Application Note

*Instrument: Pegasus® BT*

EMPOWERING RESULTS
*Elemental Analysis | GC Mass Spectrometry | Metallography*

# Monitoring of Furan Derivates and Key Aroma Species Simultaneously in Coffee and Coffee Substitutes

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## *Abstract*

The production of coffee and process development of new coffee variants and substitutes (caffeine free, alternative taste, and aroma profiles, etc.), is a huge industry globally. The ability to guarantee the safety of these products while also monitoring the flavour and aroma qualities of products to meet consumer expectations and preferences is vital. Here, a method for simultaneous analysis of Furans and Non-Target Screening (NTS) of other key compounds, such as aroma-active species, is demonstrated.

## *Introduction*

Coffee is one of the most popular beverages around the world not only because of its unique flavor, but also due to its stimulating effect. Caffeine, the major coffee alkaloid, can cause elevated blood pressure and heart rate in some individuals. There is a big market for various coffee variants or substitutes which contain zero or lower amounts of caffeine. Production of such involves a range of processing variations, or the replacement of coffee beans with roasted wheat, rye, spelt, malt, and other plant materials.[1] However, just as during the conventional roasting of coffee beans during processing, many additional flavor volatiles and toxic processing contaminants represented by furan and methylfurans (2-methylfuran, 3-methylfuran, and 2,5-dimethylfuran) can be formed. These compounds have been found in various thermally processed foods (including jarred vegetables/fruits and baby foods); their precursors are mainly ascorbic acid, carbohydrates, unsaturated fatty acids, and carotenoids.[2,3]

With regards to health concerns related to exposure to furan and methylfurans published by European Food Safety Authority (EFSA)[4], the monitoring of the presence of furan and alkylfurans in food is recommended by the EU Commission.[5]

This application note demonstrates an analytical strategy based on headspace solid phase microextraction (HS-SPME), and gas chromatography time-of-flight mass spectrometry (GC-TOFMS) for the determination of furan and methylfurans in coffee and its substitutes. A simple sample preparation in combination with LECO's *Pegasus* BT 4D allowed all the target analytes to be quantified. In addition, the entire volatile profiles were recorded.

Simultaneously, high quality, full mass range data can be collected and allows non-target screening to be performed, which allows for a variation of aroma profiles to be efficiently monitored and understood.

## Experimental

To demonstrate the applicability of the HS-SPME-GC-TOFMS method, seven samples of coffee substitutes, differing in composition, obtained from the retail market were used. To verify the trueness of this method, testing material (instant coffee) used within the proficiency testing scheme 30119 FAPAS® was purchased.

The certified standard of furan was purchased from Sigma-Aldrich; 2-methylfuran, 3-methylfuran, and 2,5-dimethylfuran were purchased from Dr. Ehrenstorfer. The CAS numbers, selected m/z, boiling points, and target analyte formulas are summarized in Table 1.

**Table 1. Target Analytes Information**

| Analyte | CAS number | Elemental formula | Boiling point | m/z of selected ions for target analysis |
|---|---|---|---|---|
| **Furan** | 110-00-9 | $C_4H_4O$ | 31 °C | 68, 39 |
| **2-Methylfuran** | 534-22-5 | $C_5H_6O$ | 63 °C | 82, 81, 53 |
| **3-Methylfuran** | 930-27-8 | $C_5H_6O$ | 66 °C | 82, 81, 53 |
| **2,5-Dimethylfuran** | 925-86-5 | $C_6H_8O$ | 93 °C | 95, 81, 53 |

To cover three different concentration ranges, 0.07, 7, and 1400 ug/ml were prepared in water and methanol, respectively. Multi point dilutions were prepared in water, using each calibration level to generate calibration curves and calculate method validation performance.

For sample preparation, 100 mg of the coffee samples were mixed with 2 mL of NaCl saturated aqueous solution in 10 ml headspace vials. To quantify target analytes, the standard addition method, depending on signal intensity, was used.

Instrumental method parameters are summarized in Table 2.

