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High-performance liquid chromatographic determination of the triphenylmethane dye, malachite green, using amperometric detection at a carbon fibre microelectrode

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ABSTRACT

The triphenylmethane dye, malachite green (Colour Index C.I. Basic Green 4) has been determined in its chromatic form in drinking and river water using high-performance liquid chromatography with amperometric detection at a carbon fibre electrode. The limit of detection of the technique was 0.07 mg/l and the linear range extended from 0.07–10 mg/l. The use of a 250-fold preconcentration on solid-phase extraction cartridges allowed the analysis of malachite green at the 0.28 μg/l level. The technique was found to be reproducible with a mean relative standard deviation of 5.4 and 3.6% at the 0.25 and 5 mg/l levels, respectively. The application of the technique to the analysis of drinking and river water samples is demonstrated. Typical recoveries from drinking water and river water samples spiked with 1 μg/l chromatic malachite green were 82 and 41%, respectively. Sorption of malachite green on to suspended organic matter in the water column is discussed. The simultaneous analysis of the chromatic malachite green and its biotransformed leuco form is described.

INTRODUCTION

Since first reported as being effective in controlling fungi on bass, trout and trout eggs [1], the triphenylmethane dye, malachite green (Colour Index C.I.; Basic Green 4), has found extensive use throughout the world in the fish farming industry as a fungicide and an ecto-parasiticide. However, it has been the subject of close scrutiny due to its suggested teratogenic properties, and, in a programme of registration of fisheries chemicals in the USA, its use has been prohibited in federal government facilities [2]. The use of malachite green is now limited to the treatment of non-food fish under an Investigational New Animal Drug Application held by the US Fish and Wildlife Service.

In Europe, malachite green is regularly used for the control of *Saprolegnia parasitica* and also in the treatment of proliferative kidney disease (PKD). Its use is controlled within the EEC by guidelines laid down in each of the individual member states. In Ireland, these state that the concentrations of malachite green in fish farm water effluent and water extracted for drinking water purposes should not exceed 100 and 1.0

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μg/l, respectively. Under the fisheries and agriculture research programme (FAR), an EEC project into the environmental fate of malachite green is presently being carried out in Trinity College, Dublin.

Malachite green and the closely related triphenylmethane dye crystal violet are reduced through biotransformation to their leuco forms in animals [3,4]. Werth and Boiteux [3], have shown that the organs of rats, injected with malachite green, contain a considerable proportion of leuco malachite green two hours after injection. Although never tested, the leuco form of malalchite green is structurally similar to classical aromatic amine carcinogens. The chromatic and leuco forms of malachite green are shown in Fig. 1.

In response to concerns regarding the health risks associated with the use of malachite green, an increasing number of methods have been developed in the past decade for its determination in environmental samples and food fish tissue.

A thin-layer chromatographic (TLC) method was first used for the analysis of malachite green residues in edible fish [5]. A methanolic solution of material extracted from fish was analysed by TLC on silica gel followed by densitometric detection and quantitation at 610 nm.

An extension of this work [6] made use of a high-performance liquid chromatographic (HPLC) separation on a LiChrosorb RP-8 or Spherisorb Octyl column with subsequent spectrophotometric detection and quantitation at 600 nm.

The simultaneous determination of malachite green and methylene blue in rainbow trout and amago muscle using HPLC with subsequent spectrophotometric detection [7] has recently been reported. Residues of malachite green were determined, using a colorimetric method, in muscle, eggs, and fry of Atlantic salmon (*Salmo salar*) and chinook salmon (*Oncorhynchus tshawytscha*) which had been treated with the chemical at fish hatcheries [8]. Allen and Meinertz [9] reported on a post-column reaction for the simultaneous analysis of chromatic and leuco forms of malachite green and crystal violet by HPLC with spectrophotometric detection at 588 nm. This system made use of a post-column reaction chamber containing PbO_2 situated between the HPLC column and the spectrophotometric detector. Separation of the leuco and chromatic forms of the dye was effected on the HPLC column, after which they passed through the post-column reaction chamber and subsequently into the spectrophotometric detector. This method has recently been applied to the analysis of the chromatic and leuco forms of malachite green in water [10].

