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A REVIEW ON SEPARATION OF BIOLOGICAL PROTEINS BY LIQUID CHROMATOGRAPHY

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ABSTRACT

After the success of human genome project, proteome is a new emerging field of biochemistry as it provides the knowledge of enzymes (proteins) interactions with different body organs and medicines administrated into human body. Therefore, the study of proteomics is very important for the development of new and effective drugs to control many lethal diseases. In proteomics study, analyses of proteome is essential and significant from the pathological point of views, i.e., in several serious diseases such as cancer, Alzheimer's disease and aging, heart diseases and also for plant biology. The separation and identification of proteomics is a challenging job due to their complex structures and closely related physico-chemical behaviors. However, the recent advances in liquid chromatography make this job easy. Various kinds of liquid chromatography, along with different detectors and optimization strategies, have been discussed in this article. Besides, attempts have been made to include chirality concept in proteomics for understanding mechanism and medication of various disease controlled by different body proteins.

KEYWORDS

Enzyme, Liquid chromatography, Alzheimer's disease and Nano-Electrospray.

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INTRODUCTION

Each cell produces thousands proteins in living organisms and a set of them is called as proteome and unlike genome, the proteome differs from cell to cell[1]. After success of human genome project, proteome is a new emerging field of biochemistry as it provides the knowledge of enzymes (proteins) interactions with different body organs and medicines, administrated into human body. Therefore, the study of proteomics is very important

for the development of new and effective drugs to control many lethal diseases. In proteomics study, analyses of proteome are very important and significant from the pathological point of views, in several serious diseases such as cancer[2-5,] Alzheimer's disease and aging[6], heart diseases[7] and plant biology[8]. The separation and identification of proteomics is a challenging job due to their complex structures and closely related physico-chemical behaviors. However, literature indicated the successful use of liquid chromatography in this concern. Most effectively used kinds of chromatography are liquid chromatography mass spectrometry (LC–MS)[9,10], nano-reversed phase liquid chromatography (nano- RPLC)[11,12] and ion exchange chromatography[13]. Platelets, having no nucleus in cells, are valuable to study hemostasis, thrombosis and heart diseases. The proteins present in platelets have been studied by multidimensional liquid chromatography followed by mass spectrometry[14]. Over last few years, the proteomic analysis reveals that it requires the combination of on-line sample preparation and analytical methods due to the diversity and complexity in proteomics structures. In view of these facts, attempts have been made to review the role of liquid chromatography in proteomics study. Various kinds of liquid chromatography, along with different detectors and optimization, have been presented in this article.

Separation methods for proteins

Since the introduction of liquid chromatography in 1980 it has become very popular in analytical science but its applications came into practice in last decade. The nano detection makes these chromatographic techniques more useful in proteomic research. Various kinds of liquid chromatography methods used in proteomic research are reversed phase, affinity, gel permeation, ligand exchange and capillary liquid chromatographies, which are discussed in this article.

Reversed phase high performance liquid chromatography

Reversed phase high performance liquid chromatography is the most popular mode of chromatography due to its wide range of applications because of the availabilities of various mobile and stationary phases. The on-line coupling of this technique with sample preparation and detection units, specially MS, makes it ideal technique in proteomics research. Nowadays, microchip based instruments are available to achieve this difficult task.

Some important separations of proteomics using RP-HPLC are discussed and analyzed critically[15]. Reported that multidimensional liquid chromatography coupled with tandem-mass spectrometry has wide range of applications in proteomics[16]. Quantified a group of 1600 gene products into 997 protein families with 830 membrane or membrane-bound proteins in normal and malignant breast cancer cells of a patient using nano-electrospray LC–MS/MS method. [17]Applied liquid chromatography with tandem-mass spectrometry for the analysis of proteome of transfected HELA cell lines having three clear single amino acid changes in a nuclear phosphoprotein, BRCA1 protein. The authors reported that Met1775Arg and the Trp1837Arg did not show effective changes in comparison to cells transected having wild type BRCA1 cDNA and only BRCA1-Ser1841 Asn mutation creates effective changes in proteomic pattern in breast cancer patients. [18]reported a nano-LC–MS method for the proteomic analysis of two murine macrophages cell lines (J774.1A and RAW 264.7), which were treated with Bacillus anthracis lethal toxin (LeTx) in anthrax infection. The authors identified five proteins as ATP synthase b-subunit, b-actin, Hsp 70, vimentin, and Hsp60 homolog, which were unregulated in above cell lines. [19]performed a quantitative neuropeptidomic study for activity of prohormone convertase-2 (PC2) in processing of hypothalamic neuropeptides and reported 53 neuropeptides or other peptides originating from 21 proteins viz. proenkephalin, proopiomelanocortin, prodynorphin, protachykinin A and B, procholecystokinin, promelanin-concentrating hormone, proneurotensin, proneuropeptide Y, provasopressin, pronociceptin/ orphanin, prothyrotropin-releasing hormone, cocaine, amphetamine - regulated transcript,

chromogranin A and B, secretogranin II, prohormone convertase 1 and 2, propeptidyl-amidating mono oxygenase, proteins designated proSAAS and VGF; after labeling by isotopic tags in extracts of mice without PC2 and wild type young ones following fractionation with RP-HPLC column. Electrospray ionization mass spectrometric method and tandem-mass spectrometry were used for analysis and identification of above said proteins, respectively.

An interaction between aquaporin and filaments was reported using liquid chromatography (LC)–tandem-mass spectrometry method. This interaction was supposed to be responsible for the lens fiber cell shape, [20]reported a LC–ESI-MS/MS and MALDI-FTICR method for the identification of tetraspanin, which were integral membrane proteins, in a model of human colon cancer. These identified proteins were integrins, Lu/BCAM, GA733, BAI2, PKC, G, proteages (ADAM10, TADG15) and syntaxins proteins, [21] reported SUMO-1 and SUMO-3 as stable modified proteins having half lives more than 20 h by LC–MS[22]. Described 12 proteins out of 37 different proteins related with Alzheimer's disease in the cortex of Tg2576 mice using matrix-assisted laser desorption/ionization-time-of flight (MALDI-TOF) and liquid chromatography–tandem-mass spectrometry. The whole phosphoproteome was studied using multidimensional liquid chromatography with electrospray mass spectrometric method in eukaryotic living beings[22,23] reported a liquid chromatography and tandem-mass spectrometry method to identify the carbolylated proteins in aged mouse brain homogenates [7]identified K7, K37 and K41 as main sites of glycation and carboxymethylation of RNase by using electrospray ionization liquid chromatography–mass spectrometry (ESI-LC–MS) method after the incubation of RNase (13.7 mg/mL, 1mM) with glucose of 0.4M concentration at 37°C for a period of 14 days in phosphate buffer. The average value of mass measurement accuracy (MMA) of apomyoglobin was reported by using nano-liquid chromatography-dual electrospray ionization-Fourier transform-ion cyclotron resonance- mass spectrometry (nano-LC-dual ESI-FT-ICR-MS) as 1.09 versus 74.5 ppm. [24]Reported 55 proteins including peptidase, ion channels, cycloskeletal proteins, enzymes of carbohydrate metabolism, regulatory enzymes etc. using PepMapTM C18 column (0.3 mm, 250 mm). Various proteins identified by LC MS/MS. performed a nano-liquid chromatography Fourier transform-ion cyclotron response mass spectrometry (nano-LC FT-ICR MS) analysis of the outer membrane protein of Helicobacter pylori, a human gastric pathogen which can create duodenal ulcers, gastric cancer diseases, using (17 cm - 50 cm) fused silica column packed with 3 lm ReproSil-Pur C18-AQ porous C18-bonded particles and identified 60 membrane associated proteins including (outer membrane protein) Omp11 and BabA proteins in each strain. The authors reported that the fragmentation efficiency in the ion trap of the nano-LC FT-ICR MS and MS/MS analysis are more reproducible; Figure No.1. [25]reported that 80 of 712 proteins in mesenchymal progenitor cell proteome create 5258 of 10506 detected peptides. Few represented mesenchymal progenitor cell (MPC) proteins create a large number of MPC peptides, which are shown in Figure. 2. A comparative study of peptides of different Caenorhabditis elegans strains, a nematode species, was performed using 0.1% trifluoroacetic acid (TFA) with 50% acetonitrile (CH_3CN) on symmetry (4.6 mm, i.d. 250 mm) C_{18} column for HPLC analysis followed by matrix-assisted laser desorption ionization- time-of-flight mass spectrometry (MALDI-TOF). Furthermore, 2-50% acetonitrile and 0.1% formic acid; in the same column; with a flow rate of 200 nL/min is used for on-line nano-liquid chromatography-quadrupole time-of-flight tandem-mass spectrometry (nano-LC-Q-TOF-MS/MS) to confirm the sequence of several naturally occurring peptides as shown in Figure No.3[26]. The authors reported that the presence of FMR Famide-like peptide (FLP) and neuropeptide like protein (NLP) in wild type strain of C. elegans was due to the activity of EGL-3 gene.

