Video Article

Fizzy Extraction of Volatile Organic Compounds Combined with Atmospheric Pressure Chemical Ionization Quadrupole Mass Spectrometry

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Abstract

Chemical analysis of volatile and semivolatile compounds dissolved in liquid samples can be challenging. The dissolved components need to be brought to the gas phase, and efficiently transferred to a detection system. Fizzy extraction takes advantage of the effervescence phenomenon. First, a carrier gas (here, carbon dioxide) is dissolved in the sample by applying overpressure and stirring the sample. Second, the sample chamber is decompressed abruptly. Decompression leads to the formation of numerous carrier gas bubbles in the sample liquid. These bubbles assist the release of the dissolved analyte species from the liquid to the gas phase. The released analytes are immediately transferred to the atmospheric pressure chemical ionization interface of a triple quadrupole mass spectrometer. The ionizable analyte species give rise to mass spectrometric signals in the time domain. Because the release of the analyte species occurs over short periods of time (a few seconds), the temporal signals have high amplitudes and high signal-to-noise ratios. The amplitudes and areas of the temporal peaks can then be correlated with concentrations of the analytes in the liquid samples subjected to fizzy extraction, which enables quantitative analysis. The advantages of fizzy extraction include: simplicity, speed, and limited use of chemicals (solvents).

Video Link

The video component of this article can be found at https://www.jove.com/video/56008/

Introduction

Various phenomena observed in nature and daily life are linked to gas-liquid phase equilibriums. Carbon dioxide is dissolved in soft and alcoholic drinks under elevated pressure. When a bottle of such a fizzy drink is opened, the pressure drops down, and gas bubbles rush to the liquid surface. In this case, effervescence improves organoleptic properties of beverages. The release of gas bubbles is also the main cause of decompression sickness ("the bends")[1]. Due to sudden decompression, bubbles form in divers' bodies. The persons suffering from the decompression sickness are treated in hyperbaric chambers.

Gas bubbles have various applications in analytical chemistry. Notably, sparging methods rely on passing gas bubbles through liquid samples to extract volatile compounds[2]. For example, a method called "purge-closed loop" is combined with gas chromatography to enable rapid analysis of dissolved volatiles[3]. While sparging can continuously extract volatiles over time, it does not confine them in space or time. The released gas-phase species need to be trapped, and-in some cases-concentrated by applying a temperature program or using sorbents. Thus, there is a need to introduce new on-line sample treatment strategies, which could reduce the number of steps, and-at the same time-concentrate volatile analytes in space or time.

To address the challenge of extracting volatile compounds from liquid samples, and performing analysis on-line, we recently introduced "fizzy extraction"[4]. This new technique takes advantage of the effervescence phenomenon. Briefly, a carrier gas (here, carbon dioxide) is first dissolved in the sample by applying overpressure and stirring the sample. Then, the sample chamber is decompressed abruptly. The sudden decompression leads to formation of numerous carrier gas bubbles in the sample liquid. These bubbles assist the release of dissolved analyte species from the liquid to the gas phase. The released analytes are immediately transferred to the mass spectrometer, producing signals in the time domain. Because the release of the analyte species is confined to a short period of time (a few seconds), the temporal signals have high amplitudes and high signal-to-noise ratios.

The pressures involved in the fizzy extraction process are very low (~150 kPa)[4]; much lower than in supercritical fluid extraction[5] (*e.g.*, ≥10 MPa). The technique does not require the use of any special consumable items (columns, cartridges). Only small volumes of solvents are used for dilution and cleaning. The extraction device can be assembled by chemists with medium technical skills using widely available parts[4]; for example, open-source electronic modules[6,7]. Fizzy extraction can be coupled on-line with modern mass spectrometers equipped with atmospheric pressure chemical ionization (APCI) interface. Because gas-phase extracts are transferred to the ion source, operation of fizzy extraction does not substantially contaminate vulnerable parts of the mass spectrometer.

The purpose of this visualized experiment article is to guide the viewers on how to implement fizzy extraction in a simple analytical task. While the core of the fizzy extraction system is as described in our previous report[4], several improvements have been introduced to make the operation

more straightforward. A microcontroller equipped with an LCD screen shield has been incorporated into the system to display the key extraction parameters in real time. All the functions are programmed in the microcontroller scripts, and there is no longer a need to use an external computer to control the extraction system.

