The determination of microcystin algal toxins in raw and treated waters by high performance liquid chromatography 1998

Methods for the Examination of Waters and Associated Materials

This booklet contains a method for the determination of a number of microcystin algal toxins in raw and treated waters by HPLC using UV detection with diode array detection.

Chromatographic methods are very sensitive to minor physical and chemical variations in the quality of materials and apparatus used. The method in this booklet reports the materials actually used in the performance testing but this does not constitute an endorsement of these particular products. Equivalent materials are acceptable and it should be understood that the performance characteristics of the method may differ if other materials are used. It is left to users to evaluate this method in their own laboratories.

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, groundwater, river and seawater, waste water and effluents as well as sewage sludges, sediments 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 reported for most parameters. 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 encompassing at least ten degrees of freedom 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. An indication of the status of the method is shown at the front of this publication on whether or not the method has undergone full performance testing.

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 in the series 'Methods for the Examination of Waters and Associated Materials' and their continuous revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are:

1.0	General principles of sampling and accuracy of results
2.0	Microbiological methods
3.0	Empirical and physical methods
4.0	Metals and metalloids
5.0	General non-metallic substances
6.0	Organic impurities
7.0	Biological monitoring
8.0	Sewage treatment methods and biodegradability
9.0	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.

Publication of new or revised methods will be notified to the technical press. An index of methods and the more important parameters and topics is available from HMSO (ISBN 0 11 752669 X).

Every effort is made to avoid errors appearing in the published text. If, however, any are found please notify the Secretary.

Dr D Westwood
Secretary

October 1997

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 any regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1988 (SI 1988/1657). 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, and these should be consulted and be readily accessible to all analysts. Amongst such publications are those produced by the Royal Society of Chemistry, namely 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', 5th edition, 1992; by Member Societies of the Microbiological Consultative Committee, 'Guidelines for Microbiological Safety', 1986, Portland Press, Colchester; and by the Public Health Laboratory Service 'Safety Precautions, Notes for Guidance'. Another useful publication is produced by the Department of Health entitled 'Good Laboratory Practice'.

Determination of microcystin algal toxins and nodularin in raw and treated waters by high performance liquid chromatography

Introduction

Microcystins are cyclic heptapeptide hepatotoxins and are produced by certain strains of various species of blue-green algae (cyanobacteria). To date, more than 50 have been characterised. Nodularin is a cyclic pentapeptide hepatotoxin and is sometimes found in brackish waters. Nodularin has been used (10.1) as an internal standard where its presence in drinking water was shown to be negligible. Algal toxins are named using a one-letter abbreviation to indicate each of two amino acid derivatives present in the molecule, see Table 4.

Stability studies have shown that microcystin-RR, microcystin-LR, microcystin-YR and nodularin are degraded quite rapidly. Reservoir water samples were spiked on five occasions over a 16-day period with microcystin-RR, microcystin-LR, microcystin-YR and nodularin so that the concentration of each compound was 10 µg l^{-1}. On day 16, all samples were analysed. The mean concentration of each toxin found in the samples is given in Table 1. Compared to the initial concentrations, the levels of all compounds were significantly lower after five days.

Algal toxins are initially produced intra-cellularly, and may be released when algal cell walls rupture. As they are potent mammalian toxins, there is concern regarding their potential presence in both raw waters and drinking waters, particularly when significant numbers of cells are present in raw water storage reservoirs.

The method, as published (10.2), describes the determination of microcystin algal toxins and nodularin dissolved in raw and treated waters (extra-cellular toxins) and within algal cells (intra-cellular toxins). Only the determination of dissolved toxins has been performance tested.

