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Characterization of Vegetable Oils by Electrospray Ionization Mass Spectrometry Fingerprinting: Classification, Quality, Adulteration, and Aging

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An improved approach for the direct infusion electrospray ionization mass spectrometry (ESI-MS) analysis of vegetable oils is described. The more polar components of the oils, including the fatty acids, are simply extracted with methanol/water (1:1) solution and analyzed by direct infusion ESI-MS in both the negative and positive ion modes. This fingerprinting analysis was applied to genuine samples of olive, soybean, corn, canola, sunflower, and cottonseed oil, to admixtures of these oils, and samples of aged soybean oil. ESI-MS fingerprints in the positive ion mode of the extracts divide the oils into well-defined groups, as confirmed by principal component analysis, whereas ESI-MS fingerprints in the negative ion mode clearly differentiate olive oil from the five other refined oils. The method is also shown to detect aging and adulteration of vegetable oils.

Vegetable oils are complex chemical mixtures. Fatty acids and di- and triglycerides are the main components, but a series of minor polar compounds are also present and their distributions are characteristic of different types of vegetable oils. The health benefits and composition of these oils depend on the vegetable, nut, or seed from which they are extracted. Olive oil is one of the most expensive vegetable oils with superior health benefits. Frequently, therefore, olive oil as well as other more valuable vegetable oils are adulterated with lower priced oils. As the authenticity of one particular type of oil is important for both health and commercial reasons, there is continued need for improved, rapid, and accurate methods to determine their provenance, quality, and adulteration. Ideal analytical methods require minimal sample preparation, rapid and accurate analysis, and straightforward automatation.

Traditionally, fatty acid composition has been used for classification and as an indicator of purity. The fatty acids are first derivatized mainly by methylation and then analyzed by gas chromatography/mass spectrometry (GC/MS).[1] Triglycerides are also analyzed by high-performance liquid chromatography (HPLC).[2] Nonvolatile polar compounds detected via HPLC with MS and ultraviolet detectors have also been used as markers of olive oil[3,4] and of low-price vegetable oils[5] added to adulterated samples. Most current methods for detecting vegetable oil provenance and adulteration have focused therefore on either the polar or nonpolar components of oils using chromatographic techniques and extraction and derivatization procedures. All together, these steps are considerably time-consuming and hard to automate. To reduce the time of analysis and to test the applicability for oil classification, direct infusion electrospray ionization-mass spectrometry (ESI-MS) has been tested to analyze samples of vegetable oils,[6,7] after dilution of the crude sample with chloroform or dichloromethane, and has been shown to detect mainly the triglycerides and fatty acids.

ESI is a soft and wide-ranging ionization technique that is best applied to polar molecules, without the need of chemical derivatization or extraction from polar solutions. Therefore, direct infusion ESI-MS has been shown to be a suitable technique able to provide fast fingerprint characterization of complex natural mixtures such as propolis,[9] beer,[10] whisky,[11] wine,[12,13] spices,[14] rum and Brazilian cachaça,[15] gasoline, [16,17] and crude oil.[18]

