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THE ANALYSIS OF FLAVOR COMPOUNDS IN GREEN TEA USING ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER (ICECLES)

BY

ABDULLAH H. ALLUHAYB

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Chemistry

South Dakota State University

2017

THE ANALYSIS OF FLAVOR COMPOUNDS IN GREEN TEA USING ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER (ICECLES)

This thesis is approved as a creditable and independent investigation by a candidate for Master of Science in Chemistry degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Brian A. Logue. Ph.D
Thesis Advisor Date

Douglas Raynie, Ph.D
Head, Department of Chemistry and Biochemistry Date

Dean, Graduate School Date

This thesis and everything I have achieved in my life is dedicated to my loving parents and my supportive family members. You have given me all the riches that life could offer and without you I would never be where I stand today. I have a special feeling of gratitude to my parents, whose encouragement has continuously provided me the motivation and inspiration in overcoming the obstacles and challenges in life. Thank you for always being there for me!

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CONTENTS

ABBREVIATIONS

ASE	Accelerated solvent extraction
SFE	Supercritical fluid extraction
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microextraction
SDME	Single-drop microextraction
DLLME	Dispersive liquid–liquid microextraction
SPE	Solid phase extraction
SPME	Solid phase micro extraction
SBSE	Stir bar sorptive extraction
ICECLES	Ice concentration linked with extractive stirrer
SDE	Simultaneous distillation and extraction
DHS	Dynamic headspace
UAE	Ultrasound assisted extraction
MAE	Microwave-assisted extraction
^{m}ICECLES	Multiple-stir bar ICECLES
PDMS	Polydimethylsiloxane
GC-MS	Gas chromatography-mass spectrometry
TDU	Thermal desorption unit
NIST	National Institute of Standards and Technology
5-HMF	5-(hydroxymethyl)furfural

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ABSTRACT

THE ANALYSIS OF FLAVOR COMPOUNDS IN GREEN TEA USING ICE CONCENTRATION LINKED WITH EXTRACTIVE STIRRER (ICECLES)

ABDULLAH H. ALLUHAYB

2017

Sample preparation of target components from food samples is one of the most difficult steps in this type of analysis. Many extraction techniques have been used for this purpose, such as liquid-liquid extraction (LLE), accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), solid-phase extraction (SPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE). Although each of these techniques works well, they each have a number of disadvantages, including selectivity, relatively high cost, long preparation time, and matrix effects. Ice concentration linked with extractive stirrer (ICECLES) is a promising new sample preparation technique, especially for the extraction of relatively polar compounds, which may prove to have widespread applicability for analytical sample preparation. ICECLES was used to prepare green tea for flavor analysis by gas chromatography-mass spectrometry (GC-MS). ICECLES produce 301 constituents, the vast majority with stronger signal to noise ratios than the 245 components found using SBSE. Therefore, 56 extra constituents were detectable via ICECLES alone, including some very important flavor compounds such as furfural, eugenol, 2-methylpyrazine, phenethyl alcohol, α-terpineol, and 2,6-dimethoxyphenol. Overall, ICECLES sample preparation followed by GC-MS showed higher extraction efficiencies for the vast majority of green tea flavor components, including relatively polar compounds, as compared to SBSE.

1. Chapter 1. Introduction

1.1. Significance

Green tea is one of the most widely consumed beverages in the world due to its beneficial medicinal properties (reduction in serum cholesterol, anti-oxidant properties, and a decreased risk of cancer) as well as its pleasant flavor. About 200 compounds in green tea have been identified and 30 compounds are related to its flavors [1]. Manufacturers of green tea analyze their products for these compounds to ensure the quality and identity of their products. Many techniques have been used to prepare green tea samples for analysis, including liquid-liquid extraction (LLE), solid phase micro extraction (SPME), and stir bar sorptive extraction (SBSE). These techniques generally suffer from low extraction efficiencies for certain compounds, especially those more polar. Moreover, those techniques which are amenable to analysis of more polar compounds (e.g., SPME with polar sorbent phases) only produce good extraction efficiencies for a narrow polarity range. Therefore, there is a critical need to develop a more comprehensive extraction technique to prepare the flavor compounds of green tea for analysis, with the ability to extract compounds with a wide polarity range.

1.2. Objective

The objective of this project was to evaluate the performance of ice concentration linked with extractive stirrer (ICECLES) sample preparation for the flavor analysis of green tea (specifically comparing to SBSE). In order to accomplish this objective, an ICECLES method for the extraction and identification of flavor compounds from green tea using ICECLES-gas chromatography-mass spectrometry

(GC-MS) was developed. Moreover, direct comparison between ICECLES and SBSE was performed.

1.3. Extractive sample preparation

1.3.1. Overview of extractive sample preparation

Figure 1.1 shows the general steps involved in analysis of samples via chromatography. All these steps can affect the results [2]. One of the most important steps is sample preparation, where an analyte is separated from the matrix interferences and typically preconcentrated for chromatographic analysis [3, 4].

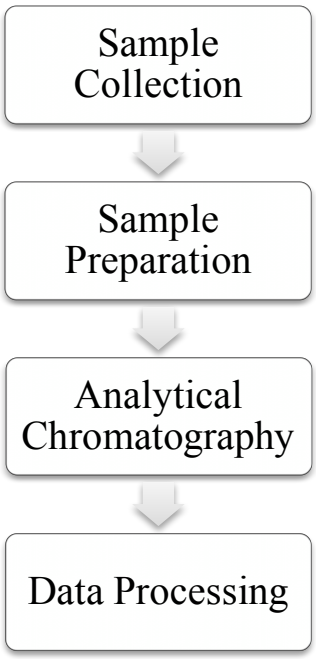

Figure 1.1 Analytical chemistry steps for chromatographic analysis.

Although scientists have focused much of their attention on analysis techniques [4], numerous sample preparation methods such as filtration or liquid-liquid extraction, have been developed over the years [3]. Most of these techniques are still in use today. Sample preparation techniques should have the following advantages [4]: 1) suitable for

trace analysis, 2) safe and environmentally friendly, 3) selective and sensitive, 4) inexpensive, 5) relatively quick, and 6) simple and easy to perform.

1.3.2. Extraction Methods

One of the most common sample preparation concepts is extraction. Extraction methods aim to separate and isolate the target analyte into an immiscible phase from the sample matrix. Many extraction techniques have been used for sample preparation, including accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), and stir bar sorptive extraction (SBSE) [4-6]. These techniques typically utilize a solvent to help to separate the target from the sample matrix [3, 4, 7]. Sample preparation techniques have been developed to be more selective and more sensitive over time for a variety of different applications such as food analysis and environmental applications.

1.3.3. Accelerated Solvent Extraction (ASE)

1.3.3.1. Basic principle of ASE

Accelerated solvent extraction (ASE) is an extraction technique first reported in 1996 by Richter et al. [6] which uses the combination of temperature and pressure to extract an analyte from a matrix. Figure 1.2 shows a schematic of an accelerated solvent extraction (ASE) instrument. ASE uses temperatures between 50-200 ^{o}C (i.e., above boiling point of the solvent) and pressures between 500-3000 psi to extract analytes from solid and semisolid samples [6, 8]. The sample is placed into an extraction chamber and pressure and heat are applied.

The extraction chamber is filled with solvent which expands its capacity to dissolve more analytes due to the elevated temperatures and pressures [8-10]. Advantages and disadvantages of ASE are listed in Table 1.1 [6, 9, 10].

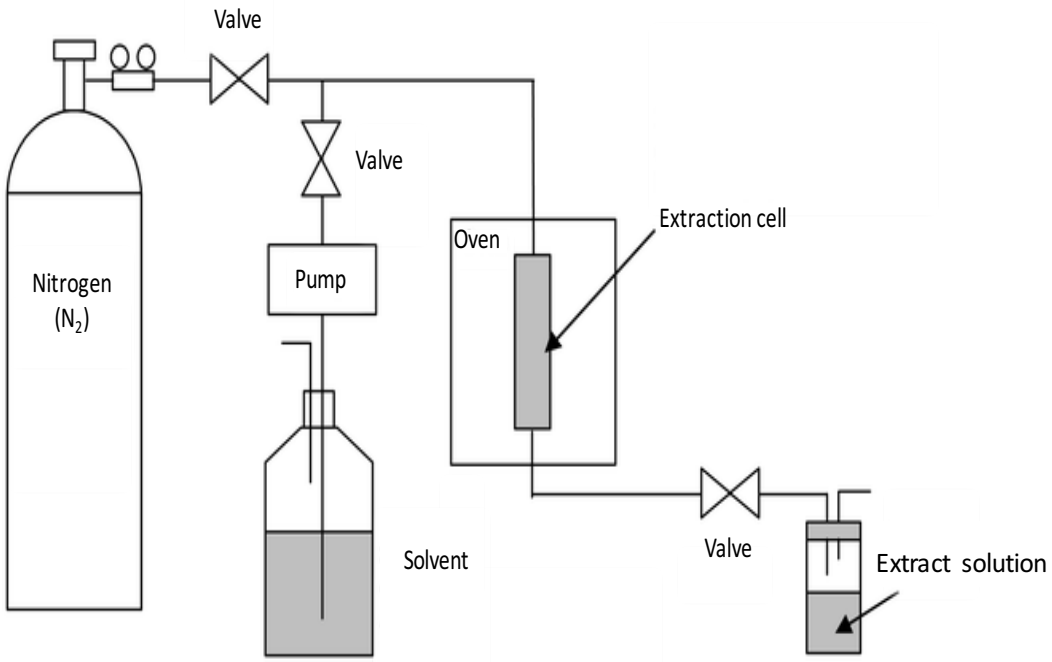

Figure 1.2 Schematic of an accelerated solvent extraction (ASE) apparatus [11].

Table 1.1 Advantages and disadvantages of ASE sample preparation.

Advantages	Disadvantages
▪ Short extraction time ▪ Low solvent consumption ▪ Sample preparation is rapid ▪ High extraction efficiency for solid and semisolid matrix	▪ Extraction is not selective ▪ Mainly useful for solids ▪ Expensive ▪ Extract steps sometimes necessary before the final analysis

1.3.3.2. Applications of ASE

Numerous analytes, including volatile and semi-volatiles, have been extracted using ASE. ASE has been mainly applied to environmental analysis. Table 1.2 summarizes some applications of ASE [8, 9, 12].

Table 1.2 Example applications of ASE sample preparation.

Compound	Matrix	Extraction conditions	Separation technique	Ref
Phenols	soils/sediments	methanol/ acetone/DCM 50-120 ^{o}C/600-1800 psi	HPLC/GC-MS	[12]
PAHs	soils/sediments	acetone-DCM or hexane 50-150 ^{o}C/ 1500-2000 psi	GC-MS/FID HPLC	[12]
Terpenes	plant (Thyme)	hexane and dichloromethane 50 ^{o}C/ 2030 psi	HPLC	[8, 13]
Flavonolignans	milk	hexane and methanol 100 ^{o}C/ 2030 psi	HPLC	[8, 13]
Flavanones	Plant (orange)	dichloromethane 100 ^{o}C	LC-photodiode	[14]
Pesticides	Solid waste	acetone-toluene or hexane 100 ^{o}C/ 2200 psi	GC-MS	[12]

1.3.4. Supercritical Fluid Extraction (SFE)

1.3.4.1. Basic principle of SFE

Supercritical fluid extraction (SFE) is an environment friendly sample preparation technique which uses a supercritical fluid (i.e., a solvent at temperature and pressure above its critical point) to extract analytes from the matrix [3, 7]. The extraction efficiency of SFE depends on the physical properties, density and viscosity, of the supercritical fluid used. Many supercritical fluids have been used in SFE, including nitrous oxide and carbon dioxide [3, 4]. Carbon dioxide (CO_2) is most commonly used

due to the high cost of xenon and the hazardous nature of nitrous oxide [4]. Figure 1.3 shows a basic schematic of supercritical fluid extraction (SFE) instrumentation.

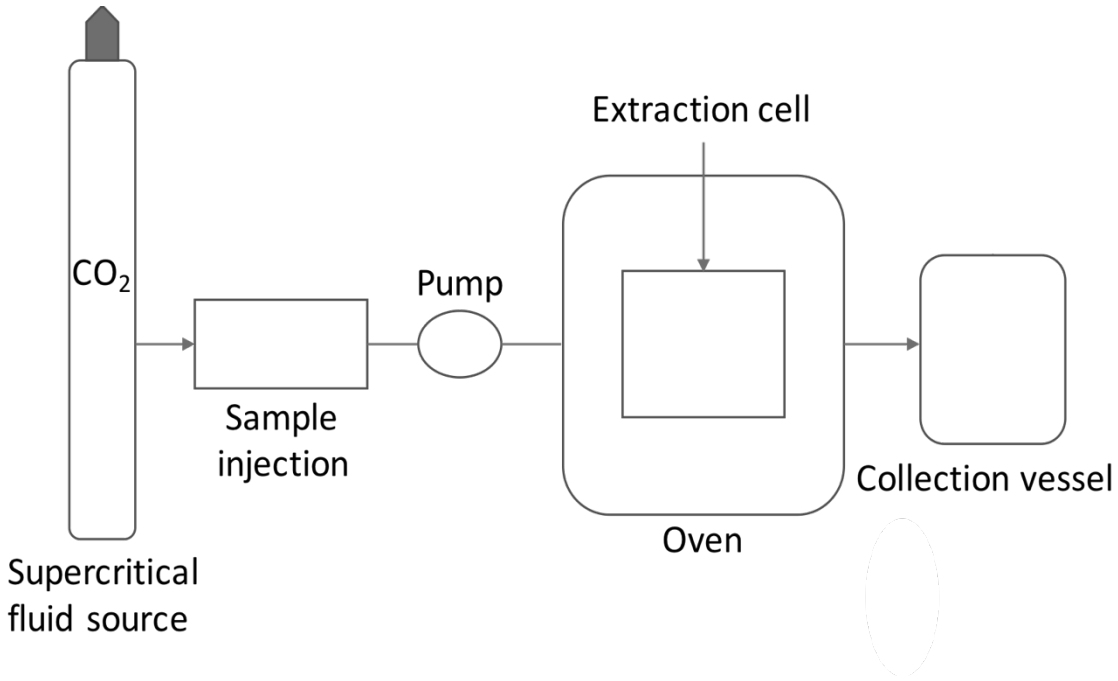

Figure 1.3 Basic schematic diagram of a supercritical fluid extraction (SFE) instrument.

