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The Determination of Selected Taste and Odour Causing Contaminants in Drinking Waters by Gas Chromatography-Mass Spectrometry

Methods for the Examination of Waters and Associated Materials

December 2017

The determination of selected taste & odour causing contaminants in drinking waters by GC MS & GC MSMS (2017)

Methods for the Examination of Waters and Associated Materials

This booklet contains 5 methods for the determination of selected taste & odour causing contaminants in drinking waters by GC MS & GC MSMS.

Limited performance data are available for the methods described in this booklet. Each method has been validated or tested in only one laboratory and consequently details are included for information purposes only, as examples of the type of procedures that are available to analysts. Information on routine multi-laboratory use of these methods would be welcomed to assess their full capabilities.

Whilst specific commercial products may be referred to in this document, this does not constitute an endorsement of these products but serves only as illustrative examples of the types of products available. Equivalent products may be available and it should be understood that the performance of the method might differ when other materials are used and all should be confirmed by validation of the method.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soils (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series “Methods for the Examination of Waters and Associated Materials” and their continuing
revision is the responsibility of the Standing Committee of Analysts (established 1972 by the Department of the Environment). At present, there are eight working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical, Inorganic and physical methods
4 Metals and metalloids
5 Solid substances
6 Organic impurities
7 Biological, biodegradability and inhibition methods
8 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. If users wish to receive copies or advanced notice of forthcoming publications or obtain details of the index of methods then contact the Secretary on the SCA’s web-page:- http://www.standingcommitteeofanalysts.co.uk/Contact.html

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary. Users should ensure they are aware of the most recent version they seek.

Rob Carter
Secretary
April 2017

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; “Safe Practices in Chemical Laboratories” and “Hazards in the Chemical Laboratory”, 1992, produced by the Royal Society of Chemistry; “Guidelines for Microbiological Safety”, 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and “Safety Precautions, Notes for Guidance” produced by the Public Health Laboratory Service. Another useful publication is “Good Laboratory Practice” produced by the Department of Health.

The determination of selected taste & odour causing contaminants in drinking waters by GC MS & GC MSMS.

1 Introduction

Water companies are responsible for ensuring the drinking water they supply is clean and wholesome. Regulation 4 of the Water Supply (Water Quality) Regulations 2016 prescribes that to be regarded as wholesome, water must not contain excess concentrations or values of particular properties, elements, organisms and substances.

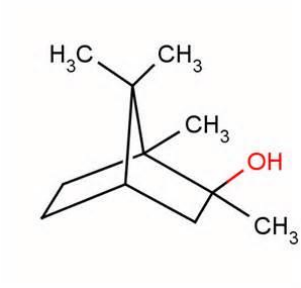
The majority of complaints related to the wholesomeness of drinking water are due to the presence of unpleasant tastes or odours. The most common cause of this is the presence of 2-Methylisoborneol (MIB) and Geosmin. These compounds are of particular concern due to the very low levels at which they can be detected by the human nose. The threshold concentrations for 2-Methylisoborneol and Geosmin are 6 ng L^{-1} and 1.3 ng L^{-1} respectively.

Areas where drinking water sources contain a high proportion of surface water often suffer from episodes of distinctly unpleasant tasting and smelling water when MIB and Geosmin are released in to the water. This is a significant problem due to the difficulty in removing these compounds using conventional water treatment techniques such as utilising activated carbon.

2-Methylisoborneol and Geosmin are n aturally occurring terpene alcohols which can be produced by blue green algae (cyanobacteria) and filamentous bacteria (actinomycetes). They produce a musty or earthy smell in the contaminated water supply that can be regarded as very off-putting.

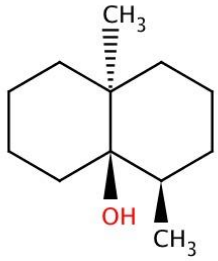
Other metabolites produced by actinomycetes, including 2-Isopropyl-3-methoxypyrazine, can produce woody-earthy and musty-mouldy potato odours.

Figure 1 Chemical structures of 2-methylisoborneol, geosmin and methoxypyrazines.



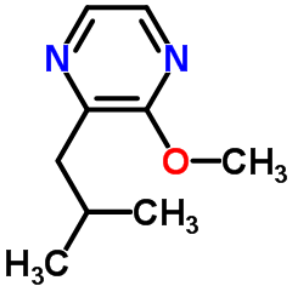
2-Methylisoborneol ($C_{11}H_{20}O$)

CAS No 2371-42-8
IUPAC: (1R,2R,4R)-1,2,7,7,-Tetramethylbicyclo[2.2.1]heptan-2-ol

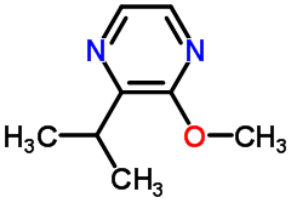


Geosmin ($C_{12}H_{22}O$)

CAS No 16423-19-1
IUPAC: (4S,4aS,8aR)-4,8a-Dimethyloctahydro-4a(2H)-naphthalenol



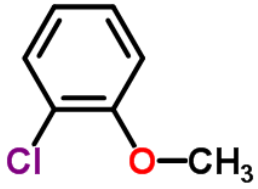
2-Isobutyl-3-methoxypyrazine ($C_9H_{14}N_2O$)
CAS: 24683-00-9



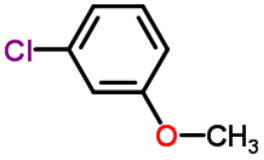
2-Isopropyl-3-methoxypyrazine ($C_8H_{12}N_2O$)
CAS: 25773-40-4

Some taste and odour issues arise from the chlorine disinfection of drinking water. Chloroanisoles have been identified as responsible for producing musty tastes and odours. They are formed by the methylation of chlorophenols, a by-product of the disinfection process, by fungi and actinomycete bacteria found in the water.

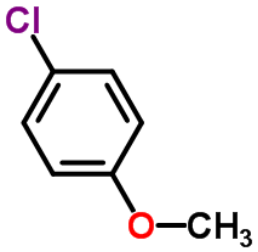
Figure 2 Chemical structures of chloro and bromo anisoles



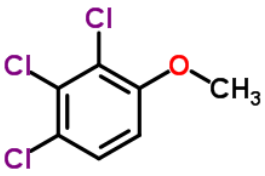
2-Chloroanisole (C_7H_7ClO)
CAS: 766-51-8
IUPAC: 1-Chloro-2-methoxybenzene



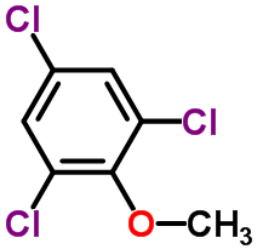
3-Chloroanisole (C_7H_7ClO)
CAS: 2845-89-8
IUPAC: 1-Chloro-3-methoxybenzene



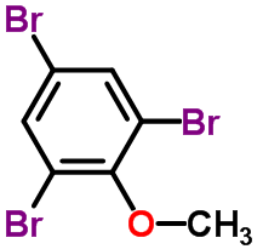
4-Chloroanisole (C_7H_7ClO)
CAS: 623-12-1
IUPAC: 1-Chloro-4-methoxybenzene



2,3,4-Trichloroanisole ($C_7H_5Cl_3O$)
CAS: 54135-80-7
IUPAC: 1,2,3-Trichloro-4-methoxybenzene

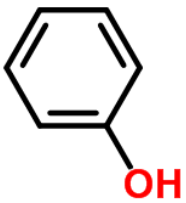


2,4,6-Trichloroanisole ($C_7H_5Cl_3O$)
CAS: 87-40-1
IUPAC: 1,3,5-Trichloro-2-methoxybenzene

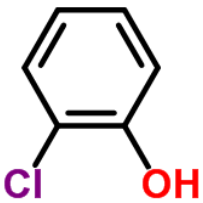


2,4,6-Tribromoanisole ($C_7H_5Br_3O$)
CAS: 607-99-8
IUPAC: 1,3,5-Tribromo-2-methoxybenzene

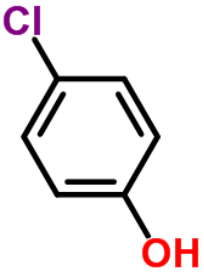
Figure 3 Chemical structures of chloro and bromo phenols



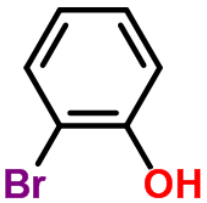
Phenol (C_6H_6O)
CAS: 108-95-2



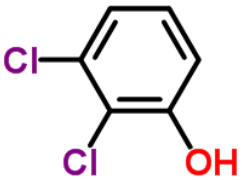
2-Chlorophenol
CAS: 95-57-8



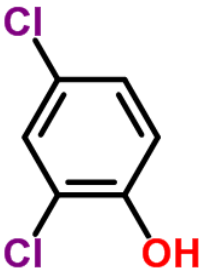
4-Chlorophenol
CAS: 106-48-9



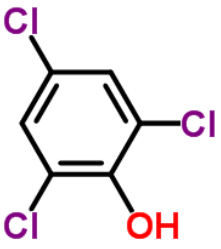
2-Bromomphenol
CAS: 95-56-7



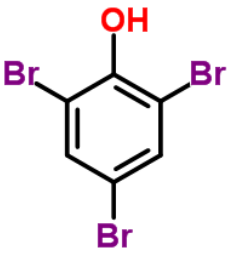
2,3-Dichlorophenol
CAS: 576-24-9



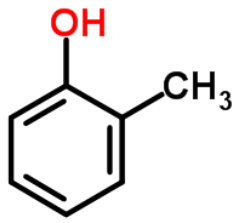
2,4-Dichlorophenol
CAS: 120-83-2



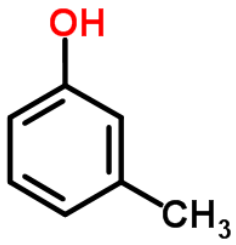
2,4,6-Trichlorophenol
CAS: 88-06-2



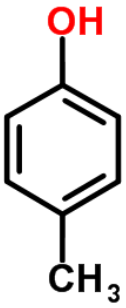
2,4,6-Tribromophenol
CAS: 118-79-6



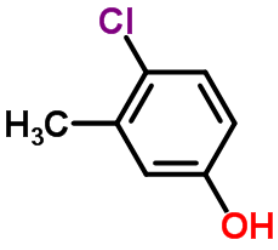
2-Methylphenol
CAS: 95-48-7



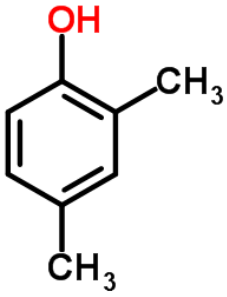
3-Methylphenol
CAS: 108-39-4



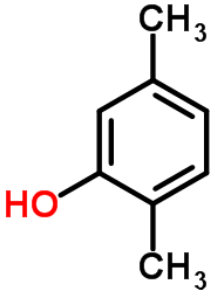
4-Methylphenol
CAS: 106-44-5



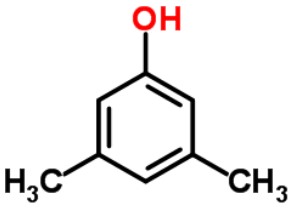
4-Chloro-3-methylphenol
CAS: 59-50-7



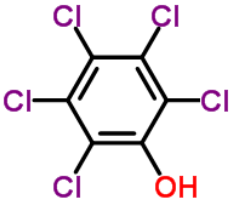
2,4-Dimethylphenol
CAS: 105-67-9



2,5-Dimethylphenol
CAS: 95-87-4



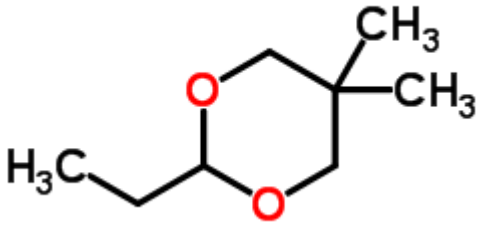
3,5-Dimethylphenol
CAS: 108-68-9



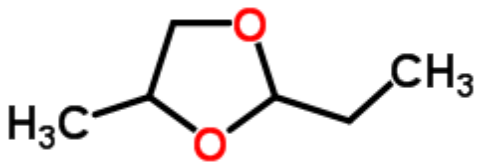
Pentachlorophenol
CAS: 87-86-5

Taste and odour incidents have also occurred due to compounds found in industrial waste. 2-Ethyl-5,5-dimethyl-1,3-dioxane (2-EDD) and 2-Ethyl-4-methyl-1,3-dioxolane (2-EMD) are waste products from resin manufacture which produce a musty, sweet nutty odour. They are small, polar and highly soluble which can make analysis difficult.

Figure 4 Chemical structures of 2-EDD and 2-EMD



2-Ethyl-5,5-dimethyl-1,3-dioxane ($C_8H_{16}O_2$)
CAS: 768-58-1
IUPAC: 2-Ethyl-5,5-dimethyl-1,3-dioxane



2-Ethyl-4-methyl-1,3-dioxolane ($C_6H_{12}O_2$)
CAS: 4359-46-0
IUPAC: 2-Ethyl-4-methyl-1,3-dioxolane

Table 1:Taste & Odour Thresholds

Compound	Source	Smell	Odour threshold µg L^{-1}	Taste threshold µg L^{-1}
2-Methylisoborneol	Actinomycetes, cyanobacteria, mirofungi	Musty/camphor	0.0063	0.0025
Geosmin	Actinomycetes, cyanobacteria, mirofungi	Musty/earthy	0.0013	0.075
2-Chloroanisole	Methylation of chlorophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	-	-
3-Chloroanisole	Methylation of chlorophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	-	-
4-Chloroanisole	Methylation of chlorophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	2.00	6.00
2,4,6-Trichloroanisole	Methylation of chlorophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	0.00008	0.025
2,3,4-Trichloroanisole	Methylation of chlorophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	-	-
2,4,6-Tribromoanisole	Methylation of bromophenols by bacteria and fungi in the water	Sweet, fruity, medicinal	-	-
2-Isobutyl-3-methoxypyrazine	Actinomycetes	Earthy, musty	<0.00005	0.0004
2-Isopropyl-3-methoxypyrazine	Actinomycetes	Earthy, musty	<0.00003	0.010
2-Ethyl-5,5-dimethyl-1,3-dioxane	Industrial waste	Musty, nutty, sweet	0.006	0.016
2-Ethyl-4-methyl-1,3-dioxolane	Industrial waste	Musty, nutty, sweet	0.01	
Phenol	Decomposition of vegatation or industrial waste	Carbolic	10	<2.00
2-Methylphenol	Disinfectant and solvent	Creosote	90	3.00
3-Methylphenol	Disinfectant and solvent	Creosote	200	2.00
4-Methylphenol	Disinfectant and solvent	Creosote	55	2.00
2-Chlorophenol	Chlorination of phenol during water treatment	Phenolic	0.09	0.14
2,5-Dimethylphenol	-	-	-	-
2,4-Dimethylphenol	-	-	-	-
3,5-Dimethylphenol	-	-	-	-
4-Chlorophenol	Chlorination of phenol during water treatment	Phenolic	10	39
4-Chloro-3-methylphenol	Pesticides, glues, paints, preservatives	Musty, damp, stale	2.5	2.5
2-Bromophenol	Pesticide metabolites, chlorination by products, naturally occuring	Medicinal	0.16	0.21
2,4-Dichlorophenol	Chlorination of phenol during water treatment	Medicinal	4.9	2.5
2,3-Dichlorophenol	Chlorination of phenol during water treatment	Medicinal	30	0.04
2,4,6-Trichlorophenol	Chlorination of phenol during water treatment	Medicinal	300	2
2,4,6-Tribromophenol	Pesticide metabolites, chlorination by products, naturally occuring	Medicinal	6.9	6.7
Pentachlorophenol	Chlorination of phenol during water treatment	Medicinal	9	8

2 References

1 Mallevialle, J., Suffet, I. H., Identification and Treatment of Tastes and Odours in Drinking Water, American Water Works Association, Denver, CO 1987, Chapter 5.