The use of electrochemical detection in conjunction with HPLC has been used for the simultaneous determination of the chromatic and leuco forms of crystal violet [11]. In view of the importance of determining malachite green in both its chromatic form, used in fungicidal and ectoparasiticidal treatment regimes, and its biotransformed leuco form, a chromatographic methodology involving electrochemical detection at a carbon fibre electrode is reported for the first time in the present paper.

In the past decade, there has been considerable interest in the development of microelectrode flow detectors, with several authors reporting on the use of carbon [12] and gold fibres [13] in such systems. In addition to the advantages offered by microelectrodes in the construction of low volume detectors, the use of such electrodes

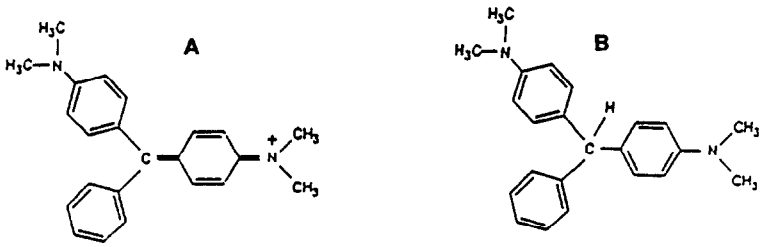

Fig. 1. Chemical structures of (A) chromatic and (B) leuco forms of malachite green.

permits a steady state to be achieved quickly [14], improved signal to noise ratios [15] and a reduced ohmic contribution.

The method proved successful for the determination of the chromatic form of malachite green in river and drinking water samples. In addition, it was also possible to carry out the simultaneous analysis of the chromatic and leuco forms without the need for post-column modification.

EXPERIMENTAL

Chemicals and reagents

Malachite green oxalate and leuco malachite green were obtained form Aldrich (Gillingham, Dorset, UK) and from BDH (Poole, UK), respectively. Acetic acid (AnalaR grade) was supplied by BDH and HPLC-grade methanol was supplied by Labscan Analytical Sciences (Dublin, Ireland). Analytical grade sodium dihydrogen orthophosphate was obtained from BDH. This compound and the supporting electrolyte were used without purification. Deionised water was used throughout this work and was obtained by passing distilled water through a Milli-Q water purification system (Millipore, Milford, MA, USA). Carbon fibres were obtained from Avco (Lowell, MA, USA). These were 14 μm in diameter and their surface had no external coating. Silver epoxy was purchased from RS components (Corby, UK).

Apparatus

Voltammetric studies were performed using an EDT potentiostat (EDT Research, London, UK) in conjunction with a JJ X-Y Model PL4 recorder (JJ Loyd Instruments, Southampton, UK). A three-electrode cell system was employed, incorporating a 0.28 cm^2 area glassy carbon electrode (EG&G Princeton Applied Research), a saturated calomel reference electrode and a platinum counter electrode.

Amperometric measurements were performed using an EG&G Model 400 EC potentiostat (EG&G Princeton Applied Research) connected to the flow cell by crocodile pins. Malachite green was detected amperometrically by employing a potential of +1.2 V at the working electrode. The resultant signals were recorded on a WPA Model CQ 95 X-t recorder (WPA, Linton, Cambridge, UK). The peak currents (measured as peak heights on the recorder) as a function of concentration were then measured for quantitative analysis.

Malachite green was separated on a 250 × 4.6 mm I.D. cyano (CN) stainless-steel column, particle size 5 μm (Hichrom, Reading, UK) using a methanol–0.1 *M* sodium acetate, pH 4.5 (70:30, v/v) mobile phase. Mobile phases containing acetonitrile were not used because previous experience had shown it to have a detrimental effect upon the long term stability of the carbon fibre. A Waters Model 501 HPLC pump was used for mobile phase delivery and sample introduction was via a Rheodyne (Cotati, CA, USA) Model 7010 injection valve, fitted with a 20-μl loop of direct injection.