SFE is well-suited for solid samples, such as herbal medicines, polymers, and some plants. However, liquid samples are difficult to extract via SFE, but may be achievable by adjusting some solvent parameters [3, 4]. The selectivity of SFE can be improved by adjusting pressure and temperature of the solvent, or by adding chemical modifiers to the solvent. For example, carbon dioxide CO_2 is relatively non-polar and its polarity can be adjusted towards more polar compounds by adding methanol [3, 4]. SFE also has some disadvantages such as the necessity for high pressure, limited polarity range, and it almost exclusively is used for solid samples. Some advantages and

disadvantages of using supercritical fluid extraction (SFE) are reported in Table 1.3 [3, 4, 15].

Table 1.3 Advantages and disadvantages of SFE.

Advantages	Disadvantages
▪ Environment friendly ▪ Effective for solid samples ▪ Flexible technique (i.e., improve the selectivity of extraction to cover a wide range of polar and non-polar compounds) ▪ Low solvent consumption ▪ High recovery	▪ Difficult to use ▪ Difficult to extract liquid samples ▪ Limited solvent types ▪ Sometimes high pressure is required ▪ Generally, only applicable towards highly nonpolar analytes

1.3.4.2. Applications of SFE

Supercritical fluid extraction (SFE) has been used in factories for many years to extract kilograms of an analyte from a sample matrix [16]. Although SFE has been applied to several fields, it has found its main application in food and agriculture. One study investigated the antioxidants produced in some plants, such as vegetables and fruits, using SFE compared to other extraction techniques, finding higher antioxidant activities via supercritical fluid extraction versus the hydrodistillation extraction technique (i.e., steam distillation) [17]. Another study used SFE followed by GC-MS and HPLC to investigate phenols in grape seeds. They used SFE in steps: 1) with pure, CO_2 they obtained high yields of antioxidants, 2) with 80% of CO_2 and 20% ethanol, they obtained high yields of agro-chemical compounds [18]. Moreover, SFE has been used in environmental analysis [19]. For example, SFE was applied to analyze pesticides in contaminated soil and obtained high recoveries [20].

1.3.5. Liquid-Liquid Extraction (LLE)

1.3.5.1. Basic principle of LLE

Liquid-liquid extraction (LLE) is one of the most common and simple sample preparation techniques used in analytical chemistry [21]. Liquid-liquid extraction typically uses an immiscible organic solvent to extract an analyte from an aqueous sample solution [3]. The extraction process in LLE is demonstrated in Figure 1.4 and usually consists of a solute (the desired analyte) transferring preferentially from an aqueous layer to organic layer.

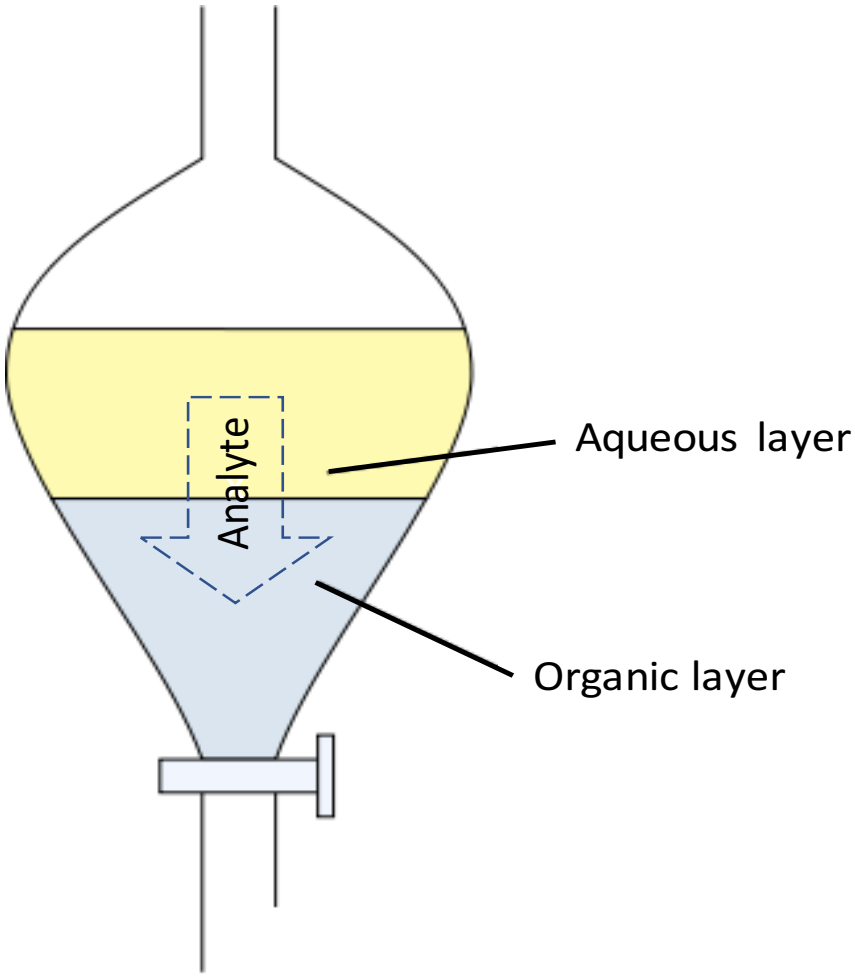

Figure 1.4 Schematic diagram of liquid-liquid extraction (LLE).

1.3.5.2. Liquid-Liquid Micro Extraction (LLME)

The isolation of the analyte from the matrix phase into the solvent phase is achieved based on the different solubility of the analyte between these phases. Although LLE is simple, organic solvents are necessary and the process can be time consuming [3, 4, 21]. Micro-liquid-liquid extraction methods have been developed to reduce the drawbacks of LLE. Multiple modes of micro-liquid-liquid-extraction have been suggested such as single-drop microextraction (SDME) and dispersive liquid–liquid microextraction (DLLME) [21, 22].

SDME is a micro LLE technique where a single droplet of an organic solvent is suspended at the end of a syringe needle. The droplet is immersed into the sample solution (DI-SDME) or held in the vial headspace above the sample (HS-SDME) to extract the analyte as illustrated in Figure 1.5. The micro-drop is pulled into the syringe and then injected into an instrument for analysis, such as gas chromatography-mass spectrometry [21-24].

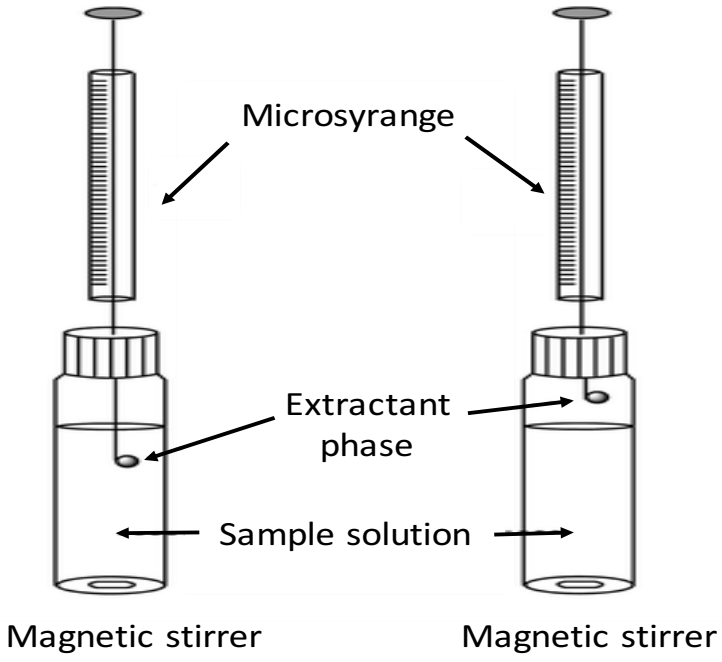

Figure 1.5 Schematic of DI-SDME and HS-SDME [24].

Dispersive liquid–liquid microextraction (DLLME), as demonstrated in Figure 1.6, is another miniaturized type of LLE. The extraction processes in DLLME occurs by injecting microliter volumes of a solvent into a solution and stirring to form a cloudy suspension. The solution is then centrifuged to obtain a small droplet at the bottom of the vail. The analyte is concentrated at the fine droplet that formed and can be analyzed by GC-MS, HPLC, or AAS [21, 25]. Some advantages and disadvantages of using LLE, SDME, and DLLME are given in Table 1.4 [3, 21, 25].

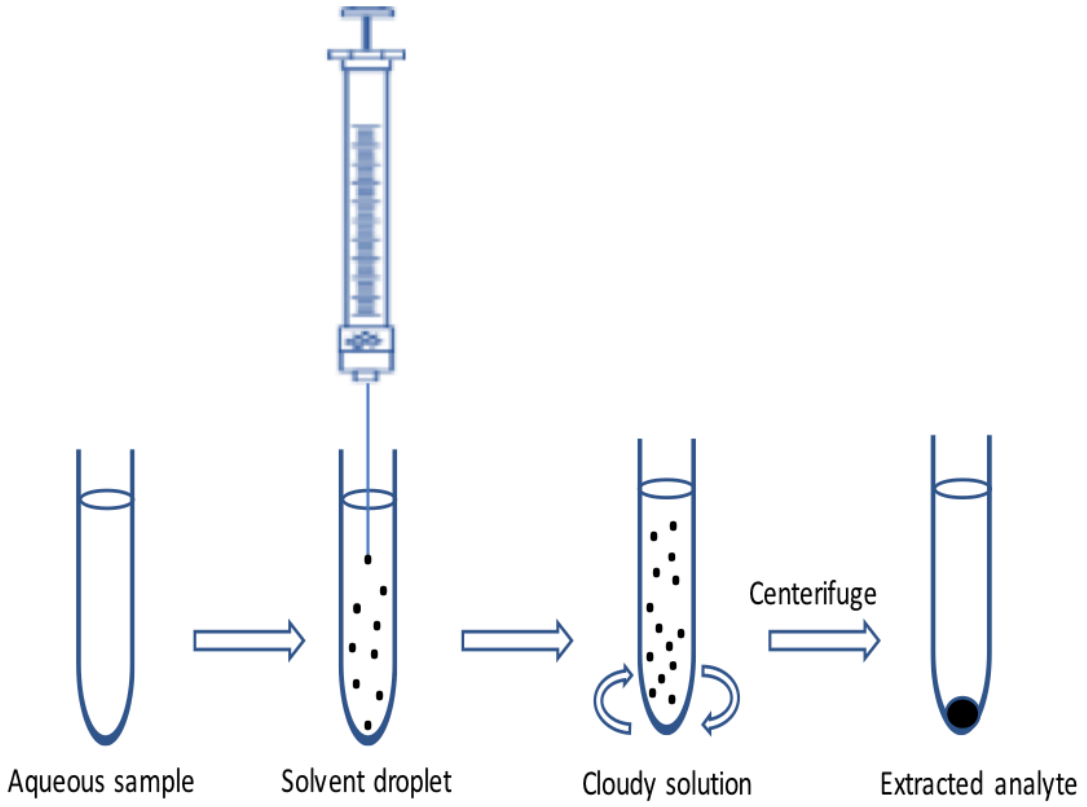

Figure 1.6 Schematic diagram of dispersive liquid–liquid microextraction (DLLME).

Table 1.4 Comparison of the advantages and disadvantages of LLE, SDME, and DLLME.

Techniques	LLE	SDME	DLLME
Advantages	▪ Inexpensive ▪ Easy to perform ▪ Large amount of analyte extracted	▪ High extraction efficiency ▪ Solvent consumption is negligible ▪ Simple	▪ Simple and rapid ▪ Inexpensive ▪ High recovery ▪ High preconcentration factors
Disadvantages	▪ Tedious and time consuming ▪ Large amount of solvent used ▪ Low selectivity	▪ The micro solvent drop sometime unstable and need some treatment ▪ Poor reducibility ▪ Limited to number of extractants ▪ Often deal with liquid samples	▪ Not suitable in complex matrix ▪ Time and reagents consuming ▪ Limited inorganic applications
	All techniques are limited to non-polar compounds.		

1.3.5.3. Applications of LLE and its Miniaturized Techniques

Liquid-liquid extraction is one of the oldest and most basic extraction techniques. Kula et al. [26] used liquid-liquid extraction at room temperature to separate enzymes and activated proteins from a mixture. Numerous applications have also been shown for miniaturized liquid-liquid techniques. Metals, organometals, and non-metals have been extracted with SDME [27]. For example, Lin et al. [28] used SDME followed by gas chromatography-flame photometric detection (GC-FPD) to extract and determine chromium (III) in water. DLLME has also been applied to extract metals and organometals such as gold, lead, cadmium, and organotin compounds [27]. For example, Rivas et al. [29] used DLLME followed by electrothermal atomic absorption spectroscopy (ETAAS) to extract lead and cadmium from aqueous samples.

1.3.6. Solid Phase Extraction (SPE)

1.3.6.1. Basic principle of SPE

SPE is a common sample preparation technique which was introduced over five decades ago. SPE has some advantages over LLE [30]. In SPE, compounds of interest are concentrated and purified from a matrix solution by partitioning or adsorbing the analytes on a solid phase which is suspended in a small column. The extraction of the analyte from a complex matrix solution is based on the partitioning of the analyte between the liquid sample and the sorbent, similar to LLE. The extraction in SPE typically requires the analytes to have higher affinity toward the solid phase than the liquid phase [30].