[27]achieved the isolation of glutathione S-transferase isoenzyme (GSTs), for the detoxification of

xenobiotics and endogenous toxicants, using a (150 mm - 4.6 mm) Vydac 214TP C_4 column with 37% acetonitrile having 0.075% trifluoroacetic acid (TFA) in water using HPLC subunit analysis of glutathione (GSH) affinity-purified human liver mitochondrial proteins. The authors identified three human liver mitochondrial GST isoenzymes namely hGSTA1 and hGSTA2 of alpha class GST and hGSTP1 of pi class GST subunits. The authors reported three GSH affinity-purified human liver mitochondrial proteins at 14.7, 19.2 and 21.5 min retention times[28]. Analyzed the peptide mixture by combination of MALDI MS/MS with off-line liquid chromatography and recognized 377 unique peptides with the identification of 93 proteins [29]reported nano-RPLC as an important method for single and multidimensional protein separation of complex protein mixtures before mass spectrometric analysis. The authors also reported the effects of various chromatographic conditions on protein separation such as alkyl chain length in the stationary phase, temperature and ion pairing agent including C_{18} column at 60°C with TFA instead of heptafluorobutyric acid (HFBA). The influence of alkyl chain length in stationary phase for model protein separation is shown in Figure No.4 at 25°C column temperature using acetonitrile as mobile phase having 0.1% TFA. [30]Identified the photosystem II (PS II) antenna proteins on Vydac protein C_4 column with 27.5-63.5% acetonitrile, 0.05% trifluoroacetic acid in water as mobile phase with 1.0 mL/min. flow rate with MS detection in arabidopsis, pea and tomato. Figure No.5 represents the ion chromatogram of pea with protein components of PS II[31]. Reported that neither surface area nor pore diameter played an important role in the application of reversed phase for HPLC for proteomics.

[32]identified various proteins by FASTA and protein Prospector software in tryptic peptide mixture of fish from sea and farm by LC-ES/MS/MS study using a narrow- bore Phenomenex Jupiter C_{18} column (250 2.1 nm) with 0.05% (v/v) TFA, 5% (v/v) formic acid in H_2O and 0.05% (v/v) TFA, 5% (v/v) formic acid in acetonitrile as solvent [33]reported a 2D-LC-MS/MS method to identify secretory proteins from rat adipose cells. The authors separated these proteins using Zorbax 300 SB-C_3 reversed phase column (150 mm - 4.6 mm) with flow rate of 700 lL/min of TFA and acetonitrile. The authors separated 33 protein complexes; called as bands; by two-dimensional LC–MS/MS usinga Mono Q HR 5/5 column with sodium chloride from 0.1 M NaCl in murine erythroleukemic cells.

A nano-HPLC-MS/MS method for the study of low abundance proteins in silico analysis of complex protein samples was reported using 5 lm Zorbax SB C_{18} using buffer A: 95% H_2O, 5% acetonitrile, 0.1% formic acid and buffer B: 90% acetonitrile, 10% water, 0.025% trifluoroacetic acid and 0.1% formic acid[34]. [35]used a nano-flow high performance liquid chromatographic (HPLC) method using 0.1% acetic acid as solvent A and 70% acetonitrile in 0.1% acetic acid as solvent B with the detection by mass spectrometer [36]identified 11 human glycosyl phosphadylinositol anchored proteins (GPIAPs) and 35 Arabidopsis thaliana GPI-APs using a 2 cm fused silica Zorbax SB-C_{18} column with solution. A having acetonitrile in 1% formic acid/0.6% acetic acid/0.005% heptafluorobutyric acid (HFBA) with 40% B solution containing 90% acetonitrile in 1% formic acid/0.6% acetic acid/0.005% HFBA as mobile phases for half an hour. Performed LC-MS/MS phosphoproteomic analysis of phosphopeptides obtained from membrane fractions of rat kidney inner medullary collecting duct (IMCD) on a C_{18} pre-column for desalting the digested peptide mixture and these peptides were subjected to a Picofrit reverse-phase analytical column which has the elution of these peptides with 0–60% acetonitrile in 0.1% formic acid maintaining 250 nL/min flow rate. Fourier transform mass spectrometer having a nanospray ion source was used to analyze the peptides. The authors reported CIC-1, LAT4, MCT2, NBC3 and NHE 1 as solute transporter proteins having new phosphorylation sites[37]. Determined the compositions of the venoms of snakes such as Bitis gabonica rhinoceros (West African gaboon viper), Bitis nasicornis (Rhinoceros viper), Bitis caudalis (Horned puff adder) using RP-

HPLC followed by N-terminal sequencing, MALDI-TOF peptide mass fingerprinting and CID-MS/MS methods. For this RP-HPLC separation, the authors used a Lichrosphere RP100 C_{18} column (25 cm · 4 mm) with 0.1% trifluoroacetic acid (TFA) in water as solution A and acetonitrile with different concentration for different times as solution B. Table 1 presents proteins of total HPLC-analyzed in venom of various snake species [38] reported iTRAQ reagents tagging in conjugation with LC–LC MS/MS analytical study advantageous for quantitative study of synaptic proteomes of wild type mice and 30UTR-calcium/calmodulin-dependent kinase II a mutant mice. The authors used 300 lL of buffer having 20% acetonitrile, 10 mM KH_2PO_4 with 2.9 pH to dissolve dried iTRAQtagged sample and injected into a Polysulfoethyl A column. The column used was of 150 mm · 100 lm i.d. with a 500 nL/min flow rate of mobile phase.

Affinity high performance liquid chromatography

Affinity HPLC is a chromatographic method capable to separate biochemical mixtures of highly specific nature. It is possibleto design a stationary phase that reversibly binds to a known subset of molecules just by combining affinity chromatography. This kind exploits a well known and defined property of analytes which can be used during purification process. The process can be considered as an entrapment with the target molecule trapped on a stationary phase while the other molecules in solution did not trap due to lack of this property.

Tumor necrosis factor receptor, factor 6 (TRAF6) binding proteins, having many heat shock proteins, in osteoclast cells were reported[39] using affinity chromatography followed by mass spectrometric technique[40] studied ubiquitin-conjugated and ubiquitin-associated proteins in human cells by immunoaffinity chromatography and LC–MS/MS. The authors reported 345 proteins as ubiquitin-related proteome in denaturing conditions (Urp-D) and 325 proteins as ubiquitin-related proteome in native conditions (Urp-N) [41]studied many potential susceptibility factors, which were occurred in the livers of SJL mice using a C_{18} pre-column (100 lm · 2 cm) followed by 5% solvent B (100% acetonitrile) for loading of isotope-coded affinity tag (ICAT)-labeled purified peptide strong cation exchange (SCX) fractions. Furthermore, the authors reported the separation of these peptides using a (75 lm · 15 cm) self packed Magic C_{18} AQ column with 250 nL/min flow rate of 99.9% H2O in 0.1% HCOOH (solvent A) and 100% acetonitrile (solvent B). Mass spectrometric analytical study has been done and studied the correlation between experimental data with theoretical spectra using a SEQUEST program. [42]reported liquid chromatography and tandem-mass spectrometry, lectin affinity chromatography, biotin/Neutr Avidin chromatography for the analysis of transmembrane proteins in human platelets and mouse mega-karyocytes. The authors reported unique peptides for 46, 68 and 22 surface membrane and intracellular membrane, respectively, and identified new plasma membrane proteins covering immunoglobulin member G6b-B, a immunoreceptor tyrosine- based inhibition motif.

Immobilized metal affinity chromatography was used for the purification of phosphopeptides from Arabidopsis root cell culture and reported 79 phosphorylation sites in 22 phosphoproteins having a central role in RNA metabolism using Pep-Map C_{18} (300 lm · 5 mm), column and 0.1% TFA with20 lL/min. flow rate in a nano-HPLC technique[15]. [9]reported up regulation of 106 phosphopeptides and 145 phosphorylation sites. Affinity chromatography was reported as an indispensable tool for the separation of complex proteins[2,10] used immobilized metal affinity chromatography coupled with electrospray ionization tandem MS and [63] described same techniques with matrix-assisted laser desorption/ionization (MALDI) MS in phosphoproteomic analysis.

Gel permeation high performance liquid chromatography

Basically, Gel Permeation High Performance Liquid Chromatography works on the principle of sizes of the compounds and in this big size molecules eluted first followed by small size molecules. It involves the transport of a liquid mobile phase through a column containing a porous material as stationary

phase. It also called as size-exclusion chromatography and affords a rapid method for the separation of polymeric species. Therefore, it is a method of choice for separation of biomolecules such as peptides, proteins, enzymes. The stationary phase is porous solid such as glass or silica, or a cross-linked gel which contains pores of appropriate dimensions to effect the separation desired. [64]reported the separation and isolation of proteins from rat liver nuclei by using microcystin- Sepharose chromatography followed by mass spectrometry. The authors also identified two novel peroxisomal proteins, one was peroxisome-specific isoform of Lon protease and the other was made up of an aminoglycoside phosphotransferase- domain with an acyl-CoA dehydrogenase domain[29].

Ligand exchange high performance liquid chromatography

Ligand exchange-HPLC is the advance version of RP-HPLC where the reversed phase column is replaced by ion exchange column. It has been used widely for the analysis of all inorganic and organic ionic species. In LE-HPLC, anion and cation exchange columns are used but, nowadays, mixed (anion and cation) columns are also available which improve the separation efficiency. In cation exchange chromatography, the stationary phase is usually composed of resins containing sulfonic acid groups or carboxylic acid groups of negative charges and, thus, cation metallic species are attracted to the stationary phase by electrostatic interactions. In anion exchange chromatography, the stationary phase is a resin, generally, containing primary or quaternary amine functional groups of positive charge and, thus, these stationary phase groups pull solutes of negative charge. It can be used effectively for the speciation of cationic, anionic and neutral species simultaneously.

