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Analysis of tetramine in sea snails by capillary electrophoresis–tandem mass spectrometry[1]

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Abstract

Tetramine (tetramethylammonium ion) is an autonomic ganglionic blocking agent that is found in several marine gastropod species. It is responsible for numerous incidents of human intoxication in Japan, Europe and Canada, due to the consumption of whelks (sea snails). This communication reports on a capillary zone electrophoresis–mass spectrometry (CZE–MS) method for the detection and quantitation of tetramine in whelk tissues. The interfacing of CZE and MS was accomplished with a coaxial sheath flow arrangement and pneumatically assisted electrospray ionization. CZE separations were evaluated using both bare fused-silica columns and capillaries coated with linear polyacrylamide, with the latter showing the better performance. Enhanced selectivity for trace level detection of tetramine in tissue extracts was achieved using CZE with on-line tandem mass spectrometry (CZE–MS–MS). Quantitation of the target analyte was facilitated using tetraethylammonium ion as an internal standard. The method has been applied to the analysis of two different *Neptunea* whelk species from Canada (*N. decemcostata* and *N. despecta tornata*) and one from Japan (*N. arthritica*). The analysis of one sample, implicated in a recent poisoning incident in Labrador, revealed high levels of tetramine (430 μg/g) as well as choline and an unusual choline derivative. This CZE–MS methodology should be useful for a number of other related cholinergic analogs bearing the quaternary amine function. © 1997 Elsevier Science B.V.

Keywords: *Neptunea* spp.; Tetramine; Tetramethylammonium cation; Toxins; Choline; Choline glycolate; Quaternary ammonium compounds

1. Introduction

Numerous poisoning incidents have been reported following the consumption of whelks (sea snails), such as *Neptunea arthritica* and *Neptunea intersculpta* in Japan, *Neptunea antiqua* in Europe and *Neptunea decemcostata* in Atlantic Canada [1]. The primary causative agent in such incidents is the tetramethylammonium (TMA) ion, also known as tetramine, which occurs naturally as the chloride salt. This toxin is an autonomic ganglionic blocking agent that is concentrated in the salivary glands of most whelk species [2,3]. Tetramine poisoning may result in a number of symptoms, which include nausea, vomiting, anorexia, weakness, fatigue, faintness, dizziness, photophobia, impaired visual accommodation and dryness of the mouth [4]. Fortunately, if the consumption is not too large, the duration of these symptoms is relatively brief (a few hours) due to rapid renal excretion [1].

Both bioassays and analysis methods have been developed for the detection of tetramine. Bioassays are usually based on dose–lethal time curves for

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mice [1], although a bioassay based on killifish has been reported [2]. The major weakness in such bioassays is the possible presence of synergistic components in extracts, which can result in unreliable quantitation. Methods of analysis that have been investigated include thin-layer chromatography [1,6], ion chromatography with conductivity detection [5], liquid chromatography with refractive index detection [7] and proton nuclear magnetic resonance (NMR) spectroscopy [8]. Most of these methods have low sensitivity and, with the exception of proton NMR, poor selectivity for identification and/or confirmation purposes.

Capillary electrophoresis (CE) has grown rapidly into an important analytical tool with the commercial availability of CE instruments and narrow-bore fused-silica capillaries. CE offers several advantages for solving analytical problems, including high resolution separations, inexpensive instrumentation, ease of operation, high speed and the possibility of trace analysis with very small amounts of sample. Several publications have reported on capillary zone electrophoresis (CZE) separations of small quaternary amines using conductivity detection [9,10] or indirect photometric detection [11–13].

Mass spectrometry (MS) is an attractive detector for CE that can provide high sensitivity, excellent selectivity for unambiguous confirmatory analysis, and even structural information for the identification of new compounds. The successful coupling of CE to MS using electrospray ionization was first reported by Olivares et al. in 1987 [14]. Since then, a number of CE–MS interfaces, including the coaxial sheath-flow [15–17], liquid junction [18,19] and sheathless [20–22] arrangements, have been developed. The successful application of CE–MS to other marine toxins, such as paralytic shellfish poisoning toxins [23], has demonstrated the potential of this technique for the identification and characterization of trace level toxins in biological extracts.

With its quaternary amine function, TMA is ideally suited for CZE separation based on its intrinsic high mobility in aqueous buffers. Its permanent charge makes it readily detectable in positive ion electrospray. These physicochemical characteristics prompted us to investigate the electrophoretic conditions conducive to the analysis of TMA using CZE–MS. In this communication, we report on the application of this technique to the detection and quantitation of TMA in whelks from Atlantic Canada and Japan. We also demonstrate that the technique is well-suited to the determination of related compounds such as choline and choline esters.

