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May 1998

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Suffield Report No. 699

A METHOD FOR THE SAMPLE HANDLING AND ANALYSIS OF BIO-ACTIVE PEPTIDES

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ABSTRACT

A method has been developed for the sample handling and analysis of bio-active peptides. Based on electrospray ionization mass spectrometry (ESI-MS), the method is applicable to the target analysis of known peptides. ESI-MS is used to determine the molecular mass of the peptide and provisional identification is based on matching the molecular mass with that of known peptides in a database. Disulfide bridge reductive alkylation is used to determine the number of cysteines in the peptide as well as the presence of disulfide bridges. The peptide is then enzymatically digested and the digestion products analyzed by ESI-MS. The resulting mass map is compared to the masses predicted from the structure of the peptide. Finally, the peptide and its enzymatic fragments are analyzed by liquid chromatography (LC) ESI-MS using collisional activated disassociation (CAD) conditions which promote the formation of product ions from which it is possible to determine the amino acid sequence of the peptide.

The developed method was applied to the analysis and identification of α-conotoxin GI. The monoisotopic molecular mass was determined to be 1436.42 ± 0.13 u (n=6). A search of the in-house database determined that the only match within one mass unit of the calculated molecular mass was α-conotoxin GI with a theoretical molecular mass of 1436.48 u. The error between the calculated and theoretical values was 42 ppm. Reductive alkylation indicated the presence of four cysteines and two intramolecular disulfide bridges which was consistent with the structure of α-conotoxin GI. LC-ESI-MS analysis of the tryptic digestion products indicated the presence of two fragments with masses of 564.18 and 1122.45 u which were in agreement with the predicted products. Under CAD conditions, Y series ions were observed from which the entire sequence of the larger tryptic fragment was determined.

Keywords

Mass Spectrometry
Electrospray
Bio-active peptides
Enzymatic Digestion
Immobilized Trypsin
Disulfide Bridge Reduction
Conotoxin
LC-ESI-MS
Collisional Activated Disassociation

Executive Summary

Title: J.R. Hancock, L.R. Provost, P.A. D'Agostino and J.Aa. Tørnes, A Method for the Sample Handling and Analysis of Bio-Active Peptides, Suffield Report No. 699, 1998, UNCLASSIFIED.

Introduction: The Canadian Forces (CF) may be called on to conduct peacekeeping or peacemaking operations in regions of the world where there is a significant threat of chemical/biological warfare agent use. To operate effectively in these theatres the CF must be able to identify the exact nature of the chemical/biological agent(s).The CB agent spectrum encompasses a wide range of materials including chemical warfare agents, bio-active peptides and biological warfare agents.

Results: A method has been developed for the sample handling and analysis of bio-active peptides which is based on electrospray ionization mass spectrometry (ESI-MS). ESI-MS is used to determine the molecular mass of the peptide and provisional identification is based on matching the molecular mass with that of known peptides in a database. The developed method was validated by the analysis and identification of α-conotoxin GI. The monoisotopic molecular mass was determined to be 1436.42 ± 0.13 u which was in excellent agreement with the theoretical value of 1436.48 u.

Significance of Results: The CF may be deployed in regions of the world where there is a significant threat of chemical/biological warfare agent use. Identification of agents is of importance as the results of such analyses would contribute to the development of strategic and political positions regarding future Canadian military operations and would facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel. The method described in this report is applicable to the target analysis of known bio-active peptides and provides the CF with a rapid means of identification of these agents.

Future Goals: The developed method will be evaluated during a NATO international training exercise for mid-spectrum agents. The training exercise held under the auspices of NATO AC/225 (LG/7-SIBCA) is planned to be held before May 1999.

INTRODUCTION

NATO may be called upon to deploy military forces in support of peacekeeping / peacemaking or battlefield operations in regions of the world where there is a significant threat of chemical/biological warfare (CBW) agent use. To operate effectively in these theatres, NATO forces must be able to detect and identify CBW agent(s). The CB agent spectrum encompasses a wide range of materials including chemical warfare agents, bio-active peptides and biological warfare agents. Analytical laboratories in the NATO countries are continually investigating new approaches for the identification of these agents.

Mass spectrometry (MS) combines exceptional sensitivity, specificity and speed for the analysis of bio-active peptides. "Growth" in this area has been extremely rapid, due to the recent development and commercial availability of two ionization techniques, electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) (1-5).The identification of bio-active peptides involves obtaining both molecular weight and amino acid sequence data from the intact peptide or peptide fragments formed following enzymatic or chemical digestion (6-17). The large number of bio-active peptides precludes the development of individual methods for all the candidate compounds. Therefore, a general approach that provides maximal structural information is required (18-19).

A method, based on ESI-MS has been developed for the sample handling and target analysis of known bio-active peptides. ESI-MS is used to determine the molecular mass of the peptide and tentative identification of the peptide is based on matching the molecular mass with that of known bio-active peptides in a database. Subsequent sample handling techniques such as disulfide bridge reductive alkylation and enzymatic digestion in conjunction with ESI-MS and liquid chromatography (LC) ESI-MS are carried out in order to confirm the identification of the peptide. This paper describes the various steps of the method and its application to the identification of α-conotoxin GI.

