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STUDY OF PAPERSPRAY IONIZATION AND ITS POSSIBLE APPLICATIONS

Master's thesis (30 EAP)

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Tartu 2012

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Abbreviations

3Q	triple quadrupole mass spectrometer
AMQ	8-amino-2methylquinoline
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
CI	chemical ionization
CID	collision induced dissociation
DESI	desorption electrospray ionization
EI	electron ionization
ESI	electrospray ionization
FT ICR	fourier transform ion cyclotron resonance
IMS	ion mobility spectroscopy
LSI	leafspray ionization
m/z	mass-to-charge ratio
MALDI	matrix-assisted laser desorption ionization
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MS^2	tandem mass spectrometry
nano-ESI	nanospray
PENT	penthrite
PSI	paperspray ionization
PTFE	polytetrafluoroethylene
RDX	hexogen
RSD	relative standard deviation
S/N	signal-to-noise ratio
stdev	standard deviation
TNT	trinitrotoluene
u	atomic weight unit

Introduction

Mass spectrometry has high selectivity and sensitivity but needs high qualification of the operator and complicated sample pre-treatment. Therefore often many possible applications for MS are unused while solving everyday problems. Complications of mass spectrometry come from the necessity of ionizing the analyte and the need to transfer analytes into vacuum environment. Therefore ionization sources with different levels of robustness and complexity are used. New ionization sources called ambient ionization sources are easy to use, need little or no sample pre-treatment and allow full use of mass spectrometry benefits such as fast analysis and superior selectivity. However these ionization sources are relatively new and therefore have not yet developed into regular use.

Paperspray ionization, a relative of electrospray ionization, is one of these new ionization techniques. The sample is transferred onto a triangular shaped paper and this paper is placed into a holder in front of mass spectrometer inlet. High voltage is applied and eluent is added to the paper in order to initiate spray from the tip of the triangle. As sample components that do not dissolve in the eluent stay on the paper the paper may act as a part of sample pre-treatment in addition to ionization. Both qualitative and quantitative analysis with paperspray ionization have been presented in literature.

Previous studies of paperspray ionization have mostly focused on researching and developing methods for measuring drugs and other compounds in blood from dried blood spots. Other possible applications like identification of illegal compounds from surfaces and quantification of pesticides have received less attention. The development of paperspray ionization has high potential in many analytical applications in a wide range of scientific fields.

The aim of this thesis was to widen the range of paperspray applications, namely:

1. studying if different papers can be distinguished and information about compounds in the paper obtained by using paperspray ionisation;
2. identification and possible quantification methods for analysis of pesticides from surfaces of fruits and vegetables;
3. analyzing trace amounts of explosives.

In order to fulfil these goals literature review was conducted and working with different mass spectrometry instruments was studied. Also paperspray ionization equipment was developed and papers were studied as paperspray materials.

1. Literature overview

1.1 Mass spectrometry

Mass spectrometry (MS) has progressed rapidly over the last decade due to its excellent sensitivity, low detection limits and diversity of its applications. Some examples for applications are in the fields of atomic physics, reaction physics, reaction kinetics, geochronology and all forms of chemical analysis. [1]

As the first step in MS the analyte is turned into gas-phase ions with an ionization source. Unlike neutral particles compounds with an electrical charge can be manipulated by electric and magnetic field. Thereafter the ions can be separated by their mass-to-charge ratio (*m/z*) using one or more mass analyzers. If compounds lose one electron or receive one proton in the ionization process the *m/z* is equivalent to the mass of the ion and therefore by detecting the ions we find the mass of the ion. The masses are expressed in atomic weight units (u) or Daltons which have the same fundamental definition. [1, 2]

Due to high energies involved in ionization the compounds can undergo fragmentation. Fragments give information about different functional groups that the precursor molecule contains. The ions that do not fragment are detected as the ions with the highest *m/z* in mass spectrum of a pure compound and are called the molecular ions. The precise measurement of molecular ions *m/z* makes it possible to calculate the accurate isotopic composition of the analyte. [1, 2]

All mass spectrometers contain the following elements:

1. Device for introduction of the compound, e.g. gas chromatography;
2. Ion source – device that produces ions from the sample;
3. One or more mass analyzers for separation of different compounds by their *m/z*;
4. Detector for measuring the abundance of ions;
5. Computer for controlling the instrument and data processing. [1]

As the ions have to move in the MS without colliding with other particles high vacuum inside the MS is required. For example to achieve the mean free pathway of 1 meter for the ion the maximum pressure possible inside the instrument is approximately 5×10^{-5} Torr. If high voltage ionization sources are used the pressure must be reduced even further to reduce the possibility of discharging. The vacuum is produced using first mechanical pumps to achieve 10^{-3} Torr of pressure. Thereafter turbomolecular, diffusion or cryogenic pumps, conjugated with the mechanical pump, are used to reduce the pressure to 10^{-6} Torr inside the MS. [1]

To characterize a MS many parameters can be given from which the most important are resolution, *m/z* accuracy, *m/z* range, sensitivity and scan speed. Resolution shows the ability of the mass analyzer to separate adjacent ions. High-resolution mass analyzers can separate ions with the same nominal mass and therefore increases the selectivity. *m/z* accuracy shows how close the true mass of the ion is to the assigned mass. Usually MS that has high resolution also has high mass accuracy. *m/z* range indicates the lowest and highest *m/z* that the MS is capable of detecting. Sensitivity shows the ability of a particular instrument to respond to a given amount of analyte. Higher sensitivity indicates that small change in amount of analyte changes strongly the response of the instrument. Scan speed shows how fast a mass spectrum can be acquired. [2]

Tandem mass spectrometry (MS/MS) is a general method for studying the structure of the analyte. The ion of interest is first separated from others and then fragmentation process takes place. During fragmentation the mass or charge of the ion is changed. These two steps can be separated in space or in time. In case of quadrupole mass analyzers for example two analyzers are connected where different parts of the process take place and therefore are separated in space. If ion trap mass analyzers are used the ion can be separated and fragmented in the same cell and therefore separated in time. [1, 2]

1.2 Ionization sources

Prior to analysis in the MS the analyte has to be ionized in the ion source. Most important considerations in the ionization techniques are the internal energy transferred during the ionization process and the physiochemical properties of the analyte. Some techniques are very energetic causing extensive fragmentation. Others are softer producing only molecular ions. Techniques like electron ionization (EI) and chemical ionization (CI) are only suitable for gas-phase ionization meaning their use is limited to compounds sufficiently volatile and thermally stable. As a large number of compounds do not meet these criteria ions must be extracted directly from the condensed phase to the gas phase. Two types of these direct ionization sources exist: liquid-phase ion sources and solid-state ion sources. In the first case the analyte is in a solution that forms droplets, created by nebulization, which are directed towards MS. Electrospray ionisation (ESI), thermospray and atmospheric pressure chemical ionization (APCI) are most common examples of liquid-phase ion sources. In case of solid-state ion sources the analyte is an involatile deposite in a solid or viscous fluid matrix which can be obtained by various sample preparation techniques. The analyte is desorbed and ionized by irradiating the deposit with energetic particles or photons which are then focused

towards the analyzer by an electric field. Matrix-assisted laser desorption ionization (MALDI), secondary ion MS, field desorption and plasma desorption sources all belong to this group of ion sources. [1]

There are different processes that create ions from neutral molecules in ionization sources. Most important are electron ejection, electron capture, protonation, deprotonation, adduct formation and transfer of charged species from condensed phase to gas phase. Ion production often implies gas-phase ion-molecule reactions. [1]

1.2.1 Electron ionization

EI is widely used for analysing organic compounds with MS. EI consists of a heated filament emitting electrons which are accelerated towards an anode and „collide“ with the gaseous molecules of the sample in the source. Unlike gases liquid samples with high vapour pressure and solids usually have to be heated to increase the vapour pressure. Although EI works well with gas-phase molecules extensive fragmentation takes place so molecular ions are not always observed. [1]

1.2.2 Chemical ionization

CI is a technique that produces ions with little excess energy and therefore has less fragmentation than EI and therefore stronger molecular ion signal. Ionization is achieved by producing ions through a collision of the analyte with primary ions present in the source. The sample is introduced into CI source similarly to EI. However a reagent gas with higher gas pressure is also introduced into the source. Thereafter electrons produced similarly to EI ionize preferentially the reagent gas. The resulting ions will collide mostly with other reagent gas molecules creating an ionization plasma through a series of reactions. Both positive and negative ions of the substance can be formed by chemical reactions with ions in the plasma causing proton transfer reactions, adduct formations, charge transfers, etc. Possible reagent gases are methane, isobutane and ammonia. [1]

1.2.3 Atmospheric pressure chemical ionization

APCI is a ionization technique that uses gas-phase ion-molecule reactions and therefore is similar to CI. Differences are that the reactions take place in atmospheric pressure and primary ions are produced by corona discharge. The sample is converted into a thin fog by a pneumatic nebulizer with high-speed nitrogen beam. The formed droplets are directed through a heated environment where the mobile phase and analyte are vaporized. As the

vaporized molecules then pass through a corona discharge ionization processes similar to CI take place. [1]

1.2.3 Electrospray ionization

ESI source consists of an atmospheric pressure region which includes a spray needle and auxiliary hardware, and vacuum interface which provides the means for ion transport into the MS. These two regions together make up the atmospheric pressure ionization (API) interface. [3]

Depending of whether positive or negative ion mode is used during the electrospray processes electrochemical oxidation or reduction takes place respectively. ESI source can be viewed as a special kind of electrochemical cell with the difference from traditional cells that charged droplets move between the spray needle and counter electrode. In the positive ion mode oxidation occurs at the needle and reduction at the counter electrode. Observed reactions depend on the current available. Compounds that are easily oxidized undergo electrochemical reaction and excess of positive charges is created. Therefore best results are achieved if the most easily oxidized species are removed. This involves removing trace elements and changing the solvent. [3]

