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Quantitative analysis of volatiles from solid matrices of vegetable origin by high concentration capacity headspace techniques: determination of furan in roasted coffee.

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Abstract

The study compares standard addition (SA), stable isotope dilution assay (SIDA) and multiple headspace extraction (MHE) as methods to quantify furan and 2-methyl-furan in roasted coffee with HS-SPME-GC-MS, using CAR-PDMS as fibre coating, d_4-furan as internal standard and in-fibre internal standardization with *n*-undecane to check the fibre reliability. The results on about 150 samples calculated with the three quantitation approaches were all very satisfactory, with coefficient of variation (CV) *versus* the U.S. Food and Drug Administration (FDA) method, taken as reference, almost always below the arbitrarily-fixed limit of 15%. Furan was detected in the 1-5 ppm range, 2-methyl-furan in the 4-20 ppm range. Moreover, experimental exponential slopes (*Q*) and linearity (r) of both furan and 2-methyl-furan MHE regression equation on 50 samples were very similar thus making possible to use the same average *Q* value for all samples of the investigated set and their quantitation with a single determination. This makes this approach very rapid and competitive in-time with SA and SIDA.

A non-separative method (HS-SPME-MS) was also developed in view of possible application on-line monitoring of furan and 2-methyl-furan in a pilot-plant with the aim of optimizing the roasting process to reduce these compounds to a minimum. Sampling times of twenty and five minutes were tested, the latter enabling total analysis time to be reduced to about nine minutes. The results on 105 samples with both SIDA and MHE approaches were again highly satisfactory most of the samples giving a CV% *versus* the conventional methods below 20%. In this case too average *Q* values for both furan and 2-methyl-furan were used for MHE.

The separative method presented very good repeatability (RSD% always below 10%) and intermediate precision over three months (RSD% always below 15%); performance were similar for the non-separative method, with repeatability (RSD%) always below 12% and intermediate precision over three months (RSD%) always below 15%. The sensitivity of both separative and non-separative methods was also very good, LOD and LOQ being in the ppb range for both furan and 2-methyl-furan, i.e. well below the amounts present in the roasted coffee samples.

1 Introduction

The ever-increasing demand for control analysis has contributed markedly to the renewal of interest in headspace (HS) sampling which has taken place over the last 10-15 years [1]. HS sampling is a solventless sample preparation technique that aims to sample the gaseous or vapour phase in equilibrium (or not) with a solid or liquid matrix in order to characterize its composition [2]. High Concentration Capacity Headspace Techniques (HCC-HS e.g. HS-SPME, HSSE, STE, SE-HSSE, etc.) are a recent approach to HS sampling, combining the main advantages of the conventional static or dynamic approaches [1,3]. HCC-HS techniques are based on the accumulation of the analytes in the vapour phase on a polymeric material, mainly by sorption and/or adsorption. They were introduced in 1993 by Zhang and Pawliszyn [4] who applied solid phase microextraction (SPME) to static headspace (S-HS) sampling (HS-SPME). These techniques offer

high sensitivity and reliability and are easy to automate, thus meeting the need for high throughput typical of the routine laboratory.

Quantitative analysis is one of the most complex task with HS sampling in particular when volatiles emitted from solid matrices have to be analyzed. Three main issues must be considered in HS quantitation of volatiles from solid matrices:

- the physical form of the matrix to be analysed, that can be sampled as such or suspended in a liquid
- the standardization and/or normalization of the accumulating polymer(s)
- the quantitation approach, which can mainly be by three methods: standard addition (SA), Stable Isotope Dilution Assay (SIDA) or Multiple Headspace Extraction (MHE).

These issues are briefly discussed at the beginning of the results and discussion section.

Furan (C_4H_4O) is an oxygenated heterocycle that, together with a series of homologues, occurs in the volatile fraction of a wide variety of foods and drinks; it is formed during thermal treatment of most food crops and drinks, as one of the Maillard reaction products [5]. Its generation is mainly due to thermal degradation of carbohydrates, oxidation of polyunsaturated fatty acids and decomposition of ascorbic acid or its derivatives [6-12]. Recently, the presence of furan in foods has been the object of a considerable attention by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) [13,14] due to its carcinogenic and cytotoxic activity in animals and to its harmful effects on human health [15,16]. The International Agency for Research on Cancer (IARC 1995) has classified furan as a possible human carcinogen (Group 2B) [17]. Although official limits have not yet been fixed, its monitoring and reduction in food is strongly recommended.

Furan and its homologues (in particular 2-methyl-furan) are formed in all foods submitted to roasting, and coffee has been found to be one of the foods containing the highest levels of these compounds, ranging from ppb to a few tenths of one ppm. One of the ways to minimize the amount of furan in coffee is to optimize the roasting process in all its steps (i.e. roasting, cooling, degassing and grinding) while, of course, leaving its organoleptic properties unaltered. Quick and automatic quantitative methods for an effective monitoring of the process are therefore necessary. In 2004, FDA introduced a static headspace-gas chromatography-mass spectrometry method (S-HS-GC-MS) to quantify furan with the standard addition approach [18,19]. This method is time-consuming because of the number of measures required, has relatively low sensitivity and requires a sampling temperature of at least 60°C, i.e. well above 40°C, the temperature at which furan starts to form spontaneously [6]. In spite of these limits, very recently Becalski et al. [20] reported the results of a survey on 176 samples in the food field, 17 of them baby food, obtained with an optimised version of the method. Starting from 2005, several groups have applied HS-SPME to sample furan in different matrices to overcome the above limits [among others 21-26]. They all used HS-SPME with a Carboxen/PDMS fibre combined on-line with GC-MS using d_4-furan as internal standard and an external calibration curve as quantitation approach and achieved higher sensitivities (ppb or fractions there-of) than S-HS, as well as lowering the sampling temperature, thus avoiding spontaneous furan formation. Furan was quantified in several food products originating from all parts of the world, in particular in coffee and related brews [21,22,24-26], in baby food [21,23,24-26], in juice, honey, sauces, pulses and in soup and broth [24-26].

The above methods are all highly reliable for routine laboratory checks but, from an objective standpoint, they are rather complex to apply directly to a pilot plant for on-line monitoring of furan formation during, for instance, a coffee roasting process. A first crucial aspect for an on-line pilot-plant analyte monitoring is the simplicity of the method and the time required for analysis. One of the possibilities is to use a non-separative method by combining directly HS-SPME and mass spectrometry (HS-SPME-MS). These methods were introduced by Marsili to study off-flavours in milk [27] and have been since then successfully applied to characterizing matrices, in particular in the food field [28-30]. These techniques in general give a reliable and diagnostic MS fingerprint of the matrix investigated, which, in combination with a suitable chemiometric elaboration, can

successfully characterize each sample within a set, and may be used for reliable and fast quality control and to detect product adulteration, and/or sample contamination or inconsistency [28], in particular when the number of samples to be analyzed routinely is large. A further advantage of mass analyzers as detectors is that they can also be used to monitor specific compounds in a set of samples, quantifying them through diagnostic target ion(s) either specific for the analytes investigated within the mass spectra profile of the sample analyzed, or after correction of their abundance by a factor representative of the contribution to the total intensity of the target ion(s) of other interfering analytes.