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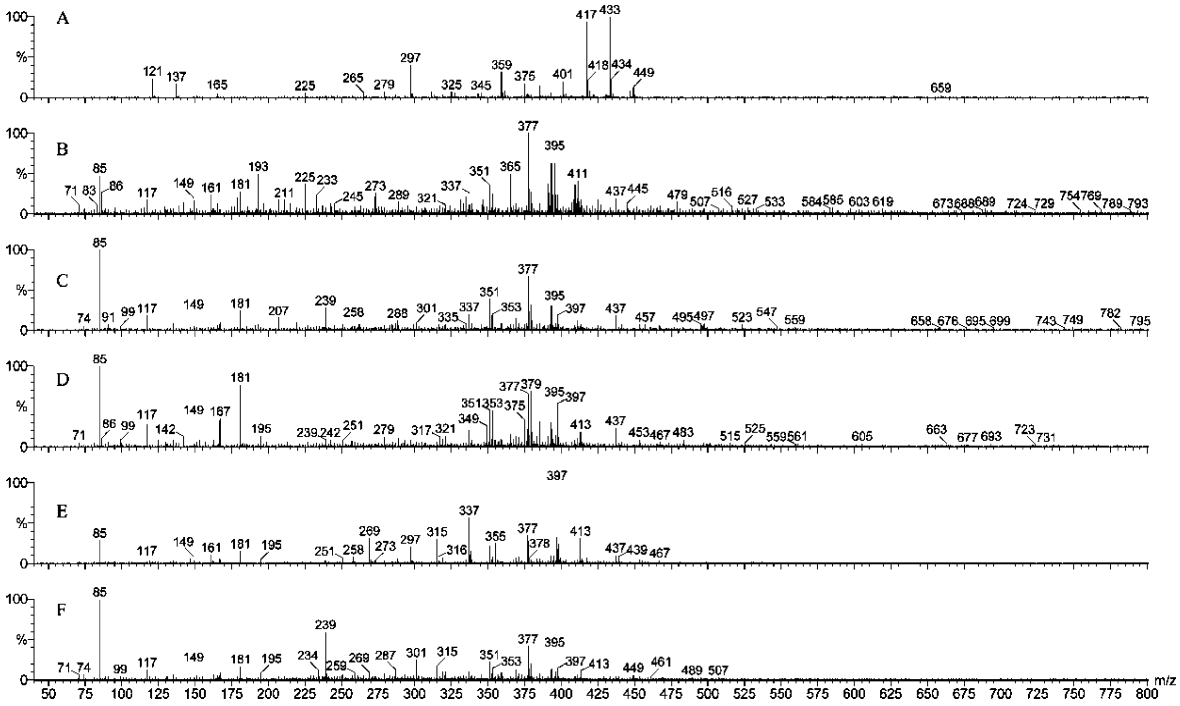

Figure 1. ESI-MS fingerprints in the positive ion mode of methanol/water extracts of the following: (A) olive, (B) soybean, (C) corn, (D) canola, (E) sunflower, and (F) cottonseed oil.

We decided therefore to systematically evaluate the applicability of direct infusion ESI-MS in both negative and positive ion modes for vegetable oil classification, adulteration, and aging. For the analysis, instead of using the crude oil, we concentrated our efforts on the more polar components of the oils by extraction with water/methanol solution, so extracting selectively major free fatty acids and phenolic compounds. Fatty acids are traditionally considered markers of purity and provenance whereas the phenolic compounds are important as they are known to be responsible for the biological antioxidant activity of vegetable oils.

EXPERIMENTAL SECTION

Chemical Reagents and Samples. All reagents used were of analytical grade. A total of 30 samples of vegetable oils were obtained from reliable sources; these included olive, soybean, corn, canola, sunflower, and cottonseed oil. For the detection of adulteration of the olive oil with other edible oils, five admixtures of the sample of extra virgin olive oil with soybean oil were prepared in triplicate containing 10, 20, 30, 40, and 50% soybean oil in the genuine olive oil sample. Altogether, 15 admixtures were prepared. These admixtures were analyzed immediately after their preparation. Five samples of soybean oil, previously stored for 24 months, were analyzed for aging detection.

General Experimental Procedures. ESI-MS fingerprints were obtained in both the positive and negative ion modes on a Q-TOF mass spectrometer (Micromass, Manchester, U.K.). Typical ESI-MS conditions were as follow: source temperature 100 °C, desolvation temperature 100 °C, capillary voltage ±3.0 kV, and cone voltage ±40 V. ESI-MS/MS of selected ions were acquired by low-energy (15−30 V) collision-induced dissociation. The oil samples (250.0 μL) were homogenized in a flask with a solution containing equal parts of water and methanol, completing the final volume of 1.0 mL. The phases were allowed to separate, and the top (hydroalcoholic) layer was removed. Formic acid (10 μL) was added to each sample for ESI(+)-MS analysis whereas 10 μL of ammonium hydroxide was added for ESI(−)-MS analysis. These solutions were then injected at a flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus), and mass spectra were acquired over the 50−2000 m/z range.

Data Handling. All data obtained from ESI-MS fingerprints of the oil samples were extracted using MassLynx 3.5 (Waters, Manchester, U.K.). Mass spectral data were accumulated over 60 s, centered, and aligned to generate a final data matrix of 30 samples and 100 mass signals (variables) ranging from m/z 70 to 500 (a range that contained all ions of interest as judged by visual inspection). To classify the oil samples after ESI-MS fingerprinting, principal component analysis (PCA) was performed on the respective ESI-MS results by the Unscrambler v. 8.0 program (CAMO Process A/S, Oslo, Norway).

