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STUDY OF ZINGIBERENE FROM *LYCOPERSICON ESCULENTUM* FRUIT BY MASS SPECTOMETRY

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The aim of the study was the recording and interpretation of the MS fragmentation spectrum for alpha-zingiberene [S–(R*,S*)]–5–(1,5–dimethylhexen–4–yl)–2–methyl–1,3–cyclohexa–1,3–diene. The mass spectra are line spectra (peaks) which contain signals, such as the line of the molecular ion, isotopic lines, base line, and lines generated by the fragmentation of the molecular ion (fragmentation peaks). The highest peak of the mass spectra of this research is alpha-zingiberene; base peak that corresponds to the long life ions which reach the detector in the highest amount. Alpha-zingiberene is identical to the molecular peak (it can be a peak generated through the fragmentation of the molecular ion M^+). The relative percentage intensity I% is considered 100% for alpha-zingiberene, and the relative intensities of the other peaks are determined relative to the base peak. Knowing the relative percentage intensity allows knowing the relative percentage abundance for each species of ions in the mass spectra. For spectra obtaining was used an ion trap spectrometer, capable of recording sequential ionization spectra, before reaching the MS^{11}. It was recorded and interpreted the fragmentation spectrum for alpha-zingiberene. For each fragment, the ionic or radical-ionic character was indicated.

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1. Introduction

The tomato (*Lycopersicon Esculentum*) contains 90% water, glucides, lycopene, organic acids: malic, pectic, citric, terpenoid compounds (alpha-zingiberene), minerals, vitamins, etc. Terpenes are among the most spread natural compounds, having different functions in the animal and vegetal organism; for the food industry they are important as flavouring components. [1].

Mono-, sesqui-, di- and sesterpenes are formed out of isoprene units linked in a head-tail bond. Triterpenes and carotenoids (tetraterpenes) contain two C15 and C20 units linked head-to-head. Mono- and sesquiterpenes are base constituents of the volatile oils and the other terpenes constituents of balms, resins, waxes and gums. Etheric oils contribute through their scent, for attracting insects, indirectly favoring pollination and may be considered plants defense products [2]. Etheric oils attenuate plant perspiration [3].

Volatile substances represent a blend of chemical bio-compounds with varied structures, obtained through different biosynthesis ways. The study of this process is based on evaluating the structure of the compounds that offer scent and aroma, and investigating their metabolism [4] during growth and maturation. Due to the lack of experimental proofs, there is a need to explain

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certain phenomena through analogy with general biochemical transformations. In the reflexogenic pharyngo-oesophageal area, by direct action on the vomiting center, alpha-zingiberene may diminish nausea.

The volatile substances synthesized in the cells, diffuse in the intracellular spaces and reach the air in the ambient. Some volatile substances are final products of the anaerobic biodegradation of glucides and are accumulated in the cells and, at a certain concentration, they have a toxic potential, determining the depreciation of tissues (browning) [5, 6]. The volatile oils accumulated in vesicles, channels or secreting bags, do not take part in the visible biodegrading processes [7, 8]. In the recent years, the issue regarding the vegetal substances biogenesis represented the object of numerous studies [9]. The research methods used for the application of radioactive isotopes and chromatographic separation lead to the classification of a large series of fundamental aspects of plant biochemistry. The classification of active principles can be done according to their chemical nature, physical-chemical properties and biological characteristics [5]. Another research method used is mass spectrometry (MS); it is used as selection method for the elucidation of molecular structures, characterizing and confirming the presence of a certain natural chemical compound. Besides the valuable information regarding the structure of the analyzed compound, mass spectrometry is characterized by high sensitivity, though it requires small samples, of micrograms level [10, 11]. The spectral analysis implies the transformation of an analyte (M) into a set of ions in the gas phase and measurement according to the mass/charge ratio (m/z). Mass spectra have applications in analysis of organic and inorganic substances, nanomaterials, impurities, geological dating, medication testing, drug identification, monitoring the processes in the oil mining industry, surface analysis, identification of the composition of some exotic compounds, etc. [12, 13].

Techniques for producing positive/negative ions have been identified, which differ through the way they can generate ions with a single elemental charge or multiple elemental charges and the extent in which, besides the ionization of the sample molecules, they can produce the break of chemical bonds (fragmentation) [14].