Protocol

This protocol assumes that all the steps are performed according to the relevant laboratory safety regulations. Some of the steps use commercial instruments - in those cases, manufacturer guidelines need to be followed. When handling toxic chemicals, MSDS guidelines need to be followed. The custom-made equipment[4] must be operated cautiously; especially, when handling pressurized gases and live electric wiring.

1. Preparation of Standard Solution

1. Prepare 6.2 x 10^{-2} M stock solution of limonene in ethanol by mixing 10 µL limonene with 990 µL ethanol.
2. Prepare 10 mL of 6.2 x 10^{-5} M limonene solution by mixing 10 µL 6.2 x 10^{-2} limonene, 490 µL ethanol, and adding pure water to the final volume of 10 mL. Shake the volumetric flask thoroughly.
3. Transfer the prepared standard solution to a 20-mL screw top headspace glass vial with septum cap. The diluted standard solution can be used for testing the system.

2. Preparation of Real Sample

1. Obtain lime juice by squeezing fresh lime fruit (cut in half) on a kitchen squeezer.
2. Prepare 10 mL of diluted lime juice by mixing 2 mL lime juice, 500 µL ethanol, and adding pure water to the final volume of 10 mL. Shake the volumetric flask thoroughly.
3. Transfer the prepared sample to a 20-mL screw top headspace glass vial with septum cap.

3. Spiking the Real Sample with Standard Solution

1. First standard addition: Prepare 10 mL of spiked sample by mixing 2 mL lime juice, 10 µL 6.2 x 10^{-2} M limonene solution, 490 µL ethanol, and adding pure water to the final volume of 10 mL. Shake the volumetric flask thoroughly.
2. Second standard addition: Prepare 10 mL of spiked sample by mixing 2 mL lime juice, 20 µL 6.2 x 10^{-2} M limonene solution, 480 µL ethanol, and adding pure water to the final volume of 10 mL. Shake the volumetric flask thoroughly.

4. Setting Up the Fizzy Extraction System

1. Put the fizzy extraction system (**Figure 1**)[4] next to the APCI source of the triple quadrupole mass spectrometer.
2. Connect the carbon dioxide gas cylinder to the gas supply inlet of the fizzy extraction system. Open the valve in the gas regulator. Set the output pressure to 1.5 bar (150 kPa).
3. Connect the extraction chamber outlet to the ion source inlet.
4. Connect the fizzy extraction system to the 12-V power supply.
5. **Set up the data acquisition software of the triple quadrupole mass spectrometer** (**Figure 2**). **Operate the instrument with the APCI source, in the positive-ion multiple reaction monitoring (MRM) mode, with argon as collision gas.**
    1. Run the data acquisition software.
    2. Select the option "LCMS8030 only".
    3. Select the option "MS On/Off".
    4. Set the desolvation line temperature to 250 °C, and the flow rate of drying gas to 15 L min^{-1}. Wait until the value of every instrument parameter becomes the same as the preset value.
    5. Select the MS data acquisition method file.
    6. Make sure the collision voltage is -20 V, the precursor ion *m/z* is 137, and the fragment ion *m/z* are 81 and 95
    7. Click on the "Start Single Run" button.
    8. Type the file name.
    9. Select the file path.
    10. Move to section 5 ("Performing fizzy extraction").
    11. Select the option "MS On/Off".
    12. Close the software window.
    13. Tick the items "Nebulizing Gas Off", "DL Heater Off", "Heat Block Off", and "Dry Gas Off". Click "OK".

5. Performing Fizzy Extraction

1. Place a sample vial in the fizzy extraction system by using the screw mount. The extraction system is operated at room temperature (~ 25 °C).
2. Press the "Start" button on the LCD shield of the fizzy extraction system.
3. Wait while the automated fizzy extraction process is executed (**Figure 3**). Observe development of ion signals on the screen of the triple quadrupole mass spectrometer.

NOTE: The following steps are executed automatically: Sample headspace is flushed with carbon dioxide during 60 s. Sample is pressurized with carbon dioxide during 60 s. Stirrer motor is on. Sample is depressurized. Multiple bubbles are formed. In the later phase, stirrer motor is on to enhance bubbling.
4. Take out (unscrew) the sample vial.
5. Wipe the sample stirring spindle with cellulose tissue.
6. Wash the stirring spindle with ethanol, and wipe it with cellulose tissue again.
7. The system is ready for analysis of another sample (repeat steps 5.1-5.6).
8. Switch off the power supply.
9. Disconnect the fizzy extraction outlet tube from the ion source.
10. Close the valve of the gas cylinder, and disconnect the gas tubing.