**Table 2. HS-SPME-GC-TOFMS Parameters**

| **HS-SPME** | |
|---|---|
| SPME Fiber | SFIB-DVB/C-WR-80/10-P5 (CTC analytics AG, Switzerland) |
| Incubation | 5 min (35 °C) |
| Extraction | 1 m (35 °C) |
| Desorption Time | 2 min |
| **GC** | **Agilent 7890A** |
| Injection Mode | Split (1:30), 240 °C |
| Column | HP-INNOWax (30 m x 0.25 mm, 0.25 µm (Agilent Technologies, USA) |
| Oven Program | 35 °C (hold 3 min), ramp 15 °C/min to 100 °C, ramp 50 °C/min to 220 °C, 220 °C 5 min |
| Transfer Line | 220 °C |
| **MS** | **LECO *Pegasus* BT** |
| EI Ion Source Temp | 230 °C |
| Mass Range | 35-600 m/z |
| Acquisition Rate | 12 spectra/s |

## Results and Discussion

*Method Validation*

The performance characteristics including linearity range, limits of quantification (LOQ), and repeatability (n=6) expressed as the relative standard deviation (RDS), were determined by standards of target analytes spiked into 2 ml of water saturated with NaCl and they are documented in Table 3. To document the trueness of generated data by the SPME–GC-MS method, the analysis of the instant coffee 30119 FAPAS® proficiency test sample was analyzed (certified matrix reference material is not available). The calculated z-scores ranged from -1.4 to 1.1 for all analytes, thus satisfactory trueness was obtained.

**Table 3. Performance Characteristics of the Method**

| Analyte | LOQ (µg/kg) | Repeatability (%) | | Linearity | | |
|---|---|---|---|---|---|---|
| | | 1.5 ng | 7 ng | ng | Regression formula | Correlation coefficient |
| **Furan** | 1 | 3 | 4 | 0.4 - 400 | y=3287632x+12903177 | 0.999 |
| **2-Methylfuran** | 1 | 19 | 8 | 0.4 - 400 | y=13020242x+5332067 | 0.998 |
| **3-Methylfuran** | 1 | 11 | 7 | 0.4 - 400 | y=13215058x+1186833 | 0.999 |
| **2,5-Dimethylfuran** | 1 | 18 | 11 | 0.4 - 400 | y=21726627x+49078191 | 0.998 |

According to the Recommendation (EU) 2022/495 on monitoring the presence of furan and alkylfurans in food, the LOQ should not be higher than 20 µg/kg and 5 µg/kg for coffee and for jarred baby food, respectively. Using this method, LOQs for all analytes were 1 µg/kg and met the requirements set in the above-mentioned document. It should be noted that by modifying the sample weight (e.g. 1 g for jarred baby food with naturally lower concentration of furans than coffee substitutes), even lower LOQs could be achieved.

Additionally, as strong matrix effects often occur in analysis of such complex sample as coffee/coffee substitutes, using the standard addition method, enables more confident analysis to be achieved.

Figure 1 shows the AIC (deconvoluted ion chromatograph) of Coffee Substitute 7. Although 2-methylfuran and 3-methylfuran are isomers, their separation is optimal.

