Recent advances in the determination of biogenic amines in food samples by (U)HPLC

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Abstract

The determination of biogenic amines (BAs) in food products stirs up an increasing interest because of the implications in toxicological and food quality issues. Apart from these aspects, in the last years, the relevance of BAs because of some organoleptic and descriptive concerns has been pointed out by several researchers. This overview aims at revising recent advances in the determination of BAs in food samples based on liquid chromatography. In particular, papers published in the last five years have been commented. Special attention has been paid in the great possibilities of ultra-high performance liquid chromatography and high-resolution mass spectrometry. Regarding applications, apart from the determination of BAs in a wide range of food matrices, novel lines of research focused on the characterization, classification and authentication of food products based on chemometrics have also been discussed.

Keywords: Biogenic amines; Food; Liquid chromatography; (U)HPLC-MS(/MS); Chemometric characterization.

1. Introduction

Biogenic amines (BAs) consist of low molecular mass compounds of basic nature that participate in metabolic pathways of living being. As shown in Fig. 1., BAs are classified of according to the number of amino groups in their structures in monoamines (e.g., histamine, tyramine, serotonin, phenylethylamine, ethanolamine and tryptamine), diamines (putrescine and cadaverine) and polyamines (spermine and spemidine). BAs are biologically active molecules that take part in multiple cellular functions. Among others, monoamines are involved in neurotransmission and regulation of the blood pressure and body temperature.[1] Polyamines are essential for the cellular proliferation and differentiation as they participate the synthesis of DNA, RNA and proteins.[2]

The principal via of formation of BAs is the degradation of the amino acids by the action of decarboxylase enzymes. In lesser extension, BAs are also produced by transamination reactions of aldehydes and ketones. Excessively high levels of amines may be harmful to living beings so that detoxification mechanisms are required. In particular, oxidases such as monoaminooxidase (MAO), diaminooxidase (DAO) and polyaminooxidase (PAO) participate in the degradation of mono-, di- and polyamines, respectively.[1,2]

Focusing our interest on food analysis, amines occur naturally in protein-rich matrices such as fish, meat, vegetables and fruits from the mentioned degradation of the corresponding amino acids. In particular, BAs are especially abundant in products subjected to the action of microorganisms like, for instance, fermented foodstuffs including dairy products, beer and wine.[3-5] Furthermore, spoiled foods and products manufactured under poor hygienic conditions commonly display high concentrations of

some BAs, especially histamine, tyramine, putrescine and cadaverine.[6] Hence, these compounds result in excellent indicators of alteration and putrefaction processes underwent by foodstuffs.

It is well known that the intake of products containing high amounts of BAs may cause toxicity episodes, especially in sensitive individuals. By far, histamine is the most studied amine since it is the most problematic one. Is has been described that histamine provokes psychoactive (headache, palpitations and itching), vasoactive (hypotension), cutaneous (rash) and gastrointestinal (nausea, vomiting and diarrhea) effects.[7,8] Its toxicity may be enhanced in combination with other components of the diet such as other BAs and alcohol.[9] Also, drugs such as monoamine oxidase inhibitors (IMAO), which are currently used for the treatment of depression and hypertension, may induce severe toxicity effects. Tyramine is another relevant monoamine related to the release of catecholamine neurotransmitters that may increase the blood pressure and cardiac frequency. In healthy people, the cardiovascular effects of tyramine from dietary sources are likely negligible although dangerous hypertensive crises that may occur in individuals taking IMAO drugs. Tyramine has also been documented as a trigger of migraine episodes.[10] Regarding adverse effects of other BAs, phenylethylamine may act as powerful migraine inductor, and spermine and spermidine seems to participate in mechanisms of cancer growth.[11]

A different aspect of dietary amines deals with their influence on the organoleptic properties of foods. In this regard, it has been reported that putrescine may be associated to rancid and dirty aromas of putrefaction and cadaverine confers meaty and vinegary odors to the foodstuffs.[12]

Recently, some authors have opened up a new research trend of great scientific impact that concerns the role of BAs as descriptors of food features.[13] In this topic,

compositional profiles of amines have been exploited as a source of analytical data to try to extract relevant information dealing with characteristics and quality of foods.

In our revision, the most significant advances on BA determination corresponding to the period 2010-2016 will be discussed. In particular, the introduction of ultra-high performance liquid chromatography (UHPLC) and high resolution mass spectrometry (HRMS) has opened up excellent possibilities to improve the figures of merit of the analytical methods. In this regard, Table 1 summarizes the publications based on high resolution chromatography, either involving core-shell column technology in conventional HPLC or UHPLC. As a different aspect to be remarked, data mining based on chemometrics has appeared in the analytical scenario to facilitate food classification and authentication studies. For such a purpose, principal component analysis (PCA) has been used extensively to carry out exploratory studies to recover the underlying information. Other methods such as partial least squares (PLS) and partial least squares-discriminant analysis (PLS-DA) have been applied to quantification of classification tasks.

2. State-of-the-art of the determination of biogenic amines in food

Rapid, simple, inexpensive and reliable analytical methods are required to satisfy the increasing demand of BA determinations in food matrices. In the last years, dozens of new analytical methods have been proposed in the scientific literature to tackle this objective. Besides, interesting revisions on this topic have been published in the last years.[14-17] Such a proliferation of methods respond to the need of resolving new challenges raised in the field of food analysis. Some advances are focused on improving figures of merit such as precision, accuracy, sensitivity and detection limits.

Complementary issues such as simplicity, cost and speed of analysis, environmental concerns, etc. are also taken into account to cover the fast-growing demand of controls of food products.[14-17]

In the 1950s, first publications on BAs reported the study of histamine in foods. Other amines such as tyramine, putrescine and cadaverine were progressively discovered and investigated in the following years. Since then, more than 20 different BAs have been identified and determined in foodstuffs.

For decades, most of the methods for the determination of BAs in food products has been based on HPLC. Nowadays, HPLC still remains as the technique of choice although other separation techniques such as capillary electrophoresis (CE) have emerged as an alternative to develop faster and cheaper methods.[11,18] New derivatization strategies and innovative on-line preconcentration procedures have contributed to improve dramatically the performance of CE methods.[19] Nowadays, the limits of detection attained by CE may be comparable to those given by HPLC. Apart from separation methods, simpler approaches have been focused on a generic biogenic amine index (BAI) as the indicator of food freshness.[5,20] Other straightforward assays can be found in the literature, typically relying on colorimetric, enzymatic and immuno-based reactions implemented as flow injection analysis (FIA) and biosensor devices.[16,21]

The following sections contain a general introduction to the key steps of liquid chromatographic methods for the determination of BAs. It has been evidenced that sample treatment procedures are recommendable to avoid interferences.[14-17] In particular, sample clean-up and analyte preconcentration are often applied to enhance the performance of analytical methods. Most of the reported methods comprise derivatization to improve the detectability of analytes resulting in electrochemical, spectroscopic or fluorimetrically active derivatives (see Table 1). Such reagents allow

126 highly sensitive detections compatible with the analyte levels typically encountered in
127 food samples. In the particular case of MS detection, however, BAs labelling is not
128 strictly needed so simpler procedures relying on direct amine detection can be
129 developed.[20]

130 A reliable optimization of analytical methods is crucial when dealing with
131 complex food samples containing a great variety of components. Optimization is often
132 conducted by a trial-and-error approach in which studies are carried out without a pre-
133 established plan of experiments. However, because of the diversity of variables that
134 are involved in the BA determination, the application of design of experiments (DOE)
135 for a more comprehensive optimization of working conditions seems to be highly
136 recommendable.[22] DOE has demonstrated to be highly effective to find out the best
137 conditions from a reduced set of experiments. In this regard, two key aspects to be
138 considered are: (i) the definition of the optimization criterion to be used and (ii) the
139 election of experimental variables to be explored.

