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High-performance liquid chromatography–electrospray ionisation–tandem mass spectrometry for the analysis of 1,2,3,4-tetrahydro-β-carboline derivatives

B. Gutsche, M. Herderich*

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

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Abstract

A rapid and sensitive method is described for the detection of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline by electrospray ionization tandem mass spectrometry coupled to liquid chromatography. In combination with selected reaction monitoring (SRM) detection limits of 3 ng ml^{-1} (ca. 75 fmol on column) were established by the use of model solutions. Due to the excellent selectivity and sensitivity of SRM no sample preparation step was required prior to analysis of food samples. In addition, any artifactual formation of tetrahydro-β-carbolines could be excluded. Application of the method revealed that all food samples analyzed contained both tetrahydro-β-carboline-carboxylic acids at ng ml^{-1} to μg ml^{-1} concentrations, whereas 1-methyl-1,2,3,4-tetrahydro-β-carboline was identified in most samples as a minor constituent.

Keywords: Food analysis; Tetrahydrocarbolines; Carbolines; Alkaloids

1. Introduction

Tetrahydro-β-carbolines (tetrahydro-9H-pyrido-[3,4-*b*]indoles) are formed by Pictet–Spengler condensation of indoleethylamine derivatives like tryptophan or tryptamine with various aldehydes. I.e., L-tryptophan yields upon reaction with formaldehyde or acetaldehyde 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) or 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (Me-THCA), respectively. Condensation of tryptamine and acetaldehyde results in the formation of 1-methyl-1,2,3,4-tetrahydro-β-carboline (Me-THC).

Tetrahydro-β-carbolines have been demonstrated to inhibit monoamine oxidase, to alter the re-uptake of biogenic amines and to interfere with the benzodiazepine receptor [1,2]. Because of their neuropharmacological effects these alkaloids have attracted much concern; thus, their participation in pathogenesis of alcoholism and psychiatric disorders is still under discussion [3,4].

Despite identification of endogenously formed tetrahydro-β-carbolines, origin of such derivatives in mammalian systems is still under debate [5–7]. In addition, food samples such as edible plants, cheese, soy sauce, smoked products and alcoholic beverages have to be taken into consideration as major exogenous sources [8–11]. But up to now, mainly analytical problems, i.e., coelution of interfering compounds and artifactual formation of tetrahydro-β-carbolines,

*Corresponding author.

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have made the analysis of food-related β-carbolines a daunting task. Therefore, a reliable analytical method for the determination of tetrahydro-β-carbolines in complex matrices could significantly clarify the role of these exogenously supplied tryptophan derivatives.

For the efficient analysis of minor compounds in complex matrices high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) is a promising analytical method with numerous yet unrevealed applications. Especially in combination with soft ionization techniques such as electrospray ionization (ESI), HPLC–MS–MS not only revolutionizes biochemical analysis [12,13] but also provides powerful approaches for the determination of low-molecular-mass trace constituents. Thus, we report for the first time a method based on HPLC–ESI-MS–MS for the profiling of THCA, Me-THCA and Me-THC in food samples. Combining sensitivity and selectivity of MS–MS, selected reaction monitoring (SRM) analysis allowed strictly reduced sample preparation, resulting in high sample-through-put and minimized risk of determining artifactually formed tetrahydro-β-carbolines. Furthermore, an effective way to monitor artifact formation during sample handling is described.

2. Experimental

2.1. Chemicals

Tryptamine, propionic acid, formaldehyde and acetaldehyde were purchased from Aldrich (Deisenhofen, Germany). Water, acetonitrile, both of HPLC gradient grade, L-tryptophan and trifluoroacetic acid (spectroscopic grade) were from Merck (Darmstadt, Germany). Synthesis of THCA, Me-THCA and Me-THC were based on the procedure of Jacobs and Craig [14]. Me-THCA was a diastereoisomeric mixture of (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid of known absolute configuration [15]. [$^2H_5$]L-Tryptophan-indole was from Cambridge Isotope Labs. (Andover, MA, USA). Membrane filters of pore size 0.2 μm were from Ziemer (Mannheim, Germany). Food samples were purchased from local markets.

2.2. Analytical procedure

2.2.1. Sample preparation

Fermented foods, seasoning sauces, yeast and fruit products were spiked with [$^2H_5$]L-tryptophan-indole (10 μg ml^{-1}) to monitor artifact formation during sample handling. Samples were filtered through membrane filters of pore size 0.2 μm and the resulting solutions were subjected to HPLC–ESI-MS–MS analysis without further sample preparation. Yeast products were diluted with water (1 g ml^{-1}) and centrifuged prior to filtration.