1 Performance characteristics of the method

1.1	Substances determined	Soluble microcystin-RR, microcystin-YR, microcystin-LR and nodularin. Other microcystins may be determined. At present, few commercially available toxins are obtainable for confirmation and standard solution preparation.
1.2	Type of sample	Raw and drinking waters.
1.3	Basis of method	Samples are filtered to remove algal cells and extracted using solid phase extraction. Following concentration, the extracts are analysed using reversed phase high performance liquid chromatography (HPLC) with gradient elution and ultraviolet (UV) photodiode array detection.
1.4	Range of application	Typically up to 10 µg l^{-1}.
1.5	Calibration curve	Linear over the range 1-10 µg l^{-1}.
1.6	Total standard deviation	See Table 3.
1.7	Limit of detection	Typically 0.5 µg l^{-1}. See Table 2.
1.8	Bias	See Table 3.
1.9	Time required for analysis	Results may be obtained within 24 hours of the commencement of sample processing. Depending on the availability of equipment, several samples may be extracted simultaneously and analysed sequentially so that several results may be available within this period.

2 Principle

Samples are filtered to remove algal cells. The filtrate is then extracted using a C_{18} solid phase extraction cartridge. The cartridge is washed with aqueous methanol before elution with acidified methanol. The acidified methanol extract is reduced to dryness; the residue is redissolved in methanol and again evaporated to dryness. The residue is taken up in 70% aqueous methanol and centrifuged (if necessary) to remove solid material. A portion of the supernatant solution is analyzed by reversed-phase high performance liquid chromatography with UV detection at 238 nm, using a photodiode array detector operating in the range 200-300 nm.

The peaks detected by HPLC-UV are compared to typical microcystin UV spectra. These spectra fall into two groups; one group having an absorption maximum at 238-240 nm and the other group (tryptophan-containing microcystins) with a maximum at 222 nm. All the peaks in the HPLC-UV chromatogram for which there is good correlation between the spectra obtained from samples and those from microcystin standards are assumed to be due to microcystins. Figure 1 shows a typical chromatogram of several microcystin algal toxins. Figure 2 shows the structure of microcystin - LR.

Quantification of microcystin responses is carried out using a standard curve based on peak areas obtained for pure microcystin standards. Results are corrected for incomplete recoveries during the extraction process.

3 Interferences

Any compounds present in a sample and extracted using the C_{18} solid phase cartridge and which are unaffected by the concentration procedure, and which elute with similar retention times as microcystins from the HPLC column under the same conditions and have similar absorption spectra to microcystins, will interfere. No interferences were detected during performance testing, but samples containing algal cells or constituents of algal cells were not tested. As cyanobacterial toxins are contained within the cell walls of living cells, and are released when the cells are ruptured, cell lysis during sample storage or sample processing may lead to release of intra-cellular toxin into the water sample. This portion will then be determined with that amount already present in solution. The recorded result will represent an over-estimation of the levels of algal toxins present in solution at the time of sampling.

4 Hazards

Microcystins and nodularin are potent mammalian toxins and appropriate precautions should be taken when handling pure toxins or standard solutions and samples. Methanol is toxic and flammable. Trifluoroacetic acid is toxic and corrosive and should be handled in a well ventilated fume cupboard. Acetonitrile is toxic and flammable.

5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the analysis. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade reagent materials are normally suitable unless otherwise specified.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other vessels found to be suitable and kept in the dark.

5.1 **Microcystin and nodularin standards.** At the time the performance data were obtained, few toxin standards were commercially available for confirmation and standard solution preparation. Those that are available can normally be obtained in septum-sealed vials containing 500 ± 10 µg. Checks can be undertaken to ascertain the comparative purity of standard toxins before use. These checks include amino acid analysis or HPLC analysis with UV detection. Vials of toxin standards should be stored at a temperature of about -18 °C.

5.1.1 **Standard solutions (1 µg µl-1).** Add 500 ± 5 µl of methanol to a septum-sealed vial containing 500 µg of toxin. Appropriate dilution with methanol (for example a 10-fold dilution) of aliquots of these solutions will provide suitable spiking solutions (for recovery tests) or standard solutions, suitable for injection into the HPLC system.

All standard solutions should be stored at -18 °C when not in use and are stable for periods of up to one year. However, they should be checked before use to ensure that their concentrations remain unchanged.

5.2 **Aqueous methanol.**

5.2.1 **Aqueous methanol (10%v/v).** Dilute 100 ± 2 ml of methanol in water and make up to 1000 ± 20 ml with water. Mix well.