140 The optimization criterion refers to the overall suitability or quality of the
141 experimental results, i.e., what it is understood as the optimal. It should be noted that
142 frequently a single objective may be insufficient to express the optimal situation of a
143 given method. For instance, in the case of the separation, the good resolution of
144 closely eluted compounds and the reduction of the run time are important objectives
145 deserving our attention. Hence, multicriteria approaches can be implemented to take
146 into account all desired objectives simultaneously.[22-24]

147 Regarding the experimental variables (factors) to be investigated, a primary aim
148 of DOE is the identification of the relevant ones using fast screening approaches. At
149 this point, the evaluation of the intensity of main effects and interactions at a reduced
150 experimental cost is fundamental. Subsequently, those factors found relevant are

submitted to a more comprehensive study while those irrelevant can be obviated. It should be highlighted that when interactions among factors are detected, simultaneous optimization of such variables should be conducted to assess the final conditions. For this purpose, surface response methodologies based on central composite, grid and other experimental designs can be used to fit the resulting data.[22]

In the last years, the number of publications exploiting DOE for a more feasible optimization of the extraction of BAs in food matrices is increasing. Some representative examples are commented as follows. For instance, Rigueira *et al*. used Plackett-Burman designs to evaluate the significance of factors that were further studied in more detail with central composite methodologies.[24] A Plackett-Burman design was also assessed to optimize variables such as reagent volume and pH in the benzoylation of nine BAs in non-alcoholic beers.[25] In another case, a Box-Behnken design was used to fit response surfaces to study the main parameters affecting the derivarization and extraction of BAs.[26] In a similar way, full factorial and central composite designs were applied to study a DLLME of dinitrobenzoyl amine derivatives using ACN and carbon tetrachloride as the solvents.[27] Response surface modeling from central composite design was established by Bashiry *et al*., to optimize the MAE-DLLME of polyamides from turkey breast meat samples.[28]

The systematic optimization of the separation entails some difficulties in the DOE definition. For instance, Fig. 2 has been adapted from reference 23 to illustrate the optimization of the separation of BA derivatives of 1,2-naphthoquinone-4-sulfonate by grid design (Fig. 2a). Slope and gradient time were chosen as the variables to create a linear gradient profile based on the percentage of methanol as the organic solvent of the mobile phase. In the example, the chromatographic resolution of three systems of close peaks and the analysis time were taken as the responses to create an

overall multi-objective function (Fig. 2b). In another example, face-centered central composite designs were used to optimize the separation of dansyl derivatives of BA in Chilean young wines.[29] Apart from DOE, sequential approaches for the simultaneous optimization of several variables can also be considered. For instance, Sanchez *et al*. studied the separation of BA by ion-exchange HPLC using the modified simplex as the optimization method in which the percentage of 2-propanol in the mobile phase and the variation of the gradient curve were the variables under study.[30]

2.1. Sample treatment

The principal tasks of sample treatment to deal with the determination of BAs in foods and beverages comprise cleanup, preconcentration and derivatization. The arsenal of strategies for sample preparation devoted to the determination of BAs and other families of food constituents have been reviewed by J. Plonka.[31] The treatment is mainly focused on removing interferences from the sample matrix, improving the detection limits and enhancing the separation and detection performance. In this revision, derivarization methods will be discussed in an independent section as they deserves especial attention.

From the point of view of the nature of interfering compounds, it should be noted that BAs are concomitant to presence of high concentrations of amino acids. Certainly, both BAs and amino acids form derivatives via amino group under similar working conditions so that mutual interferences commonly appear. It is thus crucial to overcome the amino acid interference on the BA detection either by previous removal of interferences or by selective extraction of analytes. Other families of naturally occurring food components, such as polyphenols, may also interfere with by the amine

detection because they are active spectroscopic, fluorimetric or electrochemically. In the particular case of polyphenols, their anionic nature may be exploited for a selective removal.

The sample preparation procedures depend on complexity of the food matrices as well as on the variety of compounds that occur simultaneously with the analytes.[32] For beverages such cold drinks, beer and wine, sample filtration prior HPLC analysis might be sufficient to tackle the determination of BAs. If needed, centrifugation can be used to remove suspended residue particles. For solid samples, a solvent extraction to recover the compounds of interest free of matrix components is commonly applied. Preliminary operations such as sample grinding, mashing, etc. facilitate the BA dissolution in the extraction solvent.[31]

Solvent extraction allows to obtain the amines free of most of the interfering compounds. When low polarity organic solvents such as CH_2Cl_2 and hexane are used, samples are basified to generate the neutral species of BAs that can be recovered easily the liquid-liquid extraction (LLE). Alternatively, samples can be treated with diluted acids (e.g., trichloracetic and hydrochloric acids) to dissolve the BAs as the cation (protonated) species. In any case, the corresponding extracts can be further purified by solid phase extraction (SPE) for retaining either the analytes or the interferences.[33,34] For instance, amines can be fixed efficiently by anion-exchange in commercial cartridges owing to their positive charge at acid pH values. In a different way, reversed-phase C_{18}, polymeric and anion exchange cartridges can be used for sample cleanup to retain, and thus remove, interferences from polyphenols, acids and other organic compounds.

Although conventional LLE and SPE treatments mentioned above have proved their great efficiency in terms of preconcentration and cleanup performance some weak

226 points still arise. Hence, new approaches are welcome to try simplify tedious and time-
227 consuming procedures and minimize the manipulation of large amounts of toxic and
228 expensive solvents. In this regard, the so-called salting-out assisted liquid–liquid
229 extraction (SALLE) has successfully been also introduced as a straightforward
230 methodology in the determination of BAs in food samples. For instance, in the method
231 by Jain *et al*., amines in fruit juices and alcoholic beverages were first labelled with 1-
232 naphthylisothiocyanate and derivatives were further extracted with acetonitrile (ACN).[35]
233 The separation of the organic phase was achieved by the addition of ammonium
234 sulfate which promotes the release of a clean ACN extract. An analogous methods
235 based on SALLE was applied to the BA determination in wines using dansyl-Cl as the
236 reagent and ACN as the solvent.[36] Another extraction strategy is based on matrix solid-
237 phase dispersion (MSPD). In two recent publications, Self *et al.* proposed MSPD
238 process with further analysis of extracts by UHPLC-HILIC chromatography coupled to
239 orbitrap MS for the determination of BAs in tuna and shellfish products.[37,38] Samples
240 were grinded in the presence of a CN-silica sorbent, the homogenized powder was
241 transferred into an extraction tube and analytes were eluted with a 50 mM ammonium
242 formate aqueous solution / ACN (20/80, v/v). The eluate was collected, diluted, filtered
243 and analyzed chromatographically. The incorporation of specific ligands to the sorbent
244 material has been presented as a successful modification MSPD to improve the
245 method performance. For instance, Tameem *et al*., synthetized various hydrazone-
246 based ligands to be entrapped on a silica matrix to study the extraction of BA in various
247 food samples such as ketchup, soy sauce, orange juice, etc.[39] Extraction rates were
248 higher than 96% for most of the amines. SPE or LLE have also been implemented in a
249 micro-extractive format to enhance pre-concentration while saving solvents. Apart from
250 the manipulation of reduced volumes of samples and reagents, the use of highly

selective and sensitive hydrazone ligands has been proposed.[40] Among others, benzophenone 2,4-dinitrophenylhydrazone was found to be one of the most promising agents leading to enrichment factors of 94–460 fold. As a result, figures of merit of the method, such as selectivity and detection limits, were significantly improved. In the so-called dispersive liquid-liquid microextraction (DLLME), apart from a low polarity organic solvent such as chloroform, an additional disperser solvent (e.g., acetone) is added. In the paper of Wu and coworkers, DLLME was used in combination of ultrasound assistance and fluorescence labeling with a carbonochloridate.[26] The organic phase containing the derivatives was withdrawn with a syringe and injected into the chromatograph. Detection limits achieved were below 10 ng mL^{-1}. A similar approach was followed for the extraction of BAs in fermented food products.[41] In another example, DLLME was applied to determine BAs in alcoholic beverages based on 1-dodecanol and methanol as the extraction and dispersive solvents, respectively.[42] Further separation of the organic phase was facilitated by solidification of the organic droplets in an ice bath. Apart from the afore-mentioned examples concerning DLLME, modified procedures such as surfactant-assisted emulsification and ionic liquid-based microextraction were introduced to increase sensitivities in comparison with other extraction techniques.[43]

2.2. Derivatization

More than 80% of methods proposed in the literature for BAs determination rely on fluorogenic or chromogenic dyes. From the vast list of probes suitable to react with the amino group, in the last years, dansyl chloride has become the most popular labelling one.[33,44-47] Analogously, other chlorides and related compounds (e.g., dabsyl-, 3,5-

277 dinitrobenzoyl chloride, 9-fluorenylmethyl chloroformate) have also been utilized in
278 several recent papers for similar purposes.[43,48-50] Fig. 3 shows the chemical structures
279 of various current BA labelling reagents. Regarding the derivatization mode, pre-
280 column approaches result in the most applied possibility. In contrast, the application of
281 post-column approaches is less extended due to some practical concerns of separation
282 and online derivatization. Generic schemes of on-line pre- and post-column modes are
283 depicted in Fig. 4.