2. Experimental

2.1. Chemicals

Tetramethylammonium chloride and tetraethylammonium chloride were obtained from Aldrich (Milwaukee, WI, USA). Formic acid, ammonium acetate and distilled-in-glass-grade methanol were purchased from BDH (Toronto, Canada). A Milli-Q system from Millipore (Bedford, MA, USA) was used for the preparation of de-ionized water for both sample and buffer preparation.

2.2. Sample preparation

Frozen, uncooked whelk samples from Nova Scotia (*Neptunea decemcostata*) and Japan (*N. arthritica*) were obtained from the respective inspections laboratories. Frozen samples of cooked whelk tissue implicated in a toxic event in Labrador were obtained from K. Kennedy, Fisheries and Oceans Canada, St. John's, Newfoundland. The species was identified as *N. despecta tornata*, based on shell features (personal communication, K. Kennedy).

After thawing at room temperature, tissues were pulled from the shells and homogenized using a Waring 33BL73 blender (Dynamics Corporation of America, New Hartford, CT, USA) prior to subsampling. For some of the Nova Scotia whelks, salivary glands (along with some surrounding tissue) were surgically removed first. Then the glands and the remaining tissue were homogenized separately.

Sample extraction was performed according to the method of Saitoh et al. [5] with only slight modifications. A 2-g (wet mass) portion of each homogenized tissue was mixed with 20 ml of methanol and blended using a Polytron PT3000 homogenizer equipped with a PT-DA-3012/2T generator (Brinkman Instruments, Westbury, NY, USA). The homogenate was incubated at 80°C for 30 min, cooled to room temperature, and subsequently centrifuged for

10 min at 3700 *g* using a model MP4R centrifuge with an 804S fixed-angle rotor (International Equipment, Needham Heights, MA, USA). After discarding the residue, the supernatant was evaporated to dryness. A 4-ml volume of water was used to redissolve the residue. Liquid–liquid extraction with two 4 ml aliquots of diethyl ether was used to remove lipid material. A nitrogen stream was used to evaporate residual ether. The final extract was adjusted to a volume of 5 ml with water, resulting in a concentration of 0.4 g tissue equivalents/ml.

2.3. CZE–MS

MS was performed using an API-III$^+$ triple quadrupole mass spectrometer (Perkin-Elmer SCIEX, Concord, Canada) equipped with a fully articulated IonSpray interface (pneumatically assisted electrospray) operated at atmospheric pressure. CZE was performed using a Crystal CE310 System (ATI Unicam, Madison, WI, USA). A separate power supply (EH Series, Glassman, Whitehouse Station, NJ, USA) was used to provide a voltage of 5 kV to the electrospray interface. The CZE–MS interface was based on a "sheath-flow" design using a coaxial column arrangement [14] that was subsequently modified in our laboratory [15]. A more detailed description of the interface configuration has been presented elsewhere [23]. The sheath flow solvent was 0.2% formic acid in water–methanol (3:1, v/v) pumped at 7 μl/min.

CZE–MS data acquisition was performed using a dwell time of 4 ms in full mass scan mode and 100 ms per channel in selected ion monitoring (SIM) experiments. Product ion spectra from combined CZE–MS–MS analyses were obtained using collisional activation with argon target gas in the second [radio frequency (RF)-only] quadrupole. Collision energies were typically 25 eV in the laboratory frame of reference, and the collision gas thickness was $3.5 \cdot 10^{15}$ atoms/cm^2. Tandem mass spectra were acquired using dwell times of 1 ms per step of 0.1 u in full-scan mode or 150 ms per channel in selected reaction monitoring (SRM) experiments. A Macintosh Quadra 950 Computer was used for instrument control, data acquisition and data processing.

Bare fused-silica capillaries (50 μm I.D.×180 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The coating of the inner wall of the capillary with linear polyacrylamide (LPA) was performed according to the procedure described by Huang et al. [24], with the exception that both the chlorination and the Grignard reaction steps were repeated once, to ensure better coverage of the silanol groups. Capillary lengths were typically 99 cm for bare silica columns and 90 cm for LPA-coated columns. Reconditioning of the bare fused-silica columns between runs was necessary to maintain good migration time reproducibility. This procedure involved flushing the capillaries with 0.1 *M* NaOH, water, and background electrolyte (BGE). Conditioning of the LPA-coated capillary between runs only required washing with the background electrolytes. Samples were injected at a pressure of 50 mbar for 0.3 min, corresponding to an injection volume of typically 15 nl.