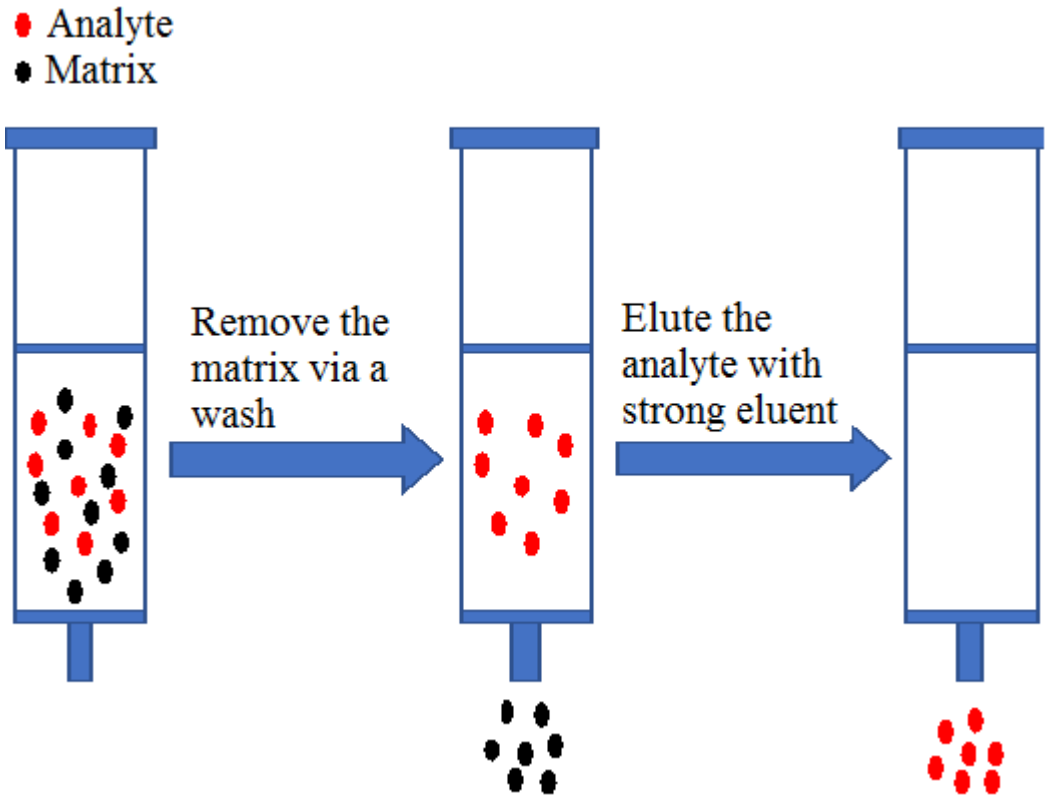

Figure 1.7 Schematic diagram of solid phase extraction (SPE).

Once the analyte is partitioned/adsorbed, it can be removed and preconcentrated into a small volume by using an extraction solvent [4, 30]. The basic sample preparation steps of SPE are shown in Figure 1.7. Currently, SPE is one of the most common sample preparation techniques. A variety of solid-phase sorbents have been used in SPE and can be classified in three main types: normal-phase sorbents, reversed-phase sorbents, and ion-exchange sorbents [31].

Normal phase sorbents include silica, alumina, and Florisil. These sorbents can adsorb polar analytes from a mixture and a gradient range of solvents from non-polar to polar are used to elute compounds. Normal phase sorbents can be chemically modified by adding polar groups such as cyano (CN), diol (COHCOH), or amino (NH_2) groups to trap analytes [31, 32]. Reversed phase sorbents include octadecyl (C_{18}), octyl (C_8), cyclohexyl, and phenyl groups bonded to silica. These sorbents can extract non-polar analytes from polar matrices and a gradient of solvents from polar to non-polar is used to elute these compounds [30-32]. Ion-exchange sorbents, including cation and anion exchangers, extract ionic analytes via ionic interactions. Cation exchanger sorbents with carboxylic acid (COOH), sulfonic acid (SO_2OH), and aromatic sulfonic acid ($ArSO_2OH$) groups can extract negatively charged analytes. Conversely, anion exchange sorbents, such as primary (NH_2), secondary (NRH), and quaternary amine (NR_2), can extract negatively charged analytes. These sorbents can extract charged analytes from the matrix and a gradient range of buffers is used for elution [31, 32]. Table 1.5 shows some advantages and disadvantages of solid phase extraction (SPE) [33, 34].

Table 1.5 Advantages and disadvantages of solid phase extraction (SPE).

Advantages	Disadvantages
▪ High extraction efficiency ▪ Low volume evaporation ▪ Low organic solvents consumption and therefore low solvents disposal ▪ Fast and easy performance ▪ No emulsions	▪ Carryover may occur ▪ Systematic and recovery errors can occur ▪ Sometimes sample stability is a problem ▪ Expensive relative to LLE

1.3.6.2. Applications of SPE

Solid-phase extraction (SPE) is one of the most accepted extraction techniques for bioanalytical, pharmaceutical, environmental, and food analysis [31, 32]. SPE has been used pharmaceutical science to investigate the effect of drugs and antibiotics in living organisms. Hu et al. [35] used SPE, followed by HPLC, to extract and determine two types of trimethoprim in human urine. Moreover, Boos and Fleischer [36] used SPE followed by HPLC for the determination of the analgesic drug, tramadol, in human plasma. SPE has also been applied in food to extract a variety of compounds. For example, Wang et al. [37] applied SPE, followed by HPLC, for the determination of caffeine and theophylline in green tea.

1.3.7. Solid-Phase Microextraction (SPME)

1.3.7.1. Basic principle of SPME

Solid-phase microextraction (SPME) is a sample preparation technique which was first reported in the 1990s. SPME is a simple and efficient extraction technique that can be used to extract, isolate, and enhance analytes, including volatile and non-volatile analytes from a matrix [3, 4]. The extraction process via SPME is shown in Figure 1.8. It proceeds by extracting a small amount of analyte via extracting phase, generally a polymer that coats the outer or internal surface of a solid-support material within a needle

housing [38]. SPME involves two main types of implementations: fiber SPME and in-tube SPME. The first developed, and most common, technique is fiber SPME. In fiber SPME, the extraction phase is a fiber, externally coated with different types of polymers which vary from non-polar to polar, depending on the analyte matrix, (e.g., polydimethyl siloxane (PDMS), polyacrylate, carboxen, and carbowax). The coated fiber is immersed either directly into the sample or in the headspace above the sample to trap analytes. In case of using direct immersion extraction, analytes are directly partitioned or adsorbed into the sorbent phase from the matrix. However, in case of extracting analytes using headspace SPME, the analytes are delivered to the extraction phase via the headspace. The extracted analytes are back-extracted into a solvent or heat, typically via a hot injection port of a GC [3, 4, 38].

In-tube SPME was developed to be more amenable with liquid chromatography. In-tube SPME consists of an open-tubular fused-silica capillary in which the internal surface is coated with sorbent. Analytes are adsorbed or partitioned into the extracting phase when the sample is drawn into the tube. Two modes are used for in-tube SPME, dynamic and active. For dynamic in-tube SPME, analytes are transported through the capillary tube via a flow of air. Conversely, the extraction of analytes via active in-tube SPME is performed without using air flow. The analytes are transferred in active in-tube SPME to the extracting phase via the gas phase present inside the system [3, 38, 39]. Table 1.6 lists some advantages and disadvantages of using solid phase microextraction (SPME) [3, 38, 40, 41].

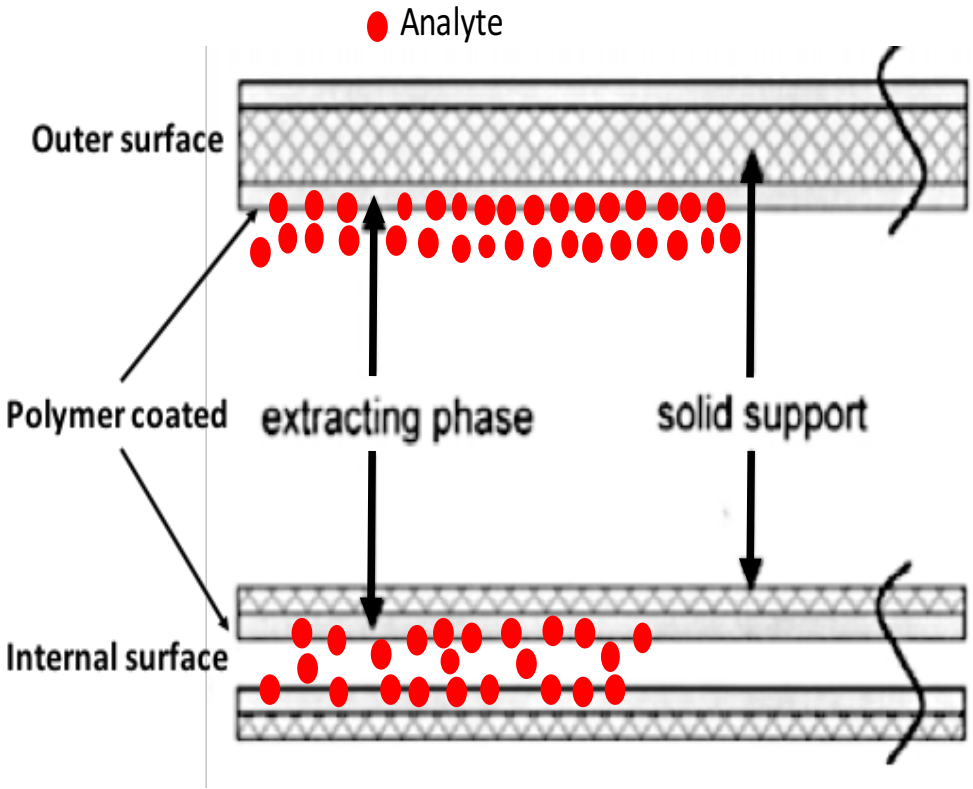

Figure 1.8 Schematic diagram of solid-phase microextraction (SPME) [38].

Table 1.6 Advantages and disadvantages of solid phase microextraction (SPME).

Advantages	Disadvantages
▪ High extraction efficiency ▪ Low volume usage ▪ The distribution of analytes in a multiphase complex can easily be studied ▪ Low solvent consumption ▪ Fast and easy to perform ▪ High accuracy and precision can be obtained	▪ Limited extraction capacity in fiber SPME ▪ Fiber SPME is not sensitive to some volatile organic sulfur compounds ▪ Fibers can be broken ▪ The GC injector temperature need to be below320 °C depending on the fiber used ▪ Carryover may be present and hard to eliminate

1.3.7.2. Applications of SPME

Solid-phase microextraction (SPME) has been used in various applications including clinical, environmental, industrial, forensic, pharmaceutical, and food

analysis. For example, Eisert et al. [42] used fiber SPME coated with PDMS followed by gas chromatography and an atomic emission detector (GC-AED) for the ultra-analysis of six organophosphorus pesticides. Furthermore, Eisert and Levsen [43] used a fiber SPME coated with polyacrylate followed by GC-MS for the determination of organophosphorus, triazines and N-heterocyclic pesticides from aqueous samples. In biomedical analysis, Guan et al. [44] used headspace SPME with PDMS and gas chromatography–electron capture detector (GC–ECD) for the determination of dinitroaniline herbicides from blood, urine, and water. Hawthorne et al. [45] and Yang et al. [46] used fiber SPME with polyimide and uncoated SPME followed by GC-MS for the analysis of caffeine and flavor and fragrance components in coffee, tea, and soft drinks.

1.3.8. Stir Bar Sorptive Extraction (SBSE)

1.3.8.1. Basic principle of SBSE

Stir bar sorptive extraction (SBSE) is a simple extraction technique which was first reported by Baltussen et al. in 1999 [47]. In SBSE, the extraction process occurs by transferring the analytes from a liquid phase to an extracting phase coated on a glass magnetic bar. The sorbent is a polymer, typically PDMS (a highly non-polar sorbent). The coated magnetic glass stir bar is introduced into the sample solution and stirred for a certain time to extract the analytes from the matrix, as shown in Figure 1.9. The extracted analytes are then desorbed via back extraction into a solvent or by heat and typically analyzed via liquid chromatography, gas chromatography, capillary electrophoresis, or inductively coupled plasma [5].

Recently, SBSE involves three main types of coatings: polydimethylsiloxane (PDMS), polyacrylate (PA), and ethylene glycol/silicone (EG/silicone). PDMS is

typically used to extract non-polar analytes. For extraction via PDMS-SBSE, an equilibrium occurs between the analyte and the coating which depends on the log K_{ow} of the compound, equilibrium is quickly achieved for non-polar compounds (i.e., compounds with high log K_{ow} reach the equilibrium in a short amount of time while compounds with low log K_{ow} spend longer time). The PA and EG/silicone were developed for the extraction of relatively-polar components [5]. Listed in Table 1.7 are some advantages and disadvantages of SBSE [47, 48].

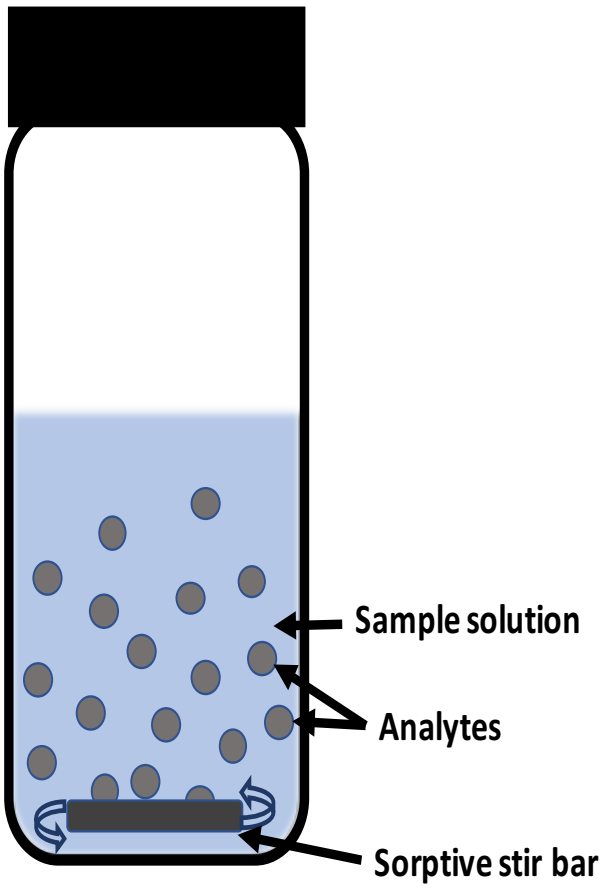

Figure 1.9 Schematic diagram of stir bar sorptive extraction (SBSE).

Table 1.7 Advantages and disadvantages of using stir bar sorptive extraction (SBSE).