6. Data Analysis

1. **Export extracted ion currents for the *m/z* 81 from the triple quadrupole mass spectrometer's data acquisition software to ASCII files (Figure 4).**
   NOTE: The ion current at the *m/z* 95 is not used in this demonstration.
   1. Run the data acquisition software. Select the option "Postrun".
   2. Select the option "Select Project (Folder)", and choose the data file.
   3. Click on the "File" menu, and select "Export Data" / "Export Data as ASCII".
   4. Select "Output File", and select the file path. Select "MS Chromatogram (MC)".

2. **Import the raw data sets into peak integration software, and measure peak areas (Figure 5). Settings: linear baseline; HVL function.**
   1. Run the peak integration software.
   2. Select the option "Import" from the "File" menu. Click "Yes" button.
   3. Select the data in X and Y column. Click "OK" button. Select the option "AutoFit Peaks I Residuals".
   4. Fit the extraction peak semi-automatically. Make sure the fitted curve follows the experimental data points. Select the option "List Peak Estimates". Select the option "ASCII Editor".
   5. Copy the fitting results to "Clipboard".

3. **Input the measured peak areas into a spreadsheet in data analysis software (Figure 6).**
   1. Run the data analysis software.
   2. Input the concentration values in X column and peak area values in Y column. Select the option "Symbol" / "Scatter" from the "Plot" menu. Select the option "Fitting" / "Fit Linear" from the "Analysis" menu.

4. Calculate the concentration of limonene in the diluted real sample based on the formula:
   $$C_{diluted} = I/S$$
   where *I* is the intercept of the linear function, while *S* is the slope.
5. Compute the concentration of limonene in the original real sample (before dilution) based on the formula:
   $$C_{Original} = DF * C_{diluted}$$
   where *DF* is the dilution factor (here, 5).

Representative Results

At the beginning, the fizzy extraction system is tested with a standard solution. Subsequently, the real sample and real sample spiked with standard are analyzed. The areas of the temporal peaks of extraction events are correlated with concentrations of the analytes in the liquid samples subjected to fizzy extraction, which enables quantitative analysis. Here, we performed double standard addition to demonstrate quantitative capabilities of the technique (**Figure 7**). The linear regression led to the following function (**Figure 8**):

$Peak\_area = (3.25 \times 10^7 \pm 0.36 \times 10^7) C_{diluted} + (2{,}770 \pm 276)$

The variable $C_{diluted}$ refers to the concentration of the added limonene standard (in moles per liter, M). Based on the obtained slope and intercept values, the concentration of limonene in the diluted lime juice sample was: $8.51 \times 10^{-5} \pm 1.26 \times 10^{-5}$ M. After multiplying that value by the dilution factor (5), the concentration of limonene in the original lime juice sample was: $4.26 \times 10^{-4} \pm 0.63 \times 10^{-4}$ M.

The main descriptors of analytical performance such as limits of detection and quantification of this method were reported previously[4]. For example, the analytical repeatability (RSD) was 6-19%. The limit of detection for limonene was ~10^{-4} M[4]. We believe that the imperfections of the fizzy extraction prototype system contribute to the signal fluctuations. These imperfections can be eliminated when the fizzy extraction apparatus is developed further and commercialized.

