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Review

Analysis of amines in plant materials

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Abstract

Biogenic amines are conveniently divided into aliphatic monoamines, aliphatic di- and polyamines and aromatic amines. These compounds are shown to fulfill an array of roles in cellular metabolism. Thus, amines are needed for growth and development and their metabolism appears to be coordinated with the cell cycle. Di- and polyamines, among which are putrescine, spermidine and spermine, are ubiquitous polycationic molecules that occur in all living cells. However, plants accumulate a number of specific related compounds under free or conjugated forms. In plant tissues, the molecular diversity combined with the fact that amine contents are highly responsive to developmental and environmental signals encouraged analysts to develop specific procedures for their isolation and characterization. The main goals were to develop high performance routine procedures in terms of selectivity, repeatability and detectability with minimum running costs. Domains of application concern not only fundamental aspects of amine biochemistry and physiology in plants but also increasing needs in the control of food and beverage quality from plant origin. The present review reports the most recent advances in extraction, identification and quantitation of amines in plant tissues with special interest in the analysis of original and uncommon metabolites. Emphasis is directed towards chromatographic and electrophoretic separation methodologies and new detection technologies of both derivatized and underivatized compounds including photometry, fluorometry, amperometry and mass spectrometry.

Keywords: Reviews; Plant materials; Amines

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1. Introduction

Among low molecular mass nitrogenous compounds, a wide variety of amines occur in higher plants [1]. These are derived from amino-acids by decarboxylation or through aldehyde transamination, especially of the simple aliphatic monoamines [2]. Amines are precursors for several alkaloids and are mainly catabolized through oxidative deamination via diamine- and polyamine oxidases [3,4]. According to criteria adopted by Smith [5,6] the compounds termed the 'biogenic amines' exclude those bearing carboxyl groups even if the substances are basic. The fundamental classes of amines are primary, secondary, tertiary and quaternary amines. Naturally occurring plant amines are commonly encountered as free or covalently bound forms. In plants the most widespread free amines are conveniently divided into three groups: aliphatic monoamines, aliphatic di- and polyamines, and aromatic amines. In many cases covalently bound forms of aliphatic polyamines and aromatic amines can account for a significant portion of the metabolic pools of plant cells [7–9]. The best characterised conjugated polyamines in plants are the hydroxycinnamic acid amides [10–12], but amines may also be found associated with fatty acids and macromolecules [13–15]. In plants, amines have been associated with many cell processes, including cell division and differentiation, synthesis of nucleic acids and proteins, membrane stability, pH and thermic or osmotic stress responses, and delay in senescence [7–9,16–26]. They also may function as allelochemical compounds and as components of the chemical and physical defences against herbivores and pathogens [12,27–30]. Aliphatic monoamines are widely distributed in higher plants and fungi, ranging from simple compounds like methylamine to *n*-hexylamine. They are volatile compounds often functioning in flowers as insect attractants [28,31,32]. The most common polyamines deriving from arginine or ornithine decarboxylation in plants that are also ubiquitously found in nature are putrescine, spermidine, spermine, and to a lesser extent, cadaverine, derived from lysine metabolism. Like bacteria, plants are also characterised by the occurrence of structurally unique and uncommon polyamines such as norspermidine, norspermine and the large molecular mass polyamines like pentamine and hexamine [33–35]. It has been hypothesized that the accumulation of both common and uncommon higher polyamines may serve specific protection roles in plants adapted to extreme environments [36,37]. Plant aromatic amines generally belong to classes of compounds derived from either histamine and other imidazole-alkylamines, tryptamine and other indole-alkylamines or phenetylamine and other phenylalkylamines. In contrast to animal tissues, their actual function in plant tissues has yet to be elucidated.

Wide interest in following the changes in the titres of a wide spectrum of amines in plants during growth and development exists, and according to the fluctuation of environmental factors, has required the development of sensitive and routine methods for their analysis. Polyamines are ubiquitous molecules that occur in all living cells and are essential for cell renewal in animals and humans where they are needed to keep the body healthy [38]. Polyamines and aromatic amines are shown to fulfill an array of roles in cellular metabolism [17,39]. It was previously believed that polyamines were synthesised by every cell in the body when required. However, recent evidence showed that, as in the case of the essential amino acids, the diet can supply sufficient amounts of amines and polyamines to support cell renewal and growth [38,40]. Moreover, demonstration of increases in polyamines and ornithine decarboxylase activity catalysing the formation of putrescine during cell growth and proliferation provoked speculation that this phenomena was relevant to problems of cancer and reinforced the idea that these metabolites need to be closely controlled in foods, especially those derived from plants [15,41].

Several techniques have been employed to analyse plant tissues for amines, including gas chromatography (GC), paper electrophoresis (PE), thin layer electrophoresis (TLE), thin layer chromatography (TLC), ion-exchange high-performance liquid chromatography (HPLC), and, more recently, reversed-phase HPLC (RP-HPLC) of derivatized polyamines and ion-pair RP-HPLC of underivatized polyamines [27,42–44]. Sensitive technologies have been developed to detect polyamines in plant extracts including reversed-phase HPLC with fluorescence or UV monitoring of dansyl, benzoyl, 9-fluoromethyl chlo-

roformate or *N*-hydroxysuccinimidyl 6-quinolyl carbamate polyamines derivatives. Ion-pair reversed-phase HPLC or ion-exchange chromatography, with fluorogenic detection following postcolumn derivatization with *O*-phthalaldehyde (OPA), is also used [45,46]. HPLC has been extended to the analysis of a large number of other naturally occurring amines in plant tissues, including polyamine homologs, heterocyclic and aromatic amines and conjugates [42]. Next to gas and liquid chromatography, capillary electrophoresis (CE) has been developed into a versatile separation technique [47]. In GC analysis of plant amines the preparation of volatile derivatives has been carried out with trifluoroacetic anhydride, trimethylsilyl chloride, ethylchloroformate or butylchloroformate [48,49]. Otherwise, pentafluoropropionyl and heptafluorobutyryl derivatives have often been used with gas chromatography–mass spectrometry (GC–MS) identification, particularly when two compounds overlapped [15]. Mass spectrometry (MS) is now widely recognized as a very reliable detection and characterization system coupled with either GC or HPLC separation [50]. Recent progress in soft ionization techniques like electrospray or matrix-assisted laser desorption has presented new opportunities for the use of MS in the analysis of biological compounds like polyamines without the use of chromatography [51].