A second important aspect is that the quantitation approach must be simple and reliable. The most widely-used approaches are SA and SIDA while multiple headspace extraction (MHE) is much less frequently applied. MHE is a quantitation approach enabling the matrix effect to be eliminated; it was introduced by Suzuky et al. [31] and McAuliffe et al. [32], further developed by Kolb et al. [2], and has recently been applied to HS-SPME. To the best of the authors' knowledge, MHE was first applied to HS-SPME by Ezquerro et al. [33] in the quantitative determination of volatiles in multilayer packaging. MHS-SPME was subsequently applied to determine volatiles in antioxidant rosemary extract [34] and in dry fermented sausages [35], haloanisoles and volatile phenols in wines [36], and aroma components in tomato samples [37].

The present study compares the headspace quantitation approaches currently available for determining furan and 2-methyl-furan in roasted coffee, with both HS-SPME-GC-MS and HS-SPME-MS, with the aim of evaluating their performance and optimizing it in view of their possible application to on-line monitoring during the roasting process. A further aim was to speed-up their determination while maintaining reliability comparable to that of existing methods.

2. Experimental

2.1. Chemicals, reagents and matrices

Furan (≥ 99%), 2-methyl-furan (99%), d_4-furan (98%), methanol (≥ 99.9%) were from Sigma Aldrich (Milan – Italy). HPLC grade water purified at 60°C under vacuum (1×10^{-3} bar) for 2 hours under stirring to eliminate volatile impurities was used. Roasted coffee samples were partly supplied by Lavazza (Turin – Italy) and partly purchased in supermarkets. A total of about 150 samples of 100% natural Arabica, 100% washed Arabica, 100% Robusta, a blend containing 50% Arabica and 50% Robusta and several commercial blends of unknown composition were analysed. SPME device and CAR/PDMS fused silica fibres from different lots were supplied by Supelco (Bellafonte, PA, USA). Before use, all fibres were conditioned as recommended by the manufacturer and tested to evaluate the consistency of their performance *versus* a reference roasted coffee sample selected in the authors' laboratory to evaluate.

2.2. Sample preparation

Static Headspace – 2 mL of HPLC grade water were added to 500 mg of ground roasted coffee in a 20 mL screw-cap glass vial and hermetically sealed with a PTFE-silicone septa and equilibrated for 20 minutes at 60°C. 1 mL of the resulting vapour phase was sampled with a gas-tight syringe and automatically injected into the GC-MS system.

HS-SPME – A suitable amount of ground roasted coffee (50 mg for SA and SIDA and 5 mg for MHE) in a 20 mL screw-cap glass vial were suspended in 2 mL of HPLC grade water and hermetically sealed with a silicone-PTFE septum. The resulting headspace was sampled by SPME with a CAR/PDMS fused silica fibre for 20 minutes at room temperature (30°C) for both separative and non-separative methods. A sampling time of 5 minutes was also tested for the non-separative method.

2.3. Analysis conditions

Analyses were carried out with a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on an Agilent 6890 GC unit coupled to an Agilent 5973N MSD (Agilent, Little Falls, DE, USA).

Separative GC-MS method - chromatographic conditions: injector temperature: 230°C, injection mode: split, ratio: 1/10; carrier gas: helium, flow rate: 1 mL/min; fibre desorption time and reconditioning: 5 min; column: MEGAWAX 20M (d_f 0.20 µm, d_c 0.20 mm, length 50 m) (Mega, Legnano (Milan), Italy). Temperature program: from 40°C (6 min) to 230°C (5 min) at 20°C.

Non-separative MS method: injector temperature: 250°C, injection mode: split, ratio: 1/10; carrier gas: helium, flow rate: 0.4 mL/min; fibre desorption time and reconditioning: 3 min; transfer column: deactivated fused silica tubing (d_c 0.10 mm, length 6.70 m) (Mega, Legnano (Milan), Italy); GC oven temperature: 250°C.

MSD conditions - analysis conditions: MS operated in EI mode (70 eV), scan range: 35 to 350 amu; SIM target ions and qualifiers: furan m/z 68, 39, 69; 2-methyl-furan m/z 82, 81, 53; d_4-furan m/z 72, 42 dwell time 40; ion source temperature: 230°C; quadrupole temperature: 150°C; transfer line temperature: 280°C.

2.4. Quantitation

Individual *stock* solutions of furan, 2-methyl-furan and d_4-furan were prepared in a 20 mL vial by adding 40 µL of pure standard to an appropriate volume of methanol (20 mL) to obtain an analyte concentration of about 2 mg/mL. An *intermediate* solution (about 11 µg/mL) and a *working* solution (about 1 µg/mL) of each analyte were then prepared by adding 120 µL of *stock* solution to 20 mL of HPLC grade water and 2 mL of *intermediate* solution to 18 mL of HPLC grade water respectively. A *spiking* solution of d_4-furan (about 23 µg/mL) was prepared by diluting 240 µL of *stock* to 20 mL of HPLC grade water. The resulting standard solutions were stored at 0°C and renewed weekly.

2.4.1 SA method – Four aliquots of each coffee sample were spiked at different concentrations (X_0, X_0 + 2.0 ppm, X_0 + 4.0 ppm and X_0 + 8.0 ppm) with appropriate volumes of *working* solutions and diluted to 2 mL with HPLC grade water. Concentrations refer to the weight of sampled ground coffee (50 mg for HS-SPME and 500 mg for S-HS). In addition, 7 µL for HS-SPME and 85 µL for S-HS of d_4-furan *spiking* solution were added to each calibration level.

2.4.2 SIDA method – An MS response factor was determined by analyzing by HS-SPME-GC-MS different calibration solutions prepared by diluting in 2 mL of water known amounts of d_4-furan, furan and 2-methyl-furan in different mass ratios, within the concentration range 50-150 ng/mL [***38***]. RF values were determined for each calibration level with the following equation (Eq. 1):

$$RF = \frac{C_{analyte}}{C_{labeled}} \Big/ \frac{A_{analyte}}{A_{labeled}} \qquad \text{Eq. 1}$$

where: $C_{analyte}$ is the concentration of furan (or 2-methyl-furan) and $C_{labeled}$ that of d_4-furan.

The average RFs obtained were 0.896 for furan and 0.538 for 2-methyl-furan. The concentration (ppb) of furan and 2-methyl-furan in coffee was calculated through the following equation:

$$C_{analyte} = \left(\frac{m_{labeled}}{m_{coffee}} x \frac{A_{analyte}}{A_{labeled}} \right) xRF \qquad \text{Eq. 2}$$

where: $m_{labeled}$ is the amount of d_4-furan added to the sample analyzed; m_{coffee} is the amount of coffee analyzed; $A_{analyte}$ is the area of furan (or 2-methyl-furan); $A_{labeled}$ is the area of d_4-furan; RF is the response factor.