2 Standing Committee of Analysts, Blue Book 233, The Determination of Taste and Odour in Drinking Water (2016).

3 UKWIR/WRc Toxicity Datasheets (2016).

4 Cheeseman, R. V., Wilson, A. L., A Manual on Analytical Quality Control for the Water Industry, NS30. Revised by Gardner, M. J., June 1989

5 Water Quality — Sampling**,** Part 3: Preservation and handling of water samples (ISO 5667-3:2012)

3 Method Summary

The table below shows a summary of the methods contained in this booklet.

Method Identifier	A	B	C	D	E
Preparation technique	Liquid/liquid	SPE	Liquid/liquid	Liquid/Liquid	Liquid/Liquid
Instrument technique	GC MSMS	GC MSMS	GC MS	GC MS	GC MS
Instrument supplier	Agilent/Waters	Agilent	Agilent	Agilent	Agilent
Determinands	2-Methyl isoborneol	2-Methyl isoborneol	2-Methyl isoborneol	-	-
	Geosmin	Geosmin	Geosmin	-	-
	Anisole			-	-
		2-Chloroanisole	2-Chloroanisole	-	-
		3-Chloroanisole	3-Chloroanisole	-	-
		4-Chloroanisole	4-Chloroanisole	-	-
	2,4,6-Trichloroanisole	2,4,6-Trichloroanisole	2,4,6-Trichloroanisole	-	-
		2,3,4-Trichloroanisole	2,3,4-Trichloroanisole	-	-
	2,4,6-Tribromoanisole	2,4,6-Tribromoanisole	2,4,6-Tribromoanisole	-	-
		2-Isobutyl-3-methoxypyrazine	2-Isobutyl 3-methoxypyrazine	-	-
	2-Isopropyl-3-methoxypyrazine	2-Isopropyl-3-methoxypyrazine	2-Isopropyl-3-methoxypyrazine	-	-
	-	-	-	-	2-Ethyl-4-methyl-1,3-dioxolane
	2-Ethyl-5,5-dimethyl-1,3-dioxane	-	-	-	2-Ethyl-5,5-dimethyl-1,3-dioxane
	-	-	Phenol	Phenol	-
	-	-	-	2-Methylphenol	-
	-	-	-	3-Methylphenol	-
	-	-	-	4-Methylphenol	-
	-	-	2-Chlorophenol	2-Chlorophenol	-
	-	-	-	2,5-Dimethylphenol	--
	-	-	-	2,4-Dimethylphenol	-
	-	-	-	3,5-Dimethylphenol	-
	-	-	-	4-Chlorophenol	-
	-	-	-	4-Chloro-3-methylphenol	-
	-	-	2-Bromophenol	-	-
	-	-	2,4-Dichlorophenol	2,4-Dichlorophenol	-
	-	-	2,3-Dichlorophenol		-
	-	-	2,4,6-Trichlorophenol	2,4,6-Trichlorophenol	-
	-	-	-	2,4,5-Trichlorophenol	-
	-	-	2,4,6-Tribromophenol	-	-
	-	-	-	Pentachlorophenol	-

4 Sample Stability

Laboratories have provided details of the stability of samples and extracts where appropriate as a guide to analysis times. A summary of the storage conditions used during the stability trails can be found in the table below.

Method identifier	A	B	C	D	E
Storage conditions	5 ± 3ºC	5 ± 3ºC	5 ± 3ºC	5 ± 3ºC	5 ± 3ºC
Preservative	Sodium thiosulphate	None	Sodium thiosulphate	Sodium thiosulphate	None
Sample prior to extraction	7 days	7 days	7 days	16 days	7 days
Extract	28 days	8 days	7 days	22 days	14 days

5 Glossary

Accuracy

Degree of agreement of the observed value with the true value of the quantity of interest. Both random and systematic errors can contribute to a reduction in accuracy.

Bias or Trueness

Systematic error – consistent difference between the mean of many measurements and the true value.

Blank

The observed value when measurement is made on a sample identical to the sample of interest, but in the absence of the determinand. Analytical or calibration blanks are used to assess (and correct for) responses other than those caused by the calibration standards.

Calibration

Comparison of responses derived from samples of known value with those from the samples under test.

Collision energy (CE)

The energy applied to the collision cell (Q2) in a triple quadrople mass selective detector to induce further fragmentation of specific ions to form product ions.

Gas Chromatography (GC)

Separation of thermally stable, volatile organic compounds from a complex mixture using an inert gas such as helium or nitrogen as the mobile phase and a liquid stationary phase usually supported on a silica based capillary column. The sample separates by differential partition of the analytes between the mobile and stationary phases, based on relative vapour pressure and solubility in the immobilized liquid stationary phase.

Injection Standard

A compound that is added in a constant amount to all samples and calibration standards after they have been extracted. It can then be used for calibration by plotting the ratio of the analyte signal to the injection standard signal as a function of the analyte concentration of the standards. This will correct for any loss of analyte during analysis.

Internal Standard

A compound that is added in a constant amount to all samples and calibration standards before extraction. It can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This will correct for any loss of analyte during sample preparation or analysis.

Limit of detection (LOD)

The smallest concentration or quantity of a substance which can be expected (at a specified probability level) to be distinguishable (again at a given probability level) from the blank measurement.

Calculated as a multiple of the within batch standard deviation of the data from a blank or low level matrix spike.

Mass Selective Detector (MSD)

Molecules are fragmented by a stream of electrons. The ionised fragments are then sorted by their mass to charge ration (m/z). The ions are then detected by an electron multipler capable of registering the presence of charged particles. Results are displayed as spectra of the relative abundance as a fuction of the mass to charge ratio. Compounds will give a characteristic fragmentation pattern which can be used for identification.

Multiple Reaction Monitoring (MRM)

A method used in a triple quadrupole mass spectrometer in which an ion of a particular mass (precursor) is selected in the first quadruple (Q1). The ion enters a collision cell (Q2) where further fragmentation occurs and a product ion is selected in the second quadrupole (Q3) for detection. Most applications will look for multiple product ions from one or more precursor ions.

Performance Data

See Validation

Precision

The degree of agreement existing between repeated measurements made under specific conditions.

Recovery

The extent to which a known, added quanity of determinand can be measured by an analytical system. It is calculated from the difference between results obtained from a spiked and unspiked aliquot of sample and is usually express as a percentage.

Relative Standard Deviation (RSD)

Relative standard deviation or coefficient of variation is defined as the ratio of the standard deviation (σ) to the mean (μ). It shows the extent of variability in relation to the mean of the population. Usually reported as a percentage.

Reporting Limit (RPL)

The smallest concentration or quantity of a substance that can be reported. Usually higher than the limit of detection to take in to account day to day variation in the method.

Retention Time (RT)

The time taken for a particular compound to travel through the column to the detector. It is calculated as the time taken from injection to detection.

Selective Ion Monitoring (SIM)

A mass spectrometry scanning mode in which only a limited mass-to-charge ratio range is transmitted/detected by the instrument, as opposed to the full spectrum range. This mode of operation typically results in significantly increased sensitivity.

Solid Phase Extraction (SPE)

A sample preparation technique which uses the affinity of solutes dissolved or suspended in a liquid for a solid through which the sample is passed. The analytes of interest in the sample are retained on the stationary phase. They are then removed from the solid phase by rinsing with an appropriate eluent.

Spike

A known quantity of determinand which is added to a sample, usually for the purpose of estimating the systematic error of an analytical system by means of a recovery calculation.

Stability trial

A trial to demonstrate that the maximum permitted delay between sampling and analysis does not result in “a material alteration in the concentration or value for the measurement or observation of which the sample is intended”. For regulatory analysis the appropriate target value is one half of the maximum permitted bias error. For many organic parameters it is 12.5%.

Surrogate Standard

Surrogates are compounds that are similar in chemical composition to the analytes of interest and spiked into all samples prior to preparation and analysis. Surrogate recoveries are used to evaluate matrix interference on a sample-specific basis.

Validation or Performance Testing

The testing required to establish the performance of an analytical system. This data is used to show the system is suitable for its intended purpose and to judge the quality, reliability and consistency of the analytical results.

‘Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO/IEC 17025).’

Water Supply (Water Quality) Regulations

The law requires that drinking water is wholesome and clean. It sets down maximum acceptable concentrations for a number of potential contaminants. Derived from EU Drinking Water Directive.

Within-batch Standard Deviation

A measurement of the precision of analytical results which have been obtained in a single batch of analysis. It is an indication of the short term (depending on how a batch of analysis has been defined) random error of analysis.

A The determination of selected taste & odour compounds in raw and potable waters using liquid/liquid extraction and GC MSMS detection.

A1 Performance characteristics of the method

A1.1	Substances determined	Anisole, 2,4,6-trichloroanisole, geosmin, 2,4,6-tribromoanisole, 2-methylisoborneol, 2-ethyl-5,5-dimethyl-1,3-dioxane and 2-isopropyl-3-methoxypyrazine. This method may be suitable for other additional taste and odour compounds.
A1.2	Type of sample	Raw waters, drinking waters and process waters.
A1.3	Basis of method	Taste & odour compounds are extracted from samples using liquid/liquid extraction using dichloromethane as the extraction solvent and are analysed using gas chromatography with a tandem mass spectrometer using MRM ion monitoring mode.
A1.4	Range of application	Typically up to 40 ng L^{-1}. The range may be extended, see section A7.12.
A1.5	Standard deviation	See Table A1.
A1.6	Limit of detection	Typically the 1ng L^{-1} for 500ml of sample. See Table A1.
A1.7	Bias	See Table A1.
A1.8	Interferences	Any compound that is extracted and elutes under the conditions used, and has similar gas chromatographic and mass spectrometric properties to the taste and odour compounds of interest will interfere.

A2 Principle

The sample, after dilution if required, is spiked with the internal standard and is extracted with dichloromethane. The dichloromethane extract is dried using anhydrous sodium sulphate and heptane is added as a keeper before the extract is evaporated down to 0.5ml. Taste & Odour compounds are determined by GC with a triple quadruple mass spectrometer using multiple reaction monitoring (MRM).

A3 Hazards

Taste & odour compounds are harmful / toxic as a solid material with an extremely strong odour and should be treated with the appropriate care, although in most laboratories they are obtained as a dilute standard in a common solvent.

Methanol is toxic and flammable; acetone and heptane are irritants and flammable. Dichloromethane is a suspect carcinogen. Sodium sulphate and sodium thiosulphate solutions are both irritants. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate.

Waste solvents should be discarded according to documented procedures.

A4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

A4.1 Methanol.

A4.2 Acetone.

A4.3 Dichloromethane.

A4.4 Heptane.

A4.5 Water. Deionised, distilled water or HPLC grade.

A4.6 Sodium sulphate (anhydrous). Dry at 400 ± 50°C for a minimum of 4 hours in a muffle furnace. Allow to cool in a desiccator prior to use. This solid should be stored in a sealed glass bottle at ambient temperature for up to 3 months.

A4.7 10% w/v Sodium thiosulphate. Dissolve 25g of sodium thiosulphate in approximately 150 ml of water, mix well to dissolve and make up to 250ml with water. This solution may be stored at ambient temperature in an amber glass bottle for up to 12 months.

A4.8 Stock calibration taste & odour standard solution (100mg L^{-1}). Commercially certified standard containing the appropriate level of anisole, 2,4,6-trichloroanisole, 2,4,6-tribromoanisole, 2-ethyl-5,5-dimethyl-1,3-dioxane, 2-isopropyl-3-methoxy pyrazine, geosmin and 2-methylisoborneol.

Similar AQC stock standard solution should be obtained, preferably from a different supplier or at least separate lot number.

A4.9 Calibration Spiking Solution (100µg L^{-1}). Add 50µl of stock calibration taste & odour into approximately 45ml of methanol. Make up to 50ml with methanol. This solution may be stored at 5 ± 3ºC for up to 3 months.

An AQC solution should be prepared, preferably by a different member of staff and using reagents from different batches or manufacturers.

A4.10 AQC Spiking Solution (100µg L^{-1}). Add 50µl of stock taste & odour standard solution into approximately 45ml of methanol. Make up to 50ml with methanol. This solution may be stored at 5 ± 3ºC for up to 3 months.

A4.11 2,3,4,5-Tetrachloronaphthalene internal standard (100mg L^{-1}). Commercially certified standards containing 2,3,4,5-tetrachloronaphthalene.

A4.12 Working 2,3,4,5-tetrachloronaphthalene internal standard (100µg L^{-1}). Add 100µl of 2,3,4,5-tetrachloronaphthalene into approximately 90ml of acetone and mix well. Make up to100ml with acetone. This solution may be stored at 5 ± 3ºC for up to 6 months.

A4.13 Blank
A procedural blank is analysed with every batch of samples. 500ml of a suitable bottled or tap water is transferred to a labelled 1 Litre clear glass bottle. This sample should be prepared on the day of use.

A4.14 Calibration standard solutions – For example, for a four point calibration, prepare a series of four 1 Litre clear glass bottles. Label them Cal 1, Cal 2, Cal 3 and Cal 4, and add 500ml of a suitable bottled or tap water to each bottle. Using the table below, add the relevant amount of spiking solution to each bottle.

Standard	Concentration ng L^{-1}	Amount of calibration spiking solution (µl)
Cal 1	2.0	10.0
Cal 2	10.0	50.0
Cal 3	20.0	100.0
Cal 4	40.0	200.0

These solutions should be prepared on the day of use.

A4.15 **AQC Sample –** An AQC sample is analysed with every batch of samples. 500ml of a suitable bottled or tap water is transferred to a labelled 1 Litre clear glass bottle. To this add the required amount of intermediate AQC spiking standard. This sample should be prepared on the day of use.

A5 Apparatus

In addition to normal laboratory glassware the following may be required.

A5.1 Muffle Furnace capable of being set to 400 ± 50°C

A5.2 Glass separating funnels and suitable drying columns.

A5.3 Blow-down apparatus. Any device capable of being set at 25 ± 3°C and can direct a gentle stream of nitrogen or air into a vial.

A5.4 GC MSMS system. Fitted with a tandem mass spectrometer capable of operating in Multiple Reaction Monitoring (MRM) mode and an appropriate data station. The performance data (see tables A1 & A2) was derived using an Agilent GC and Waters MSMS system and the conditions listed below, other systems are available but the suitability of the equipment should be evaluated.

GC Instrument conditions.

Oven/Inlet Parameters:

Initial Temp:	50°C
Initial Hold:	1 min
Ramp 1:	25.0°C min^{-1} to 100°C hold for 0.0 min
Ramp 2:	50.0°C min^{-1} to 300°C hold for 4.0 min
Total Run Time:	11.00 min
Initial Inlet Temp:	250°C
Inlet Type:	Splitless

Carrier Parameters:

Inlet Mode:	Constant Flow
Column:	DB-5MS or equivalent
Length:	30m
Diameter:	0.25mm
Film Thickness:	0.25µm
Gas Type:	Helium
Inlet Initial Flow:	1.0mL min^{-1}
Injection Volume:	2.0µL

MS Instrument conditions:

Source Temperature:	200°C
Transfer Line Temperature:	250°C

Compound	Approximate retention time (minutes)	Ions monitored		Collision energy (eV)
		Precursor	Product	
anisole	4.09	108	78	5.00
2-ethyl-5,5-dimethyl-1,3-dioxane	4.11	115	69	5.00
2-isopropyl-3-methoxypyrazine	4.78	152	137	10.00
2-methylisoborneol	5.27	108	93	10.00
2,4,6-trichloroanisole	5.74	195	167	10.00
geosmin	6.07	112	112	5.00
2,4,6-tribromoanisole	6.76	344	301	25.00
2,3,4,5-tetrachloronaphthalene (IS)	7.68	264	194	25.00

A6 Sample collection and preparation

Samples may be taken in 1 Litre clear glass bottles with caps fitted with a PTFE liner containing 0.24ml of 10% sodium thiosulphate solution. Samples may be stored for up to 7 days in glass bottles at 5 ± 3°C. The sample extracts are stable for 28 days when stored at 5 ± 3°C.