As an electric potential is applied to the needle positive and negative ions in the solution are separated. In case of positive ion mode the needle has a high positive potential relative to the vacuum orifice and therefore anions are attracted towards the needle tip and cations predominate at the meniscus surface. As the positive charges repel each other the liquid surface expands forming a “Taylor cone” until the excess positive potential overcomes the surface tension and droplets are formed. The voltage needed for electrospray process to occur can be calculated as:

$$V_{ON} \approx 0.2 \cdot \sqrt{r \cdot \gamma} \cdot \ln\left(\frac{4000 \cdot d}{r}\right), \quad (1)$$

where V_{ON} (kV) is voltage needed for onset of electrospray, r (μm) is radius of the needle, γ (N/m) is surface tension of the solvent and d (mm) is distance between the needle tip and the counter electrode. [3]

Polar and conductive solvents like water would be ideal for use in ESI. Unfortunately pure water is not suitable due to its high surface tension. Applying too high potential electric discharge can occur which can suppress the electrospray ionization process and damage the ionization source. [3]

The formed droplets carrying an excess positive charge when needle is at positive potential lose solvent as it evaporates. This process can be facilitated by using a heated gas or heating the capillary. Therefore the droplets shrink until the charge repulsion overcomes the surface tension and a stream of smaller droplets is emitted. As the droplets then continue to lose solvent another stream of droplets is emitted when columbic forces overcome the surface tension. This process can be repeated several times. [3]

There are two theories that describe the ultimate fate of the droplets neither of which are universally accepted. The charge residue mechanism suggests that ions detected in ESI-MS are charged species that remain after all the solvent has evaporated from the droplet. Second theory called ion evaporation model suggests that from droplets that have reached a radius of less than about 10 nm direct emission of desolvated ions can take place. However in both models the end result is a desolvated ion. Therefore the nature of ions observed in ESI-MS depends strongly on the next step where ions are transported by the API interface into the high vacuum region. [3]

In the next region a capillary tube is used to reduce conductance of chemical species into the vacuum region. Furthermore skimmers and vacuum pumps are used to reduce pressure. Ion optics is used for confining the ion beam and improving transmission into MS. The vacuum interface also provides the possibility of breaking up solvated ions by collision-induced dissociation (CID). CID occurs when the solvated ions are accelerated in a relatively high pressure area completing the desolvation process. Therefore CID is influenced by the electrical potential used and the pressure at the interface region. If higher potentials are used ion fragmentation can take place. Therefore the optimal conditions for analysis can be different for smaller and larger molecules. [3]

Although high eluent flow rates are convenient for use with High Performance Liquid Chromatography in some applications there are benefits in using flow rates from microliter to nanoliter per minute. Nanospray (nano-ESI) uses low flow rates and a needle with small diameter. Spray is induced only by electric potential and therefore needle diameter, positioning and applied potential are critical for initiating and maintaining a stable spray. From equation (1) it can be seen that potential needed for the onset of electrospray can be reduced by reducing the needle diameter and therefore decreasing the possibility of discharge. Therefore aqueous solutions can be analyzed more easily. Another benefit of nano-ESI technique is that very small sample quantities can be analyzed. Nano-ESI applications include interfacing with capillary electrophoresis and microcapillary liquid chromatography. A further benefit of nano-ESI is the reduction of analyte suppression by salt contamination. Due to the

formation of smaller droplets than in conventional ESI concentrations of about an order of magnitude higher can be handled as there is reduced competition between salt and analyte ions for a place on the droplet surface. [3]

1.2.4 Matrix-assisted laser desorption ionization

MALDI works in two steps. First the analyte is mixed in solvent containing small organic molecules called the matrix. These molecules are chosen so that they absorb light strongly in the used laser wavelength. As the mixture is dried from all liquid solvents a "solid-solution" is formed where the analyte molecules are embedded throughout the matrix. In the second step short intensive laser pulses are used for ablation of bulk portion of this solid-solution. The rapid heating causes localized sublimation of the matrix molecules into gas phase taking with it intact analyte molecules in the expanding matrix plume. Although there are many different physical and chemical ionization pathways suggested for MALDI the processes are still not fully understood. [1]

1.5 Ambient ionization sources

The combination of high sensitivity, selectivity and speed is the major advantages of MS. The bottleneck in MS analysis is the need to transfer sample molecules from the ambient environment into the high vacuum of MS. Moreover the target molecules are often in a condensed phase and in a complex matrix. Therefore intricate and time consuming sample pre-treatment steps are needed prior to MS analysis making the whole process complicated. To address these shortcomings a new family of ionization techniques - called ambient ionization - has emerged. [4] The definition of ambient ionization is that ionization occurs externally to the MS and that little or no sample preparation is needed. Therefore ambient ionization solves the main drawbacks of MS analysis making the analysis faster, simpler and more versatile. [5]

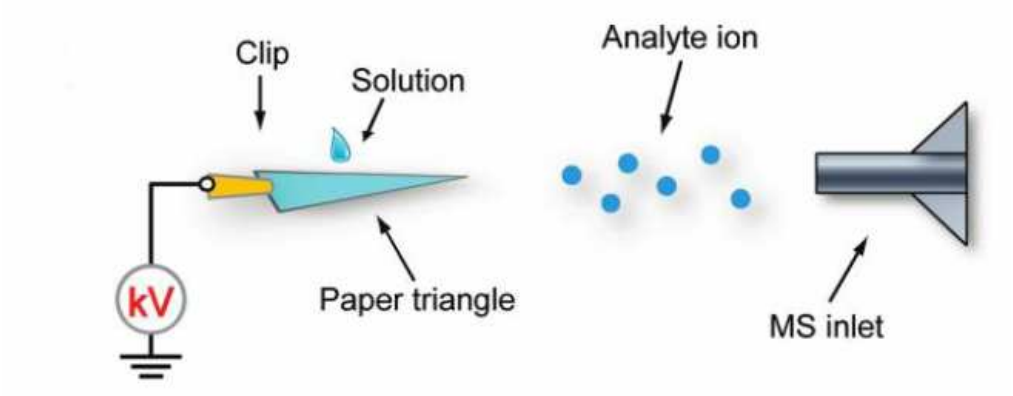
After the introduction of desorption electrospray ionization (DESI) in 2004 ambient MS techniques have evolved to include almost 30 different ionization methods. These methods use different techniques - eg spray, aerosol, laser, plasma, heating or acoustic radiation - for desorption and ionization of the sample. [5] The applications of ambient MS range from homeland security and forensic analysis, drugs and pharmaceuticals, lipids, metabolites, peptides and proteins, pathology, environmental science, fuels, drinks and beverages, crude and vegetable oils, polymers, perfumes, tissue imaging and reaction monitoring. [4]

Although with some exceptions it is also becoming clear that ambient ionization technology is not unique necessarily because of the ionization mechanisms themselves but the format in which the ion sources are designed and configured. Ambient MS techniques make use of well-established ionization principles such as ESI, CI and photoionization but in an open air direct ionization format which allows unique experiments to be performed on samples previously requiring significant sample preparation prior to MS analysis. Ambient techniques are still young and therefore fundamental studies are critically needed to understand the range of applications that can be enabled by their use. [6]

1.6 Paperspray ionization

A relative of ESI and nano-ESI by its functioning mechanism paperspray ionization (PSI) is a simple and fast ionization method. In PSI a triangular paper of approximately 5 mm in base and 10 mm in height is placed in front of the MS inlet and electrical tension of 2 – 4.5 kV is applied between the paper holder and the MS inlet. As a small amount of eluent is added to the paper the spray is induced. The analyte can be added to the paper as a solution prior to the eluent, together with the eluent or by wiping a surface of interest with the paper beforehand. Both qualitative and quantitative analysis can be performed. Many different compounds have been analyzed with PSI ranging from small organic molecules to peptides and proteins. [7] Detection limits of 20 pg have been demonstrated on the example of atenolol in bovine blood. [8]

Figure 1. Schematic illustration of PSI. [7]



Several studies of the PSI mechanism have been carried out. For example different processes by which the analyte is moving on the paper during the spray has been studied. First capillary action in the microchannels of the paper is in large part responsible for the

movement of the eluent. If excess eluent is added so that a liquid film forms on the paper the bulk eluent movement towards the spray is observed. It has also been shown that the contribution of electrophoretic flow is not significant. [9]

Also different analyte carriers – papers – have been studied. In these studies different background peaks have been observed with different papers most likely due to contaminants and additives used in the paper production. The highest signal-to-noise ratio (*S/N*) has been observed with chromatographic paper and the lowest with glass fiber with cocaine as analyte. [7]

The importance of precise positioning of the paper in front of the inlet has been studied by continuous flow of analyte solution to the paper. Signal intensity of analyte was compared in different positions. The results show that very precise positioning is not necessary. [7] However as the angle of the paper tip is increased a smaller angle of spray will be induced and therefore the precision of positioning the paper becomes more relevant. [9]

To show the versatility of PSI many different potential applications have been demonstrated. [7] PSI has shown great promise for therapeutic drug monitoring from blood to prevent under- or overdosing. Blood sample is deposited on the paper, dried thoroughly and thereafter measurements can be performed. Internal standard for measurements can be deposited to the paper prior to the analyte or added together with the eluent. The internal standard can also be added to the sample but this method is not preferred as it might not always be possible to do so. Spiked blood samples are used to produce a calibration curves. [10] Many different drugs like telmisartan, sunitinib, amitriptyline, atenolol, imatinib and sitamaquine have been analysed with PSI. [7, 10] *S/N* of approximately 4 has been achieved for analysis of atenolol in dried blood spots when 400 pg of analyte was added to the paper. Better *S/N* was achieved with 20 pg of analyte with MS/MS analysis. [7] It has been shown with different drugs that the measurement precision and accuracy are within the internationally accepted standards for bioanalytical measurements. [10] For example smaller than 5% relative standard deviation was achieved across a wide range of concentrations for analysis of imatinib in blood by spiking the blood with internal standards prior to spoting it on the paper. [7] Linear ranges of studied drugs are well within the therapeutic ranges. [7, 10]