2.4.3 Multiple HeadSpace Solid Phase Microextraction (MHS-SPME) – The total area of furan and 2-methyl-furan was estimated with three consecutive extractions of each coffee sample. A calibration curve was built up by analyzing a set of mixtures of furan and 2-methyl-furan in water under the same conditions (i.e. three consecutive extractions); the mixtures were prepared by diluting different volumes of each *intermediate* solution to 2 mL with HPLC grade water corresponding to an absolute amount of 4-800 ng for each compound or 0.8-160 ppm in coffee.

2.4. Repeatability and intermediate precision

50 mg of three coffee samples (Sample A1: Arabica, Sample R1: Robusta and Sample B1: commercial blend) were analyzed six times consecutively to evaluate the method repeatability by both HS-SPME-GC-MS and HS-SPME-MS. Intermediate precision was determined under the same conditions but the analysis were repeated every four weeks over a period of three months.

2.5. LOD and LOQ determination

The LOD and LOQ values of each analyte for all methods developed were determined by analyzing furan and 2-methyl furan in coffee, with very small amounts of the compounds, in decreasing concentrations in water (from 200 to 5 mg), thus enabling us to extrapolate a signal-to-noise ratio above three (LOD) and above ten (LOQ).

3. Results and discussion

This section is divided into three parts: 1) general discussion on the approaches adopted in this study, 2) analysis of furan and 2-methyl-furan with different quantitation approaches in commercially-available coffee samples and submitted to different technological processing by HS-SPME-GC-MS, 3) non-separative analysis of furan and 2-methyl-furan in coffee by HS-SPME-MS.

3.1 - *General considerations on the approaches investigated in the present study*

This subsection deals with some of the main aspects involved with applied methods.

3.1.1 - Physical state of the coffee samples

The headspace quantitative composition of solid matrices can be investigated with the sample either suspended in a non-volatile liquid or as such. In general, sample suspension in a liquid (in particular in water) is preferred because it affords i) reliable addition of the internal standard to the resulting suspension and ii) increased sensitivity, in particular with analyte(s) whose solubility in the solvent is low (e.g. furan in water). Solvent suspension is very useful to quantify specific analytes or groups of homologues (e.g. furan and 2-methyl-furan), although it can alter the ratios between the components in the resulting chromatogram, as a function of their solubility in the solvent, and may produce artefacts, in particular in the case of water. In such cases, the analysis must be run on the solid matrix as such. The main disadvantage with quantitative analyses directly on solid samples is the unreliability of the internal standard response mainly related to its non-consistent physical, physical-chemical and chemical interactions at the surface of the matrix.

3.1.2 - Standardization and/or normalization of the accumulating polymer(s)

The consistency of performance over time of the accumulating polymer in HCC-HS techniques is fundamental for routine quantitative analysis. Control over consistency of performance was achieved by Pawliszyn's group for SPME with the introduction of the equilibrium in-fibre internal standardization [39,40]. This approach is based on pre-loading the internal standard onto the fibre, either in vapour or in liquid phase, with a simple procedure that can easily be automated. Its use has successfully been extended to all other HCC-HS techniques (e.g. SBSE, HSSE, HS-STE, DC-STE and SE-HSSE) used in the authors' laboratory (data not reported). Pawliszyn's group developed this approach to quantify analytes of different volatility from solid and liquid matrices. In the present study, it is mainly used to monitor the reliability of fibre performance.

3.1.3 Quantitation approaches: Standard Addition (SA), Stable Isotope Dilution Assay (SIDA) and Multiple Headspace Extraction (MHE)

In this paragraph the three most widely-used approaches are briefly discussed in view of their application to the automatic determination of furan and 2-methyl-furan in coffee.

i) Standard addition (SA): this was the first approach introduced for quantitation of headspace components, but it is probably the most time-consuming because a) it requires a suitable number of measures to build a reliable calibration curve (at least seven [22]), b) it requires a calibration curve for each sample, at least until the linear response of the analyte over the concentration range of interest for the investigated matrix is confirmed, subsequently enabling a single addition to be made for routine analysis, c) it can give high uncertainty with analytes in trace amounts and/or eluting very close to others, d) the analyte standard must be available (and this is not always the case), and e) HS analysis of analytes from solid matrices are complex and can only be run with the gas phase standard addition.

ii) Stable Isotope Dilution Assay (SIDA): this method was introduced by Schieberle and Grosch **[**41] and first applied to SPME of liquid sampling by Hawthorne et al. [42] and to headspace by Steinhaus et al. [38]**.** Its characteristics are similar to those of SA but a) it requires MS as detector to discriminate between labelled standard and target analyte; b) it requires a labelled standard (in general ^{2}H or ^{13}C), which is not always available and/or may be very expensive, c) a single external calibration curve is sufficient, the labelled standard acting as target analyte when used with samples suspended in liquid or a response factor (*RF* see above) must be calculated, d) it can be used for other homologues (e.g. 2-methyl-furan) provided that a response factor (*RF*) is determined and applied. On the other hand, it is highly specific because quantitation is generally based on ions diagnostic of the analyte(s) investigated.

iii) *Multiple Headspace Extraction (MHE)*: this has been applied to HS-SPME quite recently [33-37]; it was mainly developed for analyte quantitation from the headspace of solid matrices with the aim of overcoming all the problems connected with the matrix effect, although it is relatively little used because it is (erroneously) considered to be complex and time-consuming. MHS-SPME theory is the same as that of static-MHE [2]: it too is based on a dynamic gas extraction carried out stepwise; the amount of analyte extracted by the fibre is proportional to the initial amount, and its peak area decays exponentially with the number of extractions. Quantitation is based on calculating the total area of the analyte(s) under investigation through the following equation:

$$A_T = \sum_{i=1}^{=\infty} A_i = A_1 / (1-e^{-q}) = A_1 / (1-Q) \qquad \text{(Eq. 3)}$$

where A_1 is the analyte area after the first analysis; A_T is the total area of the investigated analyte, -q is a constant that can be calculated from the linear regression analysis equation:

$$\ln A_i = -q\,(i-1) + \ln A_1 \qquad \text{(Eq. 4)}$$

A_i is the peak area obtained in the *ith* extraction and $Q = e^{-q}$. The analyte can then be quantified with an external standard procedure. The advantage of this approach is that the regression equation of several analytes can simultaneously be determined, while the main limits are that an amount of sample suitable to give linear analyte decay(s), and as a consequence significant *Q* value(s), must be analysed and that, ideally, a *Q* value for each sample should be measured. The next paragraph shows that the *Q* value tends to be constant within a relatively homogeneous set of samples, thus making it possible to process a sample in the set with a single analysis.