5.2.2 **Aqueous methanol (20%v/v).** Dilute 200 ± 5 ml of methanol in water and make up to 1000 ± 20 ml with water. Mix well.

5.2.3 **Aqueous methanol (30%v/v).** Dilute 300 ± 5 ml of methanol in water and make up to 1000 ± 20 ml with water. Mix well.

5.2.4 **Aqueous methanol (70%v/v).** Dilute 700 ± 10 ml of methanol in water and make up to 1000 ± 20 ml with water. Mix well.

5.3 **Sodium thiosulphate solution.** Dissolve 10.0 ± 0.2 g of sodium thiosulphate pentahydrate in water and make up to 1000 ± 20 ml with water. Mix well.

5.4 **Aqueous trifluoroacetic acid (10%v/v).** Dilute 100 ± 2 ml of trifluoroacetic acid in water and make up to 1000 ± 10 ml with water. Mix well.

5.5 **Methanolic trifluoroacetic acid (0.1%v/v).** Dilute 1.00 ± 0.02 ml of trifluoroacetic acid in methanol and make up to 1000 ± 10 ml with methanol. Mix well. This solution should be prepared and used on the same day.

5.6 **Solid phase cartridges.** The cartridges used to generate the performance data were Waters µBondapak C_{18} column (300 x 3.9 mm id, 10 µm) and 1 g (3 ml) Isolute C_{18} trifunctional, end-capped solid phase extraction cartridge. Similar cartridges from other manufacturers may also be suitable but should be evaluated.

5.7 **HPLC eluents.** Both eluents should be degassed before use.

5.7.1 **Eluent A (0.05% v/v aqueous trifluoroacetic acid).** Dilute 500 ± 10 µl of trifluoroacetic acid in water. Make up to 1000 ± 10 ml with water. Mix well..

5.7.2 **Eluent B (0.05% v/v trifluoroacetic acid in acetonitrile).** Dilute 500 ± 10 µl of trifluoroacetic acid in acetonitrile. Make up to 1000 ± 10 ml with acetonitrile. Mix well.

5.8 **Nitrogen.** Oxygen-free, 99.9%.

6 Apparatus

6.1 **Syringes and volumetric flasks.** Various sizes. For example, between 10 µl and 1 ml for syringes and 5 ml and 50 ml for flasks.

6.2 **Sample bottles.** Sample bottles of at least 500 ml capacity should be made of glass. Plastic screw tops are suitable provided they are fitted with polytetrafluoroethylene (PTFE) or PTFE-faced liners. Alternatively, ground-glass stoppered glass bottles may be used. Before use, the bottles should be cleaned, acid washed with, for example, hydrochloric acid (4M) and rinsed thoroughly with deionised or distilled water.

6.3 **Filtering equipment.** Porcelain Buchner filter funnels (110 mm diameter) with for example 110 mm diameter GF/C filter discs, fitted to a 1 litre Buchner flask with a Neoprene adapter ring funnel support. Alternatively, similar all-glass filtering equipment may be used. Water vacuum pumps provide a suitable vacuum.

6.4 **Extraction apparatus.** Various extraction apparatus can be used. This may be manually or automatically operated. Typically, a vacuum manifold, to which several solid phase extraction cartridges are attached, can be used. Each solid phase cartridge is fitted via a suitable connector to a reservoir tube. A reservoir volume of 6 ml is appropriate for the 1 g (3 ml) cartridges used. The reservoir can be connected to a 500 ml glass bottle containing the sample to be extracted via PTFE tubing and suitable fittings.

6.5 **Extract concentration equipment.**

6.6 **Centrifuge.**

6.7 **HPLC.** Any HPLC system capable of delivering a linear binary gradient at a flow rate of approximately 1 ml min^{-1} and equipped with a diode array detector capable of operating in the range 200-300 nm is suitable. It is advantageous if the system is also equipped with an autosampler and a computerised data handling system. A column temperature controller is required to maintain the HPLC column at a temperature of 40 °C.

