



NRC Publications Archive Archives des publications du CNRC

Determination of erythromycin A by liquid chromatography and electrochemical detection, with application to salmon tissue Janeček, Mojmir; Quilliam, Michael A.; Bailey, M. Ruth; North, David H.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. /
La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version
acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien
DOI ci-dessous.

Publisher's version / Version de l'éditeur:

[http://doi.org/10.1016/0378-4347\(93\)80446-B](http://doi.org/10.1016/0378-4347(93)80446-B)

Journal of Chromatography B: Biomedical Sciences and Applications, 619, 1, pp.
63-69, 1993-09

NRC Publications Record / Notice d'Archives des publications de CNRC:

<http://nparc.cisti-icist.nrc-cnrc.gc.ca/eng/view/object/?id=e16ee5b1-84b3-478a-886e-6d9991d23d>

<http://nparc.cisti-icist.nrc-cnrc.gc.ca/fra/voir/objet/?id=e16ee5b1-84b3-478a-886e-6d9991d23d12>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at
<http://nparc.cisti-icist.nrc-cnrc.gc.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<http://nparc.cisti-icist.nrc-cnrc.gc.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the
first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la
première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez
pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.



CHROMBIO. 7000

Determination of erythromycin A by liquid chromatography and electrochemical detection, with application to salmon tissue[☆]

Mojmir Janeček^{☆☆} and Michael A. Quilliam^{*}

Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1 (Canada)

M. Ruth Bailey and David H. North

Health Protection Branch, Health and Welfare Canada, P.O. Box 1060, Dartmouth, Nova Scotia B2Y 3Z7 (Canada)

(Received April 6th, 1993)

ABSTRACT

The chromatographic performance of erythromycin A (EA) is improved significantly over that achieved on polymeric columns by using a sterically shielded octyldiisopropylsilica (Zorbax Rx-C₈) column and a neutral mobile phase consisting of 5 mM aqueous sodium perchlorate–acetonitrile (50:50). This mobile phase facilitates electrochemical detection of EA at the 3-pmol level. Temperature control of both column and detection cell is important for minimizing detector noise and drift. A clean-up procedure, based on aminopropylsilica solid-phase extraction, allows the detection of EA in salmon flesh down to the 0.2-ppm level. Some of the metabolites of EA that retain the tertiary amine may also be detected by this method.

INTRODUCTION

Erythromycin A (EA) is a macrolide antibiotic used in human and veterinary therapy for the treatment of bacterial infections. Since bioassay procedures for the determination of EA and its metabolites in biological fluids are of limited sensitivity and selectivity, a number of chemical methods have been developed. In addition to thin-layer chromatography (TLC) [1–4], which is not well suited to quantitative work, several liquid chromatographic (LC) methods have been

described. These assays employ reversed-phase chromatography with either silica-based columns [5–9] or poly(styrene–divinylbenzene) copolymers [10,11]. Generally, the chromatographic performance reported for EA has been rather poor for all columns investigated, with efficiencies ranging from 1200 to 4000 theoretical plates per meter. Column efficiency appears to depend on both pH, with alkaline mobile phases being favored [11], and stationary phase type, with octadecylsilica giving higher efficiencies than polymeric columns. In one paper, the history of a silica-based reversed-phase column was reported to influence the quality of separation; it was found that the chromatographic behavior of EA is sensitive to slight transformations of the surface of the column packing upon aging [8]. It was

* Corresponding author.

☆ NRCC No. 34872.

☆☆ On leave from Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic.

also found that addition of tetrabutylammonium hydrogensulfate to the mobile phase as a silanol-blocking agent was important to reduce interaction between the basic EA and acidic silanol groups. Recently, Paesen *et al.* [12] have evaluated silanol-deactivated silica-based C_{18} phases and observed wide variations in column performance. Using combined liquid chromatography–mass spectrometry (LC–MS), we have shown that high column efficiencies may be achieved with silica-based reversed-phase columns using an aqueous acetonitrile mobile phase acidified with 0.2% formic acid [13]. Under acidic conditions, interaction of EA with silanols appears to be minimized.