The aim of the study was the recording and interpretation of the MS fragmentation spectrum for alpha-zingiberene [S–(R*,S*)]–5–(1,5–dimethylhexen–4–yl)–2–methyl–1,3–cyclohexa–1,3–diene from *Lycopersicon Esculentum*.

2. Materials and methods

Extraction of volatile metabolitesfrom *L. Esculentum fruits*. 20 g of fresh *L. Esculentum* fruits were cut in to small pieces and extracted with 100 mL of methyl tert–butyl ether by vigorous shaking on a shaker apparatus overnight. Each sample was supplemented with 100 μg of isobutylbenzene as an internal standard. Ethereal phase was separated off, dried with anhydrous Na_2SO_4 and concentrated to 2 mL under gentle N_2 flow. For SPME, 5 g of fresh fruits were cut into small pieces and placed in a 20 mL DuPont autosampler vial with a white solid–top polypropylene cap. Samples were overlaid with 5 mL NaCl (25%) solution, 1 g NaCl (inhibition of enzyme activity). Each sample was supplemented with 0.4μg of 2–heptenone and 0.4 μg of longifolene as internal standards. The samples were incubated at 25°C for 1 h, and then volatile compounds were collected with an SPME device PDMS–100 with a polydimethylsiloxane fiber by inserting fiber into tube and leaving it in place for 20 min at room temperature. After this incubation step, SPME fiber was injected directly into GC–MS. GC–MS analysis of *L. Esculentum* aliquot of concentrated methyl tert–butyl ether extract was injected into a GC–MSD system. Instrument was equipped with an Rtx–5 SIL column (30–m long·0.25–mm inner diameter, 0.25–μm film thickness and stationary phase 95% dimethyl–5% diphenyl polysiloxane). Helium (0.8 mL min^{-1}) was used as a carrier gas with splitless injection (temperature was 250°C; detector temperature was 280°C). The following conditions were used: initial temperature, 50°C for 1 min, followed by a ramp from 50 to 260°C at a rate of 5 C min^{-1}. A quadrupole mass detector with electron ionization at 70 eV was used to acquire MS data in range of 41 to 350 m / z. A mixture of straight–chain alkanes (C7–C23) was injected into column under afore mentioned conditions for

determination of retention times. Identification of volatiles was assigned by comparison of their retention times with those of literature, and by comparison of spectral data with standards. Quantity of component was calculated as (peak area internal standard/response factor) divided by (response factor/internal standard peak area) [15, 16].

Mass spectra were recorded with a "Brucker" spectrometer, equipped with ion trap, capable of recording spectra with sequential ionization up to MS^{11}. The acquired data were processed using the software belonging to the "Brucker" company, Dissect For Data Analysis 3.4. Electronic impact ionization was used (EI), with an electron beam with the energy of 70 eV. The reference substance, alpha-zingiberene, was obtained from Merk. All the determinations complied the ethics protocols and research ethics committees [17].

3. Results and discussion

The identification and separation of biocompounds from plants is important, separating and knowing their chemical structure being the essential step in explaining their therapeutic action. For this reason, the extraction, separation and compound identification methods, represent steps in the analysis of the chemical composition of the studied plants. The quantitative analysis methods were validated by studying parameters such as: precision, accuracy, detection limit, quantification limit, extraction yield. The organic samples are subjected to mass spectrometry analysis in order to obtain and confirm the structural information, using reduced quantities.

Mass spectrometers are based on the principle of detection and separation of positive ions (cations and radical cations) [18]. In the spectrum there can also be detected the radical cations, which come from the electronic impact on the analyzed molecules that contain the other isotopes. Molecular ions (original), correspond to the molecular mass of the analyzed compound and their identification in the spectrum is important, leading to precise determination of the molecular mass.

The more stable the molecular ion, the higher the abundance and the easier the identification will be [19]. Fragmentation ions are formed through subsequent transformations of the original molecular ion. The abundance of a fragmentation ion depends on the speed of the fragmentation reaction by which it is formed (kinetic conditioning) and the stability of the resulting cationic structure (thermodynamic conditioning) [20].

These fragmentations are favored by the stability of the resulting neutral molecules and by the stability of the new formed radical cations (they can appear as base peaks).

Rearrangement ions represent the positive ions resulted through the simultaneous fragmentation and rearrangement (i.e. isomerization, transposition) of the molecular ion (original) [21]. Table 1 shows the m/z values of the obtained signals and their relative intensity.

