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A novel method of furan determination in beer using gas chromatography with a flame ionization detector

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Abstract

Furan is considered to have a potential carcinogenic effect on human health. Its presence was found in a number of foods including beer. Thus, it is necessary to have a fast and routine method for its determination. This study describes a procedure meeting these requirements using the head-space technique associated with gas chromatography. A capillary column with the length of 60 m, inner diameter of 0.32 mm and the film thickness of 0.25 µm was used for the determination of furan. A flame-ionization detector (FID) was used for the detection. The characteristics of the procedure are presented.

Keywords: beer, furan, head-space, gas chromatography

1 Introduction

Furan is a colorless, low boiling heterocyclic compound. It definitely is a carcinogenic substance to rats and mice. It is also classified as a possible carcinogen when considering human health (International Agency for Research on Cancer, 1995; Crews and Castle, 2007).

Furan was found in a large number of different foods, especially those that were thermally treated in a sealed package (Maga, 1979). Besides, it appeared in foods such as meat, nuts, coffee and other beverages including beer (FDA, 2004; Zoller et al., 2007; Morehouse et al., 2008; Fromberg et al., 2014; EFSA, 2017).

In beer, the furan content ranged from 5 to 13 µg/L (FDA, 2004). According to a European Food Safety Authority study carried out on 102 beer samples, furan was detected at 3.0–5.2 µg/L (EFSA, 2011). Beer analyzed in Taiwan showed the furan range from 3.0 to 20.0 µg/L (Liu and Tsai, 2010). The highest furan concentration of 13 analyzed beer samples in China was 15.2 µg/L (Sijia et al., 2014).

The first quantitative method for the determination of furan in food was published by FDA (FDA, 2004). Due to the high volatility of furan, the samples are cooled to + 4 °C before handling. A headspace technique is used for the determination of furan where the sample is incubated at 80 °C. A PLOT (porous layer open tubular) column is used for the chromatographic separation. This type of column with a bound polystyrene-divinylbenzene phase can effectively separate small volatile molecules. Quantification is based on a standard addition method using deuterated furan (d4-furan) as an internal standard.

The headspace technique is based on the principle that heating of a sample releases volatile substances into the steam space. For furan, excessive heating of the sample is inappropriate and unnecessary. Becalski, Forsyth et al. (2005) showed that increasing the incubation temperature from 30 °C to 50 °C results in only a 50% increase in the furan peak area. By adding salt, the furan signal is more than doubled. Because of the risk of furan formation from its precursors, the incubation temperature of the sample at 50 °C or lower is usually used.

Some authors used the SPME (solid phase microextraction) technique instead of the classical headspace method (Cerny and Davidek, 2003; Goldmann et al., 2005; Ho et al., 2005; Bianchi et al., 2006; Condurso

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et al., 2018). In contrast to the headspace technique, where a portion of the vapor phase of the sample is directly loaded on the chromatography column, in the SPME procedure a polymer coated needle is exposed in the vapor space where volatile substances are absorbed for 10 to 60 min. Then, the SPME fiber is introduced into a gas chromatograph injector where the analytes are thermally desorbed for 1 to 5 min at 90 to 300 °C. This procedure allows higher concentration of analytes and hence, better sensitivity. The detection limit is therefore very low, namely ng/kg (Goldmann et al., 2005; Bianchi et al., 2006).

The chromatographic separation of furan from other volatile substances often takes place on PLOT columns and the temperature program starts between 20 and 50 °C (Cerny and Davidek, 2003; Reinhard et al., 2004; Becalski and Seaman, 2005; Goldmann et al., 2005; Ho et al., 2005; Senyuva and Gökmen, 2005; Bianchi et al., 2006; Hasnip et al., 2006).

Gas chromatography with a mass detector is also used for the identification and quantification of furan (Becalski et al., 2016). In this case, apart from the identification of furan using the retention time match (deviation from the standard ± 2%), the verification of the ratio of the furan molecular ion at m/z 68 to its ion fragment at m/z 39 in comparison with the standard (deviation ± 10%) is also used. A deuterated internal standard is used for the quantification. The recoveries are around 90% and the limit of detection is about 1 µg/kg. The published results show that the headspace method in combination with gas chromatography and mass detection provides excellent results (Crew and Castle, 2007).

The aim of this work was to elaborate and optimize a simple and robust method for the determination of furan in beer using the headspace method and capillary gas chromatography with the flame ionization detector.

2 Materials and methods

Furan (> 99%, Supelco, CZ) was used as the standard. Ethyl methyl sulfide at 96% purity (internal standard) was purchased from Aldrich. Ammonium sulfate in p. a. quality was purchased from Lach-Ner (CZ).

The DB-WAX capillary column with the length of 60 m, inner diameter of 0.32 mm and the film thickness of 0.25 µm was supplied by Agilent (CA, USA).
Gases in cylinders – helium in quality 5.0, hydrogen in quality 5.0, synthetic air and nitrogen in quality 4.6 supplied by Air Products (CZ).

The samples of pilsner beer produced in the Czech Republic were bought in a local store.