The following gradient was used:

	Time (min)						
	0	**10**	**40**	**42**	**44**	**46**	**55**
Eluent A (%)	70	65	30	0	0	70	70
Eluent B (%)	30	35	70	100	100	30	30

Injection volume: 25 µl.

Detection: Photodiode array detector operated over the range 200-300 nm. Microcystin-RR, microcystin-YR, microcystin-LR and nodularin have an absorption maximum at 238 nm.

7 Sample collection and preservation

Sample bottles should be thoroughly rinsed with the water to be sampled before taking the sample for analysis. Microcystin algal toxins have been shown to be biodegraded when stored in reservoir water at ambient temperatures for periods of a few days (10.3). Hence, samples should be analysed as soon as possible after being taken. If storage is unavoidable, samples should be at 4 °C, preferably after filtration.

8 Analytical procedure

Step	Procedure	Notes
8.1	**Sample pre-treatment**	
8.1.1	Using gentle suction to avoid rupturing the filter disc or any algal cells that may be present, filter (6.3) 500 ± 5ml of sample. Add 100 ± 5 µl of sodium thiosulphate solution (5.3) to the filtered sample (note a). Shake the sample vigorously and allow to stand for 2-3 minutes. Add 5.0 ± 0.2 ml of aqueous trifluoroacetic acid (5.4) and mix thoroughly. Vacuum filter through a filter disc (6.3) and add 5.0 ± 0.2 ml of methanol to the filtrate.	(a) This eliminates residual free chlorine that may be present in drinking water.
8.2	**Extraction**	
8.2.1	Condition a solid phase cartridge (5.6) by passing 10 ml of methanol through it, followed by 10 ml of water. Discard the eluates (note b). Pass the sample from step 8.1.1 through the cartridge (at a flow rate not exceeding 10 ml min^{-1}). Discard the eluate. Wash the cartridge (sequentially) first with 10.0 ± 0.5 ml of 10% aqueous methanol (5.2.1), then with 10.0 ± 0.5 ml of 20% aqueous methanol (5.2.2) and finally with 10.0 ± 0.5 ml of 30% aqueous methanol (5.2.3). Discard washings. Dry the cartridge by drawing air through it for 30 ± 5 minutes. The cartridge is then eluted with 3.0 ± 0.3 ml of methanolic trifluoroacetic acid (5.5) and the eluate collected in a vial.	(b) Do not allow the cartridge to dry out during conditioning and extraction.
8.3	**Concentration**	
8.3.1	Evaporate the extract from 8.2.1 at 45 ± 2°C using a gentle stream of dry nitrogen. The residue is dissolved in 100 ± 5µl of methanol and transferred to a micro-centrifuge tube. Rinse the vial with 100 ± 5 µl of methanol and combine with the suspension in the centrifuge tube. Evaporate the methanol using nitrogen. If the HPLC analysis is to be undertaken immediately, the residue is re-suspended in 75 ± 1 µl of methanol. If the HPLC analysis is not carried out immediately (note c) the residue may be stored at -18°C and re-suspended in 75 ± 1 µl of methanol before HPLC analysis.	(c) It has been suggested that a delay in analysing the extract leads to poor recoveries.
8.4	**HPLC analysis**	
8.4.1	If particulate matter is visible in the concentrated extract, centrifuge the tube (note d).	(d) This prevents potential problems with blockages or increased back-pressures.

Inject 25 µl of extracted samples, blanks and standard solutions into the HPLC system under the conditions described in section 6.7 (note e).

(e) Although manual injection can be used, it is more convenient to utilise an autosampler provided low-volume autosampler vials (or low-volume inserts for standard autosampler vials) are available.

Using standard solutions (5.1.1) a calibration curve for each of the microcystin algal toxins to be determined, and nodularin, should be produced. This should cover the range 0-1500 ng of each toxin injected on column.

