

1. Introduction

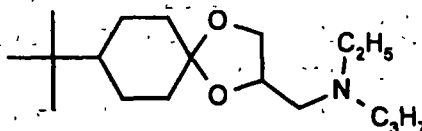
This method was developed for determination of KWG 4168 in drinking water samples and surface water samples.

1.1: Chemical and Physical Properties of KWG 4168

Trivial name : KWG 4168

Chemical designation : 8-(1,1-dimethylethyl)-N-ethyl-N-propyl-1,4-dioxaspiro[4.5]decane-2-methanamine

Structural formula



CAS-No. : 118134-30-8

Empirical formula : $C_{18}H_{35}NO_2$

Molecular weight : 297.5 g/mole

2. Principle of the Method

The method was developed in accordance to the multi residue methods of the Deutsche Institut für Normung (DIN) (3) and of the European Committee for Standardization (CEN) (4) under special consideration of the gas chromatographic standard methods F6 (5) and F14 (6).

KWG 4168 is concentrated from water samples by solid phase extraction (4-6). After drying of the cartridges KWG 4168 is eluted, concentrated to dryness and reconstituted. Identification and quantitative determination is done by gas chromatography using mass selective detection (5, 6) (see Figure 18).

3. Test System

For method validation drinking water from Monheim and surface water from the river Rhine sampled in Leverkusen-Hitdorf was used. Analytical data for the surface water are listed in Table 1.

Table 1. Analytical data for the surface water from river Rhine, sampled in Leverkusen-Hitdorf

Test	Result
Total organic carbon (TOC)	19 mg/L
Dissolved organic carbon (DOC)	2 mg/L
Conductivity at 25 °C	349 µS/cm
pH	7.7
Water hardness	6.9 °dH
Deposited substance	1.5 mL/L
Dry residue after filtration	363 mg/L

4. Instruments

Gas chromatograph	Shimadzu GC 17 A with autosampler AOC-17 / AOC-1400 and mass selective detector QP5000 Shimadzu D-47269 Duisburg
Turbo-Vap LV Evaporator	Zymark GmbH D-6270 Idstein/Taunus
SPE-Station	Vac Elut SPS 24 Varian GmbH D-63289 Darmstadt

Alternatively comparable instruments of other manufacturers can be used.

Volumetric flasks, pipettes and other common laboratory equipment.

5. Reagents

Milli-Q-water	: deionised and purified by a milli-Q-unit, Millipore Co.
Methanol	: Promochem, D-46469 Wesel
N-butyl-acetate	: Riedel de Haen, D-30926 Seelze
Ammonia solution (25%)	: Riedel de Haen, D-30926 Seelze
C ₁₈ -cartridges	: Bakerbond spe. Octadecyl (C ₁₈) (40 µm), 1 g, J.T. Baker, Phillipsburg NJ 08865 USA, Art. 7020-07
Reference substance	: KWG 4168, batch M00298, purity 98.3%, determined by GLC, identity ensured by ¹ H-NMR-spectrum expiry date July 2002

For method validation a certified reference substance of batch no. M00298 (KWG 4168, purity 98.3 %) was used. With the reference substance a primary stock solution of approx. 1000 mg/L was prepared in acetone. From this stock solution standard solutions were prepared by dilution with a mixture of 50% n-butyl-acetate, 49.5% methanol and 0.5% ammonia solution (25%) (v:v:v).

6. Safety Measures

The German guidelines for laboratories issued by the Trade Co-operative Association (e.g. Bulletin M006) or comparable guidelines in other countries must be considered when working according to this method.

The following solvents and pesticides classified as toxic and/or less toxic according to the Hazardous Substances Regulations are used.

Methanol	: toxic and easily flammable
Ammonia	: toxic
Acetic acid -n-butylester	: toxic and easily flammable
KWG 4168	: Xn harmful, R21/22

This classification is based on the German guidelines and has to be adapted to the respective national guidelines in case the method is used outside Germany.

7. Performance of Analyses

7.1. Sample Preparation

For concentration the water samples are adjusted to pH=3 with o-phosphoric acid. This should be done just before the concentration process starts because KWG 4168 is not stable in an acidic solution ($t_{1/2}$ at pH 4 >250 h, $t_{1/2}$ at pH 2.1 < 16-h). Nevertheless the acidification is necessary to increase the solubility of KWG 4168 in water and to avoid adsorption to the glass walls of the equipment.