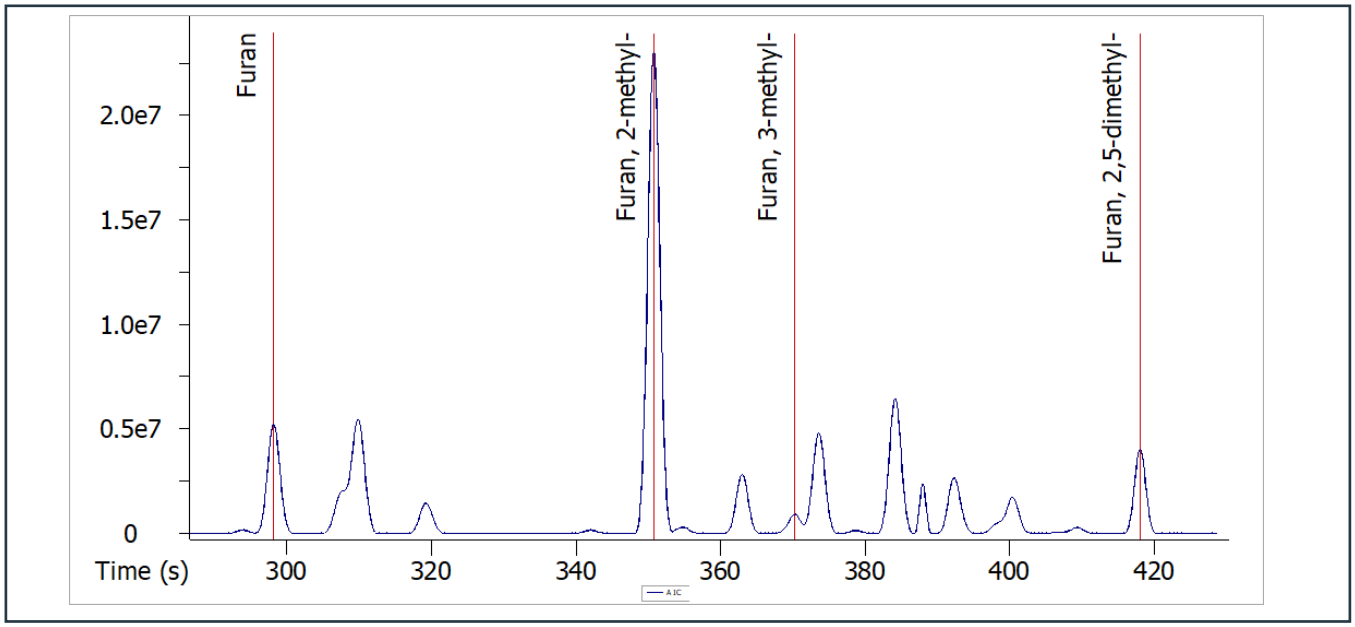


***Figure 1: AIC chromatogram of Coffee Substitute 7.***

*Furans in Coffee and Coffee Substitutes*

Figure 2 shows the concentrations of furan, 2-methylfuran, 3-methylfuran, and 2,5-dimethylfuran determined in the analyzed samples. In Coffee Substitute 5, all analytes were below LOQ. This sample was composed of sugar, glucose syrup, coconut fat, dried whey, milk protein, barley, chicory, rye, and food additives. On the other hand, the highest concentrations of all analytes were detected in Coffee Substitute 7, roasted spelt.

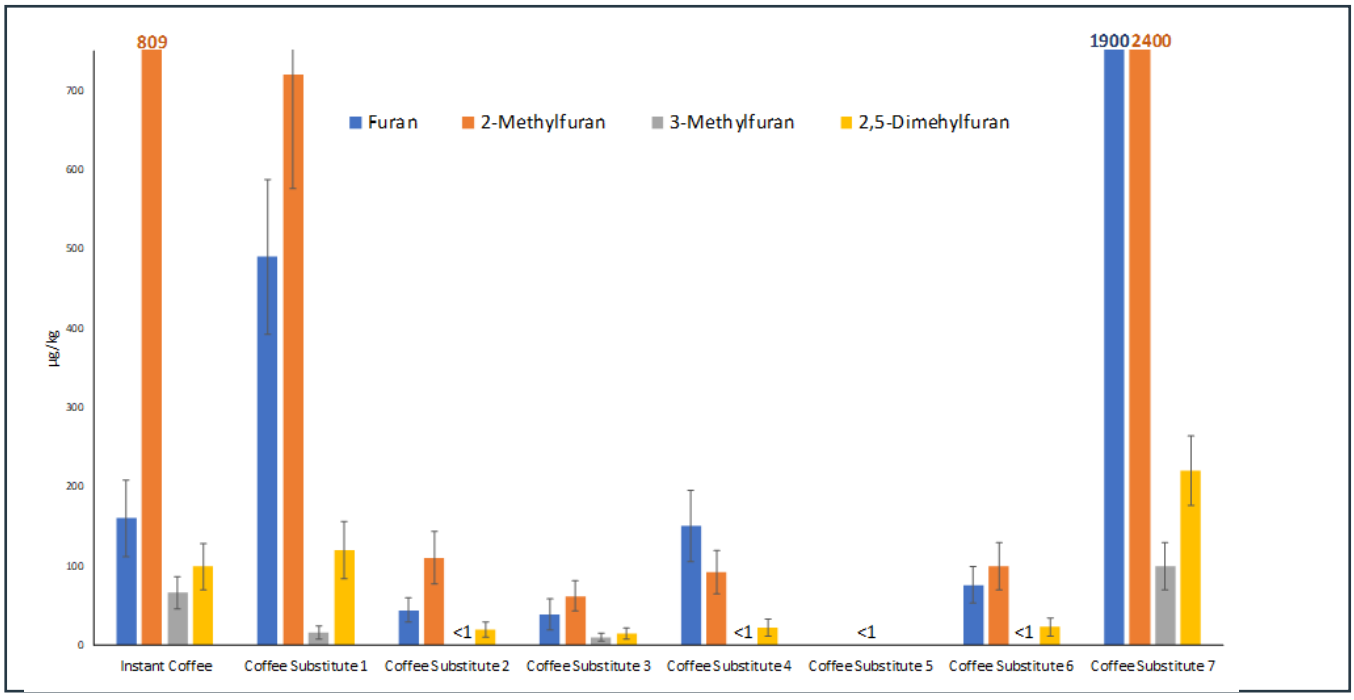


***Figure 2: concentration of furans in analyzed samples (*the error bars represent the uncertainty of the measurement).***