At the mechanistic level, it is now quite clear that the polyamines, especially putrescine, spermidine and spermine, are ubiquitous polycations which have numerous specific interactions in eukaryotic cells. Polyamines are essential for growth, and their metabolism appears to be coordinated with the cell cycle [52]. The length, charge and charge distributions of polyamines permit them to interact with large anionic molecules such as DNA, RNA and membrane phospholipids or uronic acids at the parietal level in plants [52–54]. Endogenous polyamines have been found to block and modulate, especially in plant tonoplast, a number of ion channel types [55,56]. Therefore, apart from the obvious efforts that had to be maintained for the development of more efficient quantitative techniques, studies of the mechanistic understanding of polyamines actions in cells also involved particular attention towards the development of biophysical approaches to amine interactions with plant cell structures.

The aim of this review is to present the most recent advances in isolation, characterization and quantitation of amines in plant tissues and procedures devoted to the analysis of original and uncommon metabolites. We will highlight aspects of the detection of volatile compounds, isolation of polyamine fractions (especially bound polyamine pools) and new developments in amine analyses. Special emphasis will be directed towards chromatographic and electrophoretic separation procedures and new detection technologies of both derivatized and underivatized compounds. Progress in mass spectrometry methodology will also be covered. We will not describe analytical techniques for isolation and characterization of proteins and enzymatic activities involved in amine and polyamine metabolism in plants. These subjects have been widely covered in recent years and recently reviewed by Smith [44] and Minocha et al. [57].

For general information, the functions of the polyamines in plants have been extensively reviewed by Tabor and Tabor [17]; Galston and Smith [58]; Smith [7]; Evans and Malmberg [18]; Flores et al. [8]; Bachrach and Heimer [59]; Galston and Kaur-Sawhney [19]; Flores and Arteca [60]; Slocum and Flores [61]; Kakkar and Rai [62]; Davies [63]; Minocha and Minocha [64]; Martin-Tanguy [11]; Kumar et al. [65]; Walden et al. [24]; Galston et al. [9]; Tiburcio et al. [23]; Malmberg et al. [66]; Gaspar et al. [67]; and Bouchereau et al. [26]. Methods for the estimation and characterization of a wide range of plant amines have been reviewed by Tabor and Tabor [17]; Smith [44]; and Smith and Davies [42]. Representation and distribution of plant amines can be appreciated through a few excellent phytochemical reports [1,5,68] that illustrate the versatile and powerful metabolic potential of plant tissues in the area of amine biosynthesis and accumulation.

2. Isolation of plant amines

2.1. Extraction procedures

Much of the early work on polyamines dealt with cancer in animals, since the biosynthesis of these compounds was known to greatly increase in rapidly

growing animal tissues. Moreover, in animals, the phenolamines, catecholamines, imidazolamines and indolamines are especially important as chemical signals and neurotransmitters, and although those amines are found in plants, their universal importance has not yet been established. Many of the techniques developed for these studies in animals were later adapted for research into the plant amines [44]. Some have been developed especially for amine metabolites known only in plants.

The amine may be considered to be alkaline substituted ammonia molecules, as described by Hofmann; replacement of the hydrogen atoms leading to the various classes known as primary, secondary and tertiary amines. Reactions forming a nitrogenous cation or reversion to the non-protonated amine, as a function of pH, are obviously crucial in the isolation and analysis of the natural polyamines. Thus appropriate combinations of pH and solubility of the amines and their salts in aqueous and organic media were exploited fairly early in the isolation and estimation of the polyamines [15].

The lower aliphatic monoamines are widely distributed in the plant kingdom. They are often produced in flowers at anthesis or by the fruiting bodies of fungi. Insects which carry pollen or spores are attracted by the smell of these volatile amines which may simulate the odour of rotting meat. Methylamine and ethanolamine are found ubiquitously in distillates of plant material, and in many cases they may be formed as artefacts from the degradation of larger molecules in the course of isolation [5,69]. In the *Rhodophyceae*, a wide range of amines is formed by a nonspecific amino-acid decarboxylase. In higher plants, although some simple amines are known to be produced by amino-acid decarboxylation, aldehyde amination appears to be a more common biosynthetic pathway [70]. As described by Knudsen and co-authors [71], for isolation of volatiles, the following steps are mainly used: solvent extraction, steam distillation and head-space techniques. Three types of head-space techniques are commonly used: direct scent collection without accumulation, adsorption and cold trapping. According to the authors, solvent extraction, steam distillation and direct scent collection without accumulation are 'cross sectional' methods, meaning only the volatile materials available at a single moment are isolated. On the other hand the two latter head-space methods are accumulating methods, whereby volatile substances can be enriched in time as they are produced and emitted by the plants [72]. Both solvent extractions and steam distillation can produce artefacts, the former mainly by isolating nonvolatile material from tissues, and the latter mainly by heat induced rearrangements [71]. As an example, a recent study using non-invasive cold trapping head-space of odors revealed that in *Arum maculatum* inflorescences still attached to growing plants no simple amines reported from previous invasive studies could be detected [73]. Volatile amines were previously reported to be involved in pollinator attraction [31]. Recently, both polydimethylsiloxane and mixed-carboxen polydimethylodoxane solid-phase micro-extraction fibres were found to be suitable for the head-space extraction of trimethylamine [74]. Distillation procedures efficient for amine isolation from grain, teliospores or beer before gas chromatographic analysis have also been described [75].

In other respects, research on plant aliphatic and aromatic amines has focused mostly on the pools of free compounds. It is becoming clear, however, that in many situations bound forms of di- and polyamines as well as aromatic amines can account for the major proportion of the metabolic pools [8]. As in animal tissues, free amines present either inside or outside the cells of plant tissues as cationic forms and involving ionic interactions are generally extracted after grinding with acidic solutions from perchloric (PCA), trichloroacetic (TCA) or hydrochloric acids. Routine procedures involved cold extractions with about 5% or 1 *M* of acidic extraction solvent and incubation periods lasting from a few minutes to several hours [76–80]. Physical grinding of tissues is regularly done after liquid nitrogen freezing and powdering with a mortar and pestle, or it can be homogenized in extraction solvent with a Potter-Elvehyem homogenizer [81], an omnimixer [82,83] or ruptured by sonication-shear or freeze-thawing [84]. The homogenates are then centrifuged at cold temperature and pellets reextracted. Supernatants are either submitted to immediate analysis, after neutralization, with or without derivatization procedures or subjected to fractionation steps to prevent interference with contaminating compounds like sugars or amino-acids that are generally abundant and co-extracted in plant materials [42,76,85].