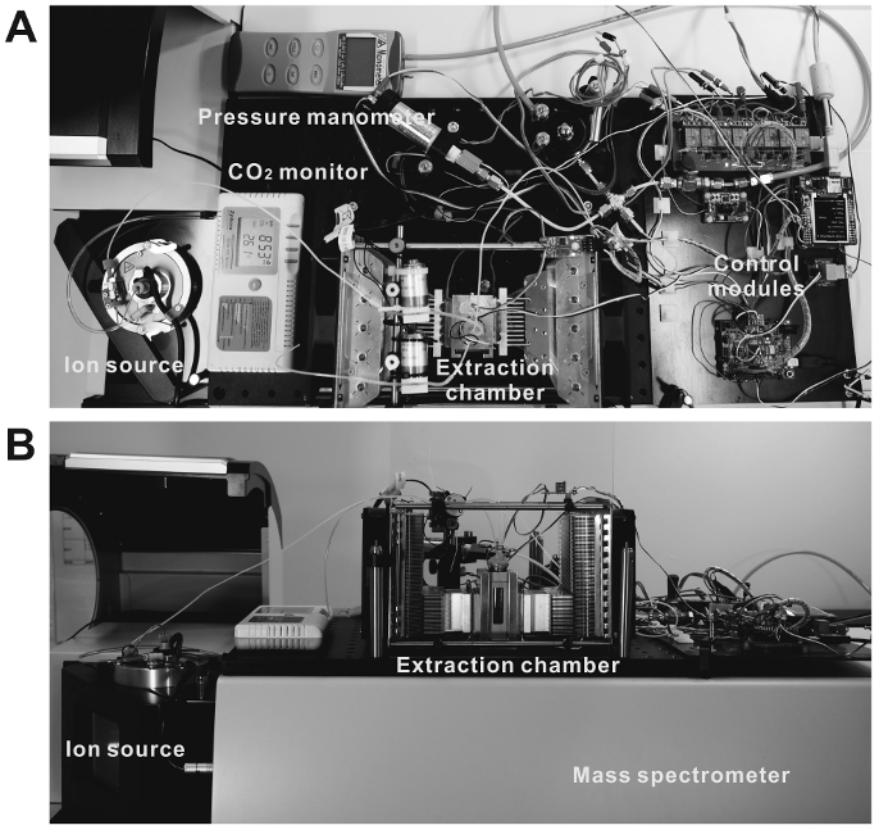

Figure 1: Photographs of the fizzy extraction system (with labels). (**A**) top view; (**B**) front view. Please click here to view a larger version of this figure.

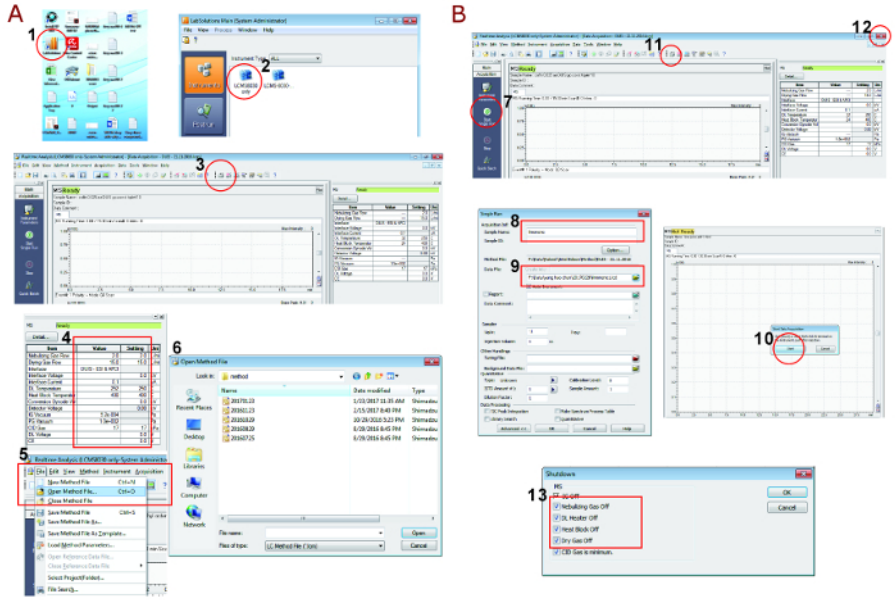

Figure 2: Setting up the data acquisition software of the triple quadrupole mass spectrometer. The consecutive steps are shown in the panels A and B. The numbers refer to the protocol step 4.5. Please click here to view a larger version of this figure.

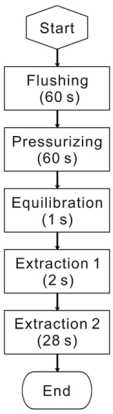

Figure 3: Workflow of typical fizzy extraction experiment. Please click here to view a larger version of this figure.