This method, using LECO's *Pegasus* BT 4D allows not only reliable quantification of target analytes at very low concentrations, but it also provides recording of the entire volatile profile. Thus, efficient NTS and the identification of 'unknown' compounds using the NIST library are possible. A selection of additionally detected and subsequently identified compounds, (based on MS similarity greater than 900), are shown in Figure 3. These include other furan derivates (e.g. 2-methoxymethylfuran, 5-methyl-2-furancarboxaldehyde), as well as other key species such as pyrazines and other substances commonly found which have an important impact on aroma, coffee.[6]

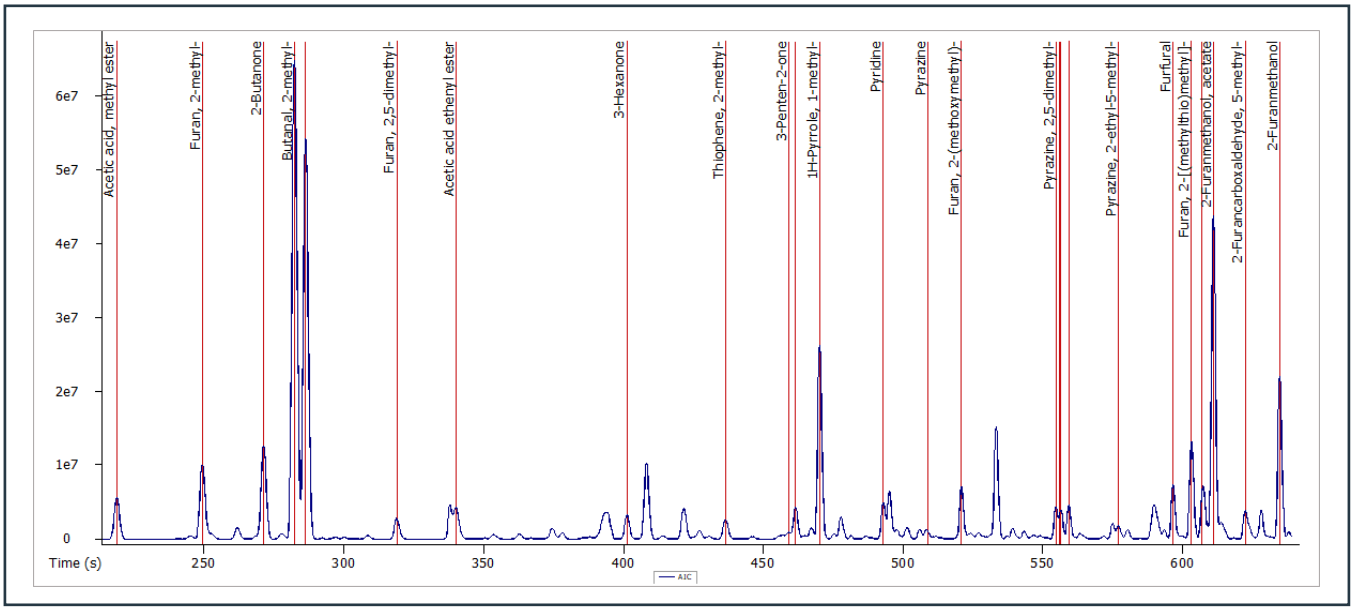


***Figure 3: Total ion chromatogram (TIC) of the coffee sample and selected analytes.***

The collection of high quality, full mass range mass spectral data at fast acquisition rates, allowed the non target data to be efficiently deconvoluted using LECO ChromaTOF® brand software. This enabled a large number of coeluting species present to be observed and identified, with good confidence. For example, Pyrazine, 2-ethyl-6-methyl-, Furan, 2-methyl-5-(methylthio)-, and Nonanal, were identified with MS library match similarities of 918, 854 and 720 respectively, despite coeluting at almost the exact retention time (Figure 4).