Due to high salt concentrations urine is usually difficult to analyse with DESI and APCI. 50 pg of heroin could be identified with PSI when dried urine spots were analyzed. Studies have also demonstrated that measurements of therapeutic drugs and disease biomarkers like lipids and hormones straight from a tissue are feasible. [7]

From other applications caffeine could be identified when 10 μL of cola drink is dispensed on the paper. Another application of PSI is “swiffer” type sample collection where a surface is wiped by a paper to identify chemicals on the surface. For example 50 ng of heroin was identified from a desktop. Also a fungicide thiabendazole could be identified by wiping a lemon or an orange. [7]

Another new ionization method derived from PSI is Leaf Spray Ionization (LSI) where the paper is exchanged with leaf of a plant. When an electrical potential is applied to the leaf a spray is induced similarly to PSI. Possibility of studying endogenous compounds present in plants has been reported. Amino acids, carbohydrates, fatty acids, alkaloids, and lipids are identified with high abundance in many tested plant tissues. [11]

1.3 Mass analyzer

Once the ions are produced in the ionization source they are separated by their *m/z* in the mass analyzer. As with ionization sources there are many different analyzers but generally they can be divided into two groups. First group of mass analyzers transmit ions of different *m/z* successively along a time scale meaning only ions with known and certain *m/z* pass the analyzer at one time. For examples magnetic sector instruments and quadrupole instruments work in this method. The second type of analyzers allows simultaneous transmission of all ions. Examples here are time-of-flight, ion trap and ion fourier transform cyclotron resonance (FT ICR) mass analyzers. In current study quadrupole and FT ICR mass analyzers were used. [1]

1.3.1 Quadrupole mass analyzers

Quadrupole mass analyzers consist of four precisely placed parallel rods called poles that are spaced around a central axis. The ions are introduced along the axis of the poles. Precisely controlled voltages (radio frequency and direct current) are applied to the opposite sets of poles and therefore only a certain *m/z* can pass the analyzer at a certain time creating a „mass filter“. Other ions with too low or high *m/z* have unstable trajectories and collide the rods. As the voltages are changed complete range of ions can be passed to the detector. [2]

This is the most common and versatile of the mass analyzers with long history in application and in the use with spectral libraries. They are particularly useful for high throughput, capability of analyzing “dirty” samples and cases where background contamination can affect the quality of the results. Although quadrupole mass analyzers have

low resolution and have the mass range of about 50 – 2000 *m/z* they require minimal vacuum. Their maximum scan speed is in range of 4000 u/sec. [2]

1.3.2 Ion cyclotron resonance mass analyzer

In an FT ICR cell ions move in a circular motion due to a high magnetic field created usually by superconductors. The frequency of the circular motion can be calculated with the following equation:

$$\omega = \frac{q \cdot B}{m}, \tag{2}$$

where ω (s^{-1}) is the ion cyclotron resonance frequency, q (C) is the charge of the ion, B (T) is the magnetic field strength and m (kg) the mass of the particle. [12]

Therefore the cyclotron resonance frequency depends on the *m/z* of the ion. Different ions with different *m/z* are therefore detected by measuring the resonance frequencies. This is done by two plates in which nanovolt-level of signal is created when a patch of ions pass close by from the plates. Therefore no separate detector is necessary for FT ICR. As all of the ions in the cell are excited at the same time a complicated signal is created which is a combination of all the cyclotron frequencies of different ions. Fourier transform analysis of this signal gives all the cyclotron resonances from where the *m/z* of the ions can be calculated. As FT ICR has the capability of measuring the *m/z* very accurately the elemental composition and structure of compounds being analyzed can be deduced from the information. [12]

1.4 Detectors and computer

The ion beam that passes through the mass analyzer is detected and transformed into a signal by a detector. Generally two different types of detectors exist: first ones measure directly the charge that reaches the detector and the second group is where intensity of the signal is amplified in the detector. [1]

Photographic plates and Faraday cylinders go under the first group. In case of photographic plates ions that have the same *m/z* reach the same place. Calibration scale is used for determining locations of certain *m/z* on the plate. The darkness on the spot gives an approximate value for the intensity of the beam. In case of Faraday cylinders the ion that has reached the cylinder will give up its charge. The discharge current is amplified and measured. Although these detectors are limited by the noise of the amplifiers they are very precise. Therefore these detectors are used for example when measurements requiring highly precise isotopic ratios are needed. [1]

Electron multipliers, photomultipliers and array detectors belong into the second group. In electron multipliers the ion reaching the plate called dynode causes the emission of several secondary particles which can be electrons, negative or positive ions or neutrals depending on the charge of the primary ion. The secondary particles are then accelerated into the continuous-dynode therefore measurable current is created at the end of the tube. [1]

Similarly in array detectors a cascade of electrons is created however small parallel tunnels in a plate are used to create the snowball effect. A semiconductor substance covering each channel ensures the electron multiplication. As the plate geometry resembles that of photographic plate ions with different *m/z* can be measured at the same time. [1]

Besides controlling the parameters of the MS computers have the task of registering the data and converting it into values of masses and peak intensities or into total ionic current, temperatures, acceleration potential values, etc. The computer can calculate the possible composition of ions of a given *m/z* taking into account only the elements in the molecular formula. Another method of identifying the analyte is by comparing the spectra with the spectra in library of the computer. Furthermore data conversion and reduction can be performed on the computer. [1]

1.5 Paper

Paper is usually a thin hydrophilic material consisting mainly of cellulose fibers. [7] Cellulose is a polysaccharide composed of glucose monomers. In paper cellulose polymer chains commonly consists of approximately 1000 glucose monomers. These chains arrange next to each other and are bond by hydrogen bonding. Approximately a hundred bonded chains form an elementary fibril that in turn form a micro fibril and approximately 2000 micro fibrils form a macro fibril. These macro fibrils are bonded together by hydrogen bonding and Van der Waals forces. [13] Therefore in paper a network of cellulose is formed that offers micro channels for liquid with dissolved analyte to be transported by capillary action. The degradation of paper can be monitored by determining low molecular weight oligomers like glucose, cellobiose and higher molecular weight oligomers. [14]

Other possible components found in paper are fillers (chalk, clay, gypsum, titanium dioxide), gluing agents (gelatine, starch, paraphin, natural gluing agents, synthetic polymers), mineral and organic coloring agents, sizing agents (pigments, binding agents, synthetic polymers) and other additives (water, metal ions). [13]

1.6 Pesticides

The use of pesticides is widespread in agricultural practice with over 800 different pesticides against insects, rodents, fungi and unwanted plants. [15] Furthermore numerous postharvest treatments including dipping and treatment with a water-emulsion wax containing fungicide (mainly imazalil, thiabendazole and prochloraz) are extensively used for preventing moisture loss during storage, shipment and marketing. [16] Although most of them leave the product or degrade some trace amounts of pesticides residues can be transferred to humans *via* the food chain. As pesticides can be potentially harmful for human health regulations on maximum residue limits for pesticide residues in foods have been established. These legislative limits have become stricter than ever due to the concerns of food safety and the demands of trade barriers, driving the demand for more sensitive and reliable analysis methods for pesticide residues. Most analytical methods used today are based on gas and liquid chromatography. Although the analytical instruments are developing rapidly their detector noise, detection limits, and final quantification are usually influenced by the interferences from food matrices. [15]

1.7 Explosives

Detection of explosives in real-time with high sensitivity and selectivity has been an area of research for decades. Major progress has been made by improving sensitivity however selectivity is still a problem. Most common chemical analysis technology for detection of explosives is ion mobility spectrometry (IMS). Although it is sensitive and robust IMS has limited chemical specifity due to inherent low separation of the ion mobility peaks. This can result in false positive signals. Therefore new approaches are needed to detect explosives with high level of confidence and a sensitivity that equals or surpasses that of an IMS. [17]

2. Experimental

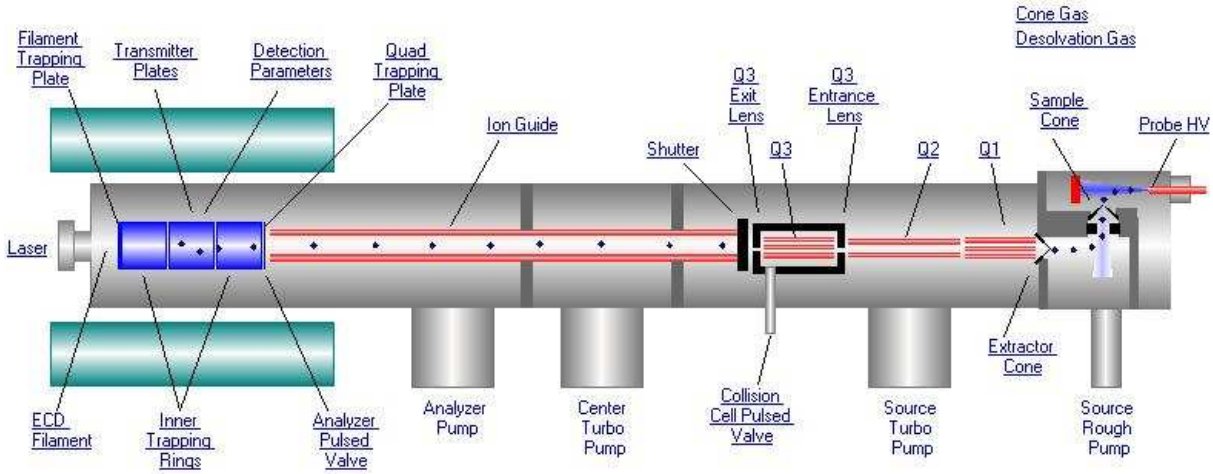
2.1 Apparatus

A scalpel and a metal template were used to cut the paper in an isosceles triangle with height of 10 mm and base of 5 mm. The paper triangle was placed into the holder which was fit for nano-ESI housing (Pictures in Appendix 1). A camera is mounted onto the API source so the spray process can be observed.