Polyamines and aromatic amines can also be released into the supernatant upon hydrolysis in 6 *M* HCl and are presumed to be linked with low molecular mass compounds [86,87]. The amides formed between di- or polyamines and hydroxycinnamic acids can be found in this fraction [8]. The pellet which remains after centrifugation of the PCA or TCA extracts can also release polyamines upon hydrolysis; these may represent linkages with high molecular-mass compounds and especially polyamines covalently bound with proteins through transglutaminases [14]. To get information on essential levels of polyamines in plants and the association of polyamines with various types of tissue (storage tissue versus growing tissue), Felix and Harr [88] examined 15 wild species and 15 crop species from 13 plant families for their polyamine content before and after germination. Three polyamine fractions were prepared accordingly from ground materials: the acid-soluble material obtained through 0.157 *M* sulfosalicylic acid extraction, the hydrolyzed acid-soluble material prepared after HCl hydrolysis of the previous fraction where RNA and DNA were precipitated with TCA, and hydrolyzed acid-insoluble material obtained after HCl hydrolysis of the initial precipitate [88]. Recently, glutamyl and acetyl derivatives of polyamines were released from isolated chloroplasts of *Helianthus tuberosus* after proteolytic digestion of their TCA-soluble and insoluble proteins [89]. Torrigiani et al. [90] reported an improved method of polyamine analysis for tobacco mosaic virus based on HPLC of sonicated-PCA-treated highly purified suspension. It is suggested that sonication acts on PCA-manipulated protein aggregates causing the release of trapped polyamine molecules. Oxidative catabolic products of putrescine, spermidine and spermine, (i.e. 1-(3-amino-propyl)-pyrrolinium, pyrroline and 1,3-diaminopropane) could also be characterized and quantitated in plant materials after TCA extraction from freshly harvested samples [78]. Root and leaf exudates (xylem and phloem respectively) of *Sinapis alba* have been analyzed for their content in free and bound putrescine after an acidic extraction of the fluids [87]. That was also performed to study long-distance translocation of polyamines in phloem and xylem of *Ricinus communis* [91].

The exhaustive extraction of the amides formed between di-poly or aromatic amines and hydroxycinnamic acids largely accumulated in plant tissues during flowering, fruit ripening and reproduction processes generally required the use of organic solvents [10,11,62,83,92]. There are two major classes of amides: water soluble basic amines of the aliphatic di- and polyamines, and water-insoluble neutral amides which include both aliphatic and aromatic amines. Detailed protocols of extraction generally involve using methanol as extractant and fractionation through ethyl acetate solubilization of neutral amides and TCA extraction of basic amides. They have been published [83,86,93] and may be considered as an exhaustive approach to the multiple chemical compartments of amines in plants.

Whatever the extraction methods, special care should be taken with potential binding of polyamines to the walls of glass apparatus or plasticware used in extraction and subsequent procedures. Several authors recommend siliconization of glassware [42]. Moreover, depending on the number of steps during the procedures, hexanediamine or isobutylamine is often added as an internal standard for estimating percentage of recovery that yields represent [77,78,94].

2.2. Purification of extracts

Amines present in crude extracts from plant tissues are often subjected to derivatization procedures or direct native analysis without pre-purification. Because of frequently occurring interfering compounds during acylation derivatization reactions for example, purification is necessary. Multiple procedures of purification through fractionation processes have been published. Most of them applied to free charged amines are based on the utilization of cationic exchange resins, with the basic fraction eluted with 6 *M* HCl or acetic acid [76,78,93,95,96]. Wehr [94] investigated the use of strong anion-exchange resins for purification of polyamines in plant samples. Results obtained indicate that anion-exchange resins produce equally good results as cation-exchange resins. Polyamines were eluted with NaOH and derivatization could be performed directly on the eluate, which resulted in enormous time savings.

The more recent technical improvements in terms of amine and polyamine extraction and purification concern the development of clean-up methods and

solid-phase extraction (SPE) procedures. Those procedures can be applied to multiple biological samples either naturally liquid, as biological fluids or beverages or after acidic extraction from solid material. High-sulfonated polymeric support, C_{18}-cartridges, and strong cation-exchanger (SCX) cartridges can be used for amine analysis [97–100]. Special care concerning checking of yield recoveries for all polyamines is also discussed. Wu and Huang [101] compared four kinds of fibers for solid-phase microextraction of aromatic amines from environmental samples (lake water) and developed optimal procedures, which could be applied to plant materials.

2.3. Derivatization procedures

For two decades, depending on desired analytes, equipment availability, costs, experience and personal taste and, above all, the domains of application, methodological developments concerning the detection and quantitation of amines after chromatographic or electrophoretic resolutions were directed either towards direct detection of native amines or detection of chemically derivatized amines. Real progress has been made in recent years in developing detection systems that do not require derivatization and which are preferred for their convenience and simplicity. Those systems mainly involve electrochemical detection like amperometric, integrated–pulsed [102,103] or mass spectrometric developments [104]. These will be discussed later in terms of their applications in amine analysis of plant materials.

Nevertheless aliphatic mono-, di and polyamines do not exhibit structural features that permit their sensitive or specific detection. Therefore, in a lot of methods currently in use for the assay of primary and secondary amines, the amino groups are utilized for the formation of derivatives suitable for sensitive determination and for improvement of separation. While the sensitivity of the detection depends on the derivatization reaction, the specificity is limited solely by the quality of the separation procedure. In practice, nearly all detection reactions have been combined with all separation procedures [45].

The objective here is not to propose an exhaustive list of all derivatization procedures used in plant amine analysis, but rather to show recent trends that have depended on the concomitant development of more efficient techniques of separation and detection. Underlying these trends is a concern for improvement of detectability, selectivity and simplicity. In fact, as has been mentioned in an excellent review on derivatization in capillary electrophoresis from Bardelmeijer and co-workers [46], the main goals of derivatization procedures include (i) improved detectability, (ii) improved separation from interfering compounds (iii), improved electrophoretic behaviour, (iv) prevention of decomposition during the separation procedure and (v) more convenient sample preparation. Frequent and current applications in plant science concern the production of colored derivatives of amines for paper and thin layer chromatographic or electrophoretic separations [45,78,105–107], UV absorbing and fluorescent derivatives of amines for thin-layer [37,108] and column chromatographic and electrophoretic separation [42,45,77,109–111], charged modified derivatives of amines for capillary electrophoresis separation [46] and stabilized or volatile derivatives of amines for gas chromatographic separations [78,112–114].