3. Results and discussion

3.1. Initial experiments

The separation of small cations on bare fused-silica columns can be quite challenging in view of the possibility of adverse effects associated with the interactions of these analytes with the negatively charged capillary wall. In addition, such cations exhibit high electrophoretic mobilities, which are in the same direction as the strong electroosmotic flow (EOF) that prevails under conventional CZE separation conditions at pH values above 4, resulting in short migration times and low resolution. Such adverse effects can be minimized by decreasing the pH of the separation buffer, thereby reducing the extent of the EOF. However, significant peak tailing can still be observed for small cationic species, even under low pH conditions, and deactivation of the capillary wall is often necessary to minimize analyte adsorption [23–25]. Previous investigations have reported the use of linear polyacrylamide-coated capillaries for the analysis of paralytic shellfish poisoning toxins with separation efficiencies exceeding 300 000 theoretical plates [23]. Therefore, initial CZE–MS experiments were conducted using LPA-coated fused-silica capillaries.

The coupling of CZE with MS is not without its

own difficulties, and judicious selection of electrolyte systems has to be made to avoid compromising the overall system performance. In order to be most compatible with MS, a buffer must carry relatively low currents, be sufficiently volatile to prevent any accumulation of residue on the orifice plate of the mass spectrometer, and have low proton affinity, to minimize the level of chemical background. In the present application, the extent of chemical background contribution was anticipated to be a significant factor, due to the low mass range of interest ($<m/z$ 200). A simple background electrolyte, 0.1 *M* formic acid, was selected for initial experiments since it has proven useful in earlier studies with LPA-coated fused-silica capillaries [23].

Since our main goal was to develop a method that was suitable for quantitative determinations, an internal standard was selected at the beginning of this project. An ideal compound would have been a stable-isotope labeled analog of TMA. However, since such material was not immediately available to us, tetraethylammonium (TEA) ion was selected as a readily available alternative. Any number of small quaternary ammonium cations would probably have served equally well, although it was important to ensure that no tetramethylammonium ion was present as an impurity in the proposed internal standard. This was established to be the case for the TEA chloride used in this study. In addition, it is also essential to establish that the proposed internal standard is not present as an endogenous compound in whelk tissue. As shown later, this was determined to be the case for TEA.

Fig. 1 shows the results of an initial experiment using full scan acquisition (m/z 30–150) mode with an LPA-coated capillary and 0.1 *M* formic acid background electrolyte. The sheathflow in the CZE–MS interface was 0.2% formic acid in water–methanol (3:1, v/v) and was operated at 7 μl/min. The total ion electropherogram (TIE) for a standard mixture of TMA and TEA (100 μ*M* each in water) is given in Fig. 1a. The TIE is very noisy because of the high background signal in the scanned mass range. A raw mass spectrum of the background electrolyte is shown in Fig. 1d. Individual reconstructed ion electropherograms for TMA (m/z 74) and TEA (m/z 130) are shown in Fig. 1b–c, respectively. TMA, being the smaller ion, eluted first, at

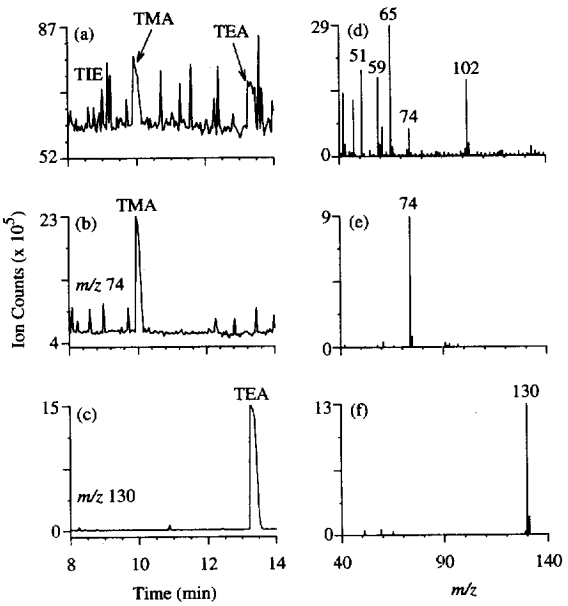

Fig. 1. CZE–MS full scan analysis of a standard mixture of TMA and TEA (100 μ*M* each). Conditions: 90 cm LPA-coated capillary with 0.1 *M* formic acid as the separation buffer. The total ion electropherogram is shown in (a) and extracted ion current profiles for m/z 74 and 130 are given in (b) and (c), respectively. The raw background mass spectrum is shown in (d), and the background-subtracted mass spectra for TMA and TEA are given in (e) and (f), respectively.