For extraction purposes, Techelut 100 mg/1.0 ml cyano solid-phase extraction cartridges (HPLC Technology, Cheshire, UK) were used in conjunction with a Techelut 12 position manifold.

Construction of carbon fibre flow cell

The carbon fibre flow cell was constructed according to the procedure described by Sagar *et al.* [16]. The carbon fibre working electrode was inserted through the centre of a 25 mm length polyethylene tubing (2 mm × 0.5 mm I.D.) as reported by Hua *et al.* [17].

Voltammetric procedures

The supporting electrolytes used for pH and cyclic voltammetric studies were prepared from a 0.1 *M* stock solution of sodium dihydrogen orthophosphate. All solutions were prepared in deionised water and were adjusted to the required pH with 0.2 *M* orthophosphoric acid or sodium hydroxide.

Cyclic voltammetry was performed on solutions containing $1 \cdot 10^{-3}$ *M* malachite green dissolved in the supporting electrolyte. Voltammetric conditions were as follows: initial potential 0.0 V; final potential +1.3 V; scan rate 50 mV/s; current range 20 μA.

Hydrodynamic voltammetric studies were carried out by injecting 5 mg/l of malachite green, dissolved in 95% aqueous methanol containing

0.05 *M* sodium acetate, pH 4.5, into the chromatograph with the detector set at different working potentials *vs.* Ag/Ag_3PO_4. Cleaning potentials of +1.3 V and −1.3 V were applied for 60 s, respectively, between each injection. Hydrodynamic voltammograms were constructed by plotting the peak current against the applied potential. The optimum potential for malachite green determination was found from the position of the plateau on the hydrodynamic wave.

Extraction procedure

Drinking water samples were collected from the Dublin city water supply and river water samples were collected from Arklow, County Wicklow. The samples were stored in plastic containers at 4°C on return to the laboratory prior to analysis on the same day.

Drinking water and river water samples were adjusted to pH 5.0 with acetic acid and spiked with 1 μg/l malachite green prior to extraction and preconcentration on the solid phase extraction cartridges. These cartridges were preconditioned with 2 ml of methanol followed by 5 ml of deionised water. An aliquot of 250 ml of the spiked water sample was eluted through the column at a flow-rate of 7 ml/min after which the retained malachite green was eluted with two 0.5-ml volumes of mobile phase. These 0.5-ml aliquots were combined and used for chromatographic analysis.

Chromatographic separation of chromatic malachite green

The liquid chromatographic analysis of the chromatic form of malachite green was based upon the method of Roybal *et al.* [11] for the determination of gentian violet. The chromatography of malachite green was investigated by varying the methanol content and the pH of the mobile phase.

Calibration, recovery and reproducibility

Calibration graphs were constructed by plotting peak current against concentration for a series of chromatic malachite green standards, prepared in mobile phase, at a potential of +1.2 V *vs.* Ag/Ag_3PO_4 and a mobile phase flow-rate of 0.9 ml/min.

Aliquots of malachite green standards, made up in 0.1 *M* sodium acetate, pH 4.5, were added to drinking water and river water samples to give final concentrations of 1.0 μg/l. Replicate spiked samples and the corresponding blanks were extracted according to the extraction procedure previously described. Recoveries of chromatic malachite green from drinking water and river water samples were assessed by comparing the peak currents obtained for the extracts with those of standard solutions.

The reproducibility of the method was ascertained by determining the relative standard deviation of five successive measurements of extracts of water samples.

Simultaneous analysis of chromatic and leuco malachite green

The chromatographic separation of the chromatic and leuco forms of malachite green was carried out on a cyano column using methanol–0.1 *M* sodium acetate, pH 4.5 (70:30, v/v) with amperometric detection at +1.2 V *vs.* Ag/Ag_3PO_4.