A7 Analytical procedure

A7.1 Rinse all glassware with dichloromethane before commencing this procedure.

A7.2 Rinse a sintered drying tube containing approximately 4 to 6cm of anhydrous sodium sulphate with approximately 20ml of dichloromethane. Discard the washings.

A7.3 Transfer 500ml of sample, using a measuring cylinder, into a labelled separating funnel. Add 100µl of working 2,3,4,5-tetrachloronaphthalene internal standard.

A7.4 Add 25ml of dichloromethane to the sample. Shake the separating funnel gently using a shaker for 5 minutes set at 175rpm. Carefully release any pressure build-up in the separating funnel.

A7.5 Allow the layers to separate for a minimum of 2 minutes. Pour the dichloromethane layer through the drying column and collect the extract in a suitable evaporating tube. If an emulsion is formed, the dichloromethane layer can be separated by centrifuging the extract; centrifuge at a speed of 2500rpm for a minimum of 5 minutes.

A7.6 Repeat stages A7.4 to A7.5 collecting the extract in the same evaporating tube.

A7.7 Rinse the drying column with 20ml dichloromethane and collect in the evaporating tube. Add 5ml of heptane to the extract and mix.

A7.8 Place the evaporating tube into the blow down apparatus which has been set at 25 ± 3°C. Evaporate the solvent in the tube to 0.5ml, this typically takes 45 minutes.

A7.9 Transfer the extract to a labelled 2ml glass vial and cap. At this stage the solution may be stored in a refrigerator for up to 28 days before analysis.

A7.10 Analyse the blank, working calibration standards and AQC using the entire procedure as described in sections A7.1 - A7.9.

A7.11 Set up the GCMS system according to manufacturer's instructions. Using the four calibration solutions i.e. 2, 10, 20 and 40 ng L^{-1}, construct a calibration graph of response versus amount of component, monitoring the ions referred to in A5.4.

A7.12 Analyse the sample extract and from the calibration graph, obtain the amount, Av, of taste and odour components in the vial and then calculate the concentration, Cs, of taste and odour compounds in the sample. If the response exceeds the calibration range, the analysis may be repeated using a smaller amount of sample and making the volume to 500 ml with ultrapure water.

A8 Calculation

From the calibration graph determine the amount, Av, of component in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = Av \times (500/Vs)$$

Cs is the concentration (ng L^{-1}) of in the sample;
Av is the amount (ng L^{-1}) of obtained from the graph; and
Vs is the sample volume taken (see section A7.12).

Blank correction is not normally necessary but should be considered if the blank control sample is above the limit of detection.

A9 Performance data

Table A1 Potable water standard (2ng L^{-1})

Compound	Mean	Std Dev	% Bias	RSD (%)	LOD
Anisole	1.94	0.2294	-3.0	11.8	1.04
2,4,6-Trichloroanisole	1.91	0.0905	-4.5	4.7	0.42
2,4,6-Tribromoanisole	2.09	0.1457	4.5	7.0	0.68
2-Ethyl-5,5-dimethyl-1,3-dioxane	1.82	0.1124	-0.9	6.2	0.52
Isopropyl-3-methoxypyrazine	1.82	0.1013	-9.0	5.6	0.47
Geosmin	1.83	0.1226	-8.5	6.7	0.57
2-Methylisoborneol	1.95	0.2406	-2.5	12.3	1.12

All values expressed as ng L^{-1} unless otherwise stated.
Std Dev is standard deviation.
RSD is relative standard deviation
LOD is limit of detection (4.53 x within batch standard deviation)
Data on a soft potable water is provided by Scottish Water Scientific Services, Edinburgh.

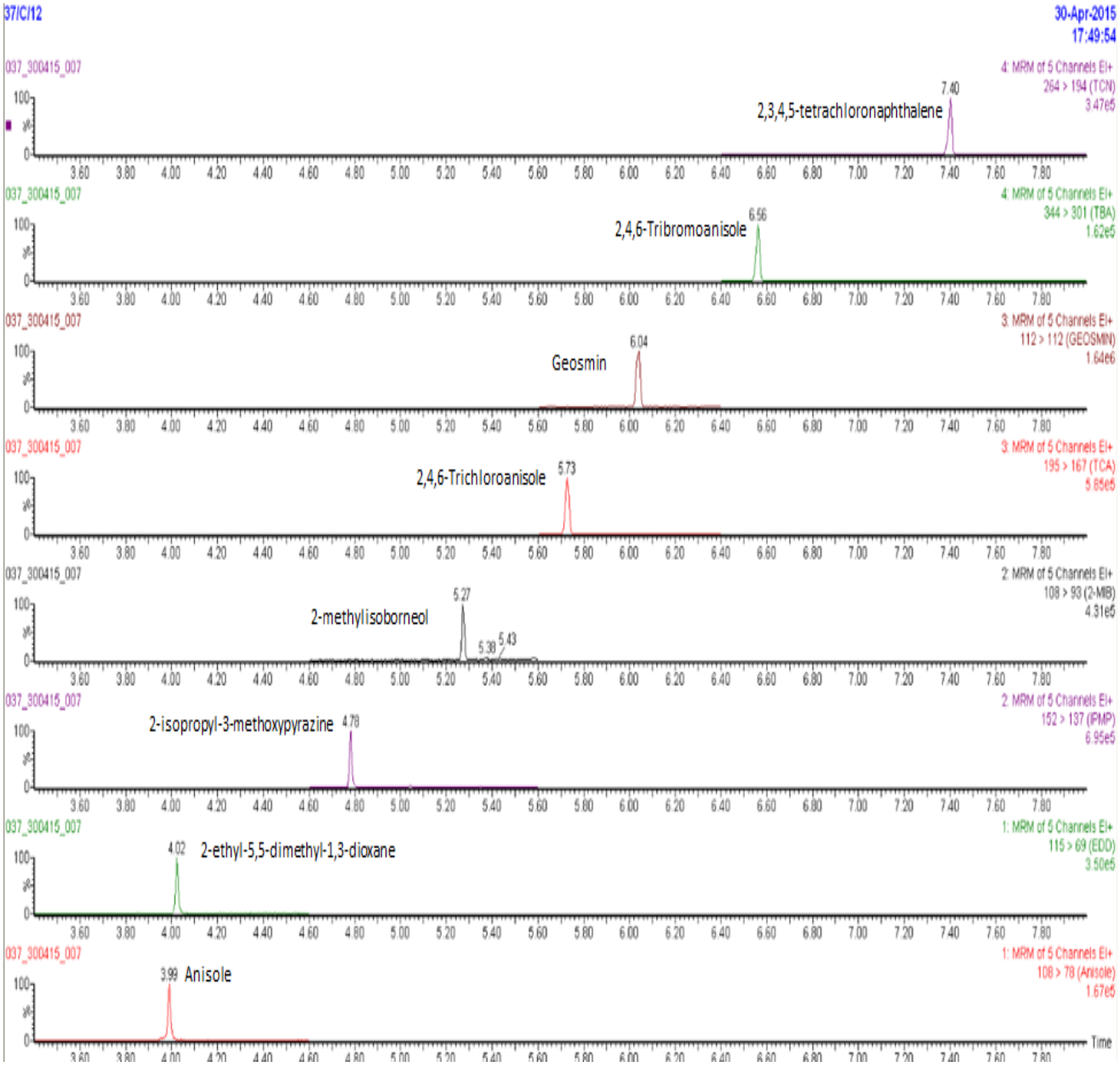
Table A2 Potable water standard (20ng L^{-1})

Compound	Mean	Std Dev	Bias (%)	RSD (%)
Anisole	19.66	1.677	-1.7	8.5
2,4,6-Trichloroanisole	19.93	0.975	-0.4	4.9
2,4,6-Tribromoanisole	20.60	0.568	3.0	2.8
2-Ethyl-5,5-dimethyl-1,3-dioxane	18.50	1.033	-7.5	5.6
Isopropyl-3-methoxypyrazine	19.28	0.927	-3.6	4.8
Geosmin	18.08	0.765	-9.6	4.2
2-Methylisoborneol	18.76	1.419	-6.2	7.6

All values expressed as ng L^{-1} unless otherwise stated.
Std Dev is standard deviation.
RSD is relative standard deviation

Data on a soft potable water is provided by Scottish Water Scientific Services, Edinburgh.

Figure A1 Typical chromatogram of taste and odour components
(Concentration of 40ng L^{-1} extracted standard – 40µg L^{-1} in extract)



B The determination of selected taste and odour causing contaminants in raw and potable waters by solid phase extraction and GC MSMS detection.

B1 Performance characteristics of the method

B1.1	Substances determined	2-methylisoborneol (2-MIB), 2-chloroanisole, 3-chloroanisole, 4-chloroanisole, geosmin, 2,4,6-trichloroanisole, 2,3,4-trichloroanisole, 2,4,6-tribromoanisole, 2-isobutyl-3-methoxy pyrazine and 2-isopropyl-3-methoxypyrazine
B1.2	Type of sample	Raw and potable waters. The method may (with suitable adaptation) be used for other types of water.
B1.3	Basis of method	An aliquot of sample is passed through a conditioned solid phase extraction cartridge (SPE). The dried cartridge is eluted with dichloromethane and internal standard added. The extract is then ready for gas chromatography (GC) with a tandem mass spectrometric (MSMS) detection operating in multiple reaction monitoring (MRM) mode.
B1.4	Range of application	Typically up to 25 ng L^{-1}, but can be extended, see section B7.11.
B1.5	Standard deviation	See Table B2.
B1.6	Limit of detection	Typically, 0.57 ng L^{-1} of 2-methylisoborneol and 0.3 ng L^{-1} of geosmin can be detected using 500ml of sample. 0.2 to 0.70 ng L^{-1} can be achieved for the other compounds, see table B1.
B1.7	Bias	See Table B2.
B1.8	Interferences	Any substance which is co-extracted under the conditions used and which exhibits similar chromatographic behaviour to compounds of interest, by producing the same mass ion fragments at the same retention time, has the potential to interfere.

B2 Principle

500ml of sample is passed through a conditioned C18 solid phase extraction cartridge. The cartridge is dried under vacuum and the analytes are eluted from the cartridge with dichloromethane and injection standard added. The extract is then ready for gas chromatography (GC) with tandem mass spectrometric (MSMS) detection using electron impact (EI) ionisation mode.

B3 Hazards

Taste & Odour compounds are harmful / toxic as a solid material with an extremely strong odour and should be treated with the appropriate care, although in most laboratories it is obtained as a dilute standard in a common solvent.

All samples are to be treated as potentially contaminated. Care should be taken and laboratory coats, safety spectacles and gloves must be worn whenever handling samples and carrying out this procedure.

Methanol is toxic and flammable. Acetone is an irritant and flammable. Dichloromethane is a suspect carcinogen. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate.

B4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

B4.1 Dichloromethane

B4.2 Methanol

B4.3 Acetone

B4.4 Calibration stock solution – Custom mix or individual solutions at 100mg L^{-1} in methanol or suitable equivalent. The mixture should contain 2-methylisoborneol, geosmin, 2-chloroanisole, 3-chloroanisole, 4-chloroanisole, 2,4,6-trichloroanisole, 2,3,4-trichloroanisole, 2,4,6-tribromoanisole, 2-isobutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine.

The solution should be stored according to manufacturer's instructions.

Similar AQC stock standard solutions should be obtained, preferably from a different supplier or at least separate lot number.

B4.5 High level calibration standard solution (100 µg L^{-1}) – Partially fill a 50ml volumetric flask with methanol and add 50µl of each of the individual stock solutions. Make up to the mark with methanol and mix by inversion. This solution may be stored at 5 ± 3°C for up to 1 month.

B4.6 Low level calibration standard solution (10 µg L^{-1}) – Partially fill a 10ml volumetric flask with methanol and add 1000µl of the high level calibration standard solution. Make up to the mark with methanol and mix by inversion. This solution may be stored at 5 ± 3°C for up to 1 month.

An AQC solution should be prepared, preferably by a different member of staff and using reagents from different batches or manufacturers.

B4.7 Working AQC standard solution (50µg L^{-1} for MIB & 100µg L^{-1} for all other compounds) – Partially fill a 50ml volumetric flask with methanol and add 25µl of 2-

methylisoborneol and 50µl of each of the remaining stock solutions. Make up to the mark with methanol and mix by inversion. This solution may be stored at 5 ± 3°C for up to 1 month.

B4.8 Stock injection standard solution (100 mg L^{-1}) – Weigh 10mg of triphenylethylene into a weighing boat and quantitatively transfer to a 100ml volumetric flask partially filled with acetone. Make up to the mark with acetone and mix thoroughly by inversion to ensure all the triphenylethylene has dissolved. This solution may be stored at 5 ± 3ºC for up to 6 months.

The solution may be available commercially and should be stored according to manufacturer's instructions.

B4.9 Working injection standard solution (10 mg L^{-1}) – Partially fill a 50ml volumetric flask with dichloromethane and add 5ml of stock injection standard solution. Make up to the mark with dichloromethane and mix thoroughly by inversion. This solution may be stored at 5 ± 3ºC for up to 3 months.

B4.10 Calibration standard solutions – For example, for a six point calibration, prepare a series of six 500 ml amber glass bottles. Label them Cal 1, Cal 2, Cal 3, Cal 4, Cal 5 and Cal 6 and add 500ml of deionised water to each bottle. Using the table below, add the relevant amount of spiking solution to each bottle.

Standard	Concentration ng L^{-1}	Amount of low level calibration solution (µl)	Amount of high level calibration solution (µl)
Cal 1	1.0	50.0	-
Cal 2	5.0	-	25.0
Cal 3	10.0	-	50.0
Cal 4	15.0	-	75.0
Cal 5	20.0	-	100.0
Cal 6	25.0	-	125.0

These solutions should be prepared on the day of use.

B4.11 Working AQC standard (5ng L^{-1} MIB & 10ng L^{-1} for all other compounds) – Take a suitably labelled 500ml amber glass bottle and add 500ml of deionised water. Add 50µl of Working AQC solution. This solution should be prepared on the day of use.

B4.12 Blank – Take a suitably labelled 500ml amber glass bottle and add 500ml of deionised water. The blank should be prepared on the day of use.

B5 Apparatus

In addition to normal laboratory glassware (grade B or better) the following will be required.

B5.1 SPE cartridges – For example, 6 ml, 200 mg C18 cartridges (Phenomenex), or suitable equivalent.

B5.2 Vacuum manifold.

B5.3 Glass vials.

B5.4 Vortex mixer

B5.5 GC MSMS system. Fitted with a tandem mass spectrometer capable of operating in Multiple Reaction Monitoring (MRM) mode and an appropriate data station.
The performance data (see tables B1 & B2) was derived using an Agilent GC MSMS system and the conditions listed below, other systems are available but the suitability of the equipment should be evaluated.

Columns: HP-5MS Ultra-inert, 30m x 0.25mm, 0.25µm, Agilent (19091S-433UI), or suitable equivalent.

Carrier gas: Helium, constant flow at 2ml per minute.

Injection volume: 10µl (Multimode inlet in solvent vent injection mode)

Temperature programmes:
Inlet: 40 °C, hold for 0.11 minutes.
Ramp 1 600ºC min^{-1} to 350ºC.