10.0 min, and TEA appeared at 13.3 min. The background-subtracted mass spectra of TMA and TEA are given in Fig. 1e–f, respectively. As expected for electrospray, the molecular ions of TMA (m/z 74) and TEA (m/z 130) dominate the mass spectra. It is clear from the noise level in the extracted TMA signal (Fig. 1b) that the presence of an abundant ion at m/z 74 in the background (Fig. 1d) severely limits the use of full scan acquisition for the detection and confirmation of TMA. The estimated limits of detection in this acquisition mode were 60 μ*M* for TMA and 5 μ*M* for TEA (at $S/N=3$). A meaningful mass spectrum could only be obtained for an on-column injection of approximately 70 pg (650 fmol) of TMA.

The selected ion monitoring (SIM) acquisition mode was investigated next, with the hope that the elimination of spectrum scanning would reduce the noise level and result in lower detection limits. Fig. 2a shows the results of an experiment similar to that

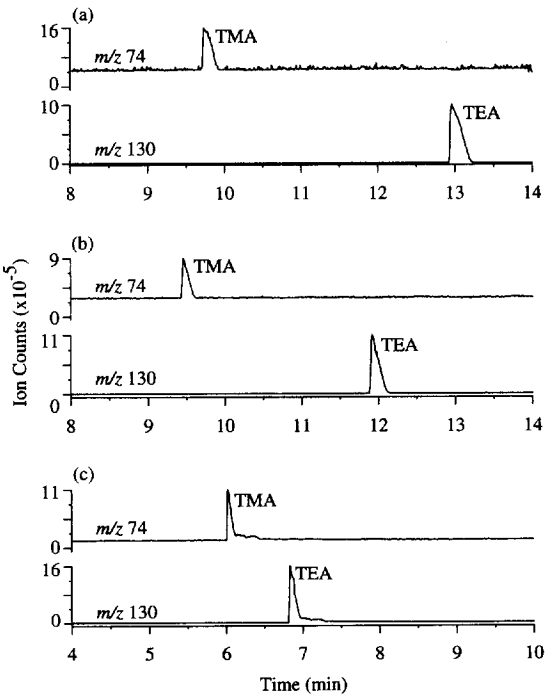

Fig. 2. Evaluation of different capillaries and background electrolytes for the CZE–MS analyses of a mixture of TMA and TEA (100 μ*M* each) using SIM and a 90-cm LPA-coated capillary with 0.1 *M* formic acid as the background electrolyte (a), a 99-cm bare fused-silica capillary with 0.1 *M* formic acid (b), or a 99-cm bare fused-silica capillary with 10 m*M* ammonium acetate at pH 7 (c).

in Fig. 1, except that only two ions were monitored; *m/z* 74 for TMA and *m/z* 130 for TEA. The background signal and noise were still very high for *m/z* 74 compared to those for *m/z* 130, although it should be noted that the frequency of the noise in the *m/z* 74 trace was higher than for the scanning experiment. The latter could probably facilitate the use of filtering techniques, but this was not explored further in this study. The estimated limits of detection using the SIM acquisition mode was 30 μ*M* for TMA and 2 μ*M* for TEA (at $S/N=3$), not a great deal better than the corresponding values for the scanning mode.

SIM acquisition was used for the investigation of different background electrolytes and capillaries. Fig. 2 shows the electropherograms from three experiments. The LPA-coated column with 0.1 *M* formic acid as the background electrolyte (Fig. 2a) gave moderate separation efficiencies ($N=33\,000$), with very reproducible migration times. The peak skewing observed in Fig. 2a was attributed mainly to the high sample concentration used in the experiment, which resulted in conductivity differences between the analyte band and the surrounding buffer and, in part, to the mobility mismatch between sample ion and buffer co-ion. Lower analyte concentrations resulted in much higher separation efficiencies and more symmetrical peaks (see below). Fig. 2b shows the results for a bare fused-silica capillary with 0.1 *M* formic acid as the background electrolyte. Due to the near-zero EOF at low pH, the migration times were similar to those with the coated capillary. Some improvements in separation efficiencies ($N=62\,000$) were observed, but very irreproducible migration times were observed if the capillary was not reconditioned between runs, a requirement that resulted in a much longer turn-around time. It was hoped that faster analyses could be achieved using a bare fused-silica capillary with a background electrolyte of 10 m*M* ammonium acetate at pH 7, where there would be an increased EOF. Although the migration times were indeed shorter, as shown in Fig. 2c, very poor peak shapes resulted from cation–wall interactions at the higher pH value. The ammonium acetate buffer did not significantly change the *m/z* 74 signal in the background spectrum. From the results of these experiments, it was concluded that the LPA-coated capillary was the best choice for further experiments.