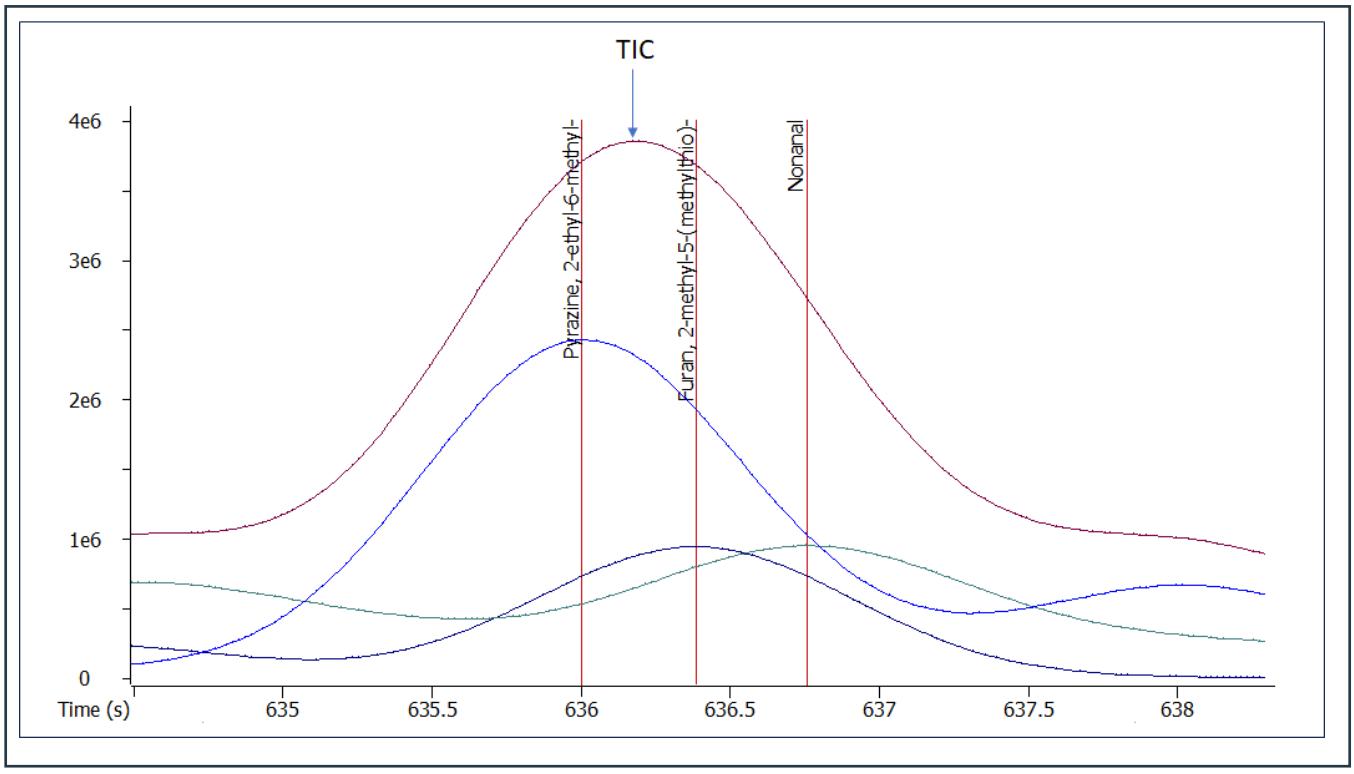


***Figure 4: Deconvoluted peaks of coeluting species, Pyrazine, 2-ethyl-6-methyl-, Furan, 2-methyl-5-(methylthio)-, and Nonanal.***

## Conclusions

A validated HS-SPME–GC–TOFMS method for analysis of coffee/coffee substitutes using the LECO *Pegasus* BT 4D instrument was demonstrated to enable reliable analysis of furan, 2-methylfuran, 3-methylfuran and 2,5-dimethylfuran. The performance characteristics of the method comply with the requirements of Recommendation (EU) 2022/495 on monitoring the presence of furan and alkylfurans in food, and, moreover, the trueness of this method was proven by analysing the FAPAS® proficiency test. Additionally, it was possible to perform high quality non-target screening simultaneously, allowing other key safety relevant and aroma active species to be detected and identified.

## References


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