STABILITY STUDIES OF RICIN, *STAPHYLOCCOCAL ENTEROTOXIN* B AND TRICHOTHECENE MYCOTOXINS ON OFFICE MATRICES

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SUMMARY

With the emerging threat from biotoxins, we study the fate of three biological warfare agents namely ricin, *staphylococcal enterotoxin B* (SEB) and T-2 mycotoxin in an indoor office environment. In this research study, we will look into the stability profile of each toxin with a suitable analytical technique over a period of 7 days. Based on the principle of amine specific isobaric tagging (i.e. iTRAQ reagents) as well as the use of nano-flow liquid chromatography-mass spectrometry, we describe here a new quantitative strategy that looks into the stability profiles of *staphylococcal enterotoxin B* (SEB) and ricin over 170 hours on carpet and parquet. This is the first paper that looks into the quantitative work of SEB and ricin using iTRAQ reagents. Using the MS/MS signature ions from the amine-derivated peptides and comparison with standard, we have shown that the study of SEB and ricin with absolute quantitation is feasible by amine isobaric tagging and analysis with nano-flow liquid chromatography-mass spectrometry (nanoLC-MS).

This quantitative methodology based on iTRAQ labeling/nanoLC-MS showed that the stability of ricin on carpet had dropped drastically to half of the spiked amount by the 8^{th} hour and had leveled down to ~20% by the end of the 7^{th} day, leaving an amount of 15.5 ug/cm^2 ricin. As for ricin on parquet, it was much stable than on carpet with ~70% remaining at the end of the 7^{th} day. SEB was very stable on parquet with no observable degradation for up to 7 days but degraded gradually on carpet with ~25% (19.5 ug/cm^2) remaining on the 7^{th} day in an indoor office environment. SEB on parquet (~100% remaining) was more stable compared to ricin on parquet (~70% remaining). Both

protein toxins were more stable on parquet than carpet with ~20% to 25% toxin remaining on carpet.

The stability profile of T-2 toxin on carpet and parquet was studied using liquid chromatography-mass spectrometry (LC-MS) based on selected ion monitoring. The stability profile of T-2 toxin was showed to be twice as stable on carpet as compared to parquet under indoor conditions up to 7 days. On the 7^{th} day, there was 0.20 mg/25 cm^2 (i.e. 20%) remaining on parquet. As for carpet, T-2 toxin was more stable and persistent with 0.34 mg/25 cm^2 (i.e. 34%) remaining. In contrast to SEB and ricin, T-2 toxin was more stable on carpet than on parquet. Parquet is an interesting indoor substrate giving extreme opposite toxin stability results for the proteinaceous toxins (i.e. SEB and ricin) and non-proteinaceous toxin (i.e. T-2 toxin) studied thus far. T-2 toxin degraded rapidly on parquet whereas SEB and ricin were extremely stable on parquet. This implied that non-protein toxin as well as protein toxins behave differently on different matrices and degrade to different extents.

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LISTS OF ABBREVIATIONS

BWA	biological warfare agents
CCD	charge-coupled device
CDC	Centers for Disease Control and Prevention
CE	capillary electrophoresis
CV	Correlation variation
CWC	Chemical Weapons Convention
diGalCer	glycosphingolipid digalactosylceramide
DTRA	Defense Threat Reduction Agency
ECD	electron capture detection
ECL	electro-chemiluminescence
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
FCL	fluorogenic-chemiluminescence
FID	flame ionisation detection
FM	microplate fluorometer
GC	gas chromatography
HFB	heptafluorobutyryl
HPLC	high performance liquid chromatography
ICAT	isotope-coded affinity tag
IMS	immunomagnetic separation
LAPS	light-addressable potentiometric sensor

LC-RI	LC-refractive index
LOD	lowest level of detection
NATO	North Atlantic Treaty Organization
MAb	monoclonal antibody
MALDI	matrix-assisted laser desorption/ionization
MS	mass spectrometry
PFP	pentafluoropropionyl
Qq TOF	Quadrupole time of flight
RIA	radioimmunoassay
SCX	Cation-ion exchange
SD	Standard deviation
SEB	*Staphylococcal enterotoxin B*
SFC	supercritical fluid chromatography
SIM	selected ion monitoring
SPME	solid phase microextraction
SPR	surface plasmon resonance
T-2 toxin	Trichothecene mycotoxins
TFA	trifluoroacetyl
TIC	Total ion chromatogram
TLC	thin layer chromatography
TMS	trimethylsilyl
TOF-MS	Time of flight-mass spectrometry
WHO	The World Health Organization

CHAPTER 1 INTRODUCTION

1.1 BIOLOGICAL WARFARE AGENTS

1.1.1 Its definition, classification and threats

Biological warfare agents (BWA) are agents that can achieve their deliberate intention by causing infectivity of diseases or intoxication through the release of microorganisms, viruses and toxins of biological origin, resulting in death, illness and incapacitation of human beings, animals or plants[1]. The pathogenicity caused by some of the BWA could be due to its own generated toxic substances. Such BWAs are known as toxins. Toxins are toxic chemical compounds produced naturally by animals, plants and microorganisms or by chemical synthesis. They could be either proteinaceous or non-proteinaceous toxins. The main characteristic difference between living organisms and toxins is that the former are alive and can reproduce and multiply in the target host whereas the latter are non-living chemical compounds from biological sources[2]. These two classes of BWA not only differ in their nature but also on their stability, toxicity, lethality, infected time and route of disease transmission. Unlike living organisms, toxins only have effects on the population that has direct exposure to them and will not transmit among human beings. The dissemination of the living organisms is much limited due to its stability[3]. As for toxins, they are mostly stable leading to simple dissemination system.

Toxins do not achieve their effects through infectivity, but rather due to toxicity. This is similar to the definition of chemical warfare agents (CWA), even though they are

biological warfare agents. Thus, toxins are known as mid-spectrum agents since they fall between the two different classes of warfare agents. The two main differences between toxins and CWA are that toxins are non-volatile and non-dermal active except for trichothecene mycotoxins[4]. Bio-toxins are believed to be the new emerging threat[5] to humans and their impact on population and casualties may be even more severe than classical CWA since bio-toxins are more lethal per kilogram than many CWA. The North Atlantic Treaty Organization (NATO) recognized the difficulty of identifying a wide diversity of mid-spectrum agents due to limitations in BWA detection, which also have diverse potencies and thus, listed out the criteria for identification of both proteinaceous and non-proteinaceous toxins[6].

Recent attempts to deploy anthrax spores and botulinum toxin aerosol in Tokyo by Japanese sect Aum Shinrikyo, followed by the 9/11 terrorist attack and mails containing anthrax spores in US had raised the alarm on the possibility of bioweapons. BWA pose a wide spectrum of threat from insignificant to massive casualties and death. The level of threat would depend on the nature of BWA used, its stability in environment, its dissemination system, its population's preparedness and its agencies' countermeasure. However, BWA do not need to be highly potent in order to be an effective weapon. For the horror, panic and confusion caused by BWA, it would have fulfilled its intended motive of psychological warfare, apart from physical injury or illness caused[7]. Thus, it is important to have the agencies and people well-educated and prepared for the possibility of bioterrorism.

1.1.2 Its potential use

The various inherent advantages of BWA are stated as follows: availability, ease of low cost production, high lethality and capability to incapacitate population, appropriate aerosol particle size which poses as the most fatal route, ease of dissemination and stability after production. At this moment of time, it is extremely difficult to detect the use of toxins as BWA immediately due to the current limitation in detection. This makes bio-toxins the most potential weapon to be used in the next era. A merging list from the Centers for Disease Control and Prevention (CDC), the North Atlantic Treaty Organization (NATO), the Defense Threat Reduction Agency (DTRA) and other entities[8] had also shown us the emerging threat from BWA. Thus, there is an urgent need to develop a rapid detection and quantitation method for BWA in order to counteract with proper medical treatment or decontamination to reduce the mortality rate.

1.2 RICIN

1.2.1 Its structure, properties & mechanism of action

Ricin is a class of proteinaceous toxin, which is also a mid-spectrum agent that can be deployed in biological warfare, other than its great promise as immunotoxins[9] for cancer therapy. Ricin toxin is found in the seeds of castor bean, *Ricinis communis*. It is one of the most toxic and easily obtainable plant toxins. Among the mid-spectrum agents, ricin takes a unique position, because it is the only protein listed under the Chemical Weapons Convention (CWC). Even though its lethal toxicity is about 10,000 fold less than botulinum toxin, ricin still has its potential for weaponization due to its worldwide

availability in bulk quantities as a by-product from castor oil production, its extreme heat stability and its ease of extraction. Before the 1990s, ricin intoxication was uncommon and thus, not much effort had been made to produce vaccination. However, with the increased possibility of using toxins for bioterrorist attack, it cannot be neglected. Effort was made by Olson et al[10] where they used protein engineering to produce an immune serum for ricin which was still under development.

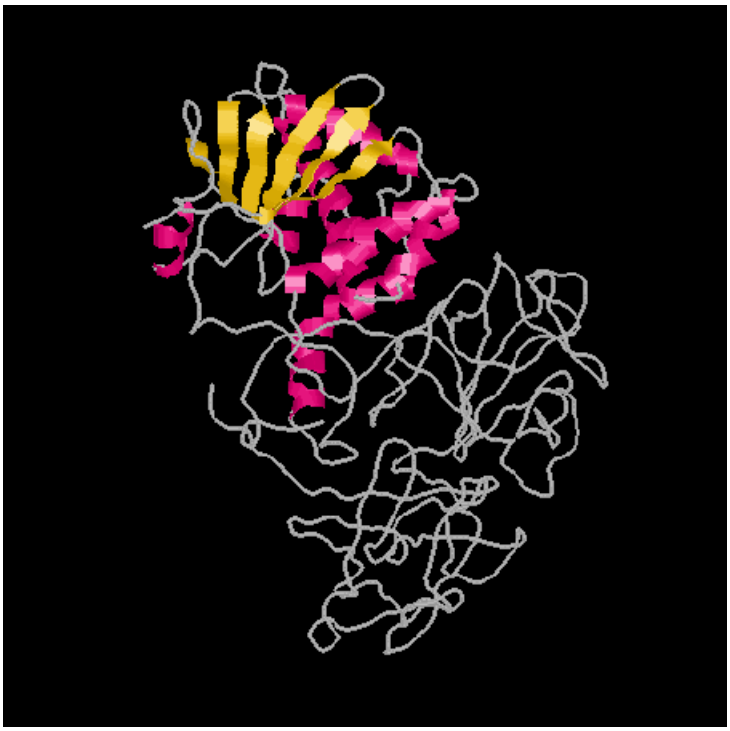


Figure 1: Structure of Ricin.
Source from Swissprot account number P02879 & RCSB protein data bank, account number 2AAI.
It is drawn using Rasmol version 2.6.

Ricin is an approximately 66 kDa globular protein consisting of two moieties, the A-chain (approximately 32 kDa) and the B-chain (approximately 34 kDa), connected by a disulfide bond as shown in Figure 1. The two chains must be associated for toxicity where one group of di-chain ribosome-inactivating proteins is specific for the

depurination of a single adenosine in ribosomal ribonucleic acid (RNA). Basically, it is the A-chain which acts as N-glycosidase that is responsible for its toxicity effect causing inhibition of protein biosynthesis.

The B-chain has lectin properties that bind to complex galactosides of cell-surface carbohydrates where this binding of B-chain to glycoside residues on glycoproteins and glycolipids would promote onset of the endocytotic uptake of the protein. Thus, it facilitates the internalization of the toxin by transporting A-chain into the cytosol within a few hours. The enzymatically active A-chain acts as a ribosome-inactivating protein by removing an adenine residue from an exposed loop of 28S ribosomal RNA once it enters into the cytoplasm of a eukaryotic cell. This causes the failure of elongation factor-2 to bind and thus, these truncated ribosomes can no longer support protein synthesis[11, 12] as the A-chain molecule inactivates ribosomes faster than the cell can make new ones.

The route of ricin poisoning could be through inhalation, intravenous injection, intraperitoneal injection, subcutaneous injection or ingestion but inhalation route is presumed to be the likeliest threat in battlefield. The toxicity of ricin depends on the route of poisoning, with the LD_{50} for mice being between 3 to 5 ug/kg for 60 hours exposure through inhalation. A longer exposure time of 90 hours would be required for mortality, following intravenous injection of 5 ug/kg, whereas 20 mg/kg would be required by ingestion. The high amount required for toxicity through injection reflects the poor absorption of ricin via the gastrointestinal tract. It has also been shown that there was no toxicity associated with dermal administration of ricin. The clinical symptoms of ricin

poisoning vary depending on the size of dose and the route of poisoning. In animal studies[13], poisoning through inhalation would generally produce physical symptoms such as sudden respiratory difficulties, eye irritation and chest tightness, and in more severe case, asthma lasting for several hours. During the first 8 hours of exposure, there would not be any damage to the lungs. By the 12th hour of exposure, increased inflammatory cell counts and total protein were observed, indicating inflammation. By the 30th hour of exposure, alveolar flooding followed by arterial hypoxemia and acidosis would be observed. Death usually occurs within 36 to 48 hours but it will depend on the size of dose being exposed. As the action of ricin is rapid, once inhaled, it is irreversible and post treatment would be difficult. For ingestion intoxication, there would be symptoms of nausea, vomiting, and abdominal pain, followed by bloody diarrhea and in severe instances, liver necrosis, rental failure, circulation collapse, coma or even death may occur. Ingestion poisoning should show all the gastrointestinal symptoms. For injection, there would be immediate localized muscle pain and regional lymph node necrosis.

As ricin has several different oligosaccharide chains linked to four glycosylation sites, it could exist in several forms by the differences in carbohydrate composition, which makes the analysis even more challenging. For environmental monitoring in bioterrorism attack as well as for medical treatment purpose, there is a need for a rapid and sensitive detection and quantitation method on trace amount of ricin in different matrices.

1.2.2 Review of analytical methods for Ricin detection

One of the earliest detection methods for low concentration ricin was radioimmunoassay (RIA) [14, 15] which used radioactive ^{125}I-labeled ricin. It could be used to quantify as low as 100 pg of ricin. Even though it had good sensitivity, its major drawbacks were long incubation time, difficulties in handling and disposing of radioisotopes. These limitations had made it less preferred compared to enzyme-linked immunosorbent assay (ELISA). ELISA involved shorter assay time compared to RIA. The principle of ELISA is based on antibody-antigen interaction. It can be in direct, competitive or sandwich format. Sandwich ELISA[16] using rabbit anti-ricin antibody was done, giving a detection limit of 40 ng/ml for ricin. This development of ricin assay had been made based on the usage of affinity-purified rabbit antiserum and avidin/biotin immuno-peroxidase complex.

Enhanced colorimetric and chemiluminescence ELISA[17], in which an affinity-purified goat polyclonal antibody was utilized to form a sandwich assay with the same antibody (biotinylated), were explored. The addition of avidin-linked alkaline phosphatase (AP) allowed colorimetric measurement at optical density of 405 nm. The enhanced colorimetric assay was made with increased biotinylated antibody content and a reduction in dilution ratio of avidin-linked AP, giving a detection of 100 pg/ml for ricin in buffer, human urine and serum. This sandwich assay could be configured to chemiluminescence's detection with quantitative range of 0.1-1 ng/ml and was subjected to greater variations compared to colorimetric assay. Further development on colorimetric detection was investigated. Monoclonal antibodies (MAb) was used in a

sandwich format with affinity purified anti-ricin B chain MAb that extracted ricin and anti-ricin A chain MAb that conjugated with peroxidase[18] to give a colorimetric detection at optical density of 450 nm. This allowed a detection of 5 ng/ml ricin in buffer, urine and human serum.

The main disadvantages of classical ELISA are its long incubation time and lengthened assay time due to several washing steps involved and also limited throughput. For an inexpensive monitoring and rapid detection of BWA, both colorimetric and chemiluminescence ELISA would not have met these requirements. With a change in the detection module to a laser-induced fluorescence, a fluorescence-based fiber optic immunoassay[19] was reported giving a detection limit of 100 pg/ml in buffer solution within a much reduced assay time of 20 minutes. This fluorescence-based assay involved the use of evanescent wave for detection and the enhanced sensitivity attributed to the avidin-biotin principle that improved the antibody binding activity. Other form of conjugation in immunoassay was explored with reduced assay time. The use of colloidal gold particles[20] as a detection reagent was used in comparison to the conventional conjugation. This allowed detection to be done in less than 10 minutes and yet gave sensitivity close to classical ELISA. The detection limit was 50 ng/ml ricin but this could be stretched down to even lower limit of 100 pg/ml with just the use of silver enhancement. The advantages of these gold particles are their superior mobility, less aggregation and commercial availability. Through coupling polyclonal antibodies with carboxylated latex particles[21], it allowed a sensitized latex agglutination test, which gave a sensitivity of 2.8 ng/0.5 ml of latex particles.

Simultaneous detection of toxins is desirable as it is not longer sufficient to have just a fluorescence-based immunoassay for detection of a single analyte since in a bioterrorist attack, we would have no idea what BWA has been released. A planar array immunosensor[22], equipped with a charge-coupled device (CCD) was reported to simultaneously analyze three toxins, namely ricin, SEB and *Yersinia pestis*. It was a simple and disposable sensing array coated with different antibodies detected through CCD. This planar array platform was able to give a detection limit of 25 ng/ml ricin, 5 ng/ml SEB and 15 ng/ml Y. *pestis*. With this detection platform, it allowed multiple analytes' detection, low quantity sample requirement and simultaneous analysis inclusive of both positive and negative controls. This array technology was extended further to analyze simultaneously six toxins[23] namely, ricin, SEB, cholera toxin, *botulinum toxoids*, trinitrotoluene and *mycotoxin fumonisin*.

From a normal sized spot on the array surface to a micrometer-sized spot, a microarray was designed. A flow-based microarray platform[24] was developed based on this microsized array where the surface was fitted to a flow module with six channels. This flow module containing the analytes' solution would flow through the microarray of fluorescence-coated antibodies and could detect low concentration of ricin rapidly in 15 minutes. Simultaneous detection of both proteins and bacteria was possible giving a detection limit of 10 ng/ml for ricin, 4 ng/ml for SEB, 8 ng/ml for cholera toxin and 6.2×10^4 cfu/ml for *Bacillus globligi*. The major advantage of microarray immunoassay

would probably be its massive number of spots on a single array surface, thus allowing several analytes to be run in parallel.

Even though fluorescence-labeled immunotechnology could give multiplexed analysis, organic fluorophores still subjected to complications such as occurrence of multiple spectral characteristics that made data analysis more challenging. By replacing such fluorophores with luminescent semiconductor nanocrystals (quantum dots)[25], it was reported to perform multiplexed fluoroimmunoassay on ricin, cholera toxin, shiga-like toxin and SEB with a good detection limit of 30 ng/ml and 3 ng/ml for ricin and SEB respectively. Quantum dots are inorganic fluorophores that are robust and span the visible spectrum. It allows particles of different sizes to be excited simultaneously at a single wavelength and yet emits multiple wavelengths. The results obtained using quantum dots were reproducible, which organic fluorophores could not. But cross reactivity may still occur within any immunoassay.

Antibodies have been the crucial part of the immunoassay technology. But due to its sensitivity to high temperature, it could be denatured easily and thus, its storage life is short. This problem could be resolved by a change of receptor to either aptamer chips[26] or glycosphinogolipids (GSLs) [27]. Aptamers are functional binding species from selected combinatorial oligonucleotide libraries, which can be chemically synthesized. The adoption of aptamer receptor into a microarray platform had various advantages like high stability of chip leading to long storage and ease of transportation, and could be regenerated without any loss of activity. But its sensitivity was only 320 ng/ml. As for

GSLs, it has strong interaction with protein toxins and its usage as recognition molecules surpasses the antibody technology since they are stable at room temperature, ease of orientation by hydrophobic and hydrophilic interaction and more binding sites available. This study was done using quartz crystal microbalance (QCM) sensor which gave a detection limit of 5 ug/ml compared to 25 ug/ml of antibody. On comparison, it showed that with a change of antibody to GSLs, it gave a better sensitivity but this detection limit was not low enough. This could be improved by a change in the detection module.

Using the principle of microchip, a three-dimensional hydrogel containing various immobilized proteins/antibodies on a microchip known as protein gel-based microchip[28] was manufactured. This gel-based microchip used in conjunction with various immunoassays was done. The detection of analyte was through fluorescence, chemi-luminescence equipped with CCD or mass spectrometry (MS). The highest sensitivity of 0.1 ng/ml for ricin and 1ng/ml for SEB was achieved by sandwich immunoassay format done individually. However when studied in parallel analysis, its sensitivity was dropped to 0.7 ng/ml of ricin. This gel-based microchip has various advantages such as good stability up to six months due to its hydrophilic condition, ease of production, and covalent immobilization of proteins within the structure, highly porous for ease of interaction and no cross reaction due to proper selection of the antibody pairs. However, it required several hours for analysis. It was mentioned in this study that the approach of MS is promising since it was a one- step procedure which requires no labeling of antibodies.

Development on the solid-phase surface of immunoassay technology was made by a replacement of the conventional microplate with magnetic microspheres[29] or gold-coated magnetoelastic sensor surface[30, 31]. In immunomagnetic microsphere surface, it involved two-phase chemiluminescence based techniques, fluorogenic-chemiluminescence (FCL) and electro-chemiluminescence (ECL) using magnetic microspheres giving detection of 1000 pg/ml ricin and 0.5 pg/ml ricin respectively. These ECL and FCL detectors could identify multiple BWA like SEB, *Botulinum* A, *Bacillus anthracis, Bacillus subtilis* and *Escherichia coli* with high sensitivity. Advantages of microsphere are large surface area for capturing antibodies which enhances sensitivity, higher reaction rates which lead to reduction in analysis time and easy detection using simple magnetic field. This technique allowed a direct separation of target analyte from a complex mixture.

Both FCL and ECL have similar formats except that FCL uses alkaline phosphatase as label and detects through measurement of fluorescence whereas ECL uses ruthenium-tribipyridal as label and detects through photo emission. For magnetoelastic sensor, the detection technology is a sandwich complex of anti-ricinus communis agglutinin antibody on the sensor surface and uses biocatalytic precipitation to cause a change in mass. This in turn, causes a shift in the resonance frequency which allowed quantitation of ricin at 5 ng/ml. This magnetoelastic sensor has sensitivity comparable to ELISA but it allows cheaper, disposable and relatively faster analysis.

Most detection techniques for ricin have been focusing on bioassay principle. From changes of various formats, labels, detection modules and receptors, the goal for

detection of ricin is clear. They aim at rapid detection with high sensitivity and ease of performance with multiplexed analysis in a single run. But for typical bioassays techniques, even though they are not ricin specific, it can also cause cell death and possibility of cross-reactivity. Only a few papers on chemical analysis have been published. They are believed to have higher specificity. Chemical analysis like Fourier transform near-infrared reflectance spectrometry[32] based on multivariate technique can give a fast and accurate quantification on ricin. Though it is a non-destructive method giving both structural and physical properties of the analyte, its low sensitivity of 1.5 mg/500 mg of wheat flour is the biggest drawback.

There have been reports of ricin analysis through the use of capillary electrophoresis (CE) detecting 10 mg/ml ricin by UV detector[33]. The attraction of CE lies in its ability to do both separation and purification of complex mixtures rapidly. Incorporating this into immunoassay where it combines the separation power of CE and the specificity of antibodies for detection results in a capability known as capillary electrophoresis-based immunoassay (CEIA)[34]. CEIA is much simpler than classical model which allows more flexibility such as custom making of analyte assay. It requires less sample and reagents' volume and yet allows simultaneous multiplexed detection and ease of result interpretation. This CE technology was improvised into a hand-held device[35] based on two microchip separations, namely gel (CGE) and zone electrophoresis (CZE). Using a microfluidic principle on chip, it allows detection of fluorescence-labeled toxin by a miniaturized laser-induced fluorescence detection module. This system was a small instrument for ease of logistic but its sensitivity was not optimized giving 25 nm for CZE

and 5 nm for CGE. Other than being miniatured, it also requires relatively less sample or solvent and at a lower cost.

Other than immunoassay, CE can be coupled to mass spectrometry (MS) [36, 37] giving a powerful analytical tool which can analyze, characterize and differentiate between various forms of ricin toxins. It provides a more precise and efficient analysis of ricin. Most proteinaceous toxins are not amenable to gas chromatography mass spectrometry analysis (GC-MS) and thus, liquid chromatography (LC) based methods are used. Mass spectrometry has emerged to be a useful tool for analysis of high molecular weight protein. During the past two decades, mass spectrometry has become established as the primary method for protein identification from complex mixtures of biological origin. This is largely attributed to the rapid instrumental advances and growth in genomic databases.

Mass spectrometers (MS) employed in proteomic analysis used are either matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI). With this combination of soft ionization methods, it provides a rapid and sensitive tool for determination of accurate molecular weight and also the possibility of amino acid sequencing for identification. However, ESI has been the standard ionization method[38] for LC-MS and LC-MS/MS. ESI is usually employed for single and triple quadrupoles and quadrupole ion traps that typically give modest resolution. The combination of a quadrupole mass selector and quadrupole collision cell with orthogonal acceleration TOF

(Qq TOF) gives high resolution (~10,000) and perhaps 5ppm mass accuracy[39]. Qq TOF has been widely used in protein/peptide analysis[40, 41, 42, 43].

Multi-dimensional (MD) LC coupled with MS is possible by having C18 desalt column or cation exchange (SCX) column in the first dimension and separation of protein/ peptides through C18 column in the second dimension, followed by MS analysis. On-line separation and purification made through MDLC coupled to ESI-MS[44, 45] allow us to identify and quantify the protein/peptides. Characterization of ricin[46, 47, 48] had been done using MS but no detection limit was reported. With the advancement of MS/MS, the power of MS could be used in different approach like the use of label such as isotope-coded affinity tag (ICAT) [49, 50, 51] for relative quantitation or iTRAQ reagents [52, 53] for absolute quantitation. In Table 1, we had summarized the various methods and lowest level of detection (LOD) for ricin. Despite the fact that we do not have any reported LOD for LC-MS, we do see MS technology as a potential for ricin detection and quantitation given their advantages in sensitivity, speed and multi-agents detection. With the labeling technology, it also allows accurate quantitation.

Table 1: summarized methods and LODs for Ricin

	Ricin Methodologies	**Lowest level of detection**
1	Radioimmunoassay	100 pg for ricin[14,15]
2	Enzyme-linked immunosorbent assay (ELISA)	5-40 ng/ml (colorimetric)[16,18] 100 pg/ml(enhanced colorimetric)[17] 0.1-1 ng/ml(chemiluminescence)[17] 50 ng/ml (colloidal gold particles)[20] 100 pg/ml(gold particles with silver enhancement)[20]
3	Fluorescence-based fiber optic immuno assay	100 pg/ml[19]
4	Fluorescence-based immunoassay using quantum dots	30 ng/ml[25]
5	Planar array immunosensor with charge-coupled device	25 ng/ml[22] 10 ng/ml (flow-based platform)[24]
6	Aptamer microarray	320 ng/ml[26]
7	GSLs with QCM	5 ug/ml[27]
8	Protein gel-based microchip immunoassay	0.7 ng/ml[28]
9	Magnetic microsphere with (a) ECL or (b) FCL	(a) 0.5 pg/ml[29] (b) 1000 pg/ml[29]
10	gold-coated magnetoelastic sensor	5 ng/ml in aqueous media[30,31]
11	Fourier transform near-infrared reflectance spectrometry	1.5 mg/500 mg of wheat flour[32]
12	Capillary electrophoresis with (a) UV or (b) MS	(a)10 mg/ml[33] (b)characterization done but no limit of detection reported[34]
13	CZE, CGE	25 nm, 5 nm[35]
14	LC-MS	Not reported

1.3 *STAPHYLOCOCCAL ENTEROTOXIN B* (SEB)

1.3.1 Its structure, properties & mechanism of action

Staphylococcal enterotoxin B (SEB) is one of the seven enterotoxins produced by strains of *Staphylococcus aureus*. Depending on the phase of cell growth cycle, pH and the glucose content, it would produce a variety of different staphylococcal toxins such as type A, B, C1, C2, C3, D and E. Their classification is based on their sequence homology. SEB belongs to a group of proteins with molecular weight ranging from 23 to 29 kDa. It is stable to heat, proteolytic digestion and pH change (pH 4 to 10) and also water-soluble. As a pyrogenic toxin, it is capable of incapacitating a person for up to two weeks with a dosage as low as 0.0004 ug/kg. It may even serve as a better incapacitant than many CWA incapacitants since its effect would be longer[2]. They are low volatile compounds and are extremely potent gastrointestinal toxins. Its structure is shown in Figure 2.

Figure 2: Structure of SEB.
Source from Swissprot account number P01552 & protein data bank account number 3SEB.
It was drawn using Rasmol version 2.6.

Due to its inherent stability, high morbidity rate, high intoxication effect and ease of dissemination, it is an attractive choice of biological aerosol weapon as considered by NATO[54]. It is classified as a B-list agent in CDC. A significantly lower quantity is required to produce the same effect as synthetic chemical agents. The mechanism of action is thought to be the activation of the immune system receptors by SEB where it causes a strong binding with the T-cells and class II molecules that would mimic the CD4 binding leading to production of large quantity of T-cells independent of antigen recognition. This could result in a massive release of cytokines such as interferon-gamma, interleukin-6 and tumor necrosis factor-alpha or histamine and leukotriene from mast cells[55]. The clinical symptoms for inhalation are fever, myalgia, dyspnoea and chest pain. As for ingestion, the usual gastrointestinal signs would be observed. With enough rest and water, it would promote recovery.

Increased awareness of its usage as BWA had raised attention from both the physicians as well as various agencies to combat any bioterrorist attack. For a fast response from them, we need an adequate and efficient detection technique, which we are now looking into with our research work.

1.3.2 Review of analytical methods for SEB detection

In the early days, there were three immunological assay formats that had been used for SEB detection, namely immunodiffusion assays, radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA). However due to its low sensitivity of 0.1 ug/ml

and long analysis time, immunodiffusion assay was not preferred. Though competitive RIA was more sensitive in detecting as low as 1 ng/ml and could be used for quantitative measurement, its limitations had been on the handling and disposal of radioactive compounds and also the low binding of the radioactive labeled toxin. Thus, ELISA has been the option for SEB detection. From classical ELISA, it transformed into a double-antibody sandwich system and detected through colorimetric[54] measurement. This sandwich system allowed relative quantitation from both culture media and food extract as well as from human urine.

A comparison study was done between monoclonal and polyclonal antibody system[56]. It showed that monoclonal antibody (Mab) system was more favored than polyclonal system. Advantages of Mab were that it provided unlimited supply of high grade reagents and adoption of cross-reactive MAbs. Despite the various improvements made in the technology of sandwich ELISA, its performance still depends heavily on the affinity and specificity of the selected antibody. Not only that, ELISA is time consuming for any analysis due to its long incubation and assay time. Reduction of analysis time was explored and a rapid and sensitive sandwich ELISA based on a highly avid anti-SEB antibody (polyclonal) [57] was developed. The avid anti-SEB antibody (polyclonal) served as the capture antibody and biotinylated antibody conjugate. By using glutaraldehyde fixation method, it concentrated the capture antibody as well as added the discrimination function of positive and negative controls. With addition of 1% polyethylene glycol, its detection sensitivity improved to 0.5 ng/ml and it could be done in 45 minutes.

A proposal of changing the antibody receptor to glycosphingolipid digalactosylceramide (diGalCer) [58] in sandwich ELISA would be able to prevent cross reactivity, which was unavoidable in classical ELISA. diGalCer was found in human kidney and proximal tubular cells which binded specifically to SEB. Since this receptor controlled the metabolic action of SEB, it could be formulated into a receptor-based immunoassay and would be of wide application due to its long shelf life. This analysis was fast, simple and sensitive to 1 ng/ml of SEB. It was comparable to existing methods in terms of sensitivity but most importantly, it was SEB specific and the results could be read with naked eye. Another approach towards the improvement of immunoassay had been the use of bidiffractive grating[59]. Detection was made through change in the refractive index and it was much faster than classical ELISA allowing multiplexed detection with lowest detection at 1 ng/ml.

With fluorescence labeling of antibody, known as fluorescence based immunoassay[60], the time of analysis would be faster by four times and yet would give sensitivity comparable to classical ELISA. . Other than introducing fluorophores into the antibody, we could also use an active electronic microchip to develop a microelectrophoresis assay[61]. This assay could be coupled with other analytical techniques such as CE and MS, hence improving the purity of sample for easier identification, as well as sensitivity. Its principle involved a combination of electrophoresis and immunoassay platform. The active electronic microchip consisted of a planar array of microelectrodes where the charged molecules transported to and from sites on the chip called microlocations. Multiplex detection was accomplished by having multiple individual assay sites present in a single microchip. The

charged molecules did not affect the solution on other parts of the array and also with the generation of an electric field, it increased the binding and improved the selectivity. Cross reactivity in classical ELISA could be resolved.

Based on the fluorescence technology, an array-based biosensor was developed that consisted of two recognition elements, which allowed simultaneous detection and quantification of multiple analytes that ranged from 4 to 20 ng/ml. The immobilized capture antibodies served as the first recognition element, followed by the recognition of the bounded analyte by fluorescent tracer antibodies. This array sensor utilized the evanescent wave where it excited the fluorescent immunocomplexes and quantified through CCD. Evanescent excitations were only sensitive to surface coated with fluorophores and thus it allowed real-time monitoring. The development of array biosensors started off with three bacterial samples and four toxin samples detection[62] followed by a nine-analyte detection[63] in automated format. This was done in 15-20 minutes. Other than its high speed, good sensitivity and multiplexed detection, this biosensor could be miniaturized for the ease of transport.

A novel fluidics cube was used in the development of a portable array biosensor[64]. This fluidics cube was constructed using poly (methyl methacrylate) where it contained six reservoirs for samples and six reservoirs for fluorescence tracer antibodies. One end was connected to a pump and the other end consisted of inlets connected to the sample and tracer. Once the air valve was relieved, the sample or tracers were loaded onto the waveguide surface and detected by CCD.

From the existing ELISA assays, it could be converted to a time-resolved fluorometry assay[65] through the use of lanthanide chelate labels, which have a unique fluorescence property. This assay had a sensitivity of 10 pg for SEB in human serum, urine, sewage water or dirt. There were other possible ways of coupling such as coupling to a silicon-based light-addressable potentiometric sensor (LAPS) [66]. Despite the fact that it gave a good sensitivity ranging from 3 to 310 pg/ml, the analysis would require an hour and the sensitivity was volume dependent.

To meet the demands of high sample throughput as well as high sensitivity, various developments had been made on the immunoassay such as immunomagnetic separation (IMS) with different detection modes. In IMS, magnetic beads coated with antibodies were used as the target antigen capturing probe. One mode of detection was through the use of a microplate fluorometer (FM) [67] in which the magnetic beads-coated antibody captured the target analyte for analysis. IMS in a microplate platform allowed efficient targets capture and served as a concentration step to give an enhanced sensitivity of 0.1 ng/ml. The advantages of this IMS-FM system are rapid analysis, direct analysis on crude sample, semiautomatic reduction of exposure of BWA to personnel, labor-saving and reduction of any non-specific false interference. IMS could be coupled to other detection modules such as flow cytometer [68, 69], allowing detection of 100 pg SEB in culture supernatant of methicillin-resistant *S. aureus* using Alex fluor 647 labeled antibodies, in less than 45 minutes. By coupling with electrochemiluminescence (ECL) [70], it provided detection by increasing its sensitivity through high luminescent signal to noise ratios that

was initiated in a controlled fashion by a voltage potential at the site of immune complex formation. This was accomplished by a heavy metal called chelate ruthenium (II) tris-bipyridal that conjugated to a detector antibody giving a high sensitivity of 1 pg/ml in serum, urine, tissue or buffer and was reproducible. This IMS-ECL assay was rapid (30 minutes), sensitive, reproducible and robust.

There are various forms of immunosensors immobilized on different platforms for examples on optical fiber[71], piezoelectric crystal[72, 73] and gold surface plasmon resonance (SPR) [74, 75, 76, 77]. The fiber optic biosensor was based on the sandwich immunoassay and quantified through optoelectronics, where a diode laser was utilized to excite the polystyrene optical probe. The emitted fluorescence would produce a signal, which is proportional to the amount of target antibody giving a detection limit of 5 ng/ml. The disadvantage of such system was the failure to give a full coating of sample and antibodies on the probes. Further research is needed to rectify this issue. In piezoelectric crystal immunosensor, it used piezoelectric crystal as the sensor. The disadvantage was the variation in the coating on the gold electrode of the crystal that would affect the signaling. The advantages are no labeling required and direct quantitation but the biggest drawback is its sensitivity that ranged from 0.1 ug/ml to 2.5 ug/ml.

In SPR sensor, the recognition antibodies were immobilized on the gold SPR by adsorption and were quantified through increase in refractive index. There are two classes of SPR sensors which are based on wavelength or angle interrogation. Wavelength interrogation implies using a fixed angle of incidence and monitors the

spectral variation. As for angle interrogation, it implies using fixed wavelength and traces the angle of reflectance. SPR sensors offer more advantages compared to the other detection methods. They allow continuous real-time monitoring of experiment, ease of removal of target analyte by low pH wash, require no consumption of reagents for direct detection, are small and compact in size for field trial and are extremely sensitive in sub-nanomolar level. With amplification, it would be in femtomolar level. A reported miniaturized wavelength based SPR sensor gave a sensitivity of 5 ng/ml for direct detection and 0.5 ng/ml with amplification in less than 10 minutes. More improvements were made such as having a dual channel SPR where the reference channel would compensate for the bulk refractive index, non-specific binding and temperature fluctuations.

All these developments had increased the potential of SPR sensors in biological and chemical agents monitoring. A recent finding for both BWA and CWA monitoring had been on the use of magnetoelastic sensor[78] where it used magnetoelastic material being immobilized onto antibodies. It was based on the change in sensor resonance frequency, which was caused by the mass change of the sensor when there was association between the analyte to the receptor immobilized on the surface of the ribbon-like magnetoelastic sensor. Both biocatalytic precipitation and biotin-avidin interaction had amplified the mass change and thus, enhanced the sensitivity to 0.5 ng/ml for an hour of incubation. The SEB sensitivity was comparable to optical and piezoelectric mass sensing except that for this magnetoelastic material, it had a very low cost and could be developed to have disposable properties. Majority of the determination methods for SEB had been based on

immunological technology. However, these methods are always dependent on the toxin amounts or concentration that could influence the sensitivity and specificity.

Alternative methods were explored and a sensitive and specific PCR-ELISA[79, 80] system was developed for simultaneous detection of enterotoxins giving a detection of 1 pg. This assay utilized the internal biotin-labeled oligonucleotides as the immobilization capture probes for capturing amplified toxin sequence and quantified using enzymatic amplification of colorimeter. The optimization of the PCR-ELISA was through the adjustment of probe concentration and hybridization time. There was no cross-reactivity and the SEB PCR-ELISA was more specific and sensitive than ELISA except that it was more time consuming.

We observe the same trend for development of immunoassay technology on both proteinaceous toxins, which are the tremendous efforts made to improve sensitivity, speed, multiplexed detection and portability of immunoassay method. However, the enormous development in analytical chemistry had opened the door to structure identification and confirmation of analyte with the use of mass spectrometry (MS) due to its molecular mass, specificity, sensitivity and ability to provide structural detail. Thus, there was increased interest in biological based chemical instrumental [81, 82, 83] analyses where it used a biomolecular recognition element like antibodies or SPR chip in conjugation with MS. This is not only specific and selective on the target analyte, it also allows identification with high mass resolution. MALDI-TOF and ESI-TOF MS are the two main areas which researchers are showed interest. It allowed analysis to be

completed in less than 1 hour and gave a detection limit of 1ng/ml. This could be adjusted to below 1 ng/ml by using a larger sample volume and a slower flow rate. The trend of analytical approach started in the early 1980s where there were reports on using high-performance liquid chromatography (HPLC) [84, 85] to separate and purify SEB. LC was then used in line with electrospray mass spectrometry [86, 87] known as LC-ESI MS. A comparison between micro-LC-ESI MS and normal mode LC-ESI MS was made and it had shown that with a smaller internal diameter, it allowed the sensitivity to increase by 30-40 folds. The determination of SEB was down to the level of 0.1-1 ng/ml. The combination of LC-ESI MS/MS allows accurate determination by the molecular mass and also by the amino acid sequencing after enzymatic digestion. In Table 2, we had summarized the lowest detection limit for various SEB methodologies. It showed that LC-MS's detection limit was comparable to ELISA. With these positive data, we do see that MS usage could be further extended for our SEB detection and quantitation study.

Table 2: summarized methods and LODs for SEB

	SEB methodologies	**Lowest level of detection**
1	Immunodiffusion assay	0.1 ug/ml[54,56]
2	Radioimmunoassay	1 ng/ml[54,56]
3	Enzyme-linked immunosorbent assay (ELISA)	0.5 ng/ml[57]
4	ELISA-glycosphingolipid digalactosylceramide	1 ng/ml[58]
5	ELISA-bidiffractive grating	1 ng/ml[59]
6	Fluorescence assay based lanthamide chelate label	10 pg/ml[65]
7	ELISA-LAPS	3-310 pg/ml[66]
8	Immunomagnetic separation (a) fluorometer (b) ECL	(a)0.01 ng/ml[67] (b)1 pg/ml[70]
9	Fiber optic-sandwich immuno assay	5 ng/ml[71]
10	Piezoelectric crystal immunosensor	0.1 ug/ml-2.5 ug/ml[72,73]
11	Surface plasmon resonance	0.5 ng/ml-5 ng/ml[74-77]
12	Magnetoelastic sensor	0.5 ng/ml[78]
13	ELISA-PCR	1 pg[79,80]
14	LC-MS	0.1 ng/ml-1 ng/ml[86,87]

1.4 Trichothecene mycotoxins (T-2 toxin)

1.4.1 Its structure, its properties & mechanism of action

Trichothecenes are a family of closely related sesquiterpenoids. Most of them have a double bond at position C-9, 10, a 12-13-epoxide ring, and a variable number of hydroxyl and acetoxy groups. In Figure 3 is the structure of T-2 toxin. This group of mycotoxin is mainly produced by various species of *Fusarium* fungi. They are non-volatile with a low molecular weight. They are relatively insoluble in water but soluble in organic solvents like acetone, ethanol, chloroform, methanol and ethyl acetate. They are ringed non-protein compounds.

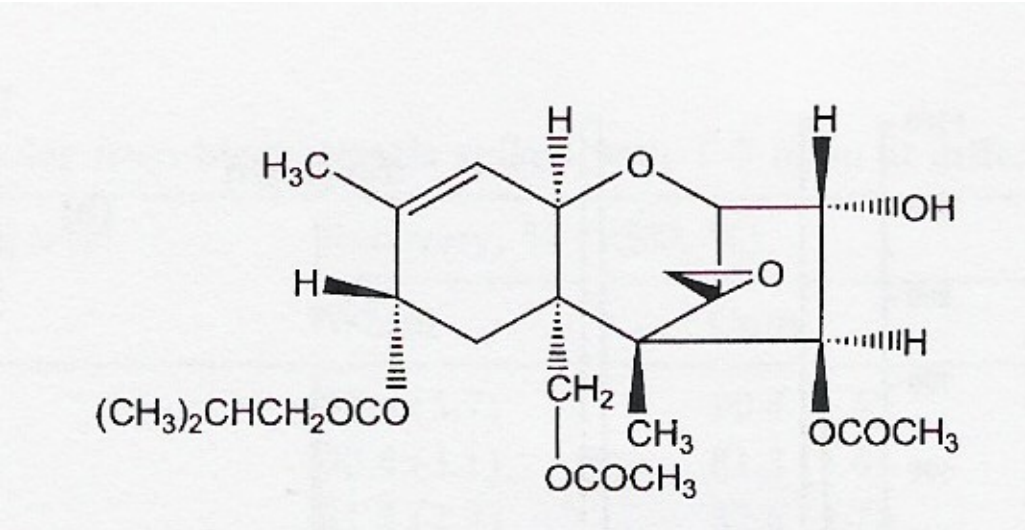


Figure 3: Structure of T-2 toxin.
Source from Journal of Chromatography A, 989 (2003), 257-264

Trichothecenes are subdivided into four different groups. Type B-trichothecenes differ from type A by the presence of α, β-unsaturated carbonyl groups. Type C are characterized by an additional epoxide and type D are macrocyclic trichothecenes.

Trichothecene mycotoxins can be disseminated in the form of dusts, droplets, aerosols, or smoke from aircraft, rockets, missiles, and artillery, mines or portable sprayers. Because of its anti-personnel properties, ease of large-scale production and apparent proven delivery by various aerial dispersal systems, the trichothecene mycotoxins (especially T-2 toxin) have excellent potential for weaponization. T-2 toxin is heat stable and not easily decontaminated by autoclaving. However, by using 5% solution of sodium hypochlorite, it could be effectively decontaminated.

T-2 mycotoxin is a type A trichothecence with a molecular weight of 466 Da. It acts primarily by inhibiting protein synthesis through binding to the ribosomal RNA, which results in disruption of cell membranes. It also inhibits either the initiation or the elongation process of translation, by interfering with peptidyl transferase activity, and can inhibit electron transport activity. T-2 toxin is about 400 times more potent than mustard during dermal exposure. The clinical symptoms[2, 88] are dose dependent, but include skin irritation and burning sensations within hours following high dermal exposure. High inhalation exposure causes vomiting, dizziness, rapid heartbeat and chest pain within an hour. Once the eyes are exposed to T-2 toxin, the response ranges from tearing to irreversible cornea damage, depending on the dosage. As for oral ingestion, sore throat, bleeding gums, abdominal pain and bloody vomit may occur that can lead to death. However, the major toxic effect from T-2 toxin as an incapacitant arises from dermal exposure. Treatment such as washing in soap and water within 3 hours effectively removes the toxin from the skin, following which calamine lotion is applied. An effective analytical method for rapid detection of T-2 toxin is essential.