Varian triple quadrupole system (3Q) (model number: MS0906A002, USA) containing different changeable API sources, vacuum interface, two quadrupole mass analyzers, a quadrupole for fragmentation and an electron multiplier detector was used. The MS/MS system is also connected to FT ICR cell by a hexapole ion guide. The cell is moved into a container (model number: JT/110/AS, Oxford, England) with superconductors that produce the necessary magnetic field for FT ICR analysis (Figure 2). The FT ICR spectra were collected and interpreted with Omega-910-ms program.

Leica DM750 microscope (Meinz, Germany) was used for picturing the papers. Other lab equipment used were Eppendorf Research automatic pipettes (Hamburg, Germany), Sartorius LA230S analytical balance (Goettingen, Germany) and Retsch GM200 (Haan, Germany) blender.

Figure 2. Scheme of ESI-MS/MS-FT ICR.



2.2 Used materials and chemicals

Filtrak 88, 91 and 390 filter papers made in German Democratic Republic and Whatman filter paper were used. Also a polytetrafluoroethylene (PTFE) filter paper, an approximately 2mm thick analytical paper and an ordinary printing paper were used.

MilliQ deionized water with electrical resistivity of approximately 18 MΩ×cm was used to prepare solvents and eluents. Also acetone (Lach-Ner, Czech Republic), acetonitrile (JT Baker, USA) and isopropanol (SigmaAldrich, USA) were used as solvents in different experiments. Formic acid (Fluke, Germany) was added to spray eluent.

From analytes pesticides thiabendazole, aldicarb, imazalil, methamyl, methiocarb and ditalymfos were acquired from Dr. Ehrenstorfer GmbH, Germany. Also five amines – triphenylamine (Reakhim, Russia), diethylamine (Fisher, USA), triethylamine (Aldrich, USA), diphenylamine (Fluka, Germany) and aniline (Sigma Aldrich, USA) – were used as analytes in this work.

Other chemicals used were 5-nitrobenzimidazole (Aldrich, USA) and 2-methylimidazole (Aldrich, USA). Furthermore 2-aminobenzimidazole and 8-amino-2-methylquinoline synthesised in Chair of Analytical Chemistry were used.

Phosphazeses used as calibrants for measurements with FT ICR positive ion mode were synthesised in Chair of Analytical Chemistry. For FT ICR calibration in negative ion mode tetrabutylammonium perchlorate (Fluka, Germany), sodium tetraphenylborate (Fluka, Germany) and pentakis(trifluoromethyl)aniline synthesised in Chair of Analytical Chemistry were used.

Small amounts of hexogen, pentrit, trotyl and tetryl with unkown purity were acquired from Estonian Forensics Science Institute.

Salts added to solvents for identification of explosives in negative ion mode were ammonium nitrate (Reakhim, Russia) and ammonium chloride (Reakhim, Russia).

2.3 PSI measurement parameters

Approximate distance between the paper tip and the MS inlet was 5 mm.

Emitter voltage of the MS/MS system was set to 3200 V and shield voltage to 300 V when PSI experiments were conducted. The shield is a part of ion optics in front of the MS inlet that helps transmittance of ions to the API capillary. Drying gas pressure was set to 8 psi and temperature 150 °C. Capillary voltage and fragmentation collision voltage were optimized for each compound with nano-ESI for MS/MS measurements. Gas pressure of 1.5 mTorr was used for fragmentation. The electron multiplier detector voltage was set to 1300 V.

For measurements in negative ion mode voltages with same values but with reversed polarities were used.

Different voltages and times of events were changed in FT ICR measurement in order to achieve sufficient signal intensity and mass range. In case of FT ICR fragmentation the intensity of laser was changed between 40 – 50 % to achieve optimal intensities of fragment ions.

Eluent used for PSI composed of 80% of acetonitrile and 20% of water containing 0.1% formic acid (by volume). The same eluent was used for preparing standard solutions. The standard solutions were kept in deep freeze for conservation.

The paper holder, shield and MS inlet were cleaned with solvent containing isopropanole and MilliQ water in a 50%/50% mixture by volume. Also to verify the cleaning a blank measurement was made regularly.

3. Results and conclusions

3.1 Triangle cutting methods

Two different methods of cutting triangles from paper for PSI measurement were tested. In both methods a CD was placed under the paper to act as a smooth surface for cutting. In the first method the scalpel was pushed on the paper moving from the back of the triangle towards the tip. In the second method the blade of the scalpel was dragged on the paper from the tip to the back. Both methods can yield sharp or split triangle tips. A picture of a triangle with a sharp tip can be seen in Figure 3. Spray was also formed when using a paper with split tip (Figure 4). Therefore both methods can be used for cutting PSI papers. However if the cutting resulted in a tip with many fibres the spray was not formed. However when a triangle with a blunt tip was used the spray still formed. Pictures of different papers and of formation of the spray are in Appendix 2.

The paper carrying the sample was placed directly in front of the MS inlet as the nano-ESI housing allowed precise positioning of the paper. The distance between the inlet and the paper tip was approximately 5 mm. After the spray emitter voltage was switched on and the mass spectrum recording was started 20 μL of eluent was dispensed on the paper with an automatic pipette. Spray formed immediately after addition of the eluent. The eluent was added as the last step so the whole spectrum could be recorded and eluent would not vaporize before spray formation.

Figure 3. Microscope picture of Whatman filterpaper.

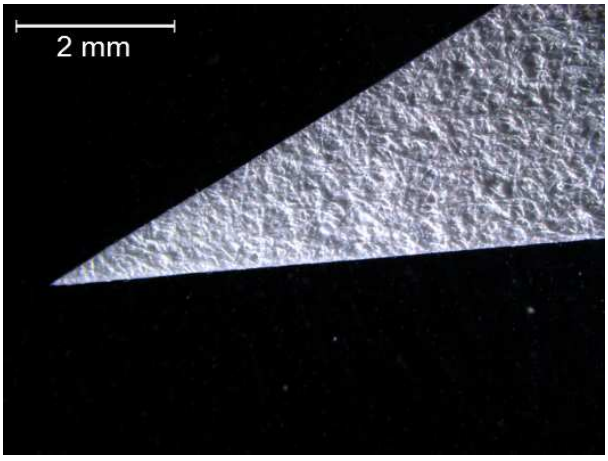
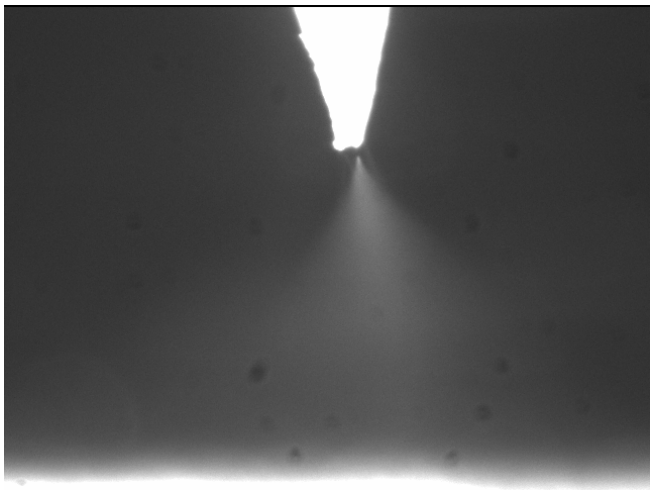


Figure 4. Spray formed using a two tip triangle.



3.2 Collecting spray ion current chronograms and spectra

The signal appeared in the collected chronogram as soon as eluent was added to the triangle when measurements were made in same steps as explained in Paragraph 4.1. However disappearance of visible spray occurred significantly earlier than the fall of MS signal intensity to noise level. This suggests that after the primary spray smaller droplets, too small to be visible, were formed or some other form of ionization takes place.

Figure 5. Total ion chronogram and ion chronograms of calibrants.

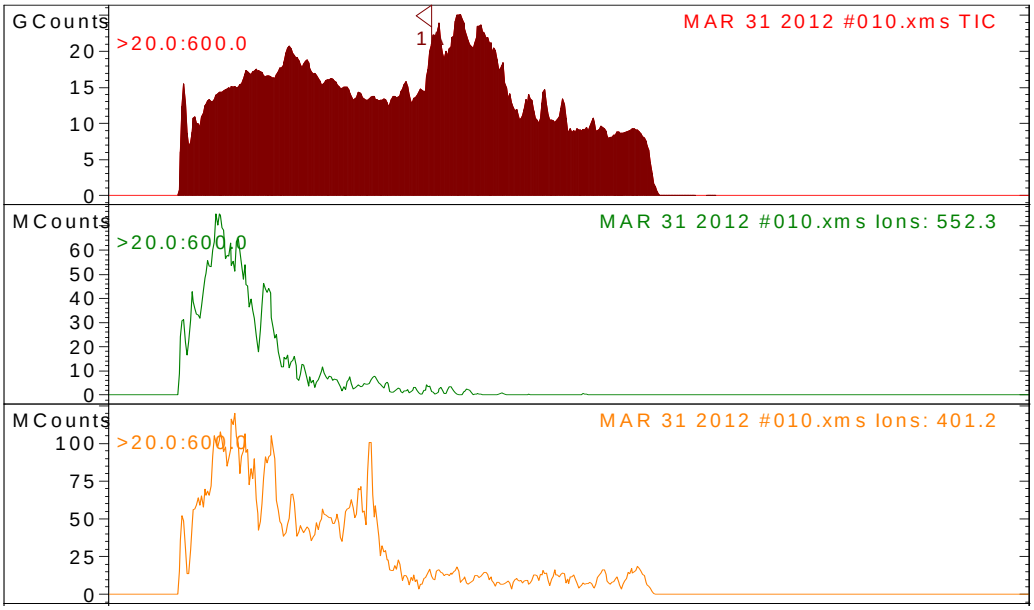


Figure 6. Mass spectrum of visible spray. Spectrum is measured with 3Q.