Two technical reviews from Smith [43] and T.A. Smith [44] detailed classical procedures of derivatization of plant amines and related separation modes. Pioneer works of Seiler and co-workers are still standard references in that domain [45,115,117]. Traditionally, HPLC of polyamines has involved the use of either UV-absorbing benzoylated- or photolabile-fluorescent dansyl (5-dimethylaminonaphtalene-1-sulphonyl) derivatives or derivatives produced by reaction with *o*-phthalaldehyde (OPA) and mercaptoethanol [77,79,110,111,118–122]. More selectively, UV-absorbing or fluorescent detection after RP-HPLC of plant biogenic amines also involves either pre-column or post-column derivatization procedures with dansyl chloride [123–125], 9-fluorenylmethyl chloroformate (FMOC) [126], fluorescamine [127], phenylisocyanate [128], carbazole-9-yl-acetyl chloride (CRA-Cl) [129], 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) [130,131], 4-fluoro-7-nitrobenzo-2-oxo-1,3-diazole (NBD-F) [132], 2-naphtyloxycarbonyl chloride (NOC) [133], 3-fluoro-3-nitrobenzotrifluoride [134], and para-nitrobenzoyloxycarbonyl chloride

(PNZ-Cl) [135], with detection limits at the fmol levels.

For dansylation procedures, which are the most commonly used, Smith [44] and references herein] brought to attention the possible interference of hydroxyl and thiol groups which are derivatized at the same time. The reaction of dansylation is generally time consuming but it can be accelerated by ultrasonics [116] or by heating [136]. Recently a rapid microwave-assisted dansylation of biogenic amines applied to plant extracts has been published that could forward interesting developments [137,138]. The biogenic amines, putrescine, cadaverine, spermidine and spermine, dansylated in a microwave oven within 5 min, were separated by high-performance thin-layer chromatography (HPTLC), with chloroform–triethylamine (2:1, v/v) as eluent and silica-gel as stationary phase. A fibre optic-based fluorescence instrument for in situ scanning was used for quantitative measurements with a detection limit of 1.8–3 ng.

Rustenbeck and co-authors [139] warned users against potential degradation of dansyl polyamines on HPTLC plates where amines apparently react with the silica gel of the plates. The fluorescence of the dansyl polyamines diminished with increasing time intervals between the application of a sample and start of the chromatographic separation. That could be the source of considerable errors in quantitation. A few years before, Smith [140] and Wettaufer and Weinstein [141] advised spraying isopropanoltriethanolamine (4:1, v/v) to intensify and stabilize fluorescence on the plate immediately after removal from the chromatographic tank. The effects of non-ionic surfactants as mobile phase additives on the fluorescence intensity of dansyl derivatives of biogenic amines in HPTLC were also investigated. The eluent consisted of chloroform–triethylamine (2:1, v/v) containing 5% of polyoxy-ethylene-10-lauryl ether (POLE). It was shown that the presence of POLE in the mobile phase enhances both the fluorescence intensity and the signal-to-noise ratio of the chromatographed dansyl derivatives. The method was applied to the determination of biogenic amines in plant tissues and beer samples [142].

A major disadvantage in application of dansylation processes in plant amine analysis is the inability to derivatize satisfactorily agmatine or *N*-carbamyl-putrescine, which are both fundamental precursors of putrescine in plants and bacteria through the arginine pathway operating concurrently with the ornithine pathway. This difficulty has been resolved by derivatizing agmatine through benzoylation procedures [47,79]. Through RP-HPLC following solid–liquid extraction and derivatization with OPA and mercaptoethanol, Raasch and co-workers [143] confirmed the presence of agmatine in mammalian tissues. Their work also raised the possibility that it could play a potential role as a neurotransmitter and/or hormone and, as is the case for plants and bacteria, serve as a polyamine precursor along a metabolic pathway previously not detected in mammals. A mitochondrial associated arginine decarboxylase activity has been characterized in animal kidney and brain tissues [66,144,145]. Thus, special efforts should be made to characterize and quantify agmatine in biological extracts. An improved RP-HPLC method has been recently proposed for the quantitation of dansylated polyamines in plants, particularly dansyl-agmatine which was characterized in cold stressed wheat [122]. A high speed analysis of dansylated polyamines in plant tissues after post derivatization clean-up was proposed by Walter and Geuns [146]. A rapid HPLC method was published for the quantitation of polyamines as their dansyl derivatives with applications for plant and animal tissues [121]. To conclude discussion of dansylation procedures which are still widely used in plant sciences and food technologies, it should be mentioned, as previously encouraged by Smith [44], that because of the frequent side reactions and potential degradation artefacts which can occur, it is essential to run a blank sample in parallel with standards and plant extracts.

In the same way Watanabe et al. [119] revealed that interfering compounds occurring during benzoylation procedures could be superimposed over those of the benzoylated polyamines in HPLC. Moreover, chromatograms obtained after benzoyl chloride can be further complicated by a wide variety of plant constituents that absorb at 245 nm or at 254 nm. The benzoylation method was compared with the derivatization procedure with OPA and mercaptoethanol for HPLC estimation of polyamines in crude extracts of *Azolla caroliniana* and other plant extracts [111]. The benzoyl chloride method was

found to vastly underestimate the amount of polyamines in some plant extracts, a problem not encountered with the OPA procedure. The latter method had a sensitivity of 1–2 picomols and completely resolved agmatine, spermine, norspermidine, spermidine, 3,5-homospermidine, 4,4-homospermidine, 1,3-diaminopropane, putrescine and cadaverine in 12 to 14 min. Pre-chromatographic derivatization with OPA followed by reversed-phase HPLC and fluorometric detection was described by the same authors as offering some advantages over other techniques through its selectivity of reacting only with primary amines [111]. Moreover, this derivatizing agent has been successfully applied using an on-column derivatization process for determination of biogenic amines in wines [100].

Simultaneous derivatization of amino-acids and biogenic amines has been exploited to develop chromatographic procedures which could resolve these two classes of metabolites during the same analyses. This was performed by RP-HPLC of the dansyl derivatives in food samples where more than 40 compounds could be separated simultaneously [125]. We have also developed a protocol of RP-HPLC separation of AQC derivatives which can be applied to crude plant extracts without prepurification steps for the concurrent characterization and quantitation of amino-acids and amines at the picomole level [131]. Moreover, monoacetyl conjugates of polyamines can be analyzed through this procedure [130]. A rapid and sensitive derivatization method, using CRA-Cl as derivatization reagent, for the simultaneous determination of amino-acids and biogenic amines in plant tissues with pre-column fluorescing derivatization via liquid chromatography was also recently proposed [129].