[56]reported anion exchange chromatography using an anion exchange column as faster and more effective technique for the separation and quantification ofnmembrane proteins of wild type Corynebacterium glutamicum and L-lysine producing strain. They also identified the proteins in the membrane of either wild type or the L-lysine. Furthermore [55] presented a significant method for the analysis of membrane proteome of a gram positive bacteria, C. glutamicum using a column (10 cm · 4.6 mm) in ion exchange chromatography. Quantities of proteins were separated from C. glutamicum membranes using different washing solutions as given in Figure: 6. The authors reported 2.5M NaBr as the best washing solution; among various lower concentration solutions of NaBr because it removes 40% of proteins. The neutral buffer (Tris–HCl, pH 8.0) or sodium carbonate (pH 11) separated 18% and 26% of total protein from membranes, respectively, while 6 M urea solution separated 70% and 4 M guanidine thiocyanate separated approximately 90% of the total protein from the membranes. [41]characterized isolated human pancreatic islet proteomes and identified 29,021 peptides equivalent with 3365 proteins using two-dimensional liquid chromatography (2D-LC) followed by ion-trap tandem-mass spectrometric (MS) study. Strong cation exchange (SCX) fractionations of enzymatic digests of proteins from human pancreatic islet have been carried out on a Polysulfoethyl A (200 · 2.1 mm) column with 10 mM ammonium formate in water having 25% acetonitrile and 500 mM ammonium formate in water having 25% acetonitrile in SCX chromatography with a flow rate of 0.2 mL/min. The protein was extracted by using urea/CHAPs or TFE.

[46,47]performed proteomic analysis of fractions of Escherichia coli lysates using combination of strong cation-exchange (SCX) or size-exclusion chromatography (SEC) coupled with RP-HPLC followed by UV and mass spectrometry detection. [68]reported a fast multidimensional chromatographic method as the combination of first-dimension ion-exchange chromatography with four reversed phase columns for the analysis of small protein and peptides of human haemofiltrate. A three-dimensional peptide fractionation approach for the quantitative proteomic study is reported [35] in which trypsin digested and isotope-coded affinity tag (ICATTM) reagent of a complete proteome lysate is fractionated [31]performed a multidimensional chromatographic separation using size-exclusion chromatography for the proteomic analysis of E. coli

(Strain BL 21). The authors used a TSKG3000SWxL 7 · 300 mm column and KH_2PO_4 50 mM and NaCl 200 mM in water as mobile phase for some aliquots and other aliquots by reversed phase C_{18}, (4.6 mm, 150 mm) column (218 TP 5415 Vydac) with linear gradient of acetonitrile and water having 0.1% TFA as mobile phase for two-dimensional separation study in SEC. The authors reported that liquid-based isoelectrofocusing-sizeexclusion chromatography (IEF-SEC) was able to separate milligrams of proteins according to isoelectric point and molecular size [71]reported a liquid chromatographic study of membrane proteins obtained from breast cancer MCF7 and BT474 cells using a fused silica strong cation exchange (SCX) column of (7.5 cm · 75 lm i.d.) having Polysulforthyl A resin. The authors identified total 313 proteins from MCF7 cell membranes, 602 proteins from BT474 cell membranes and 117 common proteins in MCF7 and BT474 cell membranes as given in Table 2 [18] studied lacrimal-specific praline-rich proteins having significant role in pathogenesis of inflammatory and autoimmune diseases, in human tear fluid with matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry followed by size-exclusion high performance liquid chromatography. The authors recognized some lacrimal-specific proteins. The success of C. elegans (a nematode), genome project gave a typical knowledge of neuropeptide signaling. Neuropeptide are originated from proprotein peptide precursor genes. [26]performed a peptidomic analysis of C. elegans using a strong cation exchange column (Bio-SCX,500 lm · 15 mm) attached with a C_{18} pre-column and 2% acetonitrile (ACN), 0.1% formic acid (FA) with water taking a flow rate of 30 lL/min. in a two-dimensional nano-scale liquid chromatography-quadrupole time-of-flight tandem-mass spectrometry (2D-nano-LC-Q-TOFMS/MS) method and reported a total ion current (TIC) chromatogram for every nano- LC–MS study shown in Figure. 7.

Capillary high performance liquid chromatography

A hybrid technique of HPLC and CE was developed in 1990 and is called as Capillary Electro-chromatography (CEC). It is expected to combine high peak efficiency which is characteristic of electrically driven separations with high separation selectivity. CEC experiments can be carried out on wall coated open tubular capillaries or capillaries packed with particulate or monolithic silica or other inorganic materials as well as organic polymers. The chromatographic and electrophoretic mechanisms work simultaneously in CEC and several combinations are possible.

The separation and identification of some proteins was performed in foam cells with capillary liquid chromatography followed by mass spectrometry[72]. [50] reported that out of 120 proteins, only 15 in cow milk fat globule membranes (MFGM), had similarity with previously studied mouse or human MFGM proteome using a micro-capillary liquid chromatography which was linked with a nanospray-tandem-mass spectrometer. [12]identified 111 human nasal mucous proteins in nasal lavage fluids (NLFs) of ten volunteers (patients) using a capillary liquid chromatography-electrospray quadrupole-time-of-flight mass spectrometric method. [36]analyzed human neutrophil granules responsible for chemotaxis, phagocytosis and bacterial killing susing two-dimensional microcapillary chromatography, reversed phase micro-capillary liquid chromatography followed by electrospray ionization tandem-mass spectrometry (2D HPLC ESI-MS/MS) technique and reported 286 proteins. [73] studied low molecular mass peptides in human cerebrospinal fluid (CSF), amyloid-like protein 1, secretogranin I, granin like neuroendocrine peptide precursor and neurosecretory protein using capillary liquid chromatography followed by quadrupole time-of-flight mass spectrometry. [6]identified 200 novel arginine-methylated proteins using micro-capillary liquid chromatography with electrospray ionization tandem-mass spectrometry. Capillary chromatography separation method was reported as a best separation method in combination with mass spectrometry for complex protein mixtures due to high sensitivity of this method. [59]Identified 145 unique peptides mapping 57 unique human serum proteins using micro-capillary liquid

chromatography electrospray ionization MS/MS method. The nano-LC-FTICR analysis of 0.5 pg of a bacterium Deinococcus radiodurans proteome was carried out using a 14.9 lm inner diameter separation capillary that was packed with 3 lm diameter stationary phase particles. [60]Reported that capillary isoelectric focusing (CIEF) in combination with FTICR-MS improved 10 throughputs for detection of proteins. [76]Reported CEC of enriched peptides, i.e., nitrotyrosine-containing peptides in complex proteome sample of mouse brain homogenate. The mobile phases used were 0.2% acetic acid, and 0.05% TFA in water as solvent A and 0.1% TFA in 90% acetonitrile as solvent B. [41]used reversed-phase capillary liquid chromatography for separation of vacuum dried peptide fractions using reversed-phase capillary column (65 cm · 150 lm) of fused silica capillary; packed with slurry of 5.0 lm Jupiter C_{18}-bonded particles. The mobile phases were 0.2% acetic acid and 0.05% TFA in water (Solvent system A) and 0.1% TFA in 90% acetonitrile in water (Solvent system B) was achieved by MS/MS. [39]reported a descriptive proteomic analysis of interphase and mitotic 14-3-3-binding proteins using 14-3-3 zeta affinity column and many new 14-3-3 binding proteins were recognized by micro-capillary high performance liquid chromatography tandem-mass spectrometry. These proteins had a significant role in cell cycle regulation, metabolism, protein synthesis, protein folding, proteolysis, nucleic acid binding etc.

Comparison of various chromatographic methods

Among various chromatographic methods used in proteomic analyses the order of application is reversed phase >gel permeation >ligand exchange > affinity. During our search of

literature it was found that the maximum papers on proteomic analyses were on reversed phase high performance liquid chromatography. It is due the fact that this kind of chromatography is well developed. There are many types of reversed phase diseases. Of course, it is very complicated issue to ascertain the mechanisms of diseases through proteomics and to the best of our knowledge there is no report available on this subject. Visualization has stationary phases available, which can be used for analyses of proteomes. Besides, the reversed phase columns are capable to work with a wide range of mobile phases, enhancing the application range of reversed phase chromatography. On the other hand, gel permeation HPLC is also useful for proteomic separation and identification due to a wide variation in the sizes of proteins. Ligand exchange is also useful as proteins have charges, which may be exploited in this kind of chromatography. Affinity and capillary electro-chromatographic techniques have also been used in proteomic area. Later technique is more useful as it needs little amount of sample and also has low detection limit. Therefore, all these techniques are important and useful for proteomics analyses depending on the type and nature of the proteins to be analyzed. They have their own merits and demerits, which cannot be discussed in detail here. However, the comparative features can be seen from Table 3 having applications of different kinds of chromatographic methods.

Chirality and chirality and protomics

It is well known-fact that millions of our bodies proteins interact among themselves and with the biological environment, i.e., with cell, tissue, organelle, protoplasm and other cellular molecules. Normally, these interactions are ideal at the time of birth but may change into abnormal during the course of time resulting into various diseases. The proteomics is more difficult phase in the process of understanding cellular biochemistry and mechanisms of disease. It is very important to mention here that proteomic interactions are stereospecific in nature[28]. These interactions define an individual's state of wellness or disease. Perhaps, the abnormal interactions of proteins occur due to change in the chiral structure of proteins. Therefore, the main root of diseases at molecular level could be due to chiral based abnormal interactions. The understanding of the mechanisms of chiral change in proteins and their interactions may be boon to control various been made for proteomes interactions and tried to establish the mechanisms of diseases evolution. Under normal situations the proteins are synthesized in cell by the direction of genomes and they interact

into the body for some fruitful purposes, i.e., growth and repair of the body. But under abnormal conditions some mutation occurred into genome resulting into deformated protein synthesis, which results into major or small change into their chiral structures. Due to change in chiral structures of proteins their mode of interactions is changed slightly giving rise abnormal behavior of cell and organs, i.e., diseases. For example the carcinoma is nothing but abnormal growth of cells. As stated above that various interactions of proteins may be reflected into an individual's state of wellness or disease. For example, a specific configuration of proteins in liver tissue could define a particular tumor.