Table 1. m/z values of the obtained signals and their relative intensity

m/z	Relative intensity
41	48
55	20
69	37
77	55
79	30
81	10
91	28
92	16
93	100
105	29
107	10
119	60
120	10
133	6
147	8
161	8
204	6

Fig 1 shows the mass spectrum of α–zingiberene. It can be observed that after the ionization, an advanced fragmentation of the molecule occurred, resulting in 17 signals. For all the compound series, the peak intensity decreases with the increase of the distance between the methylene group and the position of the double bond in the ester chain. Contrary, the relative intensities of the peaks rise with the methylene group moving away from the carbonyl group. The abundance of the ions, corresponding to the hydrogen transposition, is higher than the abundance of the ions generated after the double transposition.

Also, it was investigated the cumulative effect of the chain length and the position of the double bond, on the intensity of the transposition ion, revealing that the relative intensities of the peaks of the spectra of the terpenes are lower than those of the analogous compounds with saturated chain. A possible explanation of this behavior lays in a reduced flexibility of the hydrocarbonated chain due to the rigidity of the double and triple bonds, therefore those groups cannot reach the minimum required distance in order to achieve transfer with the same ease as in the case of linear alkenes [4]. The fragmentation of α–zingiberene resulted both in positive ions as well as radicals with odd number of electrons and with positive charge.

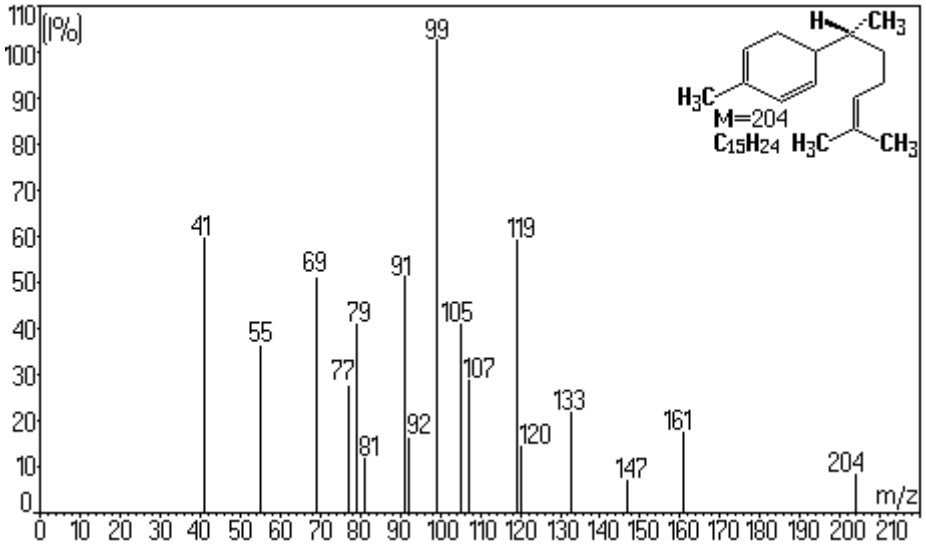

Fig. 1. MS EI (+) spectrum of α–zingiberene

As a result of the energy intake, the formed molecular ions are continuously subjected to fragmentation processes.

The formed fragments can possess enough energy to dissociate through a similar process or to be subjected to transposition reactions. In the mass spectra of some organic compounds there was also observed an intense peak "*McLafferty plus one*", corresponding to a double hydrogen transposition.

The majority of hydrogen transpositions occur by losing or effective gaining of one or more hydrogen atoms from the electrically charged fragment both unidirectional as well as reciprocal. These intra-molecular migrations of 2 hydrogen atoms, double hydrogen transpositions, occur either before or after the break of weak bonds in the molecule. The fragmentation results both in positive ions as well as radicals with odd number of electrons and with positive charge. In the case of the studied compound, the line of the molecular ion is well enough resolved. The fragmentation involves the break of allylic bond (in the β position against the double bond) with the formation of an allylic cation stabilized by resonance.

Due to the fact that both the radical and the allyl cation are stabilized by conjugation, there is a competition in their forming. Localizing the double bond in the acyclic alkenes is difficult because of the migration possibility of the double bond to the formed fragments. Figures 2 and 3 represent the fragmentation ways of α–zingiberene, taken into account when interpreting the mass spectrum.