1.4.2 Review of analytical methods for T-2 toxin detection

The classification of trichothecene analysis can be divided into two categories[89], namely the screening techniques such as thin layer chromatography (TLC) and ELISA or analytical techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC). The first method developed for the determination of T-2 toxin was based on thin layer chromatography (TLC) [90, 91, 92]. Basically, TLC operated in an offline mode and was suitable for crude sample analysis. Its advantages are adjustable detection limit simply by the use of larger sample volume and capability to detect as low as 0.005 ug/kg by using fluorescence or absorbance densitometer. Although TLC was a simple, robust and inexpensive technique, it lacked in separating power and was also unable to discriminate any possible co-extracted interference from the toxins of interest[93]. TLC is useful only as a screening test due to its high variance.

Different immunochemicals methods especially enzyme-linked immunosorbent assay (ELISA) systems[94] had also been established for T-2 toxin. For ELISA using either monoclonal or polyclonal antibodies, it was less precise than TLC. However, ELISA method gave a higher recovery, allowed more rapid analysis and was less laborious to handle compared to TLC. Improvement was made in ELISA by the use of labeled sandwich immunoassay[95] with fluorogenic-chemiluminescence (FCL) and electro-chemiluminescence (ECL) detection. The former assay reaction was determined by measuring the fluoroscence and the latter was assessed through photon emission. Even with this increased sensitivity, ELISA method has not been validated at sufficiently low

levels and also it is time-consuming since incubation period is required. The invention of membrane based immuno-filtration[96] assay for detection of T-2 toxin would speed up the analysis time taken. It uses a competitive format, which involves three layers. The first layer was a membrane layer that consisted of 36 antibody spots, the second layer was an absorbent layer and the third layer was polyethylene card. This setup allowed simultaneous analysis of different analytes and also an on-site detection by densitometer. It gave a detection of 12.5 and 25 ug/kg in wheat and poultry feed respectively in 40 minutes. With monoclonal immunoassay[97], it was able to achieve a low detection limit of 30 ng/g T-2 toxin, which was comparable to GC analysis. But the advancement on T-2 toxin detection was still on the use of GC analysis where the majority of the published papers for T-2 toxin had been on GC instrumentation.

There were two review papers [98, 99] that focused on the chemical instrumental methods for T-2 toxin. The most frequently used method was GC analysis and had been established on flame ionisation detection (FID) [100] giving a limit of quantification of 75 ug/kg, electron capture detection (ECD)[101, 102] about 40 ug/kg and mass spectrometry (MS) [103, 104, 105, 106, 107, 108] approximately 5 ng/g. GC-MS is the method that is most widely employed today since it allows simultaneous analysis of several trichothecences, capability to identify them based on their spectrum and high sensitivity in ng/g.. However almost all GC methods required derivatisation of hydroxyl groups forming trimethylsilyl (TMS), trifluoroacetyl (TFA), pentafluoropropionyl (PFP) or hepta-fluorobutyryl (HFB) derivatives in order to attain the volatility and sensitivity required for detection. And the

required derivatisation reagent was dependent on type of trichothecene involved and also what detection method was employed.

The problem with derivatized trichothecenes was that only the MS spectra of the common trichothecenes were published, although special libraries had been established. GC-MS could have incomplete derivatisation reaction and removal of water prior to derivatisation was essential. Although GC-ECD had less daily variation, the matrix effect and the problem of derivatisation were similar to GC-MS. For identification of samples, GC-MS was run in scan modes as for quantitative purpose, the GC had to be run in selected ion monitoring (SIM) mode. Some non-derivatised GC methods[109] had been explored by using on-column injection technique in conjunction with GC-MS. This was able to give a quantitative detection limit of 0.1-0.5 ug/g but the problem was that due to repeated injections, there could be contaminations from the non-volatile samples on top of the column. Alternative form without any chemical derivatisation was by using solid phase microextraction (SPME) coupled with GC-FID[110] giving detection at 10 ng/ml. This SPME principle works on a principle of aqueous and liquid phases coated on the fiber. Once immersed in sample, the sample would be absorbed and when this fiber was exposed in GC, it desorbed and analysis was done.

Although GC-MS has an important role in the confirmatory determination, scientists have diverted their attention to the use of HPLC since it is less hassle to handle and no derivatisation is required. High performance liquid chromatography (HPLC) had been used by coupling with various detectors such as LC-refractive index (LC-RI) [111, 112] and

UV [113, 114] for T-2 toxin study. But LC-UV detection was not suitable for T-2 toxin detection, as it did not contain a carbonyl function at C8 conjugation with a double bond in C9-10 so it was not for UV absorption[115]. Alternatively, fluorescent labeling reagents could be added to T-2 toxin where it could be determined through HPLC-fluorescence (F) [116] detection giving a low detection limit of 0.005 ug/ml. Immunoaffinity column was used to extract T-2 toxin, followed by pre-column derivatisation with 1-Anthroylnitrile and then detection through HPLC-F.

The trend of HPLC is moving towards MS[117, 118, 119, 120, 121] both on qualitative and quantitative works. In HPLC-MS analysis, no derivatization is required and this reduces the sample preparation time substantially. Problems arise from GC methods such as inability to obtain straight calibration curves, memory effects and matrix interferences are being resolved. With the use of selected ion monitoring (SIM) mode for HPLC, it is a quick and sensitive method as the background noise is reduced. Given the MS spectrum from LC-MS, it could confirm the analyte of interest. Based on a recent paper of HPLC-ESI-MS on T-2 toxin, it was able to give a detection limit of 5 ng/g[122]. This detection limit was comparable to GC-MS. Thus, given the various advantages of LC-MS such as no derivatisation required, good sensitivity, confirmatory identification and quantitation, there seem to be no reasons why it should not be in demand for future works. Other techniques being used for T-2 determination was supercritical fluid chromatography (SFC). But this technique of SFC was suitable only to compounds with low volatility and high polarity. Again LC-MS/MS compare to all other methods has shown to be superior due its rapid analysis time, high specificity giving structural confirmation as well as good

sensitivity and no derivatisation. In Table 3, we summarized the lowest level of detection for T-2 methodologies where LC-MS showed comparable LOD as to the common GC methods. Thus, in our study, we used LC-MS for relative quantitation of T-2 toxin for our time profile study.

Table 3: summarized methods and LODs for T-2 toxin

	T-2 methodologies	**Lowest level of detection**
1	TLC	0.005 ug/kg[90-92]
2	ELISA	12.5-25 ug/kg[96]
3	Monoclonal immunoassay	30 ng/g[97]
4	GC-FID	75 ug/kg[100]
5	GC-ECD	40 ug/kg-200 ug/kg[101,102]
6	GC-MS	~5 ng/g[103-108]
7	Non-derivated GC-MS (on-column injection technique)	0.1-0.5 ug/g[109]
8	SPME GC-FID	10 mg/ml[110]
9	HPLC-fluorescence detection	0.005 ug/g[116]
10	LC-MS	5 ng/g[122]

1.5 AIMS & OBJECTIVES

In this project, the stability profiles on carpet and parquet surfaces of three potential BWA, ricin, *staphylococcal enterotoxin B* (SEB) and trichothecene mycotoxin (T-2 toxin), have been investigated over 7 days under indoor office conditions of air temperature 22.5-25.5^{0}C and relative humidity <70%. This condition is referenced to National Environment Agency's acceptable indoor air quality. A suitable analysis method has been developed and tested for each toxin.

For SEB and ricin, the feasibility of using amine specific isobaric tagging as well as the use of nano-flow liquid chromatography-mass spectrometry would be determined since this amine labeling has not yet been reported before for both toxins. This amine derived peptides will then be analyzed by nano-flow LC-MS/MS. For T-2 toxin, we have chosen HPLC-ESI-MS system since no derivatization is required as compared to GC methods and much less cumbersome in sample preparation.

CHAPTER 2 MATERIALS AND METHODS

2.1 SUPPLY OF T-2 TOXIN, SEB AND RICIN

T-2 toxin from *Fusarium* SP (product number T4887-5 mg) was purchased from Sigma-Aldrich. SEB (Cat.number BT202, 10 mg) was purchased from Toxin Technology, Inc. Ricin was extracted and purified in-house at DSO National Laboratories.

2.2 CHEMICALS AND MATERIALS

Acetonitrile and water used were HPLC grade from TEDIA company, Inc. 50% Formic acid/50% water was from Fluka, HPLC grade. iTRAQ reagents kit was from Applied Biosystem. Trypsin enzyme was from Sigma-Aldrich. The concentrating tubes were Millipore Amicon Ultra-15 with molecular cutoff of 5 kDa and 10 kDa.

2.3 USAGE OF AMINE SPECIFIC ISOBARIC TAGGING, ITRAQ™ REAGENTS[52, 53] IN OUR STUDY

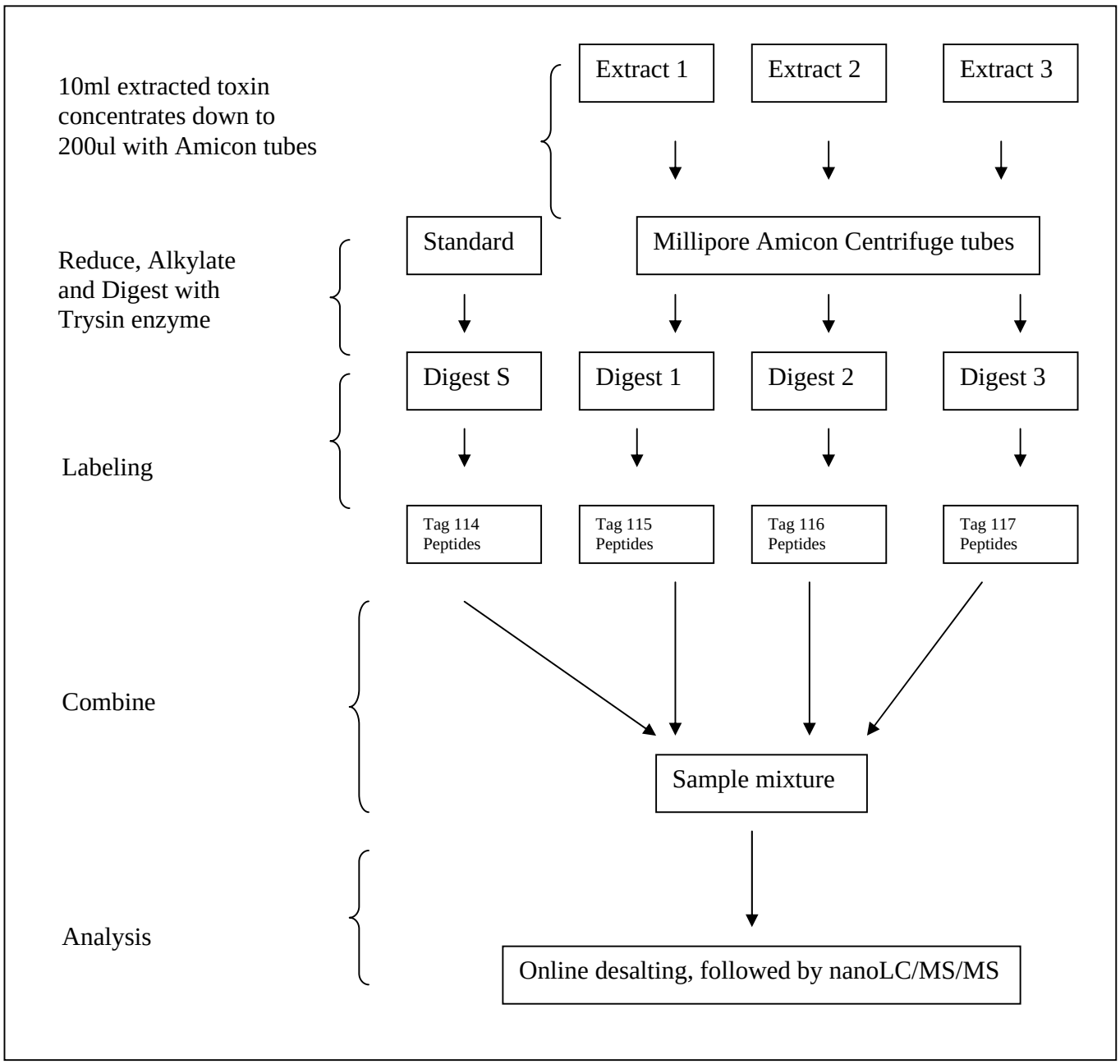
Based on our literature search on the detection methods as well as the detection limits for both ricin and SEB, we see a potential use of LC-MS/MS as well as stable isotope labeling technology for our toxin analysis. In our study, we used iTRAQ reagents as label on the peptides of the extracted samples for quantitation purpose. With the rapid evolution of stable isotope tagging reagents being made available like iTRAQ, ICAT and

SILAC, they provide additional advantages such as elimination of sample to sample recovery difference and also allow multiple samples detection. The isotope tagging methodology that we used in this research study was iTRAQ^{TM} reagents (from the Applied Biosystems Inc, CA). This novel multiplexed set of four unique isobaric tagging reagents was recently introduced in mid-2004 for quantitative protein analysis. iTRAQ reagents are amine-specific labeling reagents that allows multiplexed relative and absolute protein quantitation via MS/MS, other than identification of protein.

With a single multiplex kit, one can perform any combination of experiments based on the nature of the desired project. It can be used for a minimum of two set of experiments or up to a maximum of four set of experiments in a single analysis run. This is highly economical since running multiple samples in a single experiment can significantly reduce operating costs as well as making it less laborious. Yet, precision and accuracy are not compromised since all the processes are performed in parallel workflows as in Figure 4. Our sample preparation is in order of the schematic diagram shown in Figure 4. Extract 1, 2 and 3 represent the sample extracts from the matrices. Standard represents the control which has the identical amount of spiked toxin.

iTRAQ reagents consist of a reporter and a balancer group as shown in Figure 5. The iTRAQ methodology works on the isobaric mass labels of 114, 115, 116 and 117 being tagged onto the N termini and lysine side chains of peptides in a digest mixture.

Figure 4: Our modified iTRAQ protocol



The reporter group provides quantitation by producing MS/MS signature ions at m/z 114, 115, 116 and 117, following collision-induced dissociation. The individual ratio of the

identified peptides from the signature ion peak areas using ProQuant software (from Applied Biosystems) would allow identification and quantification of sample even if the amine derivatized peptides are isobaric and chromatographically indistinguishable. Absolute quantitation of targeted proteins can be achieved by using internal standard as one of the mass labels.

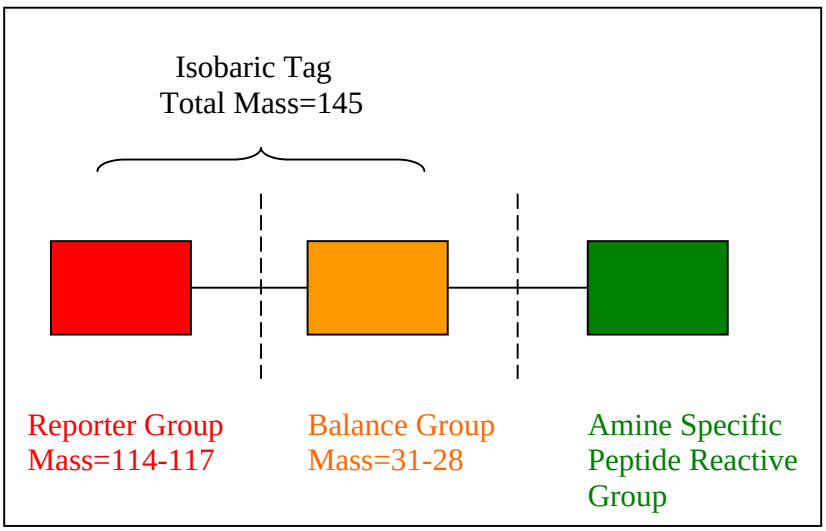


Figure 5: iTRAQ reagent's structure

(Source from Applied Biosystem's iTRAQ reagents chemistry reference)

With the use of iTRAQ reagents, the MS/MS spectra obtained allows enhanced sensitivity and increased confidence as well as the peptide coverage. The multiplex tagging techniques makes analysis of up to four different biological samples simultaneously possible and also remove any quantitative variation that occurs between sample to sample. Thus, our research study would discuss the feasibility of using iTRAQ

reagents and chemical instrumentation of nano-flow LC-MS/MS for quantitative study of BWA, SEB and ricin.

2.4 METHOD FOR RICIN

2.4.1 Nano-flow LC-MS/MS system for ricin and SEB

For both ricin and SEB study, we used the same nano-flow LC-MS/MS system. Nano-flow liquid chromatography was from Agilent 1100 series and QSTAR® XL MS/MS system was from Applied Biosystem as shown in Figure 6. The nanoLC column was self packed by using LUNA 3 μm C18 (2) 100 angstrom packing material into New Objective's PicoFrit™ self /p column of OD 360 μm, ID 75 μm with tip of 15 μm. The desalt column used was Zorbax 300SB C18, 5 μm, 5 x 0.3 mm.

Figure 6: Agilent nano-flow LC-Qstar XL MS/MS system

2.4.2 Mass Spectrometry for toxins

In our study, we used two kinds of mass spectrometers. In nano-flow LC MS/MS system (i.e. ricin and SEB studies), it involved nano-flow LC-nanosprayESI-QTOF mass spectrometer and in LC-MS system (i.e. T-2 toxin study), it involved LC-ESI-ion trap mass spectrometer. Despite that both are LC-MS methods, their representative data were different. Here, we took a selected mass spectrum from the ricin's experiment as the representative set to show how we obtain our final data in terms of the ratio of extract:control using the ProQuant software for identification and quantitation. The first data obtained from iTRAQ labeled sample injection was a total ion chromatography (TIC) as shown in Window A of Figure 8. This TIC was then sent to mass spectrometer to obtain the TOF-MS scan in Window B, which was a scan of all the mass range of the peptides found in TIC. From TOF-MS, the two most intense peaks would undergo TOF-MS/MS to obtain the product ion as shown in Window C and D of Figure 8.

In Window A, the TIC showed various small peaks. At each peak, it contained many peptides. This chromatogram obtained was different from the usual LC-MS spectrum as in T-2 toxin study using LC-MS, where one peak represented the identity of T-2 toxin. In normal LC-MS, each peak must have a good resolution touching the baseline in order to have a good quantitation (i.e. area under the curve) of T-2 toxin as shown in Figure 7. But for our iTRAQ labeled extracts, our focus would be on the 114, 115, 116 and 117 mass label peaks showed in MS/MS spectra in Window C and D. Basically, in TIC, it was a one dimensional LC separation for the peptides to undergo separation using the gradient

elution but it would not have a single peak representing one peptide as there were multiple peptides that could elute at the same time. Instead, a TOF-MS was obtained based on the TIC, followed by TOF-MS/MS for us to identify the iTRAQ labeled peaks at 114, 115, 116 and 117 of each identified peptides.

Figure 7: Normal LC-MS chromatogram of T-2 toxin using LC-MS

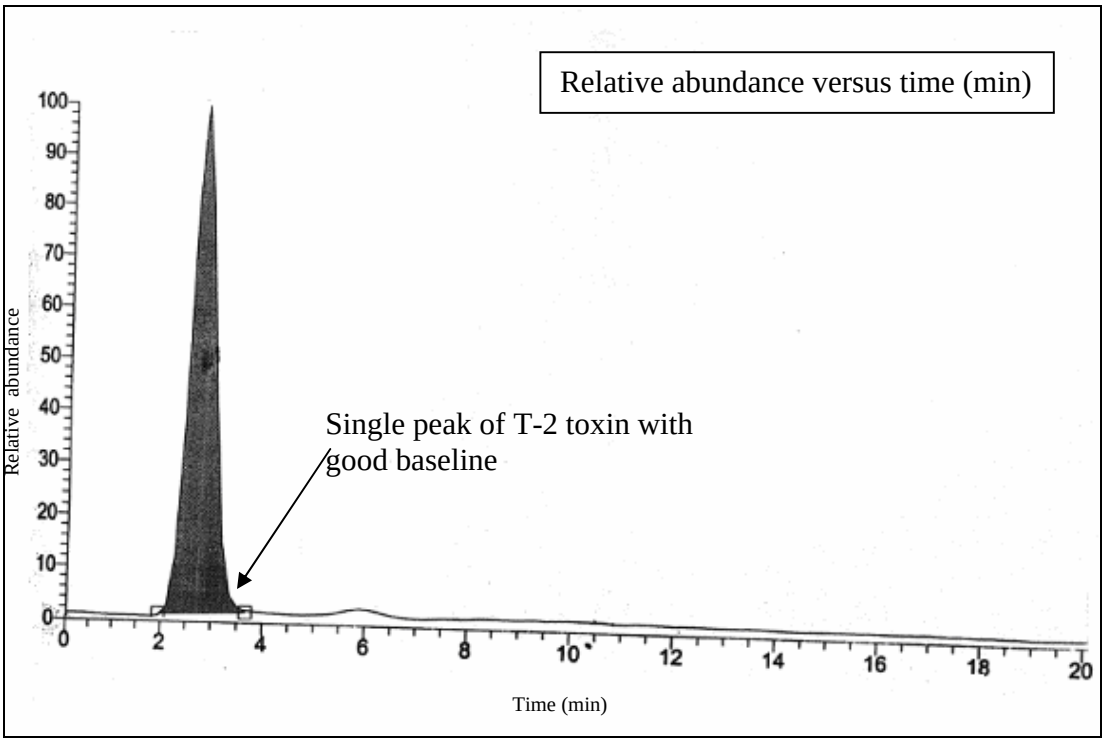
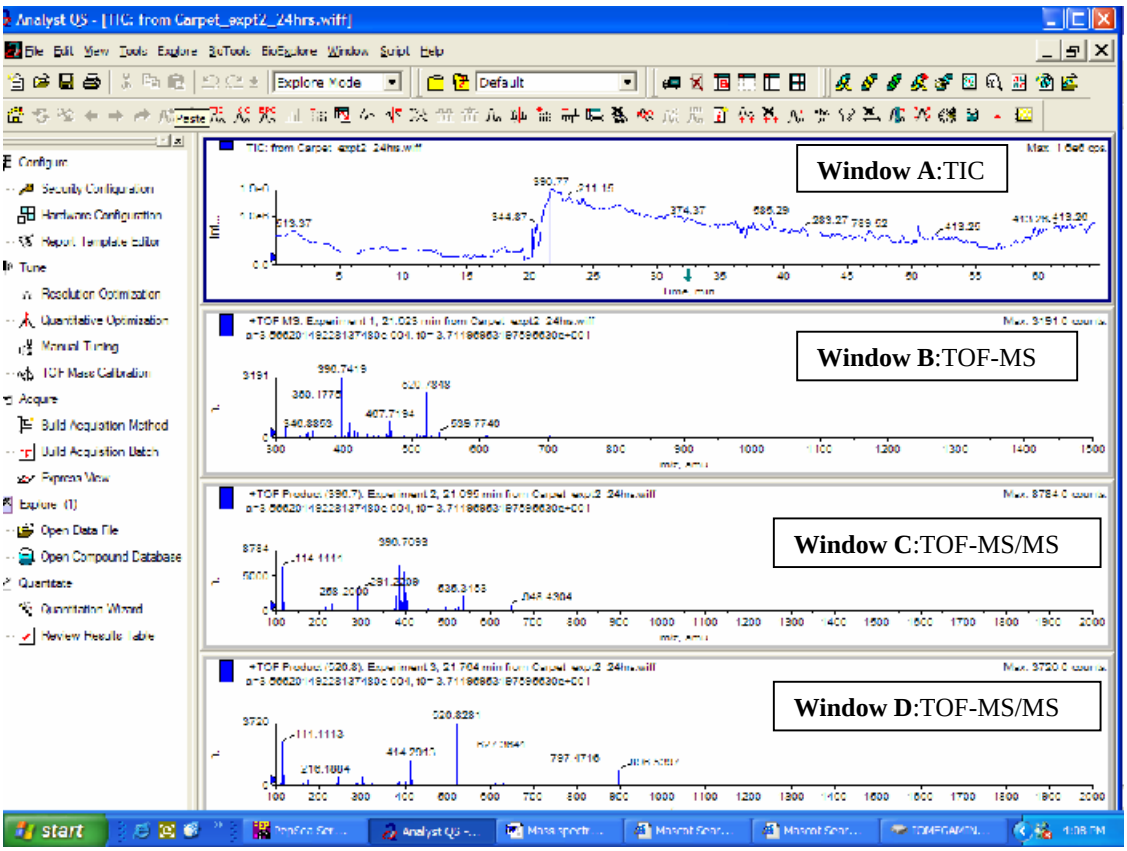


Figure 8: The data representative of the iTRAQ labeled extracts using nano-flow LC-MS/MS



Using Mascot search as shown in Figure 9, the acquired MS/MS spectra in Window C and D was sent for identification. The database used was MSDB where it contained 1019653 sequences and 321672149 residues. The enzyme selection was trypsin with no fixed modification but with variable modification set to MMTS, iTRAQ_Lys(K), iTRAQ_Tyr(Y) and N-term_iTRAQ. The peptide tolerance was set at ±0.2 Da, MS/MS tolerance at ±0.5 Da and peptides charge between +2 and +3. This search would take into consideration of iTRAQ being labeled onto peptides. The mascot result showed in Table 4 had identified ricin with Mass: 64050, Total score: 782 and Peptides matched: 34. It

showed a hit list of peptides belonging to ricin. We made comparison with the results obtained from ProQUANT 1.0 software at a particular product ion of 520.8. ProQUANT search would be used for identification and quantitation for the rest of this study and thus the confidence level and accuracy of its function must be tested. By clicking at the mascot result of 520.8 in Table 4, it showed that both b and y ions had good hit in Figure 10 and Table 5.

Figure 9: Mascot search screen

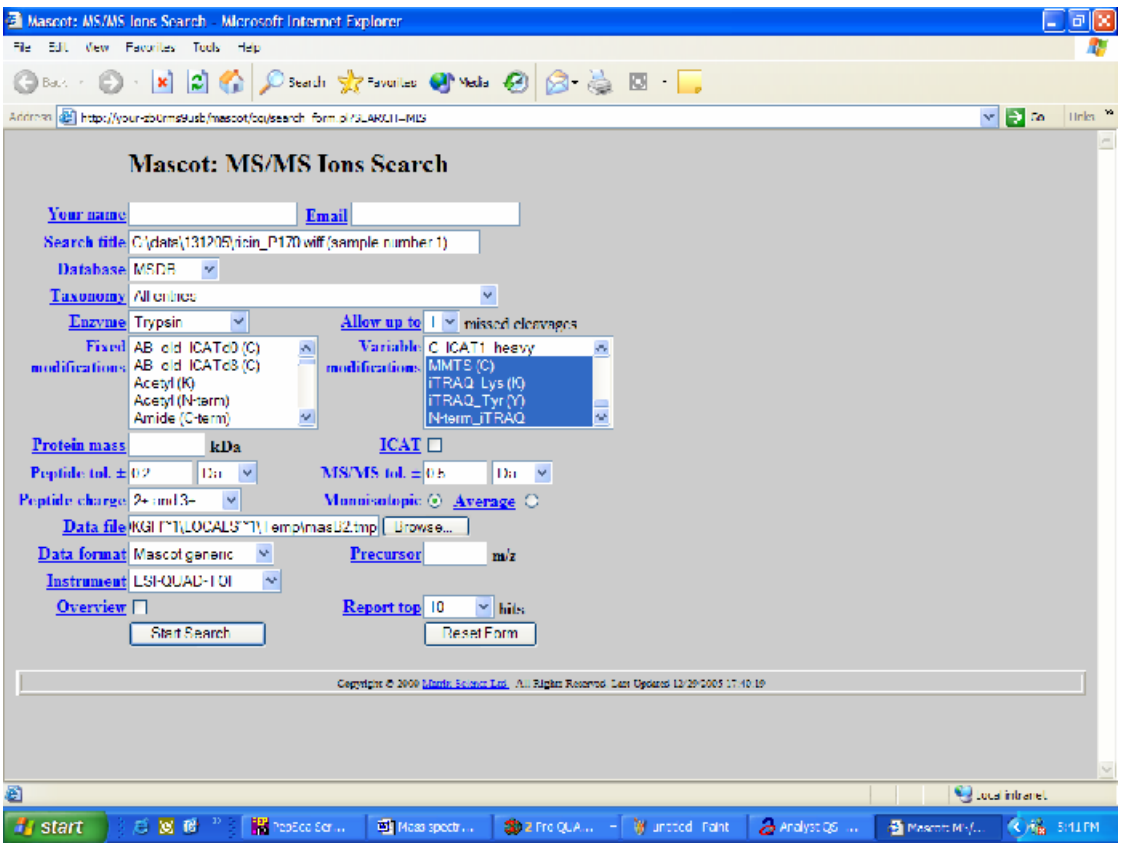


Table 4: Mascot Results of ricin extracted from parquet at 170hours

Significant hits:

RLCSD	ricin D precursor - castor bean
Q41174	Proricin A chain (EC 3.2.2.22) (rRNA N-glycosidase) (Fragment).- Ricinus communis (Castor bean).
1BR5A	ricin (EC 3.2.2.22) - castor bean
AAB22584	S40368 NID: - Ricinus communis

Product ion 520.8

1. **RLCSD** **Mass: 64050 Total score: 782 Peptides matched:34**
ricin D precursor - castor bean

Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
159	450.24	898.46	898.50	-0.04	0	22	1	WMFK + iTRAQ_Lys (K); N-term_iTRAQ
234	520.80	1039.59	1039.63	-0.04	0	46	1	VGLPINQR + N-term_iTRAQ
235	520.80	1039.59	1039.63	-0.03	0	(12)	1	VGLPINQR + N-term_iTRAQ
237	520.81	1039.60	1039.63	-0.03	0	(28)	1	VGLPINQR + N-term_iTRAQ
282	406.89	1217.65	1217.70	-0.05	0	(22)	1	HEIPVLPNR + N-term_iTRAQ
283	406.89	1217.66	1217.70	-0.04	0	35	1	HEIPVLPNR + N-term_iTRAQ
284	406.89	1217.66	1217.70	-0.04	0	(19)	1	HEIPVLPNR + N-term_iTRAQ
285	406.90	1217.67	1217.70	-0.03	0	(26)	1	HEIPVLPNR + N-term_iTRAQ
305	683.32	1364.63	1364.66	-0.04	0	25	1	ILSCGPASSGQR + MMTS (C); N-term_iTRAQ
306	683.32	1364.63	1364.66	-0.03	0	(13)	3	ILSCGPASSGQR + MMTS (C); N-term_iTRAQ
321	487.57	1459.68	1459.74	-0.06	0	28	1	FQYIEGEMR + iTRAQ_Tyr (Y); N-term_iTRAQ
322	487.57	1459.70	1459.74	-0.04	0	(22)	1	FQYIEGEMR + iTRAQ_Tyr (Y); N-term_iTRAQ

☑	348	814.38	1626.74	1626.79	-0.06	0	77	1	SFIICIQMISEAAR + MMTS (C)
☑	359	591.29	1770.84	1770.89	-0.05	0	(77)	1	SFIICIQMISEAAR + MMTS (C); N-term_iTRAQ
☑	360	591.29	1770.85	1770.89	-0.04	0	(34)	1	SFIICIQMISEAAR + MMTS (C); N-term_iTRAQ
☑	367	936.94	1871.87	1871.95	-0.08	0	123	1	SAPDPSVITLENSWGR + N-term_iTRAQ
☑	368	624.97	1871.88	1871.95	-0.07	0	(57)	1	SAPDPSVITLENSWGR + N-term_iTRAQ
☑	369	624.97	1871.90	1871.95	-0.05	0	(56)	1	SAPDPSVITLENSWGR + N-term_iTRAQ
☑	370	624.97	1871.90	1871.95	-0.05	0	(66)	1	SAPDPSVITLENSWGR + N-term_iTRAQ
☑	376	669.35	2005.02	2005.10	-0.08	0	86	1	NDGTILNLYSGLVLDVR + N-term_iTRAQ
☑	377	669.36	2005.05	2005.10	-0.05	0	(17)	1	NDGTILNLYSGLVLDVR + N-term_iTRAQ
☑	378	669.37	2005.08	2005.10	-0.02	0	(27)	1	NDGTILNLYSGLVLDVR + N-term_iTRAQ
☑	391	786.08	2355.23	2355.34	-0.11	0	79	1	FSVYDVSILIPIIALMVYR + N-term_iTRAQ
☑	394	801.74	2402.19	2402.27	-0.08	0	(85)	1	LSTAIQESNQGAFASPIQLQR + N-term_iTRAQ
☑	395	801.74	2402.19	2402.27	-0.08	0	(69)	1	LSTAIQESNQGAFASPIQLQR + N-term_iTRAQ
☑	396	801.74	2402.19	2402.27	-0.07	0	(62)	1	LSTAIQESNQGAFASPIQLQR + N-term_iTRAQ
☑	397	801.74	2402.19	2402.27	-0.07	0	89	1	LSTAIQESNQGAFASPIQLQR + N-term_iTRAQ
☑	398	807.76	2420.25	2420.34	-0.09	0	(47)	1	QIILYPLHGDPNQIWLPLF + N-term_iTRAQ
☑	399	807.76	2420.27	2420.34	-0.07	0	(44)	1	QIILYPLHGDPNQIWLPLF + N-term_iTRAQ
☑	400	807.76	2420.27	2420.34	-0.07	0	59	1	QIILYPLHGDPNQIWLPLF + N-term_iTRAQ
☑	401	817.06	2448.14	2448.23	-0.09	1	(31)	1	YTFAFGGNYDRLEQLAGNLR + N-term_iTRAQ
☑	402	817.06	2448.15	2448.23	-0.08	1	52	1	YTFAFGGNYDRLEQLAGNLR + N-term_iTRAQ
☑	404	855.79	2564.34	2564.44	-0.10	0	(50)	1	QIILYPLHGDPNQIWLPLF + iTRAQ_Tyr (Y); N-term_iTRAQ
☑	405	914.81	2741.40	2741.48	-0.09	1	71	1	WMFKNDGTILNLYSGLVLDVR + iTRAQ_Lys (K); N-term_iTRAQ

Figure 10: Mass ranges for b and y ions based on Mascot search of product ion, 520.8

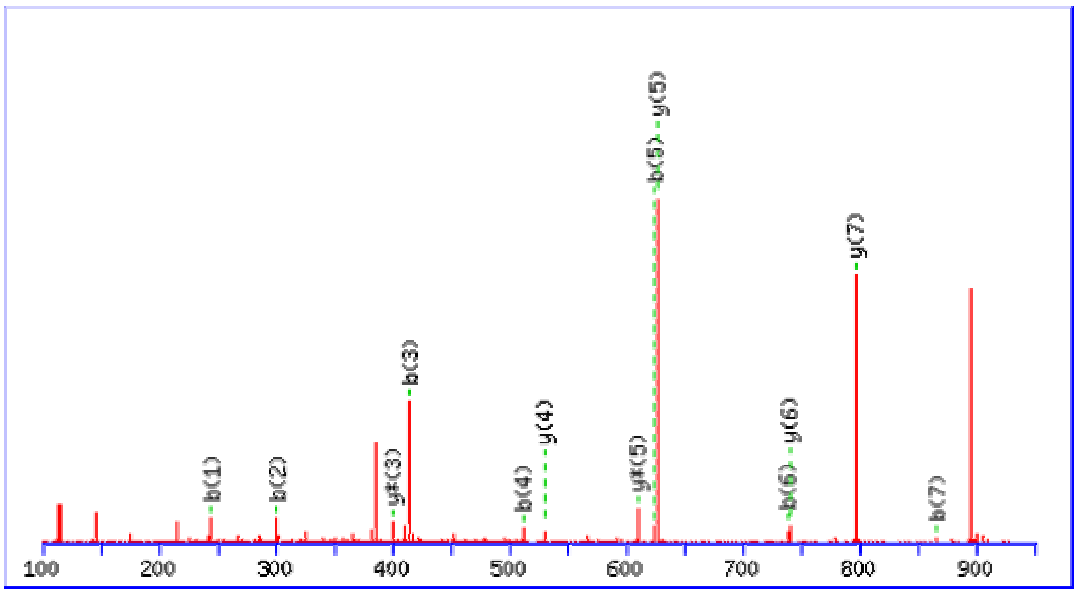


Table 5: Showed good hit for b and y ions based on Mascot search of product ion, 520.8

#	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	244.18	122.59			V					8
2	301.20	151.10			G	797.46	399.24	780.44	390.72	7
3	414.28	207.65			L	740.44	370.72	723.42	362.21	6
4	511.34	256.17			P	627.36	314.18	610.33	305.67	5
5	624.42	312.71			I	530.31	265.66	513.28	257.14	4
6	738.46	369.74	721.44	361.22	N	417.22	209.11	400.19	200.60	3
7	866.52	433.77	849.50	425.25	Q	303.18	152.09	286.15	143.58	2
8					R	175.12	88.06	158.09	79.55	1

Red implied that all the peptides found from extract met the same peptides' list as b and y ions of ricin.

ProQUANT 1.0 software was set with confidence setting at 90, tolerance setting for peptide identification at 0.3 Da for MS and 0.6 Da for MS/MS, and charge ranging from +2 to +4. The ProQUANT search screen was shown in Figure 11. The same data in Figure 8 was sent to ProQUANT search and the result identified ricin with 99 confidence, 37 peptides and a good hit of b and y ions as well for the product ion 520.8 of sequence VGLPINQR in Figure 12 and Table 6. This showed that the identification made through using ProQUANT software gave the same level of accuracy and confidence as to Mascot. ProQUANT software was used for the rest of the experiment for both the identification and quantitation purpose. The identification was based on the matched amino sequences and the quantitation was based on the area under the curve of the iTRAQ labeled peptide peaks as shown in Figure 13.

ProQUANT data analysis sequence

1. Send same MS/MS data to ProQuant – click process data

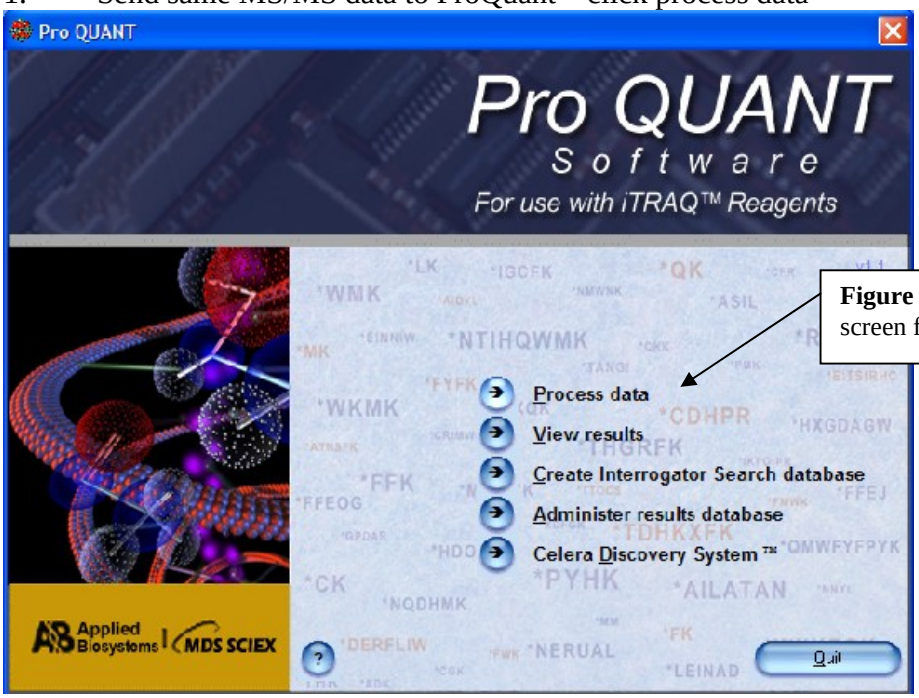


Figure 11: ProQUANT software screen for iTRAQ reagents

2. Results from ProQUANT software

It identified Ricin under the column name with 99 confidence and 37 peptides. This identification was based on the matched amino sequences. The average ratio of extract:control was shown as Avg 115:114 or Avg 116:114 or Avg 117:114. This was used for quantitation of the remaining toxin from the matrices. Using the same sequence of VGLPINQR from the product ion 520.8, it showed an equivalent good hit of b and y ions comparable to Mascot search.

Figure 12: Results from ProQUANT search

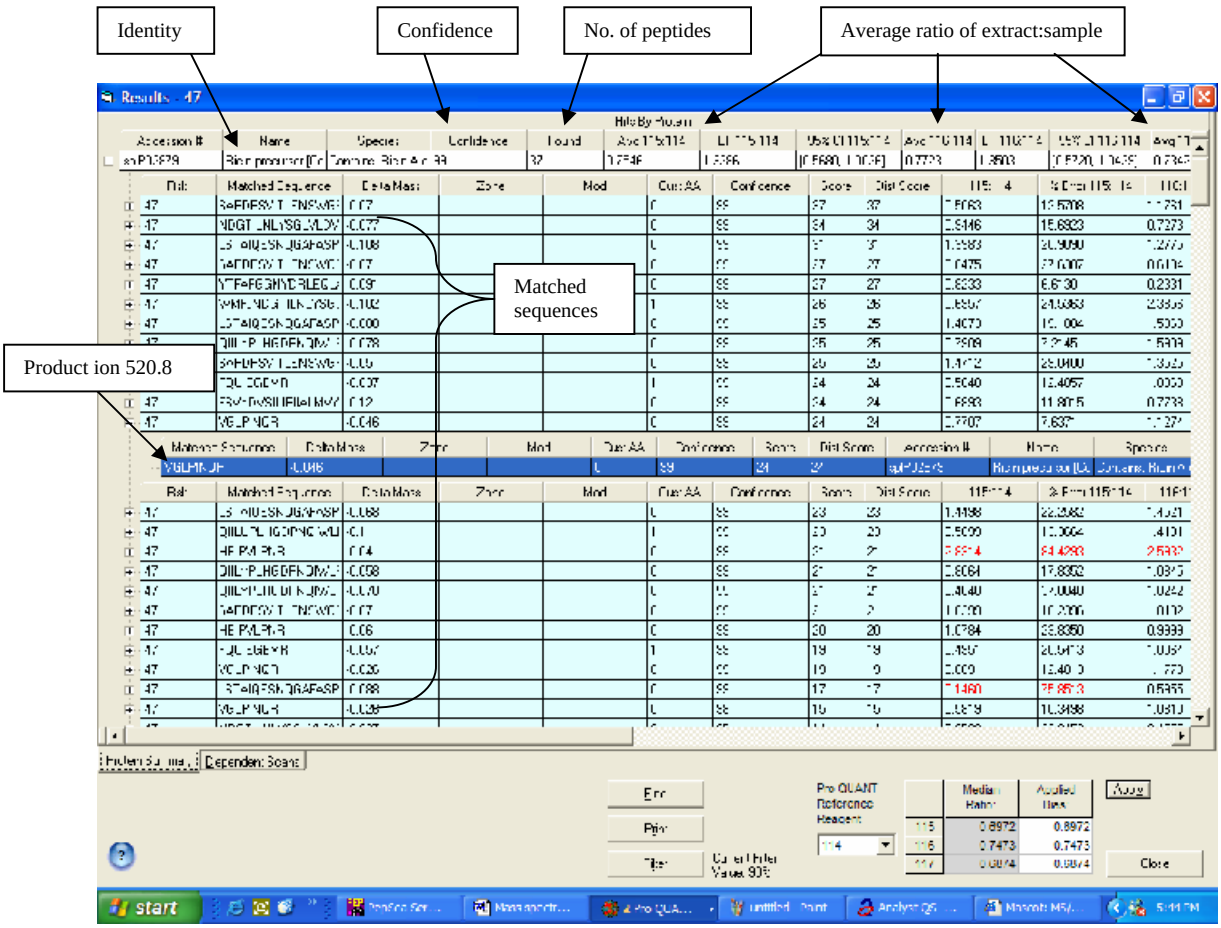
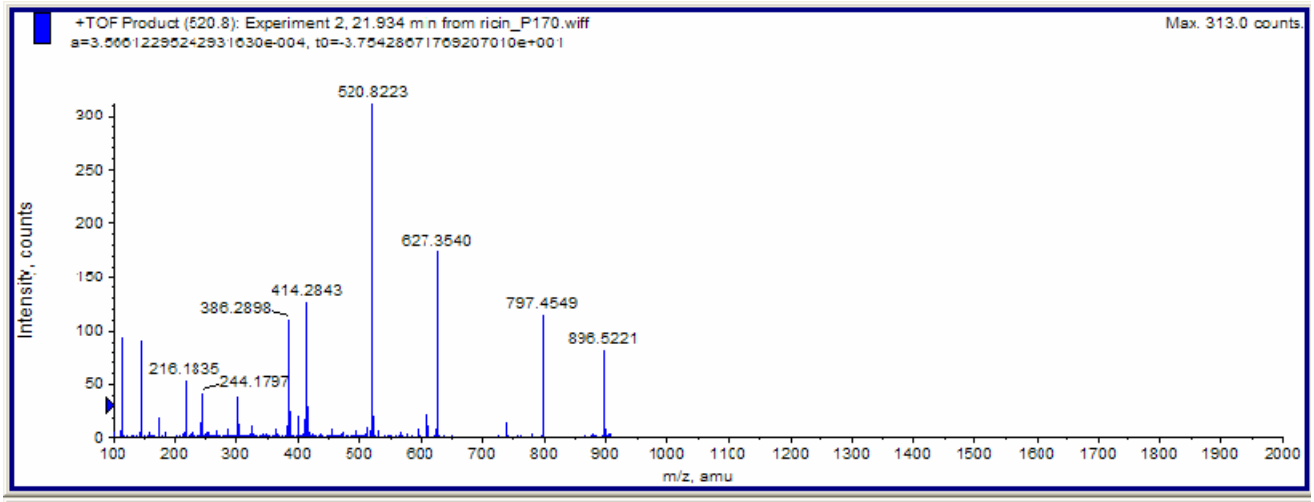


Table 6: Hit list of **b** and **y** ions using ProQUANT on product ion, 520.8



Controls

Reset to Default... | Set as Default | Sequence | Tag

Sequences and Tags +TOF Product (520.8): Experiment 2, 21.934 min from ricin_P170.wiff

Fragments [Precursor MW = 1039.5869, Charge = 2]

30 of 56 matched - 54%

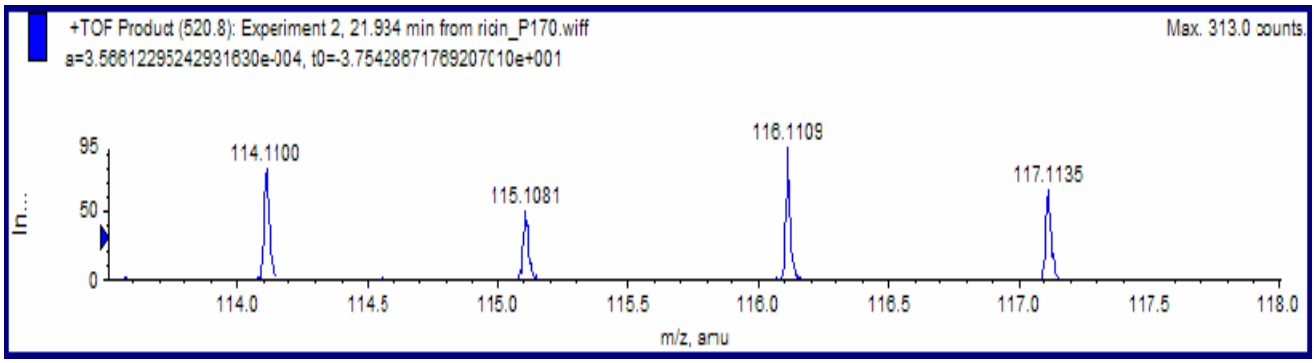
IT6 VGLPINQR -OH -0.0391 Ion charge: 1

	Residue	Mass	Immonium	a	a-NH3	b	b-NH3	y	y-NH3
1	V, Val	99.0684	72.0808	***216.1626***	199.1563	***244.1776***	***227.1512***	1040.6333	1023.6067
2	G, Gly	57.0215	30.0338	273.2043	***256.1778***	***301.1992***	***284.1727***	***797.4628***	***780.4363***
3	L, Leu	113.0841	86.0964	***386.2884***	369.2618	***414.2833***	***397.2567***	***740.4413***	***723.4148***
4	P, Pro	97.0528	70.0651	***403.3411***	386.3140	***511.3360***	494.3095	***627.3573***	***610.3307***
5	I, Ile	113.0841	86.0964	***596.4252***	579.3986	***624.4201***	607.3936	***530.3045***	***513.2780***
6	N, Asn	114.0429	87.0553	710.4681	693.4416	***738.4630***	721.4365	***417.2205***	***400.1939***
7	Q, Gln	128.0586	***101.0709***	838.5267	821.5001	***866.5216***	849.4951	***303.1775***	***286.1510***
8	R, Arg	156.1011	129.1135	994.6278	977.6013	1022.6227	1005.5962	***175.1190***	***158.0924***

Yellow highlighted implied that all the peptides found from extract met the same peptides' list as b and y ions of ricin.