RESULTS AND DISCUSSION

Voltammetric behaviour of malachite green

The voltammetric behaviour of the chromatic form of malachite green was studied over the pH range 4–12 in 0.05 *M* phosphate buffer using differential pulse voltammetry (DPV) at a glassy carbon electrode. The single anodic peak obtained in the range studied was found to be pH dependent.

A plot of pH against peak current indicated a pK_a value of 6.5. This value is in close agreement with the value of 6.9 previously reported [18].

In the cyclic voltammogram of malachite green, no cathodic peaks were present in the reverse scan, thus indicating that the oxidation process was irreversible in nature.

In order to determine the working potential used for the amperometric detection of malachite green, hydrodynamic voltammograms were constructed. The hydrodynamic voltammogram exhibited one wave, a finding which was in accordance with the results obtained from the

pH and cyclic voltammetric studies. The hydrodynamic voltammogram indicates that in the potential range studied, the response increased with increased potential. However, at potentials greater than +1.2 V, both the background current and noise levels increased rapidly. Consequently, a potential of +1.2 V was used throughout the study, as this gave the optimum signal-to-noise ratio. The hydrodynamic voltammogram obtained for malachite green is similar to that reported by Roybal *et al.* [11] for the structurally related dye gentian violet. Whilst a study of the mechanism was not carried out in this investigation, it is thought that the oxidation of the triphenylmethane to the triphenylcarbinol derivative, as suggested by Roybal *et al.* [11], is a probable reaction scheme.

Electrode pretreatment

When using an electrochemical detector it is important to take into consideration the fact that the surface of the electrode changes with time due to either the adsorption of species from solution or chemical changes on the electrode surface itself. These changes have important consequences in that they can result in variations in sensitivity, reproducibility and selectivity , and must therefore be overcome if an analytical procedure is to be ultimately successful. During the course of the developmental work on an electrochemical detection system for the determination of malachite green, it was noted that the sensitivity of the technique decreased markedly with repeated injections of malachite green standards. This represented a serious problem regarding the eventual applicability of the method, and in an attempt to overcome it, an electrochemical pretreatment step was included in the analytical methodology.

A pretreatment protocol which improved the peak current involved the pre-anodisation of the microelectrode at +1.3 V for 60 s followed by cathodisation at −1.3 V for 60 s *vs.* Ag/Ag_3PO_4, followed by equilibration at +1.2 V for 3 min. It has been proposed [19] that the improvement in performance is as a result of the removal of surface contaminants or inhibitory layers which hinder electron transport. Thus extended lifetime can be obtained after electrochemical pretreatment. In an analytical methodology without a pretreatment procedure, the working electrode could be used for 3 to 5 measurements before deterioration of the analytical response. With the use of the pretreatment procedure the lifetime of the electrode could be extended up to 30 to 40 measurements.

The effect of pretreating the electrode is to increase its current response, which is probably due to the activation of the surface, producing quinoidal functionalities [20]. It is envisaged that a greater signal-to-noise ratio would be possible with the use of a potentiostat specifically tailored to the measurement of the low currents at such microelectrodes.

Electrode stability

The stability of the electrode is of importance in order to define the period of time over which the electrode can be used without a significant decrease in the sensitivity. The response of the electrode to a 5 mg/l malachite green standard solution was measured over a 9-day period. A significant decrease in the response was observed after the fourth day.

The carbon fibre flow detector yielded peaks whose currents had relative standard deviations of 5.4, 4.8, 6.1 and 5.6% on each of the first four days, respectively. After 6 and 9 days of continuous use, the electrode response was 61 and 38%, respectively, of its initial value, with relative standard deviations of 9 and 11%. There was no leakage from the electrode during its operating lifetime and the nature of its construction made it very stable in the mobile phase.

As the carbon fibre working electrode can be easily and cost effectively replaced, this decrease in sensitivity is not of major consequence. The use of a glassy carbon macroelectrode would otherwise require extensive physical cleaning and polishing steps to renew the electrode surface and consequently regain a sensitive response.