2.2.2. Separation of tetrahydro-β-carboline derivatives by HPLC with fluorescence detection

The HPLC system consisted of two Knauer HPLC pumps 64 equipped with micro pump heads (Knauer, Berlin, Germany) and a Shimadzu RF 530 fluorescence detector (Shimadzu, Kyoto, Japan). A Knauer HPLC-data system was used to record the data. For sample injection a Rheodyne 7725 equipped with a 5 μl loop was used. Chromatographic separation was performed on an Eurospher 100 C_{18} column (250× 2.0 mm I.D., 5 μm) (Knauer). Solvent A was 0.05% (v/v) trifluoroacetic acid (TFA) in water, solvent B was 0.05% (v/v) TFA in acetonitrile. Compounds were separated using the following gradient program: t=0 min 20% solvent B, 10 min 30% solvent B, 15 min 100% solvent B, 25 min 100% solvent B. Flow-rate was 200 μl min^{-1} and injection volume was 5 μl. Fluorescence was monitored at 360 nm after excitation at 290 nm.

2.2.3. Mass spectrometric analysis of tetrahydro-β-carboline derivatives

For HPLC–ESI-MS–MS chromatographic separation was performed on an Eurospher 100 C_{18} column (250×2.0 mm I.D., 5 μm) (Knauer) with a binary gradient delivered by an Applied Biosystems 140b pump. Solvent A was 0.05% (v/v) TFA in water, solvent B was 0.05% (v/v) TFA in acetonitrile. HPLC was programmed as follows: pressurizing with 50% B, equilibration time 5 min at 10% solvent B; gradient-A: t=0 min 10% solvent B, 20 min 30% solvent B, 30 min 50% solvent B;

gradient-B: t=0 min 20% solvent B, 10 min 30% solvent B, 15 min 100% solvent B. The flow-rate was 200 μl min^{-1} and injection volume was 5 μl.

HPLC–ESI-MS–MS analysis was performed with a triple stage quadrupole TSQ 7000 mass spectrometer with ESI interface (Finnigan MAT, Bremen, Germany). Data acquisition and evaluation were conducted on a Personal DECstation 5000/33 (Digital Equipment, Unterföhring, Germany) and ICIS 8.1 software (Finnigan MAT). Nitrogen served both as sheath and auxiliary gas; argon served as collision gas.

For ESI–MS–MS analysis electrospray ionization parameters were optimized as follows: temperature of the heated capillary (220°C) serving simultaneously as repeller electrode (20 V), electrospray capillary voltage (3.5 kV), sheath gas (50 p.s.i.; 1 p.s.i.=6894.76 Pa) and auxiliary gas (20 units). For tuning of the ESI interface solutions of THCA and Me-THCA (20 μg ml^{-1} each) were continuously delivered at a flow-rate of 200 μl min^{-1} by means of a syringe pump system. The influence of TFA on signal intensity [19] was investigated as described by Ijames et al. [16] by replacing TFA by acetic acid (0.05%, v/v) or by post-column addition of a 75:25 mixture of propionic acid and isopropanol (25%, v/v).

Positive ions were detected scanning from 150 to 500 u with a total scan duration of 1.0 s. MS–MS experiments were performed at a collision gas pressure of 2.0 mTorr Ar and a collision energy of 15 eV, scanning a mass range from 20 to 400 u with a total scan duration of 3.0 s (1 Torr=133.322 Pa). From the resulting characteristic product ion spectra the most abundant product ions were chosen for the SRM experiment; positive ions were detected at a scan duration of 0.4 s each; selected ion pairs were: m/z 217/144, 231/158, 187/158. Ion pairs represent the protonated molecule $[M+H]^+$ and the most abundant product ion for each of the carbolines. Quantitative evaluations were carried out using standard solutions of reference compounds for external calibration.

Artifactual formation of $[^2H_4]$-labeled tetrahydro-β-carboline-3-carboxylic acids was controlled by monitoring the neutral loss of the characteristic Retro-Diels–Alder fragment 73 u. Neutral loss experiments were performed at a collision gas pressure of 2.0 mTorr Ar and collision energy of 15 eV scanning a mass range from 180 to 380 u with a total scan duration of 3.0 s.

3. Results and discussion

Initial experiments revealed that the electrospray process effectively ionized tetrahydro-β-carboline derivatives; as result, exclusively protonated molecules $[M+H]^+$ were obtained from each of the references.