8.5 Blanks and recoveries

8.5.1 Adequate blank values should be obtained using interference-free water before analysing samples. Adequate recovery values should be obtained using water of a similar nature to the sample being analysed. At least one reagent blank should be analysed with each batch of samples. Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard solutions (5.1.1) to separate samples of interference-free water immediately before extraction. Process these solutions under conditions identical to those used for the samples under investigation. If the responses of the extracted standard solutions are used for comparison with those of the samples, an automatic correction is obtained. If responses from un-extracted standard solutions are used, the data from previous tests should be averaged and a mean correction factor determined and used for correcting for recovery.

8.6 AQC

8.6.1 Carry out the entire procedure using distilled water accurately spiked at levels of individual algal toxin concentrations, for example 2-10 µg l^{-1}. Analyse a corresponding distilled water blank.

9 Calculation

Quantification of microcystin algal toxins and nodularin is carried out using external standard calibration based on peak area measurement obtained using un-extracted standard solutions.

The total concentration of each algal toxin is given by:

$$C = \frac{c \times v}{V} \quad \mu g\ l^{-1}$$

where

C = total concentration of the algal toxin in the original sample (µg l^{-1}),
c = concentration determined in the extract from the calibration graph (µg ml^{-1}),
V = volume of sample extracted (litres), normally 0.5 litre,
v = volume of extract injected in the determination (µl).

Alternative methods of calculation may be used provided they give equivalent results. The calculations are more easily performed using a laboratory data handling system.

10 References

10.1 Determination of microcystin-LR in drinking waters by HPLC 1994. Methods for the Examination of Waters and Associated Materials, in this series.

10.2 Lawton, L.A., Edwards, C. and Codd, G.A. (1994) Extraction and High Performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Treated Waters. *The Analyst* **119**, 1525-1529.

10.3 WRc / DWI report, number 4108 (1996). Microcystin analysis - Supervision of and results from performance testing.

Table 1 **Stability tests of spiked reservoir water** (initial concentration of each toxin was 10 µg l^{-1})

Day	Microcystin-LR	Microcystin-RR	Microcystin-YR	Nodularin
0	9.27 (0.42)	10.89 (1.00)	9.04 (1.50)	10.72 (0.97)
5	6.41 (0.83)	7.60 (1.85)	5.06 (0.21)	7.03 (1.25)
8	0.41 (0.41)	0.62 (0.24)	0.08 (0.15)	0.73 (1.24)
12	0.41 (0.28)	0.56 (0.47)	0.36 (0.13)	0.43 (0.54)
16	1.02 (0.65)	1.04 (0.46)	0.25 (0.23)	0.20 (0.29)

Each result represents a mean of four determinations and figures in brackets are standard deviations. The limit of detection was approximately 0.5 µg l^{-1}.

Table 2 **Limits of detection** (µg l^{-1})

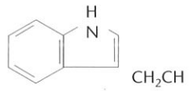
Laboratory	Microcystin-LR	Microcystin-RR	Microcystin-YR	Nodularin
1	0.3	0.6	0.6	0.4
2	0.4	0.5	0.5	0.4
3	2.0	2.8	2.1	0.9
4	0.3	0.3	0.4	0.3

Limits of detection were each obtained with 11 degrees of freedom and calculated from 4.65 x S_W where S_W is the within-batch standard deviation of a blank (deionised water) spiked at 0.5 µg l^{-1}.

Table 3 **Means (and standard deviations) of spiked samples from inter-laboratory trial**