Advantages	Disadvantages
▪ High preconcentration capacity ▪ Simple and easy to perform ▪ High recovery ▪ Applied for a large range of organic compounds applications ▪ Environmentally friendly ▪ Use a small volume of sample ▪ Can be coupled with GC, LC, CE, and ICP ▪ The PDMS -coated bar can be used for several times (hundreds of times)	▪ limited to range of polarity ▪ Need matrix modifiers to overcome the extraction of compounds with low Log K_{ow} ▪ Matrix effects are highly affect the extraction ▪ Time consuming ▪ Sorptive stir bar needs to recondition after each analysis

1.3.8.2. Applications of SBSE

SBSE has been applied to the vast majority of analytical fields, including environmental, soils, food, pharmaceutical, and clinical analysis. SBSE was successfully applied for food analysis by Li et al. [49], who used a PDMS sorptive stir bar followed by GC-ECD for the determination of 12 pyrethroid pesticides in tea samples. In addition, SBSE with PDMS followed by GC-MS has been used to identify 113 organic compounds in vinegars [50]. In the environmental field, SBSE (with PDMS coating) was coupled with HPLC-fluorescence detection (FLD) for the determination of polycyclic aromatic hydrocarbons (PAH) in a complex aqueous matrix [51]. Clinically, Unceta et al. [52] used SBSE with a PDMS sorptive stir bar followed by HPLC-FLD for the analysis of serotonin reuptake inhibitors in plasma, urine and brain tissue samples.

1.3.9. ICE Concentration Linked with Extractive Stirrer (ICECLES)

1.3.9.1. Basic principle of ICECLES

ICE Concentration Linked with Extractive Stirrer (ICECLES) is a new sample preparation technique which was first reported by Maslamani et al. [53] in 2016. ICECLES combines freeze concentration (FC) and stir bar sorptive extraction (SBSE) in one technique. With the inherent advantages of FC and SBSE such as high concentration factors, selectivity, simplicity, and robustness, ICECLES is a promising extraction technique for many analytical fields. The main advantage of ICECLES is that more polar compounds can be easily extracted using the commercially available PDMS coating. The basic procedure of extraction in ICECLES is demonstrated in Figure 1.10. Table 1.8 lists some advantages and disadvantages of ICECLES [53].

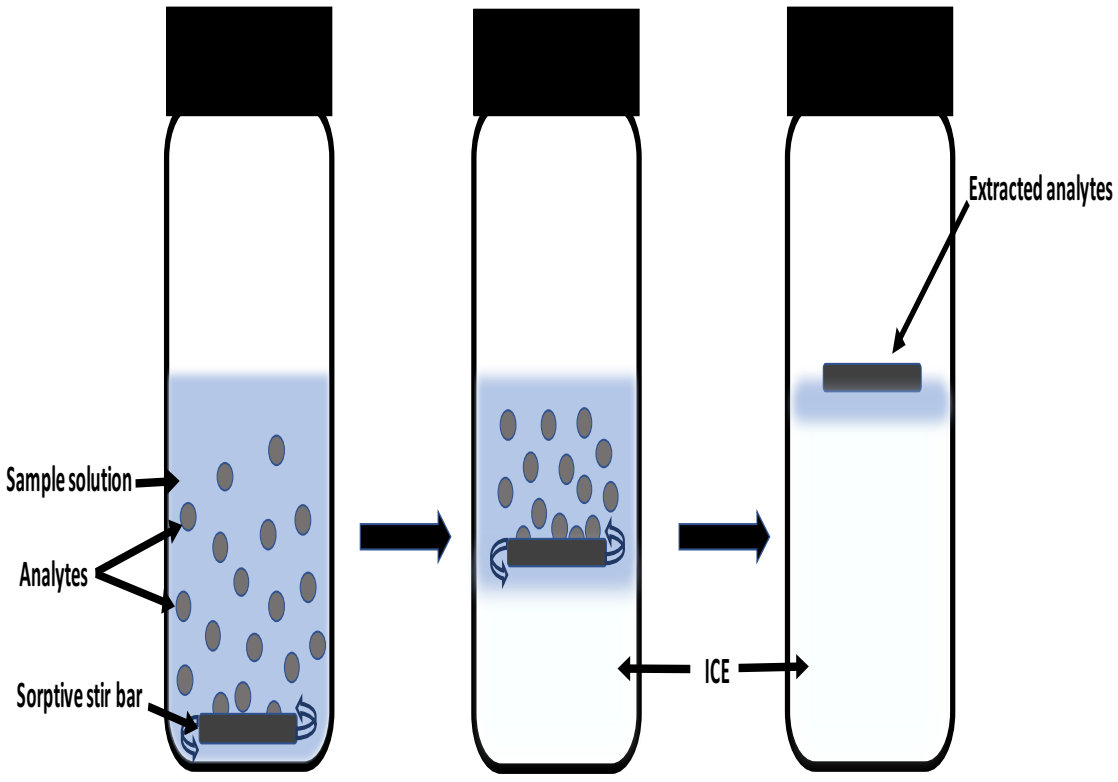

Figure 1.10 Schematic diagram of ICE concentration linked with extractive stirrer (ICECLES).

Table 1.8 Advantages and disadvantages of Ice concentration linked with extractive stirrer (ICECLES).

Advantages	Disadvantages
▪ Solventless ▪ Simple and easy to perform ▪ High selectivity ▪ Applicable over large polarity range	▪ Time consuming ▪ Limited sample volume (i.e., currently no more than 10 mL can be used)

1.3.9.2. Freeze Concentration (FC)

Freeze concentration (FC) is a process of separating analytes from a sample water solution by concentrating and crystallizing water products under freezing conditions. FC is widely uses in petroleum, food, and pulp and paper industries [54, 55].

1.3.9.3. ICECLES procedure

The extraction procedure is similar for SBSE and ICECLES, with ICECLES featuring freezing of the sample. In ICECLES, a sorptive stir is placed into the sample solution on a magnetic stir plate to extract analytes. While the sorptive stir bar is stirred, an equilibrium occurs between the analyte and stir bar coating. Freezing the sample leads the analytes to be concentrated into the aqueous solution and into the sorptive stir bar by pushing the equilibrium from the analytes towards the coated stir bar as demonstrated in Figure 1.10 [53].

1.3.9.4. Applications of ICECLES

ICECLES is a new sample preparation technique with the first and only application of ICECLES published on 2016. Maslamani et al. [53] used ICECLES for the analysis of multiple triazine pesticides in aqueous samples. The sample preparation technique performed well, producing up to 474 signal enhancement when compared to SBSE.

2. Chapter 2. The Analysis of Flavor Compounds in Green Tea Using Ice Concentration Linked with Extractive Stirrer (ICECLES)

Abstract

Worldwide, green tea is one of the most popular beverages. It has been proven to promote blood circulation, liver function, and lower the risk of cancer and cardiovascular diseases. This drink is characterized by the distinctive odors and flavors produced by its constituent compounds, with its value predicated on the amount and type of constituent components extracted from the tea leaves during brewing. Ice concentration linked with extractive stirrer (ICECLES) is a novel sample preparation technique, especially applicable for the extraction of relatively polar compounds while retaining excellent extraction efficiencies for non-polar compounds. In this study, ICECLES was used to prepare green tea for analysis of flavor compounds by gas chromatography-mass spectrometry (GC-MS). ICECLES performed very well, revealing 301 constituents as compared to 245 for SBSE. Moreover, ICECLES produced stronger signal to noise ratios for all except 4 of 301constituents, affording easier identification. Of the 56 constituents which were only detectable using ICECLES, some very important flavor and/or medicinal compounds were easily identified, including furfural, furfural alcohol, maltol, eugenol, 2-methylpyrazine, phenethyl alcohol, 2,6-dimethoxyphenol, and α-terpineol. Overall, we confirmed that ICECLES sample preparation followed by GC-MS consistently allowed more complete green tea flavor analysis, especially for relatively polar compounds, some of which are critical for flavor quality.

2.1. Introduction

Green tea is the second most consumed beverage around the world following water [56, 57]. It is made from the leaves of the *camellia sinensis* plant and has been known since ancient times to exhibit beneficial medicinal properties [58-60]. It promotes blood circulation, improves liver function, promotes metabolism of various toxins, and is more beneficial than beverages that contain large amounts of vitamin C, vitamin E, and β-carotene [57, 61, 62]. In addition, numerous studies have shown that consumption of green tea is linked to the prevention of certain types of skin, lung, and liver cancers and certain cardiovascular diseases [63-67]. The beneficial effects of green tea have been attributed to its rich abundance of antioxidant polyphenolic compounds, mainly flavonoids [68-78]. Furthermore, green tea contains other compounds that promote human health including sterols, vitamins, amino acids, and proteins [58, 70].

The distinctive flavors and aromas of green teas are due to the many volatile and semivolatile compounds extracted from green tea leaves [79-81]. These compounds generally consist of non-terpenoids and terpenoids including alcohols and aldehydes as the main source of green tea aroma [1, 82]. Approximately 200 volatile compounds have been identified and about 30 of these compounds contribute to green tea flavor [1, 83, 84]. These compounds play an important role in determining the quality of individual green teas [57, 81]. Therefore, the comprehensive analysis of green tea flavor compounds is important for researchers and tea producers to understand the makeup, quality, and identity of individual green teas [85, 86].

Various methods have been used to identify green tea flavors, including gas and liquid chromatography (GC and LC, respectively) with mass spectrometric detection (MS). For this type of analysis, sample preparation is vital but sometimes requires lengthy processing times, large sample volumes, and significant organic solvent consumption [87-89]. The objective of sample preparation for flavor analysis is to efficiently extract as many compounds from brewed green tea as possible. Liquid-liquid extraction (LLE), simultaneous distillation and extraction (SDE), dynamic headspace (DHS), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), solid-phase extraction (SPE), solid-phase micro-extraction (SPME), and stir bar sorptive extraction (SBSE) have been used to prepare green teas for analysis [1, 90, 91]. Although most of these techniques are excellent for extracting relatively hydrophobic molecules, they generally suffer from low extraction efficiencies for relatively polar compounds. For the few sample preparation techniques which are applicable to more polar compounds, they generally extract compounds in a relatively narrow polarity range [1, 5, 48].

ICE Concentration Linked with Extractive Stirrer (ICECLES) is a novel extraction technique that combines freeze concentration (FC) and SBSE. ICECLES was first reported in 2016 by Maslamani et al. [53] and showed the ability to increase the extraction efficiencies for each compound tested, but works particularly well for more polar compounds ($\log K_{ow} < 3$), without sacrificing extraction efficiency for less polar compounds ($\log K_{ow} \geq 3$). Furthermore, because ICECLES is performed at the freezing point of the sample, it is excellent for more volatile and thermally labile components. ICECLES proved to be an excellent sample preparation technique for trace analysis of

pesticides in environmental surface waters and other compounds in aqueous solution, producing enhanced LODs and signal enhancements of up to 474 times better than SBSE.

With the inherent advantages of ICECLES (i.e., excellent performance for more polar and more volatile compounds), it appears to be highly complementary to green tea flavor analysis. Therefore, the objective of the current study was to evaluate the performance of ICECLES towards green tea flavor analysis, with direct comparison to SBSE.

2.2. Materials and Methods

2.2.1. Materials and standards

2.2.1.1. Materials

Bigelow green tea classic brand bagged tea (CT, USA) was purchased from a local market. All tea samples in this study were stored in their original tea bags at room temperature before analysis. Acetic acid ($C_2H_4O_2$, ≥99.7%), 2-propanol (C_3H_8O, ≥99.9%), 2-furaldehyde ($C_5H_4O_2$, 99%), indole (C_8H_7N, 99+%), benzyl alcohol (C_7H_8O, 99%), 2,6-dimethoxyphenol ($C_8H_{10}O_3$, 99%), eugenol ($C_{10}H_{12}O_2$, 99%), 2-methylpyrazine ($C_5H_6N_2$, 99+%), phenethyl alcohol ($C_8H_{10}O$, 99%), α-terpineol ($C_{10}H_{18}O$, 96%), trans,trans-2,4-hexadienal (C_6H_8O, 95%), and toluene (C_6H_5-CH_3, 99.5 %) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). 1-pentanol ($C_5H_{12}O$, 99%), cis-2-penten-1-ol ($C_5H_{10}O$, ≥96%), theobromine ($C_7H_8N_4O_2$, ≥98), γ-undecalactone ($C_{11}H_{20}O_2$, ≥98%), 5-(hydroxymethyl)furfural ($C_6H_6O_3$, ≥99%), maltol ($C_6H_6O_3$, ≥99%), furfuryl alcohol ($C_5H_6O_2$, 98%), and benzyladehyde (C_7H_6O, 99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified water was obtained from a water PRO PS polisher (Labconco, Kansas City, KS, USA) at a resistivity of 18.2

MΩ cm. Stir bars (10 mm length) coated with PDMS (0.5-mm film thickness) were obtained from Gerstel, Inc. (Baltimore, MD, USA).

2.2.1.2. Standard solutions

Stock solutions of acetic acid (1 M), benzyl alcohol (1 M), benzyladehyde (1 M), toluene (1M), 1-pentanol (1 M), cis-2-penten-1-ol (1M), γ-undecalactone (1 M), maltol (10 mM), furfuryl alcohol, theobromine (10 mM), and indole (10 mM) were prepared in 10 mL of purified water and stored at room temperature. 2-furaldehyde (1 M), phenethyl alcohol (1 M), eugenol (1 M), trans,trans-2,4-hexadienal (1 M), 2-methylpyrazine (1 M), α-Terpineol (10 mM), 2,6-dimethoxyphenol (10 mM), and 5-(hydroxymethyl)furfural (100 mM) were prepared in 10 mL of purified water and stored at 4 °C. The stock solutions were diluted with purified water to the desired concentration for individual experiments.

2.2.2. Green tea sample preparation

Tea bags were carefully cut and green tea leaves were removed, weighed (1.25 g), and added to 200 mL of boiling water for 5 min. The solution, now yellowish-green, was covered with a watch glass and cooled for one hour at room temperature. The prepared green tea was then divided into four portions, each placed into a 50 mL capped vial, and centrifuged for 5 min at 3000 rpm. Carefully, a 10 mL aliquot of the supernatant was transferred into a 24 mL capped glass vial. Prepared green tea samples were then immediately extracted via ICECLES and SBSE.