Table No.1: Percentage of proteins reported in venoms of various families of snakes by HPLC separation.

Protein family	% of total venom proteins				
	B. g. rhinoceros	B. nasicornis	B. g. gabonica	B. a. arietans	B. caudalis
Bradykinin-potentiating peptides	0.3	-	2.8	-	-
Dimeric disintegrin	8.5	3.5	3.4	-	2.3
Long disintegrin	-	-	-	17.8	-
Kunitz-type inhibitors	7.5	-	3.0	4.2	3.2
Cystatin	5.3	4.2	9.8	1.7	-
DC-fragment	0.6	<0.1	0.5	-	-
svVEGF	-	-	1.0	-	-
PLA2	4.8	20.1	11.4	4.3	59.8
Serine proteinase	23.9	21.9	26.4	19.5	15.1
CRISP	1.2	1.3	2.0	-	1.2
C-type lectin	14.1	4.2	14.3	13.2	4.9
L-amino acid oxidase	2.2	3.2	1.3	-	1.7
Zn2+-metalloproteinase	30.8	40.9	22.9	38.5	11.5
Unknown peptides	0.8	0.7	1.2	0.9	0.3

Table No.2: HPLC identified proteins from BT474 and MCF7 cell membranes.

	BT474	% of total	MCF7	% of total	Common proteins	% of total
Protein locations	602		313		117	
Mitochondrion	15	2.5	4	1.3	2	1.7
Plasma membrane	49	8.1	24	7.7	3	2.6
Peroxisome	3	0.5	2	0.6	1	0.9
Nucleus	45	7.5	27	8.6	13	11.1
Endoplasmic reticulum	22	3.7	9	2.9	8	6.8
Cytoplasm	35	5.8	27	8.6	13	11.1
Golgi apparatus	2	0.3	1	0.3	0	0.0
Proteasome	4	0.7	1	0.3	1	0.9
Ribosome	22	3.7	22	7.0	18	15.4
Unknowns	392	65.1	195	62.3	58	49.6
Location unclear	15	2.5	1	0.3	0	0.0
	604[a]	100.00	313	100.00	117	100.00

Table No.3: A comparison of proteomic analyses on various chromatographic techniques.

Proteomes	Columns	Mobile phases	References
Reversed phase high performance liquid chromatography			
Complex protein mixture	C_{18} column	Acetonitrile–TFA	Wang et al. (2005)
Cycloskeletal proteins and enzymes	PepMap_ C_{18} (250 mm · 0.3 mm)	-	Babusiak et al. (2007)
Peptides of Caenorhabditis elegans	C_{18} (250 mm · 4.6 mm i.d.)	Acetonitrile–formic acid	Husson et al. (2006)
S-transferase isoenzyme	Vydac 214TP C_4 (150 mm · 4.6 mm)	Acetonitrile and water with TFA	Gallagher et al. (2006)
Photosystem II antenna protein	Vydac C_4	Acetonitrile–water–TFA	Zolla et al. (2003)
Proteins of rat adipose cells	Zorbax 300 SB-C_3 (150 · 4.6 mm)	Acetonitrile–TFA	Chen et al. (2005)
Complex protein mixture	Zorbax SB-C_{18}	Buffer–acetonitrile–FA Buffer–acetonitrile–TFA	Bihan et al. (2004)
GPI-APs protein	Zorbax SB-C_{18}	Acetonitrile with acids HFBA (different combinations)	Elortza et al. (2006)
Phosphopeptides of rat kidney IMCD	Picofrit RP column	Acetonitrile–FA	Hoffert et al. (2007)
Venoms of various	Lichrosphere RP100 C_{18}	Water–acetonitrile–TFA	Calvete et al. (2007)

snakes	(250 · 4 mm with 5 lm)		
Affinity high performance liquid chromatography			
Peptides of liver of mice	Magic C_{18} AQ (75 lm · 15 cm)	Water–acetonitrile–FA	Welch et al. (2005)
Phosphopeptides of Arabidopsis	PepMap C_{18} (300 lm · 5 mm)	-	de la Fuente van Bentem et al. (2006)
Ligand exchange high performance liquid chromatography			
Proteomic analysis of E. coli strain BL 21	218 TP 5415 Vydac C18 RP column (150 · 4.6 mm)	Acetonitrile–water–TFA	Lecchi et al. (2003)
Pancreatic islet Proteome	Polysulfoethyl A column (200 · 2.1 mm)	10 mM Ammonium formate buffer–water and acetonitrile	Metz et al. (2006)
Membrane proteins of breast cancer MCF7 and BT474 cells	Polysulfoethyl A resin (7.5 cm · 75 lm i.d.)	-	Xiang et al. (2004)
Peptidomic analysis of Caenorhabditis elegans	Bio-SCX column (15 mm · 500 lm)	Water–acetonitrile–FA	Husson et al. (2005)
Capillary electro-chromatography			
Protein of Helicobacter pylori	ReproSil-Pur C_{18}-AQ (17 cm · 50 lm i.d.)	-	Carlsohn et al. (2006)
Tryptic peptide mixture of fish	Phenomenex Jupiter C_{18} (250 · 2.1 nm)	Acetonitrile–TFA–formic acid	Monti et al. (2005)
Synaptic proteomes of wild type mice	Polysulfoethyl A (150 mm · 100 lm i.d.)	Buffers–acetonitrile–10 mM KH_2PO_4	Li et al. (2007)
Proteomic analysis of E. coli strain BL 21	TSKG3000SWxL (300 · 7 mm)	Water–50 mM KH2PO4–200 nM NaCl	Lecchi et al. (2003)
Vacuum dried peptides	Jupiter C_{18} RP capillary column (65 cm · 150)	Water with TFA and FA, water and acetonitrile with TFA	Metz et al. (2006)

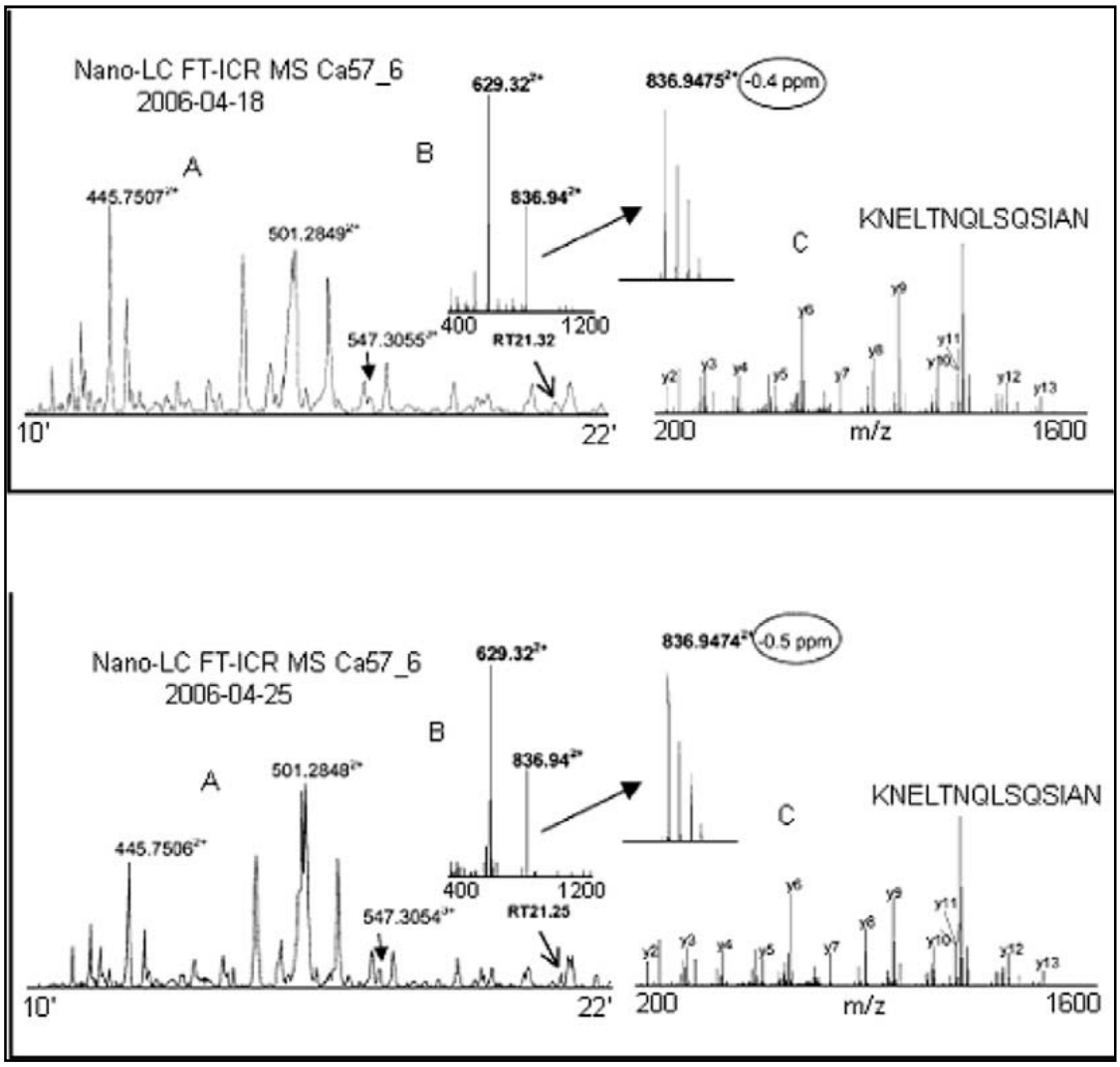

Figure No.1: Analysis of a tryptic digest of a protein band from an Outer membrane protein (Omp) at two various times. (A) In LC separation, chromatogram represents the high reproducibility of the retention time and peak distribution (B) the measurements of mass of peptides with doubly protonated at m/z 836.94 at 21.32 and 21.25 min. (C) CID spectra of doubly protonated peptide showed at m/z 836.94.