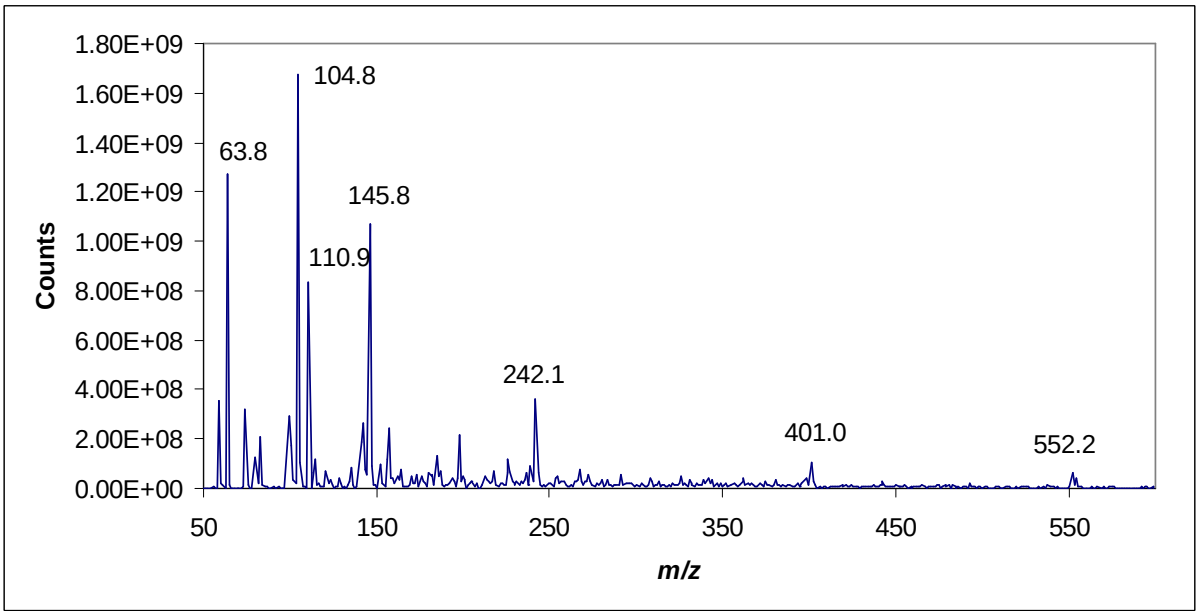


Figure 7. Mass spectrum of not visible spray. Spectrum is measured with 3Q.

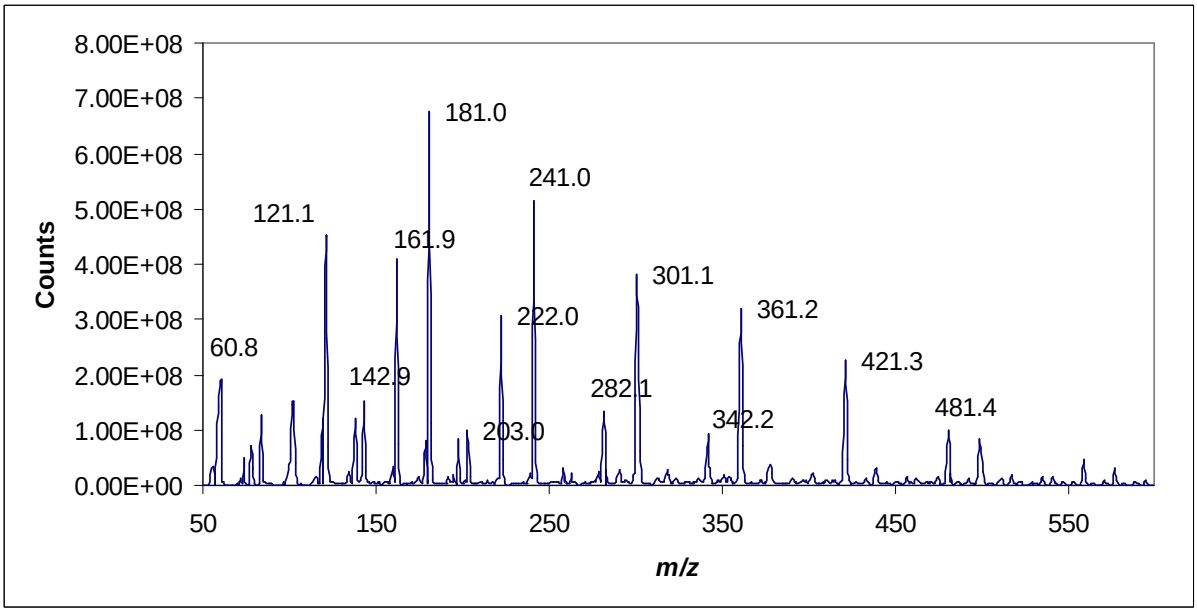
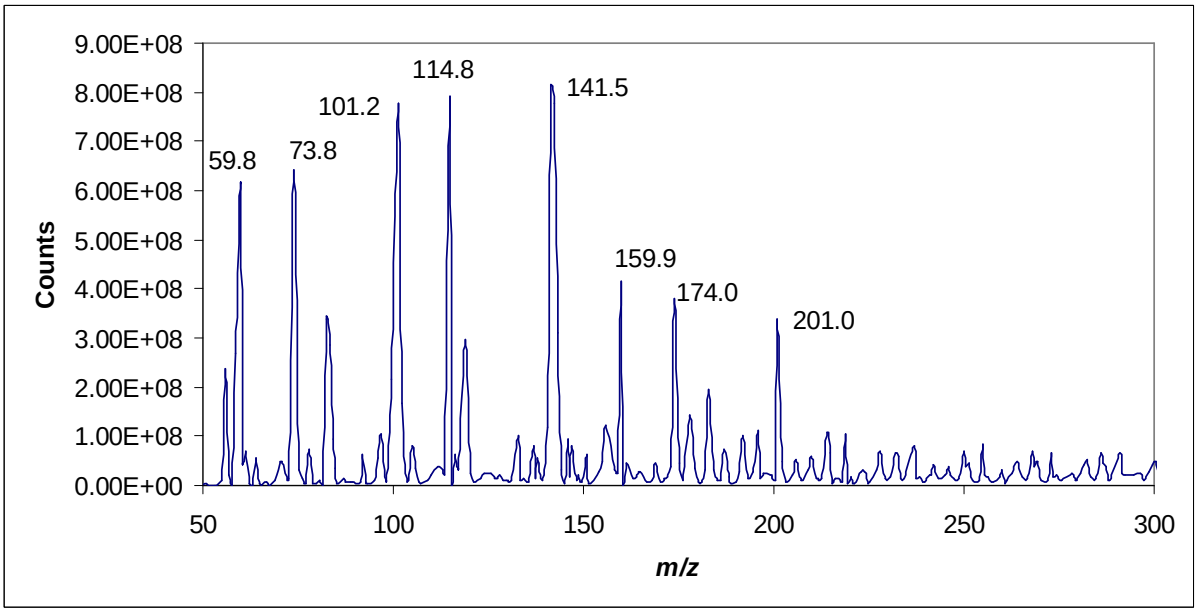


Figure 8. Mass spectrum of printing paper. Spectrum is measured with 3Q.



In order to study PSI process eluent with FT ICR calibrants was used with triangle cut from Whatman filter paper. The exact masses of calibrants can be found in Pharagraph 4.3. In Figure 5 the topmost chronogram is of the whole spray and the following chronograms correspond to calibrants. It can be seen that the intensities of calibrants decrease before the overall spray intensity decreases. If spectra are taken from the different times of measurement (Figure 6 and 7) it can be seen that mass spectra differ from each other. Figure 7 suggests that some polymer degradation products are seen in the secondary spray due to the constant mass

difference between most abundant ions in the spectrum. Furthermore when a printing paper with the same eluent was used for PSI measurements no visible spray was created and calibrants were not detected, however a mass spectrum could be recorded (Figure 8). This suggests that not all analytes can be identified from all papers. However some information might be obtained about the paper used for PSI from the secondary spray.

3.3 FT ICR spectra of different papers

FT ICR spectra of Filtrak 88, 91 and 390 filter papers and Whatman filter paper were measured in positive ion mode. Three different ranges (*m/z* 40-200, 150-400 and 100-600) were measured for every paper. Eluent containing phosphazenes with 111.09167, 242.28423, 401.207645, 552.28947 *m/z* were used as calibrants for the mass range calibration. To eliminate signals produced by contamination from handling the paper spectra was taken from a clean Whatman filter paper which was handled only with gloves. Whatman filter paper was also measured in negative ion mode. In negative ion mode compounds that produce ions with 98.94906, 319.16635 and 431.98750 *m/z* were used as calibrants.