2.2.3. ICECLES sample preparation

ICECLES was performed as previously presented [53] with minor modifications. An aliquot (10 mL) of prepared green tea, a standard solution, or blank was added to a 24

mL glass vial along with a PDMS-coated stir bar. The vial was capped and placed into an ICECLES apparatus, as shown in Figure 2.1. ICECLES was performed with coolant temperature (to modify the freezing rate) of -7, -5, and -3 ^{o}C, while stirring at 1200 rpm. After optimization of the freeze temperature, -5^{o}C was used for the remainder of the study. The green tea sample froze gradually from the bottom to the top of the vial until the entire solution was frozen. After extraction was complete, the stir bar, now located on top of the ice near the top of the vial, was magnetically removed with a clean Teflon-coated stir bar. Gently, the stir bar was dried using a clean lab wipe and then placed into a glass thermal desorption (TD) tube. It should be noted that care must be used in vial selection or the sorptive stir bars can be damaged if rounded bottom vials are used because of the high stir rate [53].

2.2.4. ^{m}ICECLES sample preparation

In this study, a multiple-stir bar (^{m}ICECLES) method was used to provide stronger signals for some compounds, which afforded easier identification of green tea components. For ^{m}ICECLES, five individual green tea samples were prepared via ICECLES as described above and analyzed via TD-GC-MS in a multi-desorption mode. In ^{m}ICECLES, the extractable green tea components in each stir bar was extracted using thermal desorption (TD) and held into cooled injection system (CIS).

2.2.5. Gas chromatography-mass spectrometry

Each prepared stir bar was extracted using a thermal desorption unit (TDU) equipped with an MPS 2 auto-sampler and a CIS 4 programmed temperature vaporization (PTV) inlet (Gerstel, Baltimore, MD, USA). The Gerstel autosampler was coupled to an Agilent Technologies 7890A gas chromatograph and a 5975C inert XL electron

ionization (EI)/chemical ionization (CI) mass selective detector (MSD) with triple-axis detector. Separation was performed on an HP-5MS capillary column (30 m x 250 μm x 0.25 μm). Following ICECLES, the glass thermal desorption tube containing the stir bar was placed into the thermal desorption unit. All prepared stir bars were thermally desorbed by performing a temperature gradient from 40 °C (held for 1 min) to 250 °C (held for 1.5 min) at 720 °C/min in splitless TDU mode. After desorption, compounds were cryo-trapped onto a deactivated cooled injection system (CIS) glass liner (filled with quartz wool) at -100 °C via liquid nitrogen. The PTV-CIS temperature was increased from -100 °C (held for 0.20 min) to 250 °C (held for 1.5 min) at 12°C/s using PTV solvent-vent mode with a purge flow of 50 mL/min (held for 1.5 min) to transfer compounds to the analytical column. The GC oven was held constant at 40 °C for 1 min and slowly increased to 250 °C (held for 3 min) at 5 °C/min within a 46-min chromatographic runtime. The mass spectrometer was operated in EI mode at 70 eV and a scan range from 35 to 550 m/z. The mass spectrometer source temperature was 230 °C and the quadrupole temperature was 150 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min and a pressure of 7.07 psi.

2.2.6. Identification of green tea components

Each peak in the ICECLES chromatogram was analyzed by comparing the mass spectrum of the compound with those of the National Institute of Standards and Technology (NIST) mass spectra reference database (the NIST/EPA/NIH Mass Spectral Library, Version 2.0d, 2005). Where possible, identification was supported by comparison of the mass spectra in ^{m}ICECLES and/or SBSE of components with the

same retention time. Furthermore, some green tea compounds were definitively confirmed by ICECLES analysis of an aqueous solution of a spiked standard compound. The retention time and mass spectra of the spiked standards were compared to those of the unknown green tea compounds to confirm their identity. In this study, all standards were prepared and analyzed simultaneously with green tea samples to eliminate day-to-day differences in retention times. To avoid run-to-run error, bias, and sorptive stir bar variability, green tea sample analysis via both ICECLES and SBSE was performed in nonuplicate under the same conditions and the chromatographic data was averaged. Automated peak selection was performed using MSD chemstation software from Agilent Technologies, Inc by setting the peak threshold to 16.1, initial area reject at 1, peak width to 0.02 minutes, and shoulder detection was off.

To consider a green tea constituent *definitively identified*, the retention time and the ion masses of the target green tea compound and the standard were matched. Moreover, an aqueous standard of the compound, analyzed alongside brewed green tea, was required to produce the same retention time (±0.1s) and identical MS fragmentation. All peaks which were not definitively identified were classified based on their probability of a spectrum match via the NIST reference database as follows: if the probability range was between 0-40, the compound classified was as unknown, if the probability range was between 41-70, the compound was classified as a medium probability, if the probability range was between 71-100 the match was classified as a high probability. Additionally, if the abundance of all mass spectrum fragments for the compound (minus the blank mass spectrum at that retention time) matched within 1% of the experimental mass fragment abundances and all fragments from the experimental mass spectrum at ≥15% of the base

peak were also present in the NIST library, the match was classified as a high probability.

2.3. Results and discussion

2.3.1. ICECLES sample preparation

ICECLES is an elegant sample preparation technique where samples are frozen while rapidly stirred with a sorptive stir bar to concentrate the sample components in the remaining aqueous layer and stir bar for follow-on analysis. As more of the liquid sample is frozen, concentration factors and extraction efficiencies can be become greatly enhanced. The advantages of ICECLES (i.e., higher extraction efficiencies, especially for more polar compounds, and ability to analyze more volatile and thermally labile compounds) are well-aligned with the main goal of green tea flavor analysis, comprehensive identification of green tea components.

In this study, ICECLES successfully preconcentrated the green tea components into a small volume. Before performing ICECLES, the components of green tea, including polar and nonpolar components, were distributed throughout the sample solution (Figure 2.1A). Green tea components initially equilibrate with the PDMS-stir bar, which is the same as with SBSE. The affinity of a PDMS-coated stir bar for nonpolar components leads the hydrophobic components (i.e., generally log $K_{ow} \geq 3$) to prefer the PDMS-coated stir bar over the aqueous green tea solution whereas the more polar components prefer the aqueous environment of the sample. During ICECLES, the sample is concentrated in progressively smaller aqueous volumes (Figures 2.1B and 2.1C). When the sample becomes almost completely frozen, the green tea components, including more polar ones, are concentrated into a very small volume at the top of the vail. This is clearly demonstrated in Figure 2.1 by the dark ring at the top of the prepared green tea sample

and the almost clear ice below it in Figure 2.1C. This concentration leads to a change in the equilibrium which encourages green tea components, even more polar ones, to concentrate into the PDMS-coated stir bar.

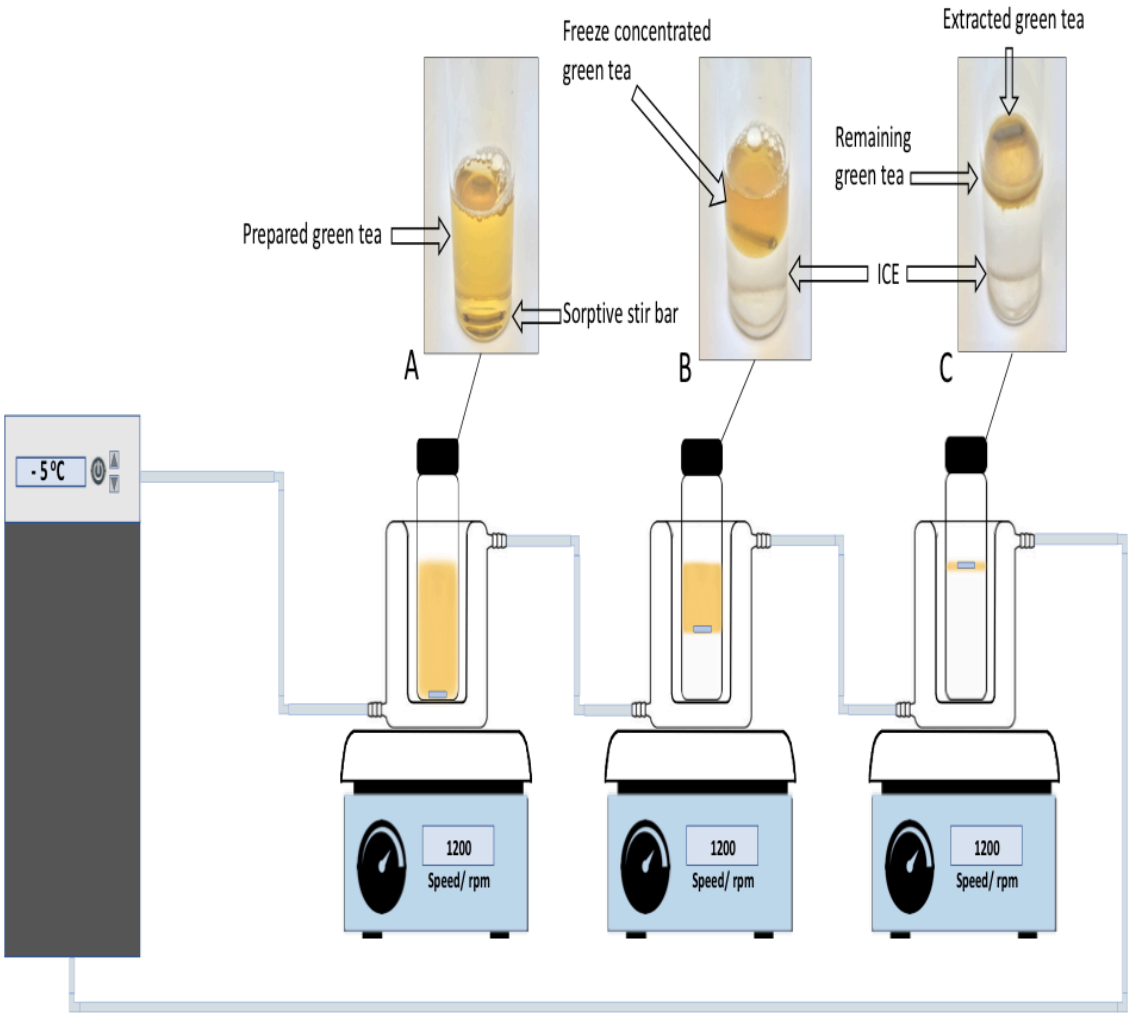

Figure 2.1 Green tea extraction via ICECLES sample preparation. The schematic and photographs show sample preparation before (A), during (B), and after (C) ICECLES. The green tea solution in (A) is clearly concentrated in a small volume of solution as the solution is progressively frozen from the bottom of the vial (B + C). After performing ICECLES, green tea components are concentrated in a sorptive stir bar (C) and analyzed by TD-GC-MS.

2.3.2. Extraction of green tea components

ICECLES was performed at different temperatures (or freeze rates) to determine the temperature that produced the best green tea extraction. The extraction efficiency

increased as the temperature increased from -7°C to -3°C. Although -3 °C produced better extraction, sample preparation took 14 hours (overnight). Therefore, since ICECLES sample preparation at -5 °C gave very similar extraction efficiencies to -3 °C, but was complete within 5.5 hrs, -5°C was used for the remainder of the study. Figure 2.2 shows the average total ion chromatograms comparing ICECLES and SBSE from nine samples each. Log K_{ow}s of the green tea compounds [48, 53, 92-101], retention times, and signal enhancements are also reported in Table S1. It is evident that signals for most components of the ICECLES prepared samples are larger than for SBSE, especially over the first 15-20 minutes of the chromatograms. Moreover, when using automated integration, the average number of components found with ICECLES was 301 peaks, not counting those peaks attributable to components in the blank, while the average number of peaks for SBSE was 245 peaks. A large number of green tea components observed in ICECLES were not detected in the SBSE prepared sample (i.e., 56). All green tea components that were detected only via ICECLES are reported in the supporting information (Table S2).

Except four components (107, 296, 297, and 300; see supporting information Table S1), signal enhancements were above 1 for each green tea component. As observed in Figure 2.2 and Table S1, high signal enhancements in ICECLES are primarily seen for higher polarity compounds, log $K_{ow} < 3$. When components have low polarity, ICECLES and SBSE show similar extraction efficiencies. It is interesting to note that some green tea components present only in the ICECLES chromatogram have log $K_{ow} \geq 3$ (Table S2). This is likely because of their relatively small concentrations, necessitating the high concentration factors afforded by ICECLES in order to be detected.

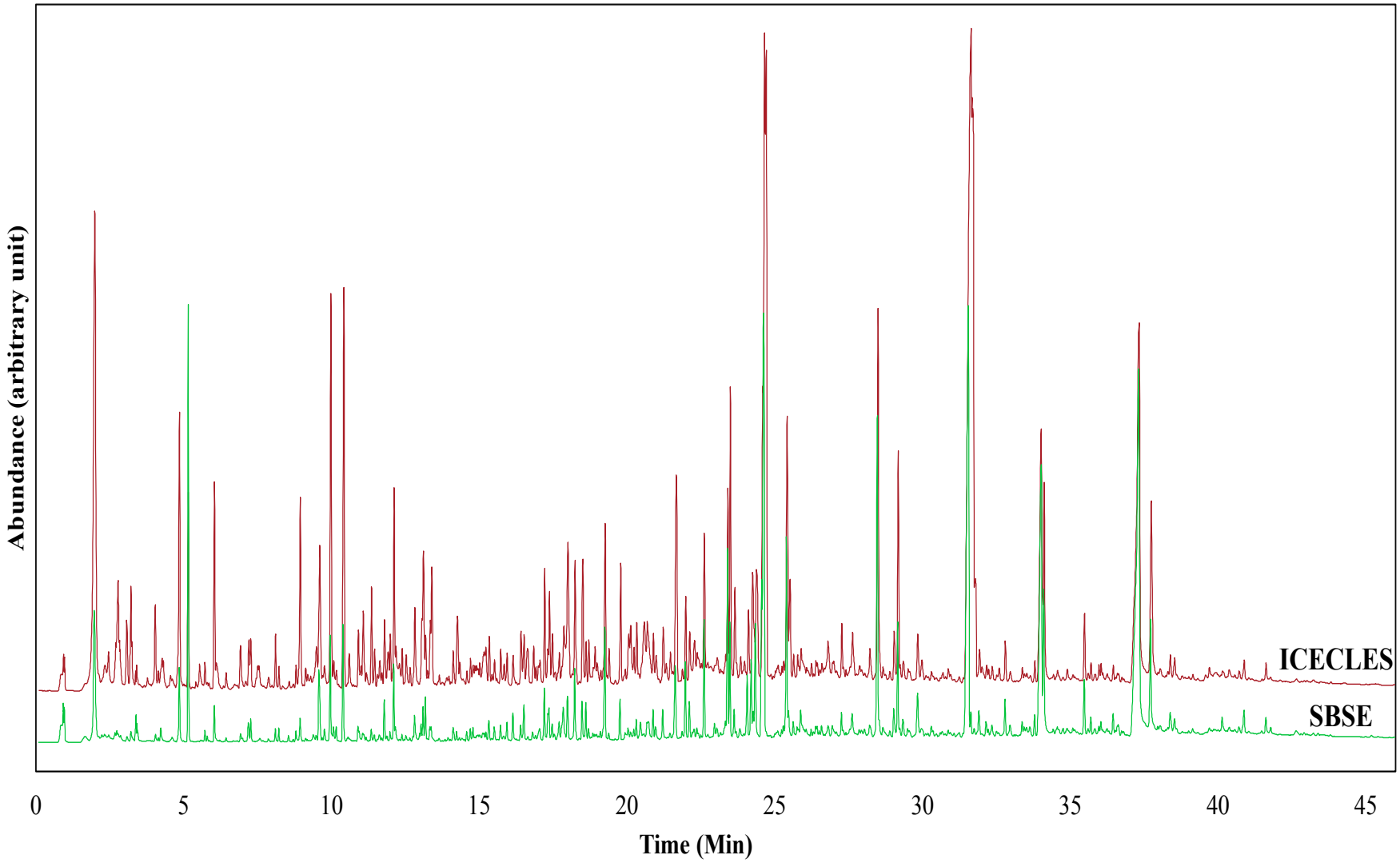

Figure 2.2 Total ion chromatograms of ICECLES and SBSE prepared green tea samples. As clearly shown in the first 15-20 minutes, ICECLES extracts the vast majority green tea components more efficiently than SBSE.