Oven Initial temperature at 35 °C for 1 minute, then 10 °C per minute to 150 °C, then 40 °C per minute to 270 °C and hold for 5 minutes,

MSMS parameters

Compound	Retention time	Precursor ion	Product ion	Dwell	Collision energy	Quantification Ion
2-Isopropyl-3-methoxypyrazine	8.17	137	109.1	30	5	
2-Isopropyl-3-methoxypyrazine	8.17	137	105	100	20	Y
3-Chloroanisole	8.21	142	77	60	25	Y
3-Chloroanisole	8.21	142	112	30	10	
4-Chloroanisole	8.36	142	99	60	25	Y
4-Chloroanisole	8.36	142	127	30	10	
2-Chloroanisole	8.61	142	99	60	25	Y
2-Chloroanisole	8.61	142	127	30	10	
2-Isobutyl-3-methoxypyrazine	9.46	124	94	100	10	Y
2-Isobutyl-3-methoxypyrazine	9.46	124	81	50	10	
2MIB	9.52	95.1	67	100	10	
2MIB	9.52	95.1	55.1	75	20	Y
2,4,6-Trichloroanisole	11.58	210	194.9	50	10	Y
2,4,6-Trichloroanisole	11.58	210	166.9	50	25	
Geosmin	12.61	112.1	97.1	60	10	Y
Geosmin	12.61	112.1	83	25	10	
2,3,4-Trichloroanisole	13.52	210	194.9	50	10	Y
2,3,4-Trichloroanisole	13.52	210	166.9	50	25	
2,4,6-Tribromoanisole	14.16	344	329	100	15	Y
2,4,6-Tribromoanisole	14.16	346	303	50	35	
Triphenylethylene	16.00	256	178	30	20	

B6 Sample collection and preservation

Samples should be collected in a 1-litre amber glass bottle. Samples should be extracted as soon as possible after being received in the Laboratory. If analysis cannot be undertaken immediately, samples may be stored at 5 ± 3ºC for a maximum of 7 days. The sample extracts can be stored for up to 8 days at 5 ± 3ºC.

B7 Analytical procedure

B7.1 Allow the samples to reach room temperature before proceeding. Transfer a suitable aliquot of sample, typically 500 ml, into a clean bottle.

B7.2 For each blank, AQC, sample and standard, condition a labelled SPE cartridge by passing approximately 5 ml of methanol through the cartridge under gravity. Do not let the meniscus of the solvent to go below the level of the cartridge packing material. Discard the washings. Repeat this process with two further washes of methanol.

B7.3 Repeat step B7.2 using three 5ml aliquots of distilled water.

B7.4 Using vacuum, pass the standards, AQC, blank and samples through the respective cartridges. The flow rate should be approximately 10 ml per minute.

B7.5 When all of the sample has passed through the cartridge, use 5 ml of deionised water to rinse the sample bottle, and allow it to pass through the cartridge.

B7.6 Dry the cartridge, this process should take about 60 minutes and may be assisted with vacuum or by passing nitrogen through the cartridge. Ensure the cartridge is thoroughly dry before continuing.

B7.7 Add 1 ml of dichloromethane to each SPE cartridge. Allow the dichloromethane to soak into the cartridge packing material for about 10 minutes. Continue the elution under gravity, collecting the eluate in a suitable container.

B7.8 Add a further 1 ml of dichloromethane collecting the eluate in the same container. Use a syringe to push out any residual solvent from the cartridge.

B7.9 Add 25µl of working injection standard solution and mix on the vortex mixer for approximately 3 seconds.

Transfer the extract to a suitably labelled auto sampler vial and cap ready for analysis.

B7.10 Set up the GC MSMS system according to manufacturer's instructions. Using the calibration standard solutions and the conditions described in section B5.5, construct a calibration graph of response versus amount for each individual compound. This equates to 0.0, 1.0, 5.0, 10.0, 15.0, 20.0 and 25.0 ng L^{-1}.

B7.11 Analyse the blank, sample and AQC extracts using the procedure described in step B7.10. If the response exceeds the calibration range, the analysis should be repeated using a smaller amount of sample (B7.1) and making the volume to 500 ml with deionised water.

B8 Calculations

Determine the concentration, Cs, in the sample using the equation:

$$Cs = Av \times (500/Vs)$$

where

Cs is the concentration (ng L^{-1}) of analyte in the sample;
Av is the amount (ng L^{-1}) of analyte obtained from the calibration graph;
Vs is the volume (ml) of sample analysed (B7.1) taking into account any dilution of the sample

Table B1 Performance data – Limit of Detection (LOD)

Compound	LOD (ng L^{-1})
2-Isopropyl-3-methoxypyrazine	0.72
3-Chloroanisole	0.28
4-Chloroanisole	0.21
2-Chloroanisole	0.23
2-Isobutyl-3-methoxypyrazine	0.21
2-Methylisoborneol	0.57
2,4,6-Trichloroanisole	0.38
Geosmin	0.30
2,3,4-Trichloroanisole	0.25
2,4,6-Tribromoanisole	0.31

Limit of detection = 3 x within batch standard deviation of water samples spiked at 1ng L^{-1}.

Table B2 Performance data – Medium Water

Low Sample	Mean (ng L^{-1})	Standard deviation	Bias (%)	Precision (%)
2-Isopropyl-3-methoxypyrazine	5.478	0.609	3.04	22.24
3-Chloroanisole	5.358	0.429	0.31	16.01
4-Chloroanisole	5.115	0.381	1.54	14.90
2-Chloroanisole	4.657	0.178	-9.62	7.65
2-Isobutyl-3-methoxypyrazine	5.115	0.212	0.09	8.31
2-Methylisoborneol	5.761	0.652	9.95	22.64
2,4,6-Trichloroanisole	5.178	0.571	1.11	22.04
Geosmin	5.406	0.347	-3.21	12.83
2,3,4-Trichloroanisole	4.933	0.260	-4.60	10.54
2,4,6-Tribromoanisole	4.711	0.299	-8.49	12.68

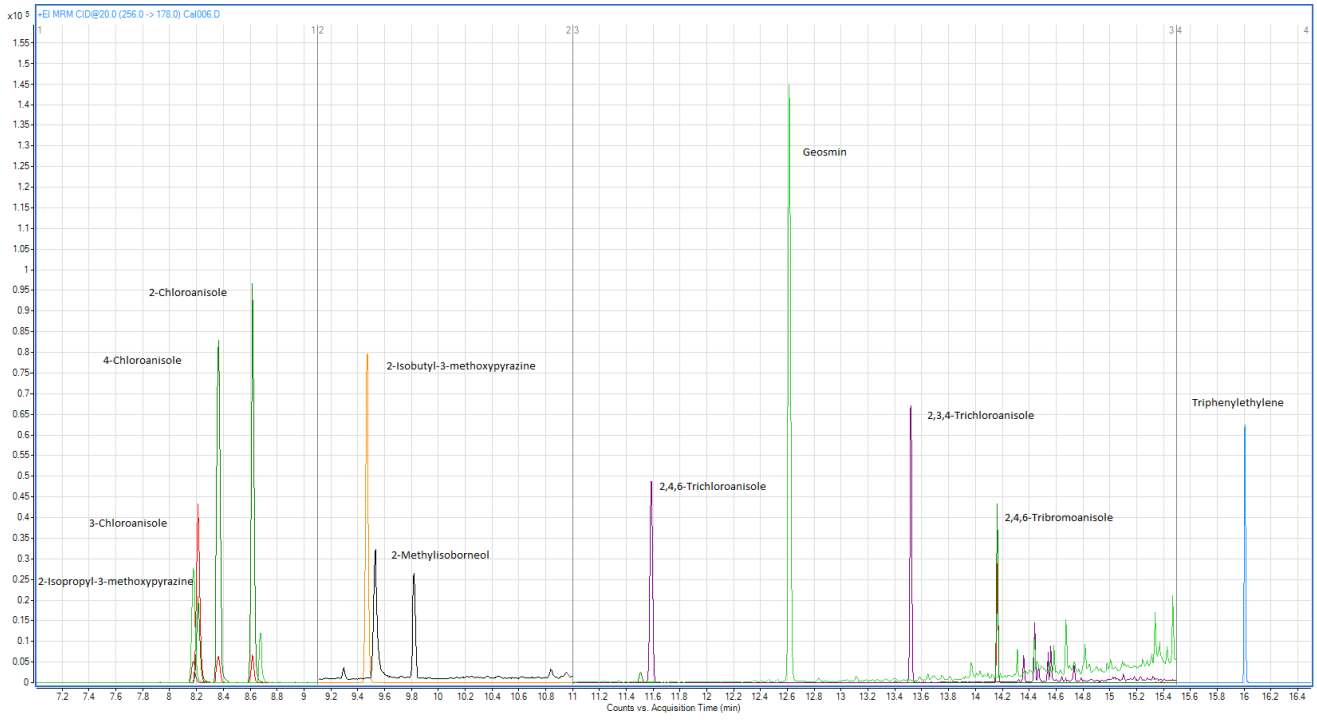
High Sample	Mean (ng L^{-1})	Standard deviation	Bias (%)	Precision (%)
2-Isopropyl-3-methoxypyrazine	20.315	1.855	-0.01	18.26
3-Chloroanisole	19.562	1.239	-3.83	12.67
4-Chloroanisole	20.061	1.208	0.12	12.04
2-Chloroanisole	17.623	1.114	-12.55	12.64
2-Isobutyl-3-methoxypyrazine	20.074	1.316	-0.19	13.12
2-Methylisoborneol	20.709	2.022	2.32	19.53
2,4,6-Trichloroanisole	20.103	1.425	-0.09	14.18
Geosmin	20.121	1.333	-2.26	13.25
2,3,4-Trichloroanisole	19.753	1.210	-2.07	12.26
2,4,6-Tribromoanisole	19.285	1.217	-4.28	12.62

Low sample was Sluvad Final Surface water spiked at 5 ng L^{-1}
High sample was Sluvad Final Surface water spiked at 20 ng L^{-1}
Bias is calculated as the percentage difference between the mean value of a large number of repeated measurements and the true value.
Precision is calculated as twice the standard deviation (within a batch and between batches) of the spread of results about the mean.

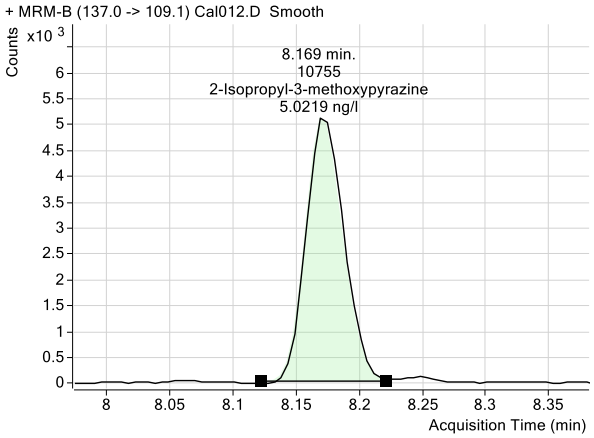
Data provided by Dwr Cymru Welsh Water.

Figure B1 Typical spectra/chromatograms

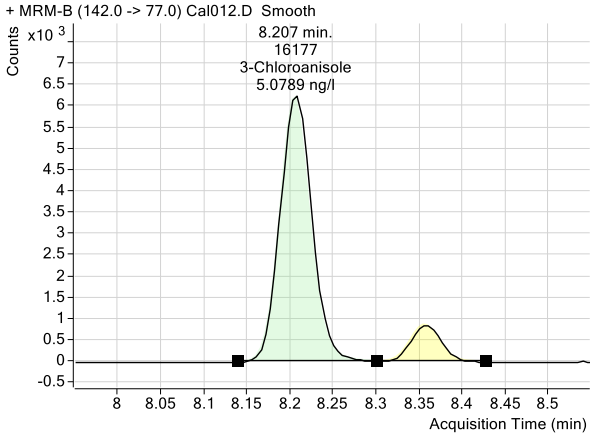
Typical Chromatogram



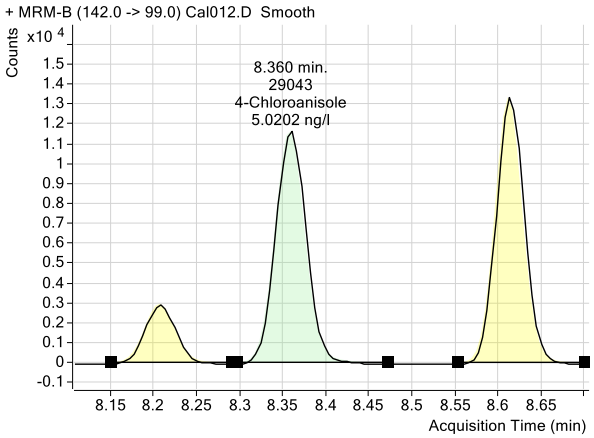
5ng L^{-1} 2-Isopropyl-3-methoxypyrazine standard



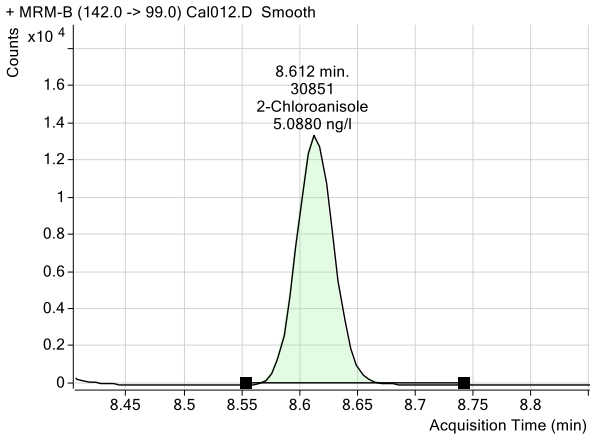
5ng L^{-1} 3-Chloroanisole standard



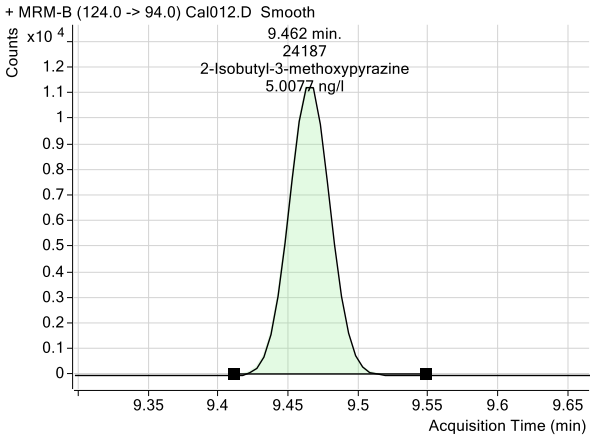
5ng L^{-1} 4-Chloroanisole standard



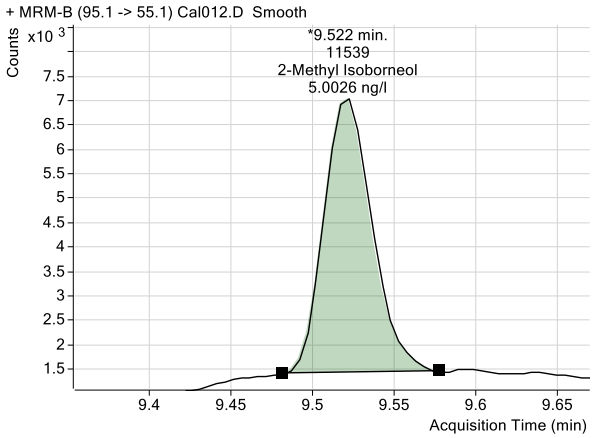
5ng L^{-1} 2-Chloroanisole standard



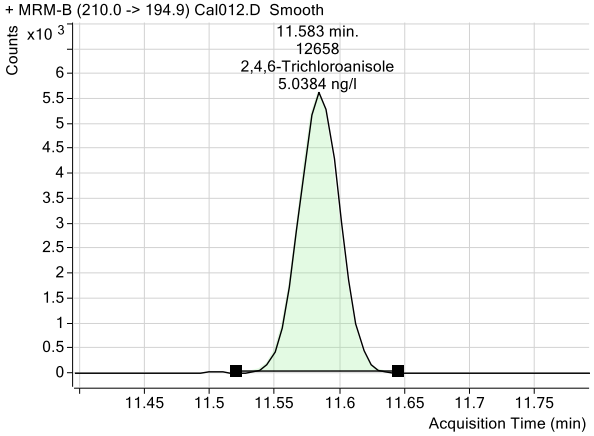
5ng L^{-1} 2-Isobutyl-3-methoxypyrazine standard