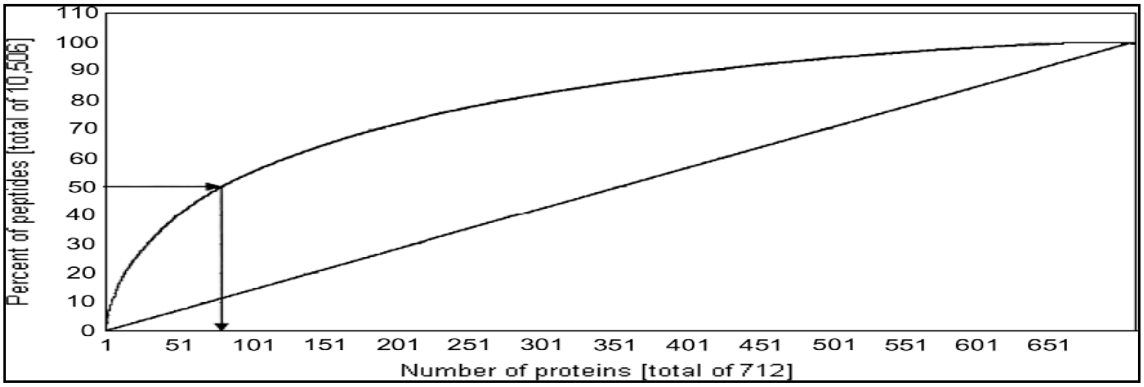

Figure No.2: Few represented MPC proteins create a large no of MPC peptides.

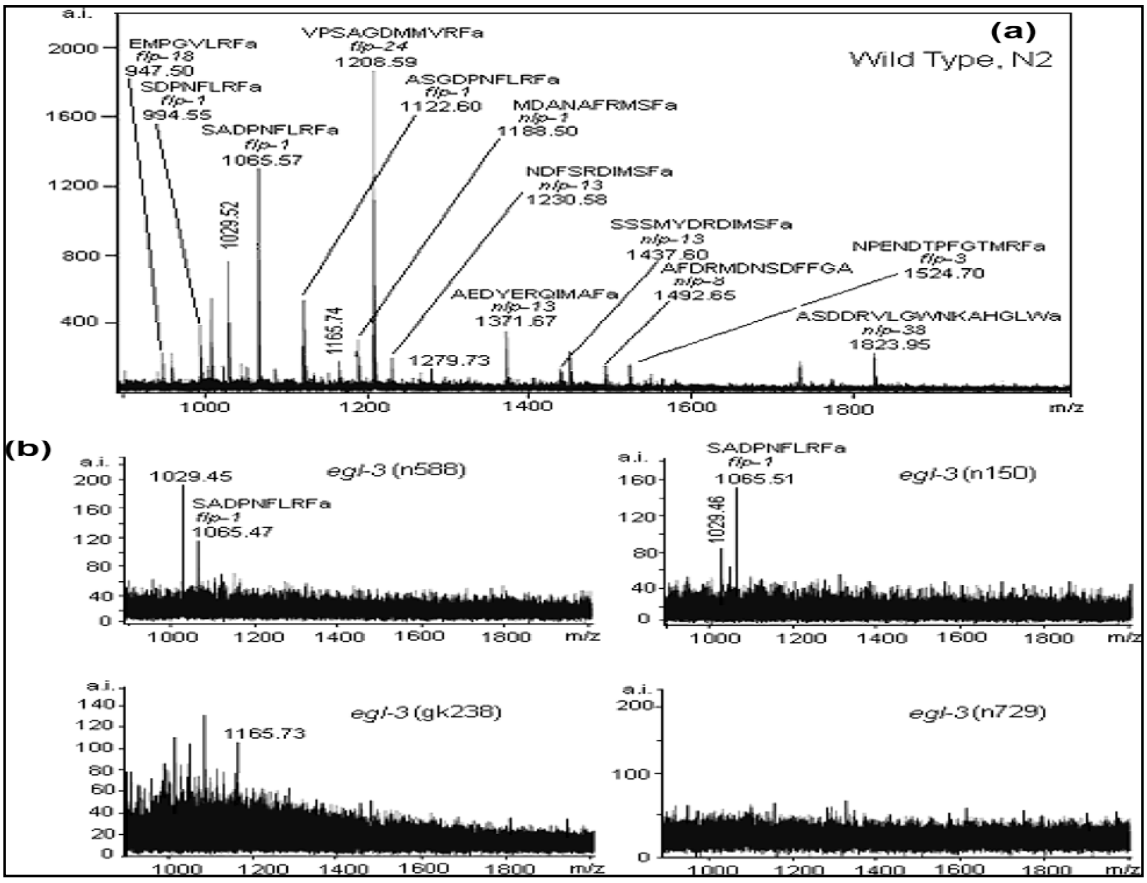

Figure No.3: Comparative study of MALDI-TOF MS spectra (a): C18 HPLC analysis of wild type C. elegans extract and obtained fractions were further analyzed by MALDI-TOF MS (only fraction 35 is shown in figure). Measured masses were compared with theoretical masses of FLP and NLP peptides. (b) The analysis of extracts of various C. elegans strains with mutated egl-3 with same procedure as with wild type strains. Zoom spectrum of fraction 35 of 4 strains namely n588, n150, n729 and gk238.

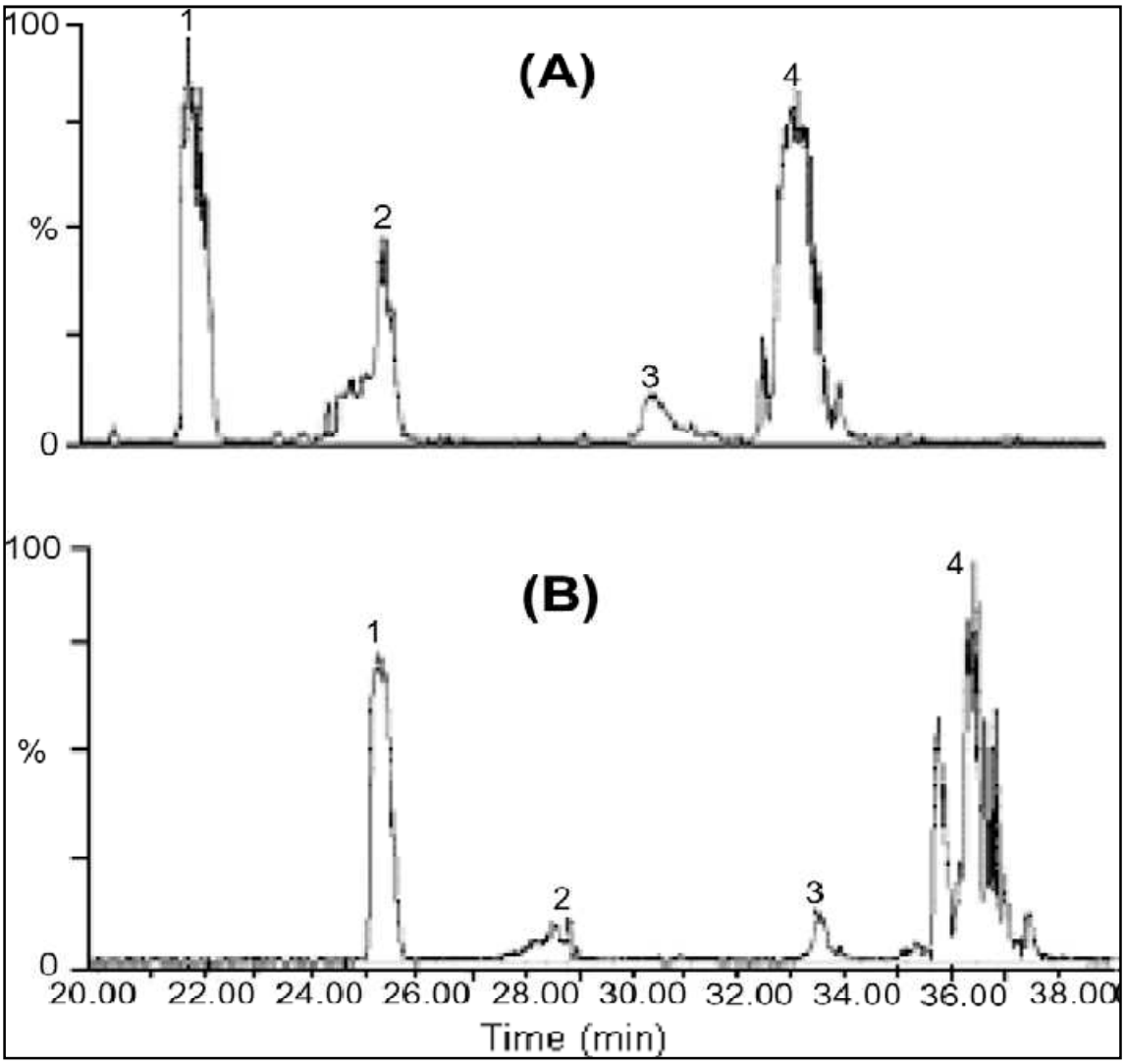

Figure No.4: Comparative study of model protein separation by using (A): C4 column (B): C18 at 25 _C with an elution order as (1) ribonuclease A, (2) cytochrome c (3) bovine serum albumin and (4) myoglobin using acetonitrile as mobile phase having 0.1% TFA with 200 nL/min flow rate.