EXPERIMENTAL

Materials

Alpha-Conotoxin GI (1 mg) was purchased from Bachem (Torrance, California) and dissolved in water (1 mL). Dithiothreitol, iodoacetamide, cysteine, TRIS HCl and TRIS base were purchased from Sigma-Aldrich Canada (Oakville, Ontario). Ammonium bicarbonate was purchased from Caledon (Georgetown, Ontario). Calcium chloride was purchased from Fisher Scientific (Nepean, Ontario).

Bulk poroszyme (20 μm) immobilized trypsin was purchased from PerSeptive Biosystems Inc. (Framingham, MA, U.S.A.) and packed into a 0.5 mm ID x 25 cm PEEK column (courtesy of Mr. L. Hogge and Mr. D. Olson, National Research Council (NRC), Saskatoon) .

Sample Handling

Reductive Alkylation

The initial step in the reductive alkylation of disulfide bridges was to measure the pH of the aqueous sample. Where necessary, up to 30μL of pH 8 ammonium bicarbonate buffer (50mM) was added to 100μL of aqueous sample in order to bring the sample to approximately pH 8. To this solution was added 10μL of dithiothreitol (45 mM) and the solution placed in a 100°C water bath for 10 minutes. The solution was then immediately placed in an ice bath for 5 minutes. Ten microlitres of iodoacetamide (100 mM) was added to the solution and allowed to react at room temperature for 15 minutes. Finally, 10μL of cysteine (200 mM) was added to the solution to react with remaining iodoacetamide. The quenched reaction was allowed to stand for 2 minutes.

Tryptic Digestion

Enzymatic digestions were carried out using an immobilized trypsin column connected to a Hewlett Packard 1090 liquid chromatograph and a Kratos Spectroflow 773 variable wavelength UV detector

(210 nm). Ammonium bicarbonate buffer (50mM) containing 5 % acetonitrile was used as the digestion buffer. Sample introduction, of pH adjusted samples, was performed using a 20μL sample loop and a flowrate of 50μL/min. Digestions were carried out under stop flow conditions at 37°C, where the flow was stopped 35-50 seconds after injection. The sample was allowed to digest on the column for 10 minutes after which the flow was re-established and three fractions (20 μL each) were collected for analysis.

Instrumental

All LC separations were performed with an Applied Biosystems Model 140B dual syringe pump (Foster City, CA) equipped with a Micro-Tech Scientific 150 mm x 0.32 ID Zorbax C_{18} SB (5μm) packed fused-silica capillary column (Sunnyvale, CA) and a Rheodyne 8125 injector with a 5μL sample loop (Cotati, CA). The following solvent compositions were prepared for sample introduction: Solvent A (0.1% trifluoroacetic acid (TFA) in water) and Solvent B (0.1% TFA in acetonitrile/water, 95:5). Chromatographic separations were performed using a 1% to 75%B gradient over 30 minutes. Rapid analysis were made through the column under isocratic conditions (75%B, 5μL/min) which ensured minimal interaction of the sample with the column. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 200μL/min and split prior to the injector such that the flow through the column was 5μL/min.

All electrospray mass spectra were acquired using a Micromass Autospec-Q hybrid tandem mass spectrometer (Manchester, UK) equipped with a Micromass VG Mark II electrospray interface. The electrospray needle was operated at 7.6 kV and ions were accelerated into the mass spectrometer at 4 kV. A sampling cone voltage range of 50-125 volts was utilized. Nitrogen (Very Dry, Liquid Carbonic Inc., Scarborough, Ont., Canada) bath gas was introduced into the interface (80 °C) at a flow rate of 500 L/hr. Nitrogen nebulizer gas was introduced at a flow rate of 14 L/hr. The electrospray interface was pumped with both a rotary and a turbomolecular pump, which enabled maintenance of a $3x10^{-6}$ and $5x10^{-8}$ Torr vacuum within the source and analyzer regions of the instrument, respectively.

ESI-MS data for the peptides and their tryptic fragments were acquired in the continuum mode from 1500 to 150u (exponential scan), at 4 s/decade with a resolution of 2000 (10 % valley definition). Five to ten scans were typically averaged to enhance the signal-to-noise ratio and the data were smoothed using Micromass OPUS software. External calibrations were performed with a solution containing polyethylene glycols (PEG) 200, 600 and 1540 (0.03mg/mL in acetonitrile).

RESULTS AND DISCUSSION

General Considerations

The identification of bio-active peptides may be carried out by ESI-MS and ESI-MS/MS. The general approach used involves acquiring monoisotopic or average molecular mass for the intact peptide. This data is then used to search peptide and protein databases. Provisional identification is based upon matching, the molecular mass of the peptide with a known bio-active peptide in the database. If the peptide in the database contains disulfide bonds, reductive alkylation is carried out in order to confirm their presence in the sample. The sample is then enzymatically digested and a mass map acquired which is then compared to the theoretical mass map of the peptide. Finally collisional activated disassociation (CAD) of the peptide and its enzymatic fragments either in the ESI interface or in the quadrupole collisional cell may be used to provide amino acid sequence data. A six step method, which is described below, has been developed for the sample handling and analysis of bio-active peptides.