RESULTS AND DISCUSSION

As Figures 1 and 2 exemplify, ESI-MS fingerprints for the oil extracts in both the positive and negative ion modes are very characteristic. Additionally, PCA of both ESI(+)-MS and ESI(−)-MS data (Figures 3 and 4) divide the samples into very well-defined groups, as visual analysis of the mass spectra indicates.

ESI(+)-MS Fingerprints. The ESI(+)-MS fingerprints (Figure 1) of the extracts permit clear differentiation between the six types of vegetable oils. In the 30−800 m/z range, each oil produces numerous diagnostic ions that allow straightforward oil classification based mainly on very pronounced changes in relative cation

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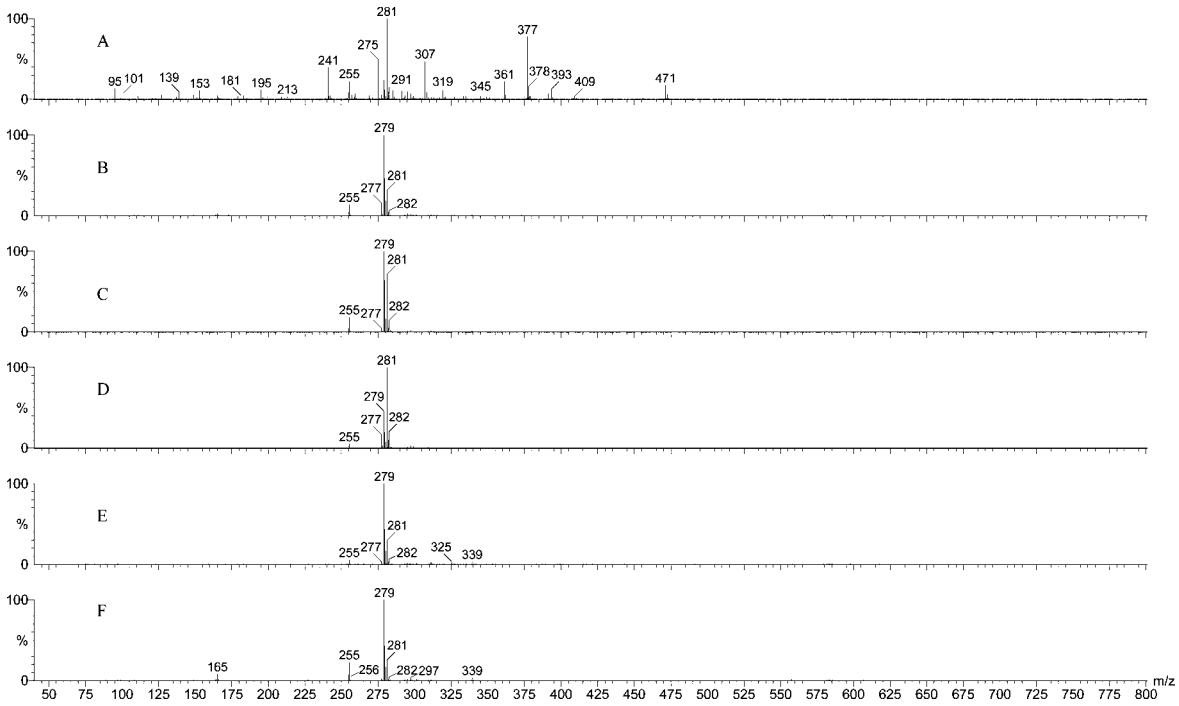

Figure 2. ESI-MS fingerprints in the negative ion mode of methanol/water extracts of the following: (A) olive, (B) soybean, (C) corn, (D) canola, (E) sunflower, and (F) cottonseed oil.

