ANNEX 4

Analytical methods and achievability

A4.1 Analytical methods

In *volumetric titration*, chemicals are analysed by titration with a standardized titrant. The titration end-point is identified by the development of colour resulting from the reaction with an indicator, by the change of electrical potential or by the change of pH value.

Colorimetric methods are based on measuring the intensity of colour of a coloured target chemical or reaction product. The optical absorbance is measured using light of a suitable wavelength. The concentration is determined by means of a calibration curve obtained using known concentrations of the determinant. The ultraviolet (UV) method is similar to this method except that UV light is used. For ionic materials, the ion concentration can be measured using an *ion selective electrode*. The measured potential is proportional to the logarithm of the ion concentration. Some organic compounds absorb UV light (wavelength 190–380 nm) in proportion to their concentration. *UV absorption* is useful for qualitative estimation of organic substances, because a strong correlation may exist between UV absorption and organic carbon content.

Atomic absorption spectrometry (AAS) is used for the determination of metals. It is based on the phenomenon that the atom in the ground state absorbs the light of wavelengths that are characteristic to each element when light is passed through the atoms in the vapour state. Because this absorption of light depends on the concentration of atoms in the vapour, the concentration of the target element in the water sample is determined from the measured absorbance. The Beer-Lambert law describes the relationship between concentration and absorbance.

In *flame atomic absorption spectrometry (FAAS)*, a sample is aspirated into a flame and atomized. A light beam from a hollow cathode lamp of the same element as the target metal is radiated through the flame, and the amount of absorbed light is measured by the detector. This method is much more sensitive than other methods and free from spectral or radiation interference by co-existing elements. Pretreatment is either unnecessary or straightforward. However, it is not suitable for simultaneous analysis of many elements, because the light source is different for each target element.

Electrothermal atomic absorption spectrometry (EAAS) is based on the same principle as FAAS, but an electrically heated atomizer or graphite furnace replaces the standard burner head for determination of metals. In comparison with FAAS, EAAS gives higher sensitivities and lower detection limits, and a smaller sample volume is required. EAAS suffers from more interference through light scattering by co-existing elements and requires a longer analysis time than FAAS.

The principle of *inductively coupled plasma atomic emission spectrometry (ICP-AES)* for determination of metals is as follows. An ICP source consists of a flowing stream of argon gas ionized by an applied radio frequency. A sample aerosol is generated in a nebulizer and spray chamber and then carried into the plasma through an injector tube. A sample is heated and excited in the high-temperature plasma. The high temperature of the plasma causes the atoms to become excited. On returning to the ground state, the excited atoms produce ionic emission spectra. A monochromator is used to separate specific wavelengths corresponding to different elements, and a detector measures the intensity of radiation of each wavelength. A significant reduction in chemical interference is achieved. In the case of water with low pollution, simultaneous or sequential analysis is possible without special pretreatment to achieve low detection limits for many elements. This, coupled with the extended dynamic range from three digits to five digits, means that multielement determination of metals can be achieved. ICP-AES has similar sensitivity to FAAS or EAAS.

In *inductively coupled plasma mass spectrometry* (ICP-MS), elements are atomized and excited as in ICP-AES, then passed to a mass spectrometer. Once inside the mass spectrometer, the ions are accelerated by high voltage and passed through a series of ion optics, an electrostatic analyser and, finally, a magnet. By varying the strength of the magnet, ions are separated according to mass/charge ratio and passed through a slit into the detector, which records only a very small atomic mass range at a given time. By varying the magnet and electrostatic analyser settings, the entire mass range can be scanned within a relatively short period of time. In the case of water with low pollution, simultaneous or sequential analysis is possible without special pretreatment to achieve low detection limits for many elements. This, coupled with the extended dynamic range from three digits to five digits, means that multielement determination of metals can be achieved.

Chromatography is a separation method based on the affinity difference between two phases, the stationary and mobile phases. A sample is injected into a column, either packed or coated with the stationary phase, and separated by the mobile phase based on the difference in interaction (distribution or adsorption) between compounds and the stationary phase. Compounds with a low affinity for the stationary phase move more quickly through the column and elute earlier. The compounds that elute from the end of the column are determined by a suitable detector.