Chromatographic analysis of chromatic malachite green

In the initial chromatographic studies, variations were made in the mobile phase composition to optimise the analysis time and peak width. A mobile phase composed of methanol–

sodium acetate buffer, pH 4.5 (70:30, v/v) yielded the optimum results.

Calibration curves prepared on each of three successive days were linear over the range 0.07–10 mg/l with correlation coefficients of greater than 0.999.

The reproducibility of the technique was determined from five replicate injections of 5 mg/l and 0.25 mg/l standard solutions. Typical reproducibilities, determined from the coefficient of variation of the peak currents, of 3.6 and 5.4% were achieved at the 5 and 0.25 mg/l levels, respectively.

The detection limit of the technique, defined as three times the signal-to-noise ratio, was 0.07 mg/l. When used in conjunction with the 250 to 1 preconcentration on the solid-phase cartridges, the method detection limit was 0.28 μg/l. As previously mentioned, a lower detection limit should be possible with the use of a potentiostat designed to specifically measure the low currents at carbon fibre microelectrodes.

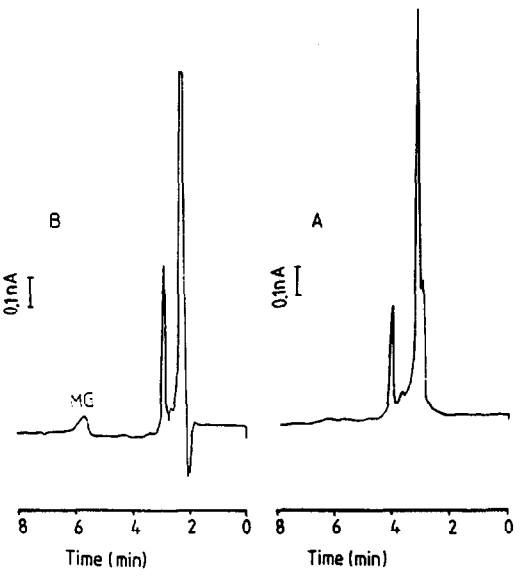

Fig. 2. HPLC chromatograms of drinking water extracts. (A) Blank drinking water sample. (B) Drinking water sample spiked with 1 μg/l malachite green (MG).

Analytical application

The chromatographic method was applied to the analysis of drinking water and river water samples spiked with 1.0 μg/l chromatic malachite green. In Fig. 2, chromatograms are shown for a blank drinking water extract and a spiked drinking water extract.

The chromatograms show well defined peaks for the chromatic form of malachite green with a retention time of 5.9 min. There were no late eluting peaks in the analysis, thus enabling successive injections to be made without the need for a wash period.

The mean recoveries for malachite green were found to be 82 and 41% in drinking water and river water samples, respectively. The reproducibility of the determination was measured by carrying out successive injections for drinking water and river water extracts. The reproducibility, as determined by the relative standard deviation, was 6.5 and 11% for drinking water and river water, respectively.

In Ireland, the current directives on malachite green in water state that the maximum permissible levels in fish farm effluent water and water extracted for drinking purposes should not exceed 100 and 1.0 μg/l, respectively. The chromatographic method described here is capable of determining malachite green at the 1.0 μg/l level stated in current directives and is comparable to the method described by Allen and co-workers [9,10].

The sample analyses were carried out in the nanoampere (nA) current range. The use of lower current ranges was prohibited by the presence of early eluting electroactive species which in the low current ranges produced large chromatographic peaks that obscured the analyte peak of interest. As part of continuing research, the incorporation of an extraction procedure into the analytical methodology is being investigated. It is hoped that this will eliminate these electroactive species thus allowing sample analyses to be carried out in the lower current range, and as a consequence improve the detection limits.