284 Some general issues of pre-column labelling deal with the stability of reagent
285 and reaction products, the formation of a unique derivative for each analyte and the
286 completeness of the derivatization. Although reagents mentioned above mostly satisfy
287 these requirements of suitability, some concerns may still arise. In general, the stability
288 of products is moderate and derivatives may undergo partial degradation at high
289 temperature and pH.[15] Another problematic aspect refers to the formation of unique
290 derivatives since, in the case of di- and polyamines, various amino groups participate in
291 the reaction. As a result, mixtures of mono-, di- and poly-derivatives of variable
292 composition might occur when working under non-optimized experimental conditions.
293 The multiplicity of derivatives may be circumvented using high reactive/analyte ratios in
294 order to fully label all active amino groups. Hence, the completeness of the reaction will
295 rely on adding a sufficient excess of reagent with respect to the overall amount of
296 amino compounds. However, it has been highlighted that the reagent excess is another
297 serious shortcoming since it may cause severe interferences in the chromatographic
298 peaks some amines. As a result, processes for removing this excess are
299 recommendable (in some cases indispensable). Other experimental conditions such as
300 pH, working temperature and reaction time are also relevant to get a quantitative
301 derivatization.[15] In general, pH values required are comprised between 9 and 11 to

keep the amine reactive (deprotonated) while minimizing side hydrolysis processes due to an excess of OH^-. Regarding temperature and time, optimal values are quite characteristic of the kinetics of each labeling agent (see below).

Pre-column labeling is usually developed in off-line format in glass vials by mixing small volumes of reagent, sample and buffer. If necessary, vials can be heated to accelerate the reaction. Organic solvents may be used to extract, purify and concentrate the resulting derivatives. In the particular case of dansyl-Cl, the sulfonyl chloride as the active group is able to react with both primary and secondary amines. The reaction requires quite long reaction times (20 – 60 min) and mild temperatures (40 – 70^{o}C). Derivatives can be monitored spectrophotometrically at 250 and 320 nm or fluorimetrically at λex = 320 nm and λem = 520 nm. [33,44-47] The intrinsic absorption or fluorescence of the reagent may cause interferences so procedures for removing its excess after labeling are recommended. Regarding other chloride reagents, such as dabsyl-Cl, 9-fluorenylmethyl chloroformate, etc., experimental conditions are quite similar to those reported for dansyl-Cl since they are related structurally and reaction mechanisms are analogous.[48-50]

Apart from off-line methods, the pre-column reaction can also be implemented on a continuous-flow manifold coupled on-line to the chromatographic system.[15] The set-up is assembled using pumping devices, reactors, T-pieces, connectors, etc. such as those typically used in flow injection analysis (FIA).[21] Among other advantages, the reaction can be automated and drawbacks dealing with degradation and by-products can be minimized.

Post-column derivatization has been proposed for circumventing some weak points of pre-column modes, especially regarding to the formation of multiple derivatives. In addition, other concerns dealing with stability and full derivatization

become irrelevant. It should be noted that although the reaction yield should be as high as possible to maximize the sensitivity, a full derivatization of analytes is not essential. In consequence, agents suitable for the post-column mode should be compatible with a sufficient development of the process in a short residence time. For such a purpose, *o*-phthaldialehyde (OPA) and NQS have been introduced due to the rather fast kinetics in the reaction with the amino compounds.[51-53,15] Post-column derivatization approaches obviously involve the separation of bare amines coupled online to a continuous flow system in which the reaction takes place. The experimental set-ups have been adapted from FIA systems using standard components and pieces of such manifolds.

Briefly, OPA is the most typical agent for to post-column derivatization although it has also been used in pre-chromatographic mode. OPA is able to react with primary amino groups in the presence of a mercapto-containing compound (e.g., 2-mercaptoethanol, 3-mercaptopropionic, etc.) at pH in the range 8.5 – 10.7 and room temperature to yield derivatives detectable by UV absorption and fluorescence spectroscopy.[51-53] In a similar way, NQS has been introduced as labeling of both primary and secondary amines through nucleophilic aromatic substitution of the sulfonate by the amino group. The reaction is developed at pH values in the range 9 to 10.5 and temperatures about 60 to 90^{o}C. NQS derivatives are detectable both spectroscopically (at 305 and 470 nm) and electrochemically via reduction of quinone moiety.[15]

Fig. 5 shows an example to illustrate the complexity of the optimization issues in the BA derivatization with NQS.[23] Variables under study were temperature and reaction time and responses selected were the peak areas of each BA. In order to perform a more comprehensive evaluation of working conditions, a DOE methodology was conducted. In the graphs of response surfaces of representative amines fitted from a

central composite design (Fig. 5a), it can be seen that the behavior was quite different depending on the analyte, for instance, with high yields at high temperature and time (histamine), at low temperature and high time (cadaverine), high temperature and low time (tryptamine) and mild temperature and time (phenylethylamine) (Fig. 5b). Under these circumstances, it was clear that a consensus solution was needed to reach a reasonable sensitivity for all the compounds. Hence, agreement relied on multi-objective functions such as the geometric mean of each amine area, e.g., overall response = $(a_{histamine} \times a_{cadaverine} \times a_{tryptamine} \times a_{phenylethylamine} \times \ldots)^{1/amines}$. The maximum of this function corresponded to optimum of the experimental conditions (Fig. 5c).

2.3. Separation

Although the most common approach for BA determination relies on the separation of amine derivatives, the separation of unlabelled BAs has also been considered as another analytical possibility. Bare BAs are sometimes separated by reversed phase HPLC using octadecylsilane (C_{18}) stationary phases specially functionalized for the retention of polar compound. This is the case of Sagratini and coworkers that separate underivatized BAs in a Synergy Hydro RP column from Phenomenex[34,54] or Renes *et al.* that carried out the separation in an Atlantis Dc18 column from Waters.[55] Even so, due to the high polarity of these compounds their direct interaction with the stationary phase is still weak so that the analytes are poorly retained and separation from other matrix components is difficult. In order to enhance the the retention of analytes, ion-pair or micellar chromatographic modes can be explored.[56,57] Alternatively, the ionic nature of these compounds in acid media has been exploited to carry out excellent separations by ion-exchange LC of underivatized BA in cheese, meat

and fish samples.[53,58] More recently, new approaches based on hydrophilic interaction liquid chromatography (HILIC) have been introduced to conduct the separation of BAs. The utility of HILIC for the determination of BA and other polar compounds has been recently reviewed by Koupparis *et al*.[59] Although less popular, some authors have assessed the possibilities of the aqueous normal phase (ANP) chromatography, with a hydrated silica stationary phase, to separate bare amines.[60]

In the case of the separation of derivatives such as those commented in section 2.2, the polarity of these molecules is notably reduced and labelled compounds are able to interact efficiently with reversed-phase stationary phases. Hence, the derivatization opens up great possibilities to improve the separation with respect to the aforementioned cation exchange, HILIC, or micellar-based modes. In general, the separation of BA derivatives is carried out in C18 columns using elution gradients created from aqueous (acidified) solutions and methanol or ACN as organic solvents.