The C₁₈-cartridges are washed with 20 mL of methanol and then conditioned with 20 mL of milli-Q-water. After the conditioning step volumes of 100 mL of the water samples are sucked through the cartridges with a flow rate of approx. 1 drop per second. After this the cartridges are dried by sucking of ambient air through the cartridges for one hour. To prevent pollution of the cartridges during the drying process activated carbon cartridges are placed on top of the C₁₈-cartridges. The suction pressure is approx. 20 mbar. After the drying procedure the cartridges are eluted with 10 mL of methanol / ammonia solution (25%) (990:10, v:v) and evaporated to dryness. The residue is dissolved in 1 mL (0.1 µg/L samples) or 10 mL (1.0 µg/L samples) of a mixture of 50% n-butyl-acetate, 49.5% methanol and 0.5% ammonia solution (25%) (v:v:v). From each solution a volume of 1 µL is directly injected into the GC/MSD.

7.2. Standard Preparation

A volume of 100 mL of surface water is adjusted to pH=3 with o-phosphoric acid (blank samples) and extracted in the same manner as described for the water samples. The residue of five blank samples is transferred into a 5-mL volumetric flask using a volume of 1 mL for each blank sample of a mixture of 50% n-butyl-acetate, 49.5% methanol and 0.5% ammonia solution (25%) (v:v:v). To this solution a defined volume of the standard stock solution KWG 4168 is added and the flask is filled to the mark with a mixture of 50% n-butyl-acetate, 49.5% methanol and 0.5% ammonia solution (25%) (v:v:v) to reach a concentration similar to the concentrated samples.

7.3. Evaluation

Evaluation is performed using a laboratory data system by comparing the peak areas of the samples to the peak areas of the external standard solutions. The concentration of the samples can be calculated according to the given formula:

$$C = \frac{A \times C_s}{A_s \times F}$$

A	=	Peak area of sample solution [area counts]
A _s	=	Peak area of standard solution [area counts]
C	=	Concentration of KWG 4168 in the sample [µg/L]
C _s	=	Concentration of KWG 4168 in the standard solution [µg/L]
F	=	Concentration factor

7.4. Chromatographic Conditions A

Column	: OV-17, length 15 m; 0.25 mm i.d.; 0.25 µm film thickness Macherey & Nagel, D-52313 Düren
Carrier gas	: Helium, delivery pressure 0.25 bar
Injection volume	: 1 µL
Injector temp.	: 240 °C
Interface temp.	: 240 °C
Column temp.	: 90 °C, 2 min, 15 °C/min to 240 °C, 240 °C 1 min
Detector	: KWG 4168 : m/z 100
Retention time	: KWG 4168 Isomer A approx. 9.9 min KWG 4168 Isomer B approx. 10.3 min

7.5. Chromatographic Conditions B

Column	: Ultra 1, length 12 m; 0.2 mm i.d.; 0.33 µm film thickness Hewlett Packard, D-61352 Bad Homburg
Carrier gas	: Helium, delivery pressure 0.25 bar
Injection volume	: 1 µL
Injector temp.	: 240 °C
Interface temp.	: 240 °C
Column temp.	: 90 °C, 2 min, 15 °C/min to 240 °C, 240 °C 1 min
Detector	: KWG 4168 : m/z 100
Retention time	: KWG 4168 Isomer A approx. 10.5 min KWG 4168 Isomer B approx. 10.9 min

10. Control Samples

In the control samples KWG 4168 was not detected (see Figures 2, 5, 9 and 12).

11. Confirmatory Chromatographic Methods

For confirmation of positive detects of KWG 4168 in drinking water samples or surface water samples different confirmatory techniques can be used.

11.1. GC/MS Detection

For validation of this method the highly specific mass selective detection has been used. Due to the mass spectroscopic properties of KWG 4168 only the ion $m/z = 100$ has been detected. Though not 3 required ions have been detected, in none of the control samples of drinking water or surface water KWG 4168 could be detected.

11.2: Pattern of the A- and B Isomers

KWG 4168 can only be considered as positively detected, if signals for both isomers A and B are detected. In none of the samples a change in the isomeric pattern could be observed.

11.3: Different Stationary Phase of Different Selectivity

For confirmation of positive detects of KWG 4168 a second GC column with different polarity was used.

Figure 18 : Flow Diagram of Analysis Procedure