Sample Handling and Analysis Procedure for Bio-Active Peptides

Step 1: Removal of Particulates/Adjust pH

In general, it is assumed that a sample containing a bio-active peptide will either be collected as an aqueous sample (from a high volume aerosol sampler) or dissolved in water prior to further sample handling or analysis. The removal of particulates from an aqueous sample is necessary prior to

analysis in order to minimize damage to analytical instrumentation. Centrifugation appears to be the best approach for the removal of particulates, since low recovery of Ile-Ser-Bradykinin, a bio-active peptide, has been reported even when using low binding filtration membranes (20).

The pH of the sample is measured at this step as subsequent sample handling steps, such as reductive alkylation or enzymatic digestion, require the pH of the sample be compatible with the reaction.

Step 2: Purity and Molecular Mass Determination

Rapid analysis using isocratic LC-ESI-MS (75% B, 5μL/min) conditions with a wide mass range scan under low resolution and low sampling cone voltage conditions may be used to determine the relative purity of the sample and monoisotopic or average molecular mass(es) of the major sample component(s). A wide mass range scan (e.g. 150-1500 daltons) is used to ensure that most components from singly charged dipeptides to multiply charged polypeptides are detected. Molecular masses determined under low resolution (e.g. 2000, 10% valley definition) are sufficiently accurate for obtaining matches for target compounds in peptide databases. Low sampling cone voltages (e.g. 50 V) are used to minimize collisionally activated disassociation in the interface.

For low molecular mass compounds (<1500 u), it may be possible to obtain amino acid sequence information by inducing collisional activated disassociation in the ESI interface. Increasing sampling cone voltages generally results in increased fragmentation leading to the formation of product ions that may be used for amino acid sequencing.

Step 3: Separation and Fraction Collection

In cases where the sample contains multiple components, it is recommended that the components be separated by HPLC and fraction collected prior to subsequent sample handling steps.

Step 4: Determining the Presence of Disulfide Bonds

The three dimensional structure of proteins, while critical for biological activity, may restrict access of reagents thereby reducing the effectiveness of digestion and derivatization techniques. One technique for denaturing peptides (i.e. cause the peptide to unfold) is immersion in a boiling water bath. To ensure that the peptide doesn't re-assume it's original three dimensional shape upon cooling, it is immediately immersed in an ice bath, as it is removed from the boiling water bath.

Disulfide bridges between cysteine residues exist either between cysteines in the peptide chain (intramolecular) or between cysteines in different chains (intermolecular). These bridges may be broken using reductive alkylation, which may be carried out during peptide denaturing. Sample handling steps such as reductive alkylation require a pH 8. Ammonium bicarbonate buffer, a volatile buffer compatible with ESI-MS, can be used to adjust the pH of a sample to approximately pH 8.

Following reductive alkylation, the reaction product(s) may be analyzed by ESI-MS. When iodoacetamide is used as the alkylating reagent, the mass increases by 58.03 u for each cysteine that reacts with the iodoacetamide . The molecular mass of the intact peptide should be compared to that of the reductively alkylated peptide(s). If the molecular mass(es) are greater than that of the intact peptide, this is indicative of intramolecular disulfide bond(s). If the molecular mass calculated from the reductively alkylated peptide is less than the intact peptide this is indicative of intermolecular disulfide bond(s).

Step 5: Enzymatic Digestion and Mass Mapping of Enzymatic Fragments

Enzymatic digestions can be performed either in solution or in a flow-through system. While digestions performed in solution may take 12 or more hours, flow-through systems digest peptides in a matter of minutes. The flow-through system used in this study, consisted of a column packed with trypsin immobilized on a support, a solvent delivery system, an injector and a detector to monitor the elution of the digestion products.

Most enzymes have a pH range within which they will optimally digest peptides. For example, trypsin works best at a pH close to 8 and prior to sample introduction it is necessary to adjust the sample pH close to this value. Typically this is performed by adding a known volume of the ammonium bicarbonate buffer solution used in the actual digestion.

ESI-MS analysis of the digestion products, will produce a mass map. This mass map should correspond to the majority of the predicted mass map for the peptide that was matched in the database. Difficulties arise in comparing mass maps when there is incomplete cleavages during the digestion. Incomplete cleavage results in fragments that are either not produced during digestion or are longer than predicted from the structure and include an additional lysine or arginine in the sequence.

Step 6: Accessing Amino Acid Primary Sequence Data

The enzymatic digestion products would generally be analyzed by LC-ESI-MS under CAD conditions that promote the formation of product ions that provide the amino acid sequence data for the tryptic fragments. CAD may be carried out either in the ESI interface, or alternatively, in a collisional cell between the first and second mass analyzer. The amount and quality of the CAD data acquired will depend on the amount of sample, the mass of the analyte and its amino acid sequence.