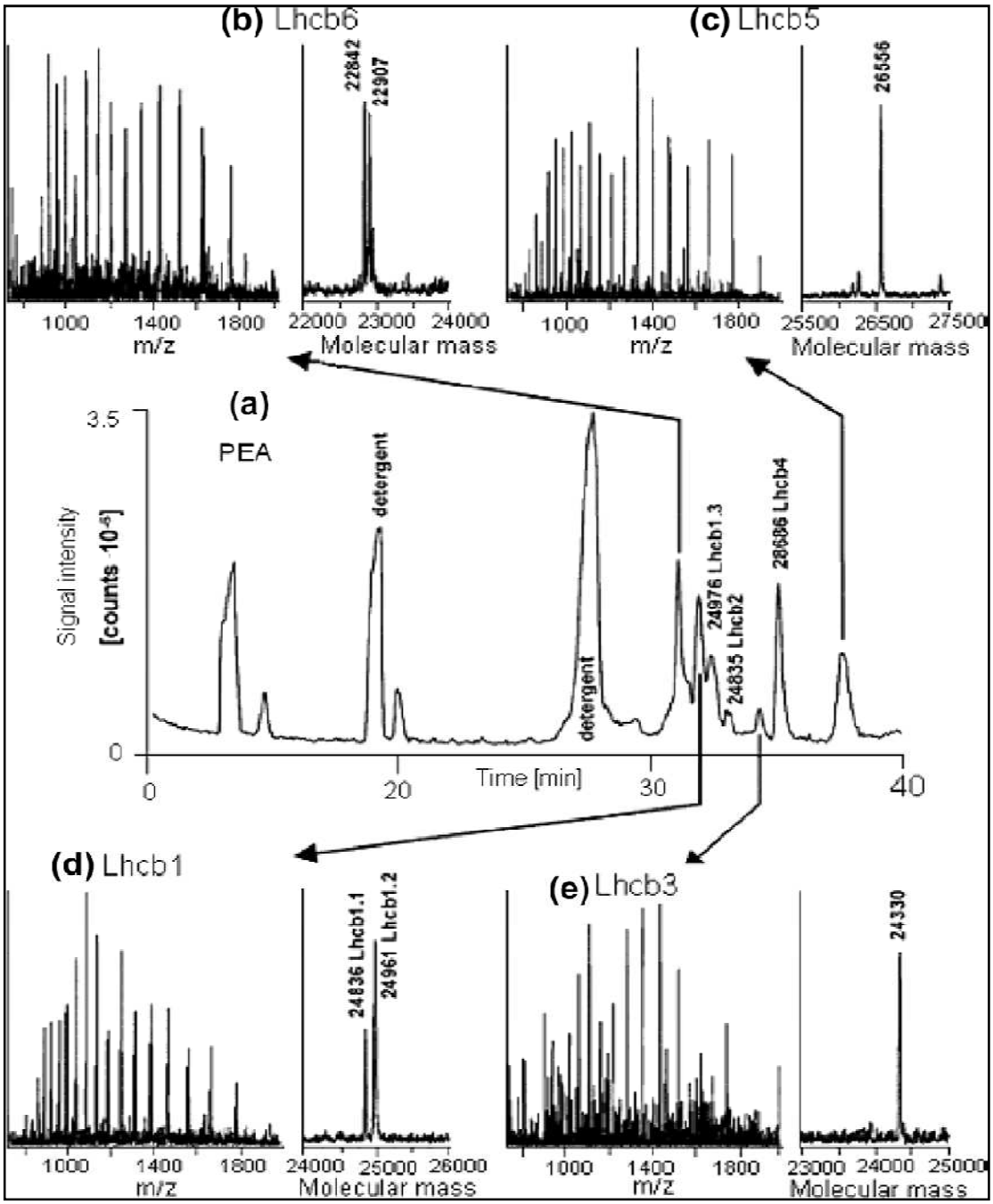

Figure No.5: Identification of protein components of photosystem II by using reversed-phase HPLC-ESI-MS.

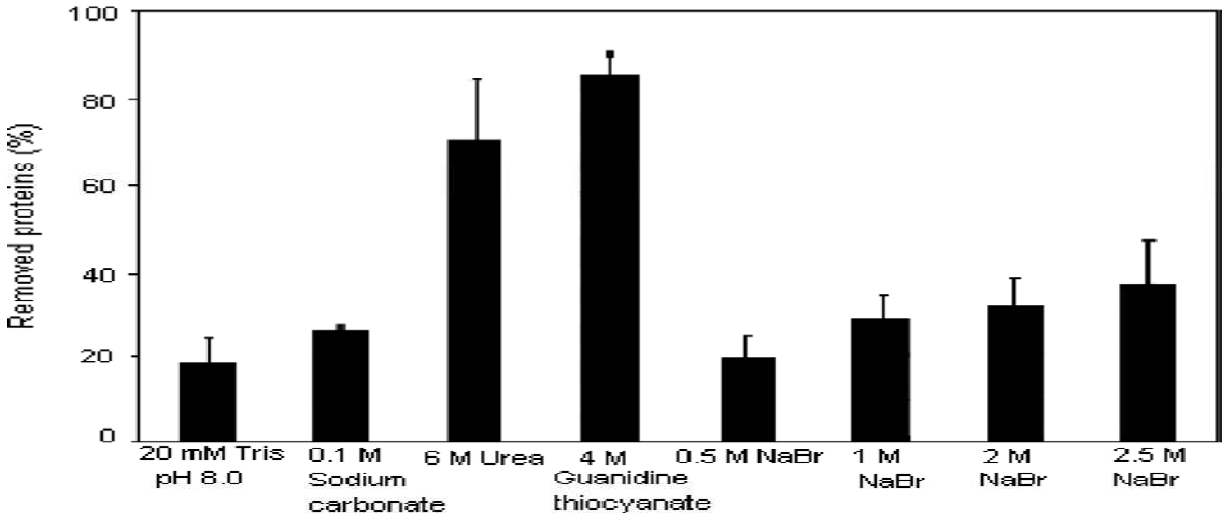

Figure No.6: Separated quantity of proteins from C. glutamicum membranes by washing with different solutions.

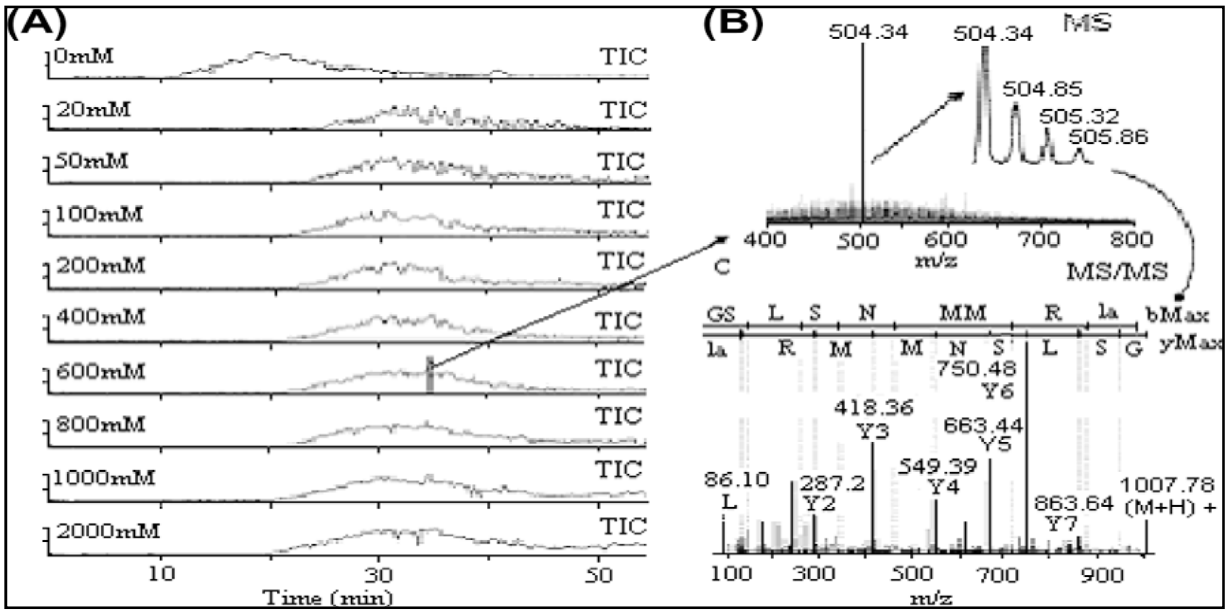

Figure No. 7: A view on 2D-nano-LC–MS/MS analysis. (A) Total ion current chromatogram of 10 SCX fractions received from a C. elegans extract after 2D-nano LC separation. (B) The ion at m/z 504.34 is selected for fragmentation of 600 mM fraction at 34.5 min. (C) GSLSNMMRI amide sequence of fragmentation spectra of selected peptides.