Among chromatographic processes applied to plant amine analysis, direct gas chromatography of alkylamines is often difficult because many column packing materials tend to absorb to polar amines and cause peak tailing and reduced sensitivity. Therefore, GC is usually preceded by derivatization of polar functional groups. Reactions usually necessitate isolation of analytes in a water and salt free form. Trifluoroacetic anhydride is probably the most widely derivatizing agent used for GC separation and flame-ionization (FID) or electron-capture (ECD) detection. The extract is generally cleaned through butanol extraction or cation-exchange chromatography [44,147]. For plant amine determination, derivatization processes for GC analyses can also be performed through trifluoracetylation [82,148], trimethysilylation [149], pentafluropropionylation [106,107,150], heptafluorobutyrylation [151], pentafluorobenzoylation [75] or *N*-ethyloxycarboxylation (with ethylchloroformate) [152–154]. Smith [44] gave some pertinent details to proceeding with some of these derivatization procedures. Interestingly, through heptafluorobutyrylation processes and GC analysis Hamana, Matsuzaki and co-workers [106,107,112–114] characterized and identified a number of common and uncommon alkylamines in leguminous seeds and plants, among which a few examples of the most unusual could be N^6-methylagmatine, homoagmatine, norspermidine, homospermidine, norspermine, thermospermine, homospermine, canavalmine, aminopropylhomospermidine, aminopropylcanavalmine, aminobutylcanavalmine, caldopentamine, homocaldopentamine, caldohexamine and homocaldohexamine [106,107,112–114]. It was reported that some of these uncommon polyamines appear to be related to stress responses in stress-tolerant plants [35]. Through the same analytical processes, triamines such as norspermidine, spermidine and homospermidine, and tetraamines such as norspermine, spermine, thermospermine and aminopropylhomospermidine were found to be distributed ubiquitously in eight extremely thermophilic bacteria (growing at 70°C) belonging to *Thermus* species [113].

3. Chromatographic monitoring of plant amines

As is the case for other biological materials, TLC is still widely used for analysis of amines in plant tissues, although it has been partially replaced nowadays by HPTLC associated with radiolabelling, pre-chromatographic derivatization and fluorimetric detection or colorimetric monitoring following post-chromatographic staining with ninhydrin or Sakaguchi reagent [43]. However, HPLC constitutes by far the most widely used technical approach for amine analysis in plants. Styles used include RP-HPLC, ion-pair reversed-phase or ion-exchange. Almost every type of detection can be coupled to the

HPLC system, including UV–Vis, fluorescence, radiochemical, electrochemical (amperometric and coulometric) and refractometric. In recent years mass spectrometric detection has become accessible in combination with HPLC (HPLC–MS), and this is probably the most powerful approach of analyte monitoring available. Detailed reviews on TLC and HPLC methods applied in plant amine biochemistry have already been published [42–44].

Since most of the HPLC procedures and detection modes devoted to the characterization of biogenic amines in animal tissues have been transposed to plant material analysis, we focus here on chromatographic processes developed specifically for the analysis of amine metabolites particular to plants, or amines specifically associated to plant cell structure or developmental programs.

Applications of HPLC to plant amine analysis have been primarily based on RP-HPLC using C_{18} columns and dansyl- or benzoyl derivatization with fluorescence and UV-monitoring, respectively [77,110]. In the case of separation of underivatized polyamines ion-pairing RP-HPLC [95] or ion-exchange chromatography with fluorogenic detection following post-column derivatization with OPA have been used [155,156]. Following pioneer works of Seiler and coworkers [117] and Smith and Best [105], Smith and Davies [77] published one of the first analytical procedures based on RP-HPLC of dansyl polyamines in plant tissues and demonstrated that fluorescence spectrophotometry (with a detection limit of 0.8 pmol of standard polyamine) was much more sensitive than the UV detection of benzoyl polyamines described a few years before by Flores and Galston [110]. These fundamental reports were succeeded by numerous studies which generated major advances in the description of amine profiles in plants, of which most were based on chromatographic analysis of dansylated products. The resolving power of the HPLC system for the separation of dansyl polyamines was the basis of many of those developments. Using acetonitrile as eluent Hayman et al. [157] were able to resolve 29 dansylamines out of the 45 tested within an elution time of 80 min, in application of the system to plant extracts. A study estimating putrescine, spermidine and spermine in lichen samples of *Evernia prunastri* L. with RP-HPLC after dansylation [118] pointed out the effectiveness of the post-derivatization initially described by Seiler and Knödgen [158]. In applying the method to lichens, it was found that about 5% (w/w) of the exogenous putrescine taken up by the thallus was unbound in the algal partner and that 60% (w/w) was conjugated in the thallus, perhaps to lichen phenolics [118]. More recently, with the need for a more rapid screening of amines in plant material and a complete resolution of 1,3-diaminopropane from other polyamines, Marcé et al. [121] described a rapid and sensitive HPLC method with pre-column derivatization using dansyl chloride for the determination of five natural polyamines, including 1,3-diaminopropane, putrescine, cadaverine, spermidine and spermine in both plant and animal tissues. The separation takes only 9 min on a Spherisorb-ODS C_{18} column with acetonitrile as eluent and provides good resolution between 1,3-diaminopropane and putrescine. 1,3-Diaminopropane and cadaverine were not detected in any of the animal tissues under investigation [121]. It should be noticed, as encouraged by Koc et al. [159], that longer HPLC protocols are required to achieve separation of the uncommon polyamines from the common polyamine counterparts. The authors have recently characterized norspermidine and norspermine in cultured tissues of maize [159]. For the special case of agmatine a simple dansylation and thin-layer chromatographic procedure was recently proposed by Bencsik et al. [122] for the separation and purification of dansyl-agmatine while attempting to determine its bioregulatory role in plant tissues and in order to standardize the RP-HPLC determination of natural polyamines. In practice, the authors observed that agmatine was relatively labile and commercial products were heavily contaminated by several impurities justifying TLC preparation of dansyl-agmatine prior to HPLC measurement. The results indicated that, among wheat genotypes that respond differently to low temperature, the significance and role of the alternative route for putrescine via agmatine may vary considerably. In the same way, we found that derivatization processes of amines combined with RP-HPLC and fluorescence detection can be efficiently applied to demonstrate the high plasticity of polyamine metabolism in response to osmotic or salt stress on a range of crop species [26,160–162]. Moreover, Outinen and co-

workers [163,164] performed intensive work on gradient elution and mobile phase developments to optimize HPLC analysis of dansylated biogenic amines in plants and embryogenic cell culture lines. The optimization was done with the PRISMA model, which is a multi solvent system which is of considerable benefit when searching the optimum mobile phase by changing both the solvent strength and its selectivity [163,164].