In *ion chromatography*, an ion exchanger is used as the stationary phase, and the eluant for determination of anions is typically a dilute solution of sodium hydrogen carbonate and sodium carbonate. Colorimetric, electrometric or titrimetric detectors can be used for determining individual anions. In suppressed ion chromatography, anions are converted to their highly conductive acid forms; in the carbonate–bicarbonate

eluant, anions are converted to weakly conductive carbonic acid. The separated acid forms are measured by conductivity and identified on the basis of retention time as compared with their standards.

High-performance liquid chromatography (HPLC) is an analytical technique using a liquid mobile phase and a column containing a liquid stationary phase. Detection of the separated compounds is achieved through the use of absorbance detectors for organic compounds and through conductivity or electrochemical detectors for metallic and inorganic compounds.

Gas chromatography (GC) permits the identification and quantification of trace organic compounds. In GC, gas is used as the mobile phase, and the stationary phase is a liquid that is coated either on an inert granular solid or on the walls of a capillary column. When the sample is injected into the column, the organic compounds are vaporized and moved through the column by the carrier gas at different rates depending on differences in partition coefficients between the mobile and stationary phases. The gas exiting the column is passed to a suitable detector. A variety of detectors can be used, including flame ionization (FID), electron capture (ECD) and nitrogen–phosphorus. As separation ability is good in this method, mixtures of substances with similar structure are systematically separated, identified and determined quantitatively in a single operation.

The *gas chromatography/mass spectrometry (GC-MS)* method is based on the same principle as the GC method, using a mass spectrometer as the detector. As the gas emerges from the end of the GC column opening, it flows through a capillary column interface into the MS. The sample then enters the ionization chamber, where a collimated beam of electrons impacts the sample molecules, causing ionization and fragmentation. The next component is a mass analyser, which uses a magnetic field to separate the positively charged particles according to their mass. Several types of separating techniques exist; the most common are quadrupoles and ion traps. After the ions are separated according to their masses, they enter a detector.

The *purge-and-trap packed column GC-MS* method or purge-and-trap packed column GC method is applicable to the determination of various purgeable organic compounds that are transferred from the aqueous to the vapour phase by bubbling purge gas through a water sample at ambient temperature. The vapour is trapped with a cooled trap. The trap is heated and backflushed with the same purge gas to desorb the compounds onto a GC column. The principles of GC or GC-MS are as referred to above.

The principle of *enzyme-linked immunosorbent assay (ELISA)* is as follows. The protein (antibody) against the chemical of interest (antigen) is coated onto the solid material. The target chemical in the water sample binds to the antibody, and a second antibody with an enzyme attached is also added that will attach to the chemical of interest. After washing to remove any of the free reagents, a chromogen is added that will give a colour reaction due to cleavage by the enzyme that is proportional to the quantity of the chemical of interest. The ELISA method can be used to determine microcystin and synthetic surfactants.

A4.2 Analytical achievability for chemicals for which guideline values have been established

Analytical achievability for chemicals for which guideline values have been established is given in Tables A4.1–A4.6.

Table A4.1 Analytical achievability for inorganic chemicals for which guideline values have been established, by source category[a]

	Field methods		Laboratory methods				
	Col	Absor	IC	FAAS	EAAS	ICP	ICP-MS
Naturally occurring chemicals							
Arsenic	+++	#		++(H)	+	++(H)	+++
Barium				++	+++	+++	+++
Boron		++				+++	+++
Chromium		#			++	++	+++
Fluoride	#	+	+++				
Manganese	#			++	+++	++[b]	+++
Selenium		#		++(H)	++	++(H)	+++
Uranium							+++
Chemicals from industrial sources and human dwellings							
Cadmium		#			++	++	+++
Mercury				+++			
Chemicals from agricultural activities							
Nitrate/nitrite	+++	+++	+++				
Chemicals used in water treatment or materials in contact with drinking-water							
Antimony				+++(H)		++(H)	+++
Copper	#	+++		+++	+++	+++	+++
Lead		#			+	+	+++
Nickel		+		+	++	++	+++

[a] For definitions and notes to Table A4.1, see below Table A4.6.
[b] For ICP-AES

Table A4.2 Analytical achievability for organic chemicals from industrial sources and human dwellings for which guideline values have been established[a]