3.2) Analysis of furan and 2-methyl-furan in commercially available coffee samples and submitted to different technological processing by HS-SPME-GC-MS with different quantitation approaches

The results given here were obtained from the analysis by HS-SPME-GC-MS of furan and 2-methyl-furan in about 150 samples of different varieties (Arabica and Robusta) or origins (Costa Rica, Nicaragua, Colombia, Brazil and Kenya), and commercial blends of coffee, submitted to different technological processing (roasting, cooling, grinding and degassing), taking the FDA method as a reference. As for the methods reported in the literature, in this case too, the analyses were carried out by suspending the coffee powder in water to achieve the required sensitivity [18-26]. All samples were analysed with the method described above and quantified with the three approaches investigated. Twelve of them (three Arabica, three washed Arabica, three Robusta samples from different origins and lots, and three commercial blends of different compositions) are employed here to illustrate the results. Fig. 1A reports the HS-SPME-GC-TIC profiles of the same Arabica coffee sample analysed as such or suspended in water. Fig 1B reports the profiles of the diagnostic ions (i.e. m/z 68, 72 and 82) adopted for the present study. Table 1 reports average concentrations (ppm) and related coefficient of variation (CV%) of furan and 2-methyl-furan calculated on three repetitions in the 12 representative samples with the three quantitation approaches investigated (SA, SIDA and MHE) *versus* the FDA method results calculated with the SA approach. The results obtained with the investigated quantitation approaches satisfactorily agreed with those obtained by the FDA method, most of them showing a CV well below 15%, arbitrarily chosen as limit of acceptance. Moreover, all methods were highly reliable, showing high repeatability: RSD never exceeded 12% for either furan or 2-methyl-furan; intermediate precision was always below 15% and sensitivity was very high (LOD and LOQ) as reported in table 2. The quantitation approach that fits the fixed CV limit of 15% most closely is MHE. In principle, this approach requires the regression equation of the analyte(s) investigated (eq. 4) to be determined for each sample to obtain the exponential slope *Q* to be used in eq. 3. Determination of eq. 4 requires at least three consecutive extractions for each sample. Roasted coffee is a relatively homogeneous matrix and, for the samples analysed here, contains concentrations of furan and 2-methyl-furan in a relatively limited range (furan: about 1-5 ppm, 2-methyl-furan: about 4-20 ppm). Table 3 reports *Q* and correlation coefficient (r) values obtained from the analysis of 34 samples of roasted coffees of different varieties and origins, as well as of the blends. The *Q* values are all within a very limited range for both analytes (0.41-0.45 for furan and 0.11-0.14 for 2-methyl-furan for all 34 samples) thus enabling the use of an average *Q* value (0.42 for furan and 0.13 for 2-methyl-furan) for the routine determination of the following samples. The reliability of *Q* is indirectly confirmed by the correlation coefficient of the regression equation, being, for all samples, above 0.9980 for furan and 0.9990 for 2-methyl-furan. As a consequence, the total area of the peak of the investigated analyte can be measured from a single determination, provided that their concentrations are in the range for which the average *Q* value has been calculated. Table 1 reports the average concentrations (ppm) and related coefficient of variation (CV%) of furan and 2-methyl-furan, calculated with the average *Q* value calculated *vs*. the FDA method. The results show that the amounts of furan and 2-methyl-furan are very similar to those calculated by MHS-SPME with the *Q* value specific for each sample, and that the CV% relative to the FDA method is likewise in all case below 15%. The possibility of HS quantitation with a single area determination makes the MHE approach very rapid and highly competitive with SA and SIDA. In addition, this method is even easier than the others because, in agreement with Kolb et al. [2], the calculation of the concentration from the total area can be run by a quick external standard determination, thus avoiding the creation of a calibration curve.

3.3) HS-SPME-MS non-separative analysis of furan and 2-methyl-furan in coffee

One of the ways to satisfy the ever increasing demand for control analyses is to develop high-speed and direct analysis methods. Non-separative methods are therefore of great interest when a large number of samples must be screened. Furan and 2-methyl-furan were here quantified in roasted coffee by a non-separative HS-SPME-MS method with SIDA and MHE approaches, and the results compared to those of the conventional separative method; SA was not considered because it requires too large a number of determinations. When used to quantify furan and 2-methyl-furan in

coffee, non-separative method is made more complex by the low m/z values of the selected diagnostic ions (m/z 68 for furan, 82 for 2-methyl-furan and 72 for d_4-furan) that are common to other components of the sample analyzed. The correction factor for the intensity of the target ions has therefore to be determined from the results of a set of conventional separative analyses; two approaches are generally used in the authors' laboratory:

a) evaluation of the average % contribution to the total intensity of each target ion of the other components containing the ions in question determined through the conventional separative analysis of a suitable number of samples. This method is particularly effective with relatively homogeneous samples, as is the case for roasted coffee. The correction factor of furan calculated over 50 samples of different varieties, origins and blends analyzed over three years was 0.82 for furan (RSD% 3.97, range 0.76-0.87) and 0.91 for 2-methyl-furan (RSD% 1.27, range 0.90-0.96);

b) mathematical correction calculated through the equation (eq. 5) introduced by Perez Pavon [43] based on the relationships between the abundance of the target ion and an extra-ion not present in the mass spectra of the target analytes (i.e. furan and 2-methyl-furan) but present in analytes whose mass spectra contains the target ions. The mathematical correction is given by the following equation:

$$I_{corr(m/z)tar} = I_{tot(m/z)} - K\, I_{(m/z)2} \qquad \text{(Eq. 5)}$$

where $I_{corr(m/z)tar}$ is the effective target ion abundance to quantify the analyte investigated (i.e. 68, 82), $I_{tot(m/z)}$ is the total abundance of the target ion in the mass profile; $I_{(m/z)2}$ is the abundance of the ion of interfering analyte(s) not present in the target analyte(s) (m/z 95 for furan, m/z 98 for 2-methyl-furan) and K is the mean of the ratio between the abundance of the analyte target ion corresponding to all interfering components (i.e. without that of the investigated analyte) and that of the extra-ion chosen for the interfering compounds, obtained from a suitable number of conventional separative analysis. The average K value calculated over 30 samples by conventional analysis was 0.06 (RSD%: 17.7, range 0.04-0.07) for furan (m/z 68/95) and 0.41 (RSD%: 10.7, range 0.34–0.47) for 2-methyl-furan (m/z 82/98).

A set of 105 samples of roasted coffee were analysed with the separative and non-separative HS-SPME-MS methods quantifying furan and 2-methyl-furan with SIDA and MHE approaches. In this case too, the results of twelve samples (four Arabica, two washed Arabica and four Robusta samples from different origins and lots, and two commercial blends of different compositions) were selected to illustrate the performance of the method. Fig. 2 reports both the HS-SPME-TIC and the mass spectrum profile of an Arabica coffee sample. Table 4 reports average concentrations (ppm) of furan and 2-methyl-furan calculated over three repetitions in the 12 representative samples quantified with SIDA and MHE, and gives the coefficients of variation (CV%) determined *vs.* the corresponding results of conventional separative HS-SPME-GC-MS method; an arbitrary CV value of 20% was taken as acceptance limit. These analyses were carried out adopting the same sampling time, 20 minutes, as for the conventional separative method. The SIDA results with the average % correction for both furan and 2-methyl-furan are satisfactory, because no samples of either furan and 2-methyl-furan presented CV% values above 20%, and many of them were below 10% compared to conventional analyses. On the other hand, three samples for furan but none for 2-methyl-furan showed CV% values above 20% for the same analyses applying the mathematical correction.