Based on the MS/MS spectra of the product ion 520.8, we zoomed into the mass range of 114 – 117 where our iTRAQ labeled peptides were quantified based on the area under the peak of 114, 115, 116 and 117 generated by statistic of ProQUANT 1.0 software.

Figure 13: Area under the peak of 114,115,116,117 label of product ion, 520.8



All the quantitation data reported here would be based on the average ratio of 115/116/117:114 (i.e. extract:control). The identification would be based on the matched amino sequences. Only ProQuant software has the capability to quantify the iTRAQ mass labels. For Mascot, it could only be used for identification purpose but not quantifying the iTRAQ mass labels.

2.4.3 Nano LC-MS/MS conditions

Separation was done using binary mobile phase gradient elution at 150 nl/min after injection of 1 ul sample. Triplicate wash in water was set before injection and blank run of water were done in between samples to prevent any carry-over of sample from the previous run. Agilent 1100 LC isocratic pump was set at 60ul/min using 5% acetonitrile

and 95% water. Agilent 1100 LC nanopump was at 150 nl/min using gradient elution of eluent A and B from 60% A to 10% A where A was 0.1% formic acid in 95% water and 5% acetonitrile and B was 0.1% formic acid in 100% acetonitrile. For the first 8 minutes, it was set at column 1 mode for desalting purpose, after which it was switched to column 2 mode to elute out the relevant peptides. All MS/MS data was acquired using information dependent acquisition in Analyst QS 1.1 with oMALDI source support and Bioanalyst^{TM} extension software from Applied Biosystem in 65 minutes.

The switching criteria were ions greater than 300 m/z, smaller than 1500 m/z with charge state from 2 to 3 that exceed 30 counts. In Experiment 1, positive TOF MS scan type was chosen and accumulation time was 1 second between TOF masses of 300 to 1500. In experiment 2 and 3, positive product ion scan type was used with accumulation time of 4 seconds between TOF masses of 100 to 2000. Nitrogen gas was used as curtain gas at 25 and collision gas at 4. Ionspray voltage was set at 2200V, declustering potential at 50, focusing potential at 190, declustering potential 2 at 15, automated collision energy, ion release delay at 6.0 and ion release width at 5.0. The voltage used will be 2200V or 2500V depending on the batch of column used with access default set at 2500V for both TOF-MS and TOF-MS/MS.

2.4.4 Preparation of labeled peptides

The sample preparation was modified from Applied Biosystem's iTRAQ protocol to prevent precipitation of toxin. 20 ul of dissolution buffer and 1 ul of denaturant were

added to 100 ug of toxin, vortexed and spin down at 2000 g. 2 ul of reducing reagent was then added, vortexed and spin down. This mixture was incubated at 50^{o}C for 60 minutes. Droplets of toxin would form on the inner cap of the eppendorf tube and thus, required to spin down before addition of 1 ul of cysteine blocking reagent. This was then allowed to incubate at room temperature for 10 minutes. Trypsin concentration of 0.2 mg/ml was prepared using HPLC grade water. 5 ug of trypsin solution was added to 100 ug of toxin giving a trypsin digestion ratio of 1:20. This was incubated at 37^{o}C for 16 hours. Once the digestion was done, 25 ug of sample was withdrawn and labeled with 20 ul of iTRAQ reagent. An incubation time of one hour was required after addition of iTRAQ reagents for the labeling reaction to take place. If there are more than two samples, each sample is labeled with a different mass label and after one-hour incubation, samples are to be mix into a single vial.

2.4.5 Verification of iTRAQ tagging on Ricin (1:1 ratio)

Two identical concentration of Ricin (100 ug) was trypsin digested based on the iTRAQ protocol as in section 2.4.4. Identical amount of digested ricin (i.e. 50 ug) was withdrawn and labeled with 30 ul of 114 and 117 respectively. This is to verify that both the theoretical and experimental results gave 1:1 ratio. Since no paper has reported the use of iTRAQ reagents on ricin analysis, this is the first paper that verifies the possibility of iTRAQ labeled ricin peptides.

2.4.6 Lowest level of detection of iTRAQ labeled Ricin

A range of 1, 5, 10 and 40 ug of digested Ricin was labeled according to the respective amount of required iTRAQ label based on the protocol that for 100 ug of digested sample, 70 ul of iTRAQ label was required. Based on Table 7, the respective amount of iTRAQ reagents were added to digested ricin. These labeled samples were run using nano-flow LC-QSTAR and the acquired data was analyzed by Mascot search. Those that identified with at least three peptides hit would be significant.

Table 7: Volume of iTRAQ reagents to be utilized

Amount of digested ricin [ug]	Volume of iTRAQ reagent required [ul]
1	2
5	5
10	10
40	30

2.4.7 Concentration factor of Millipore Amicon Ultra-15, 10 kDa molecular cut-off centrifuge tube

90 ug of Ricin was spiked into 10 ml HPLC grade water and concentrated down by Millipore Amicon Ultra-15 10 kDa centrifuge tube by spinning down at 25^{o}C, 4100 rpm for 30 minutes. The retentate volume was trypsin digested as in section 2.4.4 and labeled with 70 ul of iTRAQ mass label of 115, 116 or 117. Another set of control having identical concentration without going through Millipore Amicon Ultra-15 10 kDa

centrifuge tube was also trypsin digested and labeled as 114. Triplicate was done and the inverse of the average ratio of 115/116/117:114 would be the concentration factor.

2.4.8 Recovery efficiency of Ricin extract from matrices using Millipore Amicon Ultra-15, 10 kDa at 0hour

As solvent was required for extraction, it would need a concentrating step in order to concentrate down the diluted extract after 100 ug of ricin was extracted from the matrices. 10 ml of 100% HPLC grade water was used as the extraction solvent for carpet, followed by vortexing for 3 minutes before transferring to Millipore Amicon Ultra-15 10 kDa centrifuge tube for concentrating. It was centrifuged at 25^{o}C at 4100 rpm for 30 minutes. The retentate volume was trypsin digested and iTRAQ labeled with comparison to a control. The control had identical concentration as the retentate except that it did not go through the concentrating step. The acquired MS data was then analyzed using ProQUANT 1.0 software. For parquet extraction, 10ml of 100% HPLC grade water was used as extraction solvent and vortexed for 1 minute before centrifuged with Millipore Amicon Ultra-15, 10 kDa centrifuge tube. The inverse of the average ratio of extract:control would be the recovery efficiency factor.

2.4.9 Stability profile experiment of Ricin on carpet and parquet

100 ug of Ricin was spiked onto 1.2 cm x 1.2 cm carpet. Triplicate carpets were done for each time intervals at time 0, 2, 8, 24, 48, 72 and 170 hours. At each timing, 10 ml of

100% HPLC grade water was added and vortexed at 3 minutes for carpet. For parquet, triplicates were done on 1.2 cm x 1.2 cm parquet with lacquer painted surface at 0, 2, 24, 48, 72 and 170 hours. 10 ml of 100% HPLC grade water was used and vortexed for 1 minute. The extract was then added to Millipore Amicon Ultra-15ml centrifuge tube with 10kDa molecular weight cut-off. It was then centrifuged at 25^{o}C for 4100 rpm and 30 minutes. The retentate volume was digested by trypsin enzyme followed by iTRAQ labeling protocol in section 2.4.4. 25 ug of the digested ricin was then withdrawn and labeled with 20 ul of iTRAQ reagents. The control was labeled as 114 and the triplicates were labeled as 115, 116 and 117. The acquired MS/MS data was sent to ProQUANT 1.0 for data analysis.

2.5 METHOD FOR SEB

2.5.1 Verification of iTRAQ tagging on SEB (1:1 ratio)

Both the nano-flow LCMS condition and data analysis remained the same for SEB except that the gradient elution for composition A started from 70% to 10% and the voltage applied was 1800V or 2200V with access default of 2350V set for TOF-MS and 2400V for TOF-MS/MS. As for the preparation of iTRAQ labeled peptides of the SEB extracts, they were incubated at 50^{o}C for 50 minutes instead of 60 minutes to prevent occurrence of precipitation. 20 ug of SEB was labeled with 20 ul of iTRAQ reagents in the actual stability experiment.

Since no paper has reported the use of iTRAQ reagents on SEB, this is also the first paper that verifies the possibility of iTRAQ labeling on digested SEB. Two identical concentration of SEB (100 ug) was trypsin digested based on the iTRAQ protocol in section 2.4.4 and identical amount of 50 ug was labeled with 30 ul of 115 and 117 respectively. This is to verify that for both theoretical and experimental results, we would get 1:1 ratio.

2.5.2 Lowest level of detection of iTRAQ labeled SEB

For the lowest detectable amount of iTRAQ labeled digested SEB, the same protocol in section 2.4.6 was carried out except that the toxin used was SEB.

2.5.3 Concentration factor of Millipore Amicon ultra-15, 5 kDa centrifuge tube and Reproducibility of the nano-flow LC-QSTAR

100 ug of SEB was spiked into 10 ml HPLC grade water and concentrated down by Millipore Amicon Ultra-15 5 kDa centrifuge tube spinning at 25^{o}C at 4100 rpm for 45 minutes. The retentate volume was trypsin digested and iTRAQ labeled with 70 ul reagents. Another set of control having same concentration was also digested and labeled as 114 except that it did not go through concentrating step. The sample was injected thrice into nano-flow LC-QSTAR to validate the reproducibility of the instrument and also to determine the concentration factor for this concentrating step. The inverse of the average ratio would be the concentration factor.

2.5.4 Recovery efficiency of SEB extract from matrices using Millipore Amicon Ultra-15, 5 kDa at 0hour

10 ml of 20% acetronitrile-80% water was used as the extraction solvent for carpet, followed by vortexing for 3 minutes before transferring to Millipore Amicon Ultra-15 5 kDa centrifuge tube for concentrating down the diluted extract. It was centrifuged at 25^{o}C at 4100 rpm for 45 minutes. The retentate volume was trypsin digested and iTRAQ labeled and compared to a control of identical concentration. The MS data was then analyzed using ProQUANT 1.0. A comparison of SEB recovery between 5 kDa and 10 kDa Millipore Amicon Ultra-15 centrifuge tube based on SEB extract from carpet was done to see which molecular cut-off would be good for SEB.

For parquet extraction, 10 ml of 100% HPLC grade water was used as extraction solvent and vortexed for 1 minute before centrifuged with Millipore Amicon Ultra-15, 5 kDa centrifuge tube. The recovery efficiency would be the inverse of average ratio of extract:control.

2.5.5 Stability profile experiment of SEB on carpet and parquet

100 ug of SEB was spiked onto 1.2 cm x 1.2 cm carpet. Triplicate carpets were done for each time intervals of 0, 2, 27, 50, 74 and 170 hours. At each timing, 10 ml of 20% acetronitrile-80% water was added and vortexed for 3 minutes for carpet. For parquet, triplicates were done on 1.2 cm x 1.2 cm parquet with lacquer painted surface using 10

ml of 100% HPLC grade water and vortexing for 1 minute. The extract was then added to Millipore Amicon Ultra-15 ml centrifuge tube with 5 kDa cut-off molecular weight. It was centrifuged at 25^{o}C for 4100 rpm and 45 minutes. The proteolysis digestion and iTRAQ labeling followed exactly the same as section 2.4.9 except that 20 ug of digested SEB was labeled with iTRAQ reagents. Exact data analysis was done too.

2.6 METHOD FOR T-2 MYCOTOXIN

2.6.1 HPLC-ESI-MS system

In our study, the analysis was done by using Finnigan LCQ system fitted with electrospray source. LC separation was done by using Phenomenex Luna 150 x 2 mm 5 μm C18(2) series number 148734-2 with attached guard column of Phenomenex C18 (ODS Octadecyl) 4 mm L x 2 mm ID at gradient elution of 80% acetonitrile, 10% [1methanol:1 MilliQ water] and 10% [0.1% acetic acid in MilliQ water] for 20 minutes. The mass spectrometer was set at selected ion monitoring mode at 467 [466+H], 484 [466+H_2O] and 489 [466+Na].

2.6.2 Sample Preparation

5 mg of T-2 toxin in a standard vial was diluted with 5 ml of 30% acetonitrile/70% MilliQ water to prepare 1mg/ml of spiking vial.

2.6.3 Reproducibility, Linearity, Lowest level of detection (LOD) & quantitation (LOQ)

To check the reproducibility of the system, a standard vial of 25 ppm T-2 toxin in 84% acetonitrile/16% MilliQ water was prepared and injected eight times into the LCQ. Area under the peak was quantified for each injection.

To check the linearity of T-2 toxin, three different concentrations of 2.5 ppm, 10 ppm and 25 ppm in 84% acetonitrile/16% MilliQ water were prepared and rub using LCQ. A linearity graph was plotted based on the peak area versus the various concentrations.

Two low T-2 toxin concentrations of 0.05 ppm and 0.1 ppm standard were prepared to the check the LOD and LOQ.

2.6.4 Filter factor prior to LC

A 25 ppm T-2 toxin standard prepared in 84% acetonitrile/16% MilliQ water with an internal standard of 100 ppm of benzophenone was done to compare with another exact set but filtered through a 0.45um nylon filter. The reciprocal of the area differences would be the filter factor.

2.6.5 Recovery efficiency of T-2 toxin from Carpet and Parquet

Carpet and parquet were cut into dimension of 5 cm x 5 cm. Each matrix was placed into a LOCK & LOCK container of 180 ml. Duplicate set of three different T-2 toxin concentrations of 1 mg/ml, 0.4 mg/ml and 0.1 mg/ml were spiked onto the centre of the matrix and 40ml of 84% acetonitrile/16% MilliQ water was added instantly. It was vortexed for 3 minutes before 1 ml of the sample was withdrawn. 100 ppm benzophenone was added as internal standard for parquet. For carpet, benzophenone was not used as there was an interference peak from the carpet extract that co-eluted at the same time. The sample was then filtered through a 0.45 um filter prior to LC run. Standard vials of 2.5 ppm, 10 ppm and 25 ppm T-2 toxin in 84% acetonitrile/16% MilliQ water with and without internal standards were run concurrently to determine the consistency of extraction recovery. The average of the three concentrations was taken as the extraction recovery and the reciprocal of the average was taken as the extraction factor.

2.6.6 Stability profiles of T-2 toxin on Carpet and Parquet

Eight vials of 1 mg/ml of T-2 toxin (i.e. in 1 ml of 30% acetonitrile/70% MilliQ water) were spiked onto eight matrices and at time 0, 4, 8, 12, 26, 48, 74 and 170 hours. 40 ml of extraction solvent (i.e. 84% acetonitrile/16%MilliQ water) was added. At each time interval, it was vortexed for 3 minutes and 1 ml of sample was withdrawn and filtered through 0.45 um filter. For the purpose of consistency of experiments, the matrices were not spiked with internal standard. Samples were then run using LCQ. Triplicate set was

done for each matrix and experiments were carried out in an enclosed chamber of monitored temperature 22.5-25.5^{0}C and relative humidity <70%. On each day, a one-point external calibration of 25 ppm T-2 standard in 84% acetonitrile/16%MilliQ water was done. The recovery percentage at each time interval was calculated based on the absolute area of the peak. The amount of T-2 toxin present was quantified based on the extraction efficiency factor and filter factor. The stability profile for carpet and parquet was presented in graphs.

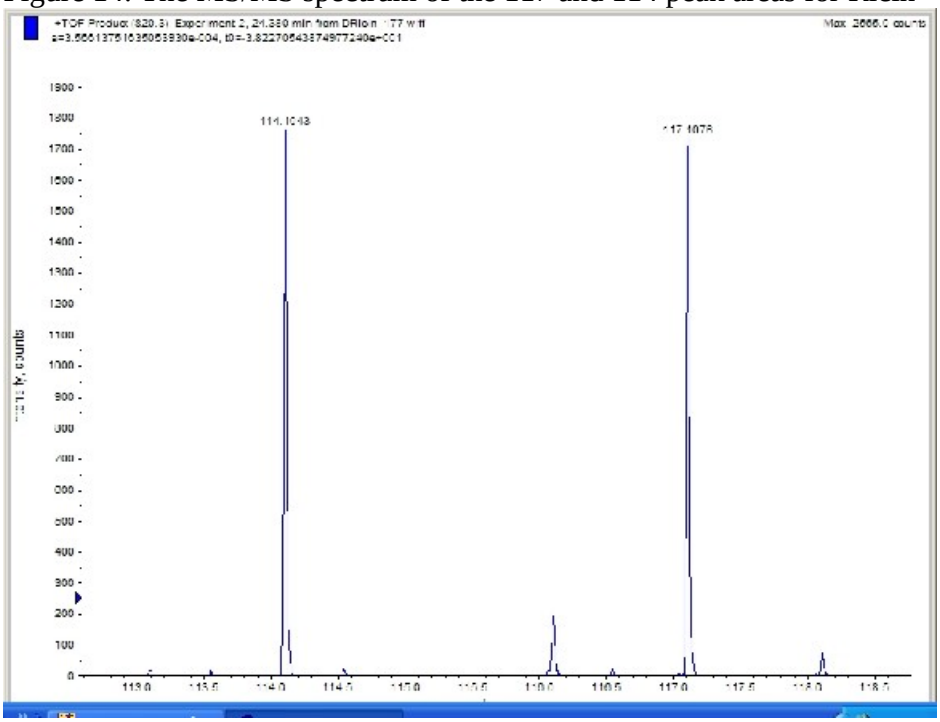
CHAPTER 3 RESULTS AND OBSERVATIONS

3.1 Ricin

3.1.1 Verification of 1:1 ratio of Ricin

Ricin was identified by analyzing the iTRAQ labeled peptides data via ProQUANT software. It identified Ricin with confidence of 99, 91 peptides and average 117:114= 0.9836. Thus, it verified that theoretical iTRAQ labeling on ricin could give an approximately 1:1 ratio experimentally. This is the first reported paper that used iTRAQ reagents on ricin for quantitation purpose. We had showed the MS/MS spectra of 117:114 peak areas at product ion 820.3 in Figure 14 where the peak area under 114 and 117 were similar.

Figure 14: The MS/MS spectrum of the 117 and 114 peak areas for Ricin



3.1.2 Lowest level of detection of iTRAQ labeled Ricin

As explained in section 2.4.5, the mascot identification is only significant when the peptide hit is at least 3 peptides. In Table 8, it showed that the lowest amount of ricin required to be effectively labeled in order to be used for quantitation purpose must be at least 5 ug where Mascot search identified 4 peptides with a score of 133.

Table 8: Lowest level of detection of iTRAQ labeled Ricin

Amount/ug	score	No. of peptides	Coverage
1		No ricin detected	
5	133	4	8%
10	127	11	14%
40	209	24	16%

3.1.3 Concentration factor of Millipore Amicon Ultra-15 10 kDa

A comparison of ricin extract with and without passing through the concentrating step of Millipore Amicon Ultra-15 10 kDa centrifuge tube was done. This allowed us to determine any loss of sample during concentrating step. The results in Table 9 showed that the recovery of ricin from Millipore Amicon Ultra-15 10 kDa centrifuge tube gave an approximate 100% recovery with CV% of 1.94%. Thus, this gave us a concentration factor of approximately 1.

Table 9: Concentration factor for Ricin using 10 kDa Millipore Amicon Ultra-15

	extract1	extract2	extract3	average	SD	CV%
10 kDa centrifuge tube	1.03	1.05	1.01	1.03	0.020	1.94

Extract 1, 2 and 3 represented the average ratio of 115/116/117:114, SD represented the standard deviation and CV% represented the correlation variation.

3.1.4 Recovery efficiency of Ricin extract from Carpet and Parquet

Based on the usage of Millipore Amicon Ultra-15 10 kDa centrifuge tube on ricin extracted out from carpet, the retentate volume was trypsin digested and labeled with iTRAQ mass label of 115, 116 and 117. These iTRAQ labeled extracts were mixed with a control (i.e. 114) without concentrating step. The extraction efficiency of ricin from carpet at time 0 was 100% recovery with CV% of 2.50% as shown in Table 10. As for ricin recovery from parquet, the recovery was also 100% with CV% of 7.88%. These results observed had revealed that the extraction efficiency based on the selected extraction solvent and developed methodology was good.

Table 10: Recovery efficiency of Ricin from carpet and parquet

	Extract 1	Extract 2	Extract 3	Average	SD	CV%
Carpet	0.98	1.01	1.03	1.01	0.025	2.50
Parquet	0.94	1.10	1.04	1.03	0.081	7.88

Extract 1, 2 and 3 represented the average ratio of 115/116/117:114, SD represented the standard deviation and CV% represented the correlation variation.

3.1.5 Stability profile of Ricin on Carpet

The stability profile for ricin spiked on carpet was shown in Table 11. Triplicate set was done at each time interval and by taking the average and the SD, a graph of ricin recovery from carpet versus time profile up to 170 hours was plotted in Figure 15. Its extraction efficiency for ricin from carpet at the point of experiment was averaged out to be 95% with correlation variation of 2.85%. Thus, the recovery factor was 1/0.95=1.06. The stability profile of ricin on carpet was observed to drop drastically to half of the spiked amount after 8 hours of residence, followed by gradual drop leaving an amount of 22.3 ug/1.44 cm^2 (15.5 ug/cm^2) ricin on the carpet at the end of 7^{th} day, taking into consideration the recovery factor and the concentration factor. We also extracted out the MS/MS spectra of a particular product ion of 579 as example where we took a look at the changes of the 114, 115, 116 and 117 peak areas as shown in Figure 16. The intensity of the peak areas had showed a rapid drop over the first 24 hours and from 48 hours onwards, it seemed to level down to a consistent recovery of ~20 % remaining until the end of the 170^{th} hour. As in this study, we focused on the quantitation study of the ricin from carpet, all other acquired data like the matched sequences used for identification purpose were filed in chapter 6 under appendix A.

Table 11: Ricin's stability on Carpet over 7 days

Hours	extract1	extract2	extract3	average	SD	CV%
0	0.98	0.93	0.93	0.95	0.03	2.85
2	0.83	0.97	0.95	0.92	0.07	8.12
8	0.53	0.40	0.44	0.46	0.07	15.02
24	0.30	0.23	0.36	0.30	0.06	21.03
48	0.21	0.20	0.32	0.25	0.07	27.60
72	0.24	0.20	0.26	0.24	0.03	12.95
170	0.28	0.18	0.17	0.21	0.06	28.58

Extract1, 2 and 3 were expressed in average ratio against SEB standard (115/116/117:114), SD = standard deviation and CV%= correlation variation.

Figure 15: Stability profile of Ricin on carpet over 7 days

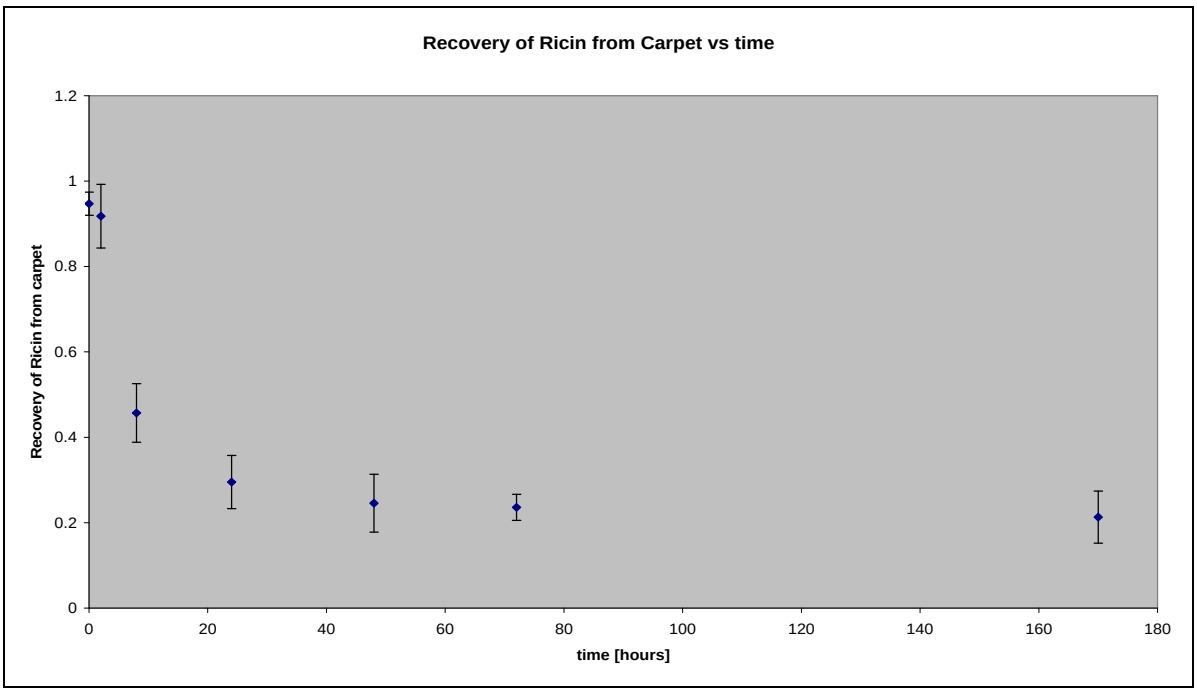
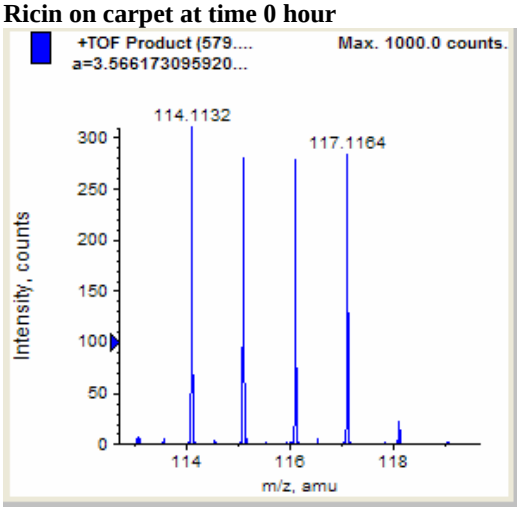
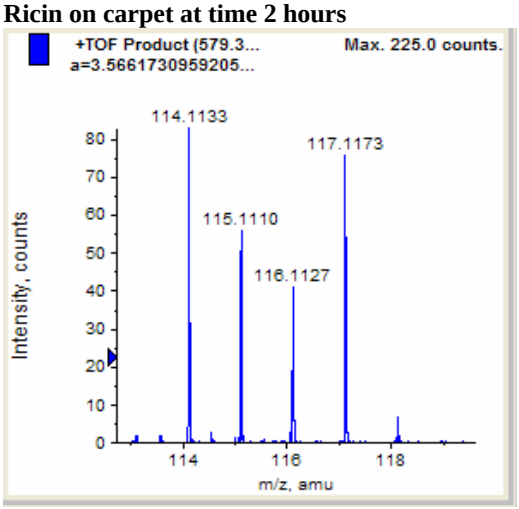


Figure 16: MS/MS spectra of the product ion, 579 over 170 hours for Ricin on carpet

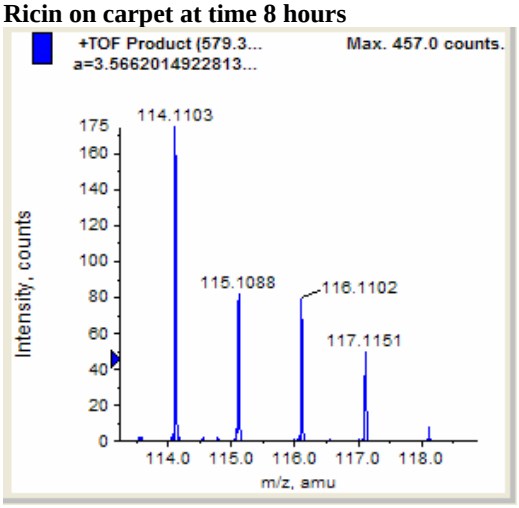
Ricin on carpet at time 0 hour



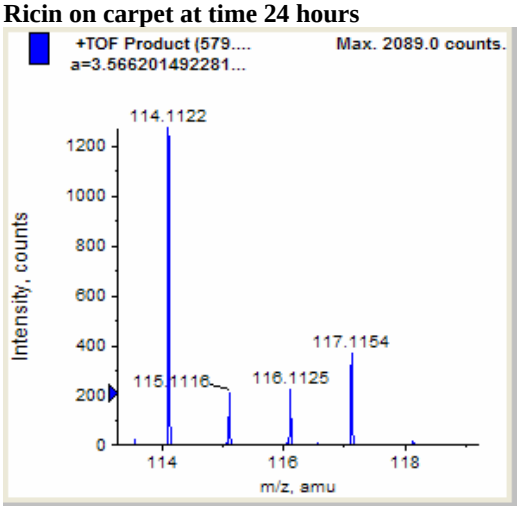
Ricin on carpet at time 2 hours



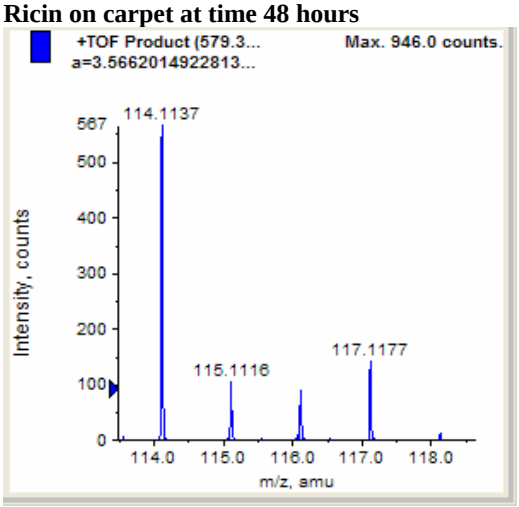
Ricin on carpet at time 8 hours



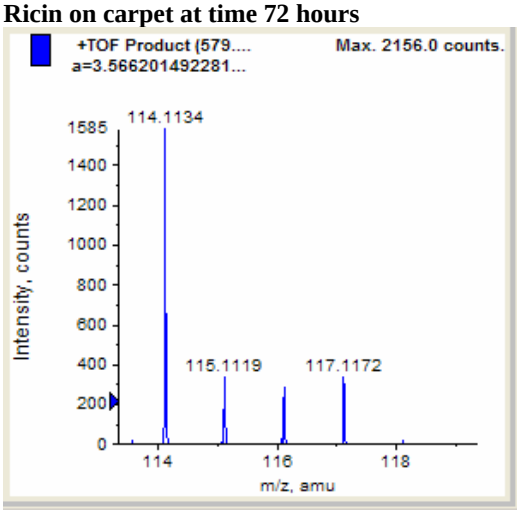
Ricin on carpet at time 24 hours



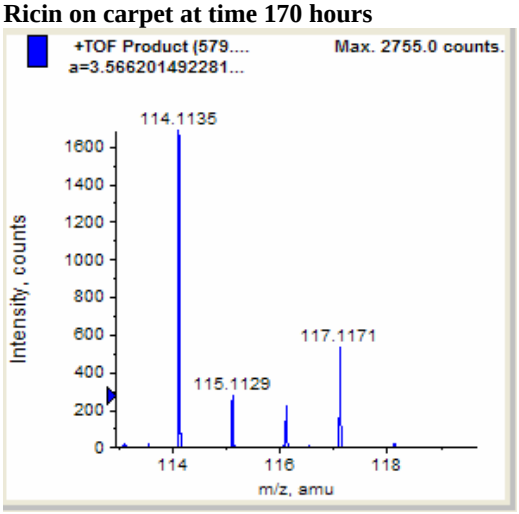
Ricin on carpet at time 48 hours



Ricin on carpet at time 72 hours



Ricin on carpet at time 170 hours



3.1.6 Stability profile of Ricin on Parquet

The stability profile for ricin spiked on parquet was shown in Table 12. A graph of ricin recovery from parquet versus the time profile up to 170 hours was plotted in Figure 17. Its extraction efficiency for ricin from parquet was approximated to be 100% with correlation variation of 7.87%. Thus, the recovery factor was 1. As compared to ricin on carpet, the stability on parquet was observed to be much more persistent on matrix and only dropped by approximately 30% by the end of 170th hour. This good stability of ricin on parquet was also observed on SEB on parquet but SEB seemed to be much stable than ricin on parquet with almost 100% remaining on parquet.

An extraction of a product ion, 450 was done from time 0 to 170 hours to see the trend in the peak areas of 114, 115, 116 and 117 in Figure 18. The trend of the peak area change followed Figure 17 except for the last time interval at 170th hour. The reason was that the ratio of 115/116/117:114 was an average of all identified product ions and at this particular product ion, the ratio at this product ion was slightly lower. However, once it was averaged against the rest of the product ions, it should be the reported average ratio as in Table 12. The matched sequences for identification at each time interval was filed under chapter 6, appendix B since our focus here was on the average ratio of 115/116/117:114.

Table 12: Ricin's stability on Parquet over 7 days

hours	extract1	extract2	extract3	average	SD	CV%
0	0.94	1.10	1.04	1.03	0.081	7.87
2	0.80	0.93	0.71	0.81	0.111	13.60
24	0.74	0.78	0.96	0.83	0.117	14.18
48	0.85	0.71	0.84	0.80	0.078	9.76
72	0.78	0.85	0.80	0.81	0.036	4.45
170	0.70	0.75	0.69	0.71	0.032	4.51

Extract1, 2 and 3 were expressed in average ratio against SEB standard (115/116/117:114), SD = standard deviation and CV%= correlation variation.

Figure 17: Stability profile of Ricin on parquet over 7 days

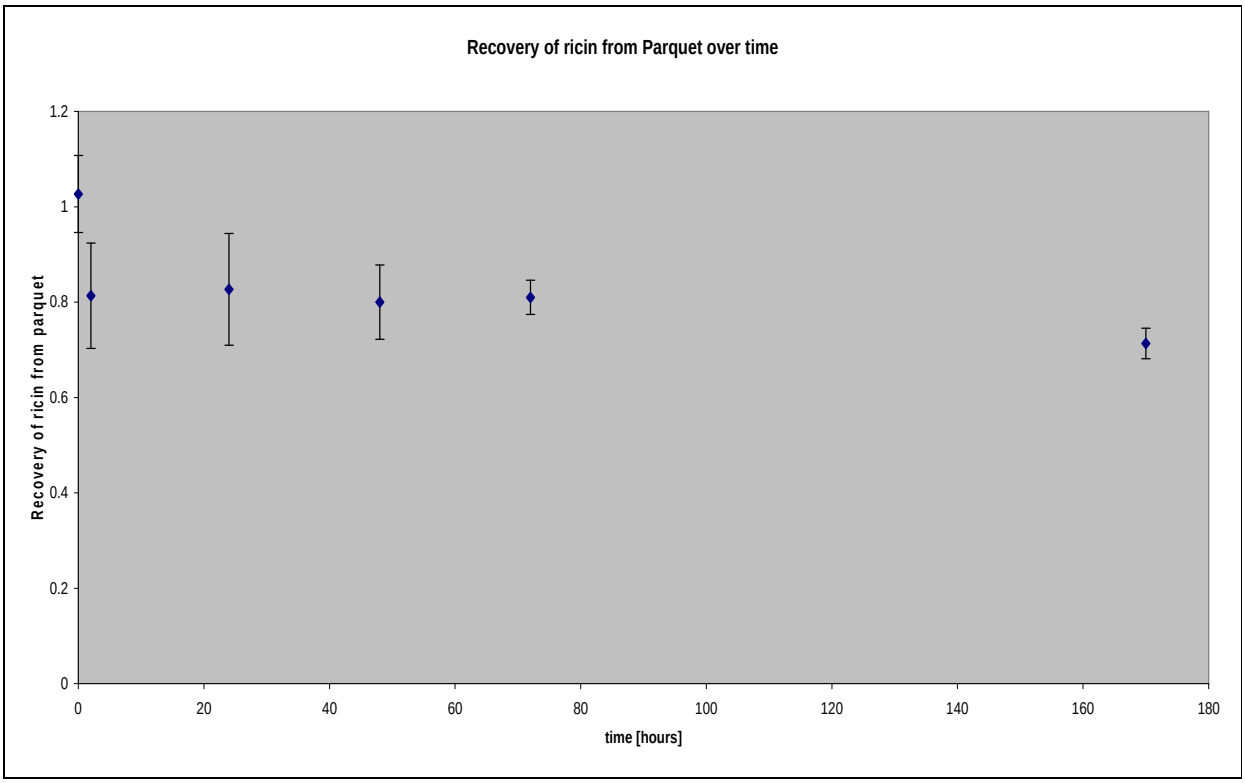
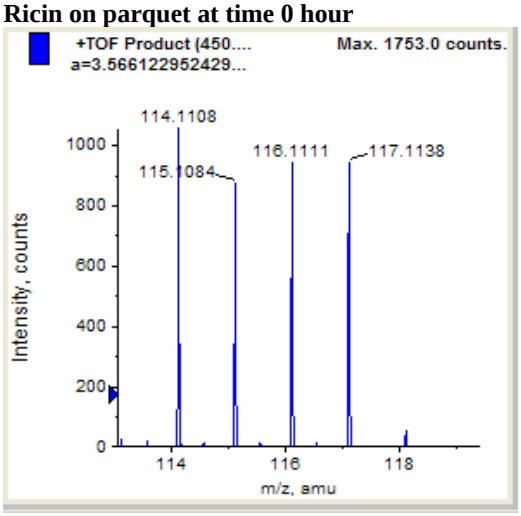
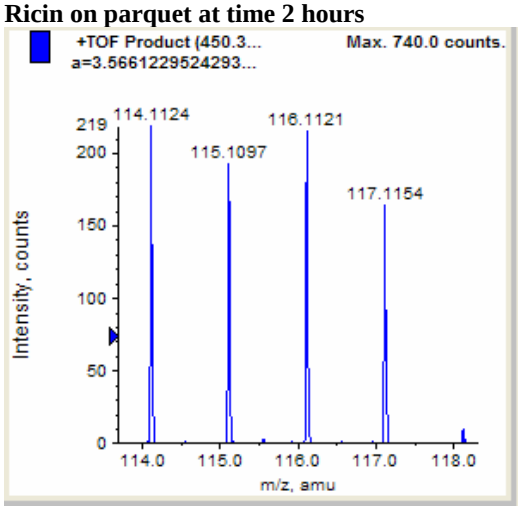


Figure 18: MS/MS spectra of the product ion, 450 over 170 hours for ricin on parquet

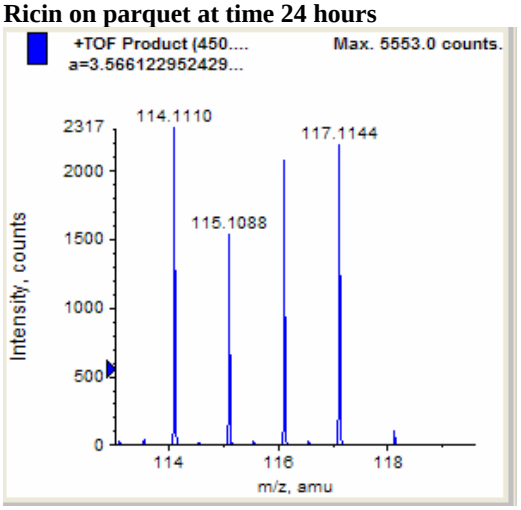
Ricin on parquet at time 0 hour



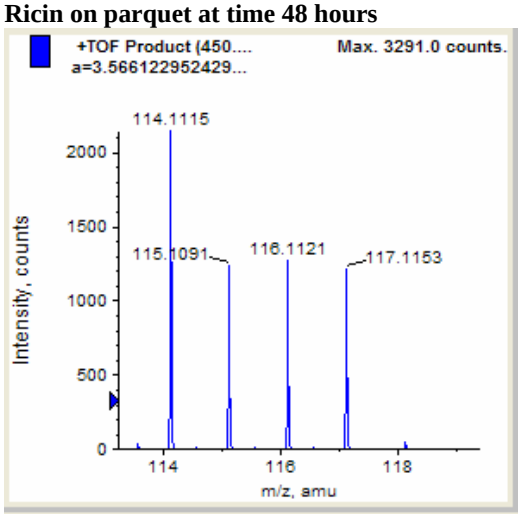
Ricin on parquet at time 2 hours



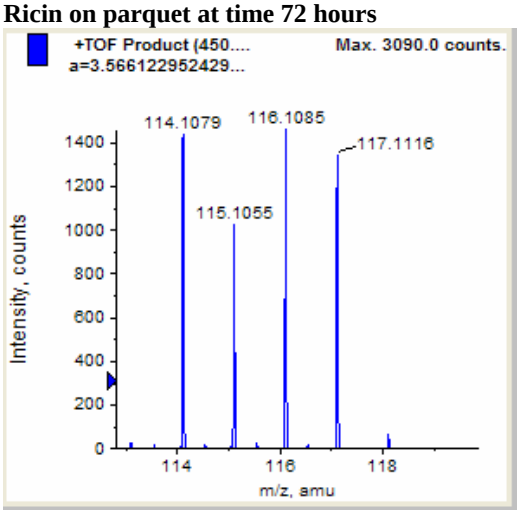
Ricin on parquet at time 24 hours



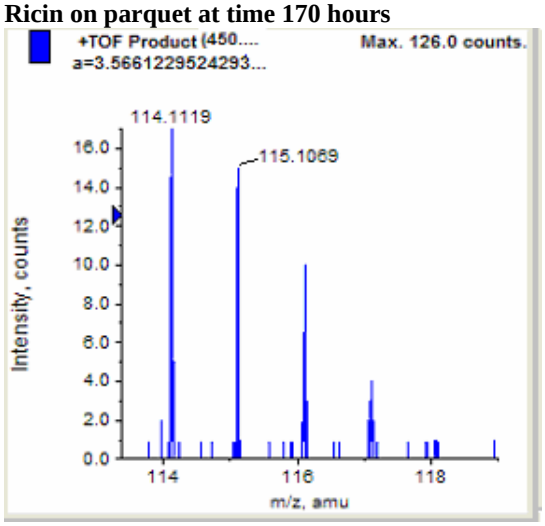
Ricin on parquet at time 48 hours



Ricin on parquet at time 72 hours



Ricin on parquet at time 170 hours

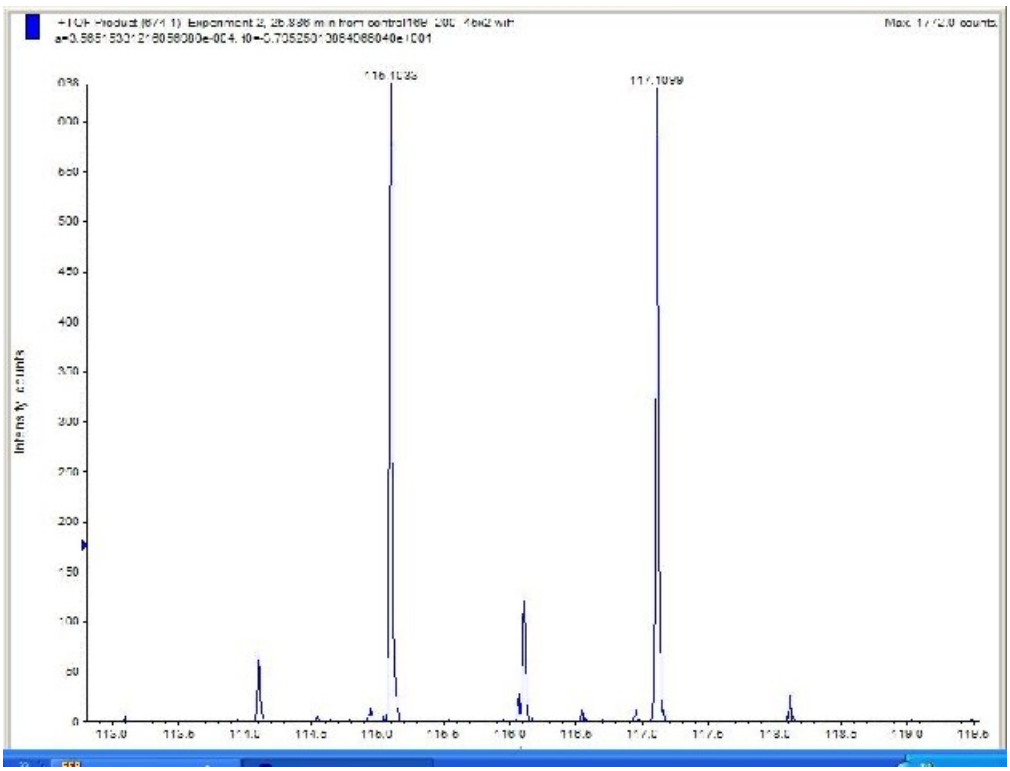


3.2 SEB

3.2.1 Verification of 1:1 ratio of SEB

SEB was identified by using iTRAQ labeled SEB peptides analyzed via ProQUANT software. It identified SEB with confidence of 99, 156 peptides and average 117:115= 0.9664. This showed that the theoretical labeling of 1:1 ratio gave a true labeling of 1:1 ratio experimentally. This was also the first reported paper on SEB using iTRAQ reagent for quantitation purpose. We showed a MS/MS spectrum of a product ion, 674.1 in Figure 19, where it showed similar peak areas under the 115 and 117 peaks.