Recently, the introduction of high resolution LC has contributed to improve the separation of BAs. In this way, ultra-high performance liquid chromatography (UHPLC) allows excellent separations in shorter analysis time compared to those obtained by conventional HPLC (i.e., using columns packed with particles bigger than 4 μm). This technology has gained importance during the last years as demonstrated the increasing the number of publications in the field of food analysis. However, as commented above, the direct separation of BAs is difficult in reversed-phase mode so that pre-column derivatization has usually been performed in UHPLC methods.[33,37,44,46,51,52,61,62] Besides, HILIC separations in UHPLC have also been reported, for example, in the quantification of BAs in tuna.[38] Alternatively to the UHPLC, the core-shell technology can provide similar resolution, sensitivity and analysis time to UHPLC as demonstrated in the determination of BAs in wines, fruit nectar, cheese, etc.[47,63-66] The most relevant applications using high

resolution LC in both core-shell and UHPLC modes for the assay of BA in foods and beverages are summarized in Table 1.

2.4. Detection

UV-vis absorption and fluorescence spectroscopies are widely spread for the detection of BAs, especially for routine and control applications.[33,37,46,50-52,61,63] It should be remarked that only few analytes such as tryptamine and phenylethylamine are readily detectable with these techniques while the rest of BAs display poor chromogenic or fluorogenic properties. Hence, derivatization is commonly required to achieve limits of detection compatible with the levels of amines in the food samples. As previously described, pre-column derivatization with dansyl chloride is widely employed to improve method sensitivity. For example, Preti *et al*. reported LOD values in the range 2 μg/L (PUT) to 23 μg/L (AGM) when fluorescence detection was used for the UHPLC determination of BAs in wine and fruit nectar samples.[47] UV-vis spectroscopy provided higher LODs (between 13 and 112 μg/L) than those achieved by fluorescence spectroscopy.[44,52] As an alternative to these spectroscopic techniques, evaporative light scattering detection has been applied for BA determination in cocoa-based products, reporting detection limits in the range 10 to 20 μg/L.[67]

In the case of mass spectrometry (MS) detection, HPLC-MS methods may include a derivatization step focused on facilitating the analyte separation by conventional reversed-phase mode while improving detection features. For example, the identification and determination of BAs in food matrices based on the use of a stable isotope-coded derivatization and LC-MS/MS analysis was reported.[64] N-hydroxysuccinimidyl ester of d_0/d_4-4-methoxybenzoic acid, which mainly functionalized

primary amines, was employed for the identification and structural characterization of BAs by tandem mass spectrometry in different foodstuffs such as sausages, cheeses and fish. The derivatives in obtained that way provided selective cleavage at the binding site between the reagent and the amine in ESI-MS/MS experiments and produced a characteristic fragment derived from the reagent moiety, allowing the simultaneous determination of labeled amines under MS/MS conditions. In general, triple quadrupole MS analyzers working in multiple reaction monitoring (MRM) acquisition mode have been described for the MS/MS determination of BAs[64] in food, and generally two selective reaction monitoring (SRM) transitions have been followed to achieve the required high selectivity and specificity of tandem mass spectrometry methods. Besides, other MS analyzers based on linear ion-trap technology have also been described for the tandem mass spectrometry determination of BAs. For instance, a QTrap instrument was employed for the LC-ESI-MS/MS determination of BAs in Gheonggukjang (a fermented Korean traditional food made from soybeans), also monitoring two SRM transitions of dansyl derivatives to achieve analyte quantitation and confirmation.[46]

In the last few years, new methods proposing the detection of unlabeled amines have appeared in the analytical scenario as a way of simplifying the procedures. In this regard, the hyphenation between HPLC and tandem mass spectrometry (MS/MS) seems to be especially attractive because of its great analytical performance providing both enhanced selectivity (chromatographic and spectral) and sensitivity. The cationic nature of amines makes recommendable the use of positive ionization often with electrospray sources. MS(/MS) is typically carried out with triple quadrupole, ion trap and QTrap analyzers using MRM as the most convenient detection mode. For instance, Sirocchi *et al*. detected and determined 10 BAs in meat samples by HPLC-

452 MS/MS.[54] Detection relied on MRM using $[M+H]^+$ as the precursor ion and $[M+H-17]^+$
453 as the product ion. In the study, both ion trap and triple quadrupole mass analyzers
454 were compared in terms of analytical performance, being the later the most sensitive
455 and selective. A similar detection strategy was used in the determination of 8 BAs in
456 fish by SPE and LC-MS/MS using a triple quadrupole mass analyzer.[34] Garrido-Frenich
457 and coworkers developed a UHPLC-MS/MS method for the simultaneous
458 determination of some biogenic and volatile amines in anchovies.[62] Again, MRM of the
459 transition of $[M+H]^+$ to $[M+H-17]^+$ was chosen for quantification (besides, other
460 confirmation transitions were also established to ensure the unambiguous detection of
461 the desired species).

462 Regarding advanced MS detection, nowadays, high resolution mass
463 spectrometry (HRMS) and accurate mass measurements are emerging as one of the
464 most powerful options for the analysis of food samples in order to guarantee
465 identification and confirmation of the targeted food contaminants. As in the case of low
466 resolution instruments, HRMS is obviously compatible with both unlabeled and labeled
467 amine detection. Basically, there are four types of HRMS instruments: magnetic sector,
468 time-of-flight (TOF), Orbitrap, and Fourier transform ion cyclotron resonance (FTICR)
469 instruments. The most frequently used with UHPLC methods are TOF and Orbitrap
470 analyzers. In general, TOF instruments present a resolution (instrument's ability to
471 measure the mass of two closely related ions precisely) of approximately 10,000 –
472 40,000 FWHM (full width half maximum) with accuracies in the mass determination of
473 1-5 ppm, while the resolution of Orbitrap instruments is in the range 10,000 – 140,000
474 with 1-2 ppm mass accuracy (for comparison, a conventional quadrupole MS
475 instrument shows a resolution of 1,000 FWHM and accuracies of 500 ppm).[68]

Recent advances in both LC-TOF-MS and LC-Orbitrap-MS methods have reduced instrument costs, simplified analysis, and improved accuracy, and today these advances offer bench-top instrumentation that is amenable to screen and identify a great variety of contaminants in food, not only the targeted ones, but also non-target or unknown contaminants.[69] So today, UHPLC-HRMS methods either using TOF[42,45,65] or Orbitrap[37,38] analyzers have also been employed for the determination of BAs in food matrices, although the potential of these techniques has not yet been fully exploited. For example, only medium resolution capabilities (10,000 FWHM) were employed for the determination of eight biogenic amines in tuna and seafood samples by LC-atmospheric pressure chemical ionization (APCI)-HRMS using an Orbitrap mass analyzer[37,38].

3. Applications

3.1 Determination of biogenic amines in food samples

The revision of the scientific literature has shown that a wide list of new methods to tackle the determination of the most common BAs is published (see examples in Table 1). In some cases, however, due to the relevance of some specific amines, more delimited methods have been addressed to quantify a given (or few) compound(s) such a histamine, putrescine and cadaverine. Apart from BAs, some methods have been focused on a wider range of components including other families of food constituents such as amino acids, volatile organic compounds, etc. as a way to enrich the information available for more comprehensive descriptions and characterizations of food products. There is an increasing interest in the assessment of the freshness or the hygienic quality of

501 foods.[20,70,71] In this context, BA contents reflect accurately these food conditions. For
502 instance, the BA index (BAI), accounted as the sum of concentrations of amines
503 (expressed in mg kg^{-1} or mg L^{-1}), results in an excellent descriptor of food quality. For
504 fresh food, BAI values lower than 5 mg kg^{-1} correspond to good conditions while BAI
505 values higher than 20 – 50 mg kg^{-1} indicate the occurrence of an extended degradation
506 and spoilage, thus suggesting poor hygiene or quality. In a similar way, the spermidine to
507 spermine ratio has also been used as a freshness index. In general, ratios lower that 0.5
508 correspond to good products while values higher than 0.7 are associated to an advanced
509 state of decomposition.