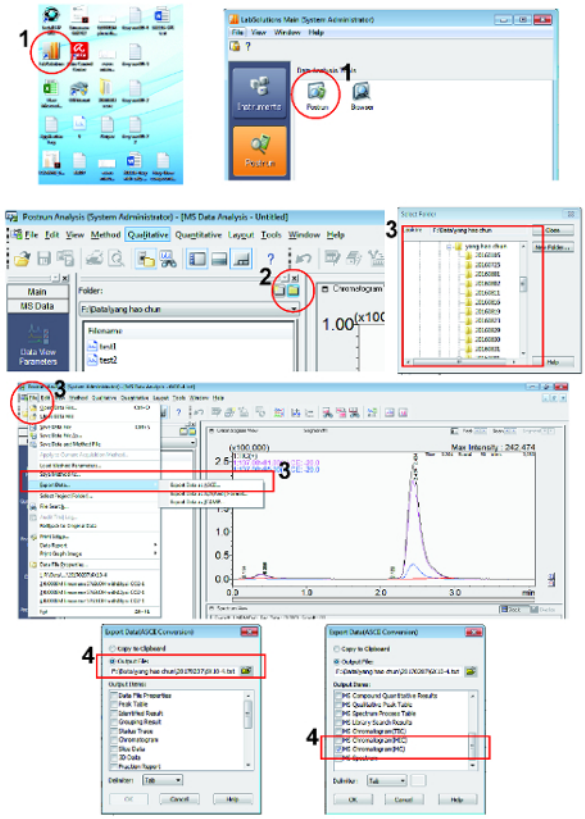

Figure 4: Exporting extracted ion currents from the data acquisition software to ASCII files. The numbers refer to the protocol step 6.1. Please click here to view a larger version of this figure.

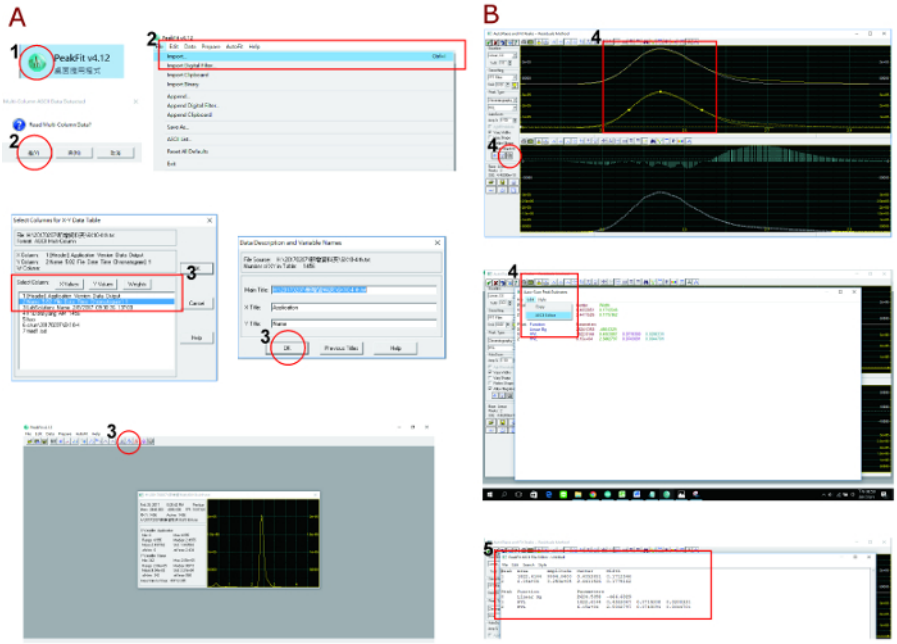

Figure 5: Importing the raw data sets into the peak integration software, and measuring peak areas. The consecutive steps are shown in the panels A and B. The numbers refer to the protocol step 6.2. Please click here to view a larger version of this figure.