Laboratory	Microcystin-RR	Microcystin-YR	Microcystin-LR	Nodularin
Blank (deionised water spiked at 0.5 µg l^{-1})				
1	0.52 (0.15)	0.58 (0.14)	0.51 (0.13)	0.52 (0.08)
2	0.40 (0.18)	0.38 (0.13)	0.31 (0.12)	0.33 (0.12)
3	1.08 (0.98)	1.72 (1.03)	1.30 (0.84)	1.08 (0.98)
4	0.67 (0.11)	0.69 (0.13)	0.66 (0.11)	0.68 (0.09)
Raw water (spiked at 2.0 µg l^{-1})				
1	1.40 (0.23)	2.57 (0.42)	2.29 (0.36)	1.97 (0.24)
2	1.72 (0.31)	1.78 (0.17)	1.94 (0.16)	1.92 (0.17)
3	1.17 (0.58)	2.35 (1.22)	1.67 (0.85)	1.78 (0.86)
4	1.98 (0.29)	2.63 (0.41)	2.07 (0.29)	2.16 (0.29)
Raw water (spiked at 8.0 µg l^{-1})				
1	5.73 (0.77)	8.88 (1.70)	8.50 (1.43)	7.67 (1.15)
2	8.46 (1.39)	7.83 (0.74)	7.89 (0.70)	8.34 (0.66)
3	5.14 (1.75)	7.77 (3.36)	6.95 (2.36)	7.35 (1.97)
4	7.67 (0.78)	8.42 (0.92)	7.91 (0.87)	8.02 (0.83)
Treated water (spiked at 2.0 µg l^{-1})				
1	1.98 (0.42)	2.24 (0.38)	2.16 (0.41)	2.06 (0.47)
2	2.23 (0.31)	1.90 (0.16)	1.86 (0.15)	2.05 (0.12)
3	1.63 (0.74)	2.58 (1.03)	2.00 (0.82)	2.09 (0.86)
4	2.03 (0.44)	2.01 (0.42)	2.01 (0.35)	2.07 (0.18)
Treated water (spiked at 8.0 µg l^{-1})				
1	7.67 (1.55)	8.99 (1.34)	8.76 (1.73)	8.10 (1.41)
2	9.59 (1.07)	8.10 (0.99)	7.88 (0.89)	8.27 (0.69)
3	5.75 (2.95)	9.69 (3.48)	7.50 (3.00)	7.59 (2.65)
4	7.93 (0.92)	7.96 (0.89)	7.90 (0.94)	7.99 (0.66)
Standard solution (spiked at 1.0 µg l^{-1})				
1	0.95 (0.19)	1.15 (0.20)	1.02 (0.21)	0.99 (0.16)
2	0.98 (0.10)	1.03 (0.11)	0.95 (0.10)	1.01 (0.09)
3	0.84 (0.48)	1.32 (0.61)	1.27 (0.97)	0.96 (0.42)
4	1.19 (0.23)	1.25 (0.26)	1.13 (0.17)	1.16 (0.19)
Standard solution (spiked) at 9.0 µg l^{-1})				
1	7.20 (0.80)	9.08 (1.48)	9.16 (1.04)	8.59 (1.21)
2	9.89 (1.51)	8.69 (0.74)	8.72 (0.69)	8.49 (0.78)
3	7.22 (4.36)	11.6 (5.42)	9.35 (4.72)	9.27 (4.31)
4	9.30 (0.97)	9.31 (1.07)	9.21 (0.90)	9.33 (0.78)

Table 4 **Structure and naming of microcystin algal toxins**

Abbreviation	Amino acid derivative	Structure
L	leucine	$(CH_3)_2CHCH_2CH$
A	alanine	CH_3CH
R	arginine	$HNC(NH_2)NHCH_2CH_2CH_2CH$
F	phenylalanine	$C_6H_5CH_2CH$
Y	tyrosine	$1,4(HO)C_6H_4CH_2CH$
W	tryphophan	

The amino acid derivatives appear in positions X and Y within the general microcystin - XY structure (see Figure 2). The abbreviation X represents the first single letter abbreviation and Y represents the second single letter abbreviation.