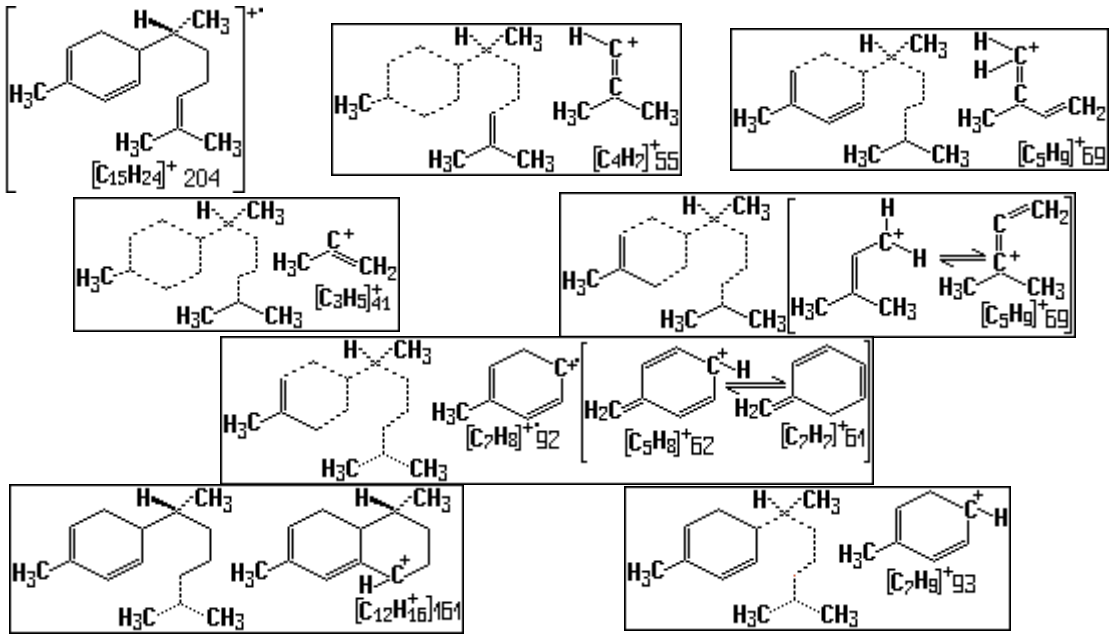

Fig. 2. Ionic and ionic–radical fragments formed subsequent to the ionization of α–zingiberene. For each fragment, the ionic or radical–ionic character is indicated.

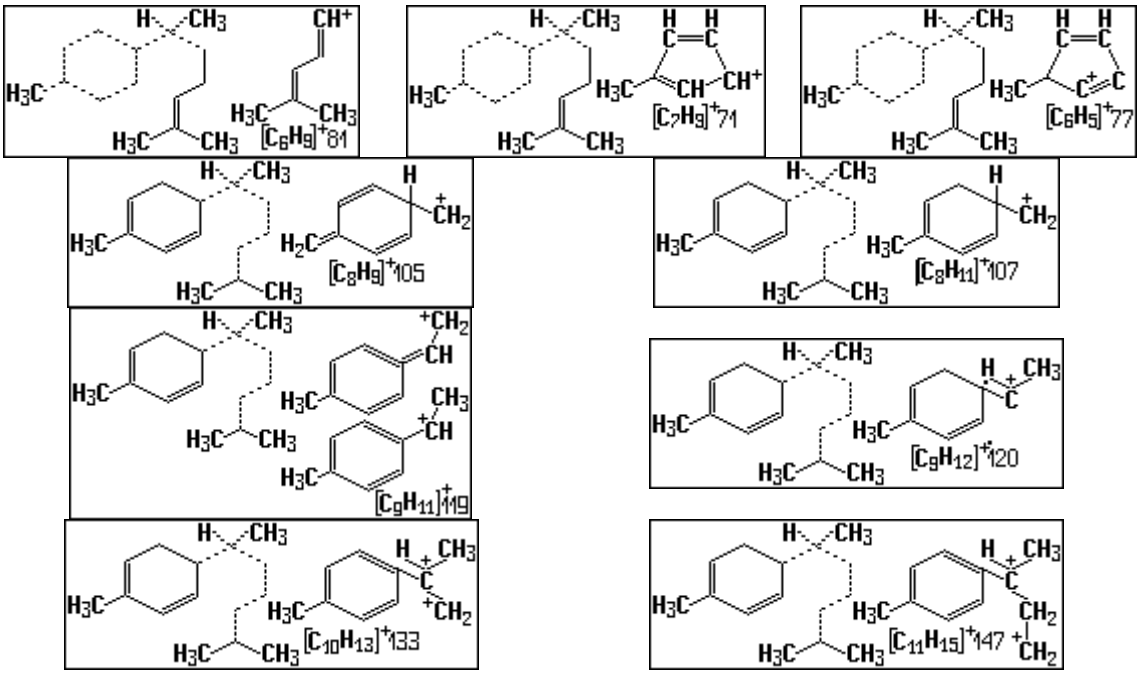

Fig. 3. Ionic and ionic–radical fragments formed subsequent to the ionization of α–zingiberene