Analysis and Identification of α-Conotoxin GI

The developed method was demonstrated during the analysis and identification of α-conotoxin GI. This toxin was selected as it; a) has intramolecular disulfide bridges, b) can be enzymatically digested with trypsin and c) has a molecular mass that could provide collisionally associated dissociation fragments in the ESI interface. Alpha-conotoxin GI, a snail toxin which acts as blocker for nictinic acetylcholine receptors in the central nervous system has the following structure:

Glu-Cys-Cys-Asn-Pro-Ala-Cys-Gly-Arg-His-Tyr-Ser-Cys-NH_2

with disulfide bridges between Cys 2-7 and Cys 3-13.

Step 1: The pH of the aqueous solution of α-Conotoxin GI was determined to be approximately six, indicating that the pH would need to be adjusted to pH 8 prior to reductive alkylation. The solution was clear and free of particulates, therefore no clarification of the sample was required.

Step 2: The monoisotopic molecular mass of the sample was determined, from two injections of the sample, under isocratic LC-ESI-MS conditions. Figure 1 illustrates a typical mass spectrum obtained under wide mass range scanning at a resolution of 2000. The monoisotopic molecular mass was calculated from the $(M+H)^+$, $(M+2H)^{2+}$ and $(M+3H)^{3+}$ ions from both injections. The calculated monoisotopic molecular mass was 1436.42 ± 0.13 u (n=6). A search of our in-house database determined that the only match within one mass unit of the calculated molecular mass was α-Conotoxin GI with a theoretical molecular mass of 1436.48 u. The error between the calculated and theoretical values was 42 ppm. Our experience with determining molecular mass under these conditions has indicated that errors are typically between 50 and 100 ppm (17). Table I lists the section of a database, under development by Canada and Norway, which contains α-Conotoxin GI and other bio-active peptides having similar molecular masses. The next closest bio-active peptide was α-Conotoxin GI (reduced) at 1440.52 u. Having provisionally identified the analyte as α-conotoxin GI, the sample was analyzed under CAD conditions that promoted fragmentation in the ESI interface in order to determine whether any amino acid sequence information could be accessed. No significant fragmentation of the intact toxin was observed.

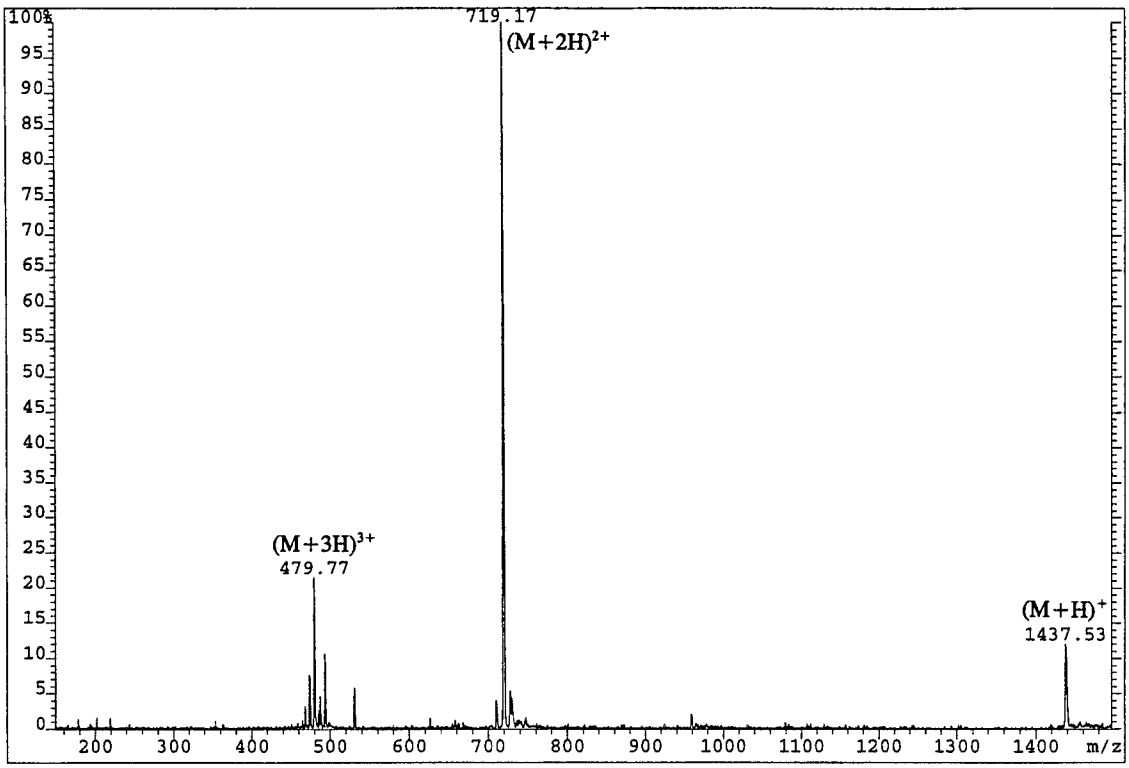

Figure 1. Electrospray mass spectrum of α-conotoxin GI obtained at 2000 resolution (10% valley definition) and a sampling cone voltage of 50 volts.