Sensitive detection of EA also presents a challenge. Since EA lacks a significant chromophore, UV detection must be performed at low wavelength (215 nm) and thus provides only low sensitivity. A sensitive fluorimetric determination has been reported but requires a complicated apparatus for post-column derivatization and extraction [6]. Ion-spray LC–MS is a very sensitive method for EA and its metabolites, but requires expensive instrumentation [13]. Several papers have reported the use of electrochemical detection (ED) in the oxidative mode [7,9,11], which allows detection of EA down to $0.2 \mu\text{mol/l}$ [11]. Oxidation of EA probably occurs at the tertiary amine of the desoaminyll sugar moiety [7].

In this paper we report on improvements in the LC analysis of EA with ED in the amperometric mode. Chromatographic performances of a wide-bore polymeric column and a narrow-bore octyldiisopropylsilica (Zorbax Rx- C_8) column are compared. Application to the analysis of EA in salmon flesh is demonstrated using a clean-up method recently developed for LC–MS of EA [14].

EXPERIMENTAL

Instrumentation

Experiments were carried out using a Hewlett-Packard (Palo Alto, CA, USA) Model HP1090M liquid chromatograph equipped with DR5 ternary pumping system and variable-volume auto-

sampler. Detection was provided by an HP1049A electrochemical detector operated in the amperometric mode using a glassy carbon working electrode with a potential of +0.9 to +1.1 V (relative to an Ag/AgCl reference electrode). Data acquisition was performed using a HP3396A integrator connected by a serial line to an MS-DOS microcomputer running ChromPerfect 2 (Justice Innovations, Palo Alto, CA, USA). Both column and detector cell were maintained at 40°C . Experiments performed with post-column addition used a microgradient syringe pump (Applied Biosystems, Santa Clara, CA, USA) for make-up flow, with a low-volume ($150 \mu\text{l}$) static mixer packed with glass beads inserted between the T-piece and the detector cell.

Chemicals

All solvents and reagents were analytical or HPLC grade materials. Distilled water was further purified using a Milli-Q system (Millipore, Bedford, MA, USA) equipped with ion-exchange and carbon filters. Acetonitrile and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA), sodium perchlorate was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and sodium borate from Sigma (St. Louis, MO, USA). EA was obtained from the Health Protection Branch Drug Repository, Health and Welfare Canada (HWC, Toronto, Canada) and anhydroerythromycin (AEA) was provided by Dr. E. G. Lovering (Bureau of Drug Research, HWC, Ottawa, Canada). Standard solutions of EA were prepared in methanol and kept at freezer temperatures until used.

Extraction of fish tissues

Ground fish tissue (25 g) was weighed into a 250-ml centrifuge bottle, acetonitrile (70 ml) was added, and the mixture was blended with a Polytron for 1 min. Afterwards, the Polytron head was rinsed with additional acetonitrile (5 ml). After centrifuging at 1000 g for 10 min, the supernatant was decanted through fluted paper into a 250-ml separatory funnel. The tissue was re-extracted by blending for 30 s with fresh acetonitrile (70 ml), centrifuging for an additional 10

min, and decanting the supernatant through the fluted paper into the 250-ml separatory funnel. Hexane (60 ml) was added and the funnel was shaken for 1 min. The acetonitrile layer was drained into a 500-ml separatory funnel, 1 M NaOH (6.6 ml) and methylene chloride (100 ml) were added, and the funnel was swirled. After adding 1% phosphate buffer (100 ml), it was shaken vigorously. After adding NaCl (5 g) and swirling, the funnel was set aside for a few minutes. Additional 1-g portions of NaCl were added until separation was achieved. The methylene chloride layer was transferred to a 500-ml separatory funnel and the aqueous portion was discarded. After washing the methylene chloride with 10% sodium chloride (100 ml), the lower methylene chloride layer was drained into a 500-ml round-bottom flask, through sodium sulfate in a powder funnel with fluted paper. The sodium sulfate was rinsed with approximately 20 ml of methylene chloride. The methylene chloride was removed using a rotary evaporator at 37°C. It was then re-evaporated with 10–20 ml of methylene chloride twice. The sample was transferred to a graduated tube and made to a volume of 25 ml with methylene chloride.