MHE quantitation was carried out by applying an average Q value calculated over 30 samples of 0.54 for furan (RSD%: 5.8, range 0.50-0.60) confirmed by a linear decay (average r: 0.9949, RSD%: 0.5) and of 0.23 for 2-methyl-furan (RSD%: 8.1, range 0.20-0.28) again with a linear decay (average r: 0.9859, RSD%: 0.9). The results obtained with MHE are similar to those with SIDA. With the average % correction, the CV% were higher than 20% compared to the conventional separative analyses for one sample in the case of furan, and for three in the case of 2-methyl-furan; with mathematical corrections, the CV% of two samples were above 20% for furan and of two for

2-methyl-furan. In this case too repeatability and intermediate precision, again determined on three coffee samples, were very good, all showing an RSD% for repeatability below 12% and below 15% for the intermediate precision. The same was for LOD and LOQ (table 2).

The non-separative methods require an MS acquisition time of about three minutes, therefore a logical step is to try to speedup the sampling time and, as a consequence, greatly reduce the total analysis time and increase analysis throughput. A set of experiments were therefore carried out, applying a sampling time of five minutes. Although the two equilibria driving HS-SPME (i.e. matrix/HS and the HS/polymer) vary, the results were nevertheless reliable, because of the highly standardized sampling conditions applied. Table 5 reports average concentrations (ppm) of furan and 2-methyl-furan calculated over three repetitions in the 12 representative samples quantified with SIDA and MHE with a sampling time of five minutes together with the coefficient of variation (CV%) determined *vs.* the corresponding results obtained with the conventional separative HS-SPME-GC-MS method (sampling time: 20 min). The results are very satisfactory because with SIDA no samples for eother furan or 2-methyl-furan had a CV% above 20%, with the average % correction, and only one in the case of furan and none in the case of 2-methyl-furan, with the mathematical correction.

MHE quantitation was carried out applying an average *Q* value calculated over 30 samples of 0.65 for furan (RSD%: 1.1, range 0.64-0.67) and 0.50 for 2-methyl-furan (RSD%: 1.7, range 0.48-0.52) These values were again confirmed by a linear decay for both furan (average r: 0.9982, RSD%: 0.2) and 2-methyl-furan (average r: 0.9999, RSD%: 0.02). The results were also good for MHE (table 5): CV% was above 20% in four samples for furan and in two for 2-methyl-furan with the average % correction, and in four samples for furan and in none for 2-methyl-furan, with mathematical correction. In this case too repeatability and intermediate precision, again determined on three coffee samples, were very good, all showing an RSD% for repeatability below 12%, and below 15% for intermediate precision and very low LOD and LOQ (table 2).

Last but not least, the consistency of the non-separative method was confirmed by the direct non-separative analysis of five different samples followed by the above separative method. The comparison of the results, in this case too, showed that CV% never exceeded 20% with either SIDA or MHE with average % correction and mathematical correction.

Conclusions

The results reported above show that all the quantitation approaches investigated can reliably be applied in combination with HS-SPME-GC-MS to quantify furan and 2-methyl-furan in roasted coffee suspended in water with high repeatability and sensitivity. MHE was also first applied to the determination of furan and 2-methyl furan, and showed that it could be successfully automated and is competitive, in terms of time, with the other most widely-used approaches, i.e. SA and SIDA, while avoiding the drawbacks related to the matrix effect. The possibility to apply an average *Q* value, determined on a significant number of samples of the same matrix, but of different origins, varieties, lots and blends for MHE, enabled us to run a single analysis for each sample, in particular when the analyte(s) to quantify is in amount(s) within the range of concentrations from which the average *Q* has been calculated. This possibility is especially valid in the case of relatively homogeneous samples, resulting from matrices processed under comparable conditions.

The results for the separative methods also made it possible to develop a quick non-separative method (HS-SPME-MS) for screening tens of samples; this opens up the possibility to monitor the roasting process on-line to a pilot plant in view of optimizing the process with the aim of minimizing furan and analogue formation. The non-separative method reduced analysis time by a factor of at least five i.e. from about 50 minutes (20 minutes for sampling + about 30 minutes for analyte thermal desorption and GC-MS analysis) to about nine minutes (5 minutes for sampling + 4 minutes for analyte thermal desorption and MS analysis). In the case of furan and 2-methyl-furan, the application of this approach is not favoured, because the target ions (m/z 68 and 82 respectively) are not specific and a correction factor to evaluate the influence of other components giving the

same fragments must be determined and applied. The reliability of the corrections factors applied is demonstrated by the fact that the CV% values calculated *vs.* the corresponding conventional analysis were almost always below 20% with both the quantitation approaches applied (SIDA and MHE). Some experiments carried out on plant matrices on analyte with highly specific ions showed ever more reliable results, with CV% values even closer to those of conventional separative analyses, provided that the whole analysis system is standardized (data not reported).

The strategy described here can be applied mainly when dozens of control analyses must be carried out, thus making it competitive to spend time developing fast methods, starting from a number of conventional analyseis producing a set of reliable data to be taken as a reference. In any case, the non-separative methods can also be used as *analytical decision makers* [44] and applied to decide which sample(s) must be analysed by conventional separative-analysis, for instance because the non-separative result is far outside the range of concentrations for which the correction factor and, in case of MHE, the *Q* values were determined or, more in general, close to an acceptance limit fixed by law.

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Captions to figures

Figure 1 – A) HS-SPME-GC-TIC profiles of an Arabica coffee sample analyzed as such or suspended in water; B) profiles of the furan, 2-methyl furan and d4-furan diagnostic ions (i.e. m/z 68, 72 and 82) used for quantitation. F: furan, MF: 2-methyl furan.

Figure 2 – HS-SPME-TIC and mass spectrum profiles of an Arabica coffee sample.

Table 1. Average concentrations (ppm) of furan and 2-methyl-furan calculated in 12 representative roasted coffee samples with the three quantitation approaches investigated (SA, SIDA and MHE) *versus* the FDA method (n = 3) and related coefficient of variation (CV%). Legend: A=Arabica; WA=washed Arabica; R=Robusta; B:blend.