3.2. Analysis using tandem mass spectrometry

MS–MS was examined as a means of introducing greater selectivity for the unambiguous identification of TMA. This was deemed necessary in view of the abundant background signal at *m/z* 74, arising from the electrolyte. Product ion spectra for TMA and TEA were obtained using on-line CZE–MS–MS with the chosen separation conditions described above. Fig. 3 shows the product ion spectra of TMA and TEA. Under low energy collisional activation, the TMA precursor ion gives an abundant fragment ion at *m/z* 58, corresponding to the loss of a neutral methane molecule. A subsequent loss of a methane molecule from *m/z* 58 affords a fragment ion at *m/z* 42, of much lower abundance. In contrast, dissociation of TEA under the same conditions results in a

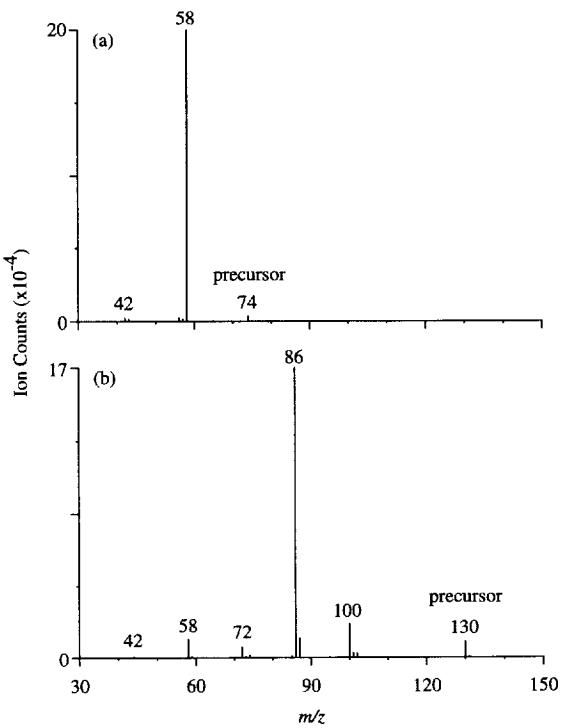

Fig. 3. Fragment ion spectra obtained from a CZE–MS–MS analysis of a mixture of TMA (a) and TEA (b). Separation conditions as for Fig. 1. The product ion scan was obtained using argon as the target gas, a collision energy of 25 eV and a collision gas thickness of $3.50 \cdot 10^{15}$ atoms/cm^2.

series of fragment ions corresponding to losses of the elements of neutral ethane plus varying numbers of methylene moieties, to give fragment ions at *m/z* 100, 86, 72, 58 and 42. The fragment ions observed for TMA and TEA, along with tentative ion structures, are listed in Table 1.

The characteristic fragment ions observed for TMA and TEA were used in SRM acquisition mode. One confirmatory reaction (*m/z* 74→*m/z* 58) and the surviving precursor ion (*m/z* 74→*m/z* 74) of TMA, and three reactions (*m/z* 130→*m/z* 100, *m/z* 130→*m/z* 86 and *m/z* 130→*m/z* 58) of TEA, were selected for this experiment. Fig. 4a shows the extracted MS–MS ion current profiles of TMA and TEA at a concentration of 1 μ*M* each. The background contribution for TMA was significantly reduced under these conditions and led to a significant improvement in concentration detection limits compared to those observed using SIM acquisition mode.