The position of the double bond can be emphasized due to the allylic break, in which case there is no possibility of migration of the double bond. The α–zingiberene, derived from the class of terpenes with 3 double bonds in the molecule, shows in its mass spectrum cations generated from the fragmentation in the allyl position and cations generated from successive fragmentations accompanied by transpositions of the double bond.

The base peak appears at m/z=93 (100%). The m/z=119 fragment is formed subsequent to a transposition of the double bond in the molecular ion followed by a break in allyl position. The

action mechanism of the monoterpenic compounds is correlated to the dissociation degree, their solutions having an acidic pH, their acidity being linked to their action level [22].

α–Zingiberene was further subjected to analysis and its mass spectrum showed molecular ion $[M^+]$ at *m/z* 204, according to formula $C_{15}H_{24}$, which characterizes compound as α–zingiberene. The initial fragmentation with loss of hexenyl radical (C_{24}) with *m/z* 81, according to formula $[C_6H_9]^+$ generates fragment at *m/z* 120 according to formula $[C_9H_{12}]^+$. The additional loss of a molecule of H with simultaneous rearrangement generates remarked base peak at *m/z* 119 according to formula $[C_9H_{11}]^+$. The peak at *m/z* 119 (according to formula $[C_9H_{11}]^+$) indicates formation of a benzene ring with alkyl side chain, cation tropilium. Despite occurrence of α–zingiberene as not the most important compound of *L. Esculentum*, this is first reported occurrence of this sesquiterpene as constituent in *L. Esculentum.*

Aiming at establishing relationships between structure/activity of α–zingiberene, this was subjected to a double bond reduction reaction by catalytic hydrogenation (after purification was subjected to analysis to confirm hydrogenation). Mass spectrum revealed a molecular ion at *m/z* 204, *i.e.*, four units of mass higher than α–zingiberene, indicating that only two double bonds were hydrogenated. Mass spectrum indicated loss of a hexyl fragment radical (*m/z* 92, according to formula $[C_7H_8]^+$) to give a monounsaturated $[C_9H_{12}]^+$ cation peak at *m/z* 120. A further fragmentation of this peak with loss of ethylene produced base peak of spectrum *m/z* 93, according to formula $[C_7H_9]^+$ (figure 2). This pattern indicated reduction of exocyclic double bond and one of ring alkenes, suggesting that partially hydrogenated product corresponds to hydrogenated zingiberene structure shown in Figure 3. α–Zingiberene was also further subjected to hydrogenation under more drastic conditions.

The pharmaco-kinetic action is based on the affinity for lipids, and their metabolism is hard to elucidate [24], therefore, in a study done on the volatile oil of *Lycopersicon Esculentum* (with a content of α–zingiberene of 2 %), the research lead to the conclusion that ketones are fixing an oxygen atom to the ethylenic bond [4], with the formation of an epoxy-derivative, leading then to the breakage of the isopropilic chain with the restoration of the monocyclic structure and then, these are eliminated. Sesquiterpens with 15 carbon atoms, which generally accompany the monoterpenic compounds in the volatile oils are liquid lipophylic substances [25], insoluble in water, have antioxidant [26], anti-inflammatory, anti-allergic and emmenagogue activity.

From the pharmaco-kinetic point of view, these substances are enzymatically linked to an oze, entering the blood stream, linking then with a seric protein, reaching the liver where a series of transformations occur, and then they are eliminated. Only a small fraction passes unmodified in the blood after the hepatocyte processes, fraction which is pharmaco-dinamically active.

4. Conclusions

The analysis of the purified product showed molecular ion at *m/z* 161 according to the formula $[C_{12}H_{16}]^+$. According to that, α–zingiberene was fully hydrogenated. Despite occurrence of α–zingiberene as not the most important compound of *L. Esculentum*, this is first reported occurrence of this sesquiterpene as constituent in *L. Esculentum*.