The spectra were interpreted according to the composition of the following elements: C, H, N, O, P, S, Cl, Na, Ca. Maximum allowable number of Na and Ca was set to one. The Double Bond Equivalents was allowed between -2 to 10. The target mass was allowed to have error of ±5 ppm. For the candidate compounds theoretical spectra were compared to the measured spectra. The chemical formula, most accurately matching both measured mass and isotope signals, was taken as the correct formula. If these criteria could not be met no formula was assigned for the measured ion.

Differences in mass spectra of the papers indicate that different compounds have been used in the production of the papers. However ions with the same *m/z* can be seen on all of the spectra, for example: 55.5458, 60.5714, 121.1427, 183.1622, 185.11430 and 200.6043. Compounds produced by cellulose degradation could not be found. Most probably no degradation had taken place yet.

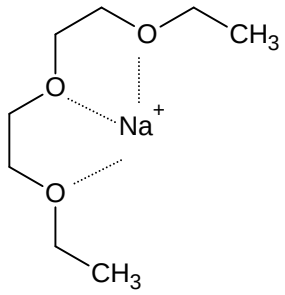
While comparing the spectra recorded from Whatman filter paper handled without gloves with one handled only with gloves spectra with significantly more peaks was observed from the former. Compounds with *m/z*'s of 74.0964, 265.2274, 297.2274, 398.2473 and 458.7918 could be found on spectra measured from usually handled Whatman filter paper however could not be found on spectra measured from the clean paper. Therefore it can be concluded that significant contamination of the paper occurs during handling.

For interpreting mass spectra collected in negative ion mode the same parameters were used however Na and Ca were removed as possible elements. Comparing to positive ion mode fewer ions could be detected. Ionization in the negative ion mode might be enhanced by using a more basic buffer.

Ion with 185.1143 *m/z* from Whatman filter paper was fragmented to determine its structure. The suggested formula of $C_8H_{18}O_3Na^+$ indicates that it can be a similar compound to diglycol methyl ether that is used to dissolve cellulose with diglyme-HCl method. Therefore the compound with 185.1143 *m/z* could be diglycol ethyl ether (Figure 9). Also a fragment with m/z 157.0835 was identified as $C_6H_{14}O_3Na^+$.

As an example FT ICR spectra of Whatman filterpaper in positive ion mode, the lowest range in negative ion mode and of the clean paper are presented in Appendix 8. Also the FT ICR spectrum of fragmentation is presented in Appendix 8.

Figure 9. Sodium aduct of diglycol ethyl ether observed in FT ICR spectrum.



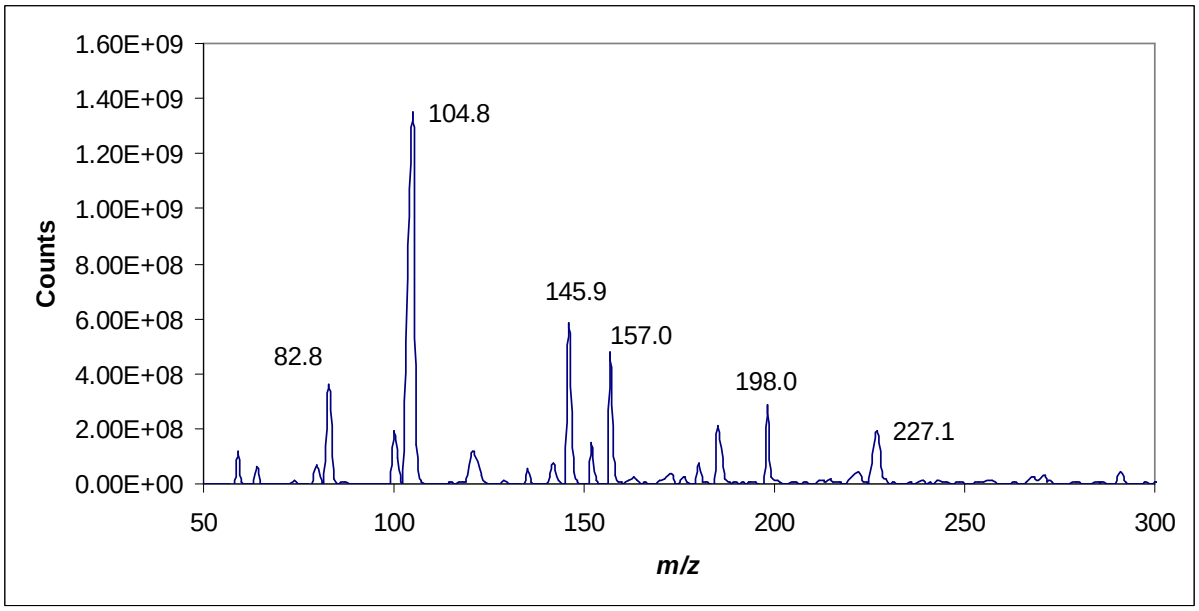
3.4 Choosing the paper for measurements

Same filter papers used in measurements with FT ICR were tested for applicability in PSI measurements with samples and analytes. Also a PTFE filter and approximately 2 mm thick analytical paper were tested. The spray and resulting signals in positive ion mode MS were compared by dispensing 20 µL of eluent on the paper. No spray was formed while using PTFE filter and the thick analytical paper. The spectrum recorded from Whatman filter paper can be found in Figure 10 and the spectra of other papers can be found in Appendix 3.

It can be concluded that although some papers were not fit for use as no spray was formed most papers were fairly similar to each other. Lack of spray in some of the papers can be explained by different structure of the paper that restricts the movement of eluent by capillary action in the paper. Moreover in case of PTFE the weight of the added eluent was too great and the paper tip dropped away from the MS inlet. The Whatman filter paper was chosen to be the best for use in PSI due to stable spray that results in stable and therefore

stronger analyte signal. Moreover the recorded signal was low resulting in higher *S/N* when analytes were to be used.

Figure 10. 3Q spectrum on Whatman filter paper.



3.5 Identifing different compounds with PSI

Several different amines and pesticides were detected with PSI. Solutions of triphenyl amine, diethylamine, triethylamine, diphenylamine and aniline with approximate concentration of 3 ppm were prepared. Respective compounds were identified by dispensing 20 µL of solution on the paper as eluent for PSI. Same experiment with solution concentration of 5 ppm was conducted to identify pesticides thiabendazole, aldicarb, imazalil, methamyl and methiocarb. The observed full-scan mass spectra of amines and pesticides are shown in Appendix 4. The MS^2 spectra are recorded by choosing a specific *m/z* with the first quadrupole, fragmentation is carried out in the second quandrupole and the whole mass spectrum is recorded with the third quadrupole.

In order to increase sensitivity and selectivity for two imazalil and thiabendazole capillary voltage and collision energies were optimized for MS/MS measurements using nano-ESI and equipment built-in optimization tools. The respective parameters were found to be 68 V and -18 V for imazalil fragment 159 *m/z* (MS^2 spectrum shown in Figure 11). For thiabendazole the respective parameters for fragment with 175 *m/z* were 76 V and -22 V (MS^2 spectrum shown in Figure 12).

Figure 11. MS^2 spectrum on imazalil fragmentation (297 *m/z*).

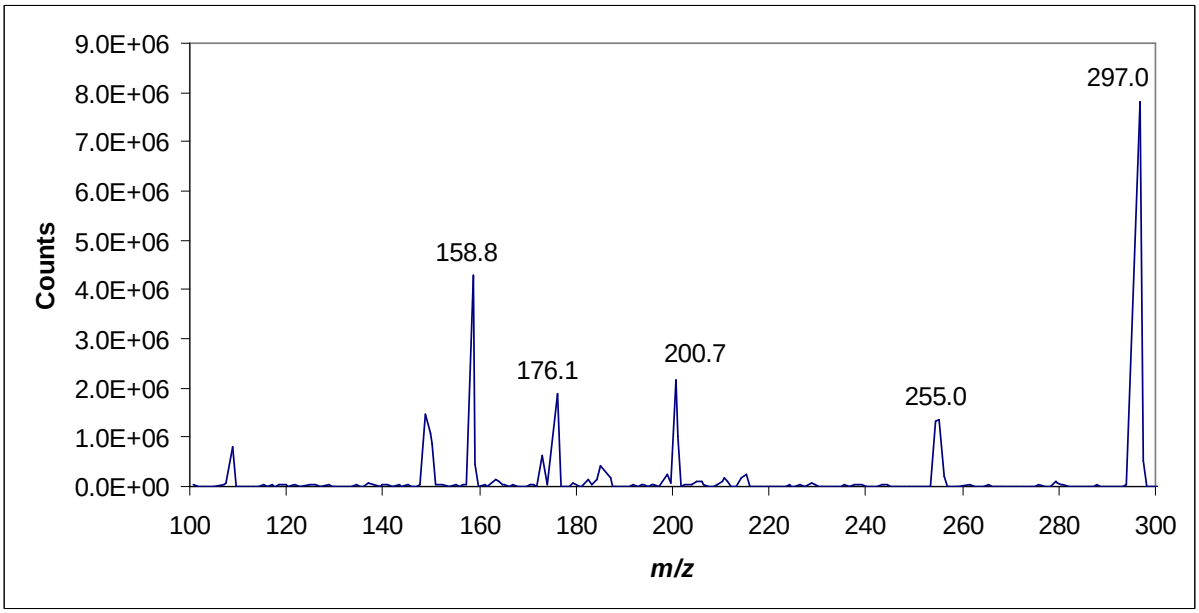
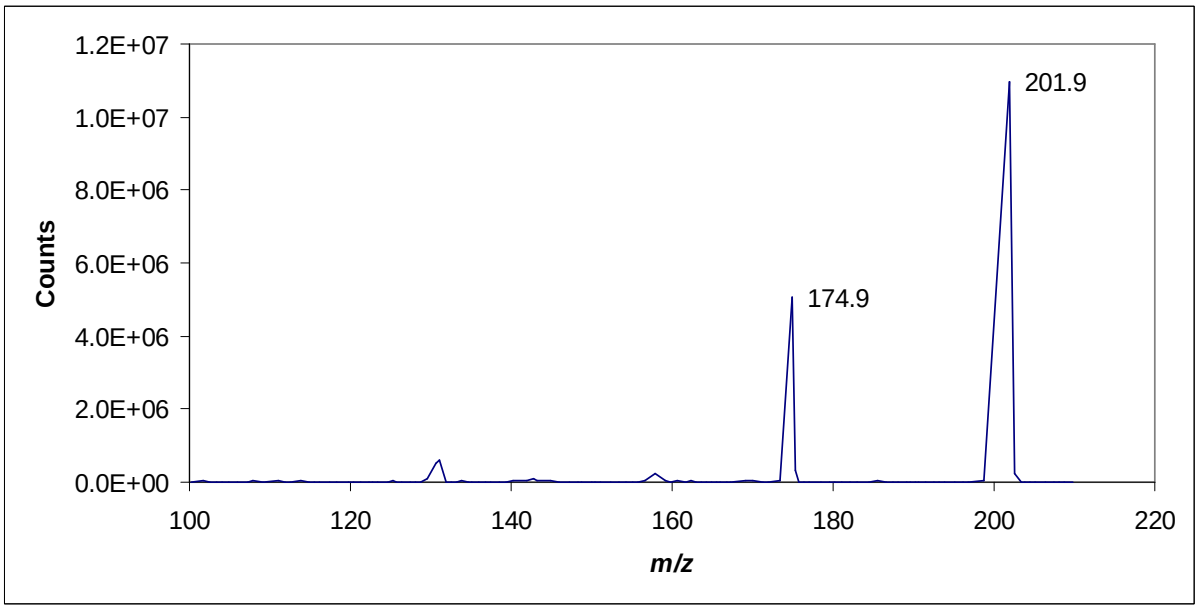