Figure 19: The MS/MS spectrum of the 117 and 115 peak areas for SEB



3.2.2 Lowest level of detection of iTRAQ labeled SEB

The results in Table 13 showed that the lowest amount of SEB required to be effectively labeled for quantitative work must be at least 1 ug using Mascot search since the lowest number of peptides hit for a protein identification to be significant was 3. At 1 ug of SEB, it gave identification of 3 peptides with score of 63.

Table 13: Lowest level of detection of iTRAQ labeled SEB

Amount/ug	score	No. of peptides	Coverage
1	63	3	9%
5	132	18	26%
10	143	21	26%
40	233	29	31%

3.2.3 Concentration factor of Millipore Amicon Ultra-15 5 kDa and Reproducibility of the nanoLC-QSTAR

The results in Table 14 showed that it was reproducible with an average of 96.2% and correlation variation of 0.165% from the same vial of sample. This was extremely precise and accurate as CV% was much less than 10%, giving us a concentration factor of 1.04 by taking the inverse of the average ratio (i.e. 1/0.962).

Table 14: Concentration factor for SEB

Sample #	1	2	3
No. of peptides	171	174	210
Ave 116:114	0.964	0.962	0.961
Standard Deviation	0.0015	CV%	0.165

3.2.4 Recovery efficiency of SEB extract from Carpet and Parquet

Using 5 kDa and 10 kDa Millipore Amicon Ultra-15 centrifuge tube for concentrating SEB extract from carpet, the concentrated volume was then proteolysis digested and iTRAQ labeled. Using 5 kDa centrifuge tube, it allowed 95% recovery with CV% of 5.17%. With 10 kDa centrifuge tube, it obtained a lower recovery of SEB of 71% with CV% of 13.6%. All these results were shown in Table 15. They were made with comparison to control of the same concentration without passing through the Millipore Amicon Ultra-15 centrifuge tube. Thus, we concluded that the use of 5 kDa Millipore Amicon Ultra-15 centrifuge tube for concentrating SEB extract from carpet would give a better recovery of approximately 100%. Since in the case of carpet, 5 kDa Millipore Amicon Ultra-15 centrifuge tube was selected due to better efficiency. The same 5 kDa Millipore Amicon Ultra-15 centrifuge tube was applied to parquet extract, which gave a recovery of 74.0% with CV% of 5.74%.

Table 15: Recovery efficiency of SEB from carpet and parquet

Carpet	Extract 1	Extract 2	Extract 3	Average	SD	CV%
5 kDa	0.93	0.92	1.01	0.95	0.049	5.17
10 kDa	0.82	0.67	0.64	0.71	0.096	13.6
Parquet						
5 kDa	0.70	0.73	0.78	0.74	0.042	5.74

Extract1, 2 and 3 were expressed in average ratio against SEB standard (116/117/118:114), SD = standard deviation and CV%= correlation variation.

3.2.5 Stability profile of SEB on Carpet

The stability profile for SEB spiked on carpet was shown in Table 16. A graph of recovery versus the time profile up to 170 hours was plotted in Figure 20. The recovery of 1 implies 100% recovery of SEB extraction from carpet. At time 0 hour, the extraction efficiency for SEB was averaged out to be 95.3% with correlation variation of 5.17%. Thus, the recovery factor was 1/0.953=1.05. Taking into consideration the recovery factor as well as the concentration factor, by the end of the 7th day it still contained 28.1 ug/1.44 cm^2 = 19.5 ug/cm^2 which was much higher than the LD_{50}. The stability of SEB on carpet dropped by half at 50^{th} hour of its spiked amount and gradually dropped over the remaining days. It settled to a remaining recovery of ~20% by the 72^{nd} hour and this was maintained until 170 hours. We extracted out the MS/MS spectrum of a product ion 575 from time 0 to 170 hours to illustrate the changes of the peak areas of 114, 115, 116 and 117 in Figure 21. It showed that the stability of SEB on carpet was better than ricin on carpet as ricin amount dropped by half on the 8^{th} hour onwards but for SEB, it only started to drop by half from 50^{th} hour onwards. But the amount remaining for both toxins

on carpet was approximately 20%. The matched sequences used for identification purpose was filed under chapter 6, appendix C.

Table 16: SEB's stability on Carpet over 7 days

Hours	extract1	extract2	extract3	average	SD	CV%
0	0.93	0.92	1.01	0.953	0.049	5.17
2	0.80	0.98	0.65	0.810	0.165	20.40
27	0.52	0.53	0.65	0.567	0.072	12.77
50	0.56	0.53	0.45	0.513	0.057	11.08
74	0.17	0.16	0.30	0.210	0.078	37.19
170	0.16	0.37	0.24	0.257	0.106	41.29

Extract1, 2 and 3 were expressed in average ratio against SEB standard (115/116/117:114), SD = standard deviation and CV%= correlation variation.

Figure 20: Stability profile of SEB on carpet over 7 days

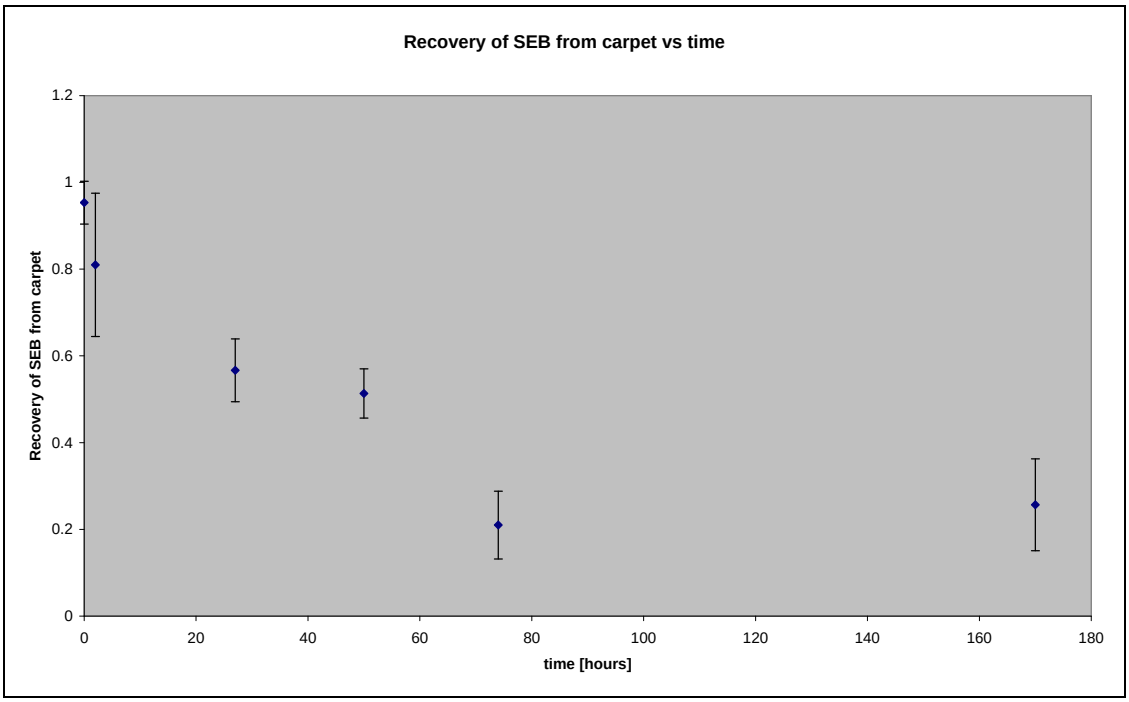
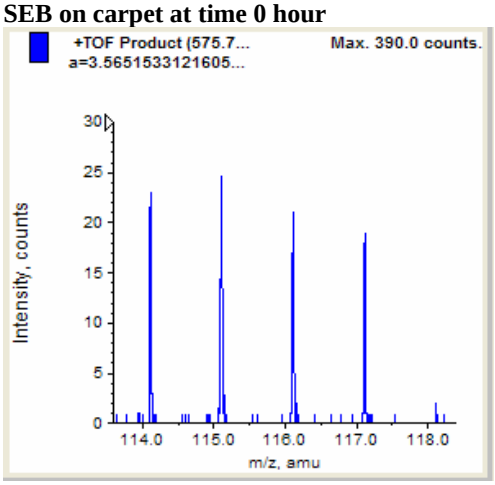
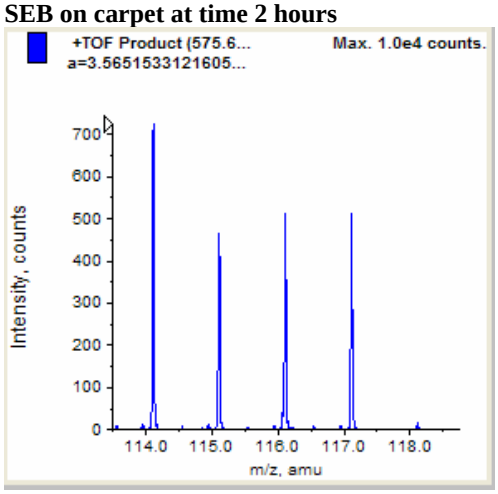


Figure 21: MS/MS spectra of the product ion, 575 over 170 hours for SEB on carpet

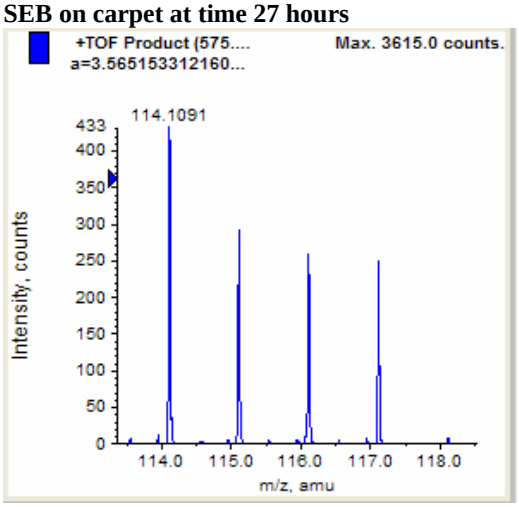
SEB on carpet at time 0 hour



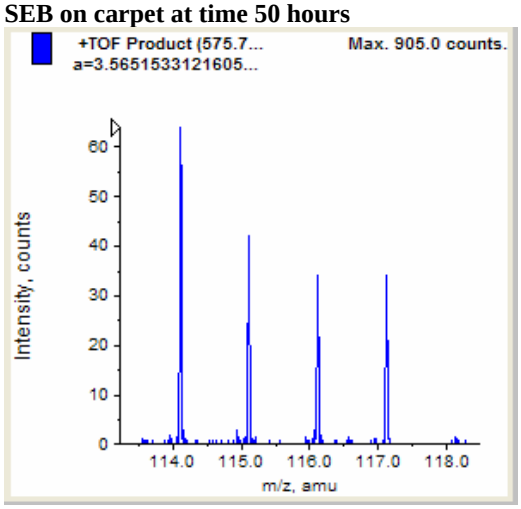
SEB on carpet at time 2 hours



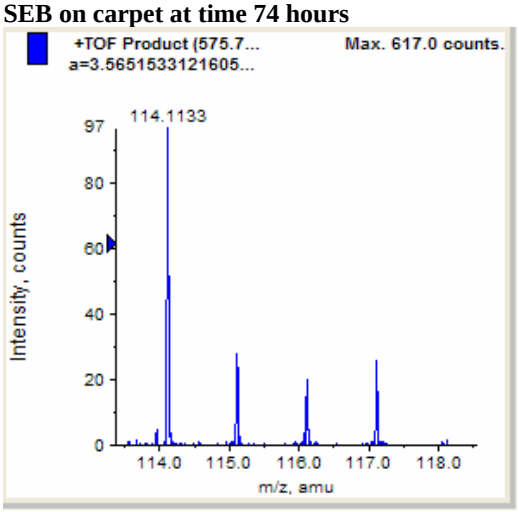
SEB on carpet at time 27 hours



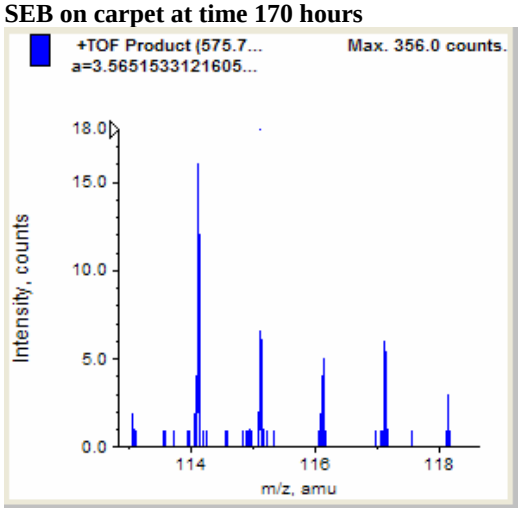
SEB on carpet at time 50 hours



SEB on carpet at time 74 hours



SEB on carpet at time 170 hours



3.2.6 Stability profile of SEB on Parquet

The stability profile for SEB spiked on parquet was shown in Table 17. A graph of SEB recovery versus the time profile up to 170 hours was plotted in Figure 22. Its extraction efficiency for SEB from parquet was averaged out to be 74% with correlation variation of 5.74%. Thus recovery factor was 1/0.74 = 1.35. SEB on parquet had showed to be much persistent and its degradation rate was almost zero even at the end of the 7^{th} day. It implied that SEB was extremely stable on parquet. By the end of the 7^{th} day, the actual amount of SEB left on parquet after multiplied by the recovery factor and concentration factor remained approximately the same as the spiked amount. We extracted out the product ion of 575 to take a look at the trend of the changes in peak areas of 114, 115, 116 and 117 in Figure 23. The intensity of the four peaks was showed to be persistent over 170 hours. On comparison to carpet, SEB seemed to be less stable on carpet.

It was noted that for most timings in parquet, the CV% were less than 10% with only one exception at 27 hours. But for carpet, the CV% deviated much more after time 0 and this could be due to the nature of the surface where the parquet had smooth surface but carpet was hollow/uneven surface allowing “interaction” of toxin with carpet or the toxin absorbed into the carpet at different extent and therefore the amount of extract differed more between each matrix at the same time interval. This CV% for carpet after time 0 were not due to the extraction technique as we already verified that at time 0, the extraction efficiency was 95.6% with good CV% of less than 10%. It was most likely due to the different “interaction” or absorption rate. This same trend of CV% was observed

for ricin as well. The matched sequence for identification purpose was filed under chapter 6, appendix D.

Table 17: SEB's stability on Parquet over 7 days

Hours	extract1	extract2	extract3	average	SD	CV%
0	0.70	0.73	0.78	0.74	0.042	5.74
2	0.77	0.74	0.79	0.77	0.026	3.45
27	0.80	0.84	0.52	0.72	0.174	24.09
50	0.77	0.84	0.77	0.79	0.042	5.34
74	0.71	0.61	0.69	0.67	0.052	7.79
170	0.73	0.67	0.79	0.73	0.058	8.00

Extract1, 2 and 3 were expressed in ratio against SEB standard, SD = standard deviation and CV%= correlation variation.

Figure 22: Stability profile of SEB on parquet over 7 days

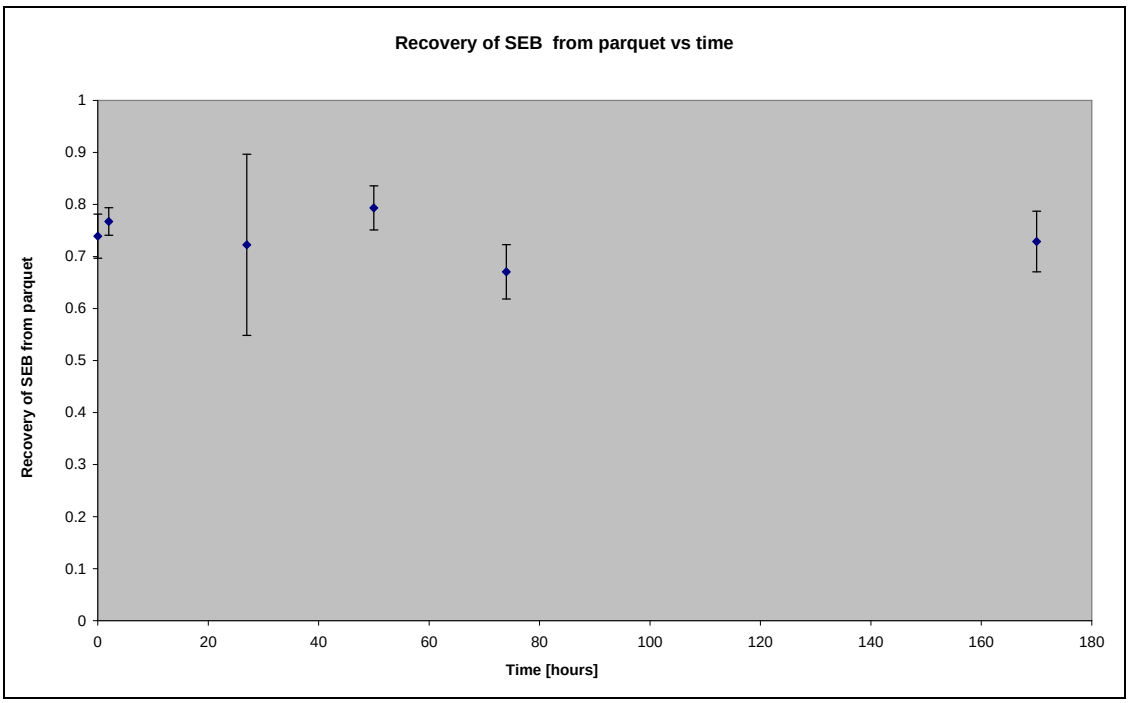
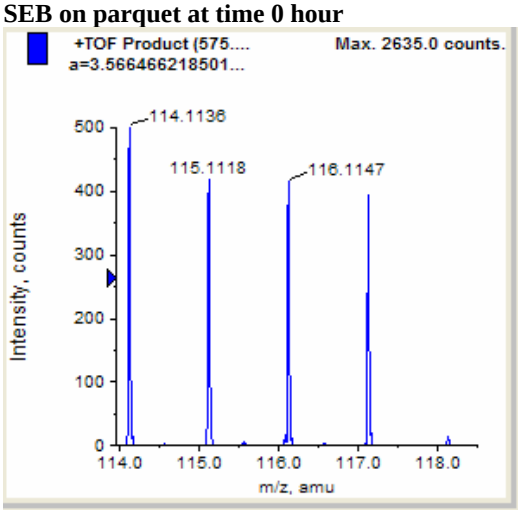
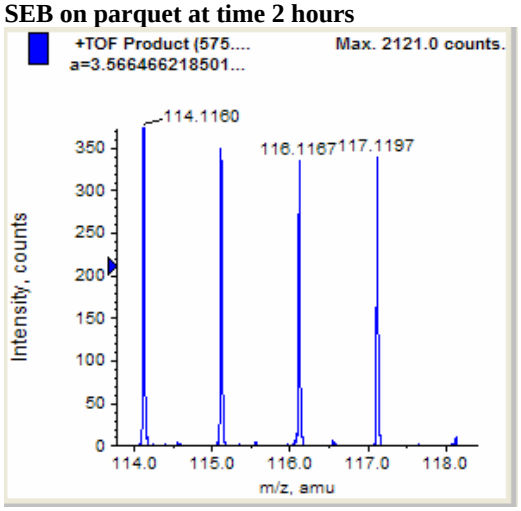


Figure 23: MS/MS spectra of the product ion, 575 over 170 hours for SEB on parquet

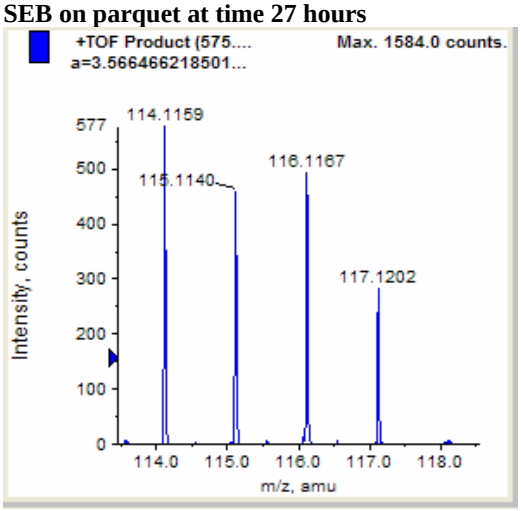
SEB on parquet at time 0 hour



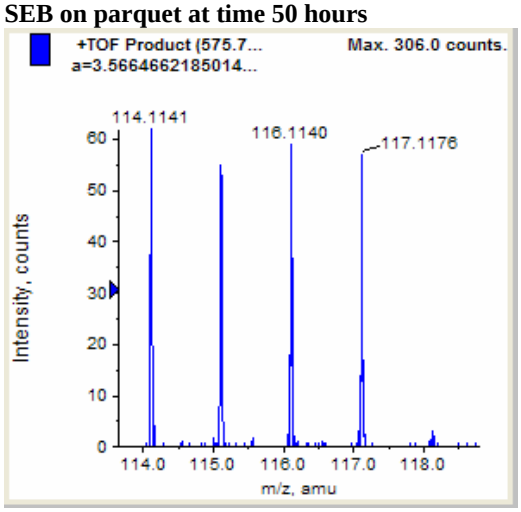
SEB on parquet at time 2 hours



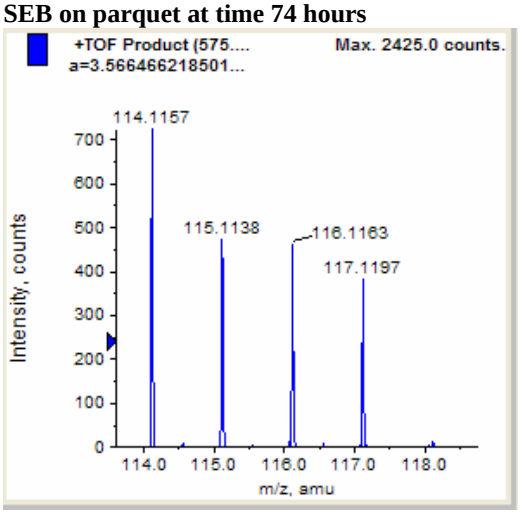
SEB on parquet at time 27 hours



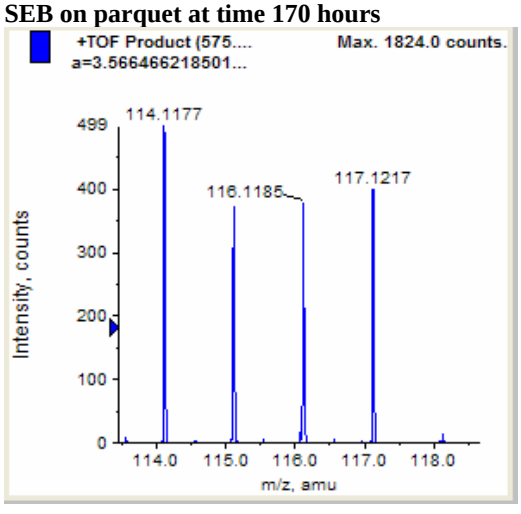
SEB on parquet at time 50 hours



SEB on parquet at time 74 hours



SEB on parquet at time 170 hours



3.3 T-2 MYCOTOXIN

3.3.1 Reproducibility

LC-MS was used to quantify the amount of T-2 toxin extracted from matrices. The reproducibility of the quantitation was assessed to ensure the stability of the LC system and validity of the T-2 toxin analysis results. In Table 18, it showed that the LC-MS had a good reproducibility of T-2 toxin giving a CV% of 5.03 for the eight repeated injections from the same vial of sample.

Table 18: Reproducibility of the LC-MS system

Sample#	1	2	3	4	5	6	7	8
Retention time[min]	2.98	2.83	2.97	2.83	2.96	2.83	2.99	2.68
Peak area	2.12E9	2.18E9	2.12E9	2.32E9	2.21E9	2.30E9	1.99E9	2.10E9

Average = 2.17E9

Standard deviation = 1.09E8

Correlation variation % = 5.03

3.3.2 Linearity

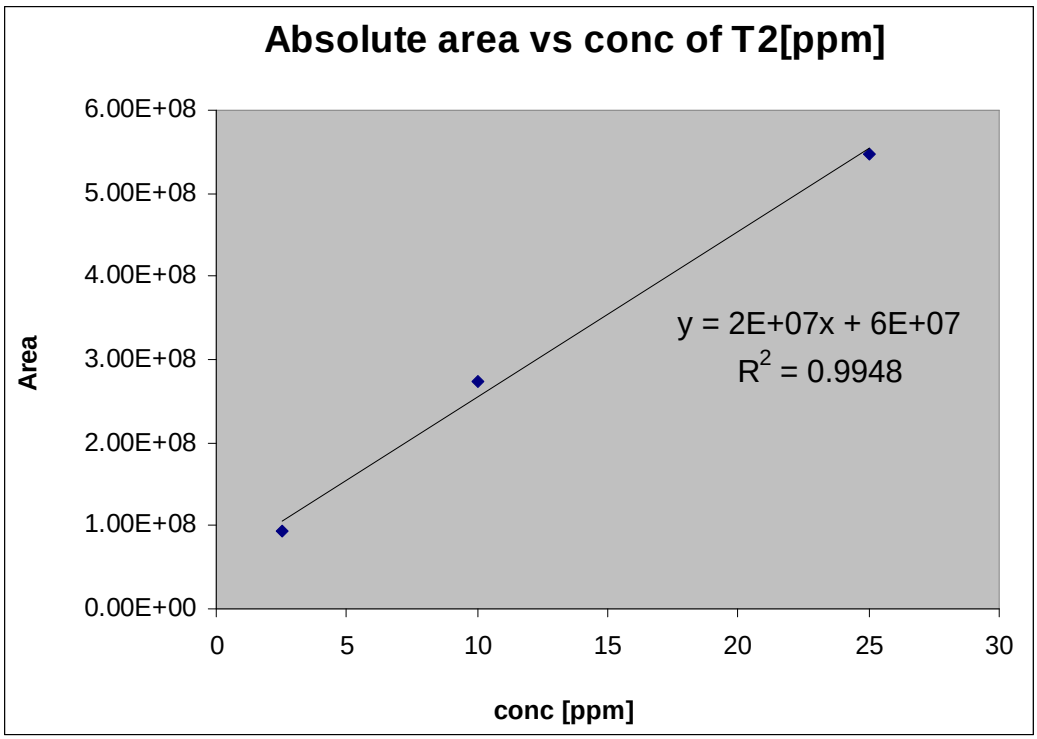
The linearity for the T-2 toxin standard calibration was done to show that the peak area of the T-2 toxin correlates to the amount of T-2 toxin present. Table 19 showed the

concentration of toxin versa area count. The plot gave a R^2 of 0.9948 as shown in Figure 24. As such, a single point external calibration will be done each day of the experiment.

Table 19: Linearity of area count versus concentration of T-2 toxin

T-2 Concentration	2.5 ppm	10 ppm	25 ppm
Retention time[min]	2.85	2.69	2.67
Peak Area	9.39E7	2.74E8	5.47E8

Figure 24: Linearity of area count versus concentration of T-2 toxin



3.3.3 Lowest level of detection (LOD) & quantitation (LOQ)

Both LOD and LOQ were analyzed to determine the sensitivity of the instrument. LOD was determined to be 0.05 ppm. LOQ was determined to be 0.1 ppm.

3.3.4 Filter factor prior to LC

Since the sample had to be filtered through a nylon filter before LC run, the loss of sample through this filtering step was determined. The recovery from nylon filter was derived by taking the average relative area (RA) of T-2 toxin through nylon filter divided by the average RA of T-2 toxin standard. This gave a recovery of 85% (i.e. 19.96/23.58*100%) and thus, the filter factor which was the inverse of recovery, gave 1.18 as shown in Table 20.

Table 20: Determination of filter factor

T-2 standard	**Extract1**	**Extract2**	**Extract3**	**Average RA**
RT(T2)	2.54	3.01	2.63	23.58
A(T2)	3.29E8	3.93E8	5.34E8	
RT(Ben)	3.38	3.84	3.52	
A(Ben)	1.25E7	1.59E7	2.71E7	
RA	26.32	24.72	19.70	
T-2 thru' Nylon filter	**Extract1**	**Extract2**		**Average RA**
RT(T2)	2.84	2.85		19.96
A(T2)	3.89E8	5.07E8		
RT(Ben)	3.68	3.84		
A(Ben)	2.09E7	2.38E7		
RA	18.61	21.30		

Where RT= retention time[min], A=peak area, Ben=Benzophenone internal standard, RA=relative area of T-2/Ben

3.3.5 Recovery efficiency of T-2 toxin from carpet

In Figure 25, it showed the extraction efficiency of T-2 toxin from carpet. With 2.5 ppm of T-2 toxin spiked on the carpet, the average recovery from it was 60.8%. When the spiking concentration was increased to 10 ppm and 25 ppm, its average recovery was 73.5% and 63% respectively. The spiked amount was 1000 ppm T-2 toxin on matrices and thus after adding 40 ml of extraction solvent, its concentration would be diluted to 25 ppm and thus, the calibration standard should be established below 25ppm. We needed to show that the calibration standard was linear for us to do relative quantitation based on peak area count of standard T-2 toxin. It had showed that the recovery of T-2 toxin from carpet was reasonably consistent over three different concentrations with a mean of 65.8% and standard deviation of 15.1% in Table 21. The carpet extraction factor was then derived to be 1.5 (i.e. 1/0.658).

Figure 25: Extraction efficiency of T-2 toxin from carpet

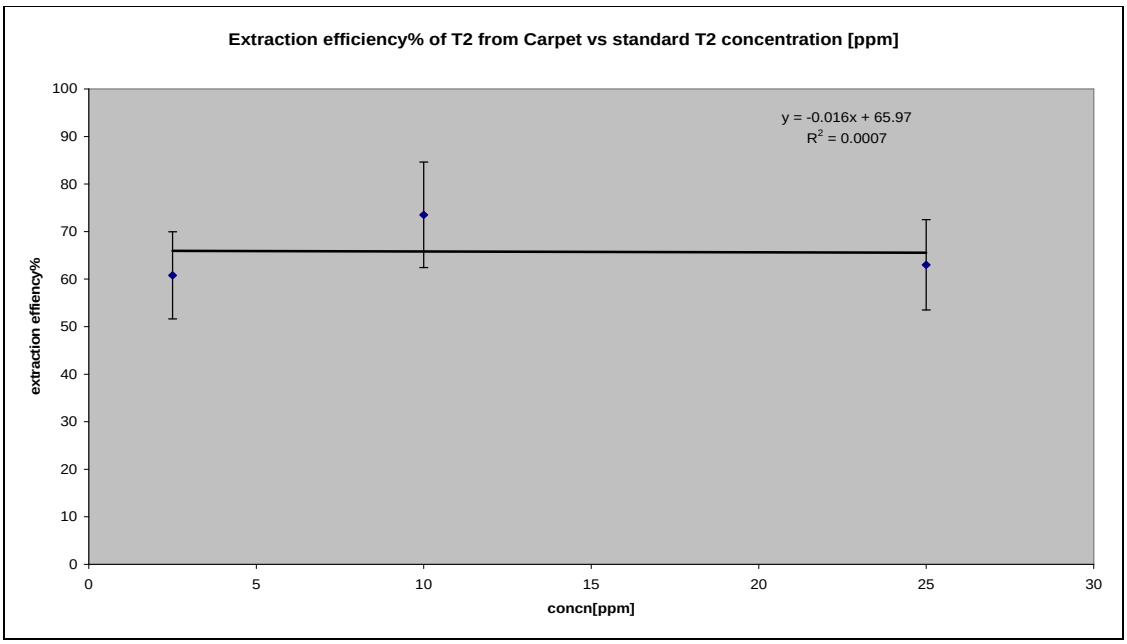


Table 21: T-2 toxin recovery from carpet

Spiked concentration	2.5ppm T-2	10ppm T-2	25ppm T-2
Ave. recovery%	60.8	73.5	63.0

Mean = 65.8%

Average standard deviation = 15.1%

Carpet extraction factor = 1.5

Refer to appendix (E) for raw data.

3.3.6 Recovery efficiency of T-2 toxin from parquet

Different matrices would give different extraction efficiency and thus, we needed to repeat the same procedure as in section 3.3.5 to determine the extraction efficiency factor of T-2 toxin from parquet. Figure 26 showed that the extraction efficiency of T-2 toxin from parquet was reasonably consistent over three different concentrations with a mean of 97.3% and standard deviation of 18.0% in Table 22. By taking the inverse of the mean recovery, the parquet extraction factor was derived to be 1.03 (i.e. 1/0.973).

Figure 26: Extraction Efficiency of T-2 toxin from Parquet

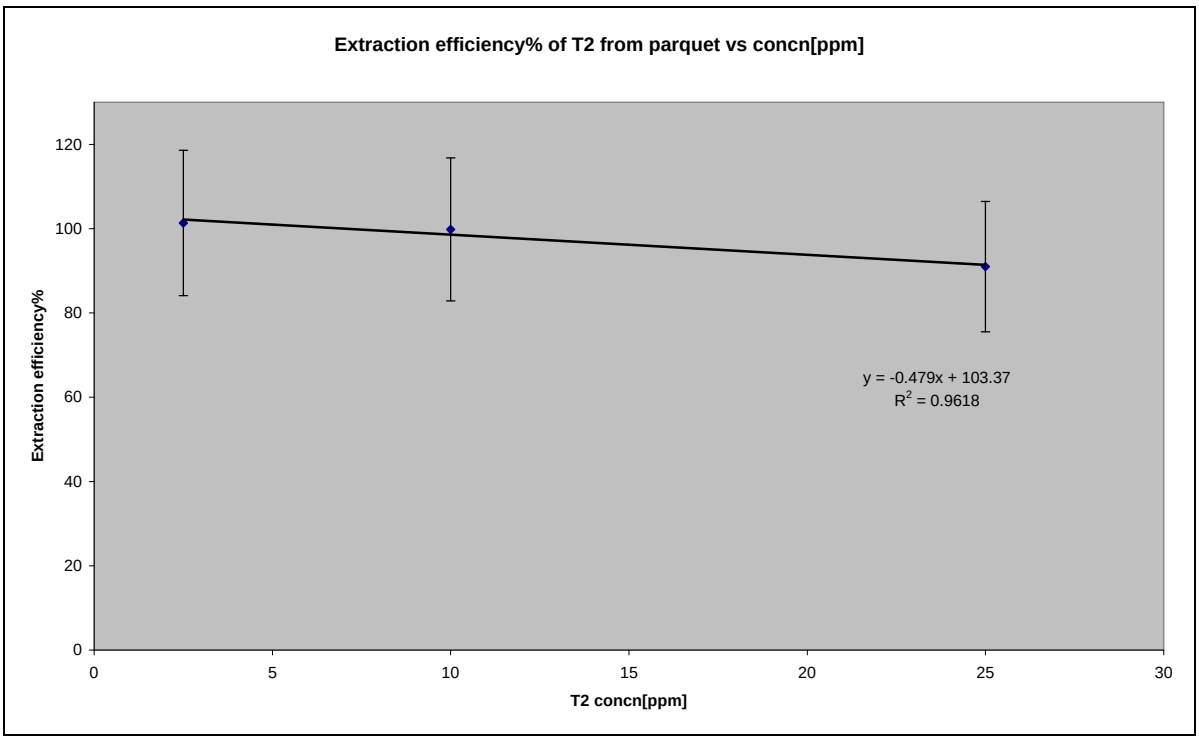


Table 22: T-2 toxin recovery from parquet

Spiked concentration	2.5ppm T-2	10ppm T-2	25ppm T-2
Ave. recovery%	101.1	99.9	90.7

Mean = 97.3%

Average standard deviation = 18.0%

Parquet extraction factor = 1.03

Refer to appendix (F) for raw data.

3.3.7 Stability profiles of T-2 toxin on Carpet and Parquet determined by HPLC-MS system

In Figure 27, T-2 toxin was observed to be more persistent on carpet showing an appreciable degradation only after 26th hour dropping to half of the spiked amount. It leveled off with an average recovery of 33.55% (taking into consideration the filter factor and extraction factor) by the end of the 7th day. Exact amount of T-2 toxin remaining from the spiked amount of 1mg was 1 x 0.34 = 0.34 mg/25 cm^2.

On comparison to parquet as shown in Figure 28, we could clearly see that the decay profile of T-2 toxin on parquet started almost instantaneously and by the 4th hour, it degraded to 50% of the initial spiked amount and leveled off from 12th hour onwards giving an average recovery of 19.50% (taking into consideration the filter factor and extraction factor). Exact amount of T-2 toxin remaining from the spiked amount of 1mg was 1 x 0.20 = 0.20 mg/25 cm^2.

We observed that T-2 toxin was not as stable on parquet compared to on carpet. The rate of degradation of T-2 toxin on parquet was much faster than carpet and the remaining amount at the end of 7th day was also lower. However, the stability of T-2 toxin on both matrices would come to a stable point where the recovery was consistent for the remaining 3 days.

Figure 27: Stability profile of T-2 toxin from Carpet using LC-MS

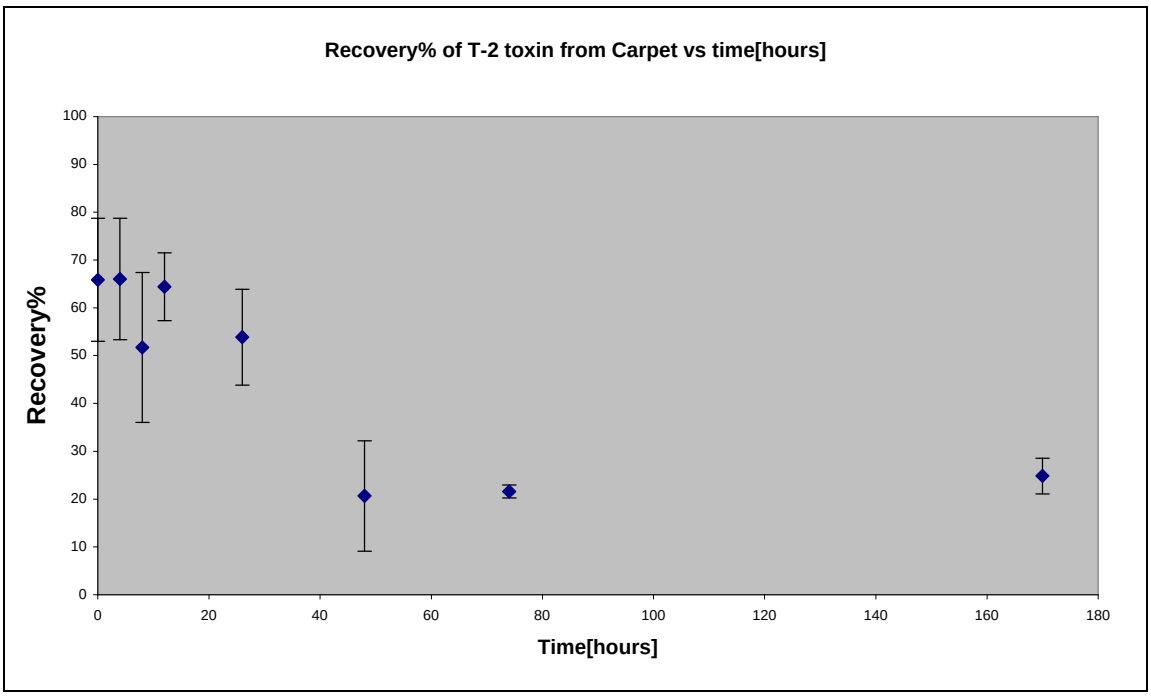
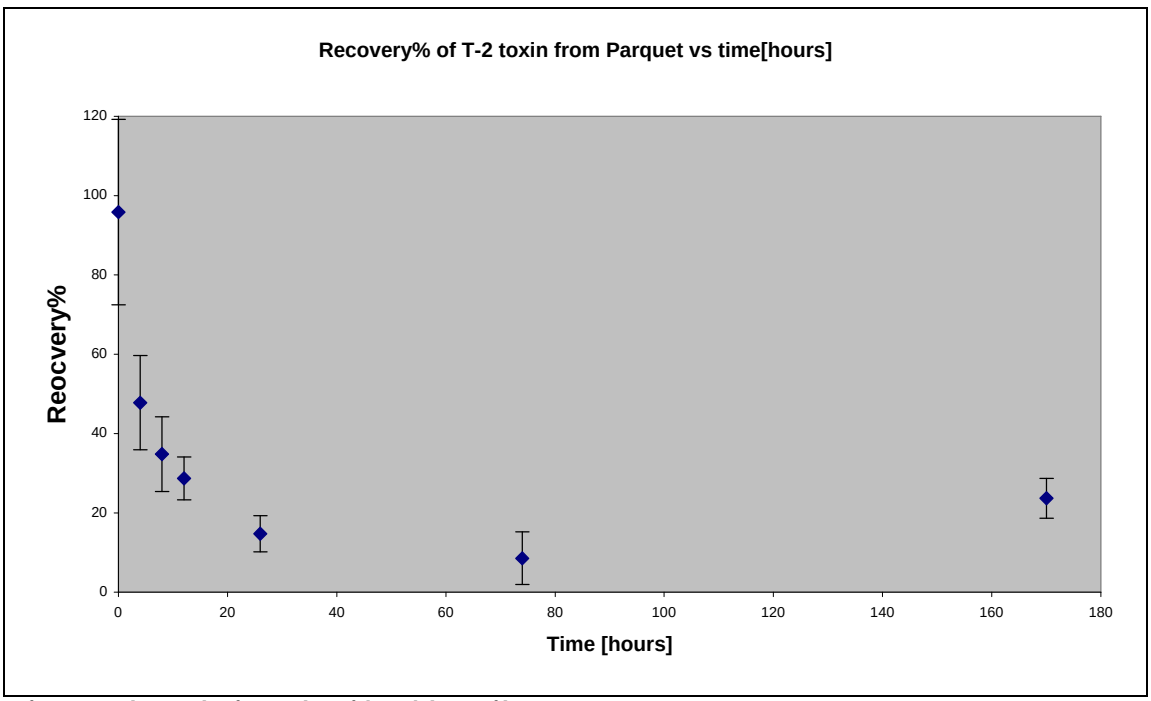


Figure 28: Stability profile of T-2 toxin from Parquet using LC-MS



Refer to appendix G and H for raw data of the stability profiles.

CHAPTER 4 DISCUSSION AND CONCLUSIONS

4.1 Stability profile of Ricin up to 170 hours on Carpet and Parquet

The stability of the toxin refers to the toxin being able to maintain its original form on the matrices. The recovery of the toxin refers to the toxin being able to extract out from the matrices. However, if the toxin was able to be recovered from the matrices and identified by its molecular mass as the respective toxin, it implied that this recovered amount of toxin was the toxin that stayed in its original form since its molecular mass was a unique characteristic of each toxin. Thus, the recovered toxin refers to the toxin that is stable in its original form on the matrices. This definition was applied to the three toxins used in this study.

The stability profile of ricin over 0, 2, 27, 50, 74 and 170 hours in an indoor office environment of 22.5-25.5^{0}C and relative humidity <70%, was studied by labeling digested sample extract with an amine isobaric tag (i.e. iTRAQ reagent) and analyzed using nano-flow liquid chromatography mass spectrometry (nano-flow LC-MS/MS). For carpet, an additional time interval at 8th hour was done as the drop between 2nd hour and 27th hour was too huge to see the trend. The verification of the feasibility of iTRAQ reagent labeling on ricin and SEB for quantitation work analyzed using nano-flow LC-MS/MS was done. It gave an approximately 1:1 ratio reading for two samples of identical concentration. This implied that iTRAQ labeling could give an accurate representation of the exact experimental amount and this labeling technique was applicable to ricin and SEB's quantitative work using nano-flow LC-MS/MS.

As the extraction was done using 10 ml of solvent, a concentration step was required before trypsin digestion. For ricin, a higher molecular weight cutoff Millipore Amicon centrifuge tube of 10 kDa was used for concentrating diluted toxin extract of 10 ml to approximately 200 ul since it had a much larger molecular weight of ~66 kDa. This concentrating step gave a concentration factor of 1 since with or without passing through the 10 kDa molecular cutoff centrifuge tube, it still quantified the same amount. Thus, there was no loss of sample during the concentrating step. The extraction solvent used was solely HPLC grade water as any content of organic solvents would result in less peptides detection. With carpet, it was required to vortex for 3 minutes after addition of 10 ml of HPLC grade water. This allowed an extraction efficiency of ricin from carpet to be 95% with CV% of 2.85%. Thus, the extraction efficiency factor was 1/0.95 = 1.06. As for parquet, it was vortexed for 1 minute and it gave an almost 100% recovery with CV% of 7.88%. This gave an extraction efficiency factor of 1 for parquet. With these two extraction factors determined for carpet and parquet, the exact amount of ricin remaining on the matrix can be determined by average iTRAQ ratio X 100ug spiked toxin X concentration factor X extraction factor.