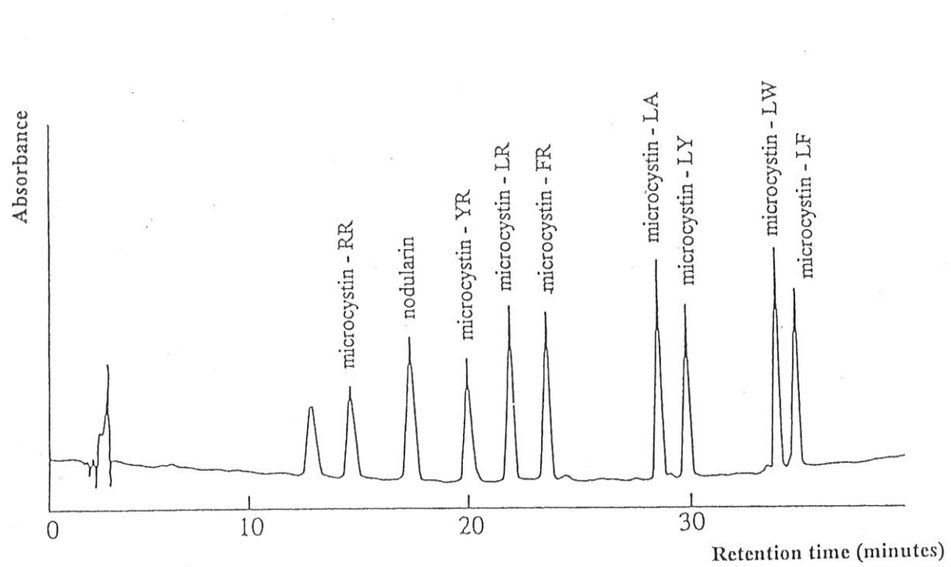


Figure 1 **Chromatogram of several algal toxin standard solutions**

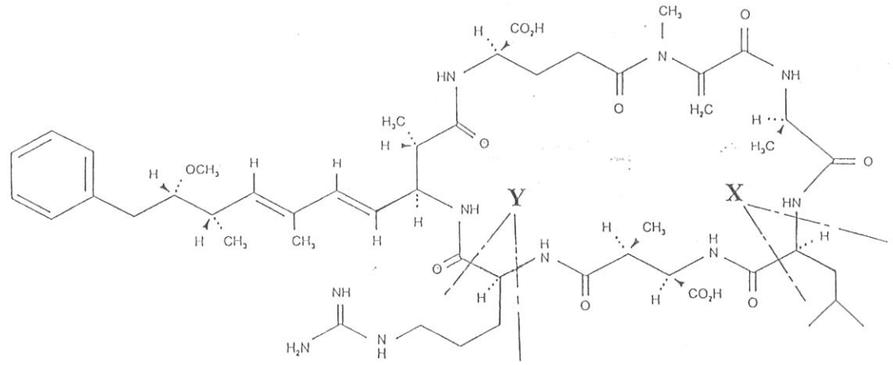


Figure 2 **Structure of microcystin - LR (see also Table 4)**

Analytical Quality Control

1 Routine control

Once a method has been selected for routine use, a system of analytical quality control should be adopted in order to validate the analysis. At least one control standard should be analysed with each batch of samples and the results plotted on a control chart. Corrective action should be taken if one value falls outside of the action limit (at ± 3s) or two consecutive values exceed the warning limit (at ± 2s). As more data are acquired, the standard deviation, s, should be updated and the control chart limits recalculated.

2 Estimation of the accuracy of analytical results using this method

None of the methods given in this booklet has been thoroughly investigated for all types of samples and before general use the accuracy achievable should be known. It would be of great value if any laboratory using or considering the use of this method would estimate the accuracy of its own analytical results and report the findings to the Secretary of the Standing Committee of Analysts.

Address for correspondence

However well a method is tested, there is always the possibility of discovering a hitherto unknown problem. Users with information on this method are requested to write to the address below:

The Secretary
Standing Committee of Analysts
Environment Agency
Steel House
11 Tothill Street
London
SW1H 9NF

Environment Agency

Standing Committee of Analysts

Members assisting with these methods

This booklet is based upon an inter-laboratory trial funded by Department of the Environment and National Rivers Authority. Comments on the report produced were made by members of the Algal Toxins panel of the Standing Committee of Analysts.

MANAGEMENT AND CONTACTS:

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For general enquiries please call your local Environment Agency office. If you are unsure who to contact, or which is your local office, please call our general enquiry line.

ENVIRONMENT AGENCY
GENERAL ENQUIRY LINE
0645 333 111

The 24-hour emergency hotline number for reporting all environmental incidents relating to air, land and water.

ENVIRONMENT AGENCY
EMERGENCY HOTLINE
0800 80 70 60

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