2.3.3. Compound identification

Multi-stir bar ICECLES (^{m}ICECLES) can be used to increase signals by preparing samples via ICECLES simultaneously, thermally extracting each in sequence and trapping the extracted compounds in a cooled injection source liner. The ^{m}ICECLES method improved the identification probability of components up to 5 times. Moreover, ^{m}ICECLES allowed detection of some components which were not detectable via a single green tea sample prepared with ICECLES. Where possible, components of ICECLES prepared green tea were definitively confirmed by standards (Table S1). Figure 2.3 shows an example of definitively identified furfural. Both furfural's retention time and mass spectra from the standard match the furfural detected using ICECLES.

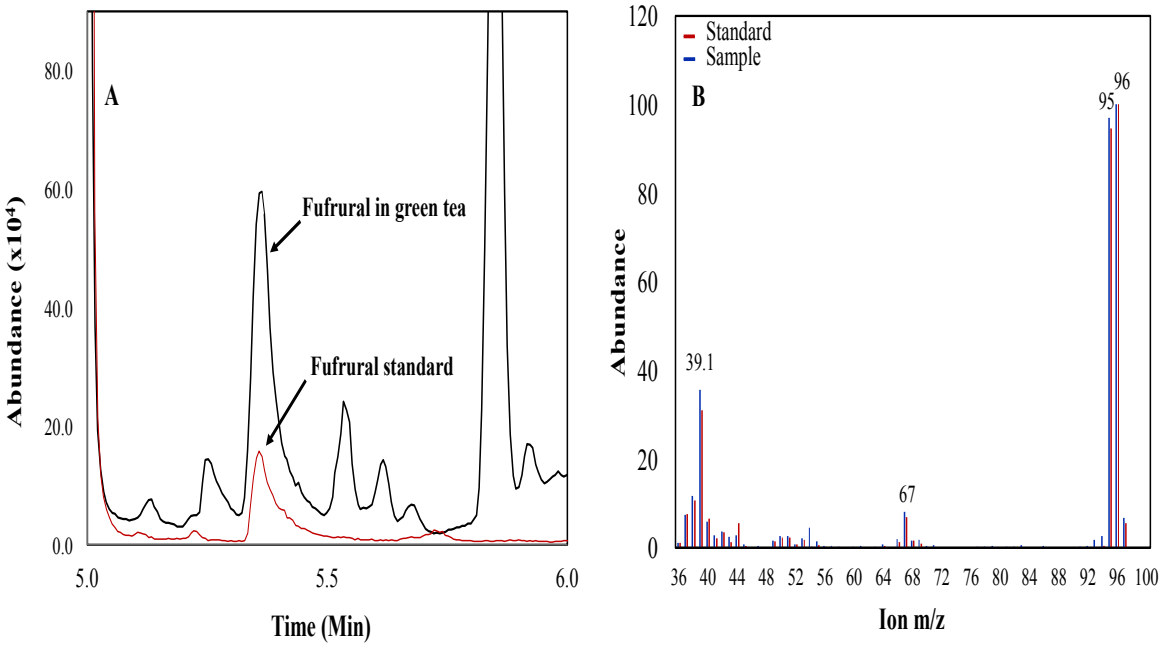

Figure 2.3 Example of definitively identified compound. A) GC-MS chromatogram of furfural extracted via ICECLES and confirmed with its standard, B) mass spectrums of furfural in green tea sample and furfural standard.

Table S1 shows the assigned identification probability with 19 compounds definitively identified, 87 compounds with a high probability of identification, and 9 compounds with a medium probability.

2.3.4. Important flavor compounds detected by ICECLES

ICECLES was able to detect many compounds which were not detectable by SBSE (i.e., 56 compounds). Four examples are shown in Figure 2.4. Aldehydes like furfural, 5-(hydroxymethyl)furfural (5-HMF), and (E,E)-2,4-hexadienal (Figure 2.4A, 2.4B, and 2.4C, respectively) make up a major group of compounds which proved difficult to detect via SBSE, but can be readily seen via ICECLES. This group of compounds is important for flavor and likely gives green tea its distinctive flavor [102]. Furfural and 5-HMF have a caramel flavor and are present in the Maillard reaction as an intermediate product, likely adding to the flavor quality of green tea [103, 104]. Furfuraldehydes have been used for assessing food quality to test the misuse of temperature and poor storage conditions in drinks such as juices and infant milks [104-106]. Another aldehyde, (E,E)-2,4-Hexadienal has a citrus odor and is used as a food additive, a fragrance agent, or as a starting material in pharmaceutical industries [107]. Pyrazine derivatives in green tea such as methyl pyrazine (Figure 2.4D) and 2,5-dimethylpyrazine are heterocyclic compounds with nutty like odor/flavor. As shown in Table S2 and Table 3, one green tea alcohol that was extracted via ICECLES but was absent in from SBSE was phenylethyl alcohol. This compound is widely consumed in food as a flavor component and is also used as ingredient for perfumes to produce a rose smell [108, 109]. Maltol (Table S2) is another flavor compound which found in green tea which does not have a remarkable odor at small concentration but is used as a potent

flavor enhancer in different types of foods [110].Maltol is widely consumed as a food additive due to its contribution to the fragrance of a variety of foods and beverages. It is also used in combination with other components in synthetic perfumes to produce a caramel smell [111].

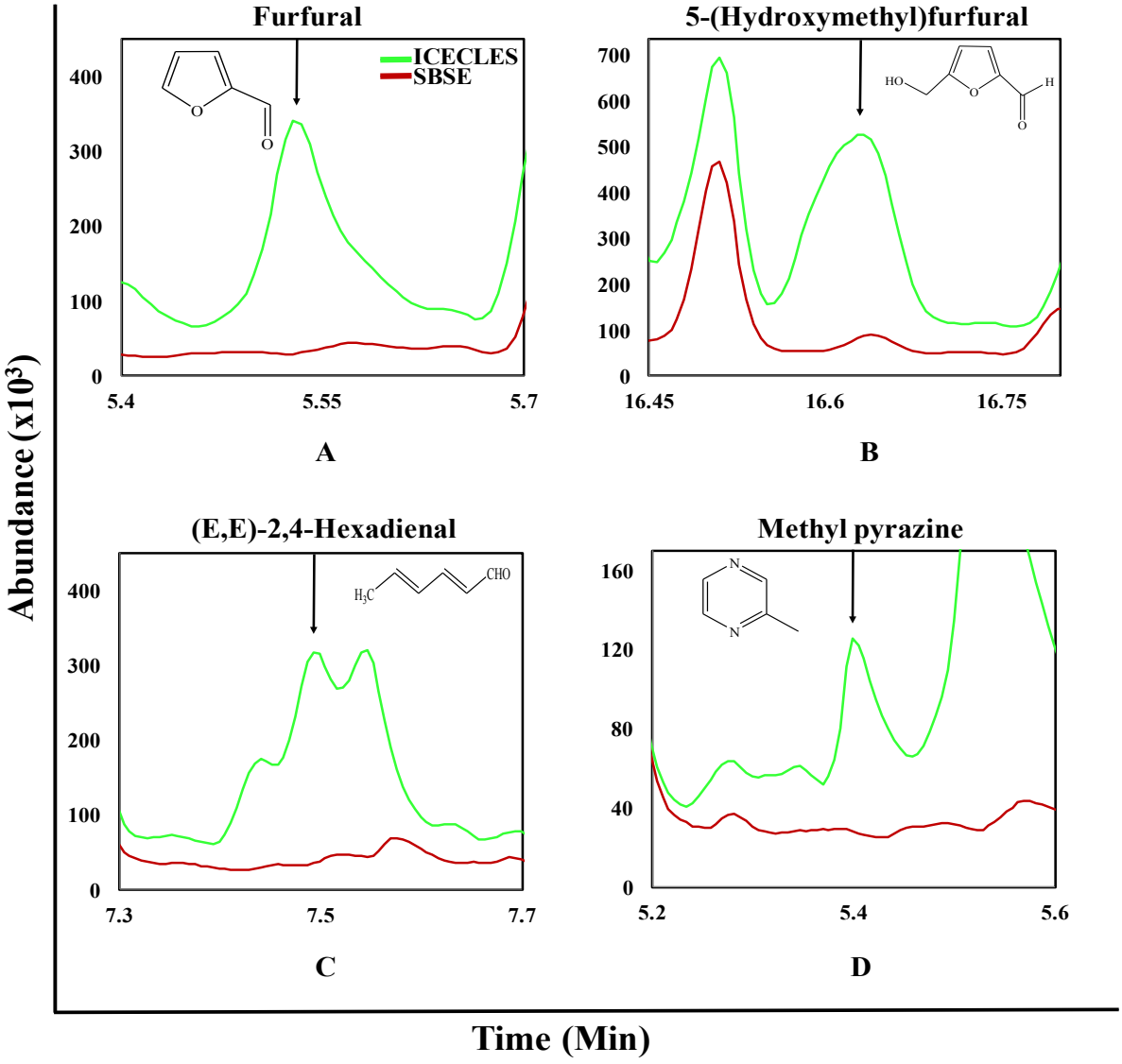

Figure 2.4 Examples of green tea components extracted via ICECLES, but undetectable via SBSE. A) furfural, B) 5-(hydroxymethyl)furfural, C) E,E-2,4-hexadienal, and D) methylpyrazine.

2.3.5. Important medicinal compounds detected by ICECLES

Beside components important for flavor, green tea contains medicinal components, including antimicrobial agents and potent antioxidants. Green tea contains several terpenoid and phenolic compounds which were only extracted via ICECLES. Terpenoids such as α-terpineol (i.e., extracted via ICECLES, Table S2) have been shown to have antibacterial effects against periodontal diseases and cariogenic bacteria [112]. Eugenol is a phenolic compound that acts as an antioxidant and an anti-inflammatory agent. It inhibits lipid-peroxidation and can treat many diseases caused by the presence of hydroxyl radicals, such as atherosclerosis, cancer and neurological disorders [113, 114]. In addition, both α-terpineol and eugenol have been used as natural antifungal agents [115, 116]. Syringol is an antioxidant compound which was also detected by ICECLES alone. Syringol is one of the main components of pyroligneous acid complex (i.e., pyroligneous acid is a complex mixture of syringol, sugar, water, aldehydes, ketones, and carboxylic acids) and has been used as sterilizing agent and antimicrobial agent [117]. Although pyrazine derivatives are used as food additives, some medicinal research proved these compounds to have pharmacological actions. For example, methyl pyrazine has been found to have a beneficial pharmacological effect, especially for tuberculosis [118-120]. Phenylethyl alcohol is also effective inhibiting agent for Gram-negative bacteria [121].

Although most green tea components with pharmacological effects are beneficial, some have shown toxicity. For example, according to the Flavor and Extract Manufacturers Association (FEMA) and National Cancer Institute (NIH), (E,E)-2,4-hexadienal is carcinogenic (LD_{50} 270 μL/kg) [107, 122]. Furthermore, maltol causes

several pains including headache and can produce nausea and vomiting, and impacts the functions of liver and kidney at high concentrations (above 200 mg kg^{-1}) [110, 111].

Table 2.1 Some important green tea components only detected via ICECLES.

Peak No.	Name	Odor	Log K_{ow}	B.P[a] (°C)
Alcohol				
14	1-Pentanol	Fruit	1.33	137.5
15	2-Penten-1-ol, (Z)	Rubber	0.9*	138
84	Phenylethyl Alcohol	Rose	1.57	218
105	3-Cyclohexene-1-methanol, α,α4-trimethyl (α-Terpineol)	Floral	3.28	218-221
Heterocyclic				
21	Pyrazine, methyl (Methyl pyrazine)	Nut	0.49	135
38	Pyrazine, 2,5-dimethyl (2,5-dimethylpyrazine)	Nut	1.03	155
Aldehyde				
22	Furfural	Caramel	0.83	161
37	(E,E)-2,4-Hexadienal	Citrus	1.37**	174
111	2-Furancarboxaldehyde, 5-(hydroxmethyl) (5-(Hydroxymethyl)furfural)	Caramel	-0.09**	114-116
Ketone				
82	Maltol	Caramel	0.02	93
91	2,6,6-Trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	Floral	1*	222
Ester				
95	Acetic acid, phenylmethylester (Benzyl acetate)	Fruit	1.96*	213
66	2-(3H)-Furanone, 5-heptyldihydro (γ-Undecalactone)	Fruit	0.7*	219
Phenol				
136	Phenol,2,6-dimethoxy (Syringol)	Phenol	1.1*	261
138	Eugenol	Clove	2.49	254

[a] Boiling point

*log K_{ow} values were calculated by using the difference between a logP value of known compound and the query compound then estimated by an additive model with well-defined correction factors [100].