After verification of these concentration and extraction factors, we did a stability time profile of ricin up to 7 days for carpet and parquet. The stability of ricin on carpet dropped drastically within 24 hours by 70% and gradually leveled off until the 7^{th} day, with 15 ug/cm^2 remaining (~20% remaining). But for ricin on parquet, it showed more stability with only a slight drop from 2 hours onwards to ~80% remaining. By the end of

7^{th} day, there was ~70% of the spiked amount remaining. Ricin showed two different trends for two different matrices implying that the stability of the same toxin could behave differently with different matrices under the same condition. Thus, the stability of ricin was matrix-dependent. The CV% was observed to be larger on carpet compared to on parquet. This is probably due to the nature of the surface that the toxin resided on. Carpet was "hollow" and absorptive compared to a smooth parquet surface and thus carpet would allow more "interaction" or absorption of toxin as the time of residence lengthened. This larger CV% was not due to the extraction technique as at time 0 hour, it had been verified that the CV% was less than 10% for both matrices.

4.2 Stability profile of SEB up to 170 hours on Carpet and Parquet

The same time intervals were used for SEB up to 170 hours in an indoor office environment. Based on iTRAQ labeling on digested SEB extracts, the concentration factor using Millipore Amicon ultra-15 5 kDa centrifuge tube for diluted extract sample of SEB gave a better reproducible recovery of 96.2% compared to 10 kDa centrifuge tube. Thus, the 5 kDa Millipore Amicon Ultra-15 centrifuge tube was used as the concentrating step for SEB. The concentration factor derived was 1.04 (i.e. 1/0.962). The extraction solvent used for carpet was 20%acetronitrile-80%water and for parquet was 100% HPLC grade water. The extraction efficiency factors for SEB from carpet and parquet were determined to be 95.3% and 74.0% respectively, giving extraction efficiency factors of 1.05 (i.e. 1/0.953) and 1.35 (i.e. 1/0.74).

SEB was found to be much more stable on parquet than carpet under indoor office environment of 22.5-25.5^{0}C and relative humidity <70% over 7 days. The SEB stability profile on carpet matrices showed a much more gradual degradation over time compared to ricin on carpet where the stability of ricin on carpet dropped to half by the 8th hour as compared to 27th hour for SEB on carpet. There was a significant amount of 19.5 ug/cm^2 SEB remaining at the end of 7th day on carpet using iTRAQ-nano-flow LC-MS/MS methodology. This was approximately 30% of the spiked amount remaining. For both toxins on carpet, the amount remaining was much higher than the LD_{50}. As for parquet, SEB showed almost no degradation in the stability profile. These stability profile experiments showed that the stability and fate of toxins were dependent on the nature of the toxins themselves as well as the matrices that these toxins were exposed to. On the whole, for proteinaceous toxins (SEB and ricin), they showed similar trends for both matrices except that SEB on parquet had been observed to be slightly more stable (i.e. ~100% remaining) compared to ricin on parquet (~70% remaining).

4.3 Advantages of our iTRAQ-nano-flow LC-MS/MS method compared to the commonly used ELISA method

With this new quantitative methodology of iTRAQ-nano-flow LC-MS/MS, it gave numerous advantages compared to the classical immunoassay method like ELISA, which had been the most commonly used method. In a single run based on iTRAQ labeled peptides obtained from the extract, it not only gave online identification of the toxin, it also allowed multiple samples detection as well as both relative and absolute

quantification in just 65 minutes. As mass spectrometry was involved, it allowed us to obtain unique characteristics of a substance, namely its molecular mass and structure. The same instrument can be used for CWA, BWA and mid-spectrum agents since each agent has its own unique molecular mass.

Using the MS/MS signature ions from the amine-derivated peptides (i.e. 114, 115, 116 and 117 peaks) of our extracts and compared with standard, the stability profile studies of SEB and ricin were feasible with quantitation since the triplicate was mixed in the same vial as the control. This new quantitative technique allowed identification of toxin based on the matched amino acid sequences obtained simultaneously as the quantitative ratio of iTRAQ labeled peptides using ProQUANT 1.0 software. It had the basic function as to ELISA for identification purpose but the time spent and the sample preparation required were much faster and easier by using iTRAQ-nano-flow LC-MS/MS. No isolation and purification were required before the analysis since it could be done online with our 1-dimensional LC. Our 1-dimensional LC consisted of a desalt column and a separation column for desalting and purifying samples. When comparison was made with a standard peptide, absolute quantitative work was possible. This could not be achieved by ELISA since it only allows relative quantitation.

However, in terms of the detection level, it was limited by the lowest detectable amount of iTRAQ labeled peptides. For iTRAQ labeled ricin to be detectable, it must be at least 5 ug and for SEB, it must be at least 1 ug. However, if we look in terms of a real

bioterrorist attack, this amount would be justifiable since the terrorists would release ample amount.

4.4 Stability profile of T-2 toxin up to 170 hours on Carpet and Parquet

The stability profile of T-2 toxin on carpet and parquet were studied using Liquid Chromatography-Mass Spectrometry (LC-MS). In our research study, we used Finngan LCQ. We had determined that the LOD for our LC-MS analysis on T-2 toxin was 0.05 ng/ul, which was comparable to GC methods. The filter factor was determined to be 1.18 since filtration was required before running the samples in LC. The extraction efficiency factors for parquet and carpet were derived at time 0 where they were 1.03 and 1.50 respectively. Benzophenone was used as internal standard for parquet but not carpet. No internal standard was used for carpet. We could not find a suitable internal standard as the elution time was near to the solvent elution time. However, as shown in Table 20, the deviation was less than 20%. We concluded that for carpet, we could do without internal standard since the purpose of internal standard was to eliminate any variation that arose and our experimental variation was within our criteria.

The stability profile of T-2 toxin was showed to be twice as stable on carpet compared to parquet under indoor conditions up to 7 days. On parquet under indoor conditions, the stability of T-2 toxin dropped by ~ 80% in 7 days. As for carpet, T-2 toxin was more stable and persistent with ~ 34% remaining after 7 days.

	Carpet	Parquet
Ave. amount of T-2 left after 7 days	0.34 mg/25cm^2	0.20 mg/25cm^2

For T-2 toxin, greater stability was observed on carpet compared to parquet where the stability on carpet dropped to half after 24 hours compared to after 4 hours on parquet. Contrary to T-2 toxin, the two proteinaceous toxins were extremely stable on parquet but degraded gradually on carpet. Parquet is an interesting indoor substrate that could 'naturally' degrade T-2 toxin to large extent (80% in 7 days) whereas SEB deposited on parquet would remain extremely stable and persistent. For ricin on parquet, the stability was good with ~70% remaining. Proteinaceous toxins (ricin and SEB) showed a completely opposite stability trend compared to non-proteinaceous toxin (T-2 toxin) on parquet. As for carpet, the percentage remaining at the end of 7th day for T-2 toxin was slightly more than the proteinaceous toxins.

4.5 Conclusions

In this research study, we had showed the feasibility of using amine isobaric labeling (i.e. iTRAQ reagents) on ricin and SEB and analysis by using nano-flow LC-MS/MS. This was the first study that showed the possibility of using iTRAQ reagents for both quantitative and identification studies on ricin and SEB using nano-flow LC-MS/MS. Two extraction factors were determined for carpet and parquet respectively and also the concentration factor for each toxin. The exact amount of ricin/SEB remaining on the matrix was calculated by taking the average iTRAQ ratio X 100ug spiked toxin X

concentration factor X extraction factor. We had determined the stability profiles for three BWA toxins namely ricin, SEB and T-2 toxin in an indoor office environment. The stability of ricin on carpet dropped drastically within 24 hours to ~30% remaining and gradually leveled off until the 7^{th} day, with 15ug/cm^2 remaining (~20% remaining). But for ricin on parquet, it showed better stability with ~70% remaining at the end of 7^{th} day.

SEB was also found to be more stable on parquet (~100% remaining) compared to carpet (~25% remaining). However, the stability profile of SEB on carpet matrices showed a more gradual drop over time compared to ricin on carpet where the stability of ricin on carpet dropped to half by the 8^{th} hour as compared to after 27^{th} hour for SEB on carpet. However, by the end of the 7^{th} day, both toxins on carpet had leveled off to ~21-25%. These were significant amounts of toxins remaining at the end of 7^{th} day which were more than the LD_{50} which we had noted to be alarming. As for T-2 toxin, greater stability was observed on carpet compared to parquet where slightly higher amount of ~35% remained on carpet compared to ~ 20% on parquet. On the carpet matrix, it was noted that the remaining percentage of the three toxins on carpet at the end of 7^{th} day was similar except that ricin's stability dropped at a faster rate compared to SEB and T-2 toxin. But on parquet, T-2 showed a completely opposite stability trend compared to ricin and SEB.

SEB was a more persistent toxin with greater environmental stability on parquet, followed by ricin and T-2 toxin. This new quantitative technique of iTRAQ-nano-flowLC-MS/MS allowed identification of ricin/SEB based on the matched amino acid

sequences obtained simultaneously with the quantitative ratio of iTRAQ labeled peptides using ProQUANT 1.0 software. It had the basic function as to ELISA for identification purpose. But it had the additional capability of obtaining absolute quantitation, which ELISA could not achieve. With this novel quantitative technique made available by iTRAQ-nano-flow LC-MS/MS, the response time for the appropriate countermeasure would be shortened since it gave identity, absolute quantity and number of different toxins being engaged but in a more rapid time frame with higher sensitivity in a single run. As for T-2 toxin, with our LC-ESI-ion trap MS, we were able to achieve the comparable LOD as common GC methods. This implied that LC-MS would be the next most potential method since it does not require derivatisation and is less laborious.

The low yield of toxins was not due to unsuitable extraction method. Each extraction method had been verified to give a good recovery of toxin with low CV% for triplicate set as shown in Table 10, 15, 21 and 22. The three toxins selected are rather stable as reviewed in Introduction but given an indoor office environment exposure for 7 days, they would probably be degraded over time. The loss of ricin and SEB would probably be due to bacterial degradation. And the loss of T-2 toxin would probably be due to fungal degradation. We could not answer whether there was any irreversible reaction with the matrices as in this study, we were only looking at the stability of toxin that still stayed in its original form on the matrices. If the toxin did have reaction with the matrices, it showed that this toxin was unstable on the matrices in addition to degradation factor.

With this iTRAQ technique, the peptides that were found in the control would be compared to the peptides that were found in the extract. We would get a comparison of the peptides that were present in both the control and extract by an average ratio reading of 115/116/117:114 (i.e. extract: control) through ProQUANT 1.0 software. As we were only interested in the “stable” toxin that stayed in its original form, the average ratio would give us the “stable” toxin amount and identification but not the degraded toxin. As for T-2 toxin, we utilized the selected ion monitoring mode looking at the particular molecular masses of 467 [466+H], 484 [466+H_2O] and 489 [466+Na] of T-2 toxin. Thus, if it degraded to other forms, we would not have observed as we did not use the scan mode which looked over the whole range of molecular masses. Only those T-2 toxin that were not degraded, would be detected by the three selected molecular masses to give a peak for quantitation.

All these data that we observed would allow us to react with proper medical action as well as counteractive measures upon any bio-terrorist attack.

4.6 Future studies

With this iTRAQ-nano-flow LC-MS/MS technology, we had achieved an understanding of the stability of three BWA namely ricin, SEB and T-2 toxin in an indoor office condition. It allowed us to deal with them in a better prospect in terms of medical or strategic planning. We could also do further study of these three BWA in a stimulated outdoor condition or even in rat blood serum for us to understand how stable these toxins would stay outdoor and in blood stream using this quantitative technology. One drawback of this iTRAQ-nano-flow LC-MS/MS technology would probably be the detection limit. However, we had explained in the discussion section that the possibility of bioterrorist attack would probably be in large quantity and thus, the detection limit of 5 ug (i.e. for ricin) and 1 ug (i.e. for SEB) would not be an issue.

Nevertheless, there is another new technique called multiple reaction monitoring-initiated detection and sequencing and in short known as "MIDAS". This technique is done by using 4000 Q-TRAP mass spectrometer from Applied Biosystem with its third quadrupole configured to quadrupole mass filter and linear ion trap by a MRM trigger. This MIDAS technique was introduced in May 2005 by Unwin, R. D. etc al [123]. In this technique, the triple quadrupole mass spectrometer first functions as a mass filter to perform MRM analysis where it selects significant MRM product ions that transmit from first quadrupole to the third quadrupole. Based on the signature peptides, it triggers a change of mode in the mass spectrometer to MS/MS product ion scan that allows generation of sequencing data and detection of peptides. MIDAS is a highly sensitive technique since MRM only allows ions of a specific m/z through the first quadrupole,

followed by fragmentation of this ion in the second quadrupole, then to the detector. Thus, the background noise would be extremely low giving an enhanced sensitivity and no stable isotope labeling would be required. This MIDAS technique would probably allow us to achieve a much lower detection limit, but it is still a relative quantitation method.

In a scenario where absolute quantitation is crucial, iTRAQ-nano-flow LC-MS/MS would probably be the choice. However, in a scenario where an extremely low amount of sample is available, MIDAS-LC-MS/MS would probably be handy but further study on its feasibility with these three BWA will still need to be verified before its usage.

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CHAPTER 6 APPENDICES

(A) Ricin on carpet over 170 hours

Pro Group Report: ricin on carpet 0hour

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (238 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	80	108	45.4
>95 (1.3)	2	2	201	238	100
>66 (0.47)	2	2	201	238	100
As shown: >95 (1.30)	2	2	201	238	100

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			-0.09	1004.48	2
1	ASDPSLK	K->R	SD	28.05	888.53	2
1	ASDPSLK			0.07	860.54	2
1	ASDPSLK	D->E	SL	14.09	874.56	2
1	ASDPSLK			80.17	940.64	2
1	CLTTUGUSPGVYVMIYDCNTAATDATRWQIWDNGTIINPR	M(Oxidation)	CLTT	16.03	4990.45	5
1	DGRFHNGNAIQLWPCJ	D->E	CJ	14.26	2157.37	4
1	DNCLTSDSNIR	D->E	SN	14.26	1394.9	2
1	DNCLTSDSNIR			14.26	1394.9	2
0	DNCLTSDSNIR			-28.02	1352.63	3
1	DNCLTSDSNIRETVVK	D->E / V->I	VVK	14.26	1951.23	3
1	DNCLTSDSNIRETVVK			0.12	1937.09	3
1	DNTIR	N(Deamidation)	DN	1.07	762.48	2
1	DNTIRSNGJ			-14.27	1277.45	3
1	DNTIRSNGK	N(Deamidation)	SN	1.05	1148.67	2

1	DNTIRSNGK			-14.05	1133.57	3
1	DNTIRSNGK			28.01	1175.63	3
1	ETVVJ			-14.04	848.5	2
1	ETVVK			-0.69	717.75	2
1	ETVVK			13.91	732.35	2
1	ETVVKILSCGPASSGQR	V->I	PASS	14.16	1889.17	4
1	ETVVKILSCGPASSGQR	K->R	SCG	28.12	1903.12	3
1	FQUIEGEMR	F->Y / M(Oxidation)	IE	15.7	1475.45	3
1	FQUIEGEMR	F->Y / M(Oxidation)	IE	15.72	1475.47	3
1	FQYIEGEMR			-26.17	1289.47	3
1	FQYIEGEMR			23.29	1338.93	2
1	FQYIEGEMR			-14.11	1301.52	3
1	FQYIEGEMR			-26.11	1289.53	3
1	FQYIEGEMR	Y->W	MR	23.29	1338.92	2
1	FQYIEGEMRTR	M->L	YI	-17.78	1555	2
99	FSVYDVSILIPIIALMVYR			-0.04	2355.3	3
1	GRLTTGADVR	R->K	TG	-27.87	1160.81	2
1	GRLTTGADVR			0.21	1188.88	2
0	GRLTTGADVR	T(O-Phosphoryl)	TGA	80.19	1268.87	2
1	HEIPVLPNR			1.08	1218.78	2
1	HEIPVLPNR			1.06	1218.76	2
1	ILSCGPASSGQRWMFJ			18.12	2073.19	3
1	ILSCGPASSGQRWMFK	F->Y / M(Oxidation)	MFK	16.28	1927.25	3
1	ILSCGPASSGQRWMFK			-13.72	1897.25	3
1	IRUNR			-28.03	980.59	2
1	IRUNR			-13.89	994.73	2
1	IRYNR			-15.7	848.8	2
1	IRYNR			-28	836.51	2
1	IRYNR			0	864.5	2
1	IVGRNGLCVDVR	N(Deamidation)	VR	1.19	1445	2
1	IVGRNGLCVDVR	N(Deamidation)	VR	1.17	1444.99	2
1	IVGRNGLCVDVR			1.17	1444.98	2
1	IVGRNGLCVDVR			1.21	1445.02	2

1	IVGRNGLCVDVR			1.15	1444.96	2
99	LEQLAGNLR			-0.03	1156.64	2
99	LEQLAGNLR			-0.01	1156.66	2
99	LEQLAGNLR			-0.05	1156.63	2
99	LEQLAGNLR			-0.03	1156.64	2
97	LEQLAGNLR	Q->E / N(Deamidation) / Q(Deamidation)	EQ	0.95	1157.62	2
1	LEQLAGNLR			18.09	1174.76	2
1	LEQLAGNLR	E->D	LAG	-14.13	1142.55	2
1	LEQLAGNLR	E->D	LAG	-14.01	1142.66	2
1	LEQLAGNLR			18.09	1174.77	2
1	LSTAIQESNQGAFASPIQLQR	Q->E / N(Deamidation) / Q(Deamidation)	TAIQ	0.97	2403.25	3
7	LTTGADVR	D->E / V->I	TT	14.01	989.57	2
1	LTTGADVR			14.01	989.57	2
1	LTTGADVR	R->K	TT	-27.93	947.62	2
1	LTTGADVR	R->K	GA	-27.91	947.64	2
1	LTTGADVR	T(O-Phosphoryl)	TTG	80.05	1055.61	2
1	NDGTILNLYSGLVLDVR	Y->H	DV	-25.8	1979.31	5
1	NDGTILNLYSGLVLDVR	Y->F	LNL	-15.8	1989.3	2
1	NGLCVDVR			1.15	1019.68	2
1	NGLCVDVR			14.13	1032.67	2
1	NGLCVDVRDGR			1.06	1347.75	3
1	NGLCVDVRDGR			17.94	1364.62	2
99	QIILUPLHGDPNQIWLPLF			-0.08	2564.37	3
99	QIILYPLHGDPNQIWLPLF			-0.08	2420.27	3
1	QIILYPLHGDPNQIWLPLF			-0.1	2420.24	5
1	RDNTIR			-13.78	903.75	2
1	RDNTIR			1.04	918.56	2
1	RDNTIR			14.18	931.7	2
1	RNGSK			28	732.41	2
1	RNGSK	R->K	GS	-27.74	676.66	2
1	RNGSK			-27.76	676.64	2

0	RSAPDPSVITLENSWGR	S(O-Phosphoryl) / T(O-Phosphoryl)	ENS	79.73	2107.78	3
99	SAPDPSVITLENSWGR			-0.03	1871.93	3
99	SAPDPSVITLENSWGR			-0.03	1871.93	3
99	SAPDPSVITLENSWGR	N(Deamidation)	TL	0.97	1872.93	3
1	SNGJ			1.17	693.59	2
1	TRIR			-13.79	674.66	2
1	UNRR	R->K	UN	-27.8	867.72	2
1	UNRR			0.2	895.73	2
1	UNRR			1.08	896.6	2
1	UTFAFGGNYDR			23.3	1621.08	2
1	UTFAFGGNYDR			23.3	1621.08	2
99	VGLPINQR			-0.05	1039.59	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.03	1039.6	2
99	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR	I->V	VG	-13.91	1025.72	3
98	WMFJ			-0.08	898.42	2
94	WMFJ			-0.02	898.48	2
1	WMFJ			0.04	898.54	2
1	WMFJ			0	898.5	2
1	WMFK			0.16	754.56	2
1	WMFK			-17.74	736.66	2
1	WMFK			-17.94	736.46	2
1	WMFK			-0.02	754.38	2
1	YNR			1.26	596.59	1
1	YNR			23.04	618.36	2
1	YNR			-27.92	567.41	1
1	YNRR			1.02	752.45	2
1	YTFAFGGNUDR	N(Deamidation)	FAF	1.24	1599.03	2

Pro Group Report: ricin on carpet 2hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (236 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	2	2	173	236	100
>95 (1.3)	2	2	173	236	100
>66 (0.47)	2	2	173	236	100
As shown: >95 (1.30)	2	2	173	236	100

Conf	Sequence	Mod	Zone	dMass	PrecMW	z
1	ASDPSLJ			-0.09	1004.49	2
0	ASDPSLJ			14.09	1018.67	2
1	ASDPSLK			80.13	940.61	2
1	ASDPSLK			28.11	888.58	2
1	ASDPSLK	K->R	SD	28.07	888.54	2
1	ASDPSLK	D->E	SL	14.07	874.55	2
1	DNCLTSDSNIR	R->K	CL	-27.72	1352.93	2
1	DNTIR	N(Deamidation)	DN	1.07	762.49	2
1	DNTIRSNGK	N(Deamidation)	SN	1.03	1148.65	2
1	DNTIRSNGK			-14.07	1133.54	3
1	DNTIRSNGK	N(Deamidation)	GK	1.13	1148.74	2
1	DNTIRSNGK			27.95	1175.56	3
1	ETVVJ	T(O-Phosphoryl)	VV	79.98	942.53	2
1	ETVVJ			-0.88	861.67	2
1	ETVVJ			-13.78	848.77	2
1	ETVVJ			-14.06	848.49	2
1	ETVVJILSCGPASSGQR	L->M	PASS	17.92	2037.03	3
1	ETVVK			0.25	718.68	2
1	ETVVK			-0.71	717.72	2
0	ETVVK			13.93	732.36	2
1	ETVVK			80.01	798.45	2
99	FSVYDVSILIPIIALMVYR			-0.04	2355.3	3
1	HEIPVLPNR			1.06	1218.76	2

1	ILSCGPASSGQRWMFJ	M->L	WM	-18.04	2037.03	3
1	IRYNR			-28.02	836.48	2
1	IRYNR			-28.12	836.38	2
1	IRYNR			-28.08	836.42	2
1	IRYNR			-28.12	836.39	2
1	IRYNR			-28	836.5	2
1	IRYNR	I->V	YN	-13.98	850.53	2
1	IRYNR			80.1	944.61	2
1	IRYNR			80.16	944.67	2
1	IRYNR			-26.02	838.48	2
1	IVGRNGLCVDVR			1.19	1445	2
1	IVGRNGLCVDVR	N(Deamidation)	VR	1.17	1444.98	2
1	IVGRNGLCVDVR	N(Deamidation)	VR	1.17	1444.98	2
1	IVGRNGLCVDVR			1.17	1444.98	2
99	LEQLAGNLR			-0.01	1156.66	2
99	LEQLAGNLR			-0.03	1156.65	2
99	LEQLAGNLR			-0.03	1156.65	2
1	LEQLAGNLR			18.09	1174.77	2
76	LEQLAGNLR			-0.03	1156.65	2
96	LEQLAGNLR			0.05	1156.72	2
99	LEQLAGNLR			-0.03	1156.65	2
1	LTTGADVR			13.97	989.52	2
1	LTTGADVR	R->K	GA	-27.93	947.63	2
1	LTTGADVR			80.05	1055.61	3
1	LTTGADVR			80.03	1055.59	2
1	NDGTILNLYSGLVLDVR	Y->F	LNL	-15.82	1989.29	2
1	NGLCVDVR			18.11	1036.65	2
1	NGLCVDVR			-27.81	990.73	2
1	NGLCVDVR	N(Deamidation)	DV	1.13	1019.67	2
1	NGLCVDVR	N(Deamidation)	DV	0.81	1019.34	2
1	NGLCVDVR			18.09	1036.63	2
1	NGLCVDVR			1.13	1019.66	2
1	NGLCVDVRDGR	N(Deamidation)	NGL	1.06	1347.75	3
1	NGLCVDVRDGR			17.96	1364.65	2
1	NGSKFSVYDVSILIPIIALMVYR	R->K	MVY	-27.73	2713.81	4
99	QIILUPLHGDPNQIWLPLF	Q->E / N(Deamidation) / Q(Deamidation)	QIW	0.94	2565.39	4

99	QIILUPLHGDPNQIWLPLF			-0.06	2564.39	3
99	QIILYPLHGDPNQIWLPLF			-0.04	2420.3	3
1	QIILYPLHGDPNQIWLPLF			-0.26	2420.08	3
1	RDNTIR			14.18	931.7	2
1	RDNTIR			0.12	917.64	3
1	RNGSJ			-27.75	820.76	2
5	RNGSK			-27.94	676.46	2
1	RNGSK			-27.76	676.65	2
29	SAPDPSVITLENSWGR	E->Q	PDP	-1.07	1870.88	3
1	SNTDANQLWTLK			13.85	1547.65	4
1	TRIR			-13.79	674.67	2
1	TRIR			-14.03	674.42	2
1	TRIR			-13.79	674.66	2
1	TRIR			-13.87	674.58	2
1	TRIR			-13.81	674.65	2
1	UNRR			-27.86	867.66	2
1	UNRR			-27.84	867.69	2
1	UNRR			1.06	896.59	2
1	UTFAFGGNYDR			23.28	1621.06	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.01	1039.62	2
99	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR	Q->E / N(Deamidation) / Q(Deamidation)	VG	1.15	1040.79	2
98	WMFJ			-0.04	898.47	2
85	WMFJ			-0.02	898.49	2
3	WMFJ			0	898.5	2
1	WMFJ			0.1	898.6	2
1	WMFJ			0	898.51	2
1	WMFJ			-17.98	880.53	2
1	WMFJ			-0.04	898.47	2
1	WMFK			0.14	754.55	2
1	YNR			23.04	618.36	2
1	YNR			1.08	596.41	1
1	YNRR			80.26	831.69	2
1	YNRR			1.02	752.45	2
1	YTFAFGGNUDR			23.28	1621.07	2
1	YTFAFGGNUDR	N(Deamidation)	FAF	1.24	1599.02	2

1	YTFAFGGNUDR	Y->W	YT	23.2	1620.99	3

Pro Group Report: Ricin on carpet 8hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (255 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	68	108	42.4
>95 (1.3)	2	2	193	255	100
>66 (0.47)	2	2	193	255	100
As shown: >95 (1.30)	2	2	193	255	100

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			-0.03	1004.55	2
1	ASDPSLJ			18.09	1022.66	2
1	ASDPSLJ			14.03	1018.6	2
1	ASDPSLK			28.07	888.55	2
1	ASDPSLK			14.11	874.59	2
1	ASDPSLK	D->E	SL	14.05	874.52	2
1	DNCLTSDSNIR			14.2	1394.84	2
1	DNCLTSDSNIRETVVJ	E->Q	NCLT	-0.71	2080.37	3
1	DNTIR			-13.71	747.71	2
1	DNTIR			-13.71	747.71	2
1	DNTIRSNGJ	I->V	RS	-14.25	1277.46	3
1	DNTIRSNGK	K->R	SN	27.93	1175.55	3
1	DNTIRSNGK	K->R	DN	27.93	1175.54	3
1	ETVVJ			-14.06	848.48	2
1	ETVVJ			-13.78	848.77	2
1	ETVVJ			13.96	876.5	2
1	ETVVJ			-0.9	861.65	2
0	ETVVK			0.17	718.6	2
0	ETVVK	E->D	VV	-13.77	704.66	2
0	ETVVK			0.17	718.61	2
1	ETVVK			0.17	718.6	2
1	ETVVK	V->I	TV	13.89	732.33	2
1	ETVVK			-0.03	718.41	2
1	ETVVK			28.25	746.68	2
0	ETVVK			0.17	718.6	2

1	ETVVKILSCGP ASSGQR	E->D / I->V	SCG	-13.86	1861.15	3
1	ETVVKILSCGP ASSGQR	Q->E	GQR	1.26	1876.26	3
1	ETVVKILSCGP ASSGQR	K->R	SCG	28.2	1903.2	3
1	ETVVKILSCGP ASSGQR	E->D / I->V	TVV	-14.16	1860.84	3
1	FHNGNAIQLWPCJ			-22.83	1792.13	3
1	FQUIEGEMR	F->Y / M(Oxidation)	IE	15.78	1475.52	3
1	FQUIEGEMRT R	E->D / I->V	FQ	-14.31	1702.59	3
1	FQYIEGEMR			-14.15	1301.49	3
1	FQYIEGEMR			15.83	1331.47	3
1	FQYIEGEMR			-14.15	1301.48	3
1	FQYIEGEMR			23.23	1338.86	2
1	FQYIEGEMR			-26.15	1289.48	3
1	FSVYDVSILIP IIALMVUR	Y->H	FS	-26.04	2473.4	4
1	GRLTTGADVR			-28.01	1160.66	2
99	HEIPVLPNR			-0.06	1217.65	3
99	HEIPVLPNR			-0.06	1217.65	3
99	HEIPVLPNR			-0.06	1217.64	3
1	IRYNR			-28.04	836.47	2
1	IRYNR			-26.04	838.46	2
99	LEQLAGNLR			-0.07	1156.61	2
99	LEQLAGNLR			-0.07	1156.61	2
99	LEQLAGNLR			-0.05	1156.62	2
1	LEQLAGNLR			18.05	1174.72	2
1	LEQLAGNLR			18.05	1174.72	2
1	LEQLAGNLR			-0.89	1155.79	2
0	LSTAIQESNQ GAFASPIQLQ R	Q->E	TAIQ	0.93	2403.2	3
1	LTTGADVR			-27.93	947.62	2
1	NDGTILNLUS GLVLDVR	L->M	DGTI	17.96	2167.17	4
0	NDGTILNLUSGLVLDVR			18.08	2167.29	4
1	NDGTILNLYSGLVLDVR			0.2	2005.3	3
43	NGLCVDVR			0.19	1018.72	2
1	NGLCVDVR			-27.91	990.63	2
1	NGLCVDVR			18.07	1036.6	2
1	NGLCVDVR	L->M	NG	18.09	1036.63	2
1	NGLCVDVR	D->E / V->I	NG	13.97	1032.5	3
1	NGLCVDVR			18.09	1036.62	2
1	NGLCVDVR			14.09	1032.63	2
1	NGLCVDVR			17.89	1036.43	3

1	NGLCVDVRDGR			17.94	1364.62	3
1	NGSKFSVYDVSILIPIIALMVYR	F->Y / M(Oxidation)	NGS	16.23	2757.76	4
99	QIILUPLHGDPNQIWLPLF			-0.14	2564.31	3
99	QIILYPLHGDPNQIWLPLF			-0.14	2420.21	3
1	RDNTIR	I->V	TI	-13.82	903.71	2
1	RDNTIR			14.1	931.63	2
1	RDNTIR			14.16	931.68	2
1	RDNTIR	I->V	TI	-13.86	903.66	2
1	RNGSK			-27.76	676.65	2
1	RNGSK			28.22	732.62	2
1	SAPDPSVITLENSWGR	E->D / I->V	PDP	-13.79	1858.17	3
1	SFIICIQMISEAAR	Q->E	AAR	1.11	1726.03	3
1	SFIICIQMISEAAR	Q->E	AAR	1.17	1726.09	3
1	TRIR			-13.81	674.64	2
1	TRIR			-13.81	674.64	2
1	TRIR			-13.81	674.64	2
1	UNRR			-27.84	867.68	2
1	UNRR			-27.88	867.64	2
1	UNRR			0.16	895.68	2
1	UTFAFGGNYDR			23.2	1620.99	2
99	VGLPINQR			-0.05	1039.58	2
99	VGLPINQR			-0.05	1039.58	2
99	VGLPINQR			-0.03	1039.6	2
3	VGLPINQR			-0.05	1039.59	2
3	VGLPINQR			-0.05	1039.59	2
1	VGLPINQR			-14.01	1025.63	3
1	VGLPINQR			0.89	1040.52	3
0	VGLPINQR			-28.05	1011.58	3
99	VGLPINQR			-0.09	1039.55	2
98	WMFJ			-0.08	898.42	2
21	WMFJ			-17.96	880.54	2
3	WMFJ			-0.04	898.46	2
0	WMFJ			-18	880.5	2
1	WMFJ			-17.76	880.75	2
1	WMFJ			-0.04	898.47	2
1	WMFJ			0.02	898.52	2
1	WMFJ			-0.02	898.48	2
1	WMFJ			-0.04	898.47	2
0	WMFJ			-17.98	880.53	2
1	WMFJNDGTILNLYSGLVLDVR	R->K	ILNL	-27.78	2713.71	4

1	WMFK			-22.72	731.69	2
1	WMFK			0.12	754.53	2
1	WMFK			0.14	754.54	2
1	YNR			23.02	618.34	2
1	YTFAFGGNYDR			15.75	1469.42	3

Pro Group Report: ricin on carpet 24hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (225 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	66	95	42.2
>95 (1.3)	1	1	66	95	42.2
>66 (0.47)	1	1	66	95	42.2
As shown: >95 (1.30)	1	1	66	95	42.2

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			-0.03	1004.55	2
1	ASDPSLK			14.13	874.6	2
1	ASDPSLK	K->R	SD	28.07	888.55	2
1	ASDPSLK			17.91	878.39	2
1	ASDPSLK	D->E	SL	14.07	874.55	2
1	ASDPSLK			18.17	878.65	2
1	DNCLTSDSNIR	D->E	DN	14.24	1394.88	2
1	DNTIR			-13.79	747.63	2
1	DNTIR			14.23	775.64	2
1	DNTIRSNGK	K->R	DN	28.05	1175.67	3
1	ETVVJ			-14.04	848.5	2
5	ETVVJILSCGPASSGQR			17.88	2036.99	3
2	ETVVK			0.19	718.62	2
1	ETVVK			28.25	746.69	2
1	ETVVK			-0.01	718.43	2
0	ETVVK			-14.13	704.31	2
1	FQUIEGEMR			-28.14	1431.6	3
1	FQYIEGEMR			-14.11	1301.52	3
1	FQYIEGEMR			23.27	1338.91	2
1	FQYIEGEMR			-14.19	1301.45	3
1	FQYIEGEMR	Y->W	MR	23.27	1338.91	2

0	FQYIEGEMR			-14.17	1301.46	3
0	FQYIEGEMRTR			0.18	1572.96	3
99	FSVYDVSILIPIIALMVYR			-0.06	2355.29	3
1	GRLTTGADVR	R->K	GR	-28.01	1160.66	2
99	HEIPVLPNR			-0.04	1217.67	3
99	HEIPVLPNR			-0.04	1217.66	3
99	HEIPVLPNR			-0.04	1217.66	3
1	ILSCGPASSGQR	Q->E	PAS	0.7	1319.38	4
1	ILSCGPASSGQR	R->K	SSG	-27.88	1290.8	3
1	ILSCGPASSGQRWMFK			-13.76	1897.21	3
1	ILSCGPASSGQRWMFK			0.22	1911.19	4
1	IRUNR			-28.09	980.52	2
1	IRYNR			-26.02	838.48	2
1	IRYNR			-28.02	836.49	2
1	IRYNR			-0.02	864.49	2
1	IRYNR	I->V	YN	-13.96	850.54	2
1	IRYNR			-28.02	836.49	2
1	IRYNR			-28	836.5	2
1	IRYNR			23.14	887.65	2
1	IRYNR			23.14	887.64	2
99	LEQLAGNLR	Q->E	EQ	0.93	1157.61	2
99	LEQLAGNLR			-0.03	1156.64	2
99	LEQLAGNLR			-0.05	1156.63	2
99	LEQLAGNLR			-0.05	1156.62	2
1	LTTGADVR	R->K	TT	-27.93	947.63	2
1	LTTGADVR			0.09	975.65	2
1	NDGTILNLYSGLVLDVR	Y->F	LNL	-15.8	1989.31	2
1	NDGTILNLYSGLVLDVR	Y->H	LVL	-25.94	1979.17	4
1	NGLCVDVR			18.17	1036.7	2
1	NGLCVDVR			13.97	1032.51	2
1	NGLCVDVR			18.07	1036.6	2
1	NGLCVDVR			0.13	1018.67	2
1	NGLCVDVR			-27.85	990.69	2
1	NGLCVDVR			18.09	1036.63	2
1	NGLCVDVR			18.11	1036.64	2
1	NGLCVDVRDGR	L->M	CV	17.88	1364.56	2
99	QIILUPLHGDPNQIWLPLF			-0.1	2564.35	3
99	QIILYPLHGDPNQIWLPLF			-0.12	2420.22	3

1	QIILYPLHGDPNQIWLPLF			-0.12	2420.23	5
1	RDNTIR			14.16	931.68	2
1	RDNTIR			-13.84	903.68	2
1	RDNTIR			14.1	931.62	2
1	RNGSJ			-28.05	820.46	2
2	RNGSK			-27.94	676.46	2
1	SAPDPSVITLENSWGR	W->Y	SA	-22.77	1849.19	3
1	SNTDANQLWTLK	W->Y	QL	-22.83	1510.97	2
1	SNTDANQLWTLK	W->Y	NT	-22.81	1510.98	2
1	TRIR			-13.81	674.65	2
1	UNRR			0.2	895.72	2
1	UTFAFGGNYDR			23.26	1621.04	2
1	UTFAFGGNYDR			23.24	1621.03	2
99	VGLPINQR			-0.05	1039.59	2
99	VGLPINQR			-0.05	1039.59	2
99	VGLPINQR			-0.07	1039.56	2
3	VGLPINQR			-28.05	1011.59	2
1	VGLPINQR			0.91	1040.54	3
1	VGLPINQR			18.07	1057.7	2
93	VGLPINQR			-0.01	1039.62	2
98	WMFJ			-0.06	898.44	2
1	WMFJ			0.1	898.6	2
1	WMFJ			-17.74	880.76	2
1	WMFJ			0.04	898.54	2
1	WMFJ			-17.84	880.67	2
1	WMFJ			-0.02	898.49	2
1	WMFJ			16.06	914.57	3
1	WMFJ			-22.74	875.76	2
1	WMFJ			-17.96	880.54	2
1	WMFJ			16.04	914.54	2
1	WMFK			0.14	754.55	2
1	WMFK			-22.72	731.69	2
0	WMFK			-17.74	736.66	2
1	YNR			23.02	618.35	2
1	YNRR			-27.72	723.71	2
1	YNRR			0.22	751.64	2

Pro Group Report: ricin on carpet 48hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (222 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	63	92	41.4
>95 (1.3)	1	1	63	92	41.4
>66 (0.47)	1	1	63	92	41.4
As shown: >95 (1.30)	1	1	63	92	41.4

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			14.11	1018.68	2
1	ASDPSLJ			0.21	1004.78	2
1	ASDPSLJ	L->M	PS	18.13	1022.7	2
1	ASDPSLK			18.11	878.59	2
1	ASDPSLK			0.07	860.54	2
1	ASDPSLK	K->R	SD	28.09	888.57	2
1	ASDPSLK	D->E	SL	14.07	874.55	2
1	AVRGR			-27.72	673.72	2
1	DNCLTSDSNIR			14.24	1394.89	2
1	DNTIRSNGJ			-0.03	1291.68	2
1	DNTIRSNGK	K->R	SN	27.93	1175.54	3
1	DNTIRSNGK	K->R	DN	27.95	1175.57	3
1	DNTIRSNGK	R->K	NT	-27.91	1119.7	2
1	ETVVJ			-14.04	848.51	2
1	ETVVJ			-0.88	861.67	2
1	ETVVJ			-14.1	848.45	2
2	ETVVJILSCGPASSGQR			17.86	2036.97	3
0	ETVVK			0.23	718.66	2
0	ETVVK	V->I	TV	14.19	732.63	2
1	ETVVKILSCGPASSGQR	E->Q	TVV	-0.8	1874.2	3
1	FHNGNAIQLWPCJ	W->Y	HN	-22.75	1792.21	3
1	FHNGNAIQLWPCJ	W->Y	GNA	-22.77	1792.19	3
1	FQUIEGEMRTR	E->D / I->V	UI	-14.25	1702.65	3
1	FQYIEGEMR			23.27	1338.9	2

1	FQYIEGEMR			-14.15	1301.49	3
99	FSVYDVSILIPIIALMVYR			-0.04	2355.3	3
0	GRLTTGADVR			0.05	1188.73	2
99	HEIPVLPNR			-0.02	1217.69	3
99	HEIPVLPNR			-0.04	1217.67	3
93	HEIPVLPNR			-0.04	1217.67	3
1	ILSCGPASSGQRWMFK			-0.2	1910.77	4
1	IRYNR	I->V	YN	-13.96	850.55	2
1	IRYNR			-13.94	850.57	2
1	IRYNR			-28	836.5	2
1	IRYNR			-28.02	836.49	2
1	IRYNR			23.16	887.67	2
1	IRYNR			23.14	887.65	2
1	IRYNR			23.12	887.63	2
1	IRYNR			23.16	887.66	2
1	IRYNR	I->V	YN	-13.98	850.53	2
1	IRYNR			0	864.5	2
1	IRYNR			-26.02	838.49	2
1	IVGRNGLCVDVR			0.07	1443.89	3
1	LEQLAGNLR	L->M	LAG	17.91	1174.59	2
99	LEQLAGNLR			-0.03	1156.65	2
99	LEQLAGNLR			-0.05	1156.63	2
99	LEQLAGNLR			-0.03	1156.65	2
1	LEQLAGNLR			18.09	1174.76	2
32	LSTAIQESNQGAFASPIQLQR	Q->E	QGAFA	0.99	2403.26	3
1	LTTGADVR			0.09	975.65	2
1	LTTGADVR			14.01	989.56	3
1	NDGTILNLYSGLVLDVR	Y->F	LNL	-15.8	1989.31	2
1	NDGTILNLYSGLVLDVR			-26	1979.11	4
1	NGLCVDVR			18.09	1036.63	2
1	NGLCVDVR			18.07	1036.61	2
1	NGLCVDVR			0.13	1018.67	2
1	NGLCVDVR			18.11	1036.64	2
1	NGLCVDVR			18.09	1036.63	2
99	QIILUPLHGDPNQIWLPLF			-0.06	2564.39	3
99	QIILYPLHGDPNQIWLPLF			-0.06	2420.28	3
1	RDNTIR	I->V	RD	-13.8	903.73	2
1	RDNTIR			14.18	931.71	2
1	RDNTIR			14.18	931.7	2
1	RDNTIR			14.16	931.69	2
1	RNGSJ			-28.05	820.47	2

1	RNGSJ			-27.75	820.76	2
1	RNGSK			-27.94	676.46	2
1	SAPDPSVITLENSWGR	E->D / I->V	WGR	-13.73	1858.23	3
1	SFIICIQMISEAAR	Q->E	AAR	1.27	1726.18	3
1	SNTDANQLWTLJR			18.15	1852.14	3
1	SNTDANQLWTLJR			0.19	1834.18	3
1	SNTDANQLWTLK	W->Y	NT	-22.81	1510.99	2
1	SNTDANQLWTLK	W->Y	NT	-22.81	1510.99	2
1	TRIR			-13.79	674.67	2
82	UNRR			0.14	895.66	2
1	UNRR			0.18	895.7	2
1	UNRR			0.18	895.7	2
1	UNRR			0.16	895.69	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.07	1039.58	2
99	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR	Q->E	VG	0.91	1040.55	3
0	VGLPINQR			-28.05	1011.58	3
98	WMFJ			-0.04	898.46	2
1	WMFJ			-22.78	875.73	2
1	WMFJ			0.04	898.55	2
1	WMFJ			0	898.5	2
1	WMFK			0.16	754.56	2
1	WMFK			0.14	754.55	2
1	YNR			23.02	618.35	2
1	YTFAFGGNUDR	Y->W	GGN	23.28	1621.07	2
1	YTFAFGGNUDR			14.2	1611.99	3

Pro Group Report: ricin on carpet 72hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (225 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	66	85	37.8
>95 (1.3)	2	2	186	225	100
>66 (0.47)	2	2	186	225	100
As shown: >95 (1.30)	2	2	186	225	100

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			14.13	1018.7	2
1	ASDPSLJ			0.01	1004.59	2
1	ASDPSLK			0.07	860.54	2
1	ASDPSLK	D->E	SL	14.07	874.55	2
1	ASDPSLK	K->R	SD	28.09	888.57	2
1	DNCLTSDSNIRETVVK	R->K	SNI	-28.14	1908.83	4
1	DNTIR			-13.77	747.64	2
1	DNTIRSNGJ	I->V	NG	-14.29	1277.42	3
1	DNTIRSNGK	K->R	SN	27.95	1175.57	3
1	ETVVJ			-14.04	848.51	2
1	ETVVJILSCGPASSGQR	E->D / I->V	TVV	-13.8	2005.3	3
2	ETVVJILSCGPASSGQR			17.88	2036.99	3
1	ETVVK			14.21	732.64	2
1	ETVVK			0.01	718.45	2
0	ETVVK			0.23	718.66	2
1	ETVVKILSCGPASSGQR	K->R	SCG	28.12	1903.13	3
1	FHNGNAIQLWPCJSNTDANQLWTLK	D->E	FH	14.13	3200.77	4
1	FQYIEGEMR			-15.79	1299.85	3
1	FQYIEGEMR			-26.23	1289.41	3
1	FQYIEGEMR			-14.13	1301.5	3
1	FQYIEGEMRTR	M->L	YI	-17.82	1554.97	3
1	FQYIEGEMRTR			-17.76	1555.03	2
1	FQYIEGEMRTR			-17.76	1555.02	2