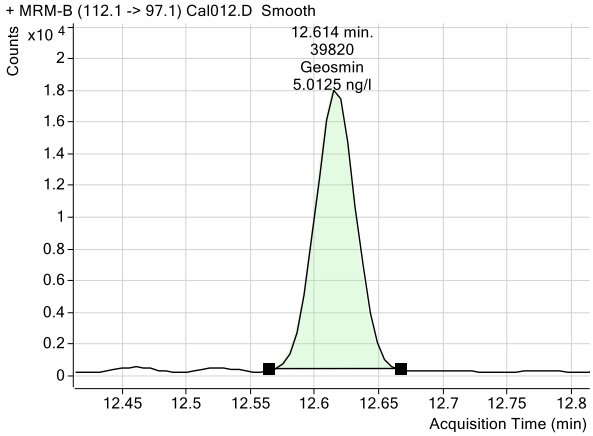
5ng L^{-1} 2-Methyl isoborneol standard



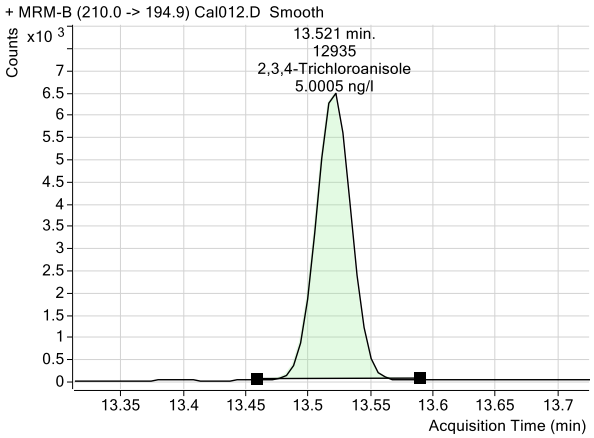
5ng L^{-1} 2,4,6-Trichloroanisole standard



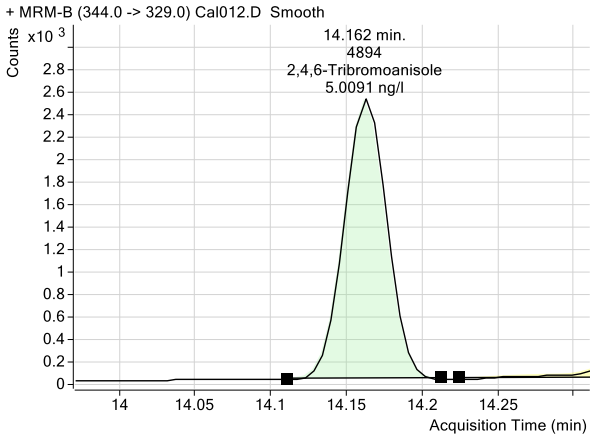
5ng L^{-1} Geosmin standard



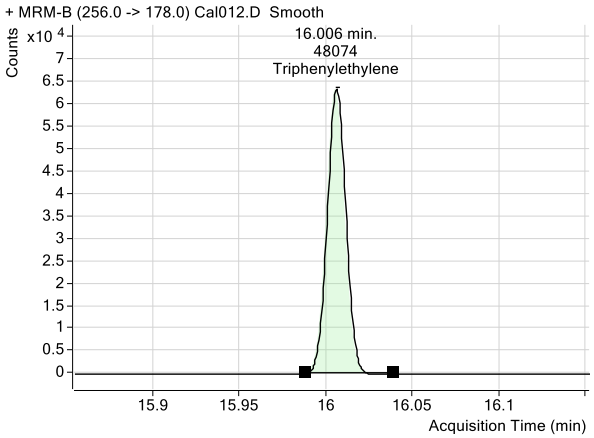
5ng L^{-1} 2,3,4-Trichloroanisole standard



5ng L^{-1} 2,4,6-Tribromoanisole standard



Triphenylethylene injection standard



C The determination of selected taste & odour compounds in raw and potable waters using liquid/liquid extraction and GCMS detection.

C1 Performance characteristics of the method

C1.1	Substances determined	phenol, 2-chlorophenol, 2-bromophenol, 3-chloroanisole, 4-chloroanisole, 2-chloroanisole, 2,4-dichlorophenol, 2-isopropyl-3-methoxypyrazine, 2,3-dichlorophenol, 2-methylisoborneol, 2-isobutyl-3-methoxypyrazine, geosmin, 2,4,6-trichloroanisole, 2,4,6-trichlorophenol, 2,6-dibromophenol, 2,3,4-trichloroanisole, 2,4,6-tribromophenol, 2,4,6-tribromoanisole. This method may be suitable for other additional taste and odour compounds.
C1.2	Type of sample	Raw, drinking and process waters. The method may (with suitable adaptation) be used for other types of water.
C1.3	Basis of method	Taste & odour compounds are extracted from the sample by a liquid/liquid extraction technique using dichloromethane as the extraction solvent. Analysis is by gas chromatography with mass spectrometric detection using Selective Ion Monitoring (SIM).
C1.4	Range of application	Typically up to 50 ng L^{-1}. The range may be extended.
C1.5	Standard deviation	See Table C1.
C1.6	Limit of detection	See Table C1 for 1000ml of sample.
C1.7	Bias	See Table C1.
C1.8	Interferences	Any compound that is extracted and elutes under the conditions used, and has similar gas chromatographic and mass spectrometric properties to the compounds of interest will interfere.

C2 Principle

900ml of sample is acidified to approximately pH 2 in the bottle and then extracted with dichloromethane. The solvent layer is collected into a turbovap tube and concentrated to 1.0ml.The sample extract is transferred to an auto sampler vial and injection standard added. The vial is capped ready for analysis. The taste & odour compounds are determined by GC with mass spectrometer using Selective Ion Monitoring (SIM).

C3 Hazards

Taste & odour compounds are harmful / toxic as a solid material with an extremely strong odour and should be treated with the appropriate care, although in most laboratories it is obtained as a dilute standard in a common solvent.

Methanol is toxic and flammable; acetone is an irritant and flammable, dichloromethane is a suspected carcinogen. Sodium sulphate and sodium thiosulphate solution are both irritants. Hydrochloric acid is corrosive and harmful. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate.

Waste solvents should be discarded according to documented procedures.

C4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

C4.1 Methanol.

C4.2 Acetone.

C4.3 Dichloromethane.

C4.4 Hydrochloric Acid (12.5% v/v)

C4.5 Water. Deionised, distilled water or HPLC grade.

C4.6 Sodium sulphate (anhydrous). Dry at 500 ± 50°C for a minimum of 4 hours in a muffle furnace. Allow to cool in a desiccator prior to use. This solid should be stored in a sealed glass bottle at ambient temperature for up to 3 months.

C4.7 Stock calibration taste & odour standard solution (100mg L^{-1}). Commercially certified standard containing the appropriate levels of phenol, 2-chlorophenol, 2-bromophenol, 2-isopropyl-3-methoxypyrazine, 3-chloroanisole, 4-chloroanisole, 2-chloroanisole, 2,4-dichlorophenol, 2,3-dichlorophenol, 2-isobutyl-3-methoxypyrazine, 2-methylisoborneol, 2,4,6-trichloroanisole, 2,4,6-trichlorophenol, 2,6-dibromophenol, geosmin, 2,3,4-trichloroanisole, 2,4,6-tribromophenol, 2,4,6-tribromoanisole in dichloromethane.

Similar AQC stock standard solutions should obtained, preferably from a different supplier or at least separate lot number

C4.8 Calibration spiking solution (100µg L^{-1}). Add 100µl of stock calibration taste & odour (100mg L^{-1}) into approximately 50ml of acetone. Make up to 100ml with acetone. This solution may be stored at 5 ± 3ºC for up to 3 months.

An AQC solution should be prepared, preferably by a different member of staff and using reagents from different batches or manufacturers.

C4.9 AQC spiking solution (100µg L^{-1}). Add 100µl of stock Taste & Odour (100mg L^{-1}) into approximately 50ml of acetone. Make up to 100ml with acetone. This solution may be stored at 5 ± 3ºC for up to 3 months.

C4.10 D8-naphthalene stock solution (2000mg L^{-1}). Commercially certified standards containing D8-naphthalene in dichloromethane.

C4.11 D8-naphthalene injection solution (10mg L^{-1}). Add 500µl of D8-naphthalene (2000mg L^{-1}) into approximately 50ml of methanol. Make up to 100ml with methanol. This solution may be stored at 5 ± 3ºC for up to 1 year.

C4.12 Blank
A procedural blank is analysed with every batch of samples. 900ml of mineral water is transferred to a suitably labelled 1 litre amber glass bottle. This sample should be prepared on the day of use.

C4.13 Calibration standard solutions – For example, for a three point calibration, prepare a series of three 1 litre amber glass bottles. Label them Cal 1, Cal 2 and Cal 3, and add 900ml of deionised water to each bottle. Using the table below, add the relevant amount of spiking solution to each bottle.

Standard	Concentration ng L^{-1}	Amount of calibration spiking solution (µl)
Cal 1	10.0	90.0
Cal 2	25.0	225.0
Cal 3	50.0	450.0

These solutions should be prepared on the day of use.

C4.14 AQC Sample – 40ng L^{-1} - An AQC sample is analysed with every batch of samples. 900ml of mineral water is transferred to a suitably labelled 1 litre amber glass bottle to which 360µl of AQC spiking solution is added. This sample should be prepared on the day of use.

C5 Apparatus

In addition to normal laboratory glassware the following may be required.

C5.1 Muffle furnace capable of being set to 500 ± 50°C

C5.2 2 litre glass separating funnel

C5.3 Blow-down apparatus. Any device (capable of being set at 40 ± 2.5 °C) that can direct a gentle stream of nitrogen or air into a vial. For example Turbovap system.

C5.4 Horizon dry disk filter apparatus and dry disk membranes

C5.5 GC system with a split/splitless injector capable of operating in pulse splitless mode, fitted with a mass selective detector capable of operating in SIM mode, with an appropriate data station. The performance data (see table C1) was derived using an Agilent GC MS system and the conditions listed below, other systems are available but the suitability of the equipment should be evaluated.

Oven/Inlet Parameters:

Initial Temp:	50°C
Initial Hold:	1 min
Ramp 1:	10.0°C min^{-1} to 150°C hold for 0.0 min
Ramp 2:	40.0°C min^{-1} to 280°C hold for 0.0 min
Total Run Time:	14.25 min
Initial Inlet Temp:	250°C
Inlet Type:	Pulse Splitless, Injection pulse pressure 20 psi until 0.4 min, Purge flow to split vent 50ml min^{-1} at 2.00 min.

Carrier Parameters:

Inlet Mode:	Constant Flow
Column:	DB-5MS or equivalent
Length:	30m
Diameter:	0.250mm
Film Thickness:	0.25µm
Gas Type:	Helium
Column pressure:	9.73 psi (approximately)
Column flow:	1.2ml min^{-1}
Injection Volume:	1.0µL

MS Instrument conditions:

Source Temperature:	230°C
Quad Temperature:	150°C
Transfer Line Temperature:	280°C

Analysis is performed in Single ion monitoring mode on the MS, with the following conditions.

Dwell time per ion	40msec (all ions)
Resolution	Low

Compound	Group	Approximate Retention Time (min)	Quantitation Ion	Qualifier ions
Phenol	1	6.49	94.0	66.0, 65.0
2-Chlorophenol	1	6.77	127.9	129.9, 64.0
2-Bromophenol	2	8.05	172.1	174.1, 65.3
2-Isopropyl-3-methoxypyrazine	2	8.31	137.3	124.0, 152.0
3-Chloroanisole	2	8.47	142.2	112.2, 99.3
4-Chloroanisole	2	8.64	142.2	127.2, 99.3
2-Chloroanisole	2	8.82	142.2	127.2, 99.3
2,4-Dichlorophenol	3	9.58	162.1	164.1, 98.9
2,3-Dichlorophenol	3	9.70	162.1	164.1
2-Isobutyl-3-methoxypyrazine	3	9.66	124.3	151.3
d8 Naphthalene	3	9.88	136.0	108.0
2-Methylisoborneol	3	10.00	95.3	108.3

2,4,6-Trichloroanisole	4	11.75	195.1	210.0
2,4,6-Trichlorophenol	4	11.97	196.1	132.2, 97.2
2,6-Dibromophenol	4	12.22	252.0	250.0
Geosmin	4	12.55	112.3	97.3, 125.3
2,3,4-Trichloroanisole	4	13.04	210.0	195.1
2,4,6-Tribromoanisole	5	13.65	345.9	347.9, 300.9
2,4,6-Tribromophenol	5	13.76	329.7	331.7, 327.6

C6 Sample collection and preservation

Samples are collected in 1 litre amber glass bottles, containing sodium thiosulphate as a preservative. The bottles stored at 5 ± 3°C on receipt at the laboratory. The samples are typically extracted within 7 days of sampling and the extracts analysed no later than 7 days after extraction.

C7 Analytical procedure

C7.1 Approximately 900ml of sample is required for extraction. If the sample bottle is received full, shake the bottle thoroughly and decant to the shoulder of the bottle. The sample is then placed on a top pan balance and the weight recorded.

C7.2 The sample is acidified to approximately pH 2.0 by the addition of 12.5% HCl solution using a Pasteur pipette.

C7.3 Approximately 100ml of dichloromethane is added to the sample bottle using a dispenser or measuring cylinder.

C7.4 The sample is shaken by hand for approximately 3-5 mins. Alternatively it can be placed on a mechanical shaker for 30 minutes, at a speed of 180rpm and then shaken by hand for approximately 1 minute to ensure complete emulsification.

C7.5 A folded filter paper is placed into either a filter funnel and filled with anhydrous sodium sulphate. The anhydrous sodium sulphate is rinsed with approximately 50ml of dichloromethane and the solvent discarded. Alternatively a dry disk apparatus can be used. A reusable dry disk is used replacing the filter paper and sodium sulphate, the sample is filtered under vacuum at 15 ± 1" Hg. The disks are reusable but should be replaced when there is evidence of water droplets in the solvent layer.

C7.6 The shaken sample is transferred into a separating funnel.

C7.7 The weight of the empty sample bottle is taken, recorded and the volume of sample used calculated.

C7.8 The sample is allowed to settle so that organic layer can be separated from the aqueous layer. The lower solvent layer is then passed through the funnel containing the sodium sulphate and collecting in a Turbovap tube labelled with the sample number. The funnel is rinsed with 20ml dichloromethane and this is added to the Turbovap tube.

C7.9 The Turbovap tube is placed into the blow down apparatus which is set at a temperature of 40 ± 2.5°C. The extract is evaporated down to 1.0ml using the sensor to detect the end point.