Furan									
Samples	**FDA**	**SA**		**SIDA**		**MHE**			
						Specific *Q*		**Average *Q***	
	ppm	**ppm**	**CV%**	**ppm**	**CV%**	**ppm**	**CV%**	**ppm**	**CV%**
A1	4.9	5.4	10.9	3.7	*-23.6*	5.4	10.5	5.5	11.5
A2	4.6	5.1	10.2	3.4	*-26.5*	4.9	6.9	5.0	8.2
A3	4.1	3.6	-13.4	3.1	*-23.8*	4.1	0.3	4.3	3.2
WA1	5.0	5.6	10.8	4.9	-3.1	5.1	1.6	5.1	0.7
WA2	4.1	5.2	*26.1*	4.3	5.1	4.2	2.0	4.2	3.2
WA3	4.3	4.5	6.2	3.1	*-26.2*	4.0	-5.6	4.1	-3.3
R1	5.3	6.0	13.5	4.5	-14.9	5.0	-4.8	4.8	-8.9
R2	4.8	5.4	13.0	4.2	-11.7	4.6	-4.1	4.5	-5.0
R3	4.8	4.9	2.6	3.8	*-20.3*	4.5	-6.1	4.4	-8.4
B1	1.6	2.0	*23.5*	1.6	0.5	1.7	2.8	1.7	5.3
B2	1.9	2.1	13.9	1.7	-10.3	1.9	1.5	1.8	-1.2
B3	4.5	4.8	7.8	3.8	-14.1	3.8	-13.8	3.5	*-20.8*
2-Methyl-furan									
A1	14.1	14.2	1.3	12.4	-12.0	14.3	2.0	14.6	3.5
A2	12.0	10.3	-14.2	10.6	-11.7	12.8	6.9	13.1	9.4
A3	10.0	10.0	0.1	10.3	3.6	9.7	-2.3	10.5	5.9
WA1	13.6	13.5	-0.7	13.0	-4.4	13.3	-2.3	13.6	0.0
WA2	11.8	12.6	7.3	12.8	9.1	10.1	-14.3	10.8	-8.0
WA3	9.2	9.5	2.4	8.5	-8.5	9.1	-2.0	9.9	7.1
R1	15.4	18.1	*17.2*	16.5	6.7	14.2	-8.2	14.2	-8.2
R2	13.5	16.0	*18.4*	15.5	14.7	12.4	-8.1	12.8	-4.9
R3	13.7	15.0	9.9	12.5	-8.3	11.2	*-18.0*	11.7	-14.5
B1	4.3	4.1	-4.5	4.3	0.2	3.9	-9.6	3.9	-7.9
B2	6.9	6.0	-11.9	5.9	-14.5	6.1	-11.8	6.0	-12.6
B3	17.6	21.5	*22.1*	22.0	*24.7*	18.7	6.4	19.0	5.2

Table 2. Repeatability and intermediate precision (RSD%) for both furan and 2-methyl-furan with the three quantitation approaches investigated (SA, SIDA and MHE) and LOD and LOQ values obtained for both separative and non-separative methods for three roasted coffee samples. Legend: A=Arabica; R=Robusta; B:blend; Rep.: repeatability; Int. prec.: Intermediate precision.

HS-SPME-GC-MS							
		SA		SIDA		MHE	
Samples	Compound	Rep.	Int. prec.	Rep.	Int. prec.	Rep.	Int. prec.
		RSD%					
A1	furan	0.5	3.9	1.8	2.6	4.7	12.2
	2-methyl-furan	2.4	8.6	6.3	10.5	2.2	8.3
R1	furan	1.7	4.6	1.5	2.0	5.8	9.4
	2-methyl-furan	3.8	6.8	7.6	10.7	6.4	11.1
B1	furan	2.2	7.7	1.1	1.9	7.8	14.8
	2-methyl-furan	2.6	8.6	8.5	13.4	2.1	3.3
HS-SPME-MS 20 minutes							
A1	furan			3.1	6.2	11.2	12.5
	2-methyl-furan			9.8	13.4	1.1	3.6
R1	furan			4.2	6.7	8.2	10.3
	2-methyl-furan			10.4	12.5	11.8	12.2
B1	furan			4.9	6.8	10.9	13.6
	2-methyl-furan			3.0	4.6	2.9	8.9
HS-SPME-MS 5 minutes							
A1	furan			2.6	4.6	3.0	4.2
	2-methyl-furan			2.3	5.8	9.8	12.5
R1	furan			0.8	3.6	6.4	9.6
	2-methyl-furan			0.3	6.5	5.1	7.4
B1	furan			3.8	8.4	4.4	5.2
	2-methyl-furan			4.7	6.9	2.2	6.5

		HS-SPME-GC-MS	HS-SPME- MS 20 minutes	HS-SPME- MS 5 minutes
LOD (ng/g)	furan	2	5	6
	2-methyl-furan	1	3	5
LOQ (ng/g)	furan	10	25	30
	2-methyl-furan	5	15	25

Table 3. Exponential slope *Q* and correlation coefficient (r) values obtained from the analysis of 34 roasted coffee samples different variety, origin and blends. Legend: A=Arabica; WA=washed Arabica; R=Robusta; B:blend.