99	FSVYDVSILIPIIALMVYR			-0.02	2355.32	3
1	GRLTTGADVR			0.13	1188.81	2
1	GRLTTGADVR	R->K	GR	-27.99	1160.69	2
99	HEIPVLPNR			-0.02	1217.68	3
99	HEIPVLPNR			-0.02	1217.68	3
0	HEIPVLPNR			-0.88	1216.83	2
1	ILSCGPASSGQR	I->V	SCG	-13.74	1304.95	2
1	ILSCGPASSGQR	Q->E	SCG	0.74	1319.42	4
1	IRYNR			-27.84	836.66	2
1	IRYNR			23.14	887.65	2
1	IRYNR	I->V	YN	-13.92	850.58	1
1	IRYNR	Y->F	YN	-15.7	848.8	2
1	IRYNR			-26.02	838.49	2
1	IRYNR	I->V	YN	-13.8	850.71	2
1	IRYNR			-28	836.51	2
1	IRYNR			-13.96	850.54	2
1	IRYNR			-25.94	838.56	2
99	LEQLAGNLR			-0.03	1156.64	2
99	LEQLAGNLR			-0.07	1156.6	2
1	LEQLAGNLR			0.01	1156.69	2
1	LEQLAGNLR			18.13	1174.8	2
57	LSTAIQESNQGAFASPIQLQR	Q->E	QGAFA	0.99	2403.27	3
1	LTTGADVR			0.11	975.66	2
1	LTTGADVR	R->K	GA	-27.93	947.63	2
1	NDGTILNLYSGLVLDVR	Y->F	LNL	-15.78	1989.32	2
1	NGLCVDVR			0.15	1018.68	2
9	NGLCVDVR			0.21	1018.74	2
99	QIILUPLHGDPNQIWLPLF			-0.06	2564.38	3
99	QIILYPLHGDPNQIWLPLF			-0.06	2420.29	3
55	QIILYPLHGDPNQIWLPLF			-0.08	2420.27	4
1	RDNTIR			14.2	931.72	2
1	RDNTIR			-13.82	903.7	2
1	RDNTIR			14.2	931.73	2
1	RDNTIR			14.18	931.71	2
1	RNGSJ			-28.05	820.47	2
1	RNGSK			-27.94	676.47	2
1	RNGSK			27.96	732.36	2
99	SAPDPSVITLENSWGR			-0.03	1871.93	3

1	SAPDPSVITLENSWGR	E->D / I->V	WGR	-13.75	1858.2	3
1	SNTDANQLWTLJ			13.77	1691.67	5
1	SNTDANQLWTLJR			18.21	1852.21	3
0	SNTDANQLWTLK			17.81	1551.61	4
1	SNTDANQLWTLK	W->Y	QL	-22.83	1510.97	2
1	TRIR			-13.79	674.66	2
1	UNRR			0.16	895.68	2
1	UNRR			-27.84	867.68	2
1	UTFAFGGNYDR			23.28	1621.07	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.01	1039.62	2
99	VGLPINQR			-0.05	1039.59	2
1	VGLPINQR	Q->E	VG	0.93	1040.56	3
1	VGLPINQR	R->K	VG	-28.01	1011.63	2
98	WMFJ			-0.06	898.45	2
85	WMFJ			-0.02	898.49	2
1	WMFJ			0.12	898.62	2
1	WMFJ			16.04	914.55	2
1	WMFJ			0	898.5	2
1	WMFJ			-22.74	875.76	2
0	WMFJ			-17.74	880.77	2
1	WMFK			0.16	754.56	2
1	YNR			23.04	618.36	2
1	YTFAFGGNUDR	Y->W	GGN	23.3	1621.08	2

Pro Group Report: ricin on carpet 170hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (224 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	72	92	41.1
>95 (1.3)	1	1	72	92	41.1
>66 (0.47)	1	1	72	92	41.1
As shown: >95 (1.30)	1	1	72	92	41.1

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			0.09	1004.66	2
1	ASDPSLJ			18.13	1022.71	2
1	ASDPSLK			0.07	860.55	2
1	ASDPSLK			14.15	874.62	2
1	ASDPSLK	D->E	SL	14.11	874.58	2
1	ASDPSLK	K->R	SD	28.09	888.57	2
1	DNCLTSDSNIR	D->E	IR	14.24	1394.89	2
1	DNTIR			-13.77	747.64	2
1	DNTIRSNGJ	R->K	RS	-28.05	1263.66	3
1	DNTIRSNGJ			-0.03	1291.68	2
1	DNTIRSNGK	R->K	NT	-27.93	1119.69	2
1	DNTIRSNGK	K->R	SN	27.95	1175.56	3
1	DNTIRSNGK			-14.07	1133.54	3
1	ETVVJ			-0.86	861.68	2
1	ETVVK			28.25	746.69	2
1	ETVVK			13.99	732.42	2
0	ETVVK			0.23	718.66	2
1	FHNGNAIQLWPCJ			-0.01	1814.94	3
1	FQUIEGEMR	F->Y / M(Oxidation)	IE	15.82	1475.57	3
1	FQYIEGEMR	Y->W	MR	23.29	1338.92	2
1	FQYIEGEMR	Y->W	MR	23.27	1338.91	2
0	FQYIEGEMR	E->D / I->V	GE	-14.13	1301.5	3
1	FQYIEGEMRTR	M->L	YI	-17.76	1555.02	2

1	FSVUDVSILIP IIALMVUR	D->E / V->I	ALMV	14.18	2657.72	4
99	FSVYDVSILIPIIALMVYR			-0.02	2355.32	3
1	GRLTTGADVR			0.09	1188.77	2
1	GRLTTGADVR			0.13	1188.81	2
1	HEIPVLPNR			17.8	1235.51	3
1	HEIPVLPNR			-0.88	1216.82	2
99	HEIPVLPNR			-0.02	1217.68	3
99	HEIPVLPNR			-0.02	1217.68	3
1	HEIPVLPNRVGLPINQR			0.05	2095.26	4
1	ILSCGPASSGQR			-13.78	1304.91	2
1	IRUNR			0.01	1008.63	2
1	IRYNR			23.18	887.69	2
1	IRYNR			23.16	887.66	2
1	IRYNR			-27.88	836.62	2
1	IRYNR			-26.02	838.49	2
1	IRYNR			23.16	887.67	2
1	IRYNR			0	864.5	2
1	IRYNR			-28	836.5	2
0	IVGRNGLCVDVR			-28.27	1415.54	4
99	LEQLAGNLR			-0.07	1156.6	2
99	LEQLAGNLR			-0.03	1156.65	2
99	LEQLAGNLR			-0.01	1156.66	2
2	LSTAIQESNQ GAFASPIQLQ R	Q->E	TAIQ	0.97	2403.25	3
0	LSTAIQESNQ GAFASPIQLQ RR	R->K	ESNQ	-28.05	2530.33	4
1	LTTGADVR			-27.91	947.64	2
1	LTTGADVR	R->K	GA	-27.91	947.65	2
1	LTTGADVR			0.11	975.66	2
1	LTTGADVR			13.99	989.55	3
1	NDGTILNLYS GLVLDVR	Y->F	LNL	-15.78	1989.33	2
1	NDGTILNLYSGLVLDVR			0.22	2005.33	3
1	NGLCVDVR			0.15	1018.68	2
1	NGLCVDVR			18.07	1036.6	2
1	NGLCVDVR			18.11	1036.64	2
1	NGLCVDVR			18.11	1036.64	2
1	NGLCVDVRDGR			18.26	1364.94	2
1	NGLCVDVRD GR	L->M	CV	17.94	1364.63	2
99	QIILUPLHGDPNQIWLPLF			-0.08	2564.37	3

99	QIILYPLHGDPNQIWLPLF			-0.04	2420.3	3
1	RDNTIR			14.18	931.7	2
1	RDNTIR	R->K	RD	-27.88	889.65	2
1	RDNTIR			14.2	931.72	2
1	RDNTIR			-13.8	903.72	2
1	RDNTIR			-13.86	903.67	2
1	RNGSJ			-27.77	820.75	2
1	RNGSK			27.96	732.36	2
1	RNGSK			-28.02	676.38	2
99	SAPDPSVITLENSWGR			-0.01	1871.94	3
1	SAPDPSVITLENSWGR	W->Y	PDP	-22.75	1849.2	3
1	SNGJCLTTUGYSPGVUVMIUDCNTAATDATR		UGYSPGV	22.72	4077.73	5
1	SNTDANQLWTLK	W->Y	NT	-22.79	1511	2
1	SNTDANQLWTLK	W->Y	NT	-22.79	1511	2
1	SNTDANQLWTLKR	W->Y	NT	-22.77	1667.13	2
1	TRIR			-13.79	674.67	2
1	UNRR			0.18	895.71	2
1	UNRR			-27.84	867.68	2
1	UNRR			0.2	895.72	2
99	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR			18.07	1057.71	2
1	VGLPINQR	Q->E	VG	0.91	1040.55	3
98	WMFJ			-0.04	898.46	2
43	WMFJ			-0.02	898.49	2
1	WMFJ			0.1	898.61	2
1	WMFJ			-17.74	880.77	2
1	WMFJ			0	898.5	2
1	WMFJ			-22.72	875.79	2
1	WMFK			0.16	754.56	2
1	YNR			23.04	618.36	2
10	YTFAFGGNYDR	Y->F	FA	-15.81	1437.87	3
0	YTFAFGGNYDRLEQLAGNLR			-27.97	2420.27	5

(B) Ricin on parquet over 170 hours

Pro Group Report:ricin on parquet 0hour

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (61 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	17	41	67.2
>95 (1.3)	1	1	17	41	67.2
>66 (0.47)	1	1	17	41	67.2
As shown: >95 (1.30)	1	1	17	41	67.2

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			0.23	1004.8	2
1	ETVVJ			-0.22	862.32	2
99	FSVYDVSILIPIIALMVYR			-0.02	2355.32	3
99	HEIPVLPNR			-0.02	1217.68	3
99	HEIPVLPNR			-0.02	1217.68	3
1	IRYNR			-0.2	864.3	2
98	LEQLAGNLR			-0.03	1156.64	2
1	LEQLAGNLR			0.01	1156.69	2
1	LEQLAGNLR			-0.05	1156.62	2
99	LSTAIQESNQGAFASPIQLQR			-0.05	2402.22	3
99	LSTAIQESNQGAFASPIQLQR			0.01	2402.28	3
99	LSTAIQESNQGAFASPIQLQR			-0.01	2402.26	3
99	LSTAIQESNQGAFASPIQLQR			-0.07	2402.21	3
99	NDGTILNLUSGLVLDVR			-0.04	2149.17	3

99	NDGTILNLYSGLVLDVR			-0.04	2005.07	3
99	NDGTILNLYSGLVLDVR			-0.02	2005.09	3
99	NDGTILNLYSGLVLDVR			-0.04	2005.07	3
99	QIILUPLHGDPNQIWLPLF			-0.04	2564.41	3
99	QIILYPLHGDPNQIWLPLF			-0.02	2420.32	3
99	QIILYPLHGDPNQIWLPLF			-0.02	2420.32	3
99	QIILYPLHGDPNQIWLPLF			-0.06	2420.29	3
99	SAPDPSVITLENSWGR			-0.03	1871.93	3
99	SAPDPSVITLENSWGR			-0.03	1871.92	3
99	SAPDPSVITLENSWGR			-0.03	1871.93	3
99	SAPDPSVITLENSWGR			-0.09	1871.86	2
99	VGLPINQR			-0.03	1039.61	2
99	VGLPINQR			-0.03	1039.61	2
95	VGLPINQR			-0.03	1039.61	2
69	VGLPINQR			-0.03	1039.61	2
3	VGLPINQR			-0.01	1039.62	2
22	WMFJ			-0.04	898.47	2
22	WMFJ			-0.06	898.45	2
3	WMFJ			-0.02	898.49	2
1	WMFJ			-0.02	898.49	2
1	WMFJ			-0.02	898.48	2
1	WMFJ			-0.02	898.49	2
1	WMFJ			-0.12	898.38	2
1	WMFJNDGTILNLYSGLVLDVR			-0.14	2741.35	4
1	WMFK			0.16	754.56	2
99	YTFAFGGNYDRLEQLAGNLR			-0.05	2448.19	3

99	YTFAFGGNYDRLEQLAGNLR			-0.05	2448.18	3

Pro Group Report: ricin on parquet 2hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (48 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	19	41	85.4
>95 (1.3)	1	1	19	41	85.4
>66 (0.47)	1	1	19	41	85.4
As shown: >95 (1.30)	1	1	19	41	85.4

Conf	Sequence	Mod	Zone	dMass	PrecMW
1	ASDPSLJ			0.23	1004.81
1	ASDPSLK			-0.29	860.18
99	FSVYDVSILIPIIALMVYR			-0.02	2355.33
1	HEIPVLPNR			0	1217.71
1	IRYNR			0.22	864.73
1	IRYNR			0.12	864.62
99	LEQLAGNLR			0.01	1156.68
99	LEQLAGNLR			-0.03	1156.65
99	LEQLAGNLR			-0.03	1156.65
99	LEQLAGNLR			-0.01	1156.66
99	LEQLAGNLR			-0.01	1156.67
99	LSTAIQESNQGAFASPIQLQR			-0.01	2402.26
99	LSTAIQESNQGAFASPIQLQR			-0.03	2402.25
99	LSTAIQESNQGAFASPIQLQR			-0.05	2402.22
1	NDGTILNLUSGLVLDVR			-0.06	2149.15
99	NDGTILNLYSGLVLDVR			-0.02	2005.09
95	QIILUPLHGDPNQIWLPLF			-0.04	2564.4
99	QIILYPLHGDPNQIWLPLF			-0.04	2420.3
69	QIILYPLHGDPNQIWLPLF			0	2420.34

1	QIILYPLHGDPNQIWLPLF			-0.04	2420.3
1	RNGSK			-0.22	704.19
99	SAPDPSVITLENSWGR			-0.01	1871.94
99	SAPDPSVITLENSWGR			-0.03	1871.93
99	SAPDPSVITLENSWGR			-0.03	1871.93
22	SAPDPSVITLENSWGR			-0.01	1871.95
99	UTFAFGGNYDR			-0.02	1597.77
99	VGLPINQR			-0.03	1039.61
99	VGLPINQR			-0.03	1039.61
98	VGLPINQR			-0.03	1039.61
95	VGLPINQR			-0.03	1039.61
69	VGLPINQR			0.01	1039.64
22	WMFJ			-0.02	898.49
9	WMFJ			-0.02	898.49
3	WMFJ			-0.02	898.49
3	WMFJ			-0.02	898.48
1	WMFJ			-0.02	898.49
1	WMFJ			0.08	898.58
1	WMFK			0.16	754.56
98	YTFAFGGNYDR			-0.03	1453.64
99	YTFAFGGNYDRLEQLAGNLR			0.01	2448.24
99	YTFAFGGNYDRLEQLAGNLR			-0.03	2448.2

Pro Group Report:ricin on parquet 24hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (42 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	13	33	78.6
>95 (1.3)	1	1	13	33	78.6
>66 (0.47)	1	1	13	33	78.6
As shown: >95 (1.30)	1	1	13	33	78.6

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	FQYIEGEMRTR			0.02	1572.81	3
99	FSVYDVSILIPIIALMVYR			-0.04	2355.3	3
99	LEQLAGNLR			-0.03	1156.64	2
22	LEQLAGNLR			-0.03	1156.65	2
22	LEQLAGNLR			-0.03	1156.64	2
9	LEQLAGNLR			-0.03	1156.65	2
1	LEQLAGNLR			0.11	1156.78	2
1	LEQLAGNLR			0.11	1156.78	2
99	LSTAIQESNQGAFASPIQLQR			-0.07	2402.2	3
99	LSTAIQESNQGAFASPIQLQR			-0.07	2402.21	3
99	LSTAIQESNQGAFASPIQLQR			-0.05	2402.23	3
86	LSTAIQESNQGAFASPIQLQR			-0.05	2402.23	3
99	NDGTILNLYSGLVLDVR			-0.04	2005.07	3
99	NDGTILNLYSGLVLDVR			-0.04	2005.07	3
1	QIILUPLHGDPNQIWLPLF			-0.04	2564.4	3
99	QIILYPLHGDPNQIWLPLF			-0.06	2420.29	3
99	SAPDPSVITLENSWGR			-0.05	1871.9	2
98	SAPDPSVITLENSWGR			-0.03	1871.92	3
99	UTFAFGGNYDR			-0.02	1597.76	3

99	VGLPINQR			-0.05	1039.59	2
69	VGLPINQR			-0.01	1039.62	2
45	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR			-0.03	1039.6	2
1	VGLPINQR			-0.03	1039.61	2
1	VGLPINQR			-0.03	1039.61	2
1	VGLPINQR			-0.05	1039.58	2
22	WMFJ			-0.04	898.46	2
9	WMFJ			-0.02	898.48	2
1	WMFJ			-0.12	898.38	2
1	WMFJ			-0.02	898.48	2
1	WMFJ			-0.04	898.47	2
1	WMFK			0.14	754.55	2
99	YTFAFGGNYDRLEQLAGNLR			-0.03	2448.2	3

Pro Group Report: ricin on parquet 48hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (44 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	17	36	81.8
>95 (1.3)	1	1	17	36	81.8
>66 (0.47)	1	1	17	36	81.8
As shown: >95 (1.30)	1	1	17	36	81.8

Conf	Sequence	Mod	Zone	dMass	PrecMW
9	FQUIEGEMR			-0.02	1459.73
99	FSVYDVSILIPIIALMVYR			-0.02	2355.33
9	HEIPVLPNR			-0.04	1217.66
1	IRYNR			-0.2	864.3
1	IRYNR			0.14	864.64
99	LEQLAGNLR			-0.05	1156.62
99	LEQLAGNLR			-0.03	1156.65
99	LEQLAGNLR			-0.01	1156.66
95	LEQLAGNLR			-0.01	1156.66
9	LEQLAGNLR			-0.01	1156.66
99	LSTAIQESNQGAFASPIQLQR			-0.03	2402.24

99	LSTAIQESNQGAFASPIQLQR			-0.03	2402.24
3	LSTAIQESNQGAFASPIQLQR			-0.03	2402.24
1	LTTGADVR			-0.27	975.28
22	NDGTILNLYSGLVLDVR			-0.08	2005.03
99	NDGTILNLYSGLVLDVR			-0.06	2005.05
99	QIILUPLHGDPNQIWLPLF			-0.04	2564.4
99	QIILYPLHGDPNQIWLPLF			-0.04	2420.3
1	QIILYPLHGDPNQIWLPLF			-0.04	2420.31
1	RNGSK			-0.22	704.18
99	SAPDPSVITLENSWGR			-0.03	1871.93
99	UTFAFGGNYDR			-0.02	1597.76
99	VGLPINQR			-0.05	1039.59
98	VGLPINQR			-0.01	1039.62
95	VGLPINQR			-0.03	1039.6
1	VGLPINQR			-0.05	1039.59
22	WMFJ			-0.02	898.48
22	WMFJ			-0.04	898.46
3	WMFJ			-0.02	898.48
1	WMFJ			-0.02	898.48
1	WMFJ			0	898.5
1	WMFJ			0	898.5
99	YTFAFGGNYDR			-0.05	1453.63
99	YTFAFGGNYDRLEQLAGNLR			-0.03	2448.21
99	YTFAFGGNYDRLEQLAGNLR			-0.05	2448.18
45	YTFAFGGNYDRLEQLAGNLR			-0.03	2448.2

Pro Group Report: ricin on parquet 72hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (49 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	16	41	83.7
>95 (1.3)	1	1	16	41	83.7
>66 (0.47)	1	1	16	41	83.7
As shown: >95 (1.30)	1	1	16	41	83.7

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ETVVK			0.21	718.65	2
1	ETVVK			0.25	718.68	2
99	FQUIEGEMR			-0.08	1459.66	3
99	FSVYDVSILIPIIALMVYR			-0.12	2355.23	3
99	HEIPVLPNR			-0.08	1217.63	3
99	HEIPVLPNR			-0.06	1217.65	3
1	ILSCGPASSGQRWMFK			-0.14	1910.82	4
99	LEQLAGNLR			-0.07	1156.6	2
99	LEQLAGNLR			-0.07	1156.61	2
99	LSTAIQESNQGAFASPIQLQR			-0.09	2402.18	3
99	LSTAIQESNQGAFASPIQLQR			-0.11	2402.16	3
99	NDGTILNLYSGLVLDVR			-0.12	2004.98	3
99	NDGTILNLYSGLVLDVR			-0.1	2005	3
99	NDGTILNLYSGLVLDVR			-0.1	2005.01	3
9	NDGTILNLYSGLVLDVR			-0.12	2004.99	3
1	NDGTILNLYSGLVLDVR			0.08	2005.18	3
99	QIILUPLHGDPNQIWLPLF			-0.12	2564.33	3
95	QIILYPLHGDPNQIWLPLF			-0.1	2420.25	3
22	QIILYPLHGDPNQIWLPLF			-0.1	2420.24	3
99	SAPDPSVITLENSWGR			-0.15	1871.81	2

99	SAPDPSVITLENSWGR			-0.07	1871.89	3
99	SAPDPSVITLENSWGR			-0.07	1871.89	3
1	UNRR			0.28	895.8	2
99	VGLPINQR			-0.07	1039.56	2
99	VGLPINQR			-0.05	1039.58	2
95	VGLPINQR			-0.07	1039.57	2
69	VGLPINQR			-0.05	1039.59	2
45	VGLPINQR			-0.05	1039.59	2
9	VGLPINQR			-0.05	1039.58	2
9	VGLPINQR			-0.05	1039.58	2
9	VGLPINQR			-0.05	1039.58	2
1	VGLPINQR			-0.05	1039.58	2
69	WMFJ			-0.04	898.46	2
22	WMFJ			-0.04	898.46	2
22	WMFJ			-0.1	898.41	2
1	WMFJ			-0.04	898.47	2
1	WMFJ			-0.04	898.47	2
1	WMFJ			-0.06	898.45	2
86	WMFJNDGTILNLYSGLVLDVR			-0.12	2741.36	3
99	YTFAFGGNYDRLEQLAGNLR			-0.13	2448.11	3
99	YTFAFGGNYDRLEQLAGNLR			-0.11	2448.13	3

Pro Group Report: ricin on parquet 170hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (41 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	16	37	90.2
>95 (1.3)	1	1	16	37	90.2
>66 (0.47)	1	1	16	37	90.2
As shown: >95 (1.30)	1	1	16	37	90.2

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	ASDPSLJ			0.21	1004.78	2
1	ASDPSLK			-0.03	860.45	2
99	FQUIEGEMR			-0.06	1459.68	3
99	FQUIEGEMR			-0.04	1459.71	3
99	FSVYDVSILIPIIALMVYR			-0.12	2355.23	3
99	HEIPVLPNR			-0.04	1217.67	3
99	HEIPVLPNR			-0.06	1217.65	3
99	LSTAIQESNQGAFASPIQLQR			-0.07	2402.21	3
99	LSTAIQESNQGAFASPIQLQR			-0.09	2402.18	3
99	LSTAIQESNQGAFASPIQLQR			-0.11	2402.17	3
99	LSTAIQESNQGAFASPIQLQR			-0.09	2402.19	3
99	NDGTILNLYSGLVLDVR			-0.08	2005.02	3
95	NDGTILNLYSGLVLDVR			-0.04	2005.07	3
9	NDGTILNLYSGLVLDVR			-0.06	2005.05	3
99	QIILUPLHGDPNQIWLPLF			-0.1	2564.35	3
99	QIILYPLHGDPNQIWLPLF			-0.06	2420.28	3
99	QIILYPLHGDPNQIWLPLF			-0.08	2420.26	3
99	QIILYPLHGDPNQIWLPLF			-0.08	2420.27	3

1	RNGSJ			-0.11	848.4	2
99	SAPDPSVITLENSWGR			-0.07	1871.88	2
99	SAPDPSVITLENSWGR			-0.07	1871.89	3
99	SAPDPSVITLENSWGR			-0.07	1871.88	3
99	SAPDPSVITLENSWGR			-0.05	1871.9	3
1	UNRR			0.26	895.79	2
99	VGLPINQR			-0.05	1039.59	2
99	VGLPINQR			-0.03	1039.6	2
98	VGLPINQR			-0.03	1039.6	2
95	VGLPINQR			-0.03	1039.61	2
69	VGLPINQR			-0.03	1039.6	2
69	VGLPINQR			-0.03	1039.6	2
22	VGLPINQR			-0.05	1039.59	2
45	WMFJ			-0.04	898.46	2
3	WMFJ			-0.02	898.48	2
1	WMFJ			-0.04	898.47	2
99	WMFJNDGTILNLYSGLVLDVR			-0.1	2741.39	3
99	YTFAFGGNYDRLEQLAGNLR			-0.09	2448.15	3

(C) SEB on carpet over 170 hours

Pro Group Report: SEB on carpet 0hour

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (184 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	89	162	88
>95 (1.3)	1	1	89	162	88
>66 (0.47)	2	2	113	184	100
As shown: >95 (1.30)	1	1	89	162	88

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DTJ			-0.02	650.36	2
99	DVJIEVYLTTJ			-0.04	1740.01	3
99	DVJIEVYLTTJ			-0.02	1740.03	3
99	DVJIEVYLTTJ			-0.06	1739.99	3
99	DVJIEVYLTTJ			0.02	1740.06	3
1	DVJIEVYLTTJ	Y->W	JIE	22.94	1762.98	4
1	DVJIEVYLTTK			27.86	1623.81	3
1	FDQSJYLMMYNDNJ	F->Y / M(Oxidation)	DQS	16.26	2244.37	4
1	FDQSJYLMMYNDNK	Y(O-Phosphoryl) / S(O-Phosphoryl)	YN	80.01	2164	4
1	FDQSK	K->R	QS	28.31	795.7	2
1	FIENENSFWUDMMPAPGDKFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.02	3328.58	4
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FWYD	1	3328.57	5
76	FIENENSFWYDMMPAPGDJFDQSJ			0	3327.56	4

13	FIENENSFWYDMMPAPGDJFDQSJ			0.02	3327.58	5
13	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.02	3328.59	5
99	FIENENSFWYDMMPAPGDJFDQSJ			-0.08	3327.49	3
37	FTGLMENMJ			-0.02	1357.69	3
7	FTGLMENMJ			0	1357.71	3
1	FTGLMENMJ			-0.84	1356.87	2
1	FTGLMENMJ			0	1357.7	3
1	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ			-0.02	1357.69	3
99	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ	N(Deamidation)	ME	1.02	1358.72	3
99	FTGLMENMJ			0	1357.71	3
99	FTGLMENMJ			-0.02	1357.69	3
99	FTGLMENMJ			-0.02	1357.69	3
99	FTGLMENMJ			-0.08	1357.62	2
99	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ			-0.02	1357.69	3
99	FTGLMENMJ			0	1357.7	3
93	FTGLMENMJ			0.02	1357.72	3
82	FTGLMENMJ			-0.02	1357.69	3
82	FTGLMENMJ			0	1357.7	3
37	FTGLMENMJ			0	1357.7	3
99	HULVJ			-0.05	1090.65	2
1	HULVJ			0.01	1090.7	3
1	HULVKNJ			18.04	1350.86	2
99	HYLVJ			0	946.59	3
99	HYLVJ			-0.04	946.54	3
99	HYLVJ			0	946.59	3
21	HYLVJ			-0.12	946.46	2
1	HYLVJNJ	Y->H	YL	-26.02	1306.81	2
1	HYLVJNK			80.08	1268.81	2
1	HYLVJNK			80.06	1268.78	2
1	HYLVKNJ			14.04	1202.77	2
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.08	1124.7	2
1	HYLVKNK			13.9	1058.52	2
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.08	1124.7	2
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.02	1124.65	2

99	IEVYLTTJ			-0.03	1253.73	2
98	IEVYLTTJ			-0.01	1253.74	3
98	IEVYLTTJ			-0.01	1253.75	3
85	IEVYLTTJ			0.01	1253.77	3
1	IEVYLTTKJ			-0.28	1381.57	3
1	JLUEFNNSPYETGYIJ			1.02	2542.39	5
1	JLYEFNNSPUETGYIK	Y->F	EFN	-15.72	2381.55	4
99	JVTAQELDYLTR			-0.03	1723.94	3
99	JVTAQELDYLTR			0.01	1723.98	3
98	JVTAQELDYLTR	E->Q	EL	-1.03	1722.94	3
1	JVTAQELDYLTR			-0.05	1723.93	3
1	KVTAQELDULTR	E->Q	VTA	-0.97	1723	3
1	LGNUDNVR	N(Deamidation)	VR	1.19	1238.86	2
1	LGNUDNVR	N(Deamidation)	VR	1.17	1238.85	2
1	LGNUDNVRVEFK			0.18	1741.13	3
1	LGNUDNVRVEFK			18.2	1759.14	3
1	LGNYDNVRVEFJ			0	1740.95	4
99	LGNYDNVRVEFJ			0.02	1740.97	4
2	LGNYDNVRVEFJ			0.02	1740.96	3
99	LGNYDNVRVEFJ			-0.12	1740.83	2
1	LUEFNNSPYETGYIK			-0.04	2125.03	3
1	LUEFNNSPYETGYIK			28.3	2153.36	3
99	LYEFNNSPUETGYIJ			-0.14	2269.03	3
10	LYEFNNSPUETGYIK			-0.08	2124.98	3
95	LYEFNNSPYETGYIJ			-0.02	2125.05	3
48	LYEFNNSPYETGYIJ			0	2125.06	3
1	LYEFNNSPYETGYIJ			0	2125.06	3
1	MUJ			-0.1	872.42	2
1	MUJ			16.12	888.65	2
1	MUJ			0.04	872.57	2
1	MUKR			16.13	900.64	2
1	MVDSJ			0.08	866.57	2
1	MVDSJDVK	K->R	VK	28.21	1236.88	2

1	MVDSK			28.23	750.61	2
1	MVDSKDVJ	M(Oxidation)	DV	16.17	1224.84	2
1	MVDSKDVJ			0.11	1208.78	2
1	MVDSKDVK			14.17	1078.75	2
1	MVDSKDVK			28.21	1092.78	2
1	MYK			16.17	600.49	2
1	MYKR			-18.01	722.41	2
1	NJDLADK			0.06	1090.68	3
1	NKDLADK	N(Deamidation)	DL	1.1	947.63	2
99	NLLSFDVQTNJ			-0.07	1565.81	2
1	NLLSFDVQTNK			1	1422.77	3
1	NLLSFDVQTNKJ			28.08	1722.04	4
1	SIDQFLUFDLIYSIKDTJ	L->M	DTJ	18.08	2658.53	4
1	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1.06	2641.5	4
1	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1	2641.44	4
88	SIDQFLUFDLIYSIKDTJ			0.04	2640.48	4
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.06	2658.51	5
3	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1.1	2641.54	4
4	SIDQFLUFDLIYSIKDTJ			0.02	2640.46	4
99	SIDQFLUFDLIYSIKDTJ			0	2640.44	4
24	SIDQFLUFDLIYSIKDTJ			0.02	2640.46	4
99	SIDQFLYFDLIUSIJ			-0.07	2296.21	3
1	SIDQFLYFDLIUSIK	L->M	FDL	18.23	2170.41	3
99	SIDQFLYFDLIUSIKDTJ			0	2640.44	4
98	SIDQFLYFDLIUSIKDTJ			-0.04	2640.41	4
95	SIDQFLYFDLIUSIKDTJ	Q->E / Q(Deamidation)	DQFL	1	2641.44	4
24	SIDQFLYFDLIUSIKDTJ	Q->E / Q(Deamidation)	DQFL	0.98	2641.43	4
4	SIDQFLYFDLIUSIKDTJ	Q->E / Q(Deamidation)	DQFL	1	2641.44	4
99	SIDQFLYFDLIYSIJ			0.01	2152.18	3

99	SIDQFLYFDLIYSIJ			0.01	2152.18	3
99	SIDQFLYFDLIYSIJ			-0.09	2152.08	2
99	SIDQFLYFDLIYSIJ			0.03	2152.2	3
99	SIDQFLYFDLIYSIJ			-0.01	2152.16	3
99	SIDQFLYFDLIYSIJ			0.05	2152.23	3
99	SIDQFLYFDLIYSIJ			0.03	2152.21	3
99	SIDQFLYFDLIYSIJ			0.03	2152.21	3
99	SIDQFLYFDLIYSIJ			0.03	2152.21	3
99	SIDQFLYFDLIYSIJ			0.01	2152.19	3
97	SIDQFLYFDLIYSIJ			0.03	2152.2	3
1	SIDQFLYFDLIYSIJ	F->Y	IJ	16.23	2168.41	3
48	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1	2641.44	4
99	SIDQFLYFDLIYSIJDTJ			0	2640.44	4
95	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.02	2641.46	4
99	SIDQFLYFDLIYSIJDTJ			0.02	2640.46	4
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	YFD	1.02	2641.47	4
99	SIDQFLYFDLIYSIJDTJ			0.04	2640.49	4
99	SIDQFLYFDLIYSIJDTJ			-0.02	2640.43	3
1	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.02	2641.47	4
3	SIDQFLYFDLIYSIK	Y->H	FL	-26.29	1981.78	3
1	SITVR			79.97	798.42	2
1	SSKFTGLMENMJ	M->L	EN	-18.18	1641.68	3
1	TCMUGGVTEHNGNQLDKYR	M->L	TC	-18.25	2454.92	3
1	TCMYGGVTEHNGNQLDJ	Y->W	TC	23.05	2177.06	4
12	TNDINSHQTDKR	Q->E / N(Deamidation) / Q(Deamidation)	IN	1.26	1573.05	2

1	TNDINSHQTDKR			1.04	1572.82	3
1	UJDJ			0.06	1128.77	2
1	UKDJ			-0.12	984.49	3
1	ULMMUNDNK	M(Oxidation)	LM	16.26	1639.08	3
1	ULMMYNDNJ	M(Oxidation)	NDN	16.2	1639.03	3
1	UVDVFGANUUYQCYFSJ	Q->E / N(Deamidation) / Q(Deamidation)	FSJ	0.87	2850.31	3
99	VFEDGJNLLSFDVQTNJ			-0.05	2385.25	3
1	VFEDGK	F->Y	DG	15.75	853.18	2
1	VFEDGK			-14.13	823.31	2
1	VFEDGK			-0.93	836.5	2
99	VLUDDNHVSAINVJ	N(Deamidation)	AIN	0.98	2019.11	4
17	VLUDDNHVSAINVJ			1	2019.13	4
1	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1	2019.13	4
1	VLUDDNHVSAINVJ			1.16	2019.28	3
1	VLUDDNHVSAINVJ			0	2018.13	4
99	VLYDDNHVSAINVJ			0.21	1874.23	3
97	VLYDDNHVSAINVJ			0.01	1874.03	3
1	VTAQELDULTRHYLVK			0.19	2236.45	3
1	YJDJ			-16.06	968.55	3
1	YJDJ			80.1	1064.71	2
1	YKDJ	D->E	KD	13.99	854.49	2
1	YLMMUNDNK			0.01	1478.72	3
96	YLMMYNDNJ			0.01	1478.72	3
1	YLMMYNDNJ			0.03	1478.74	3
99	YLMMYNDNJ			-0.13	1478.59	2

Pro Group Report: SEB on carpet 2hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (129 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	74	109	84.5
>95 (1.3)	1	1	74	109	84.5
>66 (0.47)	1	1	74	109	84.5
As shown: >95 (1.30)	1	1	74	109	84.5

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
0	DKYVDVFGANYYYQCYFSK	Q->E / N(Deamidation) / Q(Deamidation)	DK	0.95	2517.11	4
1	DLADJUK			17.72	1301.47	3
1	DLADK			18.02	722.41	2
1	DTKLGNYDNVR			-16.27	1421.47	3
1	DTKLGNYDNVR	L->M	KLG	17.83	1455.56	4
99	DVJIEVYLTTJ			0	1740.05	3
99	DVJIEVYLTTJ			-0.06	1739.99	3
99	DVJIEVYLTTJ			0	1740.04	3
1	FDQSKYLMMYNDNJ			80.05	2164.04	4
1	FDQSKYLMMYNDNJ	Y(O-Phosphoryl) / S(O-Phosphoryl)	MM	80.05	2164.04	4
9	FIENENSFWYDMMPAPGDJ	M->L	ENE	-17.85	2560.33	3
99	FIENENSFWYDMMPAPGDJFDQSJ			-0.06	3327.51	3
53	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1.04	3328.61	5
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.04	3328.61	5

99	FTGLMENMJ	N(Deamidation)	ME	1	1358.71	3
99	FTGLMENMJ			0	1357.7	3
1	FTGLMENMJ			0	1357.71	3
1	FTGLMENMJ			0.02	1357.72	3
1	FTGLMENMJ			18.04	1375.74	3
1	FTGLMENMJ			15.98	1373.68	3
99	FTGLMENMJ			-0.08	1357.63	3
99	FTGLMENMJ			0.02	1357.73	3
99	HULVJ			-0.01	1090.68	3
1	HULVJ			25.87	1116.56	2
99	HYLVJ			0	946.59	3
1	HYLVJ			26.02	972.6	3
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.06	1124.69	2
1	IEVULTTJ			-0.05	1397.8	3
99	IEVYLTTJ			-0.05	1253.7	2
1	IEVYLTTKJ			13.72	1395.56	3
1	IEVYLTTKK	Y->H	IE	-26.3	1211.44	3
99	JLYEFNNSPYETGYIJ			-0.1	2397.17	3
5	JVTAQELDULTR			-0.03	1868.05	3
99	JVTAQELDYLTR			-0.01	1723.97	4
99	JVTAQELDYLTR			0.01	1723.99	3
99	JVTAQELDYLTR			0.03	1724	3
99	JVTAQELDYLTR			-0.05	1723.93	3
12	JVTAQELDYLTR			0.01	1723.99	3
1	JVTAQELDYLTR			0.03	1724	3
3	KVTAQELDYLTR			80.23	1660.1	3
1	LGNUDNVRVEFJ			1	1886.05	4
1	LGNUDNVRVEFK	K->R	UD	28.28	1769.22	2
55	LGNYDNVRVEFJ			0	1740.95	4
1	LGNYDNVRVEFJ			0	1740.95	3
99	LGNYDNVRVEFJ			-0.06	1740.89	2
77	LGNYDNVRVEFJ			0.12	1741.06	4
1	LUEFNNSPYETGYIJ			-0.02	2269.14	4
99	LYEFNNSPUETGYIJ			-0.06	2269.1	3

99	LYEFNNSPYETGYIJ			-0.1	2124.97	3
10	LYEFNNSPYETGYIJ			-0.02	2125.05	3
99	LYEFNNSPYETGYIJ			0	2125.07	3
1	MVDSJDVJ	M(Oxidation)	DV	16.15	1368.93	2
1	MVDSJDVJ	M(Oxidation)	DV	16.19	1368.96	2
1	MVDSJDVK			0.05	1208.73	2
1	MVDSKDVJ			28.21	1236.88	3
1	MVDSKDVJ			-0.07	1208.61	3
1	MVDSKDVJ	K->R	KD	28.17	1236.84	2
1	MVDSKDVK			13.97	1078.55	2
1	MVDSKDVK			14.19	1078.76	2
1	MYK			80.09	664.41	2
3	NKDLADK	D->E	DL	13.96	960.48	2
76	NLLSFDVQTNJ	Q->E / N(Deamidation) / Q(Deamidation)	SF	0.99	1566.87	3
1	NLLSFDVQTNJ			0.05	1565.93	3
99	NLLSFDVQTNJ			-0.03	1565.84	3
99	NLLSFDVQTNJJ			-0.08	1837.99	3
53	NLLSFDVQTNJJ			0.02	1838.08	4
1	SIDQFLUFDLIUSIJ	S(O-Phosphoryl)	SI	79.75	2520.13	4
99	SIDQFLUFDLIYSIJ			0.01	2296.28	3
24	SIDQFLUFDLIYSIJDTK	Q->E / Q(Deamidation)	DQFL	1.04	2641.48	4
48	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1.04	2641.48	4
24	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1	2641.44	4
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.08	2658.52	4
1	SIDQFLUFDLIYSIKDTJ	L->M	YSIK	18.08	2658.53	5
99	SIDQFLYFDLIUSIJ	Q->E / Q(Deamidation)	LY	1.01	2297.28	4
68	SIDQFLYFDLIUSIJDTJ	Q->E / Q(Deamidation)	QFL	1.02	2785.57	5
0	SIDQFLYFDLIUSIKDTK	Y->W	DQF	22.74	2519.09	3

99	SIDQFLYFDLIYSIJ			0.01	2152.19	3
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.02	2641.47	4
99	SIDQFLYFDLIYSIJDTJ			0	2640.45	3
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.04	2641.48	4
1	SITVRVFEDGK			-0.71	1393.06	2
1	TCMYGGVTEHNGNQLDJ	H->Y	TC	26.07	2180.08	3
1	TNDINSHQTDK	Q->E / N(Deamidation) / Q(Deamidation)	TN	1.18	1416.87	3
1	UKDK			13.99	854.49	2
1	ULMMYNDNKMVDSJ	Y->H	ULM	-25.92	2157.17	4
1	ULMMYNDNKMVDSK	L->M	KMV	18.22	2057.2	5
0	ULMMYNDNKMVDSK	L->M	KMV	18.2	2057.18	5
1	URSITVR			-0.21	1181.51	3
85	VFEDGJNLLSFDVQTNJ			0.03	2385.32	4
99	VLUDDNHVSAINVJ			-0.1	2018.03	3
96	VLUDDNHVSAINVJ			-0.02	2018.11	4
3	VLUDDNHVSAINVJ			1.02	2019.15	4
3	VLUDDNHVSAINVJ			1	2019.12	4
1	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.02	2019.14	4
99	VLYDDNHVSAINVJ			-0.05	1873.97	3
62	VLYDDNHVSAINVJ			0.03	1874.05	4
62	VLYDDNHVSAINVJ			0.01	1874.02	3
7	VLYDDNHVSAINVJ			0.03	1874.05	3
1	VLYDDNHVSAINVJ	Y->W	LY	23.25	1897.26	3
99	VTAQELDULTR			-0.08	1595.81	3
1	YLMMUNDNJ			-15.74	1607.08	2
1	YLMMUNDNK			0.01	1478.72	3
1	YLMMUNDNK			-25.79	1452.93	2
99	YLMMYNDNJ			-0.07	1478.64	2
1	YLMMYNDNJ			0.01	1478.73	3

1	YLMMYNDNKMVDSJ			18.22	2057.21	3
1	YLMMYNDNKMVDSK			-15.69	1879.18	3
1	YVDVFGANYYUQCUFSJ	Y->H	YV	-25.93	2679.41	5
1	YVDVFGANYYUQCUFSJ	Y->H	YV	-25.91	2679.43	5

Pro Group Report: SEB on carpet 27hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (169 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	89	143	84.6
>95 (1.3)	1	1	89	143	84.6
>66 (0.47)	2	2	119	169	100
As shown: >95 (1.30)	1	1	89	143	84.6

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADJYJ			23.08	1306.83	2
1	DLADJYJ			23.1	1306.84	2
1	DLADK			18.02	722.41	2
1	DLADKYK			23.14	1018.69	2
1	DTKLGNYDNVR	N(Deamidation)	VR	1.17	1438.91	2
1	DTKLGNYDNVR	N(Deamidation)	VR	1.17	1438.91	2
99	DVJIEVYLTTJ			-0.1	1739.95	2
99	DVJIEVYLTTJ			-0.02	1740.03	4
99	DVJIEVYLTTJ			0	1740.04	3
99	DVJIEVYLTTJ			0	1740.05	3
99	DVJIEVYLTTJ			0	1740.04	3
1	DVKIEVYLTTJ	E->Q	LTT	-0.86	1595.08	3
1	FDQSJULMMYNDNK	F->Y / M(Oxidation)	ULM	16.26	2244.37	4
1	FDQSJYLMMYNDNK			79.99	2163.98	4
1	FDQSK			80.17	847.56	2
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FWYD	1.02	3328.58	5

53	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1	3328.56	4
2	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1	3328.57	4
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.2	3328.77	4
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1.06	3328.62	5
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.04	3328.6	5
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.04	3328.61	4
99	FIENENSFWYDMMPAPGDJFDQSJ			-0.06	3327.5	3
99	FIENENSFWYDMMPAPGDJFDQSJ			0.04	3327.6	5
62	FTGLMENMJ			-0.06	1357.65	3
82	FTGLMENMJ			0.08	1357.78	3
62	FTGLMENMJ			0	1357.71	3
1	FTGLMENMJ			0	1357.7	3
1	FTGLMENMJ			0.02	1357.72	3
99	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ			0	1357.7	3
99	FTGLMENMJ			-0.02	1357.69	3
1	FTGLMENMJ			-1.06	1356.64	3
99	FTGLMENMJ			-0.1	1357.61	3
99	FTGLMENMJ			0	1357.7	3
97	FTGLMENMJ			-0.02	1357.68	3
97	FTGLMENMJ			-0.02	1357.69	3
93	FTGLMENMJ			0	1357.71	3
1	HULVJ			-0.01	1090.68	3
1	HULVJNK			18.06	1350.88	2
1	HULVK			26	972.58	2
1	HULVKNJ	L->M	KN	18.04	1350.86	2

99	HYLVJ			0.02	946.6	3
99	HYLVJ			0.02	946.6	3
99	HYLVJ			-0.06	946.53	2
43	HYLVJ			0	946.59	3
9	HYLVJNK			80.14	1268.88	2
1	HYLVJNK			0.1	1188.82	2
1	HYLVJNK			80.12	1268.85	2
1	HYLVJNK	Y(O-Phosphoryl)	YL	80.18	1268.9	2
1	HYLVK			-15.96	786.52	2
1	HYLVKNK			14.08	1058.71	2
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.08	1124.71	2
99	IEVYLTTJ			-0.03	1253.73	2
1	IEVYLTTJ			23.07	1276.83	2
1	IEVYLTTJJ			23.02	1548.97	3
1	IEVYLTTJK			13.8	1395.65	3
99	JLUEFNNSPYETGYIK	N(Deamidation)	UEF	1	2398.26	4
99	JLYEFNNSPYETGYIJ			-0.1	2397.17	3
17	JVTAQELDULTR	Q->E / Q(Deamidation)	AQ	1.03	1869.1	4
68	JVTAQELDYLTR	E->Q	EL	-0.99	1722.98	3
99	JVTAQELDYLTR			-0.01	1723.97	3
99	JVTAQELDYLTR			0.01	1723.99	3
99	JVTAQELDYLTR			-0.07	1723.91	3
1	JVTAQELDYLTR	E->Q	EL	-0.87	1723.11	3
1	LGNUDNVR	N(Deamidation)	VR	1.15	1238.82	2
1	LGNUDNVR	N(Deamidation)	VR	1.15	1238.82	2
1	LGNUDNVR	N(Deamidation)	VR	1.19	1238.87	2
1	LGNUDNVRVEFJ			-0.1	1884.95	3
18	LGNYDNVR			0.92	1094.49	3
1	LGNYDNVR			-15.98	1077.59	2
13	LGNYDNVRVEFJ			0	1740.95	3
13	LGNYDNVRVEFJ			0.06	1741.01	4
1	LGNYDNVRVEFJ			0.08	1741.02	4
1	LGNYDNVRVEFK			0.04	1596.89	3