**log K_{ow} values were calculated by using an atom/fragment contribution method via KOWWIN™ program [101].

2.4. Conclusion

ICECLES proved to be well-suited for food flavor analysis of green tea and was more efficient for flavor analysis than SBSE for extraction for most green tea components, especially for more polar compounds (log $K_{ow} < 3$). Signal enhancements were above 1 for ICECLES for the vast majority of green tea components. Moreover, ICECLES allowed detection of 56 more constituents than SBSE, some of which were important flavor and/or medicinal compounds.

Acknowledgments

The authors would like to acknowledge Qassim University (QU) and the State of South Dakota for supporting and funding the described project. The authors also would like to thank the U.S. Joint Executive Office for Chem Bio Defense, Joint Program Management Protection Contract W911SR-09-0059, for funding aquisition of the GC-MS instrument used in this work.

3. Chapter 3. Conclusions and Future Work

3.1. Conclusions

In this study, the new sample preparation technique, ICECLES, was used to prepare green tea for analysis of flavor compounds and was compared to SBSE. ICECLES extracted 301 constituents as compared to 245 for SBSE with 56 compounds only detectable via ICECLES. Some of these compounds were very important for flavor or medicinal properties of green tea. For example, 1-pentanol, (E,E)-2,4-hexadienal, furfural, furfural alcohol, maltol, eugenol, 2-methylpyrazine, phenethyl alcohol, 2,6-dimethoxyphenol, and α-terpineol were identified via ICECLES where SBSE did not allow detection. Many of these compounds were identified with the help of the National Institute of Standards and Technology (NIST) mass spectra reference database (2005) and, where possible, standards were used for confirmation. Overall, ICECLES proved to be an excellent extraction technique for analysis of green tea due to its multiple advantages, which include the ability to extract relatively polar compounds, simplicity, and high extraction efficiencies. However, ICECLES still has some drawbacks that need to be overcome, including long extraction times (i.e., 5.5 hrs), long conditioning times for the sorptive stir bars, and the sample volume limit of 10 mL.

3.2. Future Work

Green tea is made up of multiple components, and the quality of green tea depends on the identity and concentration of these components. With the inherent advantages of ICECLES, determination of the LOD for some important flavor, medicinal, and toxic compounds in green tea are needed. Moreover, optimization of ICECLES to reduce the total extraction time is necessary.

4. References

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APPENDIX AND SUPPORTING MATERIAL

Appendix 1.

A.1.1. Table S1. Green tea components with the corresponding identification, log K_{ow}, and signal enhancement, and (common names).

A.1.2. Table S2. Green tea components extracted only by ICECLES with the corresponding retention time, identification, log K_{ow}, and boiling point.

Table 00.1. Green tea components with the corresponding identification, log K_{ow}, and signal enhancement, and (common names).

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
1	Propanoic acid, chloro-2-hydroxy	2.12	MP	0*	326557	0	NA
2	Threo-4-Hydroxy-L-homoarginine lactone	2.34	HP		594690	174609	3.40
3	Propane, 2-ethoxy-2-methyl	2.46	HP	1.92**	319468	0	NA
4	Acetic acid	2.78	DI	-0.17	3062786	603397	5.07
5		2.86	UK		599352	189323	3.16
6		3.07	UK		1318266	120612	10.92
7	Pentanal	3.21	HP	1.31	3522124	185174	19.02
8		3.65	UK		109103	0	NA
9		3.77	UK		222513	0	NA
10		3.87	UK		145901	0	NA
11	2-Pentanal, (E)	4.04	HP		1779267	155760	11.42
12		4.14	UK		280330	76079	3.68
13	Toluene	4.23	DI	2.69	499814	270270	1.84
14	1-Pentanol	4.26	DI	1.33	632067	0	NA
15	2-Penten-1-ol, (Z)	4.31	DI	0.9*	605861	0	NA
16		4.55	UK		342383	0	NA
17		4.61	UK		295275	123634	2.38
18	Hexanal	4.85	HP	1.80	6264675	1557444	4.02
19		5.00	UK		147553	0	NA
20		5.07	UK		139146	0	NA
21	Pyrazine, methyl (Methyl pyrazine)	5.40	DI	0.49	212283	0	NA
22	Furfural	5.53	DI	0.83	652725	0	NA
23	2,4-Dimethyl-1-heptene	5.71	HP	4.4*	583900	221161	2.64
24	1-Hexene-3-yne, 2,5,5-trimethyl	5.79	HP	3.8*	241821	152565	1.58
25	2-Hexenal[e]	5.85	HP	1.5*	173703	48753	3.56
26	2-Hexenal[e]	6.03	HP	1.5*	4678468	736077	6.35
27	2-Furanmethanol (Furfural alcohol)	6.14	DI	0.45	640330	0	NA
28		6.34	UK		104357	0	NA
29		6.43	UK		368507	102319	3.60
30		6.72	UK		162838	0	NA
31		6.81	UK		148768	43603	3.41
32		6.92	UK		768043	0	NA
33		7.08	UK		768043	0	NA
34	4-Heptenal,(Z)	7.20	HP	1.4*	1238977	699318	1.77

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
35	Heptanal	7.27	HP	2.29	1136474	475986	2.38
36	1-Pentanone, 1-(3-Furnayl)-4-Hydroxy	7.44	HP		284242	0	NA
37	2,4-Hexadienal, (E,E)	7.49	DI	1.37**	588011	0	NA
38	Pyrazine, 2,5-dimethyl (2,5-dimethylpyrazine)	7.54	HP	1.03	611607	0	NA
39	2-Cyclopentene-1-one, 2-hydroxy	7.87	MP	0.4*	326455	0	NA
40		7.98	UK		134963	59562	2.26
41	2,5-Dimethylhexane-2,5-dihydroperoxide	8.09	HP	0.9*	1000326	237751	4.20
42	2-Heptanone, 4-methyl	8.21	HP	2.3*	613183	275592	2.22
43	5-(3,7-Dimethylocta-2,6-dienyl)-4-methyl-2,3 dihydrothiophene 1,1-dioxide	8.34	MP		138650	0	NA
44		8.70	UK		153050	65213	2.34
45	2-Heptenal, (Z)	8.79	HP	2.1*	553156	287119	1.92
46	Benzaldehyde	8.94	DI	1.48	4404536	493092	8.9
47		9.12	UK		466290	88367	5.27
48		9.19	UK		389617	72116	5.40
49		9.29	UK		346181	0	NA
50		9.38	UK		285813	168240	1.69
51		9.48	UK		882542	130310	6.77
52	5-Hepten-2-one, 6-methyl	9.60	HP	1.9*	2261549	1452586	1.55
53		9.65	UK		2735891	0	NA
54	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) (1-Terpineol)	9.76	HP		597526	286311	2.08
55	2,4-Heptadienal[e]	9.98	HP	1.6*	9079686	2122570	4.27
56	4-Bromoheptane	10.07	HP	3.6*	709099	298221	2.37
57	Octanal	10.16	HP	2.78	457311	374965	1.21
58	2,4-Heptadienal[e]	10.42	HP	1.6*	9603923	2453768	3.91
59		10.59	UK		992998	0	NA
60	1-Hexanol, 2-Ethyl	10.91	MP	3.1*	1049786	267347	3.92
61		10.99	UK		625168	71145	8.78
62	Benzyl Alcohol	11.07	DI	1.1	2179193	211717	10.29
63		11.18	UK		420638	130489	3.22
64	Benzenacetaldehyde	11.35	HP	1.8*	2304409	290558	7.93

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
65	1H-pyrrole-2 carboxaldehyde, 1-ethyl	11.45	HP	0.8*	900733	164244	5.48
66		11.56	UK		386129	0	NA
67		11.63	UK		684595	161833	4.23
68		11.70	UK		463239	98802	4.68
69	2-Octenal, (E)	11.79	HP	2.6*	1433745	808777	1.77
70	Ethanone,1-(1H-pyrrol-2-yl)	11.91	HP	0.9*	1018258	150579	6.76
71	Acetophenone	11.98	HP	1.58*	1358595	242659	5.59
72	3,5-Octadien-2-one	12.11	HP	1.8*	4953909	1726768	2.86
73		12.18	UK		954123	324157	2.94
74	1-3's-Hydroxy-2'R-butoxy(methyl)thymine, 1'ethylhydrogenphosphate	12.30	MP		729470	80187	9.09
75		12.38	UK		980750	170564	5.75
76	Benzaldehyde, 4-methyl (p-Tolualdehyde)	12.52	HP	2.26**	637268	133099	4.78
77	α-Methyl-α(4-methyl-3pentenyl)oxirane methanol	12.66	HP		487326	106636	4.56
78	3,5-Octadien-2-one(E,E)	12.82	HP	1.8*	1917051	584176	3.28
79	1,6-Octadien-3-ol, 3,7-dimethyl (Linalool)	13.05	HP	3.38	1230696	353165	3.48
80	Ethanone, 1-(2-mehtyl-1-cyclopenten-1-yl)	13.11	HP	1*	3175588	751934	4.22
81	Nonanal	13.18	HP	3.27	1373071	1186906	1.15
82	Maltol	13.25	DI	0.02	520624	0	NA
83	3,4-Dimethylcyclohexanol	13.34	HP	2*	1550458	324494	4.77
84	Penylethyl alcohol	13.39	DI	1.57	2888171	0	NA
85		13.64	UK		354784	94908	3.73
86		13.75	UK		229449	0	NA
87		13.89	UK		366731	67901	5.40
88	2,5-Pyrrolidinedione, 1-ethyl(N-ethylsuccinimide)	13.97	HP	-0.5*	353457	0	NA
89	Benzene, 1-isocyano-2-methyl	14.11	HP		896037	0	NA
90	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	14.26	HP	-0.4*	2074374	0	NA

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
91	2,6,6-Trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	14.34	HP	1*	655810	0	NA
92	2,6-Nonadienal, (E,E)	14.57	HP	2.2*	429920	235236	1.82
93	2-(1,5-Dimethyl-hexyl)-cyclobutanone	14.70	HP		557684	187984	2.96
94	2-Nonenal, (E)	14.78	HP	3.1*	530948	314804	1.68
95	Acetic acid,phenylmethyl ester (Benzyl acetate)	14.85	HP	1.96*	653684	0	NA
96		14.90	UK		565165	160177	3.52
97		15.01	UK		736826	178426	4.12
98		15.16	UK		864139	218361	3.95
99		15.22	UK		1143442	0	NA
100	Cyclohexanol, 5-methyl-2-(1-mehtylethyl)-,(1R-(1α,2ß,5α))	15.34	HP	3*	1075905	418752	2.56
101		15.42	UK		370345	93849	3.94
102		15.51	UK		761125	408761	1.86
103		15.59	UK		292968	0	NA
104	Methylsalicylate	15.72	HP	2.55*	884496	352577	2.50
105	3-Cyclohexene-1-methanol, α,α4-trimethyl (α-Terpineol)	15.84	DI	3.28	584226	0	NA
106	1,3Cyclohexdiene-1-carboxaldehyde,2,6,6-trimethyl (Safranal)	15.93	HP	2.1*	700311	339824	2.06
107		16.14	UK		666289	816535	0.81
108		16.32	UK		110625	0	NA
109	Benzofuran, 2,3-dihydro	16.41	MP	2.1*	1183997	389996	3.03
110	1-Cyclohexene-1-carboxaldehyde,2,6,6-trimethyl (β-Cyclocitral)	16.51	HP	2.4*	943863	625920	1.50
111	2-Furancarboxaldehyde, 5-(hydroxmethyl) (5(Hydroxymethyl)furfural)	16.64	DI	-0.09**	1064983	0	NA
112	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	16.84	HP	0.5*	1110710	0	NA
113		16.92	UK		581964	170181	3.41

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
114	ß-D-Glucopyranose, 1-thio-,1-(N-hydroxybenzenepropanimidate)	17.00	HP		594384	0	NA
115		17.05	UK		683926	302507	2.26
116	2-Cyclohexen-1-one,2-methyl-5-(1-mehtylethnyl)-,(s)	17.21	HP	2.5*	2426393	1043790	2.32
117	Benzene, 1,3-bis(1,1-dimethylethyl)	17.31	HP	5.8*	816544	549630	1.48
118	2,6-Octadien-1-ol, 3,7-dimethyl (Geraniol)	17.37	HP	3.56	2067464	699953	2.95
119	Acetic acid, 2 phenylethyl ester(Phenethyl Acetate)	17.47	HP	2.57	1249730	393329	3.17
120		17.57	UK		416422	182322	2.28
121		17.70	UK		870393	462088	1.88
122		17.79	UK		558326	0	NA
123	2,6-Octadienal,3,7-dimethyl (Citral)	17.85	HP	3.45**	1439562	872031	1.65
124	Nonanoic acid	17.99	HP	3.42	3283367	1016989	3.22
125	Cyclohhexene, 3-methyl-6 (1-methylethenyl)-,(3Rtrans) ((1R)-(+)-trans-Isolimonene)	18.24	HP	4*	3046178	1603812	1.89
126	Indole	18.49	DI	2.05	2653432	812730	3.26
127	2,4-Decadienal, (E,E)	18.61	HP	3.2*	997011	744643	1.33
128	Azetidine, 1-chloro-2-phenyl	18.70	HP	2.3*	1148382	303461	3.78
129		18.91	UK		987225	188491	5.23
130		18.98	UK		629342	0	NA
131		19.11	UK		438505	224649	1.95
132		19.25	UK		3574110	2302716	1.55
133	Furan, 2,3-dihydro-3methyl	19.39	HP	1.1*	1031933	0	NA
134		19.60	UK		230455	0	NA
135		19.78	UK		3211291	1006247	3.19
136	Phenol,2,6-dimethoxy (Syringol)	19.94	DI	1.1*	498756	0	NA