Table I. Selected Entries from Bio-Active Peptide Database

Compound Name	Monoisotopic Mol. Mass	Average Mol. Mass	CAS Number	Molecular Formula	Sequence (AA 1-250)
Substance P	1346.72813	1347.64419	33507-63-0	C63H98N18O13S	RPLPQQFFGLM-NH2
α-Conotoxin SI	1352.51341	1353.63198	133605-58-0	C55H84N16O16S4	ICCNPACGPKYSC-NH2 Cys2-7, Cys3-13
α-Conotoxin SI * (reduced)	1356.54471	1357.66389	115797-06-3	C55H88N16O16S4	ICCNPACGPKYSC-NH2
α-Conotoxin GII	1419.53046	1420.68232	78277-78-8	C57H85N19O16S4	ECCHPACGKHFSC-NH2
α-Conotoxin GI	1436.48423	1437.60379	76862-65-2	C55H80N20O18S4	ECCNPACGRHYSC-NH2 Cys2-7, Cys3-13
α-Conotoxin GI (reduced)	1440.51553	1441.65769	78249-65-7	C55H84N20O18S4	ECCNPACGRHYSC-NH2
α-Conotoxin SIA	1454.49882	1455.68407	135190-31-7	C60H82N18O17S4	YCCHPACGKNFDC-NH2 Cys2-7, Cys3-13
α-Conotoxin MI	1496.58937	1497.76881	83481-45-2	C58H92N22O17S4	GRCCHPACGKNYSC-NH2

*α-Conotoxin SI (reduced) is listed incorrectly in the CAS literature as α-Conotoxin GI (reduced).

Step 3: As the sample was pure this step was not applicable.

Step 4: Under reductive alkylation conditions, using iodoacetamide, the two disulfide bridges of α-conotoxin GI would be expected to break and the side chain hydrogen on each cysteine would be replaced with an acetamide group. The resulting product containing four acetamide groups would have a mass 232.12 u higher than the original sample. Figure 2 illustrates the mass spectra acquired during analysis of the reductively alkylated sample and a reaction blank. The mass spectrum for the sample contains a $(M+2H)^{2+}$ ion at m/z 835.17, indicating a molecular mass of 1668.32 u, 232.00 u higher than the unreacted peptide. This is consistent with the structure of α-Conotoxin GI indicating that the reaction product contains four cysteines with two intramolecular disulfide bridges.

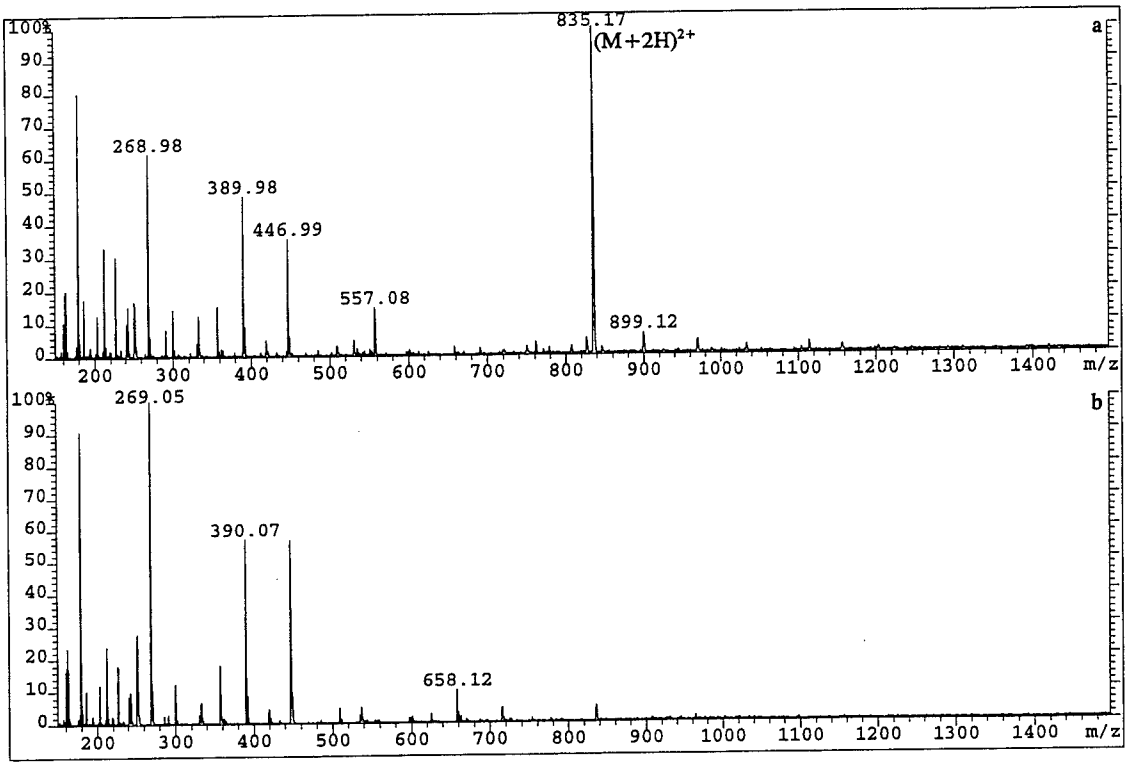

Figure 2. Electrospray mass spectra of a) reductively alkylated α-conotoxin GI and b) reductive alkylation blank obtained at 2000 resolution (10% valley definition) and a sampling cone voltage of 50 volts.