Separation of Me-THC, THCA and both diastereomers of Me-THCA by reversed-phase HPLC required addition of trifluoroacetic acid. As the deleterious effect of TFA on electrospray ionization had been demonstrated [16,19], we evaluated the influence of post-column addition of propionic acid–isopropanol on signal intensity. However, no significant improvement of the signal-to-noise ratio could be observed. In addition, substitution of TFA by acetic acid did not affect signal as well, as an increasing ion yield from the electrospray process was probably compensated by deteriorating chromatographic separation.

Due to Retro-Diels–Alder fragmentation low energy collision induced dissociation (CID) of protonated molecules revealed product ion spectra characteristic for tetrahydro-β-carbolines. As representative example, product ions of Me-THCA are outlined in Fig. 1. The most abundant product ion m/z 158 apparently was originating from the protonated molecule (m/z 231) by the neutral loss of the

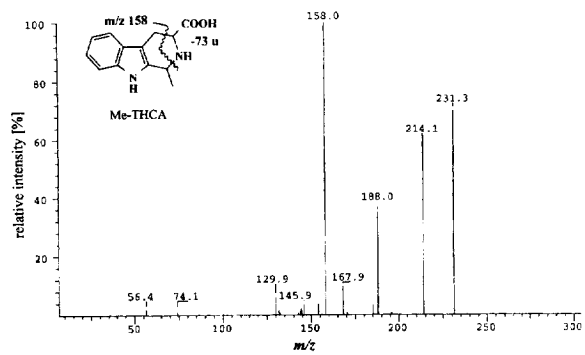

Fig. 1. Product ion spectrum (15 eV, 2.0 mTorr Ar) of Me-THCA; precursor ion m/z 231.1 $[M+H]^+$.

iminoacetic acid fragment $C_2H_3NO_2$ (−73 u) via Retro-Diels–Alder reaction. This fragmentation was confirmed by the corresponding neutral loss experiment, which clearly revealed the elimination of the iminoacetic acid moiety as neutral fragment from both Me-THCA and THCA. Retro-Diels–Alder fragmentation of Me-THC (*m/z* 187) resulted in the most abundant product ion *m/z* 158 by the loss of the CH_2=NH moiety (−29 u).

Based on characteristic fragment ions obtained from tetrahydro-β-carbolines we developed SRM experiments, resulting in high sensitivity and selectivity as well as in effective reduction of chemical noise. Besides their HPLC retention time, analytes were identified by their molecular ions $[M+H]^+$, representing a spectral filter on molecular mass information together with their characteristic product ions as structure-specific information. Reactions monitored were *m/z* 187→*m/z* 158, *m/z* 217→*m/z* 144 and *m/z* 231→*m/z* 158 for Me-THC, THCA and Me-THCA, respectively. Coeluting compounds exhibiting either different molecular mass or product ions were excluded from detection. In addition, product ion spectra enabled us to confirm positive identification of carbolines under study. Detection limits for HPLC–ESI-MS–MS analysis of tetrahydro-β-carbolines applying SRM experiments were established at 3 ng ml^{-1} (ca. 75 fmol on column).

Since the Pictet–Spengler condensation had been demonstrated to proceed readily during extraction of food samples, determination of THCA, Me-THCA and Me-THC had been a daunting task in the past. In order to obtain valuable information on the occurrence of tetrahydro-β-carbolines in food, one had to control carefully artifactual formation of tryptophan or tryptamine degradation products under study. Analytical approaches included trapping of free aldehydes by semicarbazide or removal of either tryptophan or tryptamine with fluorescamine prior to analysis [10,17]. Due to the excellent selectivity and sensitivity of HPLC–ESI-MS–MS we were able to omit sample preparation almost completely. Clean-up procedure prior to analysis of beverages, soy sauce and yeast products could be reduced to centrifugation and membrane filtration, thus minimizing the risk of analyzing laboratory-made tetrahydro-β-carbolines. Furthermore, after addition of $[^2H_5]$-L-tryptophan no deuterium-labeled tetrahydro-β-carboline derivatives could be detected by neutral loss scanning (Fig. 2, traces B and D); thus, HPLC–MS–MS clearly demonstrated that tetrahydro-β-carbolines detected were distinctive constituents of the diet (Fig. 2, traces A and C) rather than analytical artifacts.