CONCLUSION

Liquid chromatography is considered to be the back bone of the separation science. With the hyphenation of mass spectrometer detectors this technique has achieved heights in analysis work. It can detect molecules at the level of the nanomole. Hence, it is useful in proteomics and genome research. Many kinds of liquid chromatography such as reversed phase high performance liquid chromatography, affinity high performance liquid chromatography, gel permeation high performance liquid chromatography, ligand exchange high performance liquid chromatography and capillary high performance liquid chromatography have been used in proteomic research. More advance paraphernalia is required to achieve the detection at picomolar and femtomolar levels, which are required in proteomics and genome research. Besides, the mechanism and medication of various diseases can be understood by using the concept of chirality in proteomic. Chiral chromatography may be a useful tool for the proteomic interactions.

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REFERENCES

1. Andre M, Le Caer JP, Greco C, Planchon S, Nemer W, Boucheix C, Rubinstein E, Chamot-Rooke J, Le Naour F. Proteomic analysis of the tetraspanin web using LC–ESI-MS/ MS and MALDI-FTICR-MS, *Proteomics*, 6, 2006, 1437-1449.
2. Azarkan M, Huet J, Baeyens-Volant D, Looze Y, Vandenbussche G. Affinity chromatography: a useful tool in proteomic studies, *J. Chromatogr*, 849, 2007, 81-90.
3. Babusiak M, Man P, Petrak J, Vyoral D. Native proteomic analysis of protein complexes in murine intestinal brush border membranes, *Proteomics*, 7, 2007, 121-129.
4. Banfi C, Brioschi M, Wait R, Begum S, Gianazza E, Fratto P, Polvani G, Vitali E, Parolari A, Mussoni L, Tremoli E. Proteomic analysis of membrane microdomains derived from both failing and non-failing human hearts, *Proteomics*, 6, 2006, 1976-1988.
5. Bihan TL, Robinson MD, Stewart II, Figeys D. Definition and characterization of a Trypsinosome from specific peptide characteristics by nano-HPLC-MS/MS and in silico analysis of complex protein mixtures, *J. Proteome Res*, 3, 2004, 1138-1148.
6. Boisvert FM, Cote J, Boulanger MC, Richard S. A proteomic analysis of arginine methylated protein complexes, *Cell Proteomics*, 2, 2003, 1319-1330.
7. Brock JW, Hinton DJ, Cotham WE, Metz TO, Thorpe SR, Baynes J, Ames JM. Proteomic analysis of the site specificity of glycation and carboxymethylation of ribonuclease, *J.Proteome Res*, 2, 2003, 506-513.
8. Calvete JJ, Escolano J, Sanz L. Snake venomics of Bitis species reveals large intragenus venom toxin composition variation, application to taxonomy of congeneric taxa, *J. Proteome Res*, 6, 2007, 27322-745.
9. Cantin GT, Venable JD, Cociorva D, Yates JR. Quantitative phosphoproteomic analysis of the tumor necrosis factor pathway, *J. Proteome Res*, 5, 2006, 127-134.
10. Cao P, Stults JT. Mapping the phosphorylation sites of proteins using on-line immobilized metal electrophoresis/electrospray ionization multiple stage tandem mass spectrometry, Rapid Commun. *Mass Spectrom*, 14, 2000, 1600-1606.
11. Carlsohn E, Nystrom J, Karlsson H, Svennerholm AM, Nilsson CL. Characterization of the outer membrane protein profile from disease-related Helicobactor pylori isolates by subcellular fractionation and nano-LCFT-ICR MS analysis, *J. Proteome Res*, 5, 2006, 3197-3204.
12. Casado B, Pannell LK, Iadarola P, Baraniuk J. Identification of human nasal mucous proteins

using proteomics, *Proteomics*, 5, 2005, 2949-2959.

13. Chen X, Cushman SW, Pannel LK, Hess S. Quantitative proteomic analysis of the secretory proteins from rat adipose cells using a-2D liquid chromatography MS/MS approach, *J. Proteome Res*, 4, 2005, 570-577.
14. Crugliano T, Quaresima B, Gaspari M, Faniello M.C, Romeo F, Baudif, Cuda G, Costanzo F, Venuta S. Specific changes in the proteomic pattern produced by the BRCA1-Ser1841 Asn missense mutation, *Int. J. Biochem. Cell Biol*, 39, 2007, 220-226.
15. De la Fuente van Bentem S, Anrather D, Roitinger E, Djamei A, Hufnagl T, Barta A, Csaszar E, Dohnal I, Lecourieux D, Hirt H. Phosphoproteomics reveals extensive in vivo phophorylation of arabidopsis proteins involved in RNA metabolism, *Nucl. Acids Res*, 34, 2006, 3267-3278.
16. Drew JE, Rucklidge GJ, Duncan G, Lufty A, Farquharson AJ, Reid MD, Russell WR, Morrice PC, Arthur JR, Duthie GG. A proteomics approach to identify changes in protein profiles in pre-cancerous colon, *Biochem. Biophys. Res*. Commun, 330, 2005, 81-87.
17. Elortza F, Mohammed S, Bunkenborg J, Foster LJ, Nuhse TS, Brodbeck Urs Peck SC, Jensen ON. Modification specific proteomics of plasma membrane proteins: identification and characterization of glycosyl phosphatidyl inositol-anchored proteins released upon phospholipase D treatment, *J. Proteome Res*, 5, 2006, 935-943.
18. Fung KY, Morris C, Sathe S, Sack R, Duncan MW. Characterization of the in vivo forms of lacrimal-specific prolinerich proteins in human tear fluid, *Proteomics*, 4, 2004, 3953-3959.
19. Gallagher EP, Gardner JL, Barber DS. Several glutathione S-transferase isozymes that protect against oxidative injury are expressed in human liver mitochondria, *Biochem. Pharmacol*, 71, 2006, 1619-1628.
20. Garcia A, Zitzmann N, Watson SP. Analyzing the platelet proteome, *Semin. Thromb. Hemost*, 30, 2004, 485-489.
21. Garcia A, Watson S.P, Dwek R.A, Zitzmann N. Applying proteomics technology to platelet research, Mass Spectrom. Rev, 24, 2005, 918-930.
22. Garcia BA, Smalley D, Cho H, Shabanowitz J, Ley K, Hunt F. The platelet microparticle proteome, *J. Proteome Res*, 4, 2005, 1516-1521.
23. Glinski M, Weckwerth W. The role of mass spectrometry in plant systems biology, *Mass Spectrom. Rev*, 25, 2006, 173-214.
24. Wang G, Pisitkun T, Shen RF, Knepper MA. An automated platform for analysis of phosphor proteomic datasets: application to kidney collecting duct phosphor proteins, *J. Proteome Res*, 6, 2007, 3501-3508.
25. Hortin GL, Shen RF, Martin BM, Remaley AT. Diverse range of small peptides associated with high-density lipoprotein, *Biochem. Biophys. Res. Commu*, 340, 2006, 909-915.
26. Husson SJ, Clynen E, Baggerman G, De Loof A, Schoofs L. Discovering neuropeptides in Caenorhabditis elegans by two dimensional liquid chromatography and mass spectrometry, *Biophys. Res. Commun*, 335, 2005, 76-86.
27. Husson SJ, Clynen E, Baggerman G, Janssen T, Schoofs L. Defective processing of neuropeptide precursors in Caenorhabditis elegans lacking proprotein convertase mutant analysis by mass spectrometry, *J. Neurochem*, 98, 2006,1999-2012.
28. Kawamura A, Hindi S. Protein fishing with chiral molecular baits, Chirality, 17, 2005, 332-337.
29. Kikuchi M, Hatano N, Yokota S, Shimozawa N, Imanaka T, Taniguchi H. Proteomic analysis of rat liver peroxisome: presence of peroxisome-specific isozyme of Lon protease, *J. Biol. Chem*, 279, 2004, 421-428.
30. Le Naour F, Andre M, Greco C, Billard M, Sordt B, Emile JF, Lanza F, Boucheix C, Rubinstein E: Profiling of the tetraspanin web of human colan