C7.10 The final extract is transferred using a Pasteur pipette into a labelled 2ml auto sampler vial. 10µl of working injection standard mix is added using a syringe or pipette to the final extract. The vial is then capped ready for analysis or to be stored in the refrigerator at 5 ± 3ºC until required.

C7.11 Analyse the blank, working calibration standards and AQC using the entire procedure as described in sections C7.1 - C7.10.

C7.12 Set up the GCMS system according to manufacturer's instructions. Using the three calibration solutions i.e. 10, 25 and 50 ng L^{-1}, construct a calibration graph of response versus amount of component, monitoring the ions referred to in C5.5.

C7.13 Analyse the sample extracts and from the calibration graph obtain the amount, Av, of taste and odour components in the vial and then calculate the concentration, Cs, of taste and odour in the sample. If the response exceeds the calibration range, the final extract may be diluted with dichloromethane and the analysis repeated.

C8 Calculation

From the calibration graph determine the amount, Av, of component in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = Av \times (1000/Vs)$$

Where

Cs is the concentration (ng L^{-1}) of in the sample;
Av is the amount (ng L^{-1}) of obtained from the graph;
Vs is the sample volume taken (C7.1)

Blank correction is not normally necessary but should be considered if the procedural blank is above the limit of detection.

Table C1 Mean, limit of detection, standard deviations and bias

The method has not undergone formal validation or precision and bias testing, but below are some statistics from the limited testing carried out and recent performance from quality control samples. Reporting limits are based on signal to noise and not performance data.

Potable water extracted standard (40ng L^{-1})

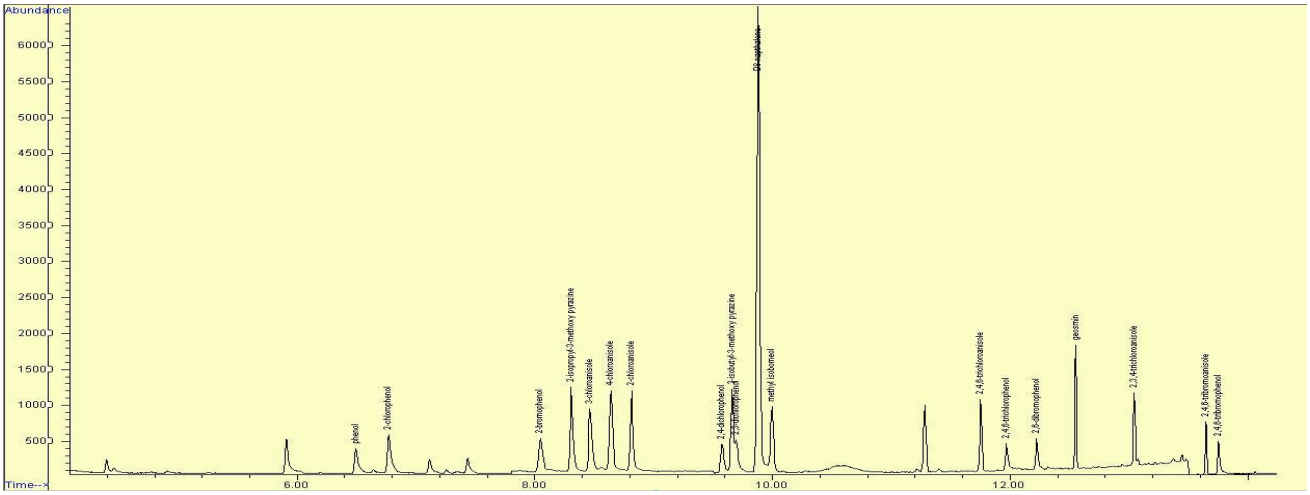
Analyte	Mean	Std Dev	% Recovery	% Bias	% RSD	RPL
phenol	33.21	4.15	83.03	-16.97	12.51	30*
2-chlorophenol	38.08	4.09	95.20	-4.80	10.73	1
2-bromophenol	38.15	4.56	95.38	-4.62	11.95	5
2-isopropyl-3-methoxypyrazine	36.87	3.93	92.17	-7.83	10.66	1
3-chloroanisole	36.99	3.78	92.47	-7.53	10.21	1
4-chloroanisole	36.54	3.86	91.34	-8.66	10.57	1
2-chloroanisole	36.64	4.23	91.60	-8.40	11.53	1
2,4-dichlorophenol	36.63	4.36	91.59	-8.41	11.90	1
2,3-dichlorophenol	36.79	3.72	91.98	-8.02	10.12	1
2-isobutyl-3-methoxypyrazine	36.58	3.45	91.44	-8.56	9.42	1
2-methylisoborneol	35.91	3.35	89.76	-10.24	9.32	1
2,4,6-trichloroanisole	36.55	4.30	91.37	-8.63	11.77	1
2,4,6-trichlorophenol	38.69	4.52	96.72	-3.28	11.68	1
2,6-dibromophenol	39.20	4.67	98.00	-2.00	11.91	1
geosmin	38.72	3.68	96.79	-3.21	9.52	1
2,3,4-trichloroanisole	37.56	4.23	93.89	-6.11	11.27	1
2,4,6-tribromoanisole	36.92	4.51	92.30	-7.70	12.22	1
2,4,6-tribromophenol	39.44	4.68	98.60	-1.40	11.87	1

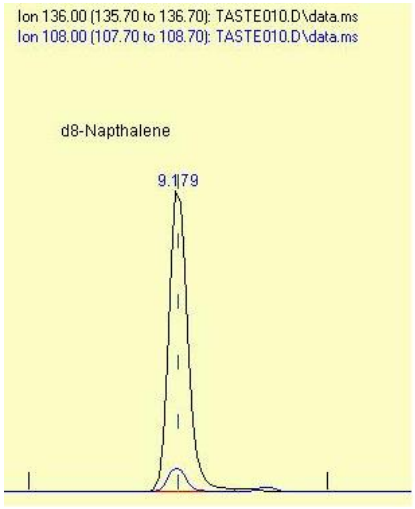
* High reporting limit (RPL) due to high background analyte levels in laboratory and procedural blanks

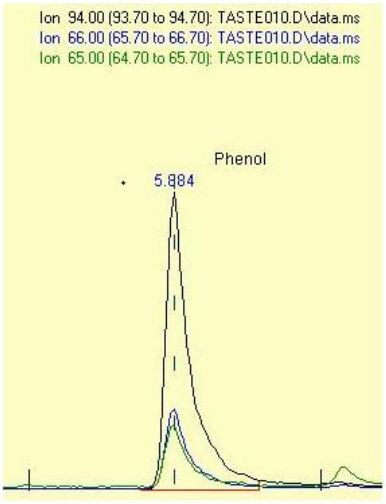
All values expressed as ng L^{-1} unless otherwise stated.
Std Dev is standard deviation.
RSD is relative standard deviation

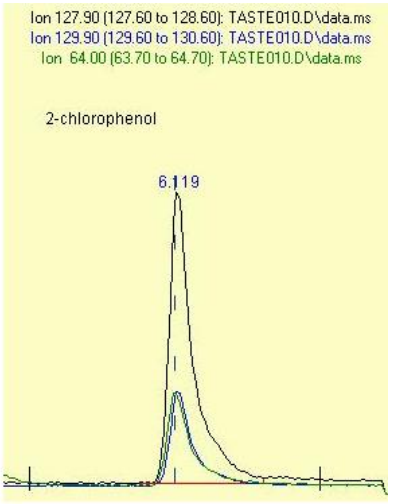
Data provided by Severn Trent Water, Bridgend Laboratory.

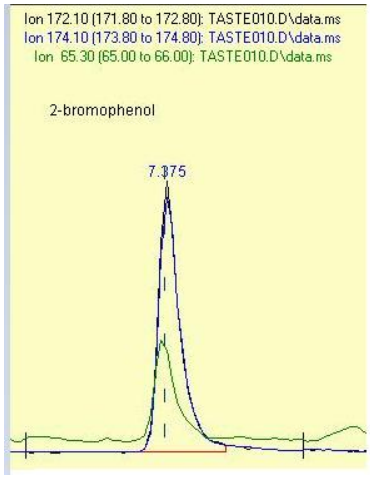
Figure C1 Typical chromatograms of taste and odour components
(Concentration of 40ng L^{-1} extracted AQC Sample – 40µg L^{-1} in extract)

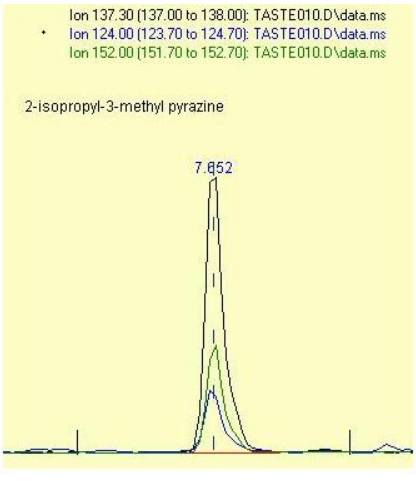


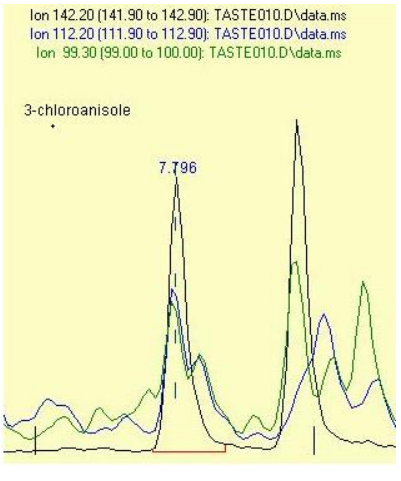


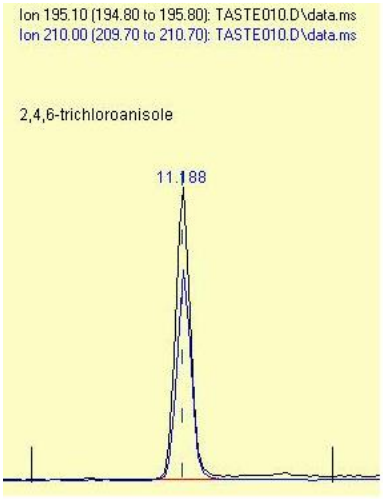








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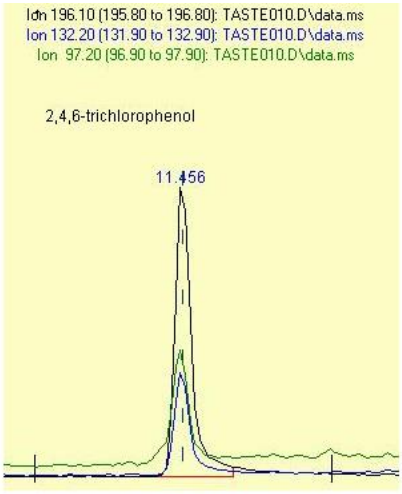
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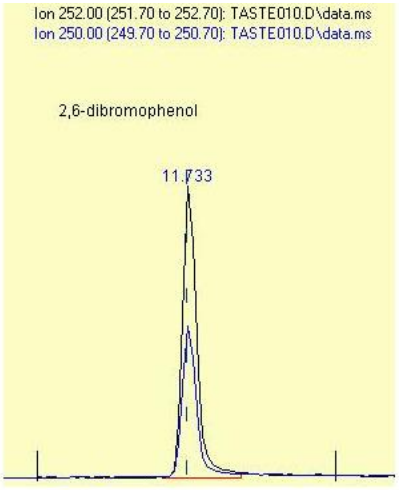
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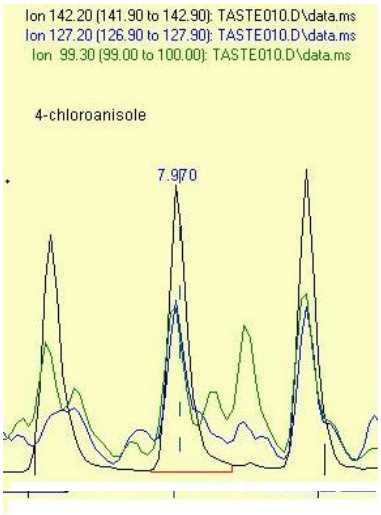
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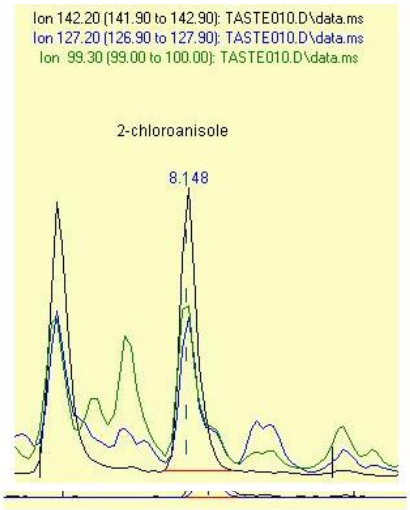
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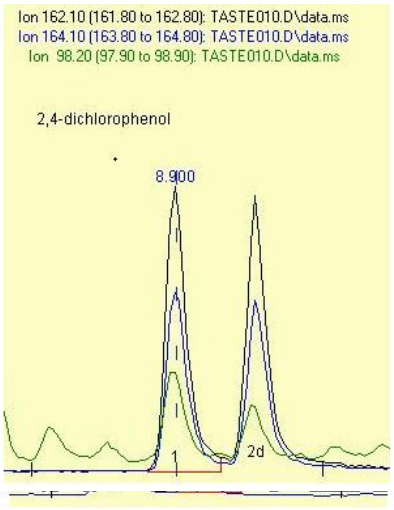
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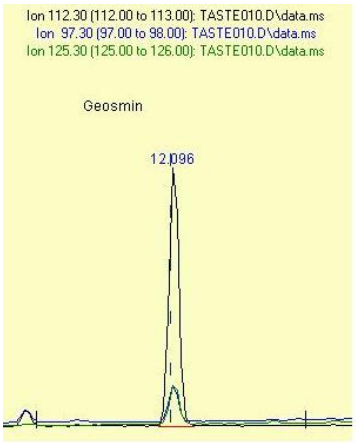
8.900
1
2d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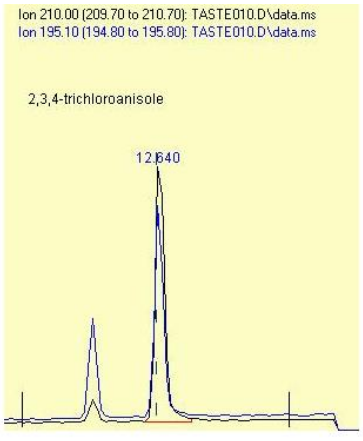
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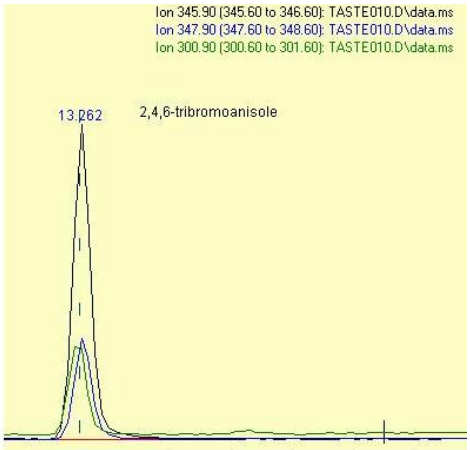
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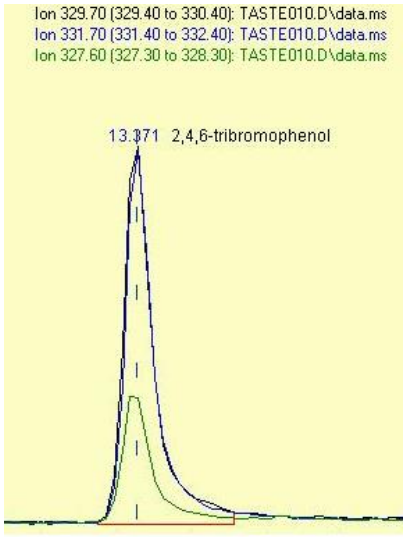
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13.371 2,4,6-tribromophenol

D The determination of phenol and selected substituted phenolic compounds in raw and potable waters using liquid/liquid extraction and GC MS detection.