The very low and variable recoveries of malachite green from river water samples was thought to be due to its sorption on organic material in the water column. The fraction of dissolved or colloidally dispersed organic macromolecules, such as humic substances or nonfilterable particles in natural waters, have been

shown to exert significant sorption properties towards organic compounds. Harrison *et al.* [21] indicated that organic compounds of low solubility exist in the water column primarily sorbed on suspended solids. The accumulation of such organic compounds in sediments has been attributed to their sorption on suspended solids and subsequent settling [22]. Similar findings were reported by Rushing and Bowman [23] for the analysis of gentian violet in wastewater. The low recoveries encountered during this analysis were attributed to the adsorption of gentian violet onto particulate matter present in the wastewater. Further chromatographic work is in progress to determine the extent to which malachite green is sorbed onto organic matter. The low recoveries from river water samples may be of use in further speciation work. This speciation work is of considerable importance in elucidating the distribution of malachite green in a water system.

Analysis of chromatic and leuco malachite green

The chromatographic separation and detection of the chromatic and leuco forms of malachite green, shown in Fig. 3, was achieved on a cyano column using a mobile phase composed of methanol–0.1 *M* sodium acetate, pH 4.5 (70:30, v/v) and amperometric detection at +1.2 V.

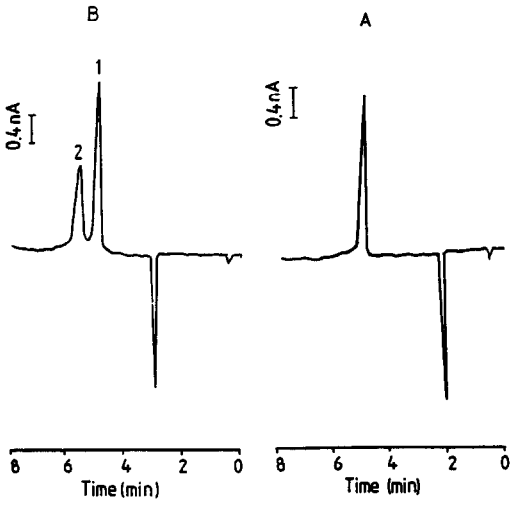

Fig. 3. Typical chromatograms for (A) 5 mg/l leuco malachite green and (B) a mixture containing 5 mg/l of (1) leuco and (2) chromatic forms of malachite green.

Under these conditions the leuco and chromatic forms of malachite green separated into two well resolved peaks with retention times of 5.0 and 5.9 min for the leuco and chromatic forms, respectively.

This ability to simultaneously analyse the chromatic and leuco forms is of great importance in studying the biotransformation of malachite green into its leuco form. In their study on the detoxication of malachite green in rats by the formation of the leuco form of malachite green, Werth and Boiteux [3] determined the distribution of leuco malachite green in tissues by carrying out the photometric titration of leucomalachite green with potassium permanganate in a weak acid solution. However, extreme care had to be exercised in order to ensure that there was quantitative oxidation whilst at the same time avoiding excess titration of potassium permanganate which would otherwise cause oxidative destruction of the molecule. In addition the background presence of the chromatic form of malachite green in the sample extract meant that its concentration had to be determined prior to the titration.

Allen and co-workers [9,10] have previously reported on the use of a post column oxidation step to allow the determination of the chromatic and leuco forms by spectrophotometric detection. This involved the packing and incorporation of a lead dioxide column into the chromatographic apparatus. Bauer *et al.* [24] also used a similar post-column oxidation step which depended upon splitting the sample in two, oxidising half to chromatic malachite green, analysing both sample aliquots for the chromatic form and determining the concentration of the leuco form by a difference method.

The use of the chromatographic method described here should allow direct analysis of a sample extract for chromatic and leuco forms of malachite green without the need for any post column modification procedure, as the use of the carbon fibre flow cell represents a small volume, easily incorporated system which can be simply maintained and replaced when the sensitivity of the response decreases. At the present time, work is being carried out as part of ongoing research in Trinity College, Dublin to adapt this

methodology to the simultaneous analysis of the chromatic and leuco forms of malachite green in tissue samples.

As the leuco form is one of the final intermediates in the production of chromatic malachite green, it is therefore a potential contaminant in commercial supplies. The use of the HPLC method described is ideally suited for rapid quality control of the final product to determine possible contamination from leuco malachite green.

