Examining The Forensic Toxicity of Ricin Using Lc/Ms Techniques

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Abstract

As one the naturally occurring toxins, Ricin is classified among the deadliest poisons available. Ricin and *Ricinus communis* agglutinin (RCA120) are derived from the castor bean plant. Ricin and its related activities can be detected using either mass spectrometric (MS) assay or Liquid Chromatography (LC). Further, the two methods can be to differentiate ricin from RCA120 because it is less toxic of the two. The sequence of Amino acid is identified by monitoring the active ricin using mass spectrometric. This study discusses how LC and MS methods can be applied to quantify, detect, and differentiate ricin from RCA120 in test samples. On the overall, the two methods were applied to tell aside ricin samples from RCA120. The study concluded that mass spectrometry is the most efficient approach in identifying ricin from RCA120.

Keywords**:** *Ricin; Chromatography; Peptide; Castor Bean; Protein; Toxin Peptide; Liquid Chromatography; RCA120; Mass Spectrometric.*

Introduction

As one of the most toxic substances, ricin is primarily obtained from the castor bean plant (*Ricinus communis*). Castor bean plant grows naturally in subtropical and tropical regions. Since the plant is easily available, ricin is easy to prepare, therefore, it can be argued that the substance can be used as a biological weapon by malicious people [1]. This makes it the only protein classified as Schedule 1 chemicals [2]. Since ricin is a protein, it is made up of both A and B chain subunits [3]. The primary responsibility of chain B lectin is binding lectors with galactose on the surface of the eukaryotic cells. By so doing, they trigger endocytosis of ricin. Chain B lectin is responsible for initiating all the deadenylase activities that are responsible for initiating an irreversible depurination reaction of 28S rRNA which terminates protein synthesis in the cell. Therefore, the fact that ricin is readily available should be a major concern because it could be either knowingly or unknowingly to contaminate global and domestic food supplies [4]. For prevention purposes, there need is develop an effective approach that can be applied to detect ricin in foods and avoid possible deaths from the same.

Ricin can get into the body through inhalation or ingestion of materials that contain the same. However, the substance can also be directly injected into the bloodstream [4]. An injection is an unlikely method because the victim must be present for the chemical to be injected into their bloodstream. In a situation where a person is attacked using the chemical, it important for the toxin to be detected as soon as possible. Detection of this chemical includes sampling of materials such as foodstuffs and soil where a case of food poisoning is suspected [5]. In regards to the nature of risk in ricin, a lot has been invested in research to help identify the most accurate and effective method that can be used to detect ricin that might be in a range of substrates. The main aim of this article, therefore, is to examine the forensic toxicity of ricin using the methods attempted and the extent of their achievement.

Materials and Method

Safety

As one of the most poisonous known substances, all the experiments and extraction processes involving ricin should be conducted in a biosafety cabinet [6]. However, the samples coming from the digestion process are not considered toxic. Therefore, when dealing with ricin, safety measures should be considered.

Preparation of Antibody-Coated Beads

Biotinylated Anti-Ricinus Communis Agglutinin

(RCA) antibodies were purchased by the researcher from Somatco in Jeddah. The antibodies were employed as intermediates to localize lectin receptors [7]. 5 mg lyophilized aliquots were bought and later restored using distilled water with a density of water of 1 mg/mL. To add to that, the researcher also bought MyOne T1 streptavidin-coated beads from ThermoFisher. The instruction of the manufacturer was used when preparing the beads. Ideally, principle of using magnetic beads is that they have an immobilized affinity to the isolated structure of ricin as the target compound [7].

Extracting Ricin and RCA120

5 mg/mL purified solutions of ricin and RCA120 agglutinin were purchased by the researcher from Somatco. The matrix sample with agglutin and toxin was incubated for 30 min and then a magnetic capture was used to recover the components. The capture beads were removed from the mixture after an hour and washed using distilled water, 0.5 mL of PBS, 1 mL of 0.05% PBST, and 1 mL of 0.01% PBST [8].

