Comparison of different analytical methods for the quantification of odour-active haloanisoles in food contact materials

Brigitte Jaksa, Thomas Lehner, Dorothea Leis, Sigrid Hager and ERICH LEITNER

Graz University of Technology, Institute of Analytical Chemistry and Food Chemistry, Stremayrgasse 9/II, A8010 Graz, Austria

Abstract

2,4,6-trichloroanisol (2,4,6-TCA), the major compound responsible for cork taint in wine, belongs to haloanisoles which are formed by microorganisms, primarily fungi [1]. These substances can contribute to off-flavour in food and food contact material at remarkably low concentrations. Odour threshold concentrations (OTCs) of haloanisoles were determined by our trained expert panel in different matrices. The fact that 2,4,6-TCA and 2,4,6-tribromoansiol (2,4,6-TBA) exhibit extremely low OTCs concludes in a challenge for analytical detection methods. Different analytical methods were developed and compared, highly sensitive and selective systems. It is shown that gas chromatography mass spectrometry (GC-MS) using negative chemical ionization (NCI) in selected ion monitoring (SIM), comprehensive GCxGC-MS using electron impact ionization (EI) in SIM and GC-MS/MS using EI in multiple reaction monitoring (MRM) mode fulfil the desired requirements.

Introduction

Haloanisoles with their musty, mouldy and earthy odour are known contaminants in recycled paper and paperboard. Using these materials for packaging food, these substances can migrate into the packed good and cause unpleasant off-flavour. Based on sensory evaluation a threshold of 10 ng/kg paperboard, which correlates with the sensitivity of the human nose and covers the OTCs of the most sensitive individuals, was chosen as reference for the required sensitivity of the analytical methods. Since the measurement with EI-GC-MS neither in scan nor in SIM mode reaches the desired sensitivity, a MRM method with increased sensitivity and selectivity was developed. With comprehensive GCxGC-MS using EI in SIM the requested sensitivity was achieved due to the process of solute focusing and reinjection in the modulator, which results in a narrow peak with higher amplitude [2]. Due to its high selectivity for electron capturing species, NCI-GC-MS operated in SIM can be used to analyse haloanisoles at very low concentrations. External calibrations of the 4 most potent haloanisoles were performed to prove sensitivity and linearity. For quantification standard addition and single point internal standard method (where applicable) were conducted. The aim of this study was to compare different gas chromatographic methods that can be used for analysis and quantification of haloanisoles in paperboard samples.

Experimental

Sensory analysis

Determinations of OTCs were performed in duplicate by Graz University of Technology sensory expert panel using a 3 alternative forced choice sample presentation method in an ascending concentration series [3]. The panel consisted of 14 persons (9 women and 5 men in the age between 27 and 50) who were trained in the standard sensory

evaluation methods and fulfilled all psychological and physical demands required for a sensory panellist. Samples were prepared the day before the trial in the specific matrix and served the panel in covered odour-free plastic cups immediately before testing. OTCs were calculated as the geometric means of the individual thresholds and were evaluated in three different matrices, water, Mygliol®812 (fat matrix) and cellulose.

Sample preparation

The sample preparation was performed by headspace solid phase microextraction (HS-SPME) with agitation by magnetic stir bars. Two different fibers, SPME fiber 50/30 µm Carboxen®/DVB/PDMS (2 cm stable flex) and PAL SPME Arrow fiber Carbon Wide Range/PDMS (CTC-ARR11-C-WR-120/20-P3), were compared to prove an alleged increase in sensitivity due to the larger stationary phase volume in the Arrow fiber.

GC-MS and GC-MS/MS

The GC-MS system consisted of a Shimadzu GCMS-TQ8050 combined with Shimadzu AOC-6000 multifunctional auto sampler, using a quadrupole mass filter and EI. The headspace exposure of the SPME fiber was performed at 100°C for 20 minutes. A 29.5 m ZB-5MSi Column with 0.25 mm id and 0.25 µm film thickness was used for GC separation. Carrier gas was helium (51.4 kPa, linear velocity, 35.0 cm/sec). Column oven temperature program was 70°C (1 min) @ 10°C/min until 200°C @ 35°C/min until 320°C (1 min). Measurements were conducted in Q3 scan (m/z 50-400, 5000 scans/sec), Q3 SIM and MRM. The m/z filtered in SIM are shown in Table 1, the transitions for the GC-MS/MS MRM method are shown in Table 2.