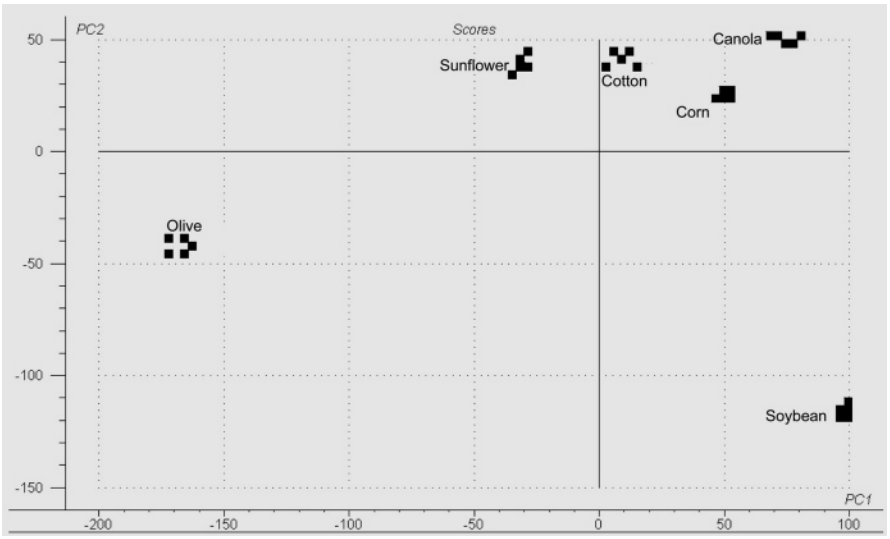

Figure 3. PCA of ESI(+)-MS data of the six samples of oil: olive, soybean, corn, canola, sunflower, and cottonseed.

abundances. The fingerprints of all the refined vegetable oils (Figure 1B–F) display seven major common ions, those of m/z 85, 149, 181, 351, 377, 395, and 397. These ions appear therefore to be characteristic for the refining process. Olive oil, which is an unrefined oil, shows diagnostic ions in its ESI-MS fingerprint, that is, those of m/z 121, 137, 359, 401, 417, 433, and 449. Major diagnostic ions for soybean oil are those of m/z 193, 225, 233, 273, 396, and 445; for canola oil those of m/z 195, 279, 317, and 349; for sunflower oil those of m/z 269 and 355, for cottonseed oil those of m/z 234, 287, and 301; and for corn oil there are no diagnostic ions.

Although not necessary for fingerprinting classification, ESI-(+)-MS/MS permits the tentative identification of some of the diagnostic ions via comparison with reported data or structurally related dissociation. For olive oil, tyrosol is probably observed as $[M + H - H_2O]^+$ of m/z 121 and hydroxytyrosol as $[M + H - H_2O]^+$ of m/z 137. The following phenolic acids are also observed in various oil samples: cinnamic (m/z 149), coumaric (m/z 165), caffeic (m/z 181), ferulic (m/z 195), and sinapic (m/z 225) acids.

ESI(−)-MS Fingerprints. Figure 2 shows the six ESI(−)-MS fingerprints of the oil extracts. Olive oil is unique since it produces numerous diagnostic ions that clearly differentiate it from all the other oils. The differentiation between the ESI(−)-MS of the refined oils is, however, less clear and relies mainly on the relative abundances of the ions of m/z 279 and 281. The ESI(−)-MS fingerprints of soybean and sunflower oil extracts are characterized by the predominance of the anion of m/z 279. Corn oil is characterized by displaying anions of m/z 279 (deprotonated linoleic acid) and 281 (deprotonated oleic acid) of close relative abundances. Canola oil is then characterized by the predominance