D1 Performance characteristics of the method

D1.1	Substances determined	Phenol, 2-methylphenol, 3-methylphenol, 4-methylphenol, 2-chlorophenol, 2,5-dimethylphenol, 2,4-dimethylphenol, 3,5-dimethylphenol, 4-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol. This method may be suitable for other additional taste and odour compounds.
D1.2	Type of sample	Raw waters and drinking waters. The method may be used for other types of water.
D1.3	Basis of method	Phenolic compounds are derivatised to their acyl form and extracted from the sample by a liquid/liquid extraction technique using isooctane as the extraction solvent. Analysis is by gas chromatography with mass spectrometric detection using Selective Ion Monitoring (SIM).
D1.4	Range of application	Typically up to 1.0 µg L^{-1}. The range may be extended, see section D7.12.
D1.5	Standard deviation	See Table D1.
D1.6	Limit of detection	See Table D1 for 250ml of sample.
D1.7	Bias	See Table D1.
D1.8	Interferences	Any compound that is extracted and elutes under the conditions used, and has similar gas chromatographic and mass spectrometric properties to the compounds of interest will interfere.

D2 Principle

250ml of sample is taken and its pH adjusted to 9.9 with a carbonate buffer. Following the addition of pentafluorobenzoyl chloride and isooctane, extractive acylation is performed. Any phenols present are converted to the corresponding acyl derivatives and extracted into the isooctane. The derivatised phenols are determined by GC with mass spectrometer using Selective Ion Monitoring (SIM).

D3 Hazards

Phenolic compounds are harmful as a solid material with an extremely strong odour and should be treated with the appropriate care, although in most laboratories it is obtained as a dilute standard in a common solvent.

Pentafluorobenzoyl chloride **reacts violently with water**.

Acetone and isooctane are irritants and flammable.

Sodium Hydroxide is corrosive and harmful.

Waste solvents should be discarded according to documented procedures.

D4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

D4.1 Isooctane (2,2,4-Trimethylpentane).

D4.2 Acetone.

D4.3 Pentafluorobenzoyl chloride (PFBC)

D4.4 Water. deionised, distilled water or HPLC grade.

D4.5 Sodium sulphate (anhydrous). Dry at 500 ± 50°C for a minimum of 4 hours in a muffle furnace. Allow to cool in a desiccator prior to use.

D4.6 Sodium hydroxide pellets.

D4.7 5.0 M sodium hydroxide solution. Weigh out 200 g of sodium hydroxide pellets into a 2 litre beaker. Carefully add 500ml of deionised water and stir slowly to dissolve the pellets. This solution will become hot.

Allow to cool and add a further 500ml of deionised water. This solid should be stored in a sealed glass bottle at ambient temperature for up to 6 months.

D4.8 Sodium bicarbonate.

D4.9 1.0 M sodium bicarbonate solution. Weigh out 84 g of sodium bicarbonate into a 2 litre beaker. Add 1000ml of deionised water and stir slowly to dissolve the solid.

This solid should be stored in a sealed glass bottle at ambient temperature for up to 6 months.

D4.10 Sodium thiosulphate pentahydrate.

D4.11 Sodium thiosulphate solution. Weigh out 46 g of sodium thiosulphate into a 1 litre bottle. Add 1000ml of deionised water and shake to dissolve the solid.

This solid should be stored in a sealed glass bottle at ambient temperature for up to 6 months.

D4.12 Stock calibration standard (2000mg L^{-1}). Commercially certified standard containing the appropriate levels of phenol, 2-methylphenol, 3-methylphenol, 4-methylphenol, 2-chlorophenol, 2,5-dimethylphenol, 2,4-dimethylphenol, 3,5-dimethylphenol, 4-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol in acetone.

Similar AQC stock standard solutions should obtained, preferably from a different supplier or at least separate lot number.

D4.13 Intermediate calibration spiking solution (20mg L^{-1}). Add 250µl of stock calibration standard (2000mg L^{-1}) into approximately 20ml of acetone. Make up to 25ml with acetone. This solution may be stored at -15 ± 3ºC for up to 12 months.

D4.14 Working calibration spiking solution (0.2mg L^{-1}). Add 250µl of intermediate calibration spiking solution (20 mg L^{-1}) into approximately 20ml of acetone. Make up to 25ml with acetone. This solution may be stored at -15 ± 3ºC for up to 12 months.

An AQC solution should be prepared, preferably by a different member of staff and using reagents from different batches or manufacturers.

D4.15 Intermediate AQC spiking solution (20mg L^{-1}). Add 250µl of stock AQC standard (2000mg L^{-1}) into approximately 20ml of acetone. Make up to 25ml with acetone. This solution may be stored at -15 ± 3ºC for up to 12 months.

D4.16 Working AQC spiking solution (0.2mg L^{-1}). Add 250µl of intermediate AQC spiking solution (20 mg L^{-1}) into approximately 20ml of acetone. Make up to 25ml with acetone. This solution may be stored at -15 ± 3ºC for up to 12 months.

D4.17 D_5-phenol internal standard. Commercially certified standard.

D4.18 Stock D_5-phenol internal standard (8000mg L^{-1}). Weigh out 0.08g of D_5-phenol into a weigh boat. Quantitatively transfer into a 10ml volumetric flask and make up to the mark with acetone. This solution may be stored at -15 ± 3ºC for up to 3 years.

D4.19 Working D_5-phenol internal standard (4mg L^{-1}). Add 25µl of stock D_5-phenol solution (8000mg L^{-1}) into approximately 20ml of acetone. Make up to 50ml with acetone. This solution may be stored at 15 ± 3ºC for up to 12 months.

D4.20 Blank - A procedural blank is analysed with every batch of samples. 100ml of water is transferred to a suitably labelled 250ml volumetric flask. This sample should be prepared on the day of use.

D4.21 Calibration standard solutions – For example, for a five point calibration, prepare a series of five 250ml volumetric flasks. Label them Cal 1, Cal 2, Cal 3, Cal 4 and Cal 5, and add 100ml of deionised water to each bottle. Using the table below, add the relevant amount of spiking solution to each bottle.

Standard	Concentration ng L^{-1}	Amount of working calibration spiking solution (µl)
Cal 1	50	25.0
Cal 2	100	50.0
Cal 3	200	100.0
Cal 4	500	250.0
Cal 5	1000	500.0

These solutions should be prepared on the day of use.

D4.22 AQC Sample – 500ng L^{-1} - An AQC sample is analysed with every batch of samples. 100ml of water is transferred to a suitably labelled 250ml volumetric flask to which 250µl of working AQC spiking solution is added. This sample should be prepared on the day of use.

D5 Apparatus

In addition to normal laboratory glassware (Grade A or better) the following may be required.

D5.1 Microlitre syringes, 500µl, 250µl, 100µl, 50µl and 25µl.

D5.2 Glass vials, 2ml with 0.2ml conical inserts, 40ml and 14ml.

D5.3 Culture tubes.

D5.4 GC system with a cool on column injector and a mass selective detector capable of operating in SIM mode, with an appropriate data station. The performance data (see table D1) was derived using an Agilent GC MS system and the conditions listed below, other systems are available but the suitability of the equipment should be evaluated.

GC Instrument conditions.

Oven/Inlet Parameters:

Equilibration time:	0.5 minutes
Initial Temp:	80°C
Initial Hold:	2 mins
Ramp 1:	20.0°C min^{-1} to 180°C hold for 5.0 min
Ramp 2:	2.0°C min^{-1} to 190°C hold for 2.0 min
Ramp 3:	20.0°C min^{-1} to 310°C hold for 5.0 min
Total Run Time:	30.00 min
Initial Inlet Temp:	80°C
Inlet Type:	Cool on column
Oven tracking:	On
Vacuum compensation:	On

Carrier Parameters:

Column:	DB-XLB or equivalent
Length:	30m
Diameter:	0.320mm
Film Thickness:	0.50µm
Gas Type:	Helium
Column pressure:	2.1 psi (approximately)
Column flow:	1.3 ml min^{-1}
Injection Volume:	2.0µL

MS Instrument conditions:

Source Temperature:	230°C
Quad Temperature:	150°C
Transfer Line Temperature:	300°C
Acquisition Mode	SIM
Resolution	Low
EM offset	300
Solvent delay	7.5 min

Compound	Group	Approximate Retention Time (min)	Quantitation Ion (m/z)*	Qualifier ions (m/z)*	Dwell
d_5-Phenol	1	8.46	293	294	100
Phenol	1	8.50	288	289	100
2-Methylphenol	2	9.35	195	302	100
3-Methylphenol	2	9.63	195	302	100
4-Methylphenol	2	9.88	195	302	100
2-Chlorophenol	3	10.80	322	195	100
2,5-Dimethylphenol	3	10.81	316	195	100
2,4-Dimethylphenol	3	11.09	316	195	100
3,5-Dimethylphenol	3	11.22	316	195	100
4-Chlorophenol	3	11.32	322	195	100
4-Chloro-3-methylphenol	4	13.55	195	336	100
Dichlorophenol	4	14.57	195	356	100
2,4,6-Trichlorophenol	5	18.02	195	390	50
2,4,5-Trichlorophenol	5	19.50	195	390	50
Pentachlorophenol	6	23.44	195	458	80

*The ions specified are for the derivatised compound

D6 Sample collection and preservation

Samples are collected in 2 litre amber glass bottles with glass stoppers, containing sodium thiosulphate as a preservative. The sample bottles should contain no headspace.

The bottles stored at 5 ± 3°C on receipt at the laboratory. The samples are typically extracted within 16 days of sampling and the extracts analysed no later than 22 days after extraction.

D7 Analytical procedure

All glassware must be washed in the glasswasher on the day of extraction or the night before and stored with the glass stoppers in place.

D7.1 Remove the samples from the fridge and allow them to reach ambient temperature.

D7.2 Shake each sample and transfer 100ml to a labelled 250ml volumetric flask.

D7.3 Add 25µl of internal standard to each flask. Stopper the flask and swirl to mix.

D7.4 Using a dispenser, add 2ml of isooctane to each flask.

D7.5 Using a measuring cylinder, add 30ml of 1.0M sodium bicarbonate solution to each flask. Stopper and swirl to mix.

D7.6 In a fume hood, add 20µl of pentafluorobenzyl chloride (PFBC) to each flask. Stopper and mix by inversion.

D7.7 Transfer the flasks to a bench top shaker and shake for 5 minutes at 190rpm.

D7.8 Using a measuring cylinder, add 10ml of 5.0M sodium hydroxide solution to each flask and allow to stand for 30 minutes with the stoppers loosened.

D7.9 Add sufficient water to each flask to facilitate the displacement of the solvent phase into the neck of the flask. Transfer approximately 1ml into a glass autosampler vial ready for analysis.

D7.10 Analyse the blank, working calibration standards and AQC using the entire procedure as described in sections D7.1 - D7.9.

D7.11 Set up the GCMS system according to manufacturer's instructions. Using the five calibration solutions i.e. 50, 100, 200, 500 and 1000 ng L^{-1}, construct a calibration graph of response versus amount of component, monitoring the ions referred to in D5.4.

D7.12 Analyse the sample extracts and from the calibration graph obtain the amount, Av, of phenols in the vial and then calculate the concentration, Cs, of phenols in the sample. If the response exceeds the calibration range, the final extract may be diluted with dichloromethane and the analysis repeated.

D8 Calculation

From the calibration graph determine the amount, Av, of component in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = Av \times (100/Vs)$$

Where

Cs is the concentration (ng L^{-1}) of in the sample;
Av is the amount (ng L^{-1}) of obtained from the graph;
Vs is the sample volume taken (D7.2)

Blank correction is not normally necessary but should be considered if the procedural blank is above the limit of detection.

Table D1 Mean, limit of detection, standard deviations and bias

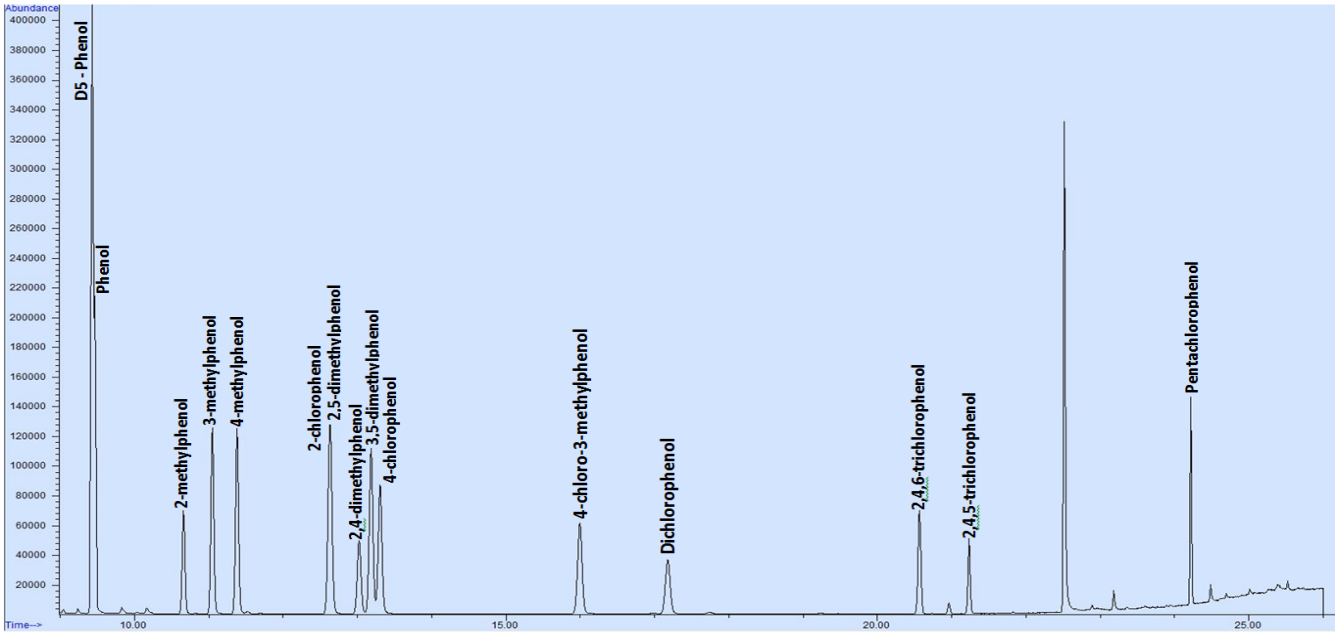
The method has not undergone formal validation or precision and bias testing, but below are some statistics from the limited testing carried out and recent performance from quality control samples

Potable water extracted standard (ng L^{-1})

Analyte	Mean	Std Dev	% Recovery	% Bias	% RSD	LOD
Phenol	478.9555	33.22	95.79	-4.21	6.936	14.125
2-Methylphenol	432.9173	39.467	86.58	-13.42	9.117	25.875
3-Methylphenol	468.4391	39.132	93.69	-6.31	8.354	11.585
4-Methylphenol	460.6564	42.264	92.13	-7.87	9.175	14.105
2-Chlorophenol	570.2296	64.11	114.05	14.05	11.243	25.825
2,5-Dimethylphenol	419.000	37.099	83.80	-16.20	8.854	18.49
2,4-Dimethylphenol	421.9914	42.114	84.40	-15.60	9.980	21.90
3,5-Dimethylphenol	488.7591	54.752	97.75	-2.25	11.202	26.805
4-Chlorophenol	571.5968	64.902	114.32	14.32	11.35	23.87
4-Chloro-3-methylphenol	561.7269	62.771	112.35	12.35	11.175	23.275
Dichlorophenol	607.2577	93.112	121.45	21.45	15.333	27.115
2,4,6-Trichlorophenol	544.9755	69.318	109.00	9.00	12.719	13.925
2,4,5-Trichlorophenol	626.5023	100.16	125.30	25.30	15.987	31.890
Pentachlorophenol	562.4541	99.262	112.49	12.49	17.648	14.925

All values expressed as ng L^{-1} unless otherwise stated.
Std Dev is standard deviation.
RSD is relative standard deviation

Data provided by Thames Water.