Aminopropylsilica SPE clean-up

Aminopropylsilica cartridges (500 mg Bond-Elut-NH₂, Analytichem) were conditioned with methanol (5 ml) followed by methylene chloride (5 ml), adjusting individual flow control valves to elute at 1 ml/min until the solvent reached the frit at the top of the column. A 5-ml aliquot of sample extract was added and allowed to flow at 1 ml/min. The cartridge was washed with methylene chloride (3 ml) and 1% methanol in methylene chloride (1 ml), while being careful to bring the solvent just to the top of the frit before adding the next portion. These washings were discarded. The EA was eluted with 2% methanol in methylene chloride (2 × 1 ml). The collected eluate was evaporated to near dryness using a stream of nitrogen with gentle warming (40°C). After adding methanol (1 ml), it was re-evaporated to near dryness, made up to a final volume of 0.5 ml with methanol, and filtered with a Millipore Ultrafree-

MC 0.22- μ m filter before transferring to a crimp-topped 0.1-ml polypropylene conical autosampler vial for injection.

LC analysis

Reversed-phase columns of two different types were employed. System 1 used two 150 mm × 4.6 mm I.D. columns (connected in series) packed with 10- μ m PRP-1 (Hamilton, Reno, NV, USA). The mobile phase consisted of 2.5 mM aqueous sodium borate–acetonitrile–methanol (40:45:15) pumped at a flow-rate of 1.0 ml/min. An injection volume of 20 μ l was used. Systems 2 and 3 used a single 150 mm × 2.1 mm I.D. column packed with 5 μ m Zorbax Rx-C₈ (Rockland Technologies, Newport, DE, USA). The mobile phase consisted of 5 mM aqueous sodium perchlorate–acetonitrile (50:50) pumped at 0.2 ml/min. For system 2, post-column addition of 1.0 mM sodium borate (0.2 ml/min) raised the pH to 9.5 prior to the detector. An injection volume of 5 μ l was used for systems 2 and 3.

RESULTS AND DISCUSSION

Electrochemical detection

The optimal detector cell potential for the oxidation of EA has been investigated previously. Hydrodynamic voltammograms showed that a working potential of +0.85 V to +1.1 V was required for maximum sensitivity [9,11]. This was confirmed for our instrumentation, and a value of +1.1 V was used in this study. At this high potential, however, the concentration of buffer in mobile phase had to be quite low to maintain a reasonable background current. Actual currents were *ca.* 200 nA with 2.5 mM sodium borate and *ca.* 220 nA with 5 mM sodium perchlorate in the mobile phase. Stable baselines were observed with a noise level of *ca.* 120 pA, but to achieve this it was necessary to thermostat both column and detector cell at 40°C due to the temperature sensitivity of the electrochemical detector. In spite of the high potential used, no significant loss of sensitivity due to poisoning of the working electrode was observed during the course of our studies.

Chromatographic performance

It is known that the pH of the mobile phase has a great effect on the chromatography of EA ($pK_a = 8.8$) [5,11]. Column efficiency and retention time, as well as electrochemical detection sensitivity, increase with increasing pH of mobile phase. Poly(styrene–divinylbenzene) copolymer stationary phases, which are stable over a wide range of pH, have been used previously to accommodate high-pH mobile phase [10,11]. Fig. 1a shows the analysis of an EA standard solution on such a column packed with the PRP-1 phase using a basic (pH 9.6) mobile phase of aqueous sodium borate (2.5 mM)–acetonitrile–methanol (40:45:15). Unfortunately, even with two 150-mm columns connected in series, a low column efficiency was observed for EA ($n = 730$). This limited both the sensitivity and the separation selectivity, and presented difficulties in the analysis of real samples (see below). Considerable baseline drift was encountered in our early experiments as is evident in Fig. 1. This drift was greatly reduced in later experiments (see below) by maintaining careful temperature control on both column and detector cell. Table I presents the detection limits estimated for this method, as well

as those in the literature. The minimum detectable quantity (MDQ) of 2 ng and the minimum detectable concentration (MDC) of 0.14 $\mu\text{mol/l}$ compare favorably with those reported by Nilsson *et al.* [11], who also used a PRP-1 column.

Fig. 1b shows the PRP-1 column analysis of an extract of flesh from a salmon that had been fed EA for nine days. The sample contained a high level of EA (*ca.* 40 $\mu\text{g/g}$) and several metabolites, the principal one being AEA. Analysis of this sample by combined LC–MS and the identification of several metabolites has been described previously [13]. As shown in Fig. 1b, both EA and AEA could be detected by LC–ED, but the PRP-1 column provided only partial resolution of the two compounds. Other metabolites were not observed; they either co-eluted with EA and AEA, or were not detected due to a lack of electrochemical activity (*e.g.*, N-demethyl metabolites).