Step 5: Tryptic digestion of reductively alkylated α-conotoxin GI would result in cleavage on the C side of arginine producing two tryptic fragments. The mass of the larger fragment would be 1122.40 u and the mass of the smaller fragment would be 564.21u. Figure 3 illustrates the total-ion-current and reconstructed-ion-current (560-570u) chromatograms obtained during gradient LC-ESI-MS (1-75%B, 5μL/min) analysis of the tryptic digest of the reductively alkylated sample. Two components were detected. Figure 4a illustrates the ESI-MS spectrum acquired for the first peak in the chromatogram. An $(M+H)^+$ ion at m/z 565.19 indicated the presence of a tryptic fragment with a mass of 564.18 u. Figure 4b illustrates the ESI-MS spectrum acquired for the second peak. The mass spectrum contains both $(M+H)^+$ and $(M+2H)^{2+}$ ions from which the molecular mass of the tryptic fragment was calculated to be 1122.45 u. This data is consistent with the two predicted products from the tryptic digestion of α-Conotoxin GI .

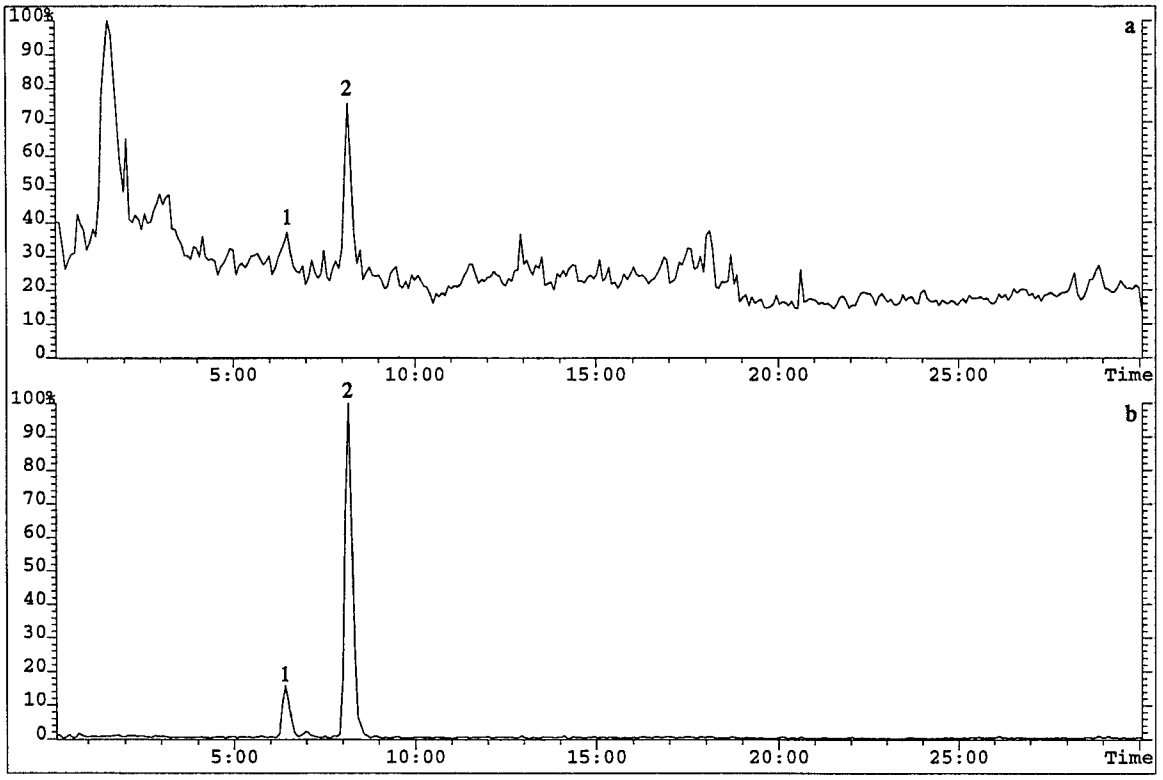

Figure 3. a) LC-ESI-MS total-ion-current and b) reconstructed-ion-current (560-570u) chromatograms of the tryptic digest of α-conotoxin GI obtained at 2000 resolution (10% valley definition) and a sampling cone voltage of 50 volts. (Peak 1; His-Tyr-Ser-Cys-NH_2 and peak 2; Glu-Cys-Cys-Asn-Pro-Ala-Cys-Gly-Arg).