Fig. 4b shows a calibration curve for the ratio of peak areas for the two principal reaction channels for TMA (*m/z* 74→*m/z* 58) and TEA (*m/z* 130→m/z 86) as a function of TMA concentration. Excellent linearity was observed (linear regression coefficient, $r^2=0.99993$), and a linear dynamic range of more than two orders of magnitude was possible. The limit of detection for TMA was 90 n*M* or 7 ng/ml (at *S/N*=3), an enhancement of more than two orders of

Table 1
Proposed structures of fragment ions of tetramine, TEA, choline and a new choline derivative in the Labrador sample

	Precursor ion	Fragment ions
Tetramine (TMA)	*m/z* 74 $(CH_3)_4N^+$	*m/z* 58 $(CH_3)_2N^+=CH_2$ *m/z* 42 $CH_2=N^+=CH_2$
TEA	*m/z* 130 $(C_2H_5)_4N^+$	*m/z* 100 $(C_2H_5)_2N^+=CHCH_3$ *m/z* 86 $(C_2H_5)_2N^+=CH_2$ *m/z* 72 $(C_2H_5)(CH_3)N^+=CH_2$ *m/z* 58 $(CH_3)_2N^+=CH_2$ *m/z* 42 $CH_2=N^+=CH_2$
Choline	*m/z* 104 $(CH_3)_3N^+CH_2CH_2OH$	*m/z* 60 $(CH_3)_3N^+H$ *m/z* 58 $(CH_3)_2N^+=CH_2$ *m/z* 45 $^+CH_2CH_2OH$
Choline derivative	*m/z* 162 $(CH_3)_3N^+CH_2CH_2OCOCH_2OH$	*m/z* 102 $(CH_3)_3N^+CH=CHOH$ *m/z* 60 $(CH_3)_3N^+H$ *m/z* 58 $(CH_3)_2N^+=CH_2$ *m/z* 43 $CH_2=C=OH^+$

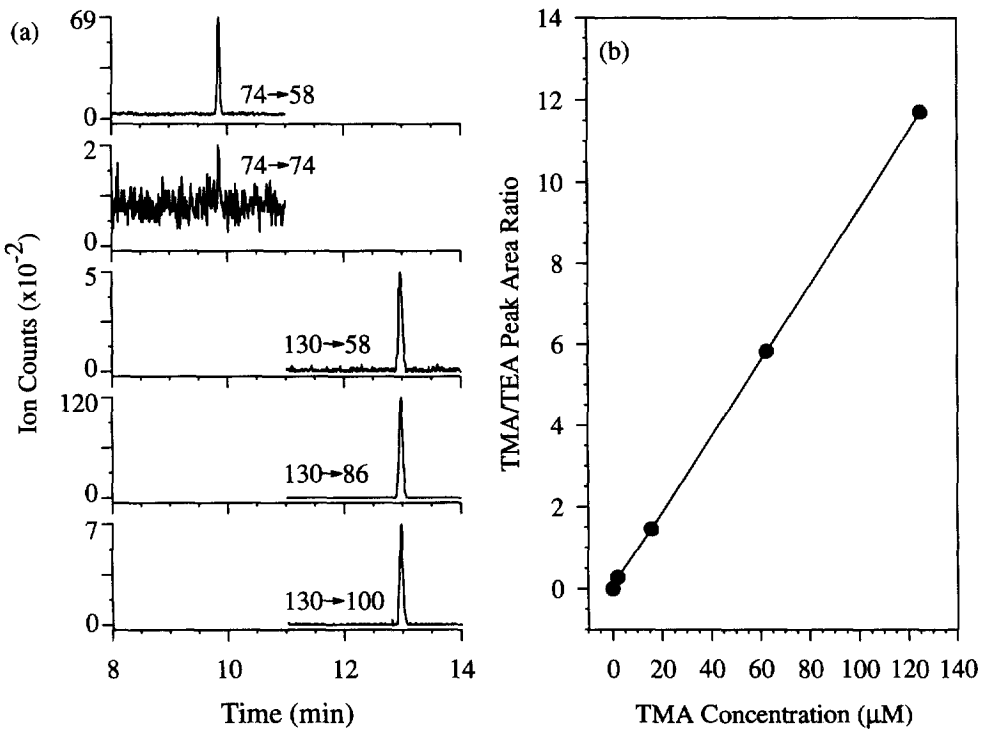

Fig. 4. CZE–MS–MS analysis of TMA and TEA (1 μ*M* each) using the SRM acquisition mode (a). The calibration curve for the ratio of the peak area of TMA (*m/z* 74→*m/z* 58) to that of TEA (*m/z* 130→*m/z* 86) is presented in (b). Conditions as for Fig. 3.

magnitude over that obtained using SIM acquisition mode. This translates to a potential method detection limit of 20 ng/g tissue when using the extraction procedure described in Section 2.