Figure 12. MS^2 spectrum on thiabendazole fragmentation (202 *m/z*).



3.7 Repeatability and internal standards

In order to perform quantitative analyses good accuracy as well as repeatability has to be achievable. Repeatability was studied for two of the pesticides – imazalil and thiabendazole – in MS/MS mode. 10 μL of solution containing approximately 6 ppm of imazalil and 1.75 ppm of thiabendazole was added to the paper triangle. The paper was dried

and the holder with the paper was placed into the API housing. Thereafter the eluent was added to initiate the spray. After the measurement the holder, shield and MS inlet were cleaned to eliminate possible cross contamination. Six replicate measurements were carried out. The sum of tandem MS signal intensity over the whole spray was taken as signal intensity of the compound. Therefore the signals of measurements are given in units of counts times minutes (Counts×min). The relative intensities were calculated by dividing the imazalil and thiabendazole intensities. From these results standard deviation (stdev) and relative standard deviation (RSD) were calculated. The results are presented in Table 1. It is seen from this data that individual repeatabilities of the analytes are very poor – up to 51% - but repeatability of relative intensities is acceptable – 9%.

It can be concluded from this data that if optimized MS/MS parameters and relative intensities are used relative standard deviation of less than 10% can be achieved. As both imazalil and thiabendazole can be present in successive measurements other internal standard must be found. In most previous studies isotope labelled internal standards have been used for quantification. However for imazalil and thiabendazole isotope labelled compounds are not available and therefore other compounds must be used for internal standard.

Ditalymfos, 5-nitrobenzimidazole, aminobenzimidazole, 2-methylimidazole, 8-amino-2-methylquinoline (AMQ) were tested for use as internal standard as these compounds are structurally similar to imazalil and thiabendazole or have been previously used in the literature. Also diphenylamine and triethylamine were tested. Ionization efficiency and fragmentation intensity of the internal standard candidates should not be too low to give measurable signal with PSI. Also carryover between measurements should be minimal.

For ditalymfos, 5-nitrobenzimidazole, 2-methylimidazole, diphenylamine ionization efficiency was not high enough. For aminobenzimidazole and triethylamine sufficient fragmentation was not observed and for AMQ carryover effect between the measurements was observed. When a measurement was made with 20 μL of eluent containing 1 ppm of AMQ the intensity of the signal was 1.758×10^{7} Counts×min. After washing the equipment a blank measurement gives signal intensity of 3.666×10^{6} Counts×min. Therefore approximately 20% of AMQ signal is measured even after cleaning the equipment. This may be due to the compound adsorbing strongly on the MS inlet and on the capillary between high vacuum and the API interface.

As none of the compounds tested were fit for use further study must be done to find a suitable internal standard for these compounds. However this study showed that the equipment is prone to contamination and therefore to avoid carryover equipment has to be

cleaned between measurements. Moreover blank measurements should be done after every measurement to verify that no contamination is present.

Table 1. Repeatability results. Peak areas are given in Counts×min.

Measurement number	Thiabendazole	Imazalil	Relative intensities
1	3.65×10^6	5.49×10^6	0.664
2	7.53×10^6	1.15×10^7	0.653
3	4.46×10^6	6.11×10^6	0.730
4	1.03×10^6	1.52×10^6	0.674
5	4.76×10^6	5.83×10^6	0.816
6	5.69×10^6	7.30×10^6	0.779
Average	4.52×10^6	6.30×10^6	0.719
stdev	2.17×10^6	3.23×10^6	0.067
RSD%	47.93%	51.26%	9.35%

3.8 Measuring pesticides directly form orange peels

In order to carry out quantitative analyses analytes form the sample have to be applied to the paper. This is problematic for samples, which are not liquids and can not be pipette on the paper. This is also the case for pesticide measurements in fruits and vegetables. Therefore different methods for measurement of imazalil and thiabendazole directly from the surface of the orange with PSI were tested. Optimized MS/MS parameters were used for imazalil and thiabendazole measurements. Oranges from local supermarket containing both imazalil and thiabendazole were used thought the experiments. It is known that these pesticides are used post-harvest and are therefore mostly on the surface of the fruits [16].

First a triangular shape of orange peel was cut and put into the PSI paper holder to test if spray could be formed directly from the peel (similar to LSI). As spray did not form it was concluded that the liquids in orange peel can not move *via* capillary action and therefore other methods should be used to analyse pesticides from fruits.

Next an arrangement where a piece of the orange peel together with the paper was placed into the holder was tested. Therefore a triangular piece of orange peel was put into the holder together with the paper and the paper was wetted with eluent. In this arrangement a spray was formed. However pesticides could not be identified as only a small part of the orange peel was in contact with the paper.

In order to increase the contact between the paper and the peel a small piece of orange peel was placed on the paper. The eluent was added so that the pesticides could be washed off

from the peel to the paper. The spray was induced and pesticides could be identified. However the signal intensities were low. When the small piece of orange peel was placed under the paper in the holder then the signals of pesticides decreased substantially.

In the next method 1.2943 g and 1.3872 g of orange peel was cut into smaller sections which were put into a centrifuge tube. 1.5 mL of 80%/20% and 60%/40% of acetonitrile/0.1% formic acid were added as solvent respectively and the mixtures were stirred with a vortex. Then 20 μL of the solvent used for extraction was then dispensed on the paper as the eluent for PSI. Pesticides could not be identified with neither of the solvents. However if 20 μL of eluent was dispensed on the paper, dried and the paper used for PSI then pesticides could be seen with low signal intensities. It was concluded that the concentration of pesticides in the eluent is too low for quantitative analysis.

Lastly a 2×2 cm paper square was cut from the paper and wetted with the eluent and the surface of the orange was wiped with the paper. When a triangle is cut from the dried square and used for PSI measurements pesticides can be identified. The paper triangle is not used for wiping due to the danger that the tip of the triangle gets damaged while wiping and the spray would not form. A blank paper was measured prior to the wiped paper. The blank gives signal intensity of 3.152×10^5 Counts×min for imazalil and 2.900×10^5 Counts×min for thiabendazole. For the wiped paper signal intensities of 1.009×10^8 Counts×min for imazalil and 1.927×10^7 Counts×min for thiabendazole. Therefore it can be said that imazalil and thiabendazole were identified from the orange with the wiping method.

3.9 Identifying pesticides from different agricultural products

The wiping method was applied to measurements of several commercially available fruits, namely orange, grapefruit, lemon, lime, mandarin, tomato, apple, pear, strawberry and sweet pepper (Appendix 5).

For citrus fruits (orange, grapefruit, lemon, lime and mandarin) candidate pesticides attempted to be identified with PSI were found on more than 2% of samples by Zhang *et al*. in [15]. Therefore identification of 16 different pesticides was attempted. For tomatoes, apples, pears and strawberries candidate pesticides were taken from Pesticide Action Network UK [18]. Identification of all cited pesticides for each sample was attempted. For sweet pepper candidate pesticides were taken from paper by Looser *et al.* [19] that were found on at least 10% of samples. Therefore identification of 19 different pesticides was attempted for sweet pepper. The mass spectra were inspected for the H^+, Na^+ and K^+ adducts of the pesticides

Imazalil and thiabendazole were identified on orange, lemon and grapefruit. Moreover only imazalil could be identified on lime. The presence of imazalil and thiabendazole for these fruits was confirmed with MS/MS measurement (Appendix 6). The wiping method is sensitive only to the pesticides directly on the surface of the fruits or vegetables. Therefore the reasons why other pesticides could not be identified, even though they might have been present, is that the pesticides might not have been on the surface of the product.