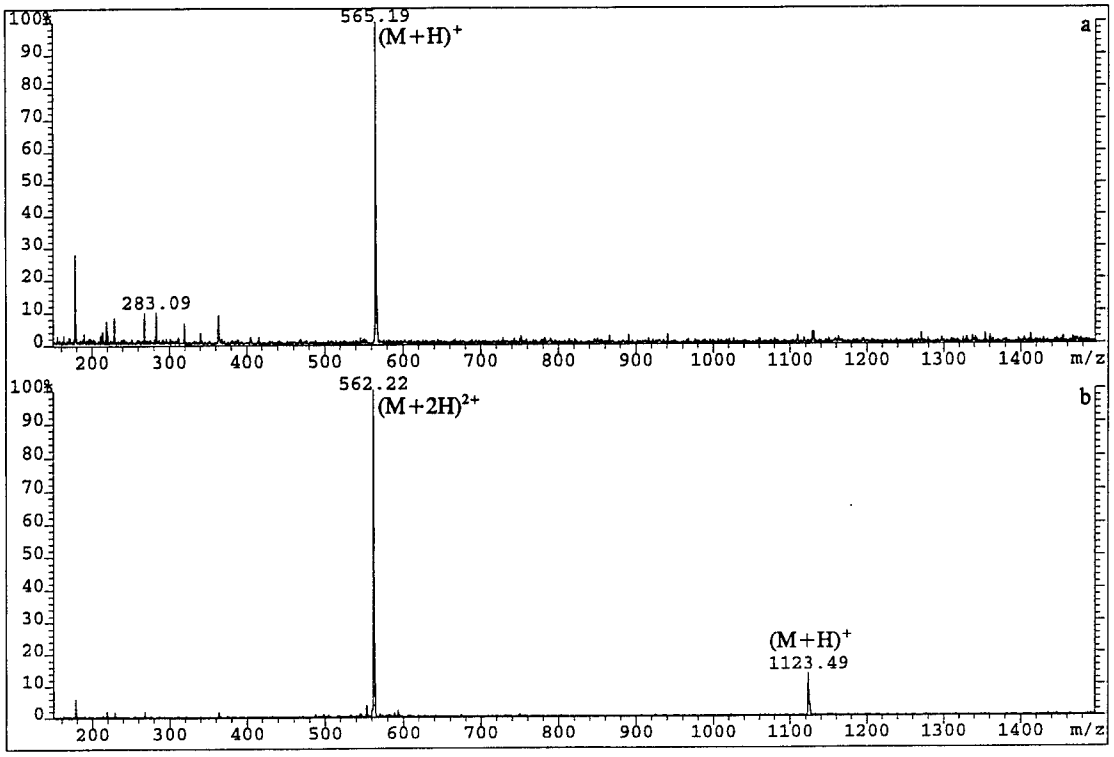

Figure 4. Electrospray mass spectra of tryptic digestion products of α-conotoxin GI a) His-Tyr-Ser-Cys-NH_2 and b) Glu-Cys-Cys-Asn-Pro-Ala-Cys-Gly-Arg obtained during gradient LC-ESI-MS analysis at 2000 resolution (10% valley definition) and a sampling cone voltage of 50 volts.

Step 6: Gradient LC-ESI-MS (1-75%B, 5μL/min) analysis of the tryptic digestion products was carried out under CAD conditions that promote the production of product ions in the ESI interface. Figure 5 illustrates the ESI-MS spectrum acquired for the larger tryptic fragment using a sampling cone voltage of 125V. Almost the complete Y series ions (Y_8 to Y_2) were observed for this fragment. These ions indicated an amino acid sequence of Glu-Cys-Cys-Asn-Pro-Ala-Cys. A Y_2 ion was observed at m/z 232.17, indicating that the final two amino acids in the sequence have a molecular mass of 231.16 u. Assuming that the tryptic fragment has an arginine at the C terminal, then the final two amino acids must be Gly-Arg (molecular mass 231.13 u). From this data the entire sequence of the larger tryptic fragment can be determined. The smaller fragment did not produce significant fragment ions under these conditions.