1	LUEFNNSPYETGYIJ			0	2269.17	4
99	LYEFNNSPUETGYIJ			-0.12	2269.05	3
99	LYEFNNSPYETGYIJ			-0.02	2125.05	3
99	LYEFNNSPYETGYIJ			0	2125.06	3
0	MUJ			16.1	888.63	2
1	MUJ			16.08	888.6	2
1	MUKR			16.11	900.63	2
2	MVDSJ			-18.02	848.47	2
1	MVDSJ	M(Oxidation)	DS	16.08	882.56	2
1	MVDSJ	D->E / V->I	DS	14.1	880.59	2
1	MVDSJDVK	M(Oxidation)	JD	16.15	1224.82	2
1	MVDSJDVK	K->R	VK	28.19	1236.86	2
1	MVDSK			28.05	750.43	2
1	MVDSKDVJ			0.09	1208.76	2
1	MVDSKDVJ	M(Oxidation)	DV	16.13	1224.81	2
1	MVDSKDVJ			0.11	1208.78	2
2	MVDSKDVK			80.17	1144.74	2
76	MVDSKDVK			80.21	1144.79	2
1	MVDSKDVK			28.17	1092.74	2
1	MVDSKDVK			28.15	1092.73	2
1	MVDSKDVK			16.13	1080.7	2
1	NJDLADJ			18.16	1252.88	2
1	NKDLADJ			0.06	1090.68	3
1	NKJ			1.21	677.67	2
99	NLLSFDVQTNJ			-0.07	1565.8	2
2	NLLSFDVQTNJ			-0.01	1565.86	3
53	NLLSFDVQTNJJ			0.04	1838.11	4
1	NLLSFDVQTNK	Q->E / N(Deamidation) / Q(Deamidation)	QT	1.18	1422.94	2
1	SIDQFLUFDLIUSIJ	S(O-Phosphoryl)	SI	79.79	2520.17	4
1	SIDQFLUFDLIUSIJ	S(O-Phosphoryl)	LU	79.81	2520.18	5
10	SIDQFLUFDLIYSIKDTJ			0.02	2640.46	4
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.08	2658.53	4

99	SIDQFLYFDLIUSIJDTJ	Q-> E / Q(Deamidation)	QFL	0.98	2785.52	5
1	SIDQFLYFDLIUSIKDTJ	L->M	USIK	18.06	2658.51	5
99	SIDQFLYFDLIYSIJ			0.03	2152.2	3
99	SIDQFLYFDLIYSIJ			0.05	2152.23	3
99	SIDQFLYFDLIYSIJ			-0.01	2152.17	3
99	SIDQFLYFDLIYSIJ			0.01	2152.19	3
99	SIDQFLYFDLIYSIJ			-0.01	2152.17	3
1	SIDQFLYFDLIYSIJ	Q->E / Q(Deamidation)	IYS	0.93	2153.11	4
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	YFD	1.02	2641.46	4
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.08	2641.52	4
99	SIDQFLYFDLIYSIJDTJ			-0.18	2640.27	4
1	SITVR			79.93	798.38	2
1	SITVRVFEDGK	S(O-Phosphoryl) / T(O-Phosphoryl)	ED	80.23	1474	2
1	TNDINSHQTDKR			1.2	1572.98	3
1	ULMMYNDNKMVDSJ			79.82	2262.9	5
1	UVDVFGANUUYQCYFSJ			0.89	2850.32	3
1	UVDVFGANUYYQCYFSJ	Y->H	UY	-25.95	2679.38	5
1	VEFK			28.21	693.61	2
1	VFEDGJ			14.04	995.58	3
1	VFEDGJNLLSFDVQTNJ			0.03	2385.33	4
99	VFEDGJNLLSFDVQTNJ			-0.05	2385.24	3
1	VFEDGK			-14.11	823.33	2
99	VLUDDNHVSAINVJ			-0.1	2018.02	3
7	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1	2019.13	4
1	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.02	2019.15	4
1	VLYDDNHVSAINVJ			-15.77	1858.24	3
99	VTAQELDULTR			-0.04	1595.85	3

1	VTAQELDYLTRHYLVJ	E->Q	VT	-1.09	2235.17	4
1	YJDJ			14.04	998.64	2
1	YKDJ	D->E	KD	13.99	854.49	2
1	YKDJ			14.13	854.62	2
1	YLMMUNDNJ			-0.06	1622.77	3
99	YLMMYNDNJ			-0.07	1478.64	2
29	YLMMYNDNJ			0.01	1478.72	3
1	YLMMYNDNK			0.27	1334.89	2

Pro Group Report: SEB on carpet 50hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (170 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	103	137	80.6
>95 (1.3)	1	1	103	137	80.6
>66 (0.47)	1	1	103	137	80.6
As shown: >95 (1.30)	1	1	103	137	80.6

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADJUK	L->M	AD	17.76	1301.5	3
1	DLADK			18.04	722.43	2
1	DTKLGNYDNVR	K->R	DT	27.75	1465.48	3
99	DVJIEVYLTTJ			0.04	1740.09	3
99	DVJIEVYLTTJ			-0.04	1740.01	3
99	DVJIEVYLTTJ			0.06	1740.11	3
1	DVKIEVYLTTJ	E->Q	LTT	-0.86	1595.08	3
1	FDQSJ			16.28	927.78	2
1	FDQSJULMMUNDNK			18.16	2390.37	4
1	FDQSJULMMUNDNK			18.16	2390.37	4
1	FDQSJYLMMYNDNJ	F-> Y / M(Oxidation)	DQS	16.26	2244.37	4
99	FIENENSFWYDMMPAPGDJ			0.07	2578.25	3
99	FIENENSFWYDMMPAPGDJFDQSJ			0.02	3327.59	3
99	FTGLMENMJ			0.04	1357.75	3

37	FTGLMENMJ			0.06	1357.77	3
1	FTGLMENMJ			18.08	1375.79	3
1	FTGLMENMJ			0.06	1357.76	3
1	FTGLMENMJ			0.04	1357.75	3
1	FTGLMENMJ			0.04	1357.74	3
99	FTGLMENMJ			-0.04	1357.66	3
1	FTGLMENMK	K->R	MK	27.78	1241.38	2
1	HULVJ			0.03	1090.73	3
99	HULVJ			0.01	1090.7	2
0	HULVKNK			13.84	1202.56	3
99	HYLVJ			0.02	946.61	3
99	HYLVJ			0	946.58	3
1	HYLVJNJ	Y->F	JN	-16.06	1316.77	4
1	HYLVJNJ			80.14	1412.96	2
1	HYLVJNK			80.14	1268.86	2
1	HYLVJNK	Y->W	YL	22.76	1211.49	3
1	HYLVJNK			80.18	1268.91	2
1	HYLVK			14.3	816.78	2
1	HYLVK			26.04	828.52	2
1	HYLVKNK			18.18	1062.81	2
1	HYLVKNK			18.16	1062.78	2
1	HYLVKNK			0.16	1044.8	2
1	HYLVKNK			26.22	1070.84	2
1	HYLVKNK			-25.86	1018.77	2
1	HYLVKNK			18.16	1062.79	2
99	IEVULTTJ			-0.03	1397.82	3
1	IEVULTTJ			0.13	1397.98	3
1	IEVULTTKJ			80.02	1605.97	3
99	IEVYLTTJ			0.03	1253.79	3
99	IEVYLTTJ			0.01	1253.77	2
98	IEVYLTTJ			0.03	1253.79	2
1	IEVYLTTKJ			13.88	1395.73	3
1	IEVYLTTKJ			13.78	1395.63	3
1	IEVYLTTKJ			23.04	1404.88	3
1	IEVYLTTKK	E->D / I->V	YL	-13.96	1223.78	2
1	IEVYLTTKK			-0.92	1236.82	3
1	IEVYLTTKK			23.22	1260.97	3
99	JLYEFNNSPYETGYIJ			-0.04	2397.22	3
1	JTCMYGGVTEHNGNQLDK	L->M	TEH	18.18	2300.29	5
1	JTNDINSHQTDK	D->E	ND	13.93	1701.81	3
1	JVTAQELDULTR	Q->E / Q(Deamidation)	AQ	1.09	1869.17	4
99	JVTAQELDYLTR			0.01	1723.99	3

99	JVTAQELDYLTR	Q->E / Q(Deamidation)	EL	1.05	1725.02	3
99	JVTAQELDYLTR			0.07	1724.05	3
99	JVTAQELDYLTR			0.07	1724.04	3
0	KVTAQELDULTR			18.05	1742.03	3
97	LGNUDNVRVEFJ			0.1	1885.14	4
99	LGNYDNVRVEFJ			0.12	1741.07	4
30	LGNYDNVRVEFJ			0.12	1741.07	4
2	LGNYDNVRVEFJ			0.1	1741.04	4
1	LGNYDNVRVEFJ			0.08	1741.03	3
1	LGNYDNVRVEFK	E->Q	NV	-0.9	1595.95	3
1	LGNYDNVRVEFK			0.08	1596.93	3
24	LUEFNNSPYETGYIJ			0.1	2269.26	4
99	LYEFNNSPUETGYIJ			-0.06	2269.11	3
99	LYEFNNSPYETGYIJ			0.06	2125.13	3
99	LYEFNNSPYETGYIJ			0.02	2125.09	3
1	LYEFNNSPYETGYIK			-13.72	1967.25	4
1	MUJR			-27.84	1000.78	2
1	MVDSJDVJ	M(Oxidation)	DV	16.17	1368.94	2
1	MVDSJDVK			0.15	1208.82	2
1	MVDSK			28.27	750.64	2
1	MVDSK			28.03	750.4	2
1	MVDSKDVJ	M(Oxidation)	DV	16.05	1224.72	2
1	MVDSKDVK			28.23	1092.8	2
1	MVDSKDVK			14.21	1078.78	2
1	MYJ			23.21	751.63	2
1	MYJR			-27.89	856.63	2
1	MYJR			16.17	900.69	2
1	MYKR			-17.69	722.72	2
1	NJDLADJ	N(Deamidation)	DL	1.04	1235.77	3
1	NKDLADJ			0.1	1090.72	3
1	NKDLADK			14.06	960.58	3
1	NKDLADK	D->E	DL	13.98	960.51	2
1	NKDLADK			1.12	947.64	2
51	NLLSFDVQTNJ			0.07	1565.94	3

99	NLLSFDVQTNJ			0.01	1565.88	3
99	NLLSFDVQTNJ			0.05	1565.93	3
63	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.1	1839.17	4
99	NLLSFDVQTNJJ			0.06	1838.12	3
99	NLLSFDVQTNJJ			1.1	1839.17	4
1	NLLSFDVQTNK			-0.28	1421.48	3
1	NLLSFDVQTNKJ			28.16	1722.12	4
1	SIDQFLUFDLIUSIJ	S(O-Phosphoryl)	SI	79.91	2520.28	5
1	SIDQFLUFDLIYSIJ	Y->F	SI	-15.99	2280.29	4
1	SIDQFLUFDLIYSIKDTK	Y->W	SI	22.8	2519.15	3
99	SIDQFLYFDLIUSIJ	Q->E / Q(Deamidation)	LY	0.89	2297.17	4
1	SIDQFLYFDLIYSIJ	Q->E / Q(Deamidation)	SI	1.09	2153.26	4
99	SIDQFLYFDLIYSIJ			0.07	2152.24	3
99	SIDQFLYFDLIYSIJ			0.09	2152.27	3
99	SIDQFLYFDLIYSIJ			0.01	2152.19	3
99	SIDQFLYFDLIYSIJDTJ			-0.02	2640.42	3
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.1	2641.54	4
1	SITVR			80.09	798.54	2
5	SSJFTGLMENMJ	N(Deamidation)	LM	0.92	1804.88	4
1	SSJFTGLMENMK	M->L	TGL	-18.14	1641.73	3
1	SSK			28.15	492.42	1
1	TCMYGGVTEHNGNQLDK			-15.83	1994.08	3
1	TCMYGGVTEHNGNQLDKYR	R->K	KY	-27.73	2301.34	4
1	ULMMUNDNJ			0	1766.93	3
7	ULMMUNDNK			0.06	1622.88	4
7	ULMMYNDNJ			0.06	1622.89	3

1	ULMMYNDNKMVDSJ	Y(O-Phosphoryl) / S(O-Phosphoryl)	MVD	79.84	2262.92	5
1	UVDVFGANUUYQCYFSK	Q->E / N(Deamidation) / Q(Deamidation)	YQC	1.25	2706.59	5
1	VEFJNK			1.25	1052.88	2
1	VEFKNK			-13.8	893.72	2
99	VFEDGJNLLSFDVQTNJ			-0.03	2385.27	3
1	VFEDGJNLLSFDVQTNJ	Q->E / N(Deamidation) / Q(Deamidation)	VF	1.11	2386.4	4
99	VLUDDNHVSAINVJ			-0.04	2018.08	3
1	VLUDDNHVSAINVJ			1.1	2019.23	5
1	VLUDDNHVSAINVJ			1.1	2019.23	5
1	VLUDDNHVSAINVJ	N(Deamidation)	VJ	1.1	2019.23	4
0	VLUDDNHVSAINVJ			14.28	2032.4	3
93	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.06	2019.19	4
99	VLYDDNHVSAINVJ			-0.03	1873.99	3
3	VLYDDNHVSAINVJ	N(Deamidation)	DDN	1.11	1875.13	4
1	VTAQELDULTRHYLVJ	E->D	ULT	-14.23	2366.12	5
1	VTAQELDYLTRHYLVJ	H->Y	VT	25.71	2261.97	5
1	YKDJ			-25.83	814.67	2
1	YKDK			-15.77	680.62	2
99	YLMMYNDNJ			-0.05	1478.66	2
1	YLMMYNDNJ			0.07	1478.78	3
1	YLMMYNDNKMVDSK			0.71	1895.59	4

Pro Group Report: SEB on carpet 74hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (130 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	76	107	82.3
>95 (1.3)	1	1	76	107	82.3
>66 (0.47)	1	1	76	107	82.3
As shown: >95 (1.30)	1	1	76	107	82.3

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADJ	L->M	AD	18.18	866.66	1
1	DTKLGNYDNVR			-16.29	1421.44	3
99	DVJIEVYLTTJ			0.04	1740.08	3
99	DVJIEVYLTTJ			0.02	1740.06	3
17	DVJIEVYLTTJ			0.06	1740.11	3
17	DVKIEVULTTJ			0.08	1740.12	3
1	FDQSJ			16.26	927.77	2
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.1	3328.67	4
99	FIENENSFWYDMMPAPGDJFDQSJ			0.04	3327.6	3
1	FTGLMENMJ			-0.82	1356.88	3
1	FTGLMENMJ			-0.82	1356.88	3
1	FTGLMENMJ			0.04	1357.75	3
1	FTGLMENMJ			-0.84	1356.86	2
1	FTGLMENMJ	E->D	FT	-14.24	1343.47	3
1	FTGLMENMJ			0.04	1357.75	3
1	FTGLMENMJ			18.08	1375.79	3
99	FTGLMENMJ			-0.04	1357.66	3
99	FTGLMENMJ	N(Deamidation)	ME	1.04	1358.75	3
99	FTGLMENMJ			0.04	1357.74	3
99	FTGLMENMJ			0.06	1357.76	3
3	FTGLMENMJ			0.08	1357.78	3
1	FTGLMENMK			27.98	1241.59	3
99	HULVJ			-0.01	1090.69	3

1	HULVJ			25.89	1116.58	2
1	HULVK			14.28	960.86	2
99	HYLVJ			-0.02	946.57	2
99	HYLVJ			0.06	946.64	3
1	HYLVJ			26.22	972.81	2
1	HYLVKNK			14.12	1058.74	2
1	HYLVKNK			18.18	1062.81	2
1	HYLVKNK			18.18	1062.81	2
1	HYLVKNK			-25.86	1018.76	2
1	HYLVKNK	Y(O-Phosphoryl)	KN	80.16	1124.78	2
21	IEVULTTJ			-0.03	1397.82	3
85	IEVYLTTJ			0.03	1253.78	2
99	IEVYLTTJ			-0.03	1253.73	2
1	IEVYLTTKJ	V->**I**	VY	13.8	1395.65	3
1	IEVYLTTKJ			13.84	1395.69	3
99	JLUEFNNSPYETGYIK			-0.02	2397.25	3
90	JLUEFNNSPYETGYIK			-0.1	2397.17	4
1	JLYEFNNSPYETGYIJ			1.1	2398.37	4
99	JVTAQELDYLTR	E->Q	EL	-0.95	1723.02	3
99	JVTAQELDYLTR			0.01	1723.99	4
99	JVTAQELDYLTR			0.03	1724.01	3
99	JVTAQELDYLTR			-0.01	1723.97	3
99	JVTAQELDYLTR			0.07	1724.04	3
1	KTNDINSHQTDJ	D->E	DIN	13.97	1701.85	3
1	KVTAQELDULTR	E->Q	VTA	-0.97	1723	3
1	LGNUDNVR	N(Deamidation)	VR	1.19	1238.87	2
1	LGNUDNVR	N(Deamidation)	VR	1.23	1238.91	2
37	LGNUDNVRVEFJ			0.06	1885.1	4
1	LGNYDNVRVEFJ			0.1	1741.04	3
99	LGNYDNVRVEFJ			0.1	1741.04	4
2	LGNYDNVRVEFJ			0.08	1741.02	3
1	LGNYDNVRVEFJ	E->Q	VE	-0.82	1740.12	4
10	LUEFNNSPYETGYIJ	N(Deamidation)	UE	1.04	2270.21	4
99	LYEFNNSPUETGYIJ			-0.02	2269.15	3

99	LYEFNNSPYETGYIJ			0.06	2125.13	3
99	LYEFNNSPYETGYIJ			-0.04	2125.03	3
1	MVDSK			28.27	750.64	2
1	MVDSKDVJ	M(Oxidation)	DV	16.13	1224.81	2
1	MVDSKDVK			28.25	1092.83	2
1	MVDSKDVK			28.23	1092.8	2
1	MYK			15.97	600.29	2
1	MYK			16.27	600.58	2
1	MYKR			-17.81	722.6	2
1	NKDLADJ			0.1	1090.73	3
7	NKDLADJ			0.12	1090.74	3
1	NKDLADK			13.98	960.51	2
89	NLLSFDVQTNJ			0.05	1565.92	3
99	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.1	1839.17	4
99	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.1	1839.16	4
99	NLLSFDVQTNJJ			0.04	1838.11	4
1	SIDQFLUFDLIUSIJ	S(O-Phosphoryl)	SI	79.99	2520.37	4
1	SIDQFLUFDLIYSIKDTJ	Q->E / Q(Deamidation)	DQFL	1.12	2641.56	4
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.1	2658.54	4
99	SIDQFLYFDLIUSIJ			0.03	2296.3	3
1	SIDQFLYFDLIUSIKDTJ	F->Y	YFD	16.06	2656.5	5
99	SIDQFLYFDLIYSIJ			0.11	2152.29	3
99	SIDQFLYFDLIYSIJ			0.03	2152.21	3
99	SIDQFLYFDLIYSIJ			0.09	2152.26	3
99	SIDQFLYFDLIYSIJDTJ			0.04	2640.49	3
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	YFD	1.1	2641.54	4
0	SITVR			0.25	718.71	2
1	TCMUGGVTEHNGNQLDJYR	E->Q	GVTE	-0.91	2616.37	4

1	TCMYGGVTEHNGNQLDJ	H->Y	TC	25.77	2179.78	3
1	TCMYGGVTEHNGNQLDJ	Y->W	TC	22.77	2176.78	3
1	ULMMUNDNJ			-0.02	1766.91	3
99	ULMMYNDNJ			0.12	1622.94	3
1	ULMMYNDNJ			0.06	1622.89	3
1	URSITVR			-0.11	1181.6	3
99	VFEDGJNLLSFDVQTNJ			-0.03	2385.26	3
1	VFEDGJNLLSFDVQTNJ	Q->E / N(Deamidation) / Q(Deamidation)	VF	1.11	2386.4	4
0	VFEDGK			27.95	865.38	2
99	VLUDDNHVSAINVJ			0.02	2018.14	4
93	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.08	2019.21	4
0	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.06	2019.19	4
99	VLYDDNHVSAINVJ			-0.05	1873.95	3
97	VLYDDNHVSAINVJ			0.09	1874.1	3
1	VLYDDNHVSAINVJ			23.15	1897.17	4
10	VTAQELDYLTR			1.01	1452.78	2
1	YJDJ			0.28	984.88	2
0	YLMMUNDNKMVDSK			-15.8	2023.19	4
99	YLMMYNDNJ			-0.01	1478.71	2
76	YLMMYNDNJ			0.05	1478.76	3
1	YRSITVR			-13.93	1023.69	2
1	YVDVFGANYUUQCUFSJ	Q->E / N(Deamidation) / Q(Deamidation)	NY	0.95	2850.38	3

Pro Group Report: SEB on parquet 170hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (109 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	74	88	80.7
>95 (1.3)	1	1	74	88	80.7
>66 (0.47)	1	1	74	88	80.7
As shown: >95 (1.30)	1	1	74	88	80.7

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DKYVDVFGANYYYQCYFSJ	Q->E / N(Deamidation) / Q(Deamidation)	DK	1.21	2661.47	5
1	DLADJYJ			-0.22	1283.52	3
1	DLADK			18.04	722.42	2
1	DLADKYK			-15.8	979.74	2
1	DLADKYK			-25.86	969.69	2
1	DTJ			14.02	664.41	2
99	DVJIEVYLTTJ			-0.04	1740	2
99	DVJIEVYLTTJ			0	1740.05	3
1	FDQSJULMMYNDNK	Q->E / N(Deamidation) / Q(Deamidation)	DNK	1.1	2229.21	4
99	FIENENSFWYDMMPAPGDJFDQSJ			-0.02	3327.54	4
99	FIENENSFWYDMMPAPGDJFDQSJ			0	3327.56	3
99	FTGLMENMJ			0.02	1357.72	3
7	FTGLMENMJ			0.02	1357.72	3
99	FTGLMENMJ			-0.08	1357.63	3
1	FTGLMENMK			-0.9	1212.71	2
99	HULVJ			-0.01	1090.69	2
1	HULVJ			0.03	1090.72	3
1	HULVJ			25.87	1116.57	2
7	HULVJ			0.01	1090.7	3
1	HULVK			13.9	960.48	2

0	HULVKNK			28.12	1216.85	2
99	HYLVJ			0	946.59	2
1	HYLVJNJ			80.22	1413.04	2
9	HYLVJNK			80.14	1268.87	2
1	HYLVKNK			80.04	1124.66	2
1	HYLVKNK			-25.86	1018.76	2
68	IEVULTTJ			-0.03	1397.83	3
1	IEVULTTJ			-14.17	1383.68	3
0	IEVULTTKK	V->I	TT	13.74	1395.58	3
99	IEVYLTTJ			-0.09	1253.66	2
1	IEVYLTTK	V->I	LT	13.89	1123.55	2
1	IEVYLTTKK			-26.26	1211.48	3
1	JLYEFNNSPYETGYIK			16.1	2269.27	4
1	JVTAQELDULTR			14.21	1882.29	3
3	JVTAQELDULTR			1.05	1869.12	4
99	JVTAQELDYLTR			0.03	1724	3
99	JVTAQELDYLTR	E->Q	EL	-1.01	1722.97	3
99	JVTAQELDYLTR			-0.07	1723.92	2
1	LGNUDNVR	N(Deamidation)	VR	1.23	1238.9	2
1	LGNUDNVR	N(Deamidation)	VR	1.21	1238.88	2
1	LGNUDNVRVEFJ			0.04	1885.09	4
1	LGNUDNVRVEFK	F->Y	FK	15.92	1756.87	4
1	LGNYDNVR			1.18	1094.75	2
1	LGNYDNVR			-15.96	1077.6	2
1	LGNYDNVRVEFJ			0.12	1741.06	4
99	LUEFNNSPYETGYIJ			0.06	2269.22	4
99	LYEFNNSPUETGYIJ			-0.08	2269.09	3
99	LYEFNNSPYETGYIJ			0.04	2125.11	3
99	LYEFNNSPYETGYIJ			-0.06	2125.01	3
10	LYEFNNSPYETGYIJ			0.04	2125.11	3
1	MVDSJDVK	K->R	VK	28.25	1236.92	2
1	MVDSKDVJ	M(Oxidation)	DV	16.17	1224.85	2
1	MVDSKDVK			13.99	1078.56	2
1	MVDSKDVK			28.21	1092.79	2
1	MYJR			0.09	884.6	2
1	NKDLADK			-0.04	946.48	2

99	NLLSFDVQTNJ			-0.05	1565.82	2
96	NLLSFDVQTNJ			0.01	1565.88	3
82	NLLSFDVQTNJJ			1.02	1839.09	4
99	NLLSFDVQTNJJ			-0.02	1838.05	2
1	NLLSFDVQTNK			-0.26	1421.51	3
1	NLLSFDVQTNKJ			80.24	1774.2	3
1	SIDQFLUFDLIYSIKDTK	Y->F	KDTK	-15.88	2480.47	5
99	SIDQFLYFDLIUSIJ	Q->E / Q(Deamidation)	DQF	0.89	2297.17	4
99	SIDQFLYFDLIUSIKDTJ			0.04	2640.49	3
99	SIDQFLYFDLIYSIJ			0.05	2152.22	3
99	SIDQFLYFDLIYSIJ			0.03	2152.2	3
10	SIDQFLYFDLIYSIJDTK	Y->W	DQF	22.82	2519.16	3
1	SITVRVFEDGK			-14.31	1379.46	2
1	TCMYGGVTEHNGNQLDJ	E->Q	TC	-1.27	2152.74	5
1	UKDK			14.01	854.5	2
1	ULMMUNDNJ			-0.02	1766.9	3
1	ULMMUNDNKMVDSJ	L->M	NDN	18.1	2345.29	4
1	VEFJNJ			-14.19	1181.55	3
99	VFEDGJNLLSFDVQTNJ			-0.07	2385.22	3
1	VFEDGJNLLSFDVQTNJ			0.03	2385.33	4
99	VLUDDNHVSAINVJ			-0.06	2018.07	3
99	VLUDDNHVSAINVJ			0	2018.13	4
99	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.04	2019.16	4
99	VLYDDNHVSAINVJ			-0.07	1873.94	3
99	VTAQELDULTR			-0.04	1595.85	3
1	YJDJ			22.98	1007.59	2
1	YKDJ			-16.25	824.24	2
1	YKDJ			23.13	863.62	2
1	YLMMUNDNJ	M(Oxidation)	MM	16.1	1638.93	3
99	YLMMYNDNJ			-0.01	1478.71	2
1	YLMMYNDNJ	Y->H	YL	-25.95	1452.77	3

1	YLMMYNDNKMVDSK			-15.69	1879.18	3

(D) SEB on parquet over 170 hours

Pro Group Report: SEB on parquet 0hour

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (160 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	81	134	83.8
>95 (1.3)	1	1	81	134	83.8
>66 (0.47)	2	2	109	160	100
As shown: >95 (1.30)	1	1	81	134	83.8

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DJUVDVFGANUYYQCYFSK	Y(O-Phosphoryl) / S(O-Phosphoryl)	DVFGA	79.99	3028.45	5
1	DLADJUK			27.82	1311.57	4
1	DLADJYK	Y(O-Phosphoryl)	JY	79.94	1219.59	4
1	DTKLGNUDNVR	L->M	TK	17.69	1599.52	5
99	DVJIEVYLTTJ			-0.08	1739.97	4
99	DVJIEVYLTTJ			0.08	1740.12	4
99	DVJIEVYLTTJ			0.14	1740.18	3
99	DVJIEVYLTTJ			0.02	1740.06	3
99	DVJIEVYLTTJ			0.08	1740.12	3
99	DVJIEVYLTTJ			0.12	1740.17	3
99	DVJIEVYLTTJ			0.08	1740.13	3
99	DVJIEVYLTTJ			0.1	1740.15	3
1	DVKIEVULTTK	E->Q	IE	-0.86	1595.08	3
1	DVKIEVYLTTK			27.76	1479.61	4

1	FDQSKYLMMUNDNK	Y(O-Phosphoryl) / S(O-Phosphoryl)	DQS	80.21	2164.21	4
1	FIENENSFWYDMMPAPGDJ			0.19	2578.37	4
90	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	MMPAPG	1.12	3328.68	4
29	FIENENSFWYDMMPAPGDJFDQSJ	E->Q	NENS	-1.2	3326.37	4
97	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ	N(Deamidation)	ME	1.1	1358.81	3
99	FTGLMENMJ			0.04	1357.75	3
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			0.08	1357.78	2
99	FTGLMENMJ			0.08	1357.79	3
99	FTGLMENMJ			-0.02	1357.69	2
99	FTGLMENMJ			0.08	1357.79	3
62	FTGLMENMJ			0.08	1357.79	3
1	FTGLMENMJ			-1	1356.71	3
1	FTGLMENMJ	E->D	NM	-14.16	1343.55	4
1	FTGLMENMJ	E->Q	ME	-0.96	1356.74	3
1	FTGLMENMK	E->D	FT	-13.98	1199.62	4
62	HULVJ			0.05	1090.75	3
37	HULVJ			0.01	1090.7	3
3	HULVJ			0.03	1090.72	3
1	HULVJ			0.05	1090.75	3
99	HYLVJ			0.04	946.63	3
85	HYLVJ			0.02	946.6	3
85	HYLVJ			0.06	946.64	3
85	HYLVJ			0.06	946.64	2
43	HYLVJ			0.04	946.62	3
1	HYLVJ			-15.86	930.73	2
9	HYLVJ			0.04	946.63	3
99	HYLVJ			0.06	946.65	3
1	HYLVJ			-26.28	920.3	2
99	HYLVJ			0.08	946.66	3
1	HYLVJ			22.92	969.5	3
1	HYLVJNJ			22.82	1355.64	3
1	HYLVKNK	K->R	LV	27.94	1072.57	2
0	IEVULTTKK	V->I	UL	13.8	1395.65	3
99	IEVYLTTJ			-0.03	1253.73	2
99	IEVYLTTJ			0.07	1253.82	2
1	IEVYLTTJJ			-26.24	1499.71	5
1	IEVYLTTJJ			-16.1	1509.84	5

1	IEVYLTTKJ	Y->H	TK	-26.22	1355.63	3
1	IEVYLTTKK			-26.24	1211.51	3
99	JVTAQELDYLTR			0.13	1724.11	3
99	JVTAQELDYLTR			0.11	1724.08	3
99	JVTAQELDYLTR			0.11	1724.08	3
99	JVTAQELDYLTR			0.03	1724	3
99	JVTAQELDYLTR			0.09	1724.06	3
99	JVTAQELDYLTR			0.11	1724.08	3
1	KTCMYGGVTEHNGNQLDJ	D->E / V->I	KT	14.28	2296.39	4
1	KTNDINSHQTDK			-14.15	1529.63	3
1	KVTAQELDYLTR	K->R	YL	27.71	1607.58	4
1	KVTAQELDYLTR			-0.19	1579.68	3
1	KVTAQELDYLTR	K->R	KVT	28.21	1608.08	3
1	LGNUDNVRVEFJ			0.12	1885.16	3
1	LGNUDNVRVEFJ			0.22	1885.27	4
2	LGNYDNVRVEFJ			0.04	1740.98	3
1	LGNYDNVRVEFJ			0.18	1741.12	4
1	LGNYDNVRVEFJ			0.18	1741.12	4
1	LGNYDNVRVEFK	Y->W	VE	22.82	1619.66	4
99	LYEFNNSPUETGYIJ			0.08	2269.25	3
99	LYEFNNSPUETGYIJ			0.14	2269.3	4
99	LYEFNNSPUETGYIK			0.16	2125.22	3
99	LYEFNNSPYETGYIJ			0.12	2125.18	3
99	LYEFNNSPYETGYIJ			0.14	2125.21	3
99	LYEFNNSPYETGYIJ			0.14	2125.2	3
96	LYEFNNSPYETGYIJ	N(Deamidation)	NNS	1.24	2126.3	3
1	LYEFNNSPYETGYIJ			18.1	2143.17	4
1	MYK			27.99	612.3	2
1	MYKR			-25.71	714.7	2
1	NJDLADJ	N(Deamidation)	AD	0.88	1235.61	3
1	NKDLADJ			0.14	1090.77	3

1	NLLSFDVQTNJ	L->M	DV	17.71	1583.58	4
1	NLLSFDVQTNJ	D->E / V->I	QTN	13.87	1579.74	4
1	NLLSFDVQTNJ	L->M	DV	17.69	1583.56	4
99	NLLSFDVQTNJJ			0.02	1838.08	3
99	NLLSFDVQTNJJ			0.1	1838.17	3
99	NLLSFDVQTNJJ			0.12	1838.18	4
99	NLLSFDVQTNJJ			0.18	1838.24	3
1	NLLSFDVQTNKK	L->M	SF	17.78	1567.65	4
1	SIDQFLUFDLIUSIKDTJ	I->V	SID	-14.1	2770.45	5
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.22	2658.66	5
1	SIDQFLUFDLIYSIKDTJ	L->M	SI	18.18	2658.63	4
99	SIDQFLYFDLIUSIKDTJ			0.22	2640.66	4
99	SIDQFLYFDLIYSIJ			0.15	2152.33	3
99	SIDQFLYFDLIYSIJ			0.11	2152.28	3
99	SIDQFLYFDLIYSIJ			0.15	2152.33	3
99	SIDQFLYFDLIYSIJ			0.09	2152.26	3
97	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.2	2641.65	4
99	SIDQFLYFDLIYSIJDTJ			0.04	2640.49	4
99	SIDQFLYFDLIYSIJDTJ			0.12	2640.56	3
99	SIDQFLYFDLIYSIJDTJ			0.1	2640.54	3
0	SITVR			80.05	798.51	2
1	SITVRVFEDGK			-14.11	1379.66	3
1	SSJ			0.13	608.5	2
1	SSKFTGLMENMK			-0.11	1515.64	4
1	TCMYGGVTEHNGNQLDJ	Y->W	MYG	23.23	2177.24	4
1	TNDINSHQTDJ	S(O-Phosphoryl) / T(O-Phosphoryl)	HQ	79.78	1639.57	4
1	UKDJ			-0.02	984.58	3
99	ULMMYNDNJ			0.1	1622.93	3

1	ULMMYNDNKMVDSJ			79.88	2262.97	5
1	UVDVFGANYYYQCUFSJ	Y->H	CU	-25.81	2679.52	4
2	VEFJ			0.09	809.58	2
1	VEFJNJ			-14.11	1181.62	3
1	VEFJNJ	F->Y	VE	15.91	1211.64	3
1	VEFKNK			16.2	923.73	2
99	VFEDGJNLLSFDVQTNJ			-0.03	2385.27	3
1	VFEDGK			14.29	851.72	2
1	VFEDGK			14.23	851.67	2
99	VLUDDNHVSAINVJ			0.12	2018.24	3
17	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.16	2019.29	4
17	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.12	2019.24	5
99	VLYDDNHVSAINVJ			0.13	1874.15	3
99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ			0.11	1874.12	3
99	VLYDDNHVSAINVJ			0.03	1874.04	3
1	VLYDDNHVSAINVK	N(Deamidation)	NVK	0.85	1730.77	4
1	VTAQELDULTR	R->K	LT	-28.3	1567.58	4
99	YLMMYNDNJ			0.05	1478.77	2
96	YLMMYNDNJ			0.11	1478.83	3
1	YLMMYNDNK			1.03	1335.64	4
1	YRSITVR			-13.87	1023.74	2

Pro Group Report: SEB on parquet 2hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (166 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	80	146	88
>95 (1.3)	1	1	80	146	88
>66 (0.47)	1	1	80	146	88
As shown: >95 (1.30)	1	1	80	146	88

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADJUK			28.02	1311.77	4
1	DLADJUK			-0.12	1283.63	3
1	DTJ			14.08	664.47	2
1	DTKLGNUDNVR	L->M	TK	17.75	1599.58	5
1	DTKLGNYDNVR	Y->F	DT	-16.17	1421.56	3
99	DVJIEVYLTTJ			0.1	1740.15	3
99	DVJIEVYLTTJ			0	1740.05	3
99	DVJIEVYLTTJ			0.1	1740.15	3
99	DVJIEVYLTTJ			0.18	1740.22	3
99	DVJIEVYLTTJ			0.12	1740.16	3
99	DVJIEVYLTTJ			0.16	1740.21	3
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FWYD	1.22	3328.78	4
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1.06	3328.63	5
1	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FIE	1.24	3328.8	4
95	FTGLMENMJ	N(Deamidation)	ME	1.12	1358.83	3
82	FTGLMENMJ			0.1	1357.8	3
5	FTGLMENMJ	E->D	GL	-14.06	1343.65	3
3	FTGLMENMJ	E->Q	NM	-0.96	1356.75	3
1	FTGLMENMJ	E->D	NM	-14.14	1343.56	4

99	FTGLMENMJ	E->Q	ME	-0.76	1356.95	3
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			-0.02	1357.69	2
1	FTGLMENMJ	E->Q	FTG	-0.94	1356.76	3
99	FTGLMENMJ			0.02	1357.73	3
99	FTGLMENMJ			0.08	1357.79	2
99	FTGLMENMJ			0.12	1357.83	3
97	FTGLMENMJ			0.12	1357.82	3
37	HULVJ			0.07	1090.77	3
7	HULVJ			0.05	1090.75	3
37	HULVJ			0.03	1090.72	3
99	HYLVJ			0.08	946.66	3
99	HYLVJ			0.06	946.65	2
99	HYLVJ			0.06	946.65	3
99	HYLVJ			0.08	946.66	3
94	HYLVJ			0.02	946.61	3
85	HYLVJ			0.06	946.64	3
1	HYLVJNJ	Y->W	HY	22.86	1355.69	3
1	HYLVJNJ			22.84	1355.67	3
1	HYLVJNJ			-15.74	1317.09	3
1	HYLVKNK	K->R	LV	27.96	1072.58	2
1	IEVULTTJ			0.11	1397.97	3
1	IEVULTTK			17.85	1271.6	3
1	IEVULTTK			17.89	1271.65	3
1	IEVULTTKJ	E->D / I->V	TTK	-14.28	1511.66	4
1	IEVULTTKK	E->D / I->V	UL	-14.2	1367.64	4
0	IEVULTTKK	V->I	UL	13.8	1395.65	3
1	IEVYLTTJ	V->I	TT	14.01	1267.77	4
85	IEVYLTTJ			-0.07	1253.68	3
94	IEVYLTTJ			0.09	1253.85	3
99	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.09	1253.84	2
99	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.03	1253.78	2
1	IEVYLTTJJ	Y->F	LTT	-16.28	1509.67	5
1	IEVYLTTKK			-14.1	1223.65	4
12	JVTAQELDYLTR	Q->E / Q(Deamidation)	EL	1.09	1725.07	3
99	JVTAQELDYLTR			0.17	1724.15	3
99	JVTAQELDYLTR			0.15	1724.12	3
99	JVTAQELDYLTR			0.11	1724.09	3
99	JVTAQELDYLTR			0.13	1724.1	3

99	JVTAQELDYLTR			0.13	1724.1	3
99	JVTAQELDYLTR	E->Q	EL	-0.91	1723.06	3
99	JVTAQELDYLTR			0.01	1723.99	3
99	JVTAQELDYLTR	E->Q	EL	-0.93	1723.05	3
99	JVTAQELDYLTR			0.03	1724.01	3
94	JVTAQELDYLTR	E->Q	EL	-0.87	1723.11	3
1	KTCMUGGVTEHNGNQLDK	D->E / V->I	KT	14.26	2296.37	4
1	KTNDINSHQTDK			-14.17	1529.6	3
1	KTNDINSHQTDK			-14.21	1529.57	5
1	KTNDINSHQTDK	S(O-Phosphoryl) / T(O-Phosphoryl)	KTN	79.73	1623.5	4
1	KVTAQELDYLTR	K->R	KVT	27.71	1607.59	4
1	KVTAQELDYLTR			28.27	1608.14	3
90	LGNYDNVRVEFJ			0.2	1741.15	4
1	LGNYDNVRVEFJ			0.2	1741.15	4
1	LGNYDNVRVEFJ			1.18	1742.13	3
1	LGNYDNVRVEFK			22.72	1619.57	5
10	LUEFNNSPYETGUIK			0.2	2269.36	4
99	LYEFNNSPUETGYIJ			0.16	2269.33	3
48	LYEFNNSPUETGYIJ	N(Deamidation)	ETGY	1.04	2270.21	4
99	LYEFNNSPYETGUIK			0.2	2125.26	3
99	LYEFNNSPYETGYIJ			0.12	2125.18	3
99	LYEFNNSPYETGYIJ			0.12	2125.18	3
88	LYEFNNSPYETGYIJ			0.2	2125.27	3
0	MVDSJ	D->E / V->I	DS	14.16	880.64	2
1	MVDSK			28.07	750.44	2
1	MYKR			-17.77	722.64	2
1	MYKR			-25.71	714.7	2
0	NJDLADJ			0.92	1235.65	3
0	NJDLADJ			1.1	1235.82	3
1	NJDLADK			0.14	1090.76	3

1	NKDLADK			13.96	960.49	2
1	NKDLADK			13.98	960.51	2
1	NLLSFDVQTNJ			17.71	1583.59	4
99	NLLSFDVQTNJ			-0.01	1565.87	2
99	NLLSFDVQTNJ			0.09	1565.97	3
5	NLLSFDVQTNJJ			0.12	1838.19	4
13	NLLSFDVQTNJJ			0.14	1838.21	4
99	NLLSFDVQTNJJ			0.12	1838.19	3
99	NLLSFDVQTNJJ			0.02	1838.09	2
99	NLLSFDVQTNJJ			0.2	1838.26	3
1	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.08	1839.15	4
1	NLLSFDVQTNKJ			28.18	1722.14	4
1	NLLSFDVQTNKJ			28.3	1722.27	3
1	NLLSFDVQTNKK			-0.14	1549.72	5
1	NLLSFDVQTNKK	L->M	SF	17.72	1567.58	4
1	NLLSFDVQTNKK			17.8	1567.67	4
99	SIDQFLUFDLIYSIJ			0.13	2296.41	3
99	SIDQFLYFDLIUSIJ			0.09	2296.37	3
99	SIDQFLYFDLIYSIJ			0.15	2152.32	3
99	SIDQFLYFDLIYSIJ			0.21	2152.38	3
99	SIDQFLYFDLIYSIJ			0.03	2152.2	3
99	SIDQFLYFDLIYSIJ			0.23	2152.41	3
99	SIDQFLYFDLIYSIJ			-0.03	2152.15	2
99	SIDQFLYFDLIYSIJ			0.23	2152.4	3
99	SIDQFLYFDLIYSIJDTJ			0.16	2640.61	3
0	SITVR			80.07	798.53	2
1	SITVRVFEDGK	E->D / I->V	SIT	-14.11	1379.67	3
1	SITVRVFEDGK			27.79	1421.57	3

1	SITVRVFEDGK			13.87	1407.65	4
1	SSJ			0.21	608.58	2
1	SSJ			0.15	608.53	2
1	TNDINSHQTDJ	S(O-Phosphoryl) / T(O-Phosphoryl)	INS	79.82	1639.6	4
1	ULMMUNDNK			17.92	1640.74	4
1	VEFJ			-13.73	795.77	2
1	VEFKNK			1.22	908.75	3
1	VFEDGJ			14.12	995.67	3
99	VLUDDNHVSAINVJ			0.02	2018.14	4
99	VLUDDNHVSAINVJ			0.14	2018.27	3
62	VLUDDNHVSAINVJ	N(Deamidation)	HVS	1.2	2019.32	4
1	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.12	2019.24	5
99	VLYDDNHVSAINVJ			0.13	1874.15	3
99	VLYDDNHVSAINVJ			0.17	1874.19	3
99	VLYDDNHVSAINVJ			0.05	1874.06	3
99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ	N(Deamidation)	HVS	1.15	1875.16	4
99	VLYDDNHVSAINVJ			0.15	1874.16	3
1	VLYDDNHVSAINVK	Y->H	SAI	-25.99	1703.92	3
16	VTAQELDULTR			0.06	1595.95	3
1	YJDJ			-26.12	958.49	2
1	YKDJ	Y(O-Phosphoryl)	YK	79.81	920.31	2
1	YKDK			80.25	776.65	2
1	YLMMUNDNJMVDSJ			17.86	2345.04	3
99	YLMMYNDNJ			0.11	1478.82	2
99	YLMMYNDNJ			0.03	1478.74	2
53	YLMMYNDNJ			0.13	1478.84	3

Pro Group Report: SEB on parquet 27hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (152 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	80	129	84.9
>95 (1.3)	1	1	80	129	84.9
>66 (0.47)	1	1	80	129	84.9
As shown: >95 (1.30)	1	1	80	129	84.9

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADJYK	Y(O-Phosphoryl)	JY	80.04	1219.69	4
1	DLADKYJ			0.06	1139.7	2
1	DLADKYK			18.16	1013.71	3
1	DLADKYK			-26.04	969.51	3
1	DTJ			14.1	664.48	2
0	DTJLGNYDNVR			80.13	1661.96	3
99	DVJIEVYLTTJ			0.02	1740.06	3
99	DVJIEVYLTTJ			0.12	1740.16	3
99	DVJIEVYLTTJ			0.14	1740.19	3
99	DVJIEVYLTTJ			0.12	1740.17	4
16	DVKIEVYLTTJ			0.04	1595.99	3
1	FDQSJYLMMYNDNK			79.99	2163.99	4
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FWYD	1.24	3328.8	4
29	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	FWYD	1.28	3328.85	4
99	FTGLMENMJ			0.12	1357.82	3
99	FTGLMENMJ			0.12	1357.83	3
99	FTGLMENMJ			0.12	1357.82	3
99	FTGLMENMJ			0.08	1357.78	3
99	FTGLMENMJ			0	1357.71	2