In previous studies, we have had excellent success in the chromatography of basic compounds using the Zorbax Rx-C₈ stationary phase. This is a sterically shielded octyldiisopropylsilane bonded to pure spherical silica with an homogenous silanol distribution [15]. Since this silica-based sorbent cannot be used with mobile phases with pH greater than 8, an attempt was made to analyze EA at neutral pH with post-column addition of base. After investigating several buffer systems, a mobile phase of 5 mM aqueous sodium perchlorate (NaClO₄)–acetonitrile (50:50; pH 6.9) was found to give good chromatographic performance (high efficiency, symmetrical peaks). The effluent was combined with an equal flow of 1 mM sodium borate in a low-volume T-union and static mixer prior to the detector cell. Final pH of the effluent was 9.5. Due to the higher conductivity of the effluent after borate addition, the potential of the working electrode had to be reduced to +0.90 V to maintain a background of *ca.* 250 nA. A good signal for EA was provided, but the electrochemical detector was much more sensitive to pump pulsations, resulting in a noisy baseline (data not shown). The use of syringe pumps for both column and make-up could have reduced this noise, but due to lack

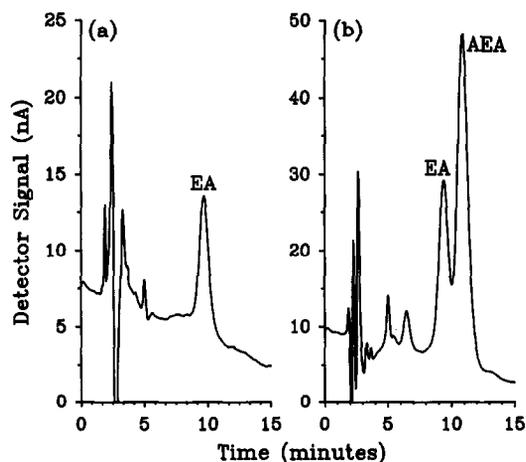


Fig. 1. LC–ED analysis of (a) an EA standard (2 $\mu\text{g/ml}$) and (b) an extract of an EA-fed fish using two 15 cm \times 4.6 mm I.D. PRP-1 columns in series. Mobile phase: 2.5 mM aqueous sodium borate–acetonitrile–methanol (40:45:15), pH 9.6; flow-rate: 1.0 ml/min; injection volume: 20 μl ; column and cell temperature: ambient; working electrode potential: +1.1 V.

TABLE I

COLUMN EFFICIENCY AND DETECTION LIMITS OF EA WITH VARIOUS CHROMATOGRAPHIC SYSTEMS USING ELECTROCHEMICAL DETECTION

Column	Mobile phase		U^b	N/m^c	MDQ ^d (ng)	MDC ^e ($\mu\text{mol/l}$)	Ref.
	Type ^a	pH					
PRP-1 (10 μm) 300 \times 4.6 mm	1	9.6	+1.1	2400	2	0.14	This study
Zorbax Rx-C ₈ 150 \times 2.1 mm	2	9.5	+0.9	19 300	7	1.8	This study
Zorbax Rx-C ₈ 150 \times 2.1 mm	3	6.9	+1.1	19 700	2	0.49	This study
PRP-1 (5 μm) 150 \times 4.6 mm	4	10.0	+1.1	5000	5	0.20	11
Nova-Pak C ₁₈ 250 \times 4.6 mm	5	7.0	+0.9	NA ^f	10	0.34	7
Ultrasphere C ₁₈ 250 \times 4.6 mm	6	6.3	+0.85	NA ^f	5	0.34	9

^a Conditions (flow-rate; mobile phase; injection volume): (1) 1 ml/min; 2.5 mM sodium borate–acetonitrile–methanol (40:45:15); 20 μl ; (2) 0.2 ml/min; 5.0 mM sodium perchlorate–acetonitrile (60:40; pH 6.9) with post-column addition of 1.0 mM sodium borate; 5 μl ; (3) 0.2 ml/min; 5.0 mM sodium perchlorate–acetonitrile (50:50); 5 μl ; (4) 1 ml/min; 50 mM potassium phosphate–acetonitrile (40:60); 20 μl ; (5) 1 ml/min; 56 mM sodium acetate–acetonitrile–methanol (56:50:4); 20 μl ; (6) 1 ml/min; 100 mM sodium acetate–acetonitrile–methanol (48:42:10); 20 μl .

^b U = potential on working electrode of electrochemical detector.

^c N/m = column efficiency (theoretical plates per meter).