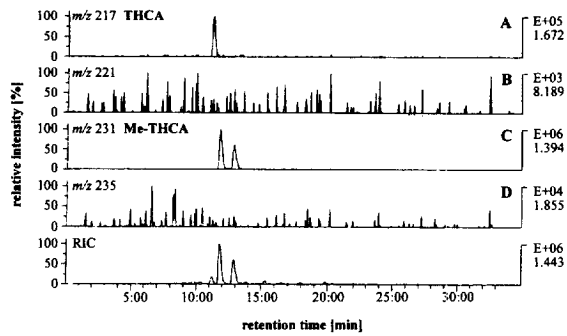

Fig. 2. HPLC–MS–MS chromatogram of vinegar, obtained by monitoring neutral loss of 73 u (19 eV, 2.0 mTorr Ar); gradient-A; (A): reconstructed ion chromatogram for precursor ion *m/z* 217 (THCA); (B): reconstructed ion chromatogram for precursor ion *m/z* 221 ($[^2H_4]$THCA); (C): reconstructed ion chromatogram for precursor ion *m/z* 231 (Me-THCA); (D): reconstructed ion chromatogram for precursor ion *m/z* 235 ($[^2H_4]$Me-THCA).

Subsequently, a number of fermented beverages, seasoning sauces, yeast and fruit products were analyzed by means of HPLC–ESI-MS–MS (Table 1). Comparison with chromatograms obtained by fluorimetric detection clearly demonstrated the selectivity of the SRM scanning (Figs. 3 and 4).

In addition, HPLC–MS–MS proved to be more reliable as tetrahydro-β-carbolines under study could successfully be differentiated from coeluting compounds. Results of the analysis of food samples are summarized in Table 1. Notably, THCA, Me-THCA and Me-THC were identified for the first time in yeast products, i.e., yeast-extract, yeast, herbal yeast and vegetarian yeast product. Concentrations of β-carbolines in alcoholic beverages and soy sauce were in good agreement with results obtained previously by HPLC with fluorimetric detection [5,10,18].

4. Conclusion

The excellent sensitivity and selectivity of HPLC–

Table 1
THCA, Me-THCA and Me-THC in food as determined by HPLC–ESI-MS–MS

Food samples	THCA	Me-THCA[a]	Me-THC
Seasoning sauce	+++	+++	+
Soy sauce	+++	+++	+
Worcester sauce	++	+++	+
White wine	+/−	++	+/−
Red wine	+/−	++	+/−
Vinegar 1	+	++	+
Vinegar 2	+	++	+
Sherry	+	++	+/−
Beer	+	+	+/−
Juice prepared from dried plums	+	+	n.d.
Fruit syrup	+	++	n.d.
Herbal yeast	++	++	+/−
Yeast extract	++	++	+/−
Yeast	+	+	−
Vegetarian yeast product	+	+	−

[a] Sum of both diastereomers.
+++: >10 μg/ml; ++: >500 ng/ml; +/−: at detection limit; −: not detectable; n.d.: not determined.

ESI-MS–MS analysis allowed specific detection of tetrahydro-β-carboline derivatives in complex matrices. Thus, no sample preparation step was necessary prior to the analysis of food samples minimizing the risk of analyzing artifactually formed tetrahydro-β-carbolines. Furthermore, addition of $[^2H_5]$L-tryptophan allowed the monitoring of laboratory-made tetrahydro-β-carbolines by the detection of $^2H_4$-labeled derivatives. As structures of even coeluting compounds were assigned successfully screening

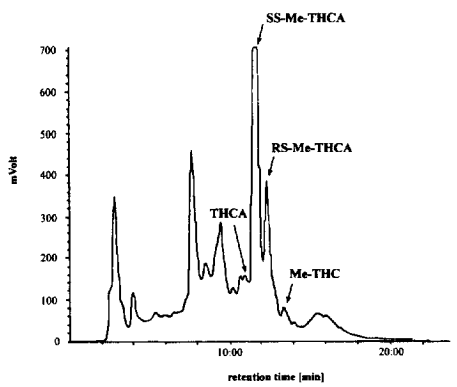

Fig. 3. HPLC–fluorescence chromatogram of seasoning sauce; excitation 290 nm, emission 360 nm. Arrows indicate retention times of tetrahydro-β-carboline derivatives as obtained by analysis of reference compounds.

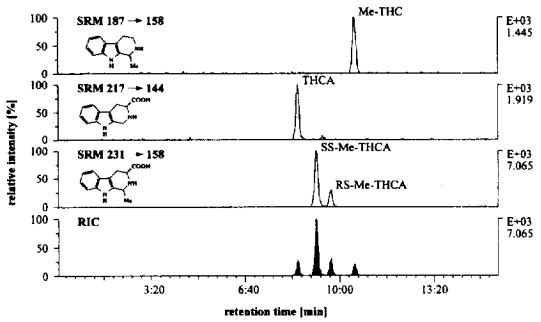

Fig. 4. HPLC–MS–MS chromatogram of seasoning sauce obtained by SRM experiment (15 eV, 2.0 mTorr Ar); gradient-B.

of tetrahydro-β-carbolines by HPLC–ESI-MS–MS proved to be more reliable than HPLC with fluorimetric detection.

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