Table 1: GC-MS measurement of haloanisoles in SIM mode, selecting following m/z

Compound	m/z	m/z	m/z
Internal Standard 2,4,6-dTCA	217	215	
2,4,6-TCA	212	210	
2,3,4,6-tetrachloroanisol (2,3,4,6-TeCA)	246	231	229
Internal Standard 2,4,6-dTBA	351	349	
2,4,6-TBA	346	344	
2,3,4,5,6-pentachloroanisol (2,3,4,5,6-PCA)	282	280	265

Table 2: GC-MS/MS method; transitions (collision energy)

Compound	transitions	transitions	transitions
2,4,6-dTCA	217.00>199.00 (15)	215.00>197.00 (15)	217.00>171.00 (30)
2,4,6-TCA	211.90>197.00 (15)	210.00>195.00 (15)	212.00>169.00 (30)
2,3,4,6-TeCA	230.90>202.90 (12)	228.90>200.90 (18)	243.90>201.90 (27)
2,4,6-dTBA	350.80>332.70 (18)	348.70>330.70 (15)	348.70>302.70 (33)
2,4,6-TBA	345.80>330.80 (18)	343.70>328.70 (15)	343.70>300.70 (27)
2,3,4,5,6-PCA	264.80>236.80 (15)	279.80>236.80 (27)	236.90>142.90 (24)

Comprehensive GCxGC-MS

The comprehensive GCxGC-MS system consisted of a Shimadzu QP2010 Ultra instrument combined with an Optic-4 injector and a Shimadzu AOC-5000 Plus auto sampler using EI and was run in SIM mode. The headspace exposure of the SPME fiber was performed at 100°C for 20 minutes. A 30 m HT1 column with 0.25 mm id and 0.25 µm film thickness was combined with a 2.5 m BPX5 column both with 0.15 mm id and

0.25 µm of film thickness. Modulation time was set with 5 seconds. Carrier gas was helium (131.1 kPa, average linear velocity 33.7 cm/sec on both columns). Column oven temperature program was 65°C (1 min) @ 5°C until 230°C. Ion source temperature was 200°C, detector voltage was 1.2 kV. M/z selected in SIM mode are the same as used in GC-MS SIM measurements (Tab.1).

Negative Chemical Ionisation (NCI)

The GC-MS system consisted of a Shimadzu QP2010 Plus system combined with AOC-5000 Plus auto sampler. The headspace-exposure of the SPME-fiber was performed at 100°C for 20 minutes. A 30 m ZB-5 MS column with 0.25 mm id and 0.25 µm film thickness was used for separation. Column oven temperature program was 50°C (1 min) @ 10°C/min until 300°C (5 min). Carrier gas was helium (45.6 kPa, linear velocity, 35.0 cm/sec). Isobutane was used for NCI, the ion source temperature was 160°C, the detector voltage 1.4 kV. In SIM mode m/z 35 and m/z 37 were filtered for compounds containing chlorine and m/z 79 and m/z 81 for bromine.

Results and discussion

Sensory analysis

OTCs of 9 different haloanisoles were determined in water. It was shown that the haloanisoles with the positions 2,4 and 6 on the benzene molecule filled with the halogen (2,4,6-TCA and 2,4,6-TBA) show the lowest OTCs. As soon as one position is exchanged or one halogen is added, the OTC increases. However, in general the matrix has great influence on the sensory threshold. To investigate this effect, OTC evaluations of the 4 most odorous compounds were conducted in Miglyol®812, a fat matrix used in the food sector as it is odour neutral due to its oxidation resistant properties. An increase in the sensory threshold of approximately 4 decades compared to the values received in water was observed. Due to the fact that haloanisoles are known to cause off-flavour in paperboard with unpleasant musty, earthy odour, the OTCs of the two most potent substances (TCA and 2,4,6-TBA) were investigated in cellulose as matrix, too. It was shown that the sensory thresholds were 3 decimal powers higher in cellulose than in water. A comparison of the OTCs in the different matrices is given in Table 3. At this point the great variations in the sensory thresholds between the individual panellists should be noticed. Values for 2,4,6-TBA for example were ranging from 0.001 ng/l to 1 ng/l (water).