Extraction of Castor Bean

The researcher purchased castor beans with approximately the same weight from M.A.B.M. Trading Est. To facilitate the process of extraction, the beans were crushed using pestle and mortar a then put in the sample matrix. The mixture was then left to incubate for 18 hours and later centrifuged to settle the particles. The beads coated with antibody were used to extract 0.5 mL aliquot of the supernatant. The analysis was then carried out using relevant methods [9].

Digestion of Protein

To facilitate the process of protein digestion during the study, the researcher purchased Rapigest SF from Somatco. Rapigest SF acted as enzymes to speed up the reactions within the solution. The enzymes were purchased in 1 mg lyophilized aliquots. These quantities were then converted in 0.1% solution using 100 mM ammonium bicarbonate. Further, the process of protein digestion was carried out using buffer exchanging magnetic beads which contained ricin in the Rapigest SF solution [8].

Preparation of Peptide

Ricin quantification peptides that combined both A and B chains were chosen by the researcher. This also helped to distinguish ricin form RCA120. However, the researcher was very keen to avoid all the peptides that contained tryptophan, methionine, and cysteine. Further, all the glycosylated peptides were omitted. Finally, the peptides that passed all the criteria were selected for synesis [7].

Liquid Chromatography

The process is very critical in separating a sample matrix into sub-components. Ideally, the separation process is initiated by various interaction of the matrix while in both mobile and stationary phases. Further, a 1200 capillary pump was used in the chromatographic separation. All the solvents were developed by Jackson and Burdick [8].

Mass Spectrometric Quantification

The method was very effective both in sensitively and selectively detecting and assigning a signal to a particular chemical even when it was in low concentrations. LTQ module was used by the researcher to actively perform mass spectrometric quantification. The sample was later put to LTQ through electrospray for ionization purpose. The process recovered all the productions that were within the mass and not exceeding a certain low mass cutoff during the Collision-induced dissociation (CID) [8].

Activity Assay

Various methods can be employed to detect ricin protein, however, the majority of these approaches in practice cannot differentiate between active and inactive ricin. The primary objective of this study was to come up with a selective and sensitive method that can be used to completely analyze ricin by taking into considerations both the structural components and biological activities of the chemical. Both MS and LC-based methods use three layers to detect ricin activities that might be present in a mixture. Therefore, the process involved various activities which include separation of ricin from the lab samples by use of antibody-coated beads. Ricin was then digested and the analyzed using MS and LS methods to identify the toxin.

Results

Ricin substrate was detected in the sample matrix spiked with 15 pmol ricin in the activity assay. However, the same did not happen in similar pure samples. Both MS and LC-based methods were effective in detecting ricin peptides and further helped to distinguish them

from RCA which has a less toxic homolog. Even though both methods were effective in detecting ricin substrates in the matrix, the main challenge was to differentiate toxic and less toxic forms. The researcher found that including four more peptides as confirmation ions did not increase ambiguity. The four additional peptides were very critical in confirming the presence of both A and B chain as well as distinguishing ricin from RCA120. The process of quantifying took into account four tryptic peptides with T7 peptide which empirically yielded charge ion and resulted in the best sensitivity. T18, T10, and T11 peptides were also unique in the experiment and they produced quantitative results similar to those from T7. Use of T7 as while quantifying the peptides lead to low limit of detection for the protein.

Differentiating Ricin form RCA120

Both substances share more than 85% sequence homology [9]. In addition to that, there are no known antibodies with a potential of binding to ricin over RCA120 [10]. Mass spectrometry method effectively and selectively differentiated the two type of toxics based on the natural sequence of proteins. Quantitative measures that employ MS/LC technique are limited because they can only be use on few tryptic peptides. Further, T7 peptide obtained from ricin can be used to tell apart ricin from a sequence of amino acids [11]. In this context, the amount of ricin produced using LC/MS experiments was constant throughout the process regardless of the amount of RCA120 employed. This was a clear indication that only ricin was being quantified and the quantity of RCA120 did not play any role in the perceived protein.