Although less common, improvements on chromatographic analyses of plant amines after benzoylation and UV detection have been applied in plant biochemistry or food technologies. The original method of Flores and Galston [110] has been widely adopted by other analysts. A simple modification of the original HPLC procedure was proposed by Slocum et al. [79], which greatly improves the separation and quantitation of putrescine and 1,3-daminopropane. It also allows the simulation analysis of phenetylamine and tyramine, which are major monoamine constituents of tobacco and other plant tissues. Moreover misidentification of the agmatine peak in the original report [110] was resolved when an agmatine sample was prepared in 10% PCA, instead of the usual 5% PCA, and then benzoylated under standard conditions [79]. With this same derivatization procedure, a narrow-bore HPLC method was successfully applied to the identification and quantitation of free, conjugated and bound polyamines from leaves of *Vitis vinifera* L. [165]. The sensitivity of the C_{18} narrow-bore column (2.1×200 mm) was about five times higher and provided a substantial savings in solvent solution of about 80% compared to the more commonly used wider bore columns.

Improved HPLC methods for estimation of polyamines derivatized with OPA and mercaptoethanol have been described [111]. The fluorescent derivatives were eluted from a C_{18} column with the dimethylcyclohexylamine–phosphate buffer derived by Skaaden and Greibickk [166]. The method had a sensitivity of 1–2 pmoles and completely resolved agmatine, spermine, norspermine, spermidine, 3,5-homospermidine, 4,4-homospermidine, 1,3-diaminopropane, putrescine and cadaverine in 12 to 14 min. Interestingly, the study compared relative efficiency of both benzoylation and derivatization with OPA for the determination of polyamines levels in a variety of plant material [111]. As an example of a recent application, an optimized method to determine biogenic amines and other amines in wine with pre-column derivatization with OPA revealed its high efficiency for food science applications [167]. Determination of the different amines has also been carried out using a polarity gradient elution technique involving methanol and sodium acetate buffer. Still with OPA as the derivatizing agent, an HPLC analytical method has been proposed for the simultaneous determination of the polyamines and 5-adenosyl-containing compounds in extracts of plant protoplasts [95]. The procedure is based on an ion-pairing RP-HPLC of native analytes, with the 5-adenosyl methionine metabolites being detected by their UV absorption at 250 nm and polyamines derivatized on-line with the OPA reagent after the eluent had passed through the UV detector. To obtain improved separation, the ion-pairing reagent initially proposed by Wagner et al. [168] was changed from octanesulfonic acid to heptanesulfonic acid [95]. On this same basis Halline and Brasitus [169] published an ion-pairing RP-HPLC method for the measurement of polyamine oxidase activity. More recently, an ion-pairing HPLC method based on the simultaneous use of heptanesulfonate and octylamine has been considered with the aim of optimizing the separation of four food-related biogenic amines and related precursor amino-acids [170]. The addition of octylamine to the eluent reduced peak asymmetry. Two decades earlier Villanueva and Adlakha [109] had proposed an automated analysis of common basic amino-acids, mono, di- and polyamines, phenolic amines and indoleamines in crude biological samples including plants. This was based on ion-exchange HPLC and post-column derivatization with OPA. This procedure was also efficiently applied to the separation and quantitation of a wide range of long chain polyamines in leguminous plants [106,107,114]. Simon-Sakadi and Holzapfel [171] characterized biogenic amines in leafy vegetables through amino-acid analysis detected following post-column colorimetry with ninhydrin. Another mode of fluorometric derivatization with OPA combined with HPLC has been developed and applied for biogenic amine determination. It consists of an in-column derivatization process achieved by pumping the OPA reagent through the column together with other

solvents used as mobile phases. The method was recently checked with several red wines from Tarragona in comparison with the standard pre-column method [100].

Although the most commonly used methods are based on pre- or post-column derivatization of amines using several derivatizing agents (as described above), detection methodology that does not require derivatization is preferred for its convenience and simplicity. This is especially true of biochemical studies of amine metabolism and interactions involving feeding experiments with radiochemicals. As an example, ion-pairing RP-HPLC on a C_8 column was recently used to confirm the covalent attachment of polyamines to plant mitochondria [172]. Accordingly, plant mitochondria from both potato and mung bean were shown to incorporate radioactive polyamines in the membrane fraction. Using a radiometric detector, HPLC of mitochondrial hydrolysates revealed that the radioactivity bound to mitochondria was attributed to polyamines and traces of acetyl polyamines [172].

New developments in electrochemical detection, especially in pulsed amperometric detection (PAD) and a new variant integrated pulsed amperometric detection (IPAD) have become quite useful for amine species [103]. As reported by Lucy [173], the emergence of biological applications of ion chromatography is mainly due to the development of pulsed amperometric detectors. Although numerous aromatic compounds including catecholamines and other aromatic amines are detected easily by anodic reactions at a constant applied potential at solid electrodes, the majority of aliphatic amines are not observed to be very electroactive under amperometric conditions. Addressing this problem resulted in interesting developments in electrochemical detection, especially with the use of multistep potential waveforms, (called PAD) for direct, sensitive and reproducible detection of numerous amino and sulphur compounds at Au and Pt electrodes [102].

Many assays that have used HPLC with electrochemical detection for amine analyses of urine, blood, cerebrospinal fluid and brain tissues have been realized both with conventional or microbore HPLC designs [174]. In contrast, very few applications for plant amine analysis have been published. Very recently, an improved IPAD method was proposed for the evaluation of biogenic amines in food of vegetable or animal origin and in fermented foods [103]. These technologies should be considered with attention by plant analysts [175,176].

Regarding other detection methodologies which do not involve derivatization procedures, the recent and highly promising development of techniques utilizing atmospheric pressure ionization, mainly atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), has pioneered the coupling of HPLC with mass spectrometry (MS). The use of HPLC with tandem MS (MS–MS) opens further dimensions in the field of amine analysis, both for quantitation and direct elucidation of the structure in complex matrices [177–181]. Uncommon conjugates of spermidine were recently discovered in the pollen of *Hippeastrum hortorum (Amaryllidaceae)* with online coupling of HPLC to atmospheric pressure chemical ionization spectrometry [182].