510 Regarding the type of samples analyzed, BAs are much more abundant in
511 fermented products than in fresh ones[72-76]. As commented in the introduction, this
512 assertion is derived from the fact that the microorganism activity associated to
513 fermentation contributes to degrade amino acids thus resulting in increased levels of
514 amines. For this reason, BAs have been much more studied in fermented and ripened
515 matrices, especially in wines (see Table 1, the review by Pena *et al*. in ref. 72 and
516 others[73-75]). BAs are mainly formed in wines during alcoholic and malolactic
517 fermentations as well as during aging process although a small amount is already
518 present in grape juice. In general, putrescine is the most abundant amine representing
519 around a 50% of the overall BA contents. Also, significant amounts of histamine,
520 tyramine and phenylethylamine can be found. The composition of BAs seems to be
521 dependent on climatic and geological factors of the wine producing regions as well as
522 enological practices such as grape variety, skin maceration, aging, yeast strains, etc.
523 As developed in the following section, compositional profiles of BAs have been
524 exploited as a source of analytical data for descriptive purposes.[77]

Other fermented products such as cheese, beer and other alcoholic beverages, vinegar, cured meat and fish have also been analyzed extensively (see examples in Table 1). In these products, the evaluation of BAs throughout the elaboration processes provides information on the evolution of the ripening. This may be of great interest from the point of view of food technology in order to better control the quality and the reproducibility of food characteristics. More sporadically, other products such as fresh meat, sauces, juices, fruits, etc., have been also subjected to BA evaluations (see references in Table 1). In these matrices, as mentioned above, BA levels may be a sign of spoiled food or products obtained under deficient sanitary conditions.

3.2. Characterization of foods through compositional profiles of biogenic amines

Some pioneering studies addressing the potential use of BAs as descriptors of food features and quality were proposed by Garcia Villar *et al.*[77] Amine contents in Spanish red wines were determined by HPLC with pre-column derivatization with NQS and the corresponding compositional profiles and fingerprints were used as the data to be treated chemometrically with PCA and PLS. Some relevant relationships among BA concentrations and wine features were deduced. Results indicated that BA contents depended on enological practices, especially on the malolatic fermentation. As a result, young wines could reasonably be distinguished from aged ones on the basis of BA profiles.[77] For instance, it was found that tyramine decreased with aging while histamine continuously increased with time.

Since then, other interesting studies have been published and various representative examples are discussed as follows. In the paper by Charlton and coworkers, compositional data, including concentrations of BAs and other chemical

parameters (e.g., rare earth elements and isotopic ratios), were exploited to tackle wine discriminations depending of geographical origins, grape varieties, harvesting seasons, etc.[78] Some chemometric methods such as discriminant partial least squares, classification and regression trees and neuro-fuzzy systems were applied to data analysis. In another example, Chilean Cabernet Sauvignon wines produced from organic and non-organic grapes were compared according to BA contents.[79] BAs were determined in the main stages of the winemaking process including must, alcoholic fermentation and malolactic fermentation. As expected, BAs were mainly formed during the malolactic fermentation. PCA and PLS-DA using BA data clearly allowed organic and non-organic wines to be discriminated. In particular, it was deduced that organic wines exhibited lower amine levels. Ordóñez and coworkers established the BA profiles of vinegars to study the amine occurrence during the elaboration process as well as to try to discriminate among varieties such as apple, balsamic and Sherry.[80] Compositional data treated by PCA showed that some vinegar types displayed characteristic BA levels which allowed the samples to be distinguished. Guarcello *et al*. published a survey on the influence of technological, microbiological and biochemical variables on BA profiles of cheeses.[81] Apart from BAs, the data matrix included amino acid concentrations, pH and water activity. PCA was used to evaluate the sample distribution as a function of the experimental variables. Despite the complex multifactorial nature of the cheese manufacturing some general patterns were envisaged. For instance, negative correlations of pH with various amine levels were found. It was also indicated that histamine concentrations increased with the amount of histidine and the activity of histidine-decarboxylase bacteria. In a similar way, Tassoni and coworkers enriched the set of data combining concentration levels of BAs, anthocyanins and other polyphenols to characterize grape berries and wines obtained

575 from different agricultural and oenological practices.[82] Although no significant
576 differences were found regarding the winemaking practices, the amine contents were
577 actually dependent on the grape varieties.

578 Recently, metabolomics has been introduced to food analysis as a powerful
579 approach to sample characterization, classification and authentication.[83] Metabolomics
580 consists of the systematic study the small organic molecules that participate in the
581 metabolic processes of living beings. In the field of food analysis, two or more groups
582 of samples can be compared to try to find out patterns and differences in metabolomic
583 profiles to be related with their features. Anyway, working with the overall metabolome
584 (i.e., all occurring metabolites) is too complex so food studies are focused on a limited
585 variety of molecules such as organic acids, alcohols, aldehydes, ketones and esters,
586 polyphenols, amino acids, saccharides and, of course, amines. Metabolomics can be
587 conducted under both targeted and untargeted modes. The term targeted
588 metabolomics has been coined to include those cases relying on concentration profiles
589 as the data. Alternatively, in the untargeted counterpart, instrumental fingerprints
590 coming from known or unknown components are analyzed. In this regard, Arbulu *et al*.
591 proposed an untargeted metabolomic method using a LC-ISE-QTOF instrument to
592 generate fingerprints of non-volatile components.[65] The search of ions on databases
593 such as METLIN provided a tentative identification of more than 400 chemical
594 candidates including sugars, amino acids, amines, polyphenols, organic acids, etc.
595 Significant differences among wine varieties on the basis of some specific biomarkers
596 were encountered.

597 As the concluding remarks, recent methods developed for BA determination rely
598 on conventional HPLC. Anyway, the introduction of UHPLC is gaining popularity
599 because of the general improvement in the method performance, especially in terms of

speed and separation capacity. Regarding the sample treatment, although the simpler matrices can be analyzed directly, in the case of meat products, seafood, cheese, etc., the application of intensive treatment procedures prior to LC injection seems to be necessary. In this sense, conventional LLE and SPE methods have demonstrated a great efficiency for cleanup processes. Some extraction novelties have been introduced to decrease the consumption of sample and solvents, to improve the recovery yield and precision, and to simplify the procedure. For instance, dispersive LLE and SPE, microwave- and ultrasound-assisted extraction methods have been applied successfully. The amine derivatization is widely used for improving the detectability and separation ability. The use of highly sensitive chromogenic and fluorogenic reagents allows excellent detection limits to be achieved, often in the order of $\mu g\ kg^{-1}$ (or $\mu g\ L^{-1}$). For this reason, LC methods with UV and fluorescence detection are fully compatible with the BA levels in food samples. Of course, in the last years, MS detection has become very popular because of its great performance and the unambiguous identification of analytes. Besides, LC-MS(/MS) has demonstrated to be highly efficient for the detection of unlabeled amines, thus resulting in an excellent way to simplify the analytical methods. Also, LC-MS(/MS) has enlarged the field of applications, especially for dealing with other families of food constituents together with BAs. The significance of BAs as efficient descriptors of food quality should not be underestimated. In particular, the freshness of food products can be assessed from BA levels. Also, the potentiality of compositional profiles of amines for descriptive purposes results in a hot topic deserving the attention of the scientific community. Hence, more comprehensive and efficient characterization, classification and authentication studies can be tackled in combination with chemometric methods using the amine amounts as the source of analytical data.

625

626 **Conflict of interests**

627 Authors declare that there is no conflict of interests.

628

629 **Acknowledgements**

630 This work was partially financed by the Generalitat de Catalunya and the Spanish
631 Ministry of Economy and Competitiveness (MEC) with projects NASSOS 2014SGR377
632 and Superfactory CTQ2014-56324, respectively.

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901

902 Figure Captions:

903

904 Fig. 1. Structures of the most relevant biogenic amines occurring in food products.

905

906 Fig. 2. Optimization of the separation of amine derivatives by central composite design.
907 Influence of the gradient profile on the overall suitability of the separation considering
908 the resolution of close peaks and the analysis time as the objective responses.

909

910 Fig. 3. Chemical structures of common reagents for pre- and post-column labelling of
911 amines. 1: dansyl chloride; 2: dabsyl chloride; 3: 9-fluorenylmethyl chloroformate; 4:
912 3,5-dinitrobenzoyl chloride; 5: 2-hydroxynaphthaldehyde; 6: 2-chloro-1,3-dinitro-5-
913 (trifluoromethyl)-benzene; 7: 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate; 8: *o*-
914 phthaldialehyde; 9: sodium 1,2-naphthoquinone-4-sulfonate; 10: naphthaldialehyde.