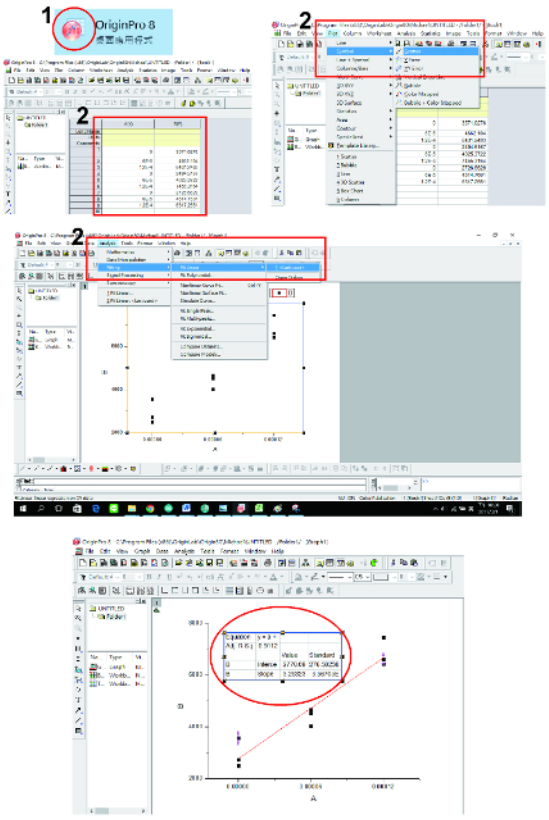

Figure 6: Inputting the measured peak areas into a spreadsheet in the data analysis software, and conducting linear regression. The numbers refer to the protocol step 6.3. Please click here to view a larger version of this figure.

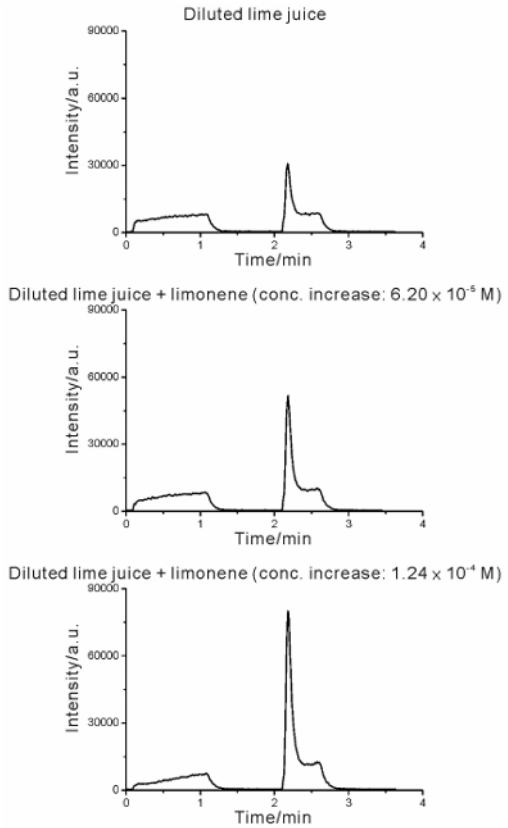

Figure 7: Typical raw data for limonene standard solution and lime juice sample.
Extracted ion currents recorded at the *m/z* 81, following fragmentation of the parent ion at the *m/z* 137 by collision-induced dissociation. Collision gas: argon. Collision voltage: -20 V. The raw data for: diluted lime juice; diluted lime juice after the first addition of limonene standard (concentration increase: 6.20 x 10^{-5} M); diluted lime juice after the second addition of limonene standard (concentration increase: 1.24 x 10^{-4} M). Please click here to view a larger version of this figure.

