid metabolism due to lipophilic nature), single-cell metabolomics to study intracellular fate have been studied. AI-assisted modeling of metabolic networks involving auraptene, and metabolite docking studies for predicting bioactivity of auraptene derivatives are also valuable methodologies to this concern.

For quality control (QC) purposes, though not yet listed in major pharmacopoeias (e.g., USP, EP), auraptene is recognized in several monographs and quality standards (e.g., Chinese Pharmacopoeia for *Fructus Aurantii*) [28]. The move toward quantitative fingerprinting and marker standardization is expected to increase auraptene's relevance in QC protocols. Auraptene quantification, combined with chemometric tools, will be effective for botanical authentication, detection of adulterants or substitution, and verification of geographic origin (terroir-based fingerprinting). To this aim, principal component analysis (PCA) of HPLC or LC-MS profiles also enhances differentiation among authentic and adulterated samples.

6. Conclusions and Future Perspectives

Auraptene, a naturally occurring oxyprenylated coumarin mainly found in *Citrus* species, has attracted considerable attention due to its wide spectrum of biological activities and its potential as a bioactive constituent in functional foods, dietary supplements, and herbal formulations. The comprehensive analytical characterization of auraptene is essential for a variety of scientific and industrial applications, including quality control, phytochemical standardization, pharmacokinetic evaluation, and metabolomic profiling. Over the years, an array of classical and advanced analytical methods have been developed and refined to meet the challenges posed by auraptene's moderate volatility, hydrophobicity, and structural similarity to other coumarins. Extraction techniques such as ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and solid-phase extraction (SPE) have proven effective in isolating auraptene from complex plant matrices, with optimizations focusing on speed, yield, environmental impact, and compound stability. Chromatographic methods, ranging from thin-layer chromatography (TLC) for preliminary screening to high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) for precise quantification, continue to play a central role in auraptene analysis. Spectroscopic techniques, particularly UV-Vis, FT-IR, NMR, and hyphenated approaches such as LC-MS/MS, have not only enabled accurate detection and structural elucidation but also metabolic and pharmacokinetic investigations. Mass spectrometry, especially in tandem and high-resolution configurations, has been instrumental in elucidating auraptene's biotransformation pathways, including hydroxylation and conjugation (glucuronidation and sulfation), with applications extending into targeted and untargeted metabolomics. These studies have revealed valuable information about auraptene's absorption, distribution, metabolism, and excretion (ADME), supporting its use as a biomarker of *Citrus* consumption and as a model compound for metabolomic studies of oxyprenylated phenylpropanoids.

In parallel, auraptene will be increasingly employed as a quality marker in the standardization of *Citrus*-derived preparations. Its quantification, when integrated with chemometric techniques such as principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), will enable authentication of plant materials, detection of adulteration, and verification of geographic origin, establishing auraptene as a reliable indicator of product integrity. Although not yet included in official pharmacopoeias such as the USP or EP, its recognition in regional standards and growing adoption in scientific quality control protocols suggests that regulatory integration is forthcoming. Looking forward, advances in analytical chemistry, including spatial metabolomics (e.g., DESI-MS imaging), single-cell metabolomics, and green analytical approaches, offer promising avenues for improving our understanding of auraptene's role in biological systems and enhancing its application across research and industry. Integration with other -*omics* platforms such as transcriptomics, proteomics, and lipidomics may further elucidate its molecular mechanisms and health-related effects. Moreover, developments in miniaturized, field-deployable LC-MS devices and AI-driven metabolomic data processing are expected to streamline auraptene analysis in both laboratory and applied contexts. Collectively, these advancements underscore the importance of continuing innovation in analytical methodologies to support the expanding interest in auraptene as a functional molecule, analytical target, and therapeutic lead.

Author Contributions

All authors contributed equally to retrieving information from the published literature, designing the layout, manuscript preparation, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest. Given the role as Editorial Board Members, Francesco Epifano had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-assisted Technologies

No AI tools were utilized for this paper.

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