915

916 Fig. 4. Scheme of online labelling system. (a) pre-column manifold; (b) post-column
917 manifold.

918

919 Fig. 5. Application of experimental design to optimize the derivatization of biogenic
920 amines with 1,2-naphthoquinone-4-sulfonate as the reagent. (a) central composite
921 design (b) Influence of temperature and reaction time on the area of histamine,
922 cadaverine, tryptamine and phenylethylamine peaks. (c) Influence of temperature and
923 reaction time on the overall multicriteria surface considering all the amines
924 simultaneously.

925

926 Table 1. Summary of methods for the determination of biogenic amines in food samples.

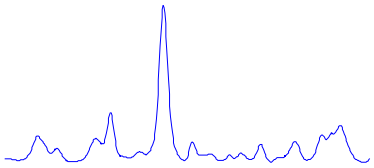
Analytes	Matrix	Pretreatment	Column	Mobile phase	Analysis time (min)	Detection	LOD	Ref
Core-shell technology								
ETA, MEA, HIS, PHE, TYR, SER, AGM, PUT, CAD, SPD, SP	Wine, fruit nectar	Pre-column Dansyl chloride	Phenomenex Kinetex XB-C18 (100 x 3 mm, 2.6 µm)	A: 0.1% formic acid in water B: ACN	13	UV (254 nm), FLD (λ_{ex}=320 nm) (λ_{em}=523 nm)	0.002-0.023 mg kg^{-1}	47
Metabolite fingerprinting	wine	Sample pre-treatment (general)	Phenomenex Kinetex XB-C18 (100 x 3 mm, 2.6 µm)	A: 0.1% formic acid in water B: ACN	40	TOF	---	65
HIS, PHE, TYR, TRP, PUT, CAD, SPD, SP	Cheese Sausages Fish	Stable isotope-coded derivatization 4-MBA-OSu	Phenomenex Kinetex C18 (50 x 2.1 mm, 1.9 µm)	A: 0.1% formic acid in water B: ACN	10	MRM	0.01-0.02 mg kg^{-1}	64
HIS, TYR, CAD, PUT	wine	AccQFluor™ derivatization	Phenomenex Kinetex PFP (100 x 4.6 mm, 2.6 µm)	A: 0.14 mol L^{-1} acetate + 0.017 mol L^{-1} TEA in water (pH 5.05) B: MeOH	10	FLD (λ_{ex}= 250 nm) (λ_{em}= 395 nm)	0.12-0.34 mg L^{-1}	63
HIS, TYR, CAD, PUT	wine	AccQFluor™ derivatization	Phenomenex Kinetex PFP (100 x 4.6 mm, 2.6 µm)	A: 0.14 mol L^{-1} acetate + 0.017 mol L^{-1} TEA in water (pH 5.05) B: MeOH	10	FLD (λ_{ex}= 250 nm) (λ_{em}= 395 nm)	0.12-0.34 mg L^{-1}	66
UHPLC								
TRP, PHE, PUT, CAD, HIS, TYR, SPD, SP	wine	Pre-column Dansyl chloride	Agilent Zorbax Extend-C18 (50 x 3.0 mm, 1.8 µm)	A: 10 % ACN (in H_2O) B: ACN	---	DAD (254 nm)	---	44
TRP, HIS, SPD, AGM, TYR, SP, PUT, CAD	Fish sauce Wine	Post-column o-phthalaldehyde	YMC-Triart C18 material (prototype) (50 x 2.0 mm, 2.2 µm) (50 x 3.0 mm, 2.2 µm)	A: 10 mM NaH_2PO_4 and 150 mM $NaClO_4$ (pH 2.0 adjusted with H_3PO_4), containing 20 mM 1-pentanesufonic acid sodium salt B: 10 mM NaH_2PO_4 and 150 mM NaClO4 (pH 2.0 adjusted with H_3PO_4)/Methanol (50/50), containing 20 mM 1-pentanesufonic acid sodium salt	7	FLD (λ_{ex}=345 nm) (λ_{em}=455 nm)	18-80 fmol	51
HIS, CAD, PUT, TMA, TEA, TYR, TPA	Anchovy	---	Waters Acquity™ UPLC BEH C_{18} (100 x 2.1 mm, 1.7 µm)	A: MeOH B: 0.1 % formic acid in water	8.5	MRM	25-60 µg kg^{-1} (LOQ)	62
TYR, TRP, PUT, CAD, HIS, PHE, SPD	wine	SPE + Pre-column Dansyl chloride	Waters Acquity™ UPLC BEH (50 x 2.1 mm, 1.7 µm)	A: 0.1% formic acid in water B: 0.1% formic acid in ACN	6.5	qTOF	3-15 ng mL^{-1}	33
TYR, TRP, PUT, CAD, HIS, PHE, SPD	Fermented food Beer Cheese Sausages	Pre-column Dansyl chloride	Waters Acquity™ UPLC BEH C_{18} (100 x 2.1 mm, 1.7 µm)	A:0.1% formic acid in water B: 0.1% formic acid in ACN	25	qTOF	0.005-0.02 µg mL^{-1}	45
TYR, TRP, PUT, CAD, HIS, PHE UCA, AGM	Tuna Seafood	Matrix solid-phase dispersion	Waters Acquity™ UPLC BEH HILIC (150 x 2.1 mm, 1.7 µm)	A: ammonium formate buffer B: ACN	15	MS (orbitrap)	0.02-2.5 ppm	37,38
AGM, CAD, PHE, PUT, SER, TRP, NE, SP, SPD, HIS, TYR, DOP	Cheonggukjang	Pre-column Dansyl chloride	Agilent Zorbax Eclipse XDB-C8 (50 x 2.1 mm, 1.8 µm)	A: 0.1% formic acid in 20 mM ammonium acetate (pH 3.5) B: 0.1% formic acid in ACN	8	MRM	10-40 µg kg^{-1}	46

AGM, PHE, CAD, HIS, PUT, SPD, SP, TRP, TYR	Seafood	Pre-column Dansyl chloride Post-column o-phthalaldehyde	Agilent Zorbax Eclipse XDB C18 (50 x 4.6 mm, 1.8 µm)	A: 0.1 mol L^{-1} ammonium acetate B: ACN	8	UV (254 nm)	0.2-1.2 mg kg^{-1}	52
ETA, MEA, BA, AGM, HIS, EA, DMA, OTA, PA, SPD, SP, DOP, TYR, PUT, CAD, TRP, PHE, IPA, Pyrrolidine, MBA	Cheese	Pre-column 6-aminoquinolul-N-Hydroxy-succinimidyl carbamate	Waters Acquity™ UPLC BEH C_{18} (50 x 2.1 mm, 1.7 µm)	A: 50mM sodium acetate in 1% tetrahydrofuran in water (adjusted to pH 6.6 with acetic acid) B: MeOH	9.5	UV (254 nm)	0.04-1.6 mg/100g	61

Agmatine (AGM), butylamine (BA), cadaverine (CAD), dimethylamine (DMA), dopamine (DOP), ethanolamine (ETA), ethylamine (EA), isopropylamine (IPA), methylamine (MEA), 3-methylbutylamine (MBA), octopamine (OTA), phenylethylamine (PHE), propylamine (PA), putrescine (PUT), serotonin (SER), tryptamine (TRP), norepinephrine (NE), spermine (SP), spermidine (SPD), histamine (HIS),tyramine (TYR), spermidine (SPD), urocanic acid (UCA), triethylamine (TEA), trimethylamine (TMA), tripopylamine (TPA).