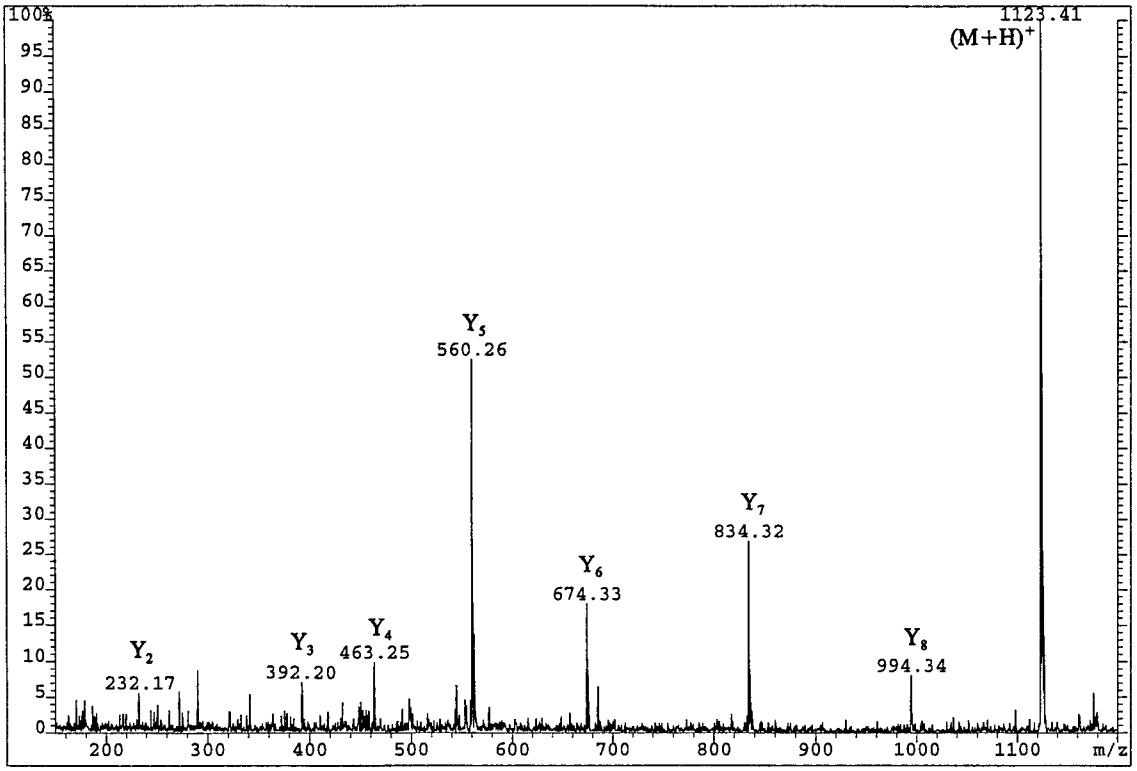

Figure 5. Product ion mass spectrum for Glu-Cys-Cys-Asn-Pro-Ala-Cys-Gly-Arg obtained during gradient LC-ESI-MS analysis at 2000 resolution (10% valley definition) and a sampling cone voltage of 125 volts.

CONCLUSIONS

A method has been developed for the sample handling and analysis of bio-active peptides. Based on electrospray ionization mass spectrometry (ESI-MS), the method is applicable to the target analysis of known peptides. The developed method was applied to the analysis and identification of α-conotoxin GI. The monoisotopic molecular mass was determined to be 1436.42 ± 0.13 u (n=6). A search of the in-house database determined that the only match within one mass unit of the calculated molecular mass was α-conotoxin GI with a theoretical molecular mass of 1436.48 u. The error between the calculated and theoretical values was 42 ppm. Reductive alkylation indicated the presence of four cysteines and two intramolecular disulfide bridges which was consistent with the structure of α-conotoxin GI. LC-ESI-MS analysis of the tryptic digestion products indicated the presence of two fragments with masses of 564.18 and 1122.45 u which were in agreement with the predicted products. Under CAD conditions,Y series ions were observed from which the entire sequence of the larger tryptic fragment was determined.

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A Method for the Sample Handling and Analysis of Bio-Active Peptides (U)

4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

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5. DATE OF PUBLICATION (month and year of publication of document)	6a. NO. OF PAGES (total containing information. Include Annexes, Appendices, etc.)	6b. NO. OF REFS (total cited in document)
May 1998	16	20

6. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Suffield Report

8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)

DRES

9a. PROJECT OR GRANT NO. (if appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant)	9b. CONTRACT NO. (if appropriate, the applicable number under which the document was written)
6ED13	

10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)	10b. OTHER DOCUMENT NOS. (Any other numbers which may be assigned this document either by the originator or by the sponsor)
SR 699	

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A method has been developed for the sample handling and analysis of bio-active peptides. Based on electrospray ionization mass spectrometry (ESI-MS), the method is applicable to the target analysis of known peptides. ESI-MS is used to determine the molecular mass of the peptide and provisional identification is based on matching the molecular mass with that of known peptides in a database. Disulfide bridge reductive alkylation is used to determine the number of cysteines in the peptide as well as the presence of disulfide bridges. The peptide is then enzymatically digested and the digestion products analyzed by ESI-MS. The resulting mass map is compared to the masses predicted from the structure of the peptide. Finally, the peptide and its enzymatic fragments are analyzed by liquid chromatography (LC) ESI-MS using collisional activated disassociation (CAD) conditions which promote the formation of product ions from which it is possible to determine the amino acid sequence of the peptide.

The developed method was applied to the analysis and identification of α-conotoxin GI. The monoisotopic molecular mass was determined to be 1436.42 ± 0.13 u (n=6). A search of the in-house database determined that the only match within one mass unit of the calculated molecular mass was α-conotoxin GI with a theoretical molecular mass of 1436.48 u. The error between the calculated and theoretical values was 42 ppm. Reductive alkylation indicated the presence of four cysteines and two intramolecular disulfide bridges which was consistent with the structure of α-conotoxin GI. LC-ESI-MS analysis of the tryptic digestion products indicated the presence of two fragments with masses of 564.18 and 1122.45 u which were in agreement with the predicted products. Under CAD conditions, Y series ions were observed from which the entire sequence of the larger tryptic fragment was determined.

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Mass Spectrometry

Electrospray

Bio-active peptides

Enzymatic Digestion

Immobilized Trypsin

Disulfide Bridge Reduction

Conotoxin

LC-ESI-MS

Collisional Activated Disassociation