Contamination of Castor Bean

During the experiment, four sample matrixes with already crushed castor beans were evaluated for ricin after they incubated in LC and MC methods. Since a castor bean is off 1.5% toxic of weight, the researcher estimated the maximum amount of ricin in a castor bean to be about 10 mg. Further, this was because the limit of ricin recovery for LC and MS method is approximated at 13 pmol/ mL [12]. However, to ensure that the collected ricin was within the established range, the tryptic samples were split to form several aliquots and then LC and MS analysis were carried out. After the dilution process, the matrixes were passed through an immune purification process and LC/MS analysis processes. The amount of ricin present in the sample was then evaluated using ricin T18, T 7, T10, and T11 peptides. Despite all the sample containing some significant amount of ricin, the amount varied from one sample to another. As the same sample of extracted ricin was used in all the matrices, pH level and complexity of the matrix did not have a significant impact on the recovery of ricin [13].

Discussion

Most methods used in forensic toxicology of ricin can be classified into three categories [14]. To begin with, the first category of the methods utilizes immunogenic interactions when detecting the presence of ricin. For example, the enzyme-linked immune-sorbent assay is one of the methods that fall under this category. Secondly, the second group makes use of the enzymatic activity of ricin. The final category of methods used to detect castor bean DNA is based on the assumption that they would contain ricin toxic as well. For instance, the polymerase reaction falls under this category. Further, these methods are very fast but have a low limit of detecting ricin [15]. In addition to that, these methods also require cheap instruments to set them up. However, despite being effective, none of the three methods represents a comprehensive assay. Further, despite polymerase chain reaction being quantitative and sensitive, the presence of toxic ricin is indirectly carried out. In addition to that, enzymatic activities in the mixture can be inefficient in scenarios when the sample contains other toxic proteins.

Almost all the biotechnology and bioscience separate, isolate and purify both peptides and proteins and other molecules in the cell. Therefore, the use of technology and separation process is very critical; this calls a further investment in the biotechnology field [16]. Hence, there is need for the stakeholders to develop a more accurate and advanced technique of separating the two toxic substances. Further, the new technique should have the capability of treating dilute solutions containing a minimum amount of the target proteins mixed in other compounds. This research was advantageous over other methods used in the separation of ricin and RCA120 because it made the use of peptides corresponding to both proteins. Therefore, LC/MS methods enable analysts to quantify ricin independently from RCA120 depending on the quantification peptide used.

Importance of Ricin Activity Measurement

Given the toxic level of ricin, it is critical for public health investigators to effectively react to any contamination cases involving ricin. Currently, various analytical methods are available to facilitate the analysis

of proteins [17]. Since the mass spectrometric techniques have been found to offer direct evidence on the structure of molecules by measuring the mass of the molecules, identifying possible modifications, and determining the sequence of amino acids, it is useful in determining whether the protein toxins is a risk factor to a certain disease [18].

Conclusion

The study analyzes the ability of MS/LC methods to detect the presence of ricin in laboratory samples. Further, the research explores selectivity and specificity of such methods in examining and quantifying the amount of ricin in a substrate. Analysis carried out using chromatography and mass spectrometry is advantageous over other methods in different ways. To begin with, they offer a direct approach to the measurement of ricin. Secondly, the approach is not easily influenced by other proteins in the sample. Further, LC/MS can successfully be used in quantifying ricin and RCA peptides. Even though LTQ approach can be used to tell apart ricin from other proteins, immune purification approach was used in the experiment because it enables the analyst to maintain a low detection limit. Further, it also ensures that a second layer is available. Finally, the choice of tryptic peptides for forensic toxicity of ricin makes it possible for the analysts to accurately and independently quantify ricin from RCA120.

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