Due to their conjugated nature to phenolics, hydroxycinnamic amides and the more basic hydroxycinnamic amine conjugates which are closely related to the flowering process, to sexual organogenesis and virus resistance in different botanical species [10], can be analyzed through HPLC without derivatization [93]. These authors demonstrated the ability of a C_{18}-RP column to separate basic hydroxycinnamic amides and hydroxycinnamic acids. This technique allowed the separation of these compounds from crude plant extracts or low purity extracts involving a clean-up procedure with Amberlite CG-50 H^+. Detection of peaks at 310 nm was very helpful in eliminating UV absorption of all co-extractable compounds able to interfere at lower wavelengths (254 or 270 nm) with aromatic amines. RP-HPLC with a C_{18} column eluted with acetonitrile and photometric detection (maxplot between 230 and 400 nm) by a photodiode array detector was recently used for the separation and quantitation of hydroxycinnamic acid amides (coumaroylputrescine, feruloylputrescine, coumaroylagmatine and feruloylagmatine) in methanolic extracts from the roots of mycorrhized barley [183]. Accordingly a preparative HPLC protocol based on the same chromatographic conditions was developed by the authors to identify the phenolic amides through positive and negative electrospray MS and ^1H and ^{13}C NMR spectroscopy. It was suggested that accumulation of

the amides in the early developmental stages of barley mycorrhization reflects initiation of a defence response [183]. Concurrently, using fluorescence detection and analytical plus preparative RP-HPLC of crude methanolic extracts, accumulation of feruloyltyramine and *p*-coumaroyltyramine was observed in tomato leaves in response to wounding. The compounds could be localized to cells at the wound sites where they are proposed to function as components of the chemical and physical defences against herbivores and pathogens [83]. Despite concentrated efforts towards the characterization and the quantitation of amine conjugates in plants, the actual functions of hydroxycinnamic amides, which have been shown to occur at high levels during cell division and cellular differentiation, is still a matter of debate among authors who consider these molecules to be storage forms and those who attribute biological activity to them (reviewed in [11]). One of the difficulties from an analytical point of view is to precisely define the chemical and the physical compartmentation of the substances at the cellular and whole plant levels in relation to developmental processes and environmental constraints. Now that quantitative procedures have been efficiently developed, this type of spatial analysis is perhaps the next big challenge for future amine research.

On a similar note for amine conjugates analysis in plant materials, a few studies have dealt with optimizing methods primarily based on HPLC and TLC for the analysis of alkylamides derived from fatty acids, which are rather concentrated in some plant species [68]. After an extraction procedure with chloroform, alkylamides can be characterized through RP-HPLC with acetonitrile–water as solvent system and UV detection at 210 and 250 nm [184,185]. A recently published separation of jasmonic acid amine conjugates has been performed by HPLC on reversed-phase and chiral stationary phases [186]. Little is known about these conjugates in terms of their physiological functions and much more analytical work needs to be performed to get clearer ideas about their distribution, representation, localization and subcellular compartmentation in plants.

Among chromatographic procedures, thin-layer chromatography was initially used extensively for characterization and even quantitation of polyamines in plant tissues. However, as described above, HPLC procedures have nowadays displaced TLC, despite the fact that TLC could still be quite useful for quantitative [187,188] or preparative purposes with MS characterization [44,79,107,189]. Some of the derivatization procedures described above, especially dansylation, are often carried out as a prerequisite for TLC or HPTLC development of amines on silica gel using simple or two-dimensional systems. That was applied by Smith and coworkers [1,189,190] to initiate the description of the distribution of cadaverine and other amines in higher plants. With TLC using cellulose plates, and Sakaguchi as chromogenic reagents the presence of the guanidino group was revealed. This same laboratory reported the occurrence of γ-hydroxyhomoarginine in pea seedlings, a significant finding since its lower homologue, γ-hydroxyarginine, is known from *Vicia* species and from *Lens culinaris* [191,192]. Agmatine derivatives such as coumaroylagmatine and hordatines, were also described in different members of the *Gramineae* according to that same procedure [105].

In this chapter of chromatographic profiling of plant amines, gas chromatographic (GC) procedures are not detailed since the isolation and derivatization processes that usually precede gas chromatography with FID, NPD, ECD or MS detection have been described in a previous paragraph of this review. An excellent recent review dealing with investigation of polyamine metabolism by HPLC and GC profiling methods pointed out that HPLC and GC of polyamines are not competitive techniques, but rather complementary [48]. It is explained that in comparison to HPLC, GC has become neglected probably because it requires laborious and unattractive prepurification. Nevertheless, at present capillary GC is said to be more suitable for profiling of polyamines and derivatives because of its higher number of theoretical plates and its easy adaptability to GC–MS. As an example, distribution profiles of polyamines derived from a compilation of analysis done by both GC and HPLC in aquatic plants has been recently published [193]. Unusual polyamines and a novel tetraamine (bis(aminopropyl)ethanediamine) have been identified through GC–MS.

4. Electrophoretic profiling of plant amines

Due to their strong positive charge at both neutral and acidic pH, amines are readily separated by electrophoresis. Paper (PE) or thin-layer (TLE) electrophoresis and ninhydrin or Sakaguchi reagent spraying is well suited for the separation and identification of the many small molecular mass guanidine compounds which are found in plants [44,194]. The mobilities of several amines are given by Smith et al. [78]. However, nowadays PE and TLE are rarely used for amine analysis.

In recent years, capillary electrophoresis (CE) has developed into a reliable and efficient separation technique well suited for the determination of amines [46]. CE separations are based on the different electrophoretic mobilities of ionic analytes present in an electrophoretic medium within a narrow bore capillary [46]. The most frequently used modes for CE are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF) and capillary isotachophoresis (cITP).

Although extremely powerful, these methods, probably due to equipment costs, are not widely used in laboratories working on plant biochemistry and plant physiology. The analytical design is mainly devoted to pharmacological approaches and food processing or quality controls from both plant and animal origins. To date, optimization of the electrophoretic separation of derivatized or non-derivatized amines has been well overcome, but the sensitivity and selectivity of the detection are still areas that need to be improved. CZE and MECC are by far the most widely used techniques [46,104,195]. The separation by the micellar electrokinetic method is routinely used with fused-silica capillaries, an electrolyte system of borate modified with sodium dodecyl sulfate, and methanol, acetone, acetonitrile or urea as organic modifiers [104,195–202]. In some cases, better separation of amines could be obtained with cyclodextrins added in the electrophoretic buffers [199,200,203]. Various modes of detection are generally found in the literature (reviewed in [46]) including fluorescein isothiocyanate (FITC) derivatization and laser induced fluorescence (LIF) detection [196,198,199,204] and other precolumn derivatizing chromophores or fluorphores such as AQC [195], benzoyl chloride [202], or dansyl chloride [197]. The latter has been applied to the determination of biogenic amines in wine and food samples [196–198,202,205]. Interestingly, trimethylamine and other low-molecular mass amines can be characterized in atmospheric aerosol samples using capillary electrophoresis with LIF detection. Such a design could conceivably be applied to plant samples where hard to handle, low molecular mass amines are well abundant but poorly characterized.