Chromatogram provided by Thames Water

E The determination of 2-EMD and 2-EDD in raw and potable waters using liquid/liquid extraction and GC MS detection.

E1 Performance characteristics of the method

E1.1	Substances determined	2-ethyl-4-methyl-1,3-dioxolane (2-EMD) and 2-ethyl-5,5-dimethyl-1,3-dioxane (2-EDD).
E1.2	Type of sample	Raw and drinking waters. The method is also applicable to final effluents, borehole waters, sewage, sewage sludge and solid materials with suitable modification.
E1.3	Basis of method	2-EMD and 2-EDD are extracted from the sample by a liquid/liquid extraction technique using dichloromethane as the extraction solvent under acidic conditions (pH 2). Analysis is by gas chromatography with mass spectrometric detection using Selective Ion Monitoring (SIM).
E1.4	Range of application	Typically up to 0.2 µg L^{-1}. The range may be extended, see section E7.14.
E1.5	Standard deviation	See Table E1.
E1.6	Limit of detection	See Table E1 for 500ml of sample.
E1.7	Bias	See Table E1.
E1.8	Interferences	Any compound that is extracted and elutes under the conditions used, and has similar gas chromatographic and mass spectrometric properties to the compounds of interest will interfere.

E2 Principle

500ml of sample is taken and its pH adjusted to 2 with 5M sulphuric acid solution. The sample is extracted by adding dichloromethane to the bottle and shaking. The solvent layer is removed and concentrated under a flow of nitrogen. The compounds of interest are determined by GC with mass spectrometer using Selective Ion Monitoring (SIM).

E3 Hazards

Taste & Odour compounds are harmful / toxic as a solid material with an extremely strong odour and should be treated with the appropriate care, although in most laboratories it is obtained as a dilute standard in a common solvent.

Methanol is toxic and flammable; acetone is an irritant and flammable. These compounds should be handled away from sources of ignition.

Dichloromethane is toxic and a suspected carcinogen. Sodium sulphate and sodium thiosulphate solutions are both irritants.

Sulphuric acid is corrosive and **can cause serious burns**.

Waste solvents should be discarded according to documented procedures.

E4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

E4.1 Water. Deionised, distilled water or HPLC grade.

E4.2 5M sulphuric acid solution.

E4.3 Dichloromethane.

E4.4 Acetone.

E4.5 Ascorbic acid

E4.6 Ascorbic acid solution. Partially fill a 50ml volumetric flask with water and add 0.2g of ascorbic acid. Swirl to dissolve and make up to the mark with water. Extract this solution with two 40ml aliquots of dichloromethane to remove any contamination and transfer the solution to a dispenser. This solution may be stored at ambient temperature for up to one month.

E4.7 Sodium sulphate (anhydrous). Dry at 800 ± 80°C for a minimum of 4 hours in a muffle furnace. Allow to cool in a desiccator prior to use.

E4.8 Stock calibration standard solution (1000mg L^{-1}). Commercially certified standard containing the appropriate levels of 2-ethyl-4-methyl-1,3-dioxolane (2-EMD) and 2-ethyl-5,5-dimethyl-1,3-dioxane (2-EDD).

Similar AQC stock standard solutions should be obtained, preferably from a different supplier or at least separate lot number.

E4.9 Intermediate Calibration Spiking Standard (10mg L^{-1}). Partially fill a 10ml volumetric with acetone and add 100µl of stock calibration standard solution (1000mg L^{-1}). Make up to the mark with acetone. This solution may be stored at -15 ± 5ºC for up to 12 months.

E4.10 Working Calibration Spiking Standard (0.2mg L^{-1}). Partially fill a 50ml volumetric flask with acetone and add 1000µl of intermediate calibration standard solution (10mg L^{-1}). Make up to the mark with acetone. This solution may be stored at -15 ± 5ºC for up to 12 months.

An AQC solution should be prepared, preferably by a different member of staff and using reagents from different batches or manufacturers.

E4.11 Intermediate AQC spiking solution (10mg L^{-1}). Partially fill a 10ml volumetric flask with acetone and add 250µl of stock solution (1000 mg L^{-1}). Make up to the mark with acetone. This solution may be stored at -15 ± 5ºC for up to 12 months.

E4.12 Working AQC spiking solution (0.2mg L^{-1}). Partially fill a 10ml volumetric flask with acetone and add 200µl of intermediate AQC spiking solution (10mg L^{-1}). Make up to the mark with acetone. This solution may be stored at -15 ± 5ºC for up to 12 months.

E4.13 D_5-chlorobenzene internal standard. Commercially certified standard.

E4.14 Stock D_5-chlorobenzene internal standard (2000mg L^{-1}). Weigh out 0.02g of D_5-chlorobenzene into a weigh boat. Quantitatively transfer into a 10ml volumetric flask and make up to the mark with acetone. This solution may be stored at -15 ± 5ºC in accordance with the manufacturer's instructions.

E4.15 Working D_5-chlorobenzene internal standard (2mg L^{-1}). Add 50µl of stock D_5-chlorobenzene solution (2000mg L^{-1}) into approximately 40ml of acetone. Make up to 50ml with acetone. This solution may be stored at -15 ± 5ºC for up to 12 months.

E4.16 Blank
A procedural blank is analysed with every batch of samples. 500ml of water is transferred to a suitably labelled 500ml Duran bottle. Add 1ml of ascorbic acid and leave for 5 minutes. This sample should be prepared on the day of use.

E4.17 Calibration standard solutions – For example, for a four point calibration, prepare a series of four 500ml Duran bottles. Label them Cal 10, Cal 50, Cal 100 and Cal 200, and add 500ml of deionised water to each bottle. Add 1ml of ascorbic acid to each bottle and leave for 5 minutes. Using the table below, add the relevant amount of spiking solution to each bottle.

Standard	Concentration ng L^{-1}	Amount of working calibration spiking solution (µl)
Cal 1	10	25.0
Cal 2	50	125.0
Cal 3	100	250.0
Cal 4	200	500.0

These solutions should be prepared on the day of use.

E4.18 **AQC Sample – 100ng L^{-1} -** An AQC sample is analysed with every batch of samples. 500ml of water is transferred to a suitably labelled 500ml Duran bottle. Add 1ml of ascorbic acid and leave for 5 minutes. Next add 250µl of working AQC spiking solution using a calibrated syringe. This sample should be prepared on the day of use.

E5 Apparatus
In addition to normal laboratory glassware (Grade A or better) the following may be required.

E5.1 Microlitre syringes, 1000µl, 500µl, 250µl, 100µl, 50µl and 25µl.

E5.2 500ml Duran bottles with PTFE lined lids.

E5.3 Glass vials, 2ml with 0.2ml conical inserts and 14ml amber glass with PTFE/Silicone screw caps.

E5.4 pH paper, pH fix 1.7 – 3.8.

E5.5 Muffle furnace, capable of being set to 800 ± 80°C

E5.6 Reciprocal bottle shaker.

E5.7 Blow-down apparatus. Any device (capable of being set at 40 ± 2.0 °C) that can direct a gentle stream of nitrogen or air into a test tube.

E5.8 GC system with a split/splitless injector and a mass selective detector capable of operating in SIM mode, with an appropriate data station. The performance data (see table E1) was derived using an Agilent GC MS system and the conditions listed below, other systems are available but the suitability of the equipment should be evaluated.

GC Instrument conditions.

Oven/Inlet Parameters:

Equilibration time:	0.5 minutes
Initial Temp:	35°C
Initial Hold:	4 mins
Ramp 1:	10.0°C min^{-1} to 80°C
Ramp 2:	50.0°C min^{-1} to 300°C hold for 10.0 min
Total Run Time:	22.90 min
Inlet Type:	Split/Splitless
Initial Inlet Temp:	250°C
Pressure:	6.8psi
Purge flow:	12.0ml/min
Purge time:	2.00 min
Total flow:	16.0ml/min
Oven tracking:	On
Vacuum compensation:	On

Carrier Parameters:

Column:	DB-5MS or equivalent
Length:	30m
Diameter:	0.25mm
Film Thickness:	0.25µm
Gas Type:	Helium
Column flow:	1.0 ml min^{-1} constant flow
Injection Volume:	2.0µL

MS Instrument conditions:

Source Temperature:	230°C
Quad Temperature:	150°C
Transfer Line Temperature:	310°C
Acquisition Mode	SIM
Resolution	Low
EM offset	+400EMV
Dwell	50ms
Solvent delay	0.5 min

Compound	Group	Approximate Retention Time (min)	Quantitation Ion	Qualifier ions
2-EMD	1	6.02	87	59, 72, 115
D5-chlorobenzene	2	7.26	117	56, 69, 143
2-EDD	2	8.87	115	56, 69, 143

E6 Sample collection and preservation

Samples are collected in 1 litre amber glass bottles. The bottles stored at 5 ± 3°C on receipt at the laboratory. The samples are typically extracted within 7 days of sampling and the extracts analysed no later than 14 days after extraction.

E7 Analytical procedure

All glassware must be washed in the glasswasher on the day of extraction or the night before and stored with the glass stoppers in place.

E7.1 Remove the samples from the fridge and allow them to reach ambient temperature.

E7.2 Shake each sample and weigh 500g in to a labelled 500ml Duran bottle.

E7.3 Add 50µl of the internal standard spiking solution to each bottle.

E7.4 Adjust the pH of the water to pH 2 by adding 5M sulphuric acid solution.

E7.5 Add 10ml of dichloromethane (DCM) to each bottle. Cap, shake briefly by hand and release the pressure build up by opening the cap.

E7.6 Place the bottle on the shaker and shake for 4 minutes at 190rpm.

E7.7 Allow the bottles to stand so that the aqueous and solvent layers separate. Use a 10ml glass pipette to remove the solvent layer and place it in a labelled test tube.

E7.8 Repeat steps E7.5 to E7.7 and place the solvent layer into a second labelled test tube.

E7.9 Inspect the test tubes containing the DCM extracts and remove any water with a Pasteur pipette. If the extract is cloudy, then add sufficient sodium sulphate until the extract becomes clear.

E7.10 Transfer the extracts to the blow down apparatus and concentrate down to approximately 200µl. During this stage combine the two extracts for each sample.

E7.11 Transfer the extract to a suitably labelled 2ml vial with insert and cap.

E7.12 Analyse the blank, working calibration standards and AQC using the entire procedure as described in sections E7.1 - E7.11.

E7.13 Set up the GCMS system according to manufacturer's instructions. Using the four calibration solutions i.e. 10, 50, 100 and 200 ng L^{-1}, construct a calibration graph of response versus amount of component, monitoring the ions referred to in E5.8.

E7.14 Analyse the sample extracts and from the calibration graph obtain the amount, Av, of 2-EMD and 2-EDD in the vial and then calculate the concentration, Cs, of 2-EMD and 2-EDD in the sample. If the response exceeds the calibration range, the final extract may be diluted with dichloromethane and the analysis repeated.

E8 Calculation

From the calibration graph determine the amount, Av, of component in the vial and determine the concentration, Cs, in the sample using the equation:

$$Cs = Av \times (500/Vs)$$

Where

Cs is the concentration (ng L^{-1}) of in the sample;
Av is the amount (ng L^{-1}) of obtained from the graph;
Vs is the sample volume (weight) taken (E7.2)

Blank correction is not normally necessary but should be considered if the procedural blank is above the limit of detection.

Table E1 Mean, limit of detection, standard deviations and bias

The method has not undergone formal validation or precision and bias testing, but below are some statistics from the limited testing carried out and recent performance from quality control samples

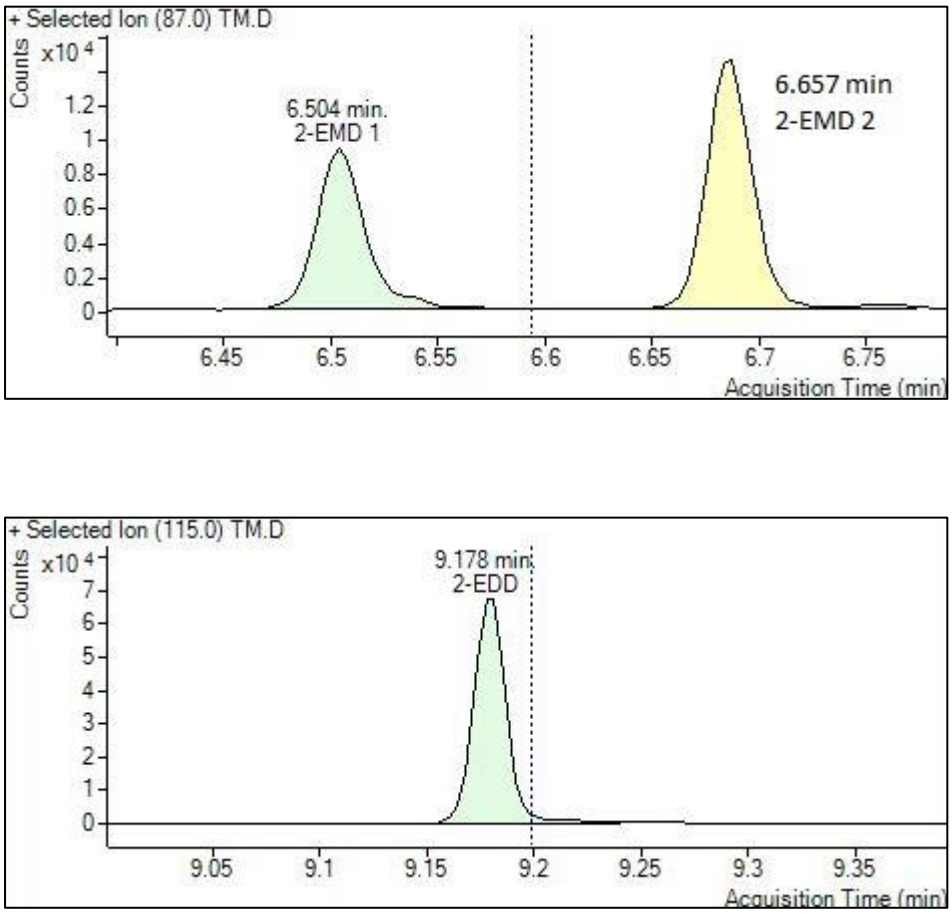
Potable water extracted standard (ng L^{-1})

Analyte	Mean	Std Dev	% Recovery	% Bias	% RSD	LOD
2-Ethyl-4-methyl-1,3-dioxolane (2-EMD)	104.018	14.155	104.018	4.018	13.608	4.0
2-Ethyl-5,5-dimethyl-1,3-dioxane (2-EDD)	88.617	7.050	88.617	-11.383	7.956	2.0

All values expressed as ng L^{-1} unless otherwise stated.
Std Dev is standard deviation.
RSD is relative standard deviation

Data provided by Thames Water.

Figure E1 – Typical chromatogram for 2-EMD and 2-EDD at 100ng L^{-1}



Chromatograms provided by Thames Water

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below. In addition, if users wish to receive advanced notice of forthcoming publications, please contact the Secretary.

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