ACN: acetonitrile, MeOH: methanol 4-MBA-Osu: N-hydroxysuccinimidyl ester of d_0/d_4-4-methoxybenzoic acid

Occurrence of biogenic amines

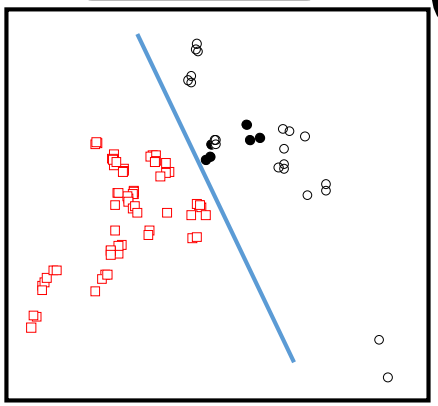


$R\text{-}NH_2$

Extraction
Derivatization

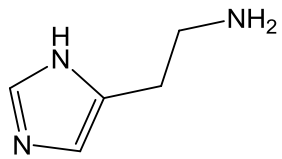
(U)HPLC analysis

Data analysis

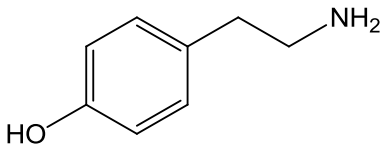


- Characterization
- Classification
- Authentication

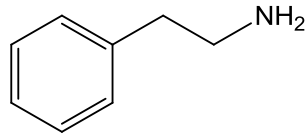
Fig. 1



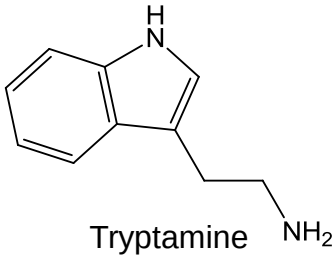
Histamine



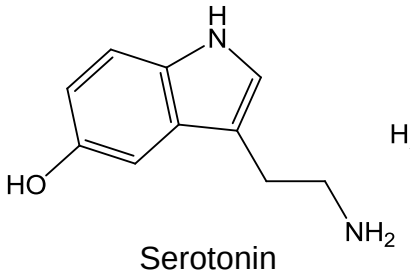
Tyramine



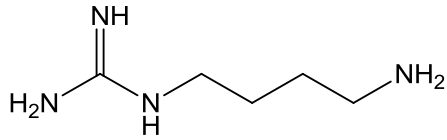
Phenylethylamine



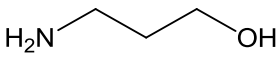
Tryptamine



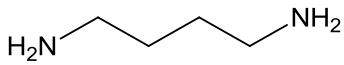
Serotonin



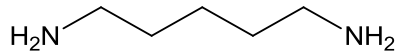
Agmatine



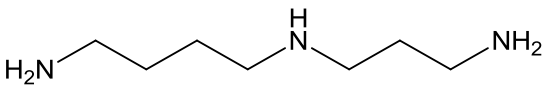
Ethanolamine



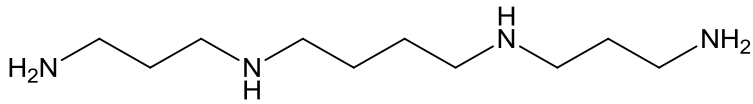
Putrescine



Cadaverine

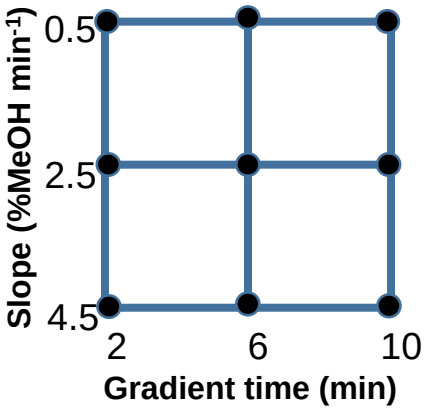


Spermidine



Spermine

Fig. 2


Slope (%MeOH min-1)
0.5
2.5
4.5
2
6
10
Gradient time (min)

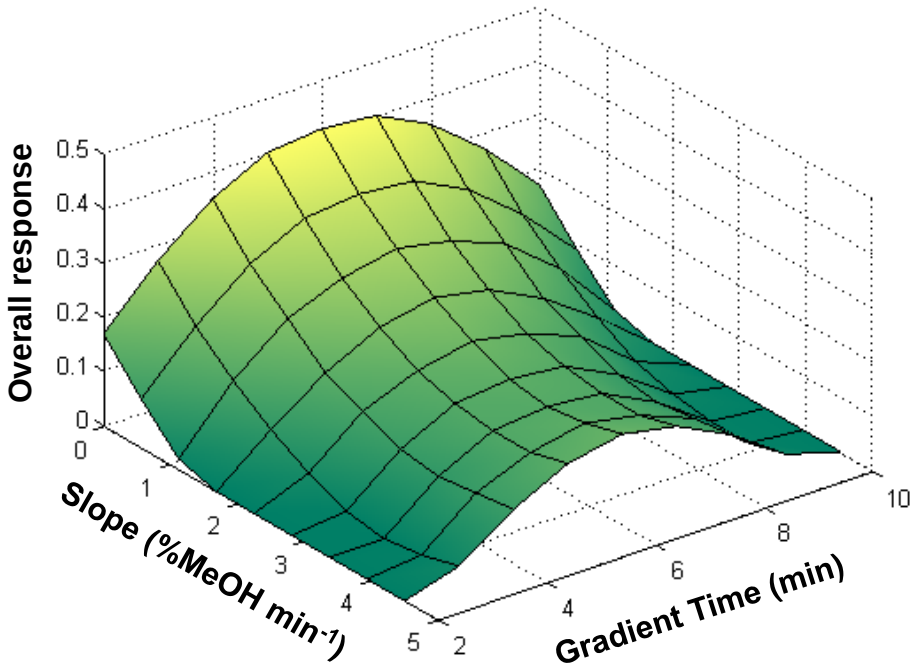
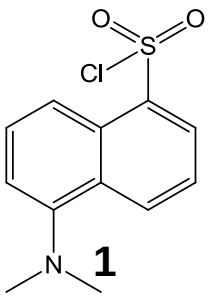

Overall response
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
Slope (%MeOH min-1)
2
4
6
8
10
Gradient Time (min)

Fig. 3

Precolumn



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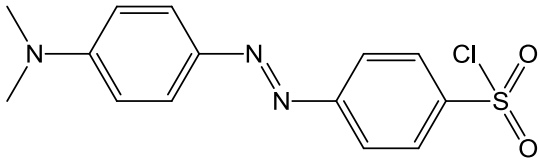

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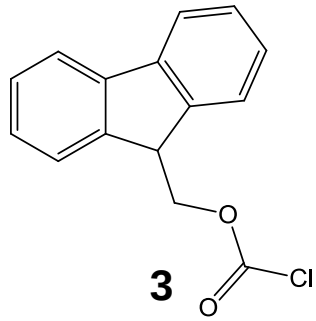

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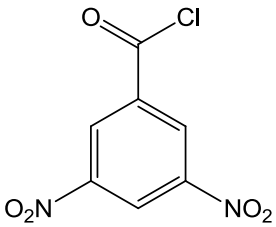

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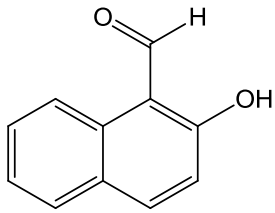

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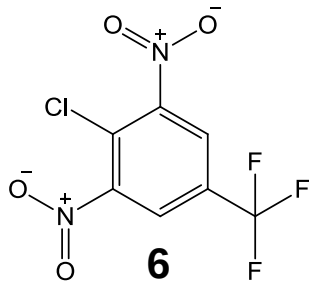

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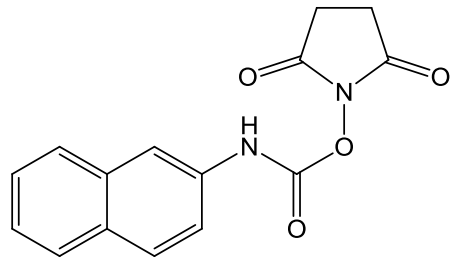
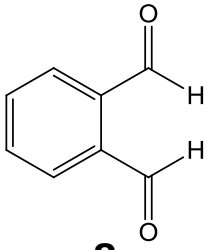


7

Pre- and postcolumn



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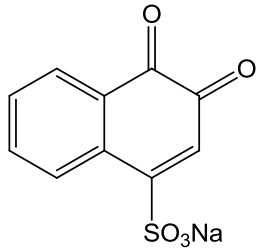