The other currently used electrophoretic methodology for amine analysis is high-performance CZE with indirect photometric detection [206–210]. Lin et al. [211] demonstrated the high sensitivity to organic modifiers in the capillary zone electrophoretic separation of aromatic amines. Meanwhile, the successful separation of tosylated aliphatic polyamines by high-performance CZE on an uncoated fused-silica capillary was reported by plant physiologists [212]. Of course, detection efficiency in capillary electrophoresis, similar to all the separation techniques described previously, can be optimized through MS coupling.

5. Concluding remarks

For three decades, almost every kind of separative methods combined to most of the detection systems have been applied to amine analysis in plant materials. The main goals of plant amine analysts were to bring these technologies into the development of high performance routine procedures in terms of selectivity, repeatability and detectability and when possible, minimum running costs. Domains of application concern fundamental aspects of amine biochemistry in plants as well as increasing needs in the control of food and beverage quality from plant origin. Considerable informative works have been performed through UV or fluorimetric detection of isolated derivatized amines after chromatographic or electrophoretic separation procedures. That led to the identification of numerous uncommon polyamines in

plants whose functions remain to be elucidated. The multiple steps often required for isolation and derivatization procedures incline to special care in terms of yield recovery. Recent improvements in electrochemical amperometric detection are worth thinking about for applications in the analysis of native underivatized amines. One of the most promising approaches in quantitating and above all characterizing plant amines is mass spectrometry applications, which are nowadays quite easy to couple to gas or liquid chromatography apparatus. Obviously, within the analytical procedure the extraction and purification of the amine fraction constitutes crucial and critical steps in plant materials which could be considered as complex matrices where numerous coextractable interfering compounds often occurred. A major difficulty depends on the multiple amine fractions often found in plants whose extractibility differs according to the nature of the chemical compartmentation of the amine compounds. All these fractions should be considered and handled with attention since these are not 'frozen' but highly fluctuating pools where amines could be interchangeable through chemical processes or enzymatic reactions. Extensive works have been done on free amine isolation in comparison to studies devoted to conjugated amine analysis, especially water soluble or insoluble amines conjugated to phenolics and fatty acids or amines covalently linked to proteins. Due to their physiological interest or their nutritional impact, more attention should be devoted to these conjugated forms.

To date, literature abounds full of descriptive data concerning quantitation of amines and polyamines (especially free amines) in numerous plant species in response to various developmental stimuli or environmental conditions. Although such correlative results are the necessary prelude to mechanistic investigation, the correlation between amine titre and a physiological response does not necessarily provide information as to their direct implications and the molecular events involved. In that way, studies focusing on the actual localization of amines at the whole plant level and on their cellular apoplastic and symplastic distribution should be encouraged. Moreover, little information is available concerning membrane translocation of amines in plants as well as their long-distance fluxes through xylem and phloem vessels for evaluating their function as inter-organ messengers. Radiochemical, cytochemical and above all immunocytochemical studies may be promising tools as far as specific antibodies can be prepared for the latter, which is not an easy task [213]. Concurrently, as previously pointed out, using these antibodies, competitive ELISA may be developed [214,215]. Concerning investigation on polyamine translocation, patch-clamp techniques have been assayed in the whole cell configuration or on membrane isolated from vacuoles and suggested polyamine channeling in protoplast and vacuole [216]. This technical approach recently demonstrated polyamine implication in regulation of vacuolar ion transport [55,56]. Thus ionic binding of amines is thought to be important in regulating the function of biological macromolecules, as well as their synthesis, in vivo. Although quite well documented in animal systems, polyamine research in plants suffered of a lack of information concerning biophysical interactions induced in situ by these compounds. In the same way, while comparing the different metabolic pathways involved in the amine content regulation, especially those related to catabolic processes, systems are less finely described in plants than in animals. Thus the regulation of amine oxidase and the metabolic fate of the catabolic products in plant cells are not well characterized. The need for analytical procedures to address these issues on plant amine metabolism should be emphasized. Taking into account the high flexibility of amine structure and metabolism in plants, future works should take advantage of technical advances performed in this area to increase understanding of the physiological functions in relation to the apprehension of concentration, structure, compartmentation and biophysical interactions.

6. Nomenclature

APCI	Atmospheric pressure chemical ionization
AQC	6-Aminoquinolyl-*N*-hydroxy succinimidyl carbamate

CE	Capillary electrophoresis
CEC	Capillary electro chromatography
CGE	Capillary gel electrophoresis
CIEF	Capillary isoelectric focusing
cITP	Capillary isotachophoresis
CRA-Cl	Carbazole-9-yl-acetyl chloride
CZE	Capillary zone electrophoresis
Dansyl	5-dimethylaminonaphtalene-1-sulphonyl
DNA	Deoxyribonucleic acid
ECD	Electron-capture detection
ESI	Electrospray ionization
FID	Flame-ionization detection
FITC	Fluorescence isothiocyanate
FMOC	9-Fluorenylmethyl chloroformate
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
IPAD	Integrated-pulsed amperometric detection
LIF	Laser induced fluorescence
MECC	Micellar electrokinetic capillary chromatography
MS	Mass spectrometry
MS–MS	Tandem mass spectrometry
NBD-F	4-Fluoro-7-nitrobenzo-2-oxo-1,3-diazole
NMR	Nuclear magnetic resonance
NOC-Cl	2-Naphtyloxycarbonyl chloride
NPD	Nitrogen–phosphorus detection
OPA	*O*-Phthalaldehyde
PAD	Pulsed amperometric detection
PCA	Perchloric acid
PE	Paper electrophoresis
PNZ-Cl	Para-nitrobenzocarbonyl chloride
POLE	Polyoxy-ethylene-10-lauryl ether
RNA	Ribonucleic acid
RP-HPLC	Reversed-phase high performance liquid chromatography
SCX	Strong cation exchanger
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TLE	Thin layer electrophoresis
Tosyl	*p*-Toluene sulfonyl
UV	Ultra-violet
UV–Vis	Ultra-violet–visible

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