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
137		20.05	UK		1633174	449165	3.63
138	Eugenol	20.12	DI	2.49	1499645	0	NA
139		20.23	UK		1011734	314468	3.21
140	2(3H)-Furanone, dihydro-5-propyl (γ-Heptalactone)	20.32	HP	1.1*	1605933	495029	3.24
141	n-Decanoic acid	20.59	HP	4.09*	1614418	482623	3.34
142		20.68	UK		2036914	587289	3.46
143		20.88	UK		1307575	727557	1.79
144		20.98	UK		788196	357363	2.20
145	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-,(Z) (cis-Jasmone)	21.22	HP	3.55	1287030	698859	1.84
146		21.32	UK		691415	121391	5.69
147		21.46	UK		771748	202260	3.81
148	2,4,7,9-Tetramethyl-5-decyn-4,7-diol (Surfynol 104)	21.67	HP	2.7*	4398238	1640929	2.68
149		21.86	UK		465316	225800	2.06
150	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl) (β-Ionone)	21.98	HP	3.84	1889633	1604913	1.17
151	Megastigmatrienone	22.11	HP	2.6*	1224327	845086	1.44
152	2H-benzopyran-2-one (Coumarin)	22.28	HP	1.51	1086358	302054	3.59
153	Butane, 1,1'-(oxybis(2,1-ethanediyloxy))bis (Butyl diglyme)	22.36	HP	1.9*	819636	350466	2.33
154		22.51	UK		687751	167840	4.09
155		22.61	UK		3225534	2293840	1.40
156		22.72	UK		670998	175181	3.83
157		23.04	UK		732816	317852	2.30
158		23.17	UK		485036	219904	2.20
159		23.33	UK		787210	432129	1.82
160	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl) (α-Ionone)	23.41	HP	3.85	4101447	3866645	1.06
161	6-Methyl-6-(5-methylfuran-2-yl)heptan-2-one	23.49	HP	2.9*	6947821	2744368	2.53
162		23.65	UK		2380989	714319	3.33
163		23.84	UK		757213	296352	2.55

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
164		23.97	UK		658129	207821	3.16
165	Phenol,2,4-bis(1,1-dimehtylethyl)	24.11	HP	4.9*	1474134	1234137	1.19
166		24.25	UK		2585816	1762767	1.46
167		24.37	UK		2510834	2170812	1.15
168		24.41	UK		2154569	0	NA
169	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (Dihydroactinidiolide)[e]	24.64	HP	2.2*	14023403	3621186	3.87
170	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (Dihydroactinidiolide)[e]	24.72	HP	2.2*	14803638	9163687	1.61
171		24.8	UK		234934	167444	1.40
172		24.94	UK		320857	0	NA
173		25.01	UK		446864	189955	2.35
174		25.06	UK		512241	222187	2.30
175		25.11	UK		580779	253955	2.28
176		25.23	UK		562957	289299	1.94
177		25.29	UK		650833	358660	1.81
178		25.41	UK		5418815	3978197	1.36
179		25.51	UK		2513703	702837	3.57
180	2-(3H)-Furanone, 5-heptyldihydro (γ-Undecalactone)	25.63	DI	0.7*	803114	0	NA
181		25.76	UK		786682	342842	2.29
182	3,5,9-Undecatrien-2-one, 6,10-dimethyl(E,E) (Geranyl acetone)	25.88	HP	3.7*	925394	674011	1.37
183		26.07	UK		521235	295649	1.76
184		26.12	UK		635640	381016	1.66
185		26.23	UK		611241	278611	2.19
186		26.31	UK		386355	230212	1.67
187		26.39	UK		627479	329167	1.90
188		26.43	UK		585097	341268	1.71
189		26.51	UK		437439	247873	1.76
190	1H-3a,7-Methanozulen-5-ol, octahydro-3,8,8-trimethyl-6-methylene (cedrenol)	26.59	HP		562766	338959	1.66

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
191		26.66	UK		563662	188765	2.98
192		26.79	UK		1158898	361815	3.20
193	Benzophenone	26.95	HP	3.18*	608991	356357	1.70
194		26.99	UK		654308	232996	2.80
195		27.09	UK		422355	233075	1.81
196	Methyl jasmonate	27.26	HP	2.76**	1492808	638455	2.33
197		27.32	UK		540480	285647	1.89
198		27.41	UK		473576	226675	2.08
199		27.53	UK		535516	180240	2.97
200	3-Oxo-ß-ionone	27.63	MP		1262551	590540	2.13
201		27.75	UK		381265	0	NA
202		27.86	UK		566052	278802	2.03
203		27.92	UK		460932	262313	1.75
204		28.04	UK		388474	281759	1.37
205		28.21	UK		976963	405207	2.41
206	Sulforidazine	28.49	HP	4.6	8536864	6899045	1.23
207		28.66	UK		537255	333563	1.61
208		28.74	UK		367146	225465	1.62
209		28.89	UK		416555	243678	1.70
210	2H-1-Benzopyran-2-one, 7-methoxy (7-Methoxycoumarin)	29.03	HP	1.9*	1287063	713482	1.80
211	3-Methylbut-2-enoic acid, 3,4-nitrophenylester	29.17	HP	2.9*	5792412	2832466	2.04
212		29.23	UK		577000	331736	1.73
213	9H-Fluoren-9-one	29.34	HP	3.58*	671573	515301	1.30
214		29.50	UK		474041	301740	1.57
215		29.55	UK		430227	261241	1.64
216		29.83	UK		1177507	985815	1.19
217		29.97	UK		668632	311776	2.14
218		30.11	UK		333686	194878	1.71
219		30.29	UK		304399	209822	1.45
220		30.36	UK		469796	386224	1.21
221		30.43	UK		275276	237818	1.15
222		30.46	UK		279767	192733	1.45
223		30.59	UK		314139	227929	1.37
224		30.68	UK		386923	234226	1.65
225		30.77	UK		311175	184435	1.68
226		30.86	UK		485593	281384	1.72
227		30.94	UK		366852	225900	1.62

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
228		31.06	UK		248914	202996	1.22
229		31.23	UK		283907	211414	1.34
230		31.36	UK		275947	196336	1.40
231	Caffeine	31.42	HP	-0.07	15402668	9726892	1.58
232		31.77	UK		561390	301367	1.86
233	1,2-Benzenedecarboxy--licacid,diundecylester (Diundecyl phthalate)	31.92	MP	11.49**	859941	644393	1.33
234	1H-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl (Theobromine)	32.01	DI	-0.78*	618206	0	NA
235		32.16	UK		562095	441182	1.27
236		32.23	UK		486416	299418	1.62
237		32.35	UK		551084	385696	1.42
238		32.51	UK		349400	252532	1.38
239	1H-Indole-3-ethanol, acetate (ester) (Ethyl 3-indoleacetate)	32.59	HP	2.1*	503746	0	NA
240	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	32.79	HP	3.8*	1078584	870929	1.23
241		32.96	UK		387211	372783	1.03
242		33.10	UK		220005	194878	1.12
243		33.18	UK		225178	209822	1.07
244		33.37	UK		461233	401050	1.15
245		33.44	UK		357643	311380	1.14
246		33.63	UK		331412	331190	1.00
247	Dibutyl phthalate	33.79	HP	4.13	623851	574032	1.08
248	n-Hexadecanoic acid (Palmitic Acid)	34.00	HP	7.17*	6722363	6144871	1.09
249		34.10	UK		4255600	3170665	1.34
250		34.42	UK		347929	299043	1.16
251		34.56	UK		454659	351704	1.29
252		34.74	UK		270278	154877	1.74
253		34.88	UK		500483	301446	1.66
254		35.08	UK		334714	319615	1.04
255		35.47	UK		1640208	1225099	1.33
256		35.59	UK		374100	292848	1.27
257		35.69	UK		557701	513346	1.08
258		35.83	UK		369740	318757	1.15
259		36.03	UK		596365	364727	1.63

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
260		36.09	UK		602677	468507	1.28
261		36.24	UK		400959	266320	1.50
262		36.45	UK		372745	306826	1.21
263		36.52	UK		277795	240563	1.15
264		36.59	UK		360766	344068	1.04
265		36.62	UK		379226	338198	1.12
266		36.73	UK		283641	249038	1.13
267		36.80	UK		272176	253058	1.07
268	9,12,15-Octadeca--trienoic acid, (Z,Z,Z)	37.32	HP	5.9*	9130163	8421613	1.08
269	Octadecanoic acid (Stearic Acid)	37.73	HP	8.28*	4265806	3373659	1.26
270		37.96	UK		401774	345431	1.16
271		38.03	UK		538349	359242	1.49
272		38.29	UK		428666	274734	1.56
273		38.38	UK		713800	574090	1.24
274		38.52	UK		728523	503216	1.44
275		38.60	UK		351083	299553	1.17
276		38.78	UK		339341	232951	1.45
277		38.86	UK		316611	250586	1.26
278		39.00	UK		349040	300023	1.16
279		39.14	UK		273998	213703	1.28
280		39.30	UK		376583	0	NA
281		39.56	UK		616871	292144	2.11
282		39.69	UK		458364	343789	1.33
283		39.91	UK		430291	388052	1.10
284		40.15	UK		551601	354386	1.55
285		40.37	UK		338553	280673	1.20
286		40.56	UK		442743	334789	1.32
287		40.70	UK		703042	691431	1.01
288		40.87	UK		298289	240628	1.23
289		41.03	UK		271357	0	NA
290		41.15	UK		256548	230403	1.11
291	2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E) (Pinostrobin Chalcone)	41.47	MP	3.5*	379750	299922	1.26
292		41.61	UK		267438	234693	1.13
293		41.76	UK		213890	188173	1.13
294		42.10	UK		202433	124852	1.62
295		42.23	UK		230719	193581	1.19

S1. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	Signal[c]		SE[d]
					ICECLES	SBSE	
296		42.63	UK		173823	241133	0.41
297		42.80	UK		105873	206117	0.51
298		42.91	UK		113212	93372	1.21
299		43.00	UK		115295	105200	1.09
300		43.22	UK		107580	116008	0.92
301		43.79	UK		187102	169299	1.10

[a] RT: retention time.

[b] ID: identification.

1) DI: definitively identified, 2) HP: high probability, 3) MP: mid probability, and 4) UK: unknown.

[c] Signal peak height of ICECLES and SBSE.

[d] SE: signal enhancement.

[e] Isomers.

[*] log K_{ow} values were calculated by using the difference between a logP value of known compound and the query compound then estimated by an additive model with well-defined correction factors [100].

[**] log K_{ow} values were calculated by using an atom/fragment contribution method via KOWWIN™ program [101].

Table S0.2. Green tea components extracted only by ICECLES with the corresponding retention time, identification, log K_{ow}, and boiling point.

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	B.P[c]
1	Propanoic acid, chloro-2-hydroxy	2.12	MP	0*	
3	Propane, 2-ethoxy-2-methyl	2.46	HP	1.92**	73 °C
8		3.65	UK		
9		3.77	UK		
10		3.87	UK		
14	1-Pentanol	4.26	DI	1.33	137.5 °C
15	2-Penten-1-ol, (Z)	4.31	DI	0.9*	138 °C
16		4.55	UK		
19		5.00	UK		
20		5.07	UK		
21	Pyrazine, methyl (Methyl pyrazine)	5.40	DI	0.49	135 °C
22	Furfural	5.53	DI	0.83	162 °C
27	2-Furanmethanol (Furfural alcohol)	6.14	DI	0.45	170 °C
28		6.34	UK		
30		6.72	UK		
32		6.92	UK		
33		7.08	UK		
36	1-Pentanone, 1-(3-Furnayl)-4-Hydroxy	7.44	HP		
37	2,4-Hexadienal, (E,E)	7.49	DI	1.37**	174 °C
38	Pyrazine, 2,5-dimethyl (2,5-dimethylpyrazine)	7.54	HP	1.03	155 °C
39	2-Cyclopenten-1-one, 2-hydroxy	7.87	MP	0.4*	244.80 °C
43	5-(3,7-Dimethylocta-2,6-dienyl)-4-methyl-2,3 dihydrothiophene 1,1-dioxide	8.34	MP		
49		9.29	UK		
53		9.65	UK		
59		10.59	UK		
66		11.56	UK		
82	Maltol	13.25	DI	0.02	93 °C
84	Penylethyl alcohol	13.39	DI	1.57	219-221 °C
86		13.75	UK		
88	2,5-Pyrrolidinedione, 1-ethyl (N-ethylsuccinimide)	13.97	HP	-0.5*	
89	Benzene, 1-isocyano-2-methyl	14.11	HP		

S2. Continued

Peak No.	Name	RT[a]	ID[b]	Log K_{ow}	B.P[c]
90	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	14.26	HP	-0.4*	
91	2,6,6-Trimethyl-2-cyclohexene-1,4-dione (Ketoisophorone)	14.34	HP	1*	222 ˚C
95	Acetic acid, phenylmethylester (Benzyl acetate)	14.85	HP	1.96*	206 ˚C
99		15.22	UK		
103		15.59	UK		
105	3-Cyclohexene-1-methanol, α,α4 trimethyl (α-Terpineol)	15.84	DI	3.28	220 ˚C
108		16.32	UK		
111	2-Furancarboxaldehyde, 5-(hydroxmethyl) (5-(Hydroxymethyl)furfural)	16.64	DI	-0.09**	114-116 ˚C
112	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	16.84	HP	0.5*	253.81 ˚C
113		16.92	UK		
114	ß-D-Glucopyranose, 1-thio-,1-(N-hydroxybenzenepropanimidate)	17.00	HP		
122		17.79	UK		
130		18.98	UK		
133	Furan, 2,3-dihydro-3-methyl	19.39	HP	1.1*	
134		19.60	UK		
136	Phenol,2,6-dimethoxy (Syringol)	19.94	DI	1.1*	261 ˚C
138	Eugenol	20.12	DI	2.49	225 ˚C
168		24.41	UK		
172		24.94	UK		
180	2-(3H)-Furanone, 5-heptyldihydro (γ-Undecalactone)	25.63	DI	0.7*	220 ˚C
201		27.75	UK		
234	1H-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl (Theobromine)	32.01	DI	-0.78*	290-295 ˚C
239	1H-Indole-3-ethanol, acetate (ester) (Ethyl 3-indoleacetate)	32.59	HP	2.1*	164-166 ˚C
280		39.30	UK		
289		41.03	UK		

[a] RT: retention time.

[b] ID: identification.

1) DI: definitively identified, 2) HP: high probability, 3) MP: mid probability, and 4) UK: unknown.

[c] Boiling point

* log K_{ow} values were calculated by using the difference between a logP value of known compound and the query compound then estimated by an additive model with well-defined correction factors [100].

** log K_{ow} values were calculated by using an atom/fragment contribution method via KOWWIN™ program [101].