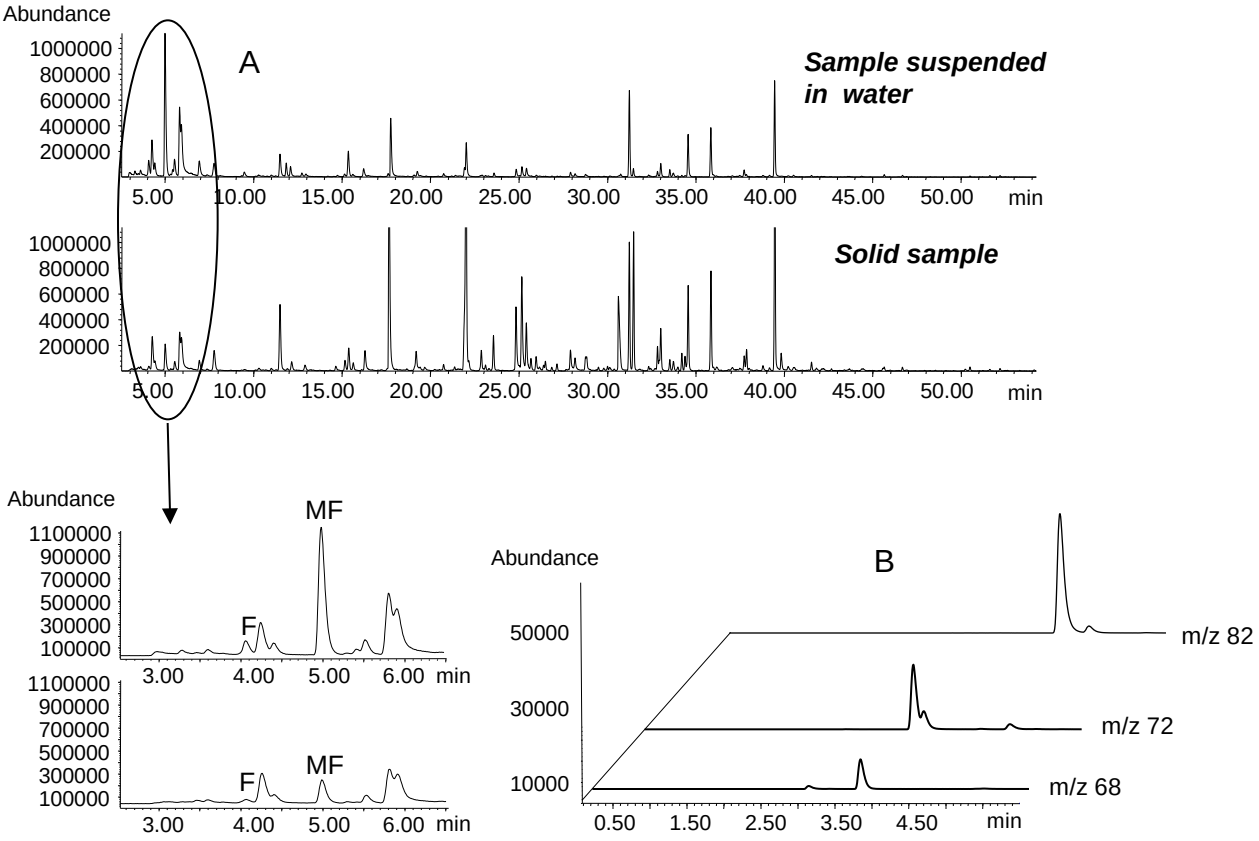
	Furan			**2-Methyl-furan**		
Samples	**ppm**	***Q***	**r**	**ppm**	***Q***	**r**
A1	5.4	0.41	0.9987	14.3	0.13	0.9994
A2	4.9	0.41	0.9981	12.8	0.13	0.9996
A3	4.1	0.41	0.9990	9.7	0.12	0.9996
A4	1.2	0.44	0.9994	3.3	0.12	1.0000
A5	1.3	0.41	0.9992	3.9	0.13	1.0000
A6	1.4	0.41	0.9994	4.7	0.12	0.9997
A7	2.3	0.42	0.9998	7.2	0.13	0.9999
A8	1.5	0.42	0.9992	5.6	0.11	0.9990
A9	1.5	0.41	0.9974	6.0	0.13	0.9997
WA1	5.1	0.43	0.9982	13.3	0.13	0.9993
WA2	4.2	0.41	0.9991	10.1	0.13	0.9994
WA3	4.0	0.41	0.9996	9.1	0.13	0.9998
WA4	1.3	0.42	0.9996	4.2	0.12	0.9995
WA5	2.1	0.43	0.9979	7.8	0.13	0.9992
WA6	2.9	0.43	0.9981	12.0	0.13	0.9996
WA7	1.2	0.41	0.9997	3.6	0.12	1.0000
WA8	2.3	0.45	0.9987	8.8	0.13	1.0000
WA9	2.6	0.44	0.9973	10.1	0.13	0.9998
R1	5.0	0.45	0.9980	14.2	0.14	0.9995
R2	4.6	0.43	0.9950	12.4	0.13	0.9989
R3	4.5	0.43	0.9993	11.2	0.14	0.9996
R4	1.8	0.41	0.9997	4.5	0.12	0.9998
R5	2.2	0.42	0.9997	6.4	0.13	1.0000
R6	3.0	0.43	0.9993	9.6	0.13	1.0000
R7	2.6	0.43	0.9989	9.6	0.14	0.9994
B1	1.7	0.41	0.9989	3.9	0.11	0.9994
B2	1.9	0.44	0.9984	6.1	0.13	1.0000
B3	3.8	0.41	0.9951	18.7	0.13	0.9998
B4	1.2	0.41	0.9994	3.8	0.11	0.9994
B5	1.2	0.41	0.9999	4.0	0.13	1.0000
B6	1.7	0.41	0.9982	6.0	0.12	0.9998
B7	1.6	0.41	0.9984	5.2	0.13	0.9995
B8	2.5	0.43	0.9985	10.7	0.13	0.9996
B9	1.4	0.42	0.9992	5.3	0.12	0.9999
Average		**0.42**	**0.9987**		**0.13**	**0.9997**
Std Dev		**0.01**	**0.0011**		**0.01**	**0.0003**
RSD%		**3.0**	**0.1148**		**5.9**	**0.0298**

Table 4. Average concentrations (ppm) of furan and 2-methyl-furan (n = 3) in 12 roasted coffee samples quantified with SIDA and MHE together with the CV% determined *vs.* the separative HS-SPME-GC-MS method. Legend: *Av. % corr.*: Average % correction; *Mathem. corr.*: Mathematical correction; A=Arabica; WA=washed Arabica; R=Robusta; B:blend.

20 MIN	**SIDA**					**MHE**				
	HS-SPME GC-MS	**HS-SPME-MS**				**HS-SPME GC-MS**	**HS-SPME-MS**			
		Av. % corr.		*Mathem. corr.*			*Av. % corr.*		*Mathem. corr.*	
	ppm	ppm	CV%	ppm	CV%	ppm	ppm	CV%	ppm	CV%
Furan										
A4	1.2	1.4	17.9	1.6	*31.9*	1.2	1.1	-6.6	1.1	-4.8
A5	1.4	1.7	18.5	1.9	*33.1*	1.3	1.4	9.5	1.5	17.4
A6	1.8	1.9	5.9	2.1	17.0	1.4	1.6	9.8	1.8	*28.2*
A7	1.9	2.2	15.0	2.3	*22.0*	2.3	2.1	-6.2	2.6	15.4
WA6	2.6	2.6	-1.0	3.0	13.1	2.9	2.5	-14.2	2.5	-13.7
WA9	2.3	2.4	4.4	2.7	19.6	2.6	2.1	-19.7	2.3	-8.2
R4	1.8	1.7	-8.4	2.0	8.8	1.8	1.6	-7.8	2.0	14.1
R5	2.2	2.1	-4.1	2.5	13.7	2.2	2.1	-4.8	2.6	17.7
R6	3.0	2.6	-12.6	3.1	3.3	3.0	2.8	-6.0	3.5	15.2
R7	3.3	2.9	-10.7	3.4	4.5	2.6	2.8	7.3	3.3	*24.3*
B7	1.9	2.1	9.7	2.3	18.9	1.6	1.8	17.6	1.9	19.8
B8	2.3	2.1	-6.3	2.4	6.7	2.5	1.9	*-24.4*	2.2	-12.2
2-Methyl-furan										
A4	2.8	3.0	7.4	3.1	9.3	3.3	3.9	16.1	3.1	-5.7
A5	3.8	4.2	9.6	4.3	12.0	3.9	4.7	*21.7*	4.9	*26.9*
A6	5.3	5.4	2.0	5.7	7.5	4.7	5.8	*24.7*	4.8	1.9
A7	5.9	6.2	4.4	6.4	8.3	7.2	7.6	6.1	6.3	-12.1
WA6	14.0	11.3	-19.5	11.9	-14.9	12.0	10.2	-15.4	9.8	19.1
WA9	10.8	9.4	-12.7	9.9	-8.3	10.1	9.2	-9.5	8.4	-17.2
R4	4.5	4.8	7.3	5.1	14.3	4.5	5.2	15.5	4.8	6.4
R5	6.5	7.3	13.0	7.7	18.6	6.4	7.3	14.0	6.7	4.7
R6	10.3	11.1	7.9	11.8	15.0	9.6	10.3	7.1	9.6	-0.5
R7	13.8	14.0	1.8	14.9	8.1	9.6	11.5	*19.4*	11.2	15.9
B7	6.0	7.1	19.5	7.1	18.7	5.2	6.9	*33.0*	6.1	18.1
B8	11.4	10.1	-11.0	10.6	-7.1	10.7	8.6	-19.8	7.9	*-26.3*

Table 5. Average concentrations (ppm) of furan and 2-methyl-furan (n = 3) in 12 roasted coffee samples quantified with SIDA and MHE with a sampling time of five minutes together with the CV% determined *vs.* the separative HS-SPME-GC-MS method (sampling time: 20 min). Legend: *Av. % corr.*: Average % correction; *Mathem. corr.*: Mathematical correction; A=Arabica; WA=washed Arabica; R=Robusta; B:blend.