The determination was performed on an Agilent Technologies 7890B gas chromatograph equipped with a split/splitless injector and flame-ionization detector (Agilent, CA, USA). The gas chromatograph was equipped with a PAL RSI 85 automatic headspace sampler and a Clarity data station.

The chromatographic column was heated to the initial temperature of 47 °C, which was maintained for 0.7 min after the sample injection. A temperature gradient of 30 °C/min to 200 °C then followed and this constant temperature was maintained for 6 min. The sample (600 µl) was injected in the split mode with a split ratio of 1:10. Both injector and detector temperatures were equal, namely 220 °C. Helium 5.0 was used as the carrier gas and the head pressure was set to 200 kPa at 120 °C.

An internal standard method was used for the quantification, using ethyl methyl sulfide as the internal standard.

3 Results and discussion

The dependence of the furan signal response on the incubation temperature of the sample was tested during the optimization. Ten milliliters of the sample in a 20 mL headspace vial was shaken at 250 rpm for 13 min in the incubator of the headspace sampler. The incubation time was chosen according to the length of chromatographic analysis, including the system conditioning. The addition of 4 g ammonium sulfate was used to promote the transfer of furan to the vapor space. The results are shown in Table 1. An increase in the incubation temperature from 30 °C to 70 °C results in an only about 50% increase in the furan response. To prevent a possible increase in the furan content by its formation from precursors (Crew and Castle, 2007), the temperature of 50 °C was chosen for further experiments

Table 1 *Dependence of furan signal response on incubation temperature*

Incubation temperature (°C)	Relative response of furan (%)
30	100
50	126
70	148

Linearity, recovery and repeatability of the method were verified within the method validation. For this purpose, standard solutions of the analytes of interest were prepared in a 5% (v/v) ethanol solution.

The calibration curve was measured over a concentration range from 0.936 to 18.72 µg/L. The correlation coefficient showed a value of 0.999 using linear regres-

sion. The method is thus characterized by high linearity within a given concentration range.

The accuracy of the method was verified by recovery. First, the furan content of five real beer samples was measured. Then, the same beer samples were enriched by the addition of furan at the concentration levels of 4.7 µg/L and 18.7 µg/L. Furan recovery at 4.7 µg/L and 18.7 µg/L reached 85% and 91%, respectively.

The repeatability of the method was determined by repeating the whole procedure five times within one day on one beer sample, which was enriched by furan at two concentration levels – 2.7 µg/L and 12.8 µg/L. The relative standard deviations (RSD) were 7.2% and 5.5%, respectively. Since the enrichment of a beer sample with furan alone contributes to the deterioration in the repeatability value, the RSD results can be considered as fully acceptable.

The limit of detection was determined as a threefold of a standard deviation of the blank, and it showed a value of 0.05 µg/L. The limit of determination was determined as a tenfold of a standard deviation of the blank noise and is 0.18 µg/L.

Figure 1 shows the chromatogram of a real beer sample. It shows good interference-free furan separation.

Attention was also paid to the repeatability of retention times. For this purpose, the same sample was measured thirteen times. Table 2 shows relative standard deviations of retention times for furan and ethyl methyl sulfide. The results show that the reproducibility, expressed as relative standard deviation of retention times, is better than 0.12%.

Figure 1 *The chromatogram of a real beer sample*

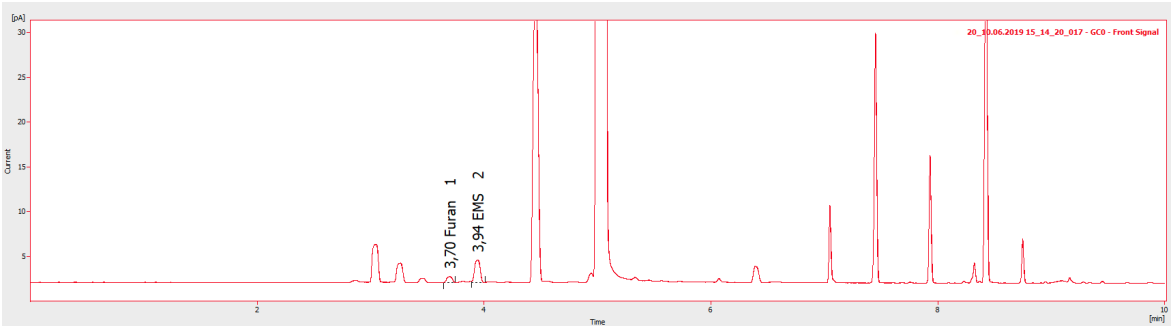

Table 2 *Relative standard deviation of retention times (RSD tr) of furan and ethyl methyl sulfide measured on column DB WAX 60 m, 0.32 mm I.D., 0.25 µm.*

Compound	RSD tr (%)
Furan	0.11
Ethyl methyl sulfide	0.11

Furan was determined by this method in 55 samples of pilsner lagers. The determined content ranged from 0.2 to 5.1 µg/L.

4 Conclusion

This study shows that the headspace/gas chromatographic method for furan determination in beer using ethyl methyl sulfide as an internal standard represents a reliable and fast procedure suitable for routine analysis. The application of ethyl methyl sulfide as an internal standard allows the use of an inexpensive, conventional flame ionization detector.

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