	Col	GC	(PT-) GC-PD	(PT-) GC-ECD	GC-FID	GC-FPD	GC-TID	GC-MS	PT-GC-MS	HPLC	HPLC -FD	HPLC-UVPAD	EAAS	IC-FD	IC-SCD	LC-MS
Benzene			+++						+++							
Carbon tetrachloride				+++					+++							
1,2-Dichlorobenzene			+++	+++				+++	+++							
1,4-Dichlorobenzene			+++	+++				+++	+++							
1,2-Dichloroethane				+++					+++							
1,2-Dichloroethene			+++	+++					+++							
Dichloromethane				+++					+++							
Di(2-ethylhexyl)phthalate								++								
1,4-Dioxane								+++								
Edetic acid								+++								
Ethylbenzene			+++						+++							
Hexachlorobutadiene			++	++					++							
Nitrilotriacetic acid		+++						+++								
Pentachlorophenol				+++				+			+					
Perchlorate															++	+++
Styrene			+++						+++							
Tetrachloroethene			+++	+++				+++	+++							
Toluene			+++						+++							
Trichloroethene			+++	+++				+++	+++							
Xylenes			+++						+++							

[a] For definitions and notes to Table A4.2, see below Table A4.6.

Table A4.3 Analytical achievability for organic chemicals from agricultural activities for which guideline values have been established[a,b]

	Col	GC	(PT-) GC-PD	(PT-) GC-ECD	GC-FID	GC-FPD	GC-TID	GC-MS	PT-GC-MS	HPLC	HPLC-FD	HPLC-UVPAD	EAAS	IC-FD
Alachlor				+++				+++						
Aldicarb											+++			
Aldrin and dieldrin				++				++						
Atrazine and its chloro-*s*-triazine metabolites				+++				+++				+++		
Carbofuran		++												
Chlordane				+++				+++						
Chlorotoluron								+++				+++		
Cyanazine				+++				+++				+		
2,4-D				+++				+++				++		
2,4-DB				+++				++				++		
1,2-Dibromo-3-chloro-propane				+++				+++	+++					
1,2-Dibromoethane				++				++	+++					
1,2-Dichloropropane				+++					+++					
1,3-Dichloropropene				+++					+++					
Dichlorprop				+++				+++						
Dimethoate								+++						
Endrin				+++				+++						
Fenoprop				+++								+		
Hydroxyatrazine							+++					+++		
Isoproturon								+++				+++		
Lindane				+++				+++						

Table A4.3 (continued)

	Col	GC	(PT-) GC-PD	(PT-) GC-ECD	GC-FID	GC-FPD	GC-TID	GC-MS	PT-GC-MS	HPLC	HPLC-FD	HPLC-UVPAD	EAAS	IC-FD
Mecoprop				+++				+++						
Methoxychlor								+++						
Metolachlor				+++				+++						
Molinate		+++						+++						
Pendimethalin								+++						
Simazine				+++				+++						
2,4,5-T				+++								+		
Terbuthylazine								+++				++		
Trifluralin		+++		+++				+++						

[a] For definitions and notes to Table A4.3, see below Table A4.6.
[b] LC-MS is also applicable for many of these agricultural chemicals.

Table A4.4 Analytical achievability for chemicals used in water treatment or from materials in contact with water for which guideline values have been established[a]

	Col	GC	(PT-) GC-PD	(PT-) GC-ECD	GC-FID	GC-FPD	GC-TID	GC-MS	PT- GC-MS	HPLC	HPLC-FD	HPLC-UVPAD	EAAS	IC
Disinfectants														
Monochloramine	+++													
Chlorine	+++													
Sodium dichloroisocyanurate							+++	+++				+++		
Disinfection by-products														
Bromate														++
Bromodichloromethane				+++				+++	+++					
Bromoform				+++				+++	+++					
Chlorate														+++

Table A4.4 (continued)