3.10 Quantifying pesticides from orange by wiping method

As two of the pesticides could be confirmed on the surface with MS/MS method it becomes of interest if quantitative or half-quantitative measurements could be carried out with wiping method. The previously optimized MS/MS parameters were used for imazalil and thiabendazole measurements. Unfortunately all of the following measurements suffer from lack of internal standard (Paragraph 4.7) therefore high variability from measurement to measurement was observed.

Table 2. Calculated partition coefficienciesbetween phase 1 and 2.

		log(P)	
Phase 1	**Phase 2**	**Imazilil**	**Thiabendazole**
Hexane	Acetone	2.25	2.99
	Acetonitrile	1.47	2.32
	0.1% HCOOH	-1.79	-0.68
	Acetonitrile + 0.1% HCOOH 80:20	1.77	2.31
	Acetonitrile + 0.1% HCOOH 50:50	1.01	1.64
	Acetonitrile + 0.1% HCOOH 20:80	-0.49	0.41
	Acetone + 0.1% HCOOH 80:20	2.33	2.72
	Acetone + 0.1% HCOOH 50:50	1.29	1.76
	Acetone + 0.1% HCOOH 20:80	-0.33	0.43

To test the efficiency of wiping the pesticides from an orange to a paper two different solvents for wetting the paper were used. Solvents for wetting the paper were chosen by calculating the partition coefficient of imazalil and thiabendazole between the solvent and a hexane phase representing the wax on the surface of the orange (Table 2). The coefficients were calculated using COSMO program by Karl Kaupmees. Based on this data 80% acetone with 20% of 0.1% formic acid and 80% acetonitrile with 20% of 0.1% formic acid were chosen for wiping solvent. An orange was cut in half and each half was wiped 5 times with one of the solvents. PSI conditions were kept constant.

The first paper square of both solvents was measured three times to determine weather a difference in wiping efficiency exists between the solvents. The results are in Table 3. It can be concluded that the solvent containing acetonitrile has at least the same wiping efficiency as with the solvent containing acetone. It was observed that significantly more visible matter was on the paper used for wiping with acetone based solvent. Therefore it can be assumed that co-extracted material suppresses the ionization of analytes more for the solvent containing acetone and therefore lower signals were observed.

Table 3. Wiping results of different solvents. Peak areas are given in Counts×min.

	ACN + 0.1% HCOOH 80:20		**Acetone + 0.1% HCOOH 80:20**	
	Imazalil	**Thiabendazole**	**Imazalil**	**Thiabendazole**
1	7.29×10^{7}	1.17×10^{8}	1.48×10^{6}	2.96×10^{6}
2	8.26×10^{6}	7.49×10^{6}	7.99×10^{5}	2.04×10^{6}
3	7.53×10^{6}	1.09×10^{7}	1.93×10^{6}	2.67×10^{6}
Average	2.96×10^{7}	4.53×10^{7}	1.40×10^{6}	2.55×10^{6}

Rest of the five paper squares used for wiping with solvent containing acetonitrile were measured only once. The results are presented in Table 4. Result of the first paper square is averaged from the three measurements presented in Table 3. As it can be seen the intensities of the signals do not have a decreasing trend. It can be concluded that significantly more than 5 wipings would be needed for measuring all of the pesticide on the peel. This would make the method too time-consuming for quantitative analyses. Therefore the wiping method could not be used for quantification of pesticides from the surface of the orange in the scope of this study.

Table 4. Wiping results of different solvents. Peak areas are given in Counts×min.

Wipings	**Imazalil**	**Thiabendazole**
1st	2.96×10^{7}	4.53×10^{7}
2nd	2.16×10^{6}	1.79×10^{6}
3rd	5.32×10^{6}	6.56×10^{6}
4th	3.94×10^{7}	3.69×10^{7}
5th	2.06×10^{7}	1.99×10^{7}

3.11 Identifying explosives with PSI

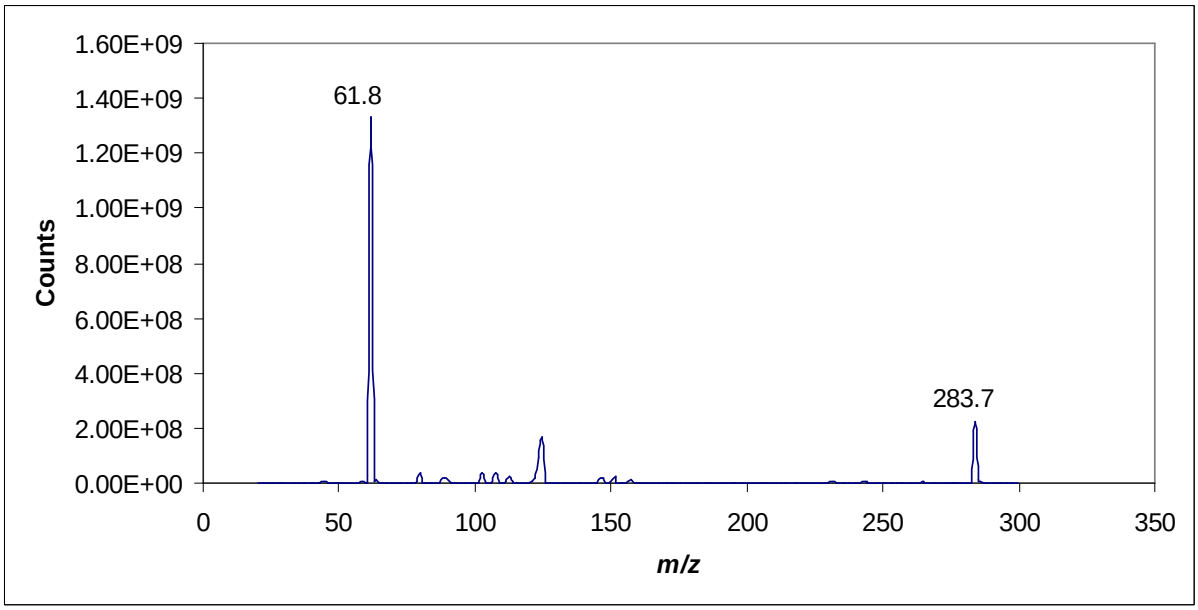
Possible use of PSI for detecting traces of explosives was studied. Identification of penthrite (PENT), trotyl (TNT), hexogen (RDX) and tetryl were tested. As indicated in by Ma *et al.* [20] and Toss *et al.* [21] RDX and PENT ionize by forming an adduct with NO_3^- and

Cl^-. However as indicated by Ma *et al*. [20] TNT ionization occurs by losing NO^- giving an ion with *m/z* of 197. Tetryl similarly to RDX and PENT has $-NO_2$ functional groups in the structure and was therefore expected to ionize by forming an adduct with NO_3^- or Cl^-. Solution of 20% 0.1 mM ammonium nitrate in MilliQ water and 80% acetonitrile was used as solvent and eluent. Similar solution where ammonium nitrate was replaced with ammonium chloride was also tested for solvent and eluent. Approximately 1000 ppm stock solutions of each explosive and working solutions of approximately 1 ppm were prepared. The measurements were made in negative ion mode. Nano-ESI was used to identify and optimize capillary voltage and fragmentation energy for each compound.

Measurements with nano-ESI were performed to optimize the measurement parameters. With the solvent containing ammonium nitrate the analytes formed adducts: [PENT$-NO_3^-$] with *m/z* of 378, [RDX$-NO_3^-$] with *m/z* of 284 (Figure 13) and [tetryl$-NO_3^-$] with *m/z* of 349. However TNT ionization did not occur. All identified adducts with corresponding masses could be identified with nano-ESI (Appendix 7). Due to fragmentation an ion with mass of 61.8 was detected for all three compounds. This indicates that the compounds loses the NO_3^- and as the compound then loses its charge further dissociation could not be determined. The optimized capillary voltage and fragmentation energy for PENT, RDX and tetryl were respectively: 30 V and 8.5 V, 30 V and 8.5 V, 30 V and 8.5 V.

Also measurements with nano-ESI were performed to optimize the measurement parameters when using solvent containing ammonium chloride. The following adducts were formed: [PENT$-Cl^-$] with *m/z* of 316, [RDX$-Cl^-$] with *m/z* of 257 and [tetryl$-Cl^-$] with *m/z* of 322. All three were identified with nano-ESI (Appendix 7). However only tetryl gave product ions in fragmentation with masses of 245.6, 198.6, 256.7, 209.6, and 168.7. The respective optimized collision energies of these product ions were 5, 6, 5, 6 and 13 V respectively. The optimized capillary voltage of PENT, RDX and tetryl were -30, -48 and -32 V respectively.

Figure 13. [RDX–NO_3^-] MS^2 spectrum with nano-ESI. Parent ion is 284 *m/z*.



For PSI measurements the previously found optimized parameters were used. When 20 µL of solution with concentration of 1 ppm of analyte were used as eluent of PSI none of the compound adducts could be identified. Also relatively intensive noise was noted (Figure 14). When MS/MS measurements were performed with eluent containing ammonium nitrate the NO_3^- products could be detected. This indicates formation of eluent molecule clusters that are fragmented and NO_3^- is produced. To break up the clusters temperature and pressure of the drying gas was raised to 300 °C and 18 psi respectively. This however further increased the *S/N* of the measurements. Also it was tested if rising the capillary voltage would decrease the noise. However this did not produce needed results. Furthermore increasing the fragmentation energy by increasing the collision voltage to 37 V was tested. Although a decrease in nois intensity was noted it was not suficient.

As an alternative explanation the malfunction of first quadrupole of the MS was suspected. However in order to confirm this the measurements have to be run again.

Under the conditions of these experiments only very high concentrations could be detected form the paper. When a solution with PENT concentration of 10 ppm in the eluent containing ammonium nitrate the compound could be identified in the spectrum (Figure 15).

Figure 14. PSI spectrum of eluent containing NO_3^-. Spectrum is measured with 3Q.