99	FTGLMENMJ			0.1	1357.8	2
1	FTGLMENMJ			18.02	1375.72	4
72	FTGLMENMJ	N(Deamidation)	ME	1.12	1358.83	3
62	FTGLMENMJ			0.12	1357.83	3
3	FTGLMENMJ			0.1	1357.8	3
1	FTGLMENMJ	M->L	ME	-17.86	1339.84	4
1	FTGLMENMK			1.2	1214.8	3
1	FTGLMENMK	F->Y / M(Oxidation)	FT	16.06	1229.67	3
1	HULVJ			0.09	1090.79	3
7	HULVJ			0.07	1090.76	3
37	HULVJ			0.03	1090.73	3
99	HULVJ			0.07	1090.77	3
1	HULVK			13.96	960.54	2
1	HULVKNJ			13.96	1346.78	2
43	HYLVJ			0.06	946.64	3
1	HYLVJ			-26.28	920.31	2
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.06	946.65	2
85	HYLVJ			0.04	946.62	3
1	HYLVJNJ	Y->W	HY	22.86	1355.69	3
1	HYLVJNJ			22.82	1355.64	3
1	HYLVJNJ			22.9	1355.73	3
1	HYLVKNK			27.98	1072.6	2
1	HYLVKNK			23.14	1067.76	3
1	IEVULTTKJ	E->D / I->V	TTK	-14.26	1511.69	4
1	IEVULTTKK	E->Q	UL	-1.2	1380.64	2
99	IEVYLTTJ			0.03	1253.79	2
99	IEVYLTTJ			0.09	1253.85	3
99	IEVYLTTJ			0.09	1253.84	2
85	IEVYLTTJ			0.11	1253.87	3
1	IEVYLTTJ	E->D / I->V	TT	-13.87	1239.88	4
1	IEVYLTTJ			17.89	1271.65	3
1	IEVYLTTJ	L->M	YL	17.87	1271.63	3
1	IEVYLTTKK			-14.1	1223.65	4
1	IEVYLTTKK			18.02	1255.77	4
1	JTNDINSHQTDK	S(O-Phosphoryl) / T(O-Phosphoryl)	NS	79.67	1767.54	4
99	JVTAQELDYLTR			0.15	1724.12	3
99	JVTAQELDYLTR			0.03	1724.01	3
99	JVTAQELDYLTR			0.11	1724.08	3

99	JVTAQELDYLTR			0.13	1724.11	3
99	JVTAQELDYLTR			0.13	1724.1	3
99	JVTAQELDYLTR			0.13	1724.1	3
99	JVTAQELDYLTR			0.17	1724.14	3
1	KTNDINSHQTDK	I->V	KTN	-14.19	1529.59	5
1	KTNDINSHQTDK			-14.13	1529.64	3
1	LGNUDNVRVEFJ			-28.06	1856.99	4
1	LGNYDNVR			14.12	1107.69	2
30	LGNYDNVRVEFJ			0.18	1741.12	3
2	LGNYDNVRVEFJ			0.06	1741.01	3
1	LGNYDNVRVEFK			22.72	1619.56	5
99	LYEFNNSPUETGYIJ			0.12	2269.29	3
24	LYEFNNSPUETGYIJ	N(Deamidation)	ETGY	1.16	2270.32	4
72	LYEFNNSPUETGYIK			0.2	2125.27	3
99	LYEFNNSPYETGYIJ			0.14	2125.2	3
99	LYEFNNSPYETGYIJ			0.16	2125.22	3
1	MVDSJ	D->E / V->I	DS	14.16	880.64	2
1	MYJ			15.91	744.32	2
1	MYK			28.01	612.32	2
1	NJDLADJ			1.12	1235.84	3
1	NJDLADK			13.76	1104.39	2
0	NKDLADK			1.18	947.71	2
1	NKDLADK			1.18	947.71	2
1	NLLSFDVQTNJ	L->M	DV	17.71	1583.58	4
96	NLLSFDVQTNJ			0.09	1565.96	3
99	NLLSFDVQTNJ			-0.03	1565.85	2
99	NLLSFDVQTNJ			0.11	1565.99	3
99	NLLSFDVQTNJJ			0.04	1838.11	3
99	NLLSFDVQTNJJ			0.12	1838.19	3
76	NLLSFDVQTNJJ			0.1	1838.16	4

82	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.14	1839.2	4
1	NLLSFDVQTNKK			-0.14	1549.73	5
99	SIDQFLYFDLIYSIJ			0.21	2152.39	3
99	SIDQFLYFDLIYSIJ			0.07	2152.25	3
99	SIDQFLYFDLIYSIJ			0.11	2152.28	3
99	SIDQFLYFDLIYSIJ			0.23	2152.41	3
99	SIDQFLYFDLIYSIJ			0.19	2152.36	3
99	SIDQFLYFDLIYSIJ			0.23	2152.4	3
99	SIDQFLYFDLIYSIJDTJ			0.12	2640.56	3
1	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	IJ	1.24	2641.69	4
99	SIDQFLYFDLIYSIK			0.13	2008.2	2
0	SITVR			80.05	798.51	2
1	SITVRVFEDGJ	R->K	VF	-28.15	1509.72	5
1	SITVRVFEDGK			27.83	1421.6	3
1	SITVRVFEDGK	E->D / I->V	RV	-14.09	1379.68	3
1	SSKFTGLMENMJ	M->L	EN	-18.1	1641.76	3
1	TCMUGGVTEHNGNQLDKYR	Y->W	QLD	22.81	2495.98	3
1	TCMYGGVTEHNGNQLDJ	Y->W	GVTE	23.27	2177.29	4
82	ULMMYNDNJ			0.16	1622.98	3
1	ULMMYNDNJ	Y->H	ND	-25.78	1597.05	3
1	ULMMYNDNJ			17.94	1640.77	4
1	ULMMYNDNKMVDSJ			79.92	2263	5
1	UVDVFGANUUYQCYFSK	D->E / V->I	YFSK	14.09	2719.43	5
1	VEFJ			14.27	823.76	2
1	VEFJNK			16.07	1067.7	3
1	VEFKNK			16.22	923.75	2
99	VLUDDNHVSAINVJ			0.14	2018.27	3
17	VLUDDNHVSAINVJ	N(Deamidation)	DDN	1.16	2019.29	4
1	VLUDDNHVSAINVJ			0.18	2018.31	4

99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ			0.15	1874.17	3
97	VLYDDNHVSAINVJ			0.19	1874.2	3
82	VLYDDNHVSAINVJ			0.09	1874.1	3
37	VLYDDNHVSAINVJ			0.15	1874.16	4
1	VLYDDNHVSAINVK	Y->H	SAI	-26.01	1703.91	3
13	VTAQELDULTR			1.12	1597	3
1	VTAQELDYLTRHYLVJ	H->Y	VT	25.73	2261.98	5
99	YLMMYNDNJ			0.09	1478.81	2
99	YLMMYNDNJ			0.11	1478.82	3
13	YLMMYNDNJ			0.13	1478.85	3
1	YVDVFGANYYYQCYFSKJ	Y->H	CYF	-25.86	2519.37	4

Pro Group Report: SEB on parquet 50hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (120 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	61	98	81.7
>95 (1.3)	1	1	61	98	81.7
>66 (0.47)	1	1	61	98	81.7
As shown: >95 (1.30)	1	1	61	98	81.7

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DLADKYJ			79.94	1219.58	2
1	DLADKYK			18.18	1013.72	3
1	DTKLGNYDNVR			17.93	1455.67	4
99	DVJIEVYLTTJ			0.08	1740.13	3
99	DVJIEVYLTTJ			0.1	1740.15	3
1	FDQSJ			16.28	927.79	2
1	FDQSJ			16.28	927.79	2

1	FDQSJYLMMYNDNK	Y(O-Phosphoryl) / S(O-Phosphoryl)	LMM	80.15	2164.15	4
1	FDQSK			1.21	768.61	2
99	FIENENSFWYDMMPAPGDJFDQSJ			0.18	3327.75	4
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1.24	3328.8	5
99	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	NENS	1.22	3328.79	4
99	FTGLMENMJ			0.06	1357.77	3
1	FTGLMENMJ	M->L	FT	-18.22	1339.48	3
99	FTGLMENMJ			0.06	1357.77	2
99	FTGLMENMJ			0.1	1357.8	2
99	FTGLMENMJ			0.08	1357.79	3
99	FTGLMENMJ			-0.82	1356.88	3
1	FTGLMENMK			0	1213.61	3
1	HULVJ			0.05	1090.75	3
97	HULVJ			0.05	1090.74	3
1	HULVK			25.82	972.41	2
1	HYLVJ			-15.9	930.69	2
1	HYLVJ			25.82	972.4	2
1	HYLVJ			-15.9	930.68	2
99	HYLVJ			0.06	946.65	3
99	HYLVJ			0.06	946.64	2
43	HYLVJ			0.04	946.63	3
43	HYLVJ			0.06	946.64	3
1	HYLVK			-15.88	786.6	2
1	IEVULTTJ	E->D / I->V	UL	-14.21	1383.65	4
1	IEVULTTJJ			0.03	1670.08	4
1	IEVYLTTJ	L->M	YL	17.83	1271.58	3
99	IEVYLTTJ			0.07	1253.82	3
99	IEVYLTTJ			0.05	1253.81	2
99	IEVYLTTJ			0.07	1253.83	2
1	IEVYLTTJJ			-26.24	1499.7	5
1	IEVYLTTJJ			-26.22	1499.72	5
99	JVTAQELDYLTR			0.11	1724.09	3
99	JVTAQELDYLTR			0.09	1724.06	3
99	JVTAQELDYLTR	E->Q	EL	-0.91	1723.07	3

99	JVTAQELDYLTR			0.11	1724.08	3
89	JVTAQELDYLTR			0.07	1724.04	3
1	JVTAQELDYLTR	Q->E / Q(Deamidation)	EL	1.09	1725.06	4
1	KLUEFNNSPUETGYIJ			0.16	2541.52	4
1	KLYEFNNSPYETGYIK	Y->W	FNN	23.31	2132.37	4
1	KTCMYGGVTEHNGNQLDJ	D->E / V->I	KT	14.26	2296.36	4
1	KTCMYGGVTEHNGNQLDK	Y->F	NGNQ	-16.04	2121.97	4
1	LGNUDNVRVEFJ			0.14	1885.18	3
99	LGNYDNVRVEFJ			0.08	1741.03	3
5	LGNYDNVRVEFJ			0.16	1741.1	4
2	LGNYDNVRVEFJ			0.12	1741.07	3
99	LYEFNNSPUETGYIJ			0.14	2269.3	3
99	LYEFNNSPUETGYIJ			0.16	2269.32	4
99	LYEFNNSPYETGYIJ			0.14	2125.21	3
99	LYEFNNSPYETGYIJ			0.14	2125.21	3
99	LYEFNNSPYETGYIJ			0.12	2125.19	3
1	MVDSJ	D->E / V->I	DS	14.16	880.64	2
1	MYK			15.99	600.3	2
0	NJDLADJ			1.02	1235.74	3
1	NJDLADJ			0.88	1235.61	3
1	NJDLADK			1.12	1091.74	3
0	NKDLADK			1.18	947.71	2
89	NLLSFDVQTNJ			0.11	1565.98	3
99	NLLSFDVQTNJ			0.09	1565.96	3
99	NLLSFDVQTNJ			0.05	1565.93	2
99	NLLSFDVQTNJJ			0.12	1838.18	3
99	NLLSFDVQTNJJ			0.06	1838.13	3
29	NLLSFDVQTNJJ			0.12	1838.19	4
1	NLLSFDVQTNJJ			1.12	1839.19	4
93	NLLSFDVQTNJJ			0.22	1838.28	3

99	SIDQFLUFDLIYSIJ			0.15	2296.43	3
99	SIDQFLYFDLIYSIJ			0.13	2152.31	3
99	SIDQFLYFDLIYSIJ			0.11	2152.28	3
99	SIDQFLYFDLIYSIJ			0.17	2152.34	3
99	SIDQFLYFDLIYSIJDTJ			0.16	2640.6	3
1	SITVR			0.27	718.73	2
1	SITVR			0.27	718.72	2
3	ULMMYNDNJ	M->L	MY	-17.92	1604.9	4
1	VEFJ			-13.73	795.76	2
1	VEFJ			14.25	823.75	2
1	VEFJ			14.27	823.76	2
1	VEFJNJ			-14.19	1181.54	3
1	VEFJNK	N(Deamidation)	NK	1.27	1052.9	2
1	VEFKNJ			-13.91	1037.72	3
1	VEFKNK			0.22	907.74	2
99	VLUDDNHVSAINVJ			0.12	2018.24	3
1	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.12	2019.25	4
99	VLYDDNHVSAINVJ			0.13	1874.14	4
99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ			0.11	1874.12	3
1	VLYDDNHVSAINVK			-26.01	1703.91	3
1	YKDJ			23.27	863.77	2
1	YKDJ	Y(O-Phosphoryl)	YK	79.79	920.29	2
99	YLMMYNDNJ			0.09	1478.81	3
99	YLMMYNDNJ			0.09	1478.8	2
76	YLMMYNDNJ			0.11	1478.82	3

Pro Group Report: SEB on parquet 74hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (275 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	128	226	82.2
>95 (1.3)	1	1	128	226	82.2
>66 (0.47)	2	2	188	275	100
As shown: >95 (1.30)	1	1	128	226	82.2

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
0	DLADK	K->R	LA	28.02	732.4	2
1	DLADKYK			-26.02	969.52	3
1	DLADKYK			0.26	995.8	2
99	DVJIEVYLTTJ			0.1	1740.15	3
99	DVJIEVYLTTJ			0.04	1740.08	3
99	DVJIEVYLTTJ			0.14	1740.18	3
1	FDQSKYLMMYNDNK			14.15	1954.04	3
37	FTGLMENMJ			0.12	1357.82	3
7	FTGLMENMJ			-1.16	1356.55	2
1	FTGLMENMJ			1.1	1358.8	3
1	FTGLMENMJ			0.1	1357.81	2
1	FTGLMENMJ			1.1	1358.81	3
1	FTGLMENMJ			-13.86	1343.85	3
99	FTGLMENMJ			0.08	1357.78	2
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			0.02	1357.73	2
99	FTGLMENMJ			0.08	1357.79	3
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			0.1	1357.81	3
97	FTGLMENMJ			0.12	1357.82	3
93	FTGLMENMJ			0.12	1357.82	3
88	FTGLMENMJ	N(Deamidation)	ME	1.12	1358.83	3
62	FTGLMENMJ			-0.22	1357.48	3
62	FTGLMENMJ			-0.22	1357.49	3
38	FTGLMENMJ	E->Q	ME	-0.94	1356.76	3
1	FTGLMENMK	E->Q	FT	-0.76	1212.84	2

1	FTGLMENMK	F->Y / M(Oxidation)	FT	16.14	1229.74	3
1	FTGLMENMK	E->Q	FT	-0.76	1212.85	2
1	FTGLMENMK	E->Q	FT	-0.76	1212.85	2
1	FTGLMENMK	E->Q	GL	-1.02	1212.58	2
1	HULVJ			0.07	1090.77	3
93	HULVJ			0.05	1090.75	3
97	HULVJ			0.05	1090.74	2
97	HULVJ			0.05	1090.74	3
1	HULVK			14.18	960.76	2
1	HULVK			0.12	946.7	2
1	HYLVJ			14	960.58	2
99	HYLVJ			0.06	946.65	3
85	HYLVJ			0.04	946.63	3
85	HYLVJ			0.04	946.63	3
94	HYLVJ			0.06	946.65	3
98	HYLVJ			0.06	946.65	3
99	HYLVJ			0.06	946.64	2
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.06	946.65	3
99	HYLVJ			0.08	946.66	3
99	HYLVJ			0.06	946.65	3
99	HYLVJ			0.06	946.65	3
99	HYLVJ			0.06	946.65	3
1	HYLVKNJ	H->Y	VK	26.06	1214.79	2
1	HYLVKNK	K->R	LV	27.94	1072.56	2
1	HYLVKNK			18.24	1062.86	2
1	IEVULTTJ	E->D / I->V	TT	-14.15	1383.7	4
1	IEVULTTJ			-14.19	1383.67	4
1	IEVULTTJJ			13.77	1683.83	2
97	IEVYLTTJ			0.23	1253.98	3
97	IEVYLTTJ			0.23	1253.98	3
93	IEVYLTTJ			0.23	1253.98	3
1	IEVYLTTJ	L->M	YL	17.87	1271.62	3
1	IEVYLTTJ	V->I	TT	14.03	1267.79	4
1	IEVYLTTJ	E->D / I->V	TT	-13.95	1239.8	4
1	IEVYLTTJ			23.23	1276.98	2
3	IEVYLTTJ	E->D / I->V	YL	-13.87	1239.88	3
62	IEVYLTTJ			0.23	1253.98	3
93	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.07	1253.83	2
99	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.09	1253.84	3
99	IEVYLTTJ			0.11	1253.86	2
99	IEVYLTTJ			0.23	1253.98	3

99	IEVYLTTJ			0.05	1253.8	2
99	IEVYLTTJ			0.23	1253.98	3
1	IEVYLTTJJ			-15.98	1509.96	5
1	IEVYLTTJJ			-16.02	1509.92	5
1	IEVYLTTK			-25.89	1083.77	2
1	IEVYLTTKJ			13.82	1395.67	3
1	IEVYLTTKK	K->R	TT	28.24	1265.98	3
1	IEVYLTTKK	E->D / I->V	IE	-14.06	1223.68	4
1	JLUEFNNSPUETGYIK			-13.76	2527.6	3
1	JTCMYGGVTEHNGNQLDK	Y->W	NGNQ	22.76	2304.86	5
1	JTNDINSHQTDK	H->Y	HQ	25.91	1713.78	2
90	JVTAQELDULTR			0.17	1868.24	4
99	JVTAQELDYLTR			0.11	1724.08	3
99	JVTAQELDYLTR			0.13	1724.1	3
99	JVTAQELDYLTR			0.09	1724.06	3
5	JVTAQELDYLTR	Q->E / Q(Deamidation)	EL	1.15	1725.13	3
1	JVTAQELDYLTR			0.11	1724.08	3
99	JVTAQELDYLTR	E->Q	EL	-0.85	1723.13	3
99	JVTAQELDYLTR	E->Q	EL	-0.89	1723.08	3
99	JVTAQELDYLTR			0.11	1724.08	3
1	KLUEFNNSPUETGUIK	E->D / I->V	UETG	-13.82	2527.55	3
1	KLYEFNNSPYETGYIK	N(Deamidation)	ETG	0.91	2109.97	5
1	KLYEFNNSPYETGYIK			-0.05	2109	4
1	KTCMUGGVTEHNGNQLDK	D->E / V->I	KT	14.3	2296.41	4
1	KTNDINSHQTDK			14.19	1557.97	3
1	KTNDINSHQTDK			-14.11	1529.66	3
1	KVTAQELDYLTR	Y(O-Phosphoryl) / T(O-Phosphoryl)	VTA	80.11	1659.99	4
1	LGNUDNVRVEFJ			0.1	1885.15	3
0	LGNUDNVRVEFK			0.22	1741.16	3
1	LGNYDNVRVEFK			79.7	1676.55	4

1	LGNYDNVRVEFK	E->Q	RVE	-0.9	1595.95	3
1	LGNYDNVRVEFK	Y->W	NVR	22.92	1619.77	4
1	LYEFNNSPUETGUIK	N(Deamidation)	ETG	1.18	2270.34	4
1	LYEFNNSPUETGUIK	N(Deamidation)	YEF	1.2	2270.36	4
88	LYEFNNSPUETGYIJ	N(Deamidation)	YEF	1.16	2270.33	4
99	LYEFNNSPUETGYIJ			0.1	2269.26	3
1	LYEFNNSPUETGYIK			0.2	2125.26	3
99	LYEFNNSPYETGUIK			0.18	2125.24	3
99	LYEFNNSPYETGUIK			0.16	2125.22	3
24	LYEFNNSPYETGYIJ			0.18	2125.24	3
99	LYEFNNSPYETGYIJ			0.12	2125.19	3
99	LYEFNNSPYETGYIJ			0.12	2125.18	3
1	LYEFNNSPYETGYIK			17.96	1998.93	3
0	MVDSJ	D->E / V->I	DS	14.18	880.67	2
0	MVDSJ	D->E / V->I	DS	14.18	880.66	2
1	MVDSJ			-17.8	848.68	2
1	MYJ			15.81	744.22	2
1	MYJ			-18.09	710.32	1
1	MYJ			15.81	744.23	2
1	MYJR			-15.91	868.6	2
1	MYJR			-25.93	858.59	2
1	MYK			28.23	612.54	2
1	MYK			27.99	612.3	2
1	MYK			16.23	600.54	2
1	MYKR			-17.79	722.62	2
1	MYKR			-25.73	714.69	2
0	NJDLADJ			0.9	1235.62	3
1	NJDLADJ			14.04	1248.77	4
1	NJDLADJ			0.9	1235.63	3
1	NJDLADJ			1.1	1235.82	3
1	NJDLADK			1.14	1091.77	3
1	NJJ			-0.23	820.32	2
1	NKDLADK			28.2	974.72	2
0	NKDLADK			1.2	947.72	2
1	NKDLADK			1.2	947.72	2
1	NKJ			0.15	676.61	2
1	NLLSFDVQTNJ			17.81	1583.68	3

98	NLLSFDVQTNJ			0.13	1566	3
98	NLLSFDVQTNJ			0.13	1566	3
99	NLLSFDVQTNJ			0.09	1565.97	3
99	NLLSFDVQTNJ			0.11	1565.98	3
99	NLLSFDVQTNJ			0.11	1565.99	3
99	NLLSFDVQTNJ			0.01	1565.89	2
99	NLLSFDVQTNJ			0.11	1565.99	3
63	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	LLS	1.14	1839.21	4
99	NLLSFDVQTNJJ			0.1	1838.17	3
99	NLLSFDVQTNJJ			0.12	1838.18	3
99	NLLSFDVQTNJJ			0.3	1838.37	4
29	NLLSFDVQTNJJ			0.1	1838.17	4
1	NLLSFDVQTNK			0.14	1421.91	3
1	SIDQFLUFDLIUSIK			0.17	2296.45	4
99	SIDQFLYFDLIUSIJ			0.11	2296.38	3
5	SIDQFLYFDLIUSIK	Q->E / Q(Deamidation)	FLY	1.21	2153.38	3
3	SIDQFLYFDLIUSIK	Q->E / Q(Deamidation)	FDL	1.17	2153.35	3
99	SIDQFLYFDLIYSIJ			0.17	2152.35	3
99	SIDQFLYFDLIYSIJ			0.17	2152.34	3
99	SIDQFLYFDLIYSIJ			0.19	2152.36	3
99	SIDQFLYFDLIYSIJ			0.19	2152.36	3
99	SIDQFLYFDLIYSIJ			0.19	2152.36	3
96	SIDQFLYFDLIYSIJ			0.21	2152.38	3
90	SIDQFLYFDLIYSIJ			0.19	2152.37	3
90	SIDQFLYFDLIYSIJ			0.19	2152.37	3
13	SIDQFLYFDLIYSIJ	Q->E / Q(Deamidation)	FDL	1.25	2153.42	3

99	SIDQFLYFDLIYSIJ			0.19	2152.36	3
3	SIDQFLYFDLIYSIJ	Q->E / Q(Deamidation)	FLY	1.17	2153.35	3
99	SIDQFLYFDLIYSIJ			0.03	2152.2	3
99	SIDQFLYFDLIYSIJ			0.13	2152.3	3
99	SIDQFLYFDLIYSIJDTJ	Q->E / Q(Deamidation)	DQFL	1.16	2641.61	4
99	SIDQFLYFDLIYSIJDTJ			0.14	2640.59	4
99	SIDQFLYFDLIYSIJDTJ			0.14	2640.59	3
1	SIDQFLYFDLIYSIKDTK	Q->E / Q(Deamidation)	SI	0.92	2353.17	4
1	SITVR			0.27	718.73	2
1	SITVR			14.25	732.7	2
1	SITVR			-27.77	690.69	2
0	SITVR			0.27	718.73	2
1	SITVRVFEDGJ	R->K	EDG	-27.93	1509.94	5
1	SITVRVFEDGK			79.79	1473.57	4
1	SITVRVFEDGK			27.81	1421.59	3
1	SSKFTGLMENMJ	M->L	EN	-18.08	1641.78	3
1	SSKFTGLMENMK			80.09	1595.84	4
1	TCMYGGVTEHNGNQLDJ	Y->W	MYG	23.27	2177.28	4
1	TCMYGGVTEHNGNQLDJYR	Y->W	MYGG	22.81	2495.98	3
1	TNDINSHQTDJ			80.14	1639.92	5
1	TNDINSHQTDJ			80	1639.79	5
1	TNDINSHQTDK			-0.08	1415.61	4
1	TNDINSHQTDK	Q->E / N(Deamidation) / Q(Deamidation)	TN	0.9	1416.58	3
1	TNDINSHQTDK	Q->E / N(Deamidation) / Q(Deamidation)	DIN	0.96	1416.64	2
1	UJDJ			-0.02	1128.69	3
1	UKDJ			-0.02	984.58	3
1	ULMMUNDNK			17.94	1640.77	4

1	ULMMUNDNK			17.96	1640.79	4
1	ULMMUNDNK			0.1	1622.93	3
2	ULMMYNDNJ	Y->H	LM	-25.82	1597	3
1	ULMMYNDNJ			17.9	1640.72	4
1	ULMMYNDNK			1.17	1479.88	3
1	ULMMYNDNKMVDSJ			-18.02	2165.06	4
1	UVDVFGANYYYQCYFSJK			28.3	2717.63	4
1	VEFJNJ			13.87	1209.61	3
1	VEFJNJ	E->D	JN	-14.11	1181.63	3
1	VFEDGJ			14.22	995.76	3
1	VLUDDNHVSAINVJ			0.1	2018.22	4
99	VLUDDNHVSAINVJ			0.08	2018.21	3
97	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.06	2019.19	4
1	VLUDDNHVSAINVJ			0.02	2018.15	4
99	VLYDDNHVSAINVJ			0.15	1874.17	3
99	VLYDDNHVSAINVJ			0.13	1874.14	3
99	VLYDDNHVSAINVJ			0.05	1874.07	3
37	VLYDDNHVSAINVJ	N(Deamidation)	DDN	1.13	1875.14	4
1	VLYDDNHVSAINVK			-26.01	1703.91	3
1	VLYDDNHVSAINVK			18.09	1748.01	4
1	YKDJ			-0.25	840.24	2
1	YKDJ			0.07	840.56	2
29	YLMMUNDNK			0.11	1478.82	3
1	YLMMUNDNK	N(Deamidation)	YL	1.09	1479.81	3
99	YLMMYNDNJ			0.11	1478.83	3
1	YLMMYNDNJ	N(Deamidation)	MM	1.13	1479.85	3
99	YLMMYNDNJ			0.11	1478.82	3
99	YLMMYNDNJ			0.05	1478.76	2
1	YLMMYNDNJ			0.13	1478.84	3
5	YLMMYNDNJ			0.13	1478.85	3
1	YLMMYNDNKMVDSJ			-18.2	2020.78	3
1	YRSITVR	I->V	SI	-13.89	1023.73	2
1	YVDVFGANUUUQCYFSK	F->Y	YFSK	15.75	2721.08	5
2	YVDVFGANYYYQCUFSKJ	Y->H	YV	-26.16	2663.17	4
1	YVDVFGANYYYQCYFSJK	Y->F	YV	-15.96	2529.27	4

Pro Group Report: SEB on parquet 170hours

Report Parameters: ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2

Report Statistics (204 total spectra):

Confidence (ProtScore) Cutoff	Proteins Identified	Proteins before Grouping	Distinct Peptides	Spectra Identified	% of Total Spectra
>99 (2.0)	1	1	79	176	86.3
>95 (1.3)	1	1	79	176	86.3
>66 (0.47)	1	1	79	176	86.3
As shown: >95 (1.30)	1	1	79	176	86.3

Conf	Sequence	Mod	Zone	dMass	PrecMW	Z
1	DKYVDVFGANYYYQCYFSK			-0.07	2516.08	3
97	DVJIEVYLTTJ			0.16	1740.21	4
99	DVJIEVYLTTJ			0.16	1740.21	3
99	DVJIEVYLTTJ			0.18	1740.22	3
99	DVJIEVYLTTJ			0.14	1740.18	3
99	DVJIEVYLTTJ			0.16	1740.21	3
99	DVJIEVYLTTJ			0.16	1740.2	3
1	FDQSJULMMUNDNJ	M->L	FD	-18.18	2498.13	3
1	FIENENSFWUDMMPAPGDJFDQSK	F->Y / M(Oxidation)	FDQSK	16	3343.56	4
1	FIENENSFWUDMMPAPGDK	M->L	APGD	-17.77	2560.41	3
29	FIENENSFWYDMMPAPGDJFDQSJ	Q->E / N(Deamidation) / Q(Deamidation)	MMPAPG	0.8	3328.37	4
99	FTGLMENMJ			0.06	1357.77	2
97	FTGLMENMJ	E->Q	FTG	-1.1	1356.61	2
97	FTGLMENMJ			0.12	1357.82	3
99	FTGLMENMJ			0.14	1357.84	3
99	FTGLMENMJ			0.12	1357.83	3
99	FTGLMENMJ			0.12	1357.82	3
99	FTGLMENMJ			0.04	1357.75	2
99	FTGLMENMJ			-1.12	1356.59	2
99	FTGLMENMJ			0.12	1357.83	3
99	FTGLMENMJ			0.1	1357.81	3
99	FTGLMENMJ			0.12	1357.82	3

99	FTGLMENMJ			0.14	1357.84	3
1	FTGLMENMK			1.2	1214.8	3
1	FTGLMENMK	F->Y / M(Oxidation)	MK	16.08	1229.68	3
1	FTGLMENMK	E->Q	FT	-0.78	1212.82	2
1	HULVJ			0.07	1090.77	3
17	HULVJ			0.09	1090.79	3
82	HULVJ			0.09	1090.79	3
82	HULVJ			0.09	1090.79	3
98	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.66	3
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.66	2
4	HYLVJ			-26.3	920.29	2
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.67	3
99	HYLVJ			0.08	946.66	3
43	HYLVJ			0.08	946.67	3
1	HYLVJNJ			-15.72	1317.11	3
1	HYLVJNJ			22.88	1355.71	3
1	HYLVKNJ			13.88	1202.61	3
1	HYLVKNK			27.96	1072.58	2
1	HYLVKNK			-16.14	1028.48	2
3	IEVULTTJ			0.13	1397.98	3
9	IEVULTTJ			0.07	1397.93	3
1	IEVULTTJJ	E->Q	EV	-0.85	1669.21	4
1	IEVULTTJJ	E->Q	EV	-0.91	1669.15	4
0	IEVULTTKK	V->I	UL	13.86	1395.7	3
99	IEVYLTTJ			0.11	1253.86	3
1	IEVYLTTJ	L->M	YL	17.87	1271.63	3
99	IEVYLTTJ			0.11	1253.87	3
99	IEVYLTTJ			0.09	1253.84	2
1	IEVYLTTJ			22.83	1276.58	2
99	IEVYLTTJ			0.11	1253.87	3
43	IEVYLTTJ			0.11	1253.86	3
98	IEVYLTTJ			0.09	1253.85	3
99	IEVYLTTJ			0.23	1253.98	3
99	IEVYLTTJ			0.13	1253.88	2
99	IEVYLTTJ			0.11	1253.87	2
1	IEVYLTTJJ	Y->H	LTT	-26.24	1499.71	5
1	IEVYLTTKK	E->Q	YL	-0.86	1236.88	3
1	JTNDINSHQTDK	S(O-Phosphoryl) / T(O-Phosphoryl)	JT	79.71	1767.59	4

99	JVTAQELDULTR			0.15	1868.23	4
99	JVTAQELDYLTR			0.15	1724.13	3
99	JVTAQELDYLTR			0.17	1724.15	3
99	JVTAQELDYLTR	E->Q	EL	-0.87	1723.09	3
99	JVTAQELDYLTR	E->Q	EL	-0.83	1723.14	3
99	JVTAQELDYLTR			0.17	1724.15	3
99	JVTAQELDYLTR			0.17	1724.14	3
99	JVTAQELDYLTR			0.13	1724.11	3
99	JVTAQELDYLTR			0.17	1724.14	3
99	JVTAQELDYLTR			0.15	1724.12	3
99	JVTAQELDYLTR			0.17	1724.14	3
99	JVTAQELDYLTR			0.15	1724.13	3
99	JVTAQELDYLTR			0.15	1724.12	3
43	JVTAQELDYLTR	E->Q	TAQ	-0.87	1723.11	3
1	KTNDINSHQTDJ			14.31	1702.18	3
1	KTNDINSHQTDJ			25.91	1713.78	2
1	KVTAQELDYLTR	K->R	YL	28.29	1608.16	3
97	LGNUDNVRVEFJ			0.2	1885.25	4
1	LGNUDNVRVEFK			0.12	1741.07	2
90	LGNYDNVRVEFJ			0.2	1741.14	3
57	LGNYDNVRVEFJ	N(Deamidation)	YDN	1.18	1742.12	3
2	LGNYDNVRVEFJ			0.26	1741.21	3
99	LUEFNNSPYETGYIK			0.2	2125.26	3
99	LYEFNNSPUETGYIJ			0.14	2269.3	3
10	LYEFNNSPUETGYIJ	N(Deamidation)	YEF	1.22	2270.39	4
99	LYEFNNSPYETGUIK			0.2	2125.27	3
99	LYEFNNSPYETGUIK			0.2	2125.27	3
99	LYEFNNSPYETGYIJ			0.2	2125.27	3
99	LYEFNNSPYETGYIJ			0.22	2125.28	3

99	LYEFNNSPYETGYIJ			0.16	2125.23	3
99	LYEFNNSPYETGYIJ			0.22	2125.29	3
95	LYEFNNSPYETGYIJ			0.2	2125.26	3
1	MVDSJ	D->E / V->I	DS	14.22	880.7	2
1	MVDSK			28.31	750.68	2
1	MVDSK			28.07	750.44	2
1	MVDSK			16.21	738.59	2
1	MVDSKDVK			28.07	1092.64	2
1	MYK			27.99	612.31	2
1	MYK			27.99	612.3	2
1	MYKR			-17.79	722.63	2
29	NJDLADJ			-0.24	1234.48	3
1	NJDLADJ			1.14	1235.86	3
1	NJDLADJ			0.92	1235.65	3
1	NJDLADJ			1.12	1235.85	3
0	NJDLADJ			1.12	1235.85	3
0	NJDLADJ			1.12	1235.85	3
1	NJDLADK			1.16	1091.78	3
1	NJDLADK			1.16	1091.78	3
1	NKDLADJ			13.76	1104.39	2
99	NLLSFDVQTNJ			0.13	1566.01	3
75	NLLSFDVQTNJ			0.15	1566.02	3
99	NLLSFDVQTNJ			0.11	1565.99	3
99	NLLSFDVQTNJ			0.15	1566.02	3
99	NLLSFDVQTNJ			0.05	1565.92	2
99	NLLSFDVQTNJ			0.15	1566.02	3
99	NLLSFDVQTNJ			0.17	1566.04	3
99	NLLSFDVQTNJ			0.13	1566.01	3
99	NLLSFDVQTNJ			0.15	1566.02	3
99	NLLSFDVQTNJJ			0.12	1838.18	2
2	NLLSFDVQTNJJ			0.18	1838.24	3
7	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	LLS	1.2	1839.26	4
38	NLLSFDVQTNJJ			1.16	1839.23	4

63	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.18	1839.25	4
90	NLLSFDVQTNJJ			0.18	1838.24	4
99	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	FD	1.18	1839.25	4
99	NLLSFDVQTNJJ	Q->E / N(Deamidation) / Q(Deamidation)	LLS	1.18	1839.24	4
99	NLLSFDVQTNJJ			0.16	1838.22	4
99	NLLSFDVQTNJJ			0.3	1838.37	4
99	NLLSFDVQTNJJ			0.18	1838.24	3
99	NLLSFDVQTNJJ			0.18	1838.25	3
99	SIDQFLYFDLIUSIJ			0.17	2296.44	3
37	SIDQFLYFDLIUSIKDTJ	Q->E / Q(Deamidation)	DQFL	1.28	2641.72	4
99	SIDQFLYFDLIYSIJ			0.21	2152.38	3
99	SIDQFLYFDLIYSIJ			0.21	2152.39	3
99	SIDQFLYFDLIYSIJ			0.21	2152.38	3
99	SIDQFLYFDLIYSIJ			0.15	2152.32	3
99	SIDQFLYFDLIYSIJ			0.21	2152.38	3
99	SIDQFLYFDLIYSIJ			0.21	2152.38	3
99	SIDQFLYFDLIYSIJ			0.23	2152.4	3
99	SIDQFLYFDLIYSIJ			0.01	2152.18	3
99	SIDQFLYFDLIYSIJ			0.17	2152.35	3
99	SIDQFLYFDLIYSIJDTJ			0.22	2640.66	3
99	ULMMYNDNJ			0.16	1622.98	3
1	UVDVFGANUUYQCYFSK	D->E / V->I	UU	14.11	2719.44	5
1	VEFJNJ			14.23	1209.96	3
1	VEFKNJ			28.11	1079.74	2
1	VFEDGK	E->Q	DG	-1.15	836.28	2
0	VFEDGK	K->R	DG	27.95	865.38	2

7	VLUDDNHVSAINVJ	N(Deamidation)	AIN	1.18	2019.3	4
99	VLUDDNHVSAINVJ			0.2	2018.32	3
99	VLYDDNHVSAINVJ			0.15	1874.17	3
99	VLYDDNHVSAINVJ			0.17	1874.19	3
99	VLYDDNHVSAINVJ			0.15	1874.17	3
99	VLYDDNHVSAINVJ			0.17	1874.19	3
99	VLYDDNHVSAINVJ			0.19	1874.21	3
93	VLYDDNHVSAINVJ	N(Deamidation)	DDN	1.17	1875.18	4
3	VLYDDNHVSAINVJ	N(Deamidation)	HVS	1.11	1875.13	3
1	VLYDDNHVSAINVK	Y->H	SAI	-25.95	1703.96	3
2	VTAQELDULTR			0.12	1596.01	3
1	VTAQELDULTR	D->E / V->I	QEL	13.76	1609.64	5
1	VTAQELDULTRHULVK	T(O-Phosphoryl)	HU	80.25	2460.6	5
1	YJDK			23.31	863.8	2
1	YKDJ			23.31	863.8	2
1	YKDJ	D->E	KD	13.83	854.33	2
99	YLMMYNDNJ			0.11	1478.82	2
99	YLMMYNDNJ			0.13	1478.85	3
99	YLMMYNDNJ			0.15	1478.86	3
90	YLMMYNDNJ			0.13	1478.84	3
29	YLMMYNDNJ			0.15	1478.86	3
17	YVDVFGANYUUQCUFSJ	Q->E / N(Deamidation) / Q(Deamidation)	DVFGA	1.13	2850.56	3

(E) Determination of carpet's extraction factor

	2.5ppm			10ppm			25ppm		
standard	1	2		1	2		1	2	
RT(T2)	2.84	2.54		2.68	2.84		2.69	2.68	
A(T2)	2.59E+08	5.67E+08		6.87E+08	9.27E+08		1.44E+09	1.54E+09	
Extract	1	2	3	1	2	3	1	2	3
RT(T2)	2.69	2.69	2.69	2.54	2.69	2.53	2.68	2.69	2.53
A(T2)	1.39E+08	1.87E+08	3.21E+08	6.17E+08	5.68E+08	4.45E+08	9.20E+08	7.12E+08	1.16E+09
Recovery	53.62	72.13	56.62	89.84	82.7	48.03	64.04	49.56	75.4
average	60.79			73.52			63		
SD	9.93			22.36			12.95		

Note that the extract 1 & 2 were made reference to standard 1 and extract 3 to standard 2. Where RT=retention time[min], A=peak area, SD=standard deviation

(F) Determination of parquet's extraction factor

	2.5ppm			10ppm			25ppm		
A(T2)	8.29E+07	9.18E+07	9.23E+07	1.90E+08	2.07E+08	2.35E+08	3.34E+08	3.24E+08	3.22E+08
RT(Ben)	3.72	3.71	3.7	3.69	3.53	3.67	3.82	3.67	3.67
A(Ben)	2.52E+07	3.30E+07	3.69E+07	4.16E+07	3.44E+07	3.88E+07	3.90E+07	3.90E+07	3.48E+07
Extract	1	2		1	2		1	2	
RT(T2)	2.69	2.69		2.85	2.69		2.7	2.7	
A(T2)	7.71E+07	6.07E+07		1.86E+08	1.38E+08		2.24E+08	2.12E+08	
RT(Ben)	3.55	3.71		3.85	3.54		3.53	3.67	
A(Ben)	2.42E+07	2.67E+07		2.44E+07	3.25E+07		3.13E+07	2.34E+07	
average	101.1			99.9			90.96		
SD	9.6			31.4			18.3		

Where RT= retention time[min], A=peak area, Ben=Benzophenone internal standard, RRT=relative retention time of T-2/Ben, RA=relative area of T-2/Ben, SD=standard deviation

(G) Raw data for Carpet's stability profile

25ppm T2 std

RT(T2)	2.83	2.97
A(T2)	2.18E+09	2.12E+09
ave	2.15E+09	

0hr	C1	C2	C3
	2.77	2.92	2.78
	1.47E+09	1.08E+09	1.05E+09
Recovery	68.37	50.23	48.84
Actual	80.68	59.27	57.63
Ave	65.86		
SD	12.86		

4hr	C1	C2	C3
	2.62	2.80	2.95
	1.14E+09	1.01E+09	1.46E+09
Recovery	5.30E+01	4.70E+01	6.79E+01
Actual	62.57	55.43	80.13
Ave	66.04		
SD	12.71		

8hr	C1	C2	C3
	3.11	2.77	2.79
	1.27E+09	8.13E+08	7.45E+08
Recovery	59.07	37.81	34.65
Actual	69.70	44.62	40.89
Ave	51.74		
SD	15.67		

12hrs	C1	C2	C3
	2.78	2.77	2.78
	1.28E+09	1.03E+09	1.21E+09
Recovery	59.53	47.91	56.28
Actual	70.25	56.53	66.41
Ave	64.40		
SD	7.08		

25ppm T2 std

RT(T2)	2.83	2.96
A(T2)	2.32E+09	2.21E+09
ave	2.27E+09	

1day	C1	C2	C3
	2.95	2.78	3.11
	1.17E+09	8.98E+08	1.25E+09
Recovery	51.66	39.65	55.19
Actual	60.95	46.78	65.12
Ave	53.87		
SD	10.02		

2 day	C1	C2	C3
	2.79	3.06	2.9
	1.41E+08	5.38E+08	5.11E+08
Recovery	6.23	23.75	22.56
Actual	7.35	28.03	26.62
Ave	20.67		
SD	11.56		

25ppm T2 std

RT(T2)	2.68
A(T2)	3.19E+09

3day	C1	C2	C3
	2.84	2.84	2.67
	5.43E+08	5.92E+08	6.15E+08
Recovery	17.02	18.56	19.28
Actual	20.09	21.90	22.75
Ave	21.58		
SD	1.36		

25ppm T2 std

RT(T2)	2.68
A(T2)	1.84E+09

7 day	C1	C2	C3
	2.69	2.68	2.67
	4.51E+08	3.74E+08	3.37E+08
Recovery	24.51	20.33	18.32
Actual	28.92	23.98	21.61
Ave	24.84		
SD	3.73		

Where RT=retention time[min], A=peak area, Actual=recovery% x filter factor of 1.18, ave=average of the actual, SD=standard deviation.

(H) Raw data for Parquet's stability profile

25ppm T2 std

RT(T2)	2.69
A(T2)	1.28E+09

0hr	P1	P2	P3
	2.68	2.68	2.69
	1.27E+09	7.68E+08	1.08E+09
Recovery	99.22	60	84.38
Actual	117.08	70.80	99.56
Ave	95.81		
SD	23.37		

25ppm T2 std

RT(T2)	2.81	2.82
A(T2)	2.14E+09	1.70E+09
Ave	1.92E+09	

4hr	P1	P2	P3
	2.69	2.83	2.67
	9.69E+08	5.82E+08	7.82E+08
Recovery	50.47	30.31	40.73
Actual	59.55	35.77	48.06
Ave	47.79		
SD	11.89		

8hr	P1	P2	P3
	2.85	2.69	2.82
	4.95E+08	4.63E+08	7.42E+08
Recovery	25.78	24.11	38.65
Actual	30.42	28.46	45.60
Ave	34.83		
SD	9.38		

25ppm T2 std

RT(T2)	2.99	2.68
A(T2)	1.99E+09	2.10E+09
Ave	2.05E+09	

12hr	P1	P2	P3
	2.85	2.68	2.69
	3.94E+08	5.24E+08	5.75E+08
Recovery	19.27	25.62	28.12
Actual	22.73	30.24	33.18
Ave	28.72		
SD	5.39		

1 day	P1	P2	P3
	2.85	2.85	2.7
	1.73E+08	2.65E+08	3.29E+08
Recovery	8.46	12.96	16.09
Actual	9.98	15.29	18.98
Ave	14.75		
SD	4.52		

3 day	P1	P2	P3
	2.84	2.69	2.86
	1.04E+08	4.37E+08	1.54E+08
Recovery	3.26	13.70	4.83
Actual	3.85	16.16	5.70
Ave	8.57		
SD	6.64		

25ppm T2 std

RT(T2)	2.84
A(T2)	1.67E+09

7 days	P1	P2	P3
	2.7	2.85	2.84
	2.85E+08	4.16E+08	3.05E+08
Recovery	17.07	24.91	18.26
Actual	20.14	29.39	21.55
Ave	23.69		
SD	4.99		

Where RT=retention time[min], A=peak area, Actual=recovery% x filter factor of 1.18, ave=average of the actual, SD=standard deviation.