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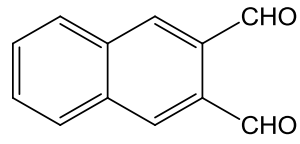
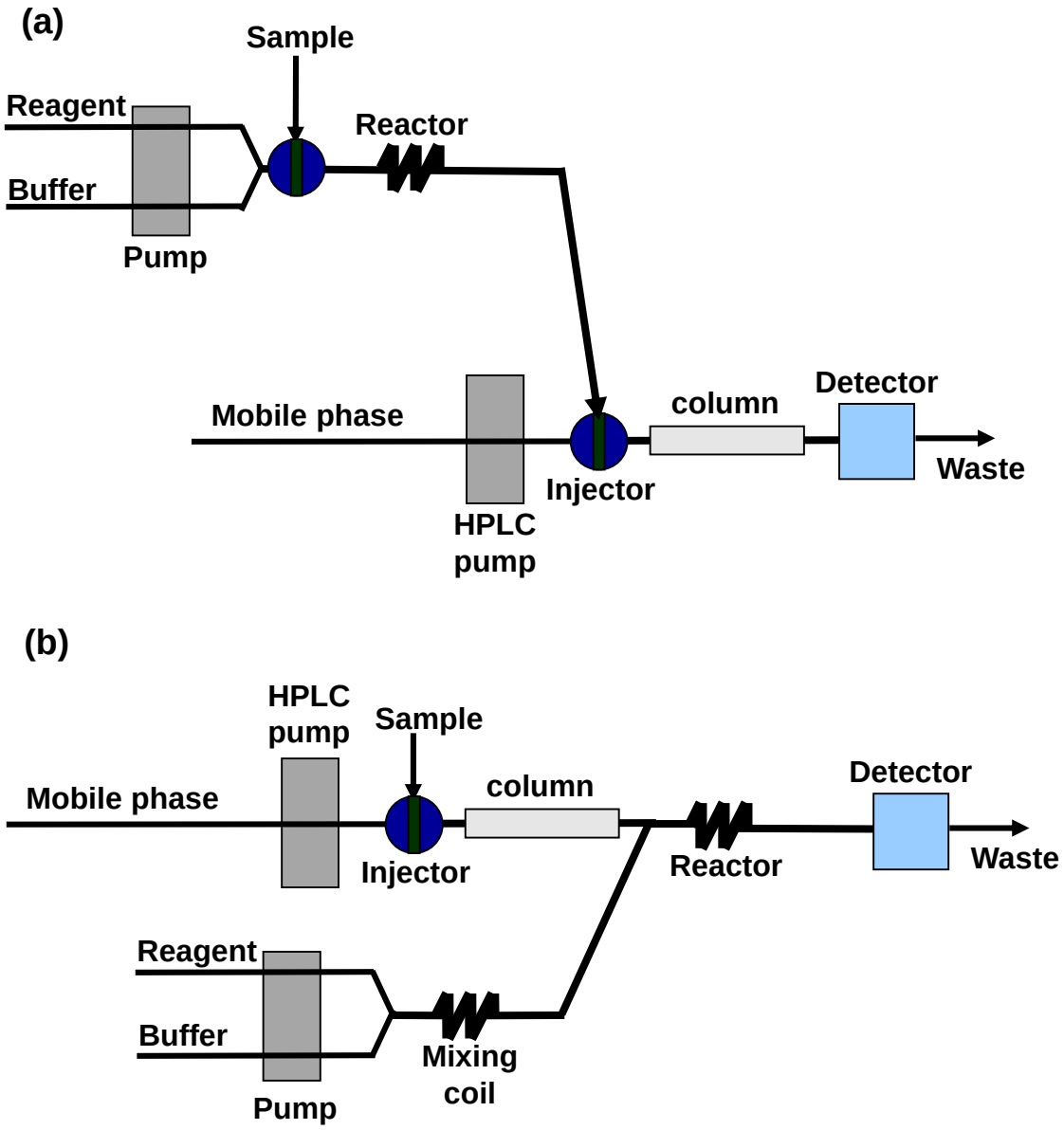


10

Fig. 4


(a)
Sample
Reagent
Buffer
Pump
Reactor
Mobile phase
HPLC pump
Injector
column
Detector
Waste
(b)
HPLC pump
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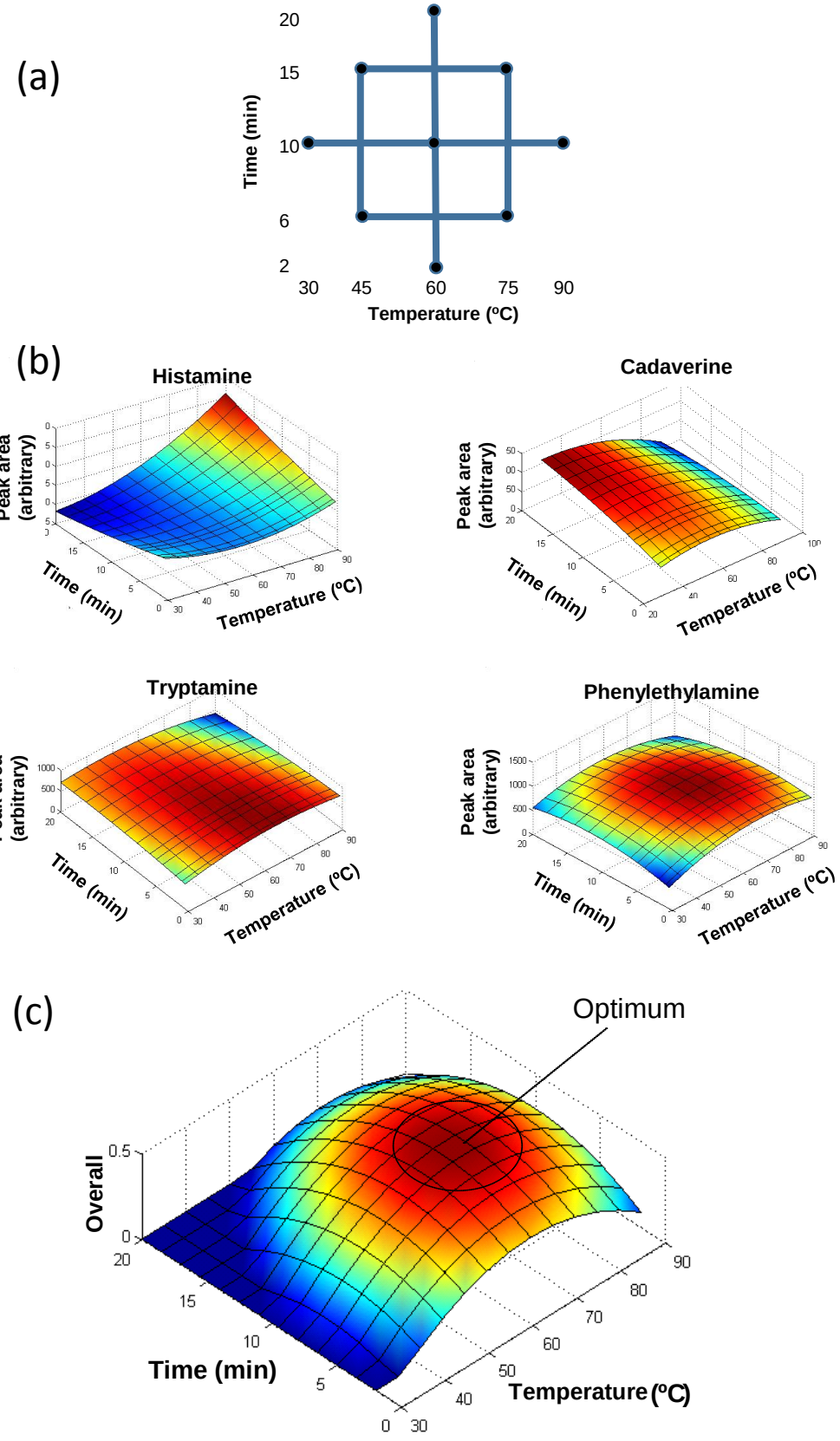
Mobile phase
Injector
column
Detector
Waste
Reactor
Reagent
Buffer
Pump
Mixing coil

Fig. 5


(a)
Time (min)
20
15
10
6
2
30 45 60 75 90
Temperature (°C)
(b)
Histamine
Cadaverine
Tryptamine
Phenylethylamine
Peak area (arbitrary)
Time (min)
Temperature (°C)
(c)
Optimum
Overall
Time (min)
Temperature (°C)