^d MDQ = minimum detectable quantity (signal-to-noise ratio = 3).

^e MDC = minimum detectable concentration (signal-to-noise ratio = 3).

^f NA = not available.

of equipment this route was not pursued. The resulting detection limits (MDQ = 7 ng; MDC = 1.8 $\mu\text{mol/l}$) with this method were considerably poorer than with the PRP-1 column mainly because of the baseline noise.

Finally, this same Rx-C₈ column and neutral perchlorate mobile phase were used without post-column addition of base. Avoiding the post-column arrangement allowed an increase in the working potential back up to +1.1 V. Baseline drift and noise were greatly reduced by maintaining careful temperature control (40°C) for both the column and the detector cell. Column temperature control also helped to increase retention time reproducibility. Fig. 2a shows the analysis of an EA standard solution with this system. The relatively high chromatographic efficiency ($n = 2950$) and low baseline noise helped to compensate for the lower sensitivity of the detector at

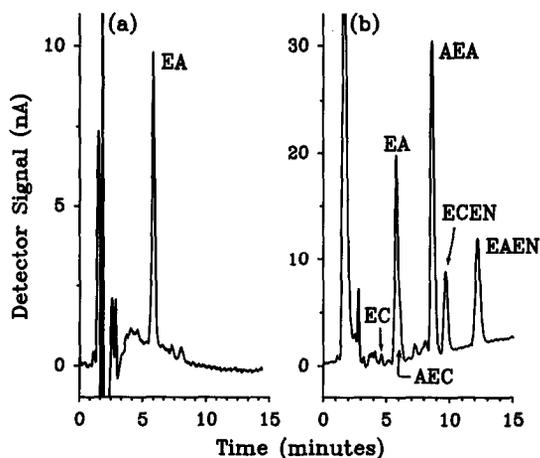


Fig. 2. LC-ED analysis of (a) an EA standard (2 $\mu\text{g/ml}$) and (b) an extract of an EA-fed fish using a 15 cm \times 2.1 mm I.D. Zorbax Rx-C₈ column. Mobile phase: 5 mM aqueous sodium perchlorate–acetonitrile (50:50), pH 6.9; flow-rate: 0.2 ml/min; injection volume: 5 μl ; column and cell temperature: 40°C; working electrode potential: +1.1 V.

neutral pH and facilitated a detection limit (MDQ = 2 ng = 3 pmol; MDC = 0.49 $\mu\text{mol/l}$), comparable to that achieved with a basic mobile phase on the PRP-1 column (system 1). The smaller injection volume (5 μl) used with the narrow-bore (2.1 mm I.D.) column also meant that less extract was required for the analysis.

Fig. 2b shows analysis of the EA-fed salmon extract with this system. The improved separation over that on the PRP-1 column (Fig. 1b) is clearly apparent. Several peaks are observed in the chromatogram including those due to EA and AEA. The identities of the extra peaks were established by an ion-spray LC-MS experiment [13] using exactly the same chromatographic conditions. The latest eluting peak was assigned as the enol ether of EA (EAEN), an isomer of AEA. Also observed was erythromycin C (EC), an impurity in the EA originally fed to the fish, and two metabolites of EC tentatively assigned as anhydroerythromycin C (AEC) and the enol ether of EC (ECEN). Unfortunately, AEC was not completely separated from EA under the conditions used. None of the N-demethyl metabolites of EA or EC gave signals in LC-ED, supporting the hypothesis that oxidation occurs at the tertiary amine of the desoaminy sugar moiety [7].

Trace level analysis

The application of LC-ED to trace levels of EA in tissue samples presents a more significant challenge. Although the official Canadian regulatory level for EA has not yet been established for fish products, a tolerance level of 0.1 ppm (100 ng/g flesh) is applied to terrestrial meat products. Therefore, it would be desirable to have a detection limit as low as 10 ng/g.