3.3. Application to whelk samples

The SRM acquisition mode was used to quantify the levels of TMA present in two different *Neptunea* whelk species from Canada (*N. decemcostata* from Nova Scotia and *N. despecta tornata* from Labrador) and one from Hokkaido, Japan (*N. arthritica*). The results are summarized in Table 2. TEA was used as an internal standard for all measurements and analyses were performed in triplicate. The results generally showed excellent precision. Notable differences in TMA concentration in the whole tissue were observed between the Canadian samples (400–500 μg/g) and the Japanese sample (65 μg/g).

For some of the *N. decemcostata* samples, the

Table 2
Quantitation of tetramine in different whelk tissue samples using CZE–MS–MS in the SRM acquisition mode

Location	Species	Tissue	TMA concentration μg/g[a] ± S.D. (n=3)
Nova Scotia	*Neptunea decemcostata*	Whole	460±19
		Meat only	370±17
		Salivary gland only	1280±29
Hokkaido	*N. arthritica*	Whole	65±20
Labrador	*N. despecta tornata*	Whole (cooked)	430±18

[a] Concentration expressed as μg of $(CH_3)_4N^+$ per gram of tissue.

salivary glands (along with some surrounding tissue) were surgically removed first. The resulting samples of glands (about 5% of the whole tissue) and the remaining tissues were analyzed separately to determine the distribution of TMA in the animals. This was of interest because some reports have stated that the TMA is mostly confined to the salivary glands of whelks [1], while other studies have reported TMA to be widely distributed throughout the animal (e.g., for *N. antiqua* [3]). Our quantitation results (Table 2) showed that, although higher levels of TMA were found in the salivary glands of *N. decemcostata* (1280 μg/g), the remaining tissue still had substantial TMA concentrations (370 μg/g). However, since the samples were frozen, it is possible that the surrounding tissues were contaminated by diffusion of TMA from the salivary glands during storage. Further studies are warranted, but it is clear that, in these samples, over 90% of the TMA was located outside of the salivary glands, suggesting that excision of the glands is not a suitable way to detoxify the whelks.

One of the Canadian samples, the *N. despecta tornata*, was particularly interesting because it was cooked material taken from the meal of a family in Labrador suffering from a severe case of tetramine poisoning. This whelk species occurs at a depth of 5–500 fathoms from the Gulf of St. Lawrence to Cape Cod. In this case, the whelks were taken as a by-catch from scallop dragging. The electropherograms for the analysis of this material are shown in Fig. 5. It should be noted that the TMA was so concentrated in this sample that the extract had to be diluted twenty-fold to avoid overloading the system. The correct migration time combined with the selective detection provided by SRM gave a clear confirmation of the presence of TMA.

Due to the known toxicity of this particular sample, additional analyses were performed to determine whether any related compounds were present that might have had a synergistic effect in the poisoning episode. Fig. 6 shows the electropherogram from a CZE–MS analysis of the extract using full scan data acquisition. TMA (peak 1) was further confirmed by both migration time (9.8 min) and its characteristic mass spectrum, shown in Fig. 6b. Additional components were also observed in the extract, giving rise to five other peaks. The principal

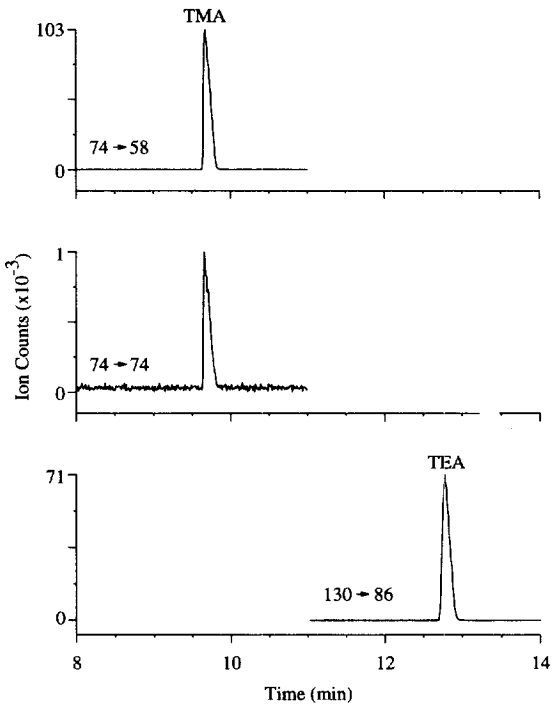

Fig. 5. CZE–MS–MS (SRM) analysis of an extract of a cooked toxic whelk sample taken from the meal of a Labrador family suffering from tetramine poisoning. The sample was diluted twenty-fold with separation buffer and TEA was added as the internal standard. The conditions were the same as in Fig. 4.

ions observed in the corresponding extracted mass spectra for these peaks were *m/z* 104 (peak 2, see Fig. 6c), *m/z* 147 (peak 3), *m/z* 156 and 175 (peak 4), *m/z* 118 (peak 5) and *m/z* 162 (peak 6, see Fig. 6d). Acquisition of fragment ion spectra in a separate CZE–MS–MS experiment provided some useful information on these compounds. Only peaks 2 and 6 seemed to be related to tetramine through the presence of the characteristic *m/z* 58 ion in the spectra. The fragment ion spectra for peaks 1 (TMA), 2 and 6 are given in Fig. 6e–g, respectively.