Table 3: BET concentrations [ng/L] / [ng/kg] in different matrices; (n.d.: not determined)

Compound	BET [ng/L] in water	BET [ng/L] in Miglyol®812	BET [ng/kg] in cellulose
2,4,6-trichloroanisol	0.1	800	360
2,4,6-tribromoanisol	0.1	5000	480
2,3,4,6-tetrachloroanisol	2.5	11000	n.d.
2,3,4,5,6-pentachloroanisol	60	284000	n.d.
2,3,5,6-tetrachloroanisol	250	n.d.	n.d.
2,3,4,5-tetrachloroanisol	1000	n.d.	n.d.
2,4-dibromoanisol	1500	n.d.	n.d.
2,3,4-trichloroanisol	36000	n.d.	n.d.
3,5-dibromoanisol	54000	n.d.	n.d.

Calibrations

In the progress of method development external calibrations (10 ng/kg; 100 ng/kg; 500 ng/kg and 1000 ng/kg) were performed. With GC-MS (SIM) the desired sensitivity of 10 ng/kg could not be achieved, but was realised with GC-MS/MS in MRM (Figure 1). An improvement of sensitivity of the Arrow fiber compared to the traditional SPME fiber in the MRM measurements could not be shown. Linearity of the calibrations curves of the 4 haloanisoles was shown using the correlation coefficients, where values of > 0.998 (SPME-fiber) and > 0.995 (arrow-fiber) were received.
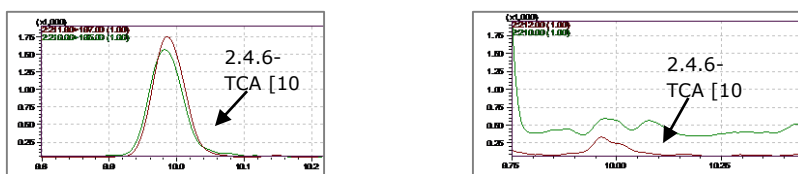

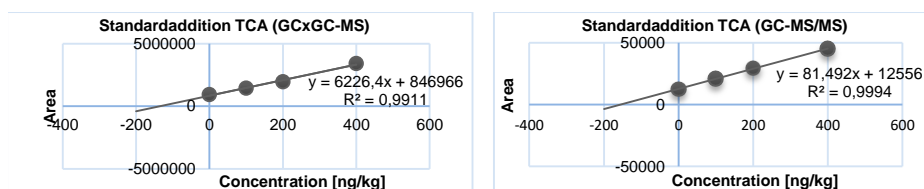**Figure 1**: 2,4,6-TCA [10 ng/kg]; left: EI-GC-MS/MS in MRM; right: EI-GC-MS in SIM

Figure 2: Calibration curves showing linear ranges for 2,4,6-TCA using comprehensive GCxGC-MS (left) and GC-MS/MS (right)

Quantifications

To investigate if the methods are applicable for the quantification of haloanisoles in real life samples, recycling paperboard samples (100 mg) were analysed. As expected, haloanisoles could not be detected with EI-GC-MS, but with comprehensive GCxGC-MS using EI in SIM, EI-GC-MS/MS in MRM and NCI-GC-MS in SIM (Table 4). Standard addition was conducted to investigate the influence of the matrix. Due to its better applicability single point internal standard method is going to be conducted in routine analysis except for the NCI-GC-MS measurements, where chlorine/bromine ions are selected in SIM and consequently the deuterated standard cannot be separated from the analyte.

Table 4: Quantification of the two most odorous compounds, 2,4,6-TCA and 2,4,6-TBA in an example paperboard sample using standard addition and * single point internal standard method

Compound	*GCxGC-MS*	*GC-MS/MS*	*GC-MS/MS**	*GC-MS/NCI*
2,4,6-TCA [ng/kg]	172 ± 9	154 ± 17	177 ± 13	137
2,4,6-TBA [ng/kg]	1536	1104 ± 102	1449 ± 234	987

References

1. Buser H.R., Zanier, C., Tanner, H. (1982) J. Agric. Food Chem. 30 (2). 359–362.
2. Berscht W. (2000) J. High Resol. Chromatogr. 23 (3) 167-181.
3. ASTM E679; Standard Practice for Determination of Odor Concentration Series Method of Limits