Fig. 3a shows the LC-ED analysis of an extract of salmon tissue sample spiked with EA at the 1 $\mu\text{g/g}$ level. This extract was prepared using an acetonitrile extraction followed by a liquid-liquid partitioning clean-up (see Experimental). This procedure was shown previously by LC-MS analysis to give acceptable recoveries (>90%) [13]; however, it was also found that the clean-up was not adequate and necessitated the use and frequent changing of a guard column to protect

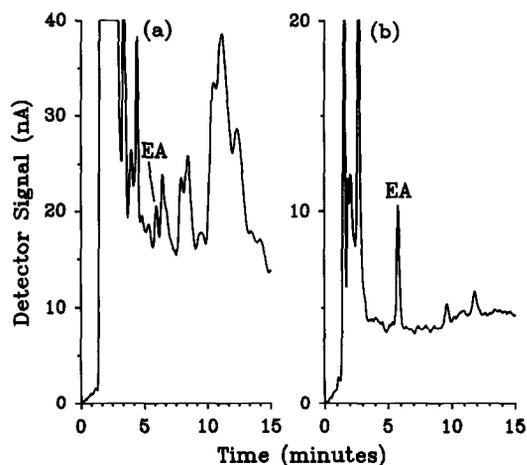


Fig. 3. LC-ED analysis of salmon flesh, spiked with 1 $\mu\text{g/g}$ EA, before (a) and after (b) an aminopropylsilica clean-up. Chromatographic conditions same as in Fig. 2.

the main column from build-up of co-extractives. It is clear from Fig. 3a that some of these co-extractives are electrochemically active and interfere with the detection of EA. The peak for EA is very difficult to discern among the large interfering peaks and analysis at the sub-ppm level is impractical. Also, co-extractives continued to elute from the column for up to 1 h and necessitated a column wash with a stronger solvent between analyses.

Recently, we have developed a solid-phase extraction (SPE) clean-up procedure for the rapid LC-MS and MS-MS analysis of EA in salmon [14]. This procedure, based on an aminopropylsilica cartridge, gives reproducible, high recoveries (>90%) at sub-ppm levels. Fig. 3b shows the LC-ED analysis of the same extract as that used to obtain Fig. 3a after further clean-up with this SPE method. EA is easily confirmed at this 1 $\mu\text{g/g}$ spike level. There are still many small peaks due to residual co-extractives, however, and we estimate the detection limit to be about 100 ng/g. Clearly further work is required if the method is to be useful at levels down to 10 ng/g. It is possible that another stage of SPE clean-up may reduce interferences even further and allow additional pre-concentration. However, the present method does represent a substantial improve-

ment over existing methods. It should be useful for a number of studies, such as determining elimination rates of EA from animals.

ACKNOWLEDGEMENTS

The authors thank Mr. W. R. Hardstaff for technical assistance, Drs. D. D. Kitts and K. McErlane (University of British Columbia) for samples of fish treated with EA, and the Bureau of Veterinary Drugs, Health and Welfare Canada for financial support.

REFERENCES

- 1 I. O. Kibwage, E. Roets and J. Hoogmartens, *J. Chromatogr.*, 256 (1983) 164.
- 2 M. Petz, R. Solly, M. Lymburn and M. H. Clear, *J. Assoc. Anal. Chem.*, 70 (1987) 691.
- 3 Th. Cachet, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.*, 403 (1987) 343.
- 4 I. O. Kibwage, E. Roets, A. Verbruggen, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.*, 434 (1988) 177.
- 5 K. Tsuji and J. F. Goetz, *J. Chromatogr.*, 147 (1978) 359.
- 6 K. Tsuji, *J. Chromatogr.*, 158 (1978) 337.
- 7 D. Croteau, F. Vallee, M. G. Bergeron and M. LeBel, *J. Chromatogr.*, 419 (1987) 205.
- 8 Th. Cachet, I. Quintens, E. Roets and J. Hoogmartens, *J. Liq. Chromatogr.*, 12 (1989) 2171.
- 9 S. Laakso, M. Scheinin and M. Anttila, *J. Chromatogr.*, 526 (1990) 475.
- 10 I. O. Kibwage, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.*, 330 (1985) 275.
- 11 L. G. Nilsson, B. Walldorf and O. Paulsen, *J. Chromatogr.*, 423 (1987) 189.
- 12 J. Paesen, P. Claeys, E. Roets and J. Hoogmartens, *J. Chromatogr.*, 630 (1993) 117.
- 13 S. Pleasance, J. Kelly, M. D. LeBlanc, M. A. Quilliam, R. K. Boyd, D. D. Kitts, K. McErlane, M. R. Bailey and D. H. North, *Biol. Mass Spectrom.*, 21 (1992) 675.
- 14 R. Bailey, D. North, M. D. LeBlanc and M. A. Quilliam, in preparation.
- 15 M. A. Stadalius, J. S. Berus and L. R. Snyder, *LC · GC*, 6 (1988) 494.