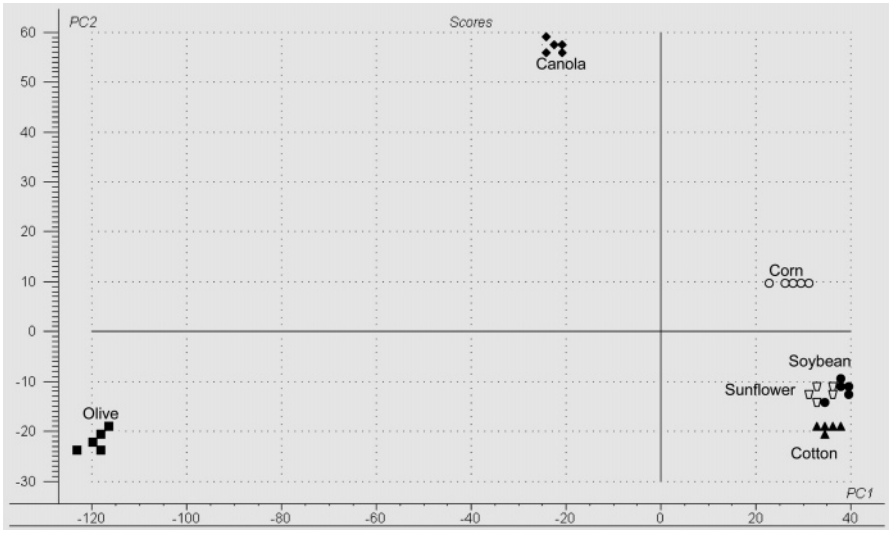

Figure 4. PCA of ESI(−)-MS data of the six samples of oil: ■ olive, ● soybean, ○ corn, ◆ canola, ▽ sunflower, and ▲ cottonseed.

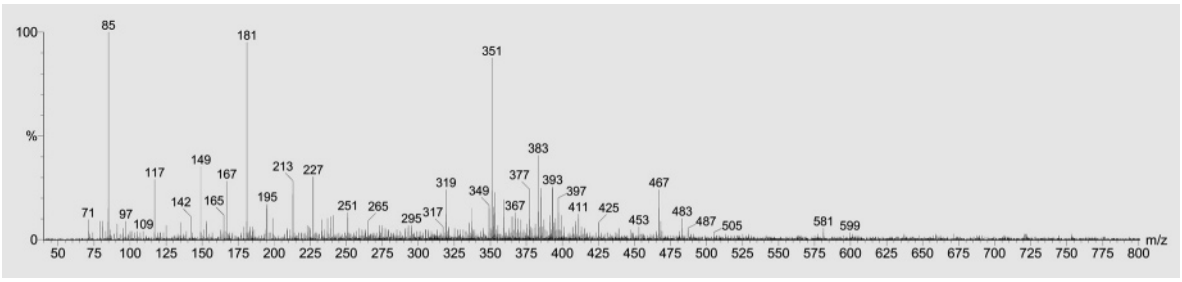

Figure 5. ESI(+)-MS fingerprint of the methanol/water (1:1) extraction solution of aged soybean oil. Compare to Figure 1B for the fresh soybean oil.

of the anion of m/z 281. These characteristic relative abundances of linoleic and oleic acids (see below) have been also determined by chromatographic analysis.[1,19]

The diagnostic anions common for the six oils are those of m/z 255, 277, 279, and 281. These are the deprotonated molecules $[M - H]^-$ of well-known fatty acids: palmitic (m/z 255), linolenic (m/z 277), linoleic (m/z 279), and oleic (m/z 281) acids as confirmed by ESI-MS/MS (not shown). Other compounds likely identified by ESI(−)-MS/MS are as follow: dihydroxybenzoic acid (m/z 153), dimethoxybenzoic acid (m/z 181), homoveriatric acid (m/z 195), elenolic acid (m/z 241), 4-norlempein aglicone (m/z 319), and hydroxytyrosillenoate (m/z 377). Although the fatty acids dominate the ESI(−)-MS fingerprints of the oil extracts, as for the ESI(−)-MS fingerprints collected by Wu et al. using the diluted crude oils,[7] the water/methanol extraction permits the simultaneous observation of the phenolic acids, which are of prime importance for the biological antioxidant effect that olive oil is particularly famous for.

Chemometric Analysis. To mathematically test the reliability of ESI-MS for statistical oil classification, PCA data treatment was performed. Figure 3 shows a scatterplot of PC1 versus PC2 for ESI(+)-MS in which each of the six types of oils are indeed very clearly grouped whereas olive oil and soybean oil are well isolated. As expected, PCA treatment of the ESI(−)-MS data also separates the samples in six groups, but less distinctly (Figure 4). By far the principal use of ESI(−)-MS is to separate olive oil from the other five refined oils, although canola oil and corn oil are also considerably isolated from the three other refined oils.