CONCLUSIONS

A high-performance liquid chromatographic method has been developed for the analysis of chromatic malachite green in drinking water and river water, based on solid-phase extraction, reversed-phase chromatographic separation on a cyano column and amperometric detection using a carbon fibre flow cell. The method compares favourably with that reported by Allen *et al.* [10]. The use of electrochemical detection in conjunction with high-performance liquid chromatography permits the sensitive and reproducible determination of the chromatic form of malachite green in drinking water samples at those levels required under current legislation. In terms of detection limit, a lower value should be obtainable with the use of a potentiostat specifically designed for the measurement of the smaller currents associated with microelectrodes.

Low recoveries of malachite green from river water samples were due to its sorption onto organic material suspended in the water column.

The carbon fibre flow cell used in this study showed excellent response characteristics over a four-day working period after which the sensitivity decreased. However, this does not represent a serious problem as the electrode can be easily and cost effectively replaced. The use of electrochemical detection for the analysis of malachite green shows potential for the simultaneous determination of its chromatic and leuco forms.

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REFERENCES

1 F.J. Foster and L. Woodbury, *Prog. Fish-Cult.*, 18 (1936) 7.
2 D.J. Alderman, *J. Fish Dis.*, 8 (1985) 289.
3 G. Werth and A. Boiteux, *Arzneimittel Forsch.*, 18 (1968) 39.
4 J.J. McDonald and C.E. Cerniglia, *Drug Metab. Dispos.*, 12 (1984) 330.
5 M. Edelhaeuser and E. Klein, *Dtsch. Lebensm.-Rundsch.*, 82 (1986) 386.
6 E. Klein and M. Edelhaeuser, *Dtsch. Lebensm.-Rundsch.*, 84 (1988) 77.
7 Y. Kasuga, M. Hishida and N. Tanahashi, *Shokuhin Eiseigaku Zasshi*, 32 (1991) 137.
8 J.L. Allen, *Invest. Fish Control*, 101 (1990) 1.
9 J.L. Allen and J.R. Meinertz, *J. Chromatogr.*, 536 (1991) 217.
10 J.L. Allen, J.R. Meinertz and J.E. Gofus, *J. Assoc. Off. Anal. Chem.*, 75 (1992) 646.
11 J.E. Roybal, R.K. Munns, J.A. Hurlbut and W. Shimoda, *J. Chromatogr.*, 467 (1989) 259.
12 C. Hua, D. Jagner and L. Renman, *Anal. Chim. Acta*, 197 (1987) 257.
13 K. McLaughlin, D. Boyd, C. Hua and M.R. Smyth, *Electroanalysis*, 4 (1992) 689.
14 K. Oldham, *J. Electronal. Chem.*, 323 (1992) 53.
15 H. Emons and F. Matysik, *Electroanalysis*, 4 (1992) 501.
16 K. Sagar, C. Hua and M.R. Smyth, *J. Chromatogr.*, 577 (1992) 109.
17 C. Hua, K. Sagar, K. McLaughlin, M. Jorge, M. Meaney and M.R. Smyth, *Analyst*, 116 (1991) 1117.
18 R.J. Goldacre and J.N. Philips, *J. Chem. Soc.*, 7 (1949) 1724.
19 W. Blaedal and R. Jenkins, *Anal. Chem.*, 46 (1974) 1952.
20 T. O'Shea, A. Garcia, P. Tunon and M.R. Smyth, *J. Electroanal. Chem.*, 307 (1991) 63.
21 R.M. Harrison, R. Perry and R.A. Wellings, *Environ. Sci. Technol.*, 10 (1978) 1151.
22 C.W. Schauberger and R.B. Wildman, *Bull. Environ. Contam. Toxic.*, 17 (1977) 534.
23 L.G. Rushing and M.C. Bowman, *J. Chromatogr. Sci.*, 18 (1980) 224.
24 V.K. Bauer, H. Dangschat, H.O. Knoppler and J. Neudegger, *Arch. Lebensmittelhyg.*, 39 (1988) 97.