cancer cells, *Mol. Cell Proteomics*, 5, 2006, 845-857.
31. Lecchi P, Gupte AR, Perez RE, Stockert LV, Abramson F. Size-exclusion chromatography in multidimensional separation schemes for proteomic analysis, *J. Biochem. Biophys*. Methods, 56, 2003, 141-152.
32. Li KW, Miller S, Klychnikov O, Loos M, Stahl-Zeng J, Spijker S, Mayford M, Smit AB. Quantitative proteomics and protein network analysis of Hippocampal synapses of CaMKIIa mutant mice, *J. Proteome Res*, 6, 2007, 3127-3133.
33. Liang X, Zhao J, Hajivandi M, Wu R, Tao J, Amshey JW, Pope RM. Quantification of membrane and membranebound proteins in normal and malignant breast cancer cells isolated from the same patient with primary breast carcinoma, *J. Proteome.Res*, 5, 2006, 2632-2641.
34. Lindsey Rose KM, Gourdie RG, Prescott AR, Quinlan RA, Crouch RK, Schey KL. The C terminus of lens aquaporin interacts with the cytoskeletal proteins filensin and CP49. Invest, Ophthalmol. *Vis. Sci*, 47, 2006, 1562-1570.
35. Link AJ. Multidimensional peptide separations in proteomics, *Trends Biotechnol*, 20, 2002, 8–13.
36. Lominadze G, Powell DW, Luerman GC, Link AJ, Ward RA, McLeish KR. Proteomic analysis of human neutrophil granules. *Mol. Cell Proteomic,s* 4, 2005, 1503–1521.
37. Martinovic S, Berger SJ, Pasa-Tolic L, Smith RD. Separation and detection of intact noncovalent protein complexes from mixtures by on-line capillary isoelectric focusing-mass spectrometry, *Anal. Chem*, 72, 2000, 5356–5360.
38. Matsumoto M, Hatakeyama S, Oyamada K, Oda Y, Nishimura T, Nakayama KI. Large-scale analysis of the human ubiquitin-related proteome, *Proteomics*, 5, 2005, 4145–4151.
39. Meek SE, Lane WS, Piwnica-Worms HJ. Comprehensive proteomic analysis of interphase and mitotic 14-3-3-binding proteins, *Biol. Chem*, 279, 2004, 32046–32054.
40. Metodiev MV, Timanova A, Stone DE. Differential phosphoproteome profiling by affinity capture and tandem matrix-assisted laser desorption/ionization mass spectrometry *Proteomics*, 4, 2004, 1433–1438.
41. Metz TO, Jacobs JM, Gritsenko MA, Fontes G, Qian WJ, Camp 2nd DG, Pointout V, Smith RD. Characterization of the human pancreatic islet proteome by two-dimensional LC/MS/MS, J. *Proteome Re*, 5, 2006, 3345–3354.
42. Monti G, Napoli LD, Mainolfi P, Barone R, Guida M, Marino G, Amoresano A: Monitoring food quality by microfluidic electrophoresis, gas chromatography, and mass spectrometry techniques: effects of aquaculture on the sea bass *Anal. Chem*, 77, 2005, 2587–2594.
43. Montine TJ, Woltjer RL, Pan C, Montine KS, Zhang J. Liquid chromatography with tandem mass spectrometry-based proteomic discovery in aging and Alzheimer's disease, *Neuro. Rx*, 3, 2006, 336–343.
44. Nepomuceno AI, Muddiman DC, Bergen 3rd HR, Craighead JR, Burke MJ, Caskey PE, Allan JA. Dual electrospray ionization source for confident generation of accurate mass tags using liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry, *Anal. Chem*, 75, 2003, 3411–3418.
45. Neverova I, Van Eyk JE. Role of chromatographic techniques in proteomic analysis, *J. Chromatogr*, 815, 2005, 51–63.
46. Opiteck CJ, Lewis KC, Jorgenson JW, Anderegg RJ. Comprehensive on-line LC/LC/MS of proteins, *Anal. Chem*, 69, 1997, 1518–1524.
47. Opiteck GJ, Ramirez SM, Jorgenson JW, Moseley MA. Comprehensive two-dimensional high-performance liquid chromatography for the isolation of over expressed proteins and proteome mapping, *Anal. Biochem*, 258, 1998, 349–361.
48. Pan H, Che FY, Peng B, Steiner DF, Pintar JE, Fricker L.D: The role of prohormone convertase-2 in hypothalamic neuropeptide processing: a quantitative neuropeptidomic study, *J. Neurochem*, 98, 2006, 1763–1777.
49. Reh E, Hahn B, Lamotte S. Evaluation of stationary phases for 2-dimensional HPLC of

proteins part 1. Validation of commercial RP-columns, *J. Chromatogr*, 844, 2006, 204–212.
50. Reinhardt TA, Lippolis JD. Bovine milk fat globule membrane proteome, *J. Dairy Res*, 73, 2006, 406–416.
51. Righetti PG, Castagna A, Antonioli P, Cecconi D, Campostrini N, Righetti SC. Proteomic approaches for studying chemoresistance in cancer. Expert Rev, *Proteomics*, 2, 2005, 215–228.
52. Rosas-Acosta G, Russell WK, Deyrieux A, Russell DH, Wilson VG. A universal strategy for proteomic studies of SUMO and other ubiquitin-like modifiers, *Mol. Cell Proteomics*, 4, 2005, 56–72.
53. Ryu J, Kim H, Lee SK, Chang EJ, Kim HJ, Kim HH. Proteomic identification of the TRAF6 regulation of vacuolar ATPase for osteoclast function, *Proteomics*, 5, 2005, 4152–4160.
54. Sapra R, Gaucher SP, Lachmann JS, Buffleben GM, Chirica GS, Comer JE, Peterson JW, Chopra AK, Singh AK. Proteomic analyses of murine macrophages treated with Bacillus anthracis lethal toxin, *Microb. Pathog*, 41, 2006, 157–167.
55. Schluesener D, Fischer F, Kruip J, Rogner M, Poetsch A. Mapping the membrane proteome of Corynebacterium glutamicum, *Proteomics*, 5, 2005, 1317–1330.
56. Schluesener D, Rogner M, Poetsch A. Evaluation of two proteomics technologies used to screen the membrane proteomes of wild-type Corynebacterium glutamicum and an L-lysine producing strain, *Anal. Bioanal. Chem*, 389, 2007, 1055–1064.
57. Senis YA, Tomlinson MG, Garcia A, Dumon S, Heath VL, Herbert J, Cobbold P, Spalton JC, Ayman S, Antrobus R, Zitzmann N, Bicknell R, Frampton J, Authi K, Martin A, Wakelam MJ, Watson SP. A comprehensive proteomics and genomics analysis reveals novel transmembrane proteins in human platelets and mouse megakaryocytes including G6b-B, a novel immunoreceptor tyrosine-based inhibitory motif protein, *Mol. Cell Proteomics*, 6, 2007, 548–564.
58. Seshi B. An integrated approach to mapping the proteome of the human bone marrow stromal cell, *Proteomics*, 6, 2006, 5169–5182.
59. Shen Y, Smith RD. Proteomics based on high-efficiency capillary separations, *Electrophoresis*, 23, 2002, 3106–3124.
60. Shen Y, Tolic N, Masselon C, Pasa-Tolic L, Camp II DG, Hixson KK, Zhao R. Ultrasensitive proteomics using high-efficiency on-line micro-SPE-nano LC-nanoESI MS and MS/ *MS, Anal. Chem*, 76, 2004, 144–154.
61. Shin SJ, Lee SE, Boo JH, Kim M, Yoon YD, Kim SI, Mook-Jung I. Profiling proteins related to amyloid deposited brain of Tg2576 mice, *Proteomics*, 4, 2004, 3359–3368.
62. Soreghan BA, Yang F, Thomas SN, Hsu J, Yang A. High throughput proteomic-based identification of oxidatively induced protein carbonylation in mouse brain, *J. Pharm. Res*, 20, 2003, 1713–1720.
63. Stensballe A, Andersen S, Jensen ON. Characterization of phosphoproteins from electrophoretic gels by nanoscale Fe(III) affinity chromatography with off-line mass spectrometry analysis, *Proteomics*, 1, 2001, 207–222.
64. Tran HT, Ulke A, Morrice N, Johannes CJ, Moorhead GB. Proteomic characterization of protein phosphatase complexes of the mammalian nucleus, *Mol. Cell Proteomics*, 3, 2004, 257–265.
65. Tyan YC, Guo R, Liu CY, Liao PC. Proteomic profiling of human urinary proteome using nano-high performance liquid chromatography/ ionization tandem mass spectrometry, *Anal. Chem. Acta*, 579, 2006, 158–176.
66. Vanrobaeys F, Van Coster R, Dhondt G, Devreese B, Van Beeumen J. Profiling of myelin proteins by 2D-gel electrophoresis and multidimensional liquid chromatography coupled to MALDI-TOF-TOF mass spectrometry, *J. Proteome Re*, 4, 2005, 2283– 2293.
67. Vasilescu J, Smith JC, Ethier M, Figeys D. Proteomic analysis of ubiquitinated proteins from human MCF-7 breast cancer cells by immunoaffinity purification and mass

spectrometry, *J. Proteome Res*, 4, 2005, 2192–2200.

68. Wagner K, Miliotis T, Marko-Varga, G, Bischoff R, Unger KK. An automated on-line multidimensional HPLC system for protein and peptide mapping with integrated sample preparation, *Anal. Chem*, 74, 2002, 809–820.
69. Wang, Y, Balgley BM, Rudnick PA, Lee CS. Effects of chromatography conditions on intact protein separations for topdown proteomics, *J. Chromatogr*, 1073, 2005, 35–41.
70. Welch K, Wen B, Goodlett DR, Yi EC, Lee H, Reilly TP, Nelson SD, Pohl LR. Proteomic identification of potential susceptibility factors in drug-induced liver disease, Chem. Res. Toxicol, 18, 2005, 924–933.
71. Xiang R, Shi Y, Dillon D.A, Negin B, Horvath C, Wilkins JA. 2D LC/MS analysis of membrane proteins from breast cancer cell lines MCF7 and BT 474, *J. Proteome Res*, 3, 2004, 1278–1283.
72. Yang PY, Rui YC, Yang PY, Yu YL. Proteomic analysis of foam cells, *Methods Mol. Biol*, 357, 2007, 297–305.
73. Yuan X, Desiderio DM. Human cerebrospinal fluid peptidomics, *J. Mass Spectrom*, 40, 2005, 176–181.
74. Yuan Q, Zhao FK. New frontiers in the proteome research quantitative, *proteomics*, 33, 2001, 477–482.
75. Zhang H, Li XJ, Martin DB, Aebersold R. Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry, *Nat. Biotechnol*, 21, 2003, 660–666.
76. Zhang Q, Qian WJ, Knyushko TV, Clauss TRW, Purvine S, Moore RJ, Sacksteder CA, Chin M, Smith D, Camp II DG, Bigelow DJ, Smith RD. A method for selective enrichment and analysis of nitrotyrosine – containing peptides in complex proteome samples, *J. Proteome Res*, 6, 2007, 2257– 2268.
77. Zolla L, Timperio AM, Walcher W, Huber CG. Proteomics of light-harvesting proteins in different plant species. Analysis and comparison by liquid chromatography – electrospray ionization mass spectrometry. *Plant Physiol*. 131, 2003, 198–214.