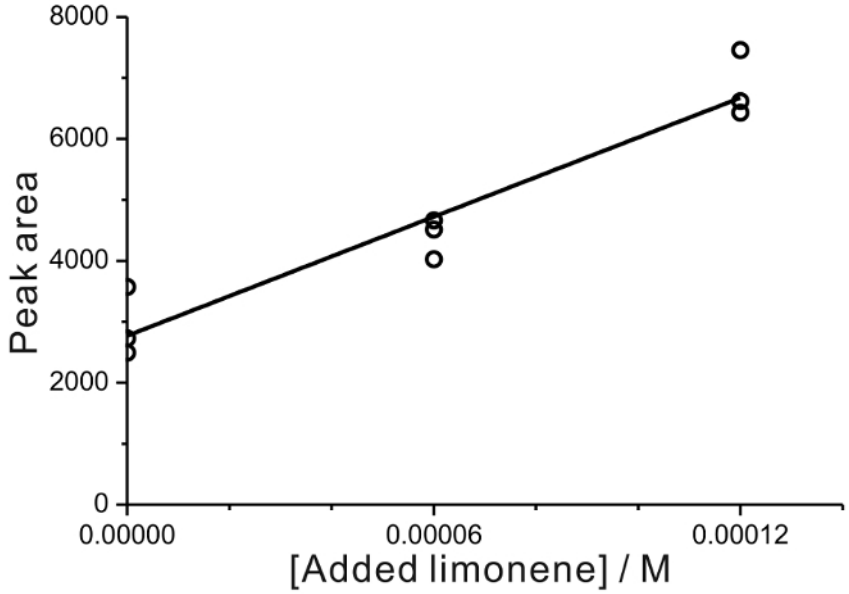

Figure 8: Plot relating temporal peak areas with concentration of limonene standard added to diluted lime juice sample. Please click here to view a larger version of this figure.

Discussion

Several smart ways to deliver samples to a mass spectrometer were developed in the studies conducted during the past three decades (*e.g.*, references[8,9,10,11,12,13,14]). One of the goals of those studies was to simplify preparation of samples for analysis. To achieve that goal, various modifications were introduced to the ion source design. In some cases, the newly developed ion sources allowed chemists to analyze matrix-rich samples, which could normally produce spectral interferences. In an alternative approach, modifications to the conventional ionization schemes are minimized, while the sample preparation (extraction) is conducted on-line and automated (*e.g.*, references[15,16,17,18]). The measurement system presented here[4] exemplifies that notion because the extraction process is combined with a conventional ionization technique (*i.e.*, APCI[19,20], *cf.* **Figure 1**). However, in the future, coupling fizzy extraction with other ion sources or detectors should not be excluded, which could potentially increase the range of detectable species.

The fizzy extraction apparatus is simple to operate, and can be used in quantitative analyses. In this demonstration, we show the possibility to detect and quantify a volatile compound present in a real sample (lime juice) by implementing a double standard addition method. The obtained concentration of limonene (along with its isomers) in fresh lime juice was estimated to 4.26×10^{-4} M, which is very close to the concentration range of limonene in this kind of matrix, as reported in the literature (4.4×10^{-4}-5.1×10^{-4} M)[21]. Certainly, the concentration of limonene in lime fruit is expected to vary depending on the cultivar, growth conditions, harvest time, storage conditions, and the method of obtaining juice for analysis - to name just a few factors. It would be ideal to implement an isotopically labeled internal standard to compensate for the experimental variability. However, such isotopologue standards are expensive, and they are not available for most analytes of interest.

The critical steps in the presented fizzy extraction protocol include: (i) setting up the fizzy extraction device (connecting power supplies, carrier gas cylinder, triple quadrupole mass spectrometer); (ii) adjusting pressure of the carrier gas; (iii) setting up the software of the triple quadrupole mass spectrometer for data acquisition; (iv) placing the sample vial in the fizzy extraction system; (v) cleaning the stirrer spindle; and (vi) data processing (peak integration).

For example, if the stirrer spindle is not cleaned well, this may lead to carryover of the analyte, and decrease analytical accuracy. Moreover, attention should be paid to the level of bubble foam formed during extraction. If some of this foam accidentally gets into the carrier gas tubing or extract tubing, the system may become contaminated. In such cases, the tubing has to be cleaned thoroughly with ethanol.

Carbon dioxide was the first-choice carrier gas because it has high solubility in water. It is also used in the production of fizzy drinks. In fact, fizzy extraction was inspired by observation of bubbles-and the release of aroma-from fizzy drinks. However, in a follow-up study, we will verify the possibility to use other gases as carrier gas.

Overall, the advantages of fizzy extraction include: simplicity, speed, and limited use of chemicals (*i.e.*, solvents for extraction or dilution). One disadvantage of the experimental system presented here is the use of a low-resolution quadrupole mass spectrometer. The possible spectral interferences limit the applicability of the technique. Thus, it is appealing to couple fizzy extraction with a high-resolution mass spectrometer equipped with ion cyclotron resonance or orbital ion trap analyzer. In the present demonstration, the low resolution of the mass analyzer (quadrupole) is compensated for (to some extent) by applying multiple reaction monitoring, which slightly increases selectivity of the mass spectrometric detection.

We foresee that fizzy extraction will find new applications in the near future. For example, it may be suitable for detection of volatile organic compounds in foodstuffs, beverages, cosmetic and household products, as well as environmental samples.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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