On-line hyphenated capillary electrophoresis and tandem mass spectrometry used for the analysis of selected biogenic amines in grape leaves

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Introduction

The quality and safety of food products have a great impact on human health. The grape and its products contain a wide range of substances. Biogenic amines (BA) are nitrogen-containing low molecular weight organic bases with the aliphatic (putrescine, cadaverine), aromatic (phenylethylamine, tyramine) or heterocyclic (histamine) structure, which are commonly found in various foodstuff. BA can be formed in food by decarboxylation of corresponding amino acids by microbial enzymes. Under normal conditions, exogenous amines ingested as part of the diet are absorbed from the food and quickly detoxified in the organism via amine oxidases or by conjugation. However, if normal catabolic routes of amines are inhibited or if the amount uptaken is large, this can result in several physiological effects (migraines, headaches, nausea, hypo- or hypertension, cardiac palpitations and anaphylactic shock)[1]. Histamine and tyramine are known to be the main cause of food intoxication, although other amines such as putrescine, cadaverine and phenylethylamine may intensify the undesirable effect of histamine. The content of biogenic amines in food should be monitored for their potential toxicity and the fact that the quantity of biogenic amines can be used as the food quality marker, while it is difficult to degrade BAs by high temperature treatment. Due to the current importance of food BAs in quality control and consumer safety, there is still a challenge to develop new methods for their fast, reliable analysis in samples of different foods[1–3].

Experimental methods

On-line combination of capillary electrophoresis with mass spectrometry (CE-MS) is an interesting combination of two attractive analytical techniques with numerous applications and advantages including high peak efficiency, short analysis time, small amount of reagents needed, good compatibility with aqueous samples, almost no need for extensive sample pretreatment and high detection selectivity and sensitivity. Moreover, MS detection enables the structural analysis and the determination of co-migrating compounds with different mass-to-charge (*m/z*) ratios[1].

A capillary electrophoretic analyzer, namely Agilent 7100 Capillary Electrophoresis System, was used in this work in a CE-ESI-MS/MS hyphenation for performing the CZE runs. The CZE column was provided with a 50 μm I.D. uncoated fused silica capillary tube of 820 mm total length. The samples were injected hydrodynamically by applying a pressure of 50 mbar for 10 s. CZE analyses were carried out in the cationic regime of the separation. A separation voltage of +30 kV was progressively applied to the capillary. The resulting current was fixed at 10 μA. The experiments were performed in a constant temperature mode at 20 °C.

A mass spectrometer Agilent 6410 Series Triple Quadrupole was used with a triple quadrupole MS tandem equipped with an electrospray ionization source (ESI). CE-MS coupling was carried out using a sheath liquid coaxial interface (Agilent). The sheath liquid was delivered by a pump Agilent 1260 Infinity and flowed through a splitter set at a ratio of 1 : 100 into the sprayer. The optimal protruding length of the CE capillary from the interface was about 0.2 mm.

Results and discussion

Optimization of the CE-ESI-MS/MS method for the simultaneous analysis of five selected BA (histamine, tyramine, putrescine, cadaverine and phenylethylamine) consisted in the optimization of all three steps of analysis. Optimization of the CE step focused on the selection of the background electrolyte system (BGE), taking into consideration several separation aspects (resolution of the analytes, analysis time, reproducibility, and thermal dispersion). Because of the MS detection, the volatile buffers with low salt concentrations were needed. The best compromise between resolution, analysis time, reproducibility, and detection sensitivity was obtained with BGE composed of 50 mmol/L formic acid with pH 2.05. In our study, CE-MS was carried out applying ESI using a sheath liquid interface, which is relatively robust and easy to implement. The sheath liquid composition and flow rate of the sheath liquid in ESI interface were optimized to enhance signal intensities of the analytes. 50% v/v methanol in water containing 0.1% v/v formic acid at a flow rate of 800 μL/min was used as the optimum sheath liquid in our work. Several operation modes were used to find the optimum fragmentor voltage (80–160 V) and collision energy (5–20 eV) and to find parent and product ions of the analytes. The sensitivity of

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the MS detection is highly dependent on several parameters related to the ESI-MS interface, such as the nebulizing gas pressure, the drying gas (temperature, flow rate), and the capillary voltage that were optimized too. The nebulizing gas (N_2) pressure was set at 10 psi. The optimum drying gas temperature was 300 °C and its flow rate was 5 L/min. The capillary voltage of +5000 V was set in the MS detector.

Five selected BA were analyzed in about 6 minutes by the proposed CE-ESI-MS/MS method. The relative standard deviations of migration time and peak area were for all analytes less than 0.5% and 10%, respectively. Limits of detection were in the range of 18.18 to 69.77 ng/mL. The method shows acceptable linearity and efficiency. The proposed method was applied to the identification and determination of selected biogenic amines in grape leaves. Cadaverine, histamine, phenylethylamine and tyramine were identified in different combinations almost in all samples of grape leaves. Putrescine was identified in all samples of grape leaves and in most of them quantified as well. The content of putrescine was in the range 0.1–13.5 μg/g.

Conclusions

This work demonstrated an analytical potential of the hyphenated CE-ESI-MS/MS method for the analysis of five selected biogenic amines (putrescine, histamine, cadaverine, phenylethylamine, tyramine) in grape leaves in the single electrophoretic run. Five biogenic amines were identified in all grape samples by the proposed method. Only putrescine was quantified by the proposed method and the content was at μg/g levels.

Tandem MS detectors, although more expensive, offer better sensitivity and specificity for the determination of biogenic amines and they can measure biogenic amines that do not contain UV sensitive chromophores, such as putrescine and cadaverine. Moreover, the used tandem MS/MS detection has an enhanced analytical information value and allows us to determine also co-migrating analytes.

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Conflicts of interest: none.

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