Based on its fragment ion spectrum, peak 2 was identified as choline. The spectrum is dominated by losses of the neutral fragments C_2H_4O and C_2H_6O (ethanol) from the precursor ion, *m/z* 104 (see Table 1 for ion assignments). The spectrum and migration time matched those obtained for a choline standard.

The product ion spectrum of *m/z* 162 (peak 6, Fig. 6g) was characterized by the loss of a neutral 60 u

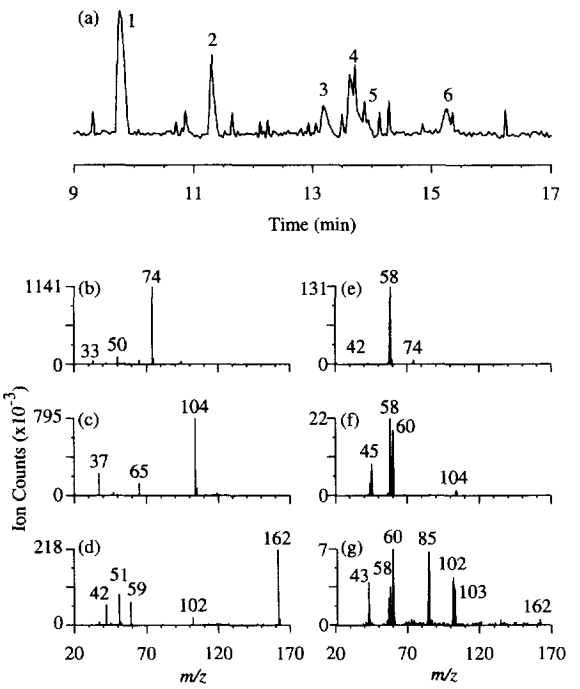

Fig. 6. CZE–MS (full scan) and CZE–MS–MS (full scan) analysis results for the same toxic whelk sample from Labrador as for Fig. 5. The sample was diluted twenty-fold with separation buffer; TEA was not added as an internal standard. Separation conditions as for Fig. 4. The principal ion electropherogram (the sum of *m/z* 74, 104, 118, 147, 156, 162 and 175) is given in (a). The background-subtracted mass spectra (b–d) [acquired from (a)] and the fragment ion spectra (e–g) (acquired from a separate run) are given for peak 1 (TMA) in (b) and (e), for peak 2 (choline) in (c) and (f), and for peak 6 in (d) and (g), respectively.

residue, tentatively assigned as $C_2H_4O_2$, followed by subsequent losses of 42 and 44 u residues, affording fragment ions at *m/z* 60 and 58, respectively. The appearance of these fragment ions, similar to those of choline, suggested that these two compounds were structurally related. A list of fragment ions and proposed structures is presented in Table 1. The structure proposed is choline glycolate. This compound has never been reported as a natural product, to our knowledge, and only two papers have referenced this compound in specialized studies, where it was synthesized [26,27]. Proof of this structure will require synthesis. This will also be necessary to establish whether this compound is toxic or has any synergistic effect on tetramine poisoning. It would also be interesting to determine if this compound is present in other snail species. A number of choline esters have been reported in marine gastropods and it has been proposed that they may have toxic effects [28]. In addition, a number of unusual shellfish toxins with the quaternized trimethylamine function have been reported in recent years [29–31]. It is likely that CZE–MS would be a very useful tool for investigating all of these compounds.

4. Conclusions

CZE–MS has been shown to be a useful method for trace level detection and quantitation of tetramine and related quaternary amines in shellfish. CZE–MS–MS is especially useful for confirmatory analyses, as was demonstrated in the case of the Labrador sample. The techniques developed here should also be useful for a range of related compounds, such as choline and its esters.

Although CZE–MS is an expensive method of analysis, it should be possible to use CZE without MS as a less expensive method for screening samples. Detection by conductivity or by indirect photometric detection should prove suitable.

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