5 MIN	**SIDA**					**MHE**				
	HS-SPME GC-MS	**HS-SPME-MS**				**HS-SPME GC-MS**	**HS-SPME-MS**			
		Av. % corr.		*Mathem. Corr.*			*Av. % corr.*		*Mathem. corr.*	
	ppm	ppm	CV%	ppm	CV%	ppm	ppm	CV%	ppm	CV%
Furan										
A4	1.2	1.3	14.6	1.5	*27.7*	1.2	1,5	*25,2*	1.6	35,4
A5	1.4	1.5	5.8	1.7	19.1	1.3	1,7	*30,8*	1.7	33.0
A6	1.8	1.7	-5.0	2.0	14.2	1.4	1.9	*34,4*	1.9	34,4
A7	1.9	1.8	-4.6	2.2	14.2	2.3	2,1	-7,8	2.1	-7,8
WA6	2.6	2.5	-5.9	2.8	8.3	2.9	2,4	-18,9	2.5	-13,5
WA9	2.3	2.1	-6.8	2.5	10.2	2.6	2,4	-6,0	2.8	9,7
R4	1.8	1.5	-16.2	1.8	1.5	1.8	1,9	7,1	2.1	18,4
R5	2.2	1.9	-16.0	2.2	1.2	2.2	2,3	3,9	2.6	19.3
R6	3.0	2.4	-19.4	2.8	-6.3	3.0	2,7	-10,7	3.0	-0,7
R7	3.3	2.7	-17.8	3.2	-0.7	2.6	3,0	14,8	3.3	26,2
B7	1.9	1.7	-12.2	2.0	4.7	1.6	1.9	*22.4*	1.9	19.8
B8	2.3	2.0	-9.7	2.4	6.3	2.5	2,2	-10,6	2.5	1,6
2-Methyl-furan										
A4	2.8	2.9	1.6	3.0	5.9	3.3	3.5	3.6	3.4	2.7
A5	3.8	3.5	-8.3	3.7	-2.9	3.9	3.9	1.7	4.0	3.3
A6	5.3	4.4	-17.1	4.6	-11.9	4.7	4.9	4.2	4.7	1.1
A7	5.9	5.0	-15.3	5.3	-10.1	7.2	5.8	-19.7	5.8	-19.2
WA6	14.0	12.9	-7.8	13.7	-2.3	12.0	8.3	*-31.2*	9.7	-19.5
WA9	10.8	8.7	-19.1	8.8	-18.2	10.1	8.2	-19.2	9.2	-9.3
R4	4.5	3.7	-17.4	4.0	-11.4	4.5	4.7	2.8	5.1	12.0
R5	6.5	5.4	-17.0	5.8	-11.4	6.4	6.0	-6.0	6.6	2.8
R6	10.3	8.3	-19.4	8.3	-19.0	9.6	8.5	-12.0	7.8	-19.0
R7	13.8	11.1	-19.4	11.0	-19.9	9.6	11.0	12.6	11.5	19.4
B7	6.0	5.3	-11.3	5.6	-6.1	5.2	4.4	-14.9	5.6	8.6
B8	11.4	9.2	-19.1	9.3	-18.2	10.7	8.0	*-25.6*	8.9	-16.5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600000
400000
200000
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
min
Abundance
MF
F
1100000
900000
700000
500000
300000
100000
3.00
4.00
5.00
6.00
min
B
Abundance
50000
30000
10000
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m/z 72
m/z 68
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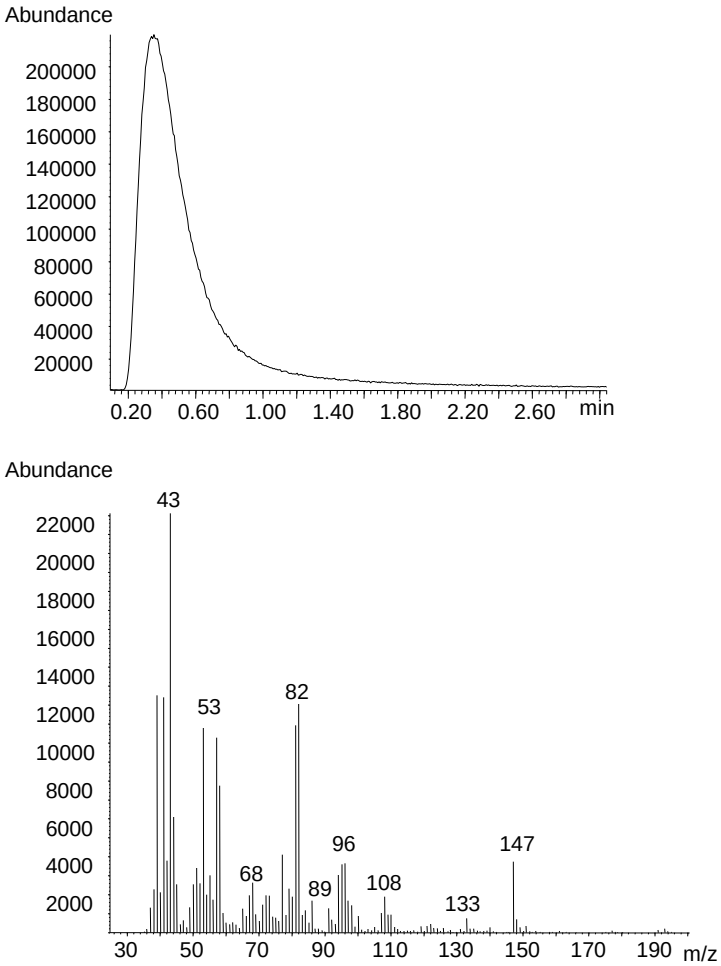
1.50
2.50
3.50
4.50
min

Figure 2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160000
140000
120000
100000
80000
60000
40000
20000
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0.60
1.00
1.40
1.80
2.20
2.60
min
Abundance
43
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
53
82
68
89
96
108
133
147
30
50
70
90
110
130
150
170
190
m/z