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References

[1] T.J.Gianfagna, C.D.Carter, J.N.Sacalis, Plant Physiol **100**, 1403 (1992).
[2] M.L.Campos, M.de Almeida, M.L.Rossi, A.P.Martinelli, C.G.Litholdo Junior, A.Figueira, F.T.Rampelotti-Ferreira, J.D.Vendramim, V.A.Benedito, L.E.Peres,J Exp Bot **60**, 4347(2009).
[3] P.M. Bleeker, P.J. Diergaarde, K. Ament, J. Guerra, M. Weidner, S. Schütz, M.T. de Both, M.A. Haring, R.C. Schuurink,Plant Physiol **151**, 925 (2009).
[4] D.D. Bou, J.H. Lago, C.R. Figueiredo, A.L. Matsuo, R.C. Guadagnin, M.G. Soares, P. Sartorelli,Molecules **18**, 9477 (2013).
[5] H.J. Koo, D.R. Gang,PLoS One**7(12)**:e51481. doi: 10.1371/journal.pone.0051481. Dec 18.(2012).
[6] M.Butnariu, A. Caunii, S. PutnokyChemistry Central Journal,**6,** 146 (2012).
[7] P.M. Bleeker, R. Mirabella, P.J. Diergaarde, A. VanDoorn, A. Tissier, M.R. Kant, M. Prins, M. de Vos, M.A. Haring, R.C. Schuurink. Proc Natl Acad Sci USA **109**(49)**,**20124 (2012).
[8] M. Butnariu, A. Caunii, Annals of agricultural and environmental medicine, **20**, 736 (2013).
[9] E.E. Stashenko, S. Andrés Ordóñez, N.A. Marín, J.R. Martínez, Vahl. J Chromatogr Sci **47,**817 (2009).
[10] M.A. El–Marzouq, Banat's Journal *of* Biotechnology **5,** 47 (2013).
[11] R. Szmigielski, M. Cieslak, K.J. Rudziński, B. Maciejewska, Environ Sci Pollut Res In t**19,**2860 (2011).
[12]X. Luo, Y. Jiang, F.R. Fronczek, C. Lin, E.B. Izevbigie, K.S. Lee, Pharm Biol **49,**464 (2011).
[13]G. Andreea, I.Samfira, M. Butnariu, M. Corneanu, Journal of Biotechnology **161,**35,(2012).
[14] D. Cuthbertson, J. Piljac-Žegarac, B.M. Lange, Biomed Chromatogr **26,**1191 (2012).
[15] P.J. Weathers, M.J. Towler. Planta Med **78**, 1024 (2012).
[16] A. Trendafilova, C. Chanev, M. Todorova, Pharmacogn Mag **6,**234 (2010).
[17] G.L. Liu, H.H. Guo, Y.M. Sun, Int J Mol Sci **13**, 6292 (2012).
[18] S. Kečkeš, U. Gašić, T.Ć. Veličković, D. Milojković-Opsenica, M. Natić, Ž. Tešić, Food Chem **138**, 32 (2013).
[19] R.G. Torde, A.J. Therrien, M.R. Shortreed, L.M. Smith, S.M. Lamos, Molecules **18**, 14977 (2013).
[20] S. Saha, M.K. Mandal, L.C. Chen, S. Ninomiya, Y. Shida, K. Hiraoka. Mass Spectrom (Tokyo)**2**(Spec Iss):S0008. doi: 10.5702/massspectrometry.S0008 (2013).
[21] S.K. Kailasa, H.F. Wu,Curr Neuropharmacol **11**, 436,(2013).
[22] X. Cheng, J. Hiras, K. Deng, B. Bowen, B.A. Simmons, P.D. Adams, S.W. Singer, T.R. Northen, Front Microbiol**4**, 365 (2013).
[24] H. Chen, T.A. Parks, X. Chen, N.D. Gillitt, C. Jobin, S. Sang, J Chromatogr A**1218,**7297, (2011).
[25] X.J. Zhang, J.F. Qiu, L.P. Guo, Y. Wang, P. Li, F.Q. Yang, H. Su, J.B. Wan, Molecules 1**8,**15329 (2013).
[26] S. Plattner, R. Erb, J.P. Chervet, H. Oberacher, Anal Bioanal Chem **406**, 213 (2014).