Vegetable Oil Aging. Aging of vegetable oils is also clearly evidenced by ESI-MS fingerprinting, as exemplified by the fingerprint of a sample of soybean oil (used as a proof-of-principle sample) kept for two years at room temperature (25 °C) in a closed tin can, not exposed to air or light. The ESI(+)-MS fingerprint of the water/methanol extract of the aged oil (Figure 5) displays many additional ions (mainly those of m/z 167, 195, 213, 251, 319, 349, 359, 367, 369, 383, 467, and 483) as compared to that of the fresh oil. These ions appears therefore to be diagnostic of soybean oil aging. Furthermore, several diagnostic ions present in the ESI-(+)-MS of fresh soybean oil, that is, mainly the ions of m/z 149, 161, 193, 225, 273, 395, 409, 437, 445, and 479, are absent in the fingerprints of the aged oils. Aging also causes substantial changes in the relative abundances of cations as seen when Figure 1B and Figure 5 are compared. Further studies are underway to characterize the aging polar products and to determine the possible chemical reactions responsible for these changes.

Olive Oil Adulteration. Admixture adulteration of vegetable oils is also clearly detected by ESI-MS fingerprinting. This ability is exemplified for adulteration of olive oil with soybean oil, which is the most common adulteration of olive oil in Brazil. Admixtures of olive oil/soybean oil were prepared in triplicate containing 10, 20, 30, 40, and 50% soybean oil. The relative intensities of the ions observed in the ESI(−)-MS fingerprints of these admixture extracts, when compared to that of pure olive oil, show quite distinct differences, mainly for the relative intensities of six pairs

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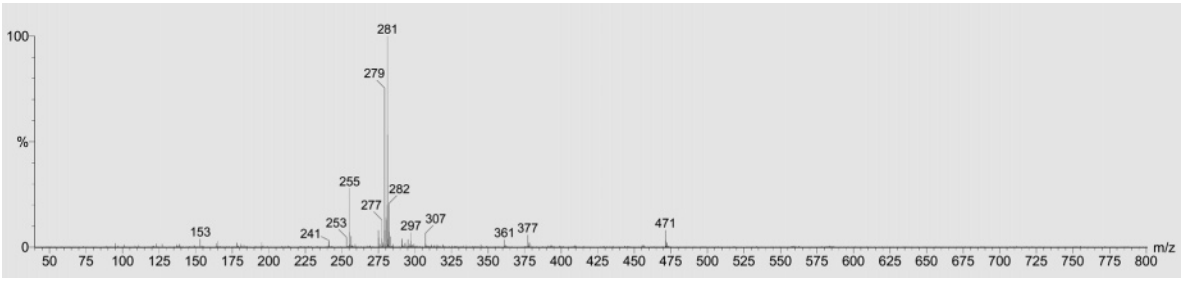

Figure 6. ESI(−)-MS fingerprint of the methanol/water (1:1) extracts of adulterated olive oil containing 10% soybean oil. Compare to Figure 2A for the pure olive oil.

of ions: (1) m/z 241 and 279, which is 2:1 in the pure oil and changes to 1:12 the 10% adulterated sample; (2) m/z 275 and 279 (2.5:1−1:5); (3) m/z 279 and 281 (1:5−1:1); (4) m/z 279 and 307 (1:2.5−7.5:1); (5) m/z 279 and 377 (1:4−7.5:1); and (6) m/z 279 and 471 (1:1−3:1). As the ions of m/z 279 and 281 are the most intense in the spectrum, the changes in their relative intensities are considered the most characteristic to detect adulteration.

CONCLUSION

ESI-MS fingerprinting of water/methanol extracts, which has the advantage of focusing on the more polar components, is a fast and selective technique to classify, to control quality (polar contaminants), to evaluate aging, and to detect adulteration of vegetable oils. Sample preparation is minimal, and the water/methanol extraction permits the simultaneous detection of both the fatty acids and the polar phenolic compounds, two of the most important classes of polar compounds found in vegetable oils. The statistical reliability of the method has been confirmed by PCA analysis.

We envisage that the method can be used to establish a library of ESI-MS fingerprints of pure vegetable oils from different origins and geographical regions, which could then be used for comparison with suspicious samples. ESI-MS with direct infusion can also be easily automated particularly when using robotized sample injection systems based on nanoESI chips, which eliminate cross-contamination and minimize ion suppression, permitting very high-throughput analysis.[20]

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