	Col	GC	(PT-) GC-PD	(PT-) GC-ECD	GC-FID	GC-FPD	GC-TID	GC-MS	PT- GC-MS	HPLC	HPLC-FD	HPLC-UVPAD	EAAS	IC
Chlorite														+++
Chloroform				+++				+++	+++					
Dibromoacetonitrile				+++				+++						
Dibromochloromethane				+++				+++	+++					
Dichloroacetic acid				+++				+++						
Dichloroacetonitrile				+++				+++						
Monochloroacetic acid				+++				++						
N-Nitrosodimethylamine								+++						
Trichloroacetic acid				+++				+++						
2,4,6-Trichlorophenol				+++				+++						
Trihalomethanes[b]				+++				+++	+++					
Organic contaminants from treatment chemicals														
Acrylamide							+				+			
Epichlorohydrin				+++	+++				+					
Organic contaminants from pipes and fittings														
Benzo[*a*]pyrene								++			++			
Vinyl chloride			++	++					+					

[a] For definitions and notes to Table A4.4, see below Table A4.6.
[b] See also individual trihalomethanes.

Table A4.5 Analytical achievability for pesticides used in water for public health purposes for which guideline values have been established[a]

	Col	GC	GC-PD	GC-EC	GC-FID	GC-FPD	GC-TID	GC-MS	PT-GC-MS	HPLC	HPLC-FD	HPLC-UVPAD	EAAS	IC/FD
Chlorpyrifos				+++		++	++	+++						
DDT (and metabolites)				++				++						

[a] For definitions and notes to Table A4.5, see below Table A4.6.

Table A4.6 Analytical achievability for cyanobacterial toxins for which guideline values have been established[a,b,c]

Cyanotoxin group	PPA	RBA	ELISA	HPLC-UVPAD	LC-MS/PAD	HPLC-FD with pre- or post-column derivatization	LC-MS/MS[d]
Anatoxin-a variants		+	++			+++	+++
Cylindrospermopsins			++	++	++		+++
Microcystins	+		++	++	++		+++
Saxitoxins		++	+			+++	+++

[a] For definitions and notes to Table A4.6, see below this table.
[b] Note that most cyanotoxins are contained within the cell material and prior extraction is crucial. See the chapter 12 fact sheets for further details.
[c] Note that for anatoxin-a variants, although the data were inadequate to establish a guideline value, a provisional reference value was derived.
[d] Quantitative reference standards are needed for congeners. See the chapter 12 fact sheets for further details.

Definitions to Tables A4.1–A4.6

Absor	Absorptiometry	HPLC-UVPAD	High-performance liquid chromatography–ultraviolet photodiode array detector
Col	Colorimetry	IC	Ion chromatography
EAAS	Electrothermal atomic absorption spectrometry	IC-FAAS	Ion chromatography–flame atomic absorption spectrometry
ELISA	Enzyme-linked immunosorbent assay	IC-FD	Ion chromatography–fluorescence detector
FAAS	Flame atomic absorption spectrometry	IC-SCD	Ion chromatography-suppressed conductivity detection
GC	Gas chromatography	ICP	Inductively coupled plasma
GC-ECD	Gas chromatography–electron capture detector	ICP-AES	Inductively coupled plasma atomic emission spectrometry
GC-FID	Gas chromatography–flame ionization detector	ICP-MS	Inductively coupled plasma mass spectrometry
GC-FPD	Gas chromatography–flame photodiode detector	LC-MS	Liquid chromatography–mass spectrometry
GC-MS	Gas chromatography–mass spectrometry	LC-MS/MS	Liquid chromatography–tandem mass spectrometry
GC-PD	Gas chromatography–photoionization detector	LC-MS/PAD	Liquid chromatography–mass spectrometry-photodiode array detector
GC-TID	Gas chromatography–thermal ionization detector	PPA	Protein phosphatase assay
HPLC	High-performance liquid chromatography	PT-GC-MS	Purge-and-trap gas chromatography–mass spectrometry
HPLC-FD	High-performance liquid chromatography–fluorescence detector	RBA	Receptor-binding assay

Notes to Tables A4.1–A4.6

+	The detection limit is between the guideline value and 1/10th of its value.
++	The detection limit is between 1/10th and 1/50th of the guideline value.
+++	The detection limit is under 1/100th of the guideline value.
#	The analytical method is available for detection of the guideline value concentration, but it is difficult to detect the concentration of 1/10 of the guideline value.
(H)	This method is applicable to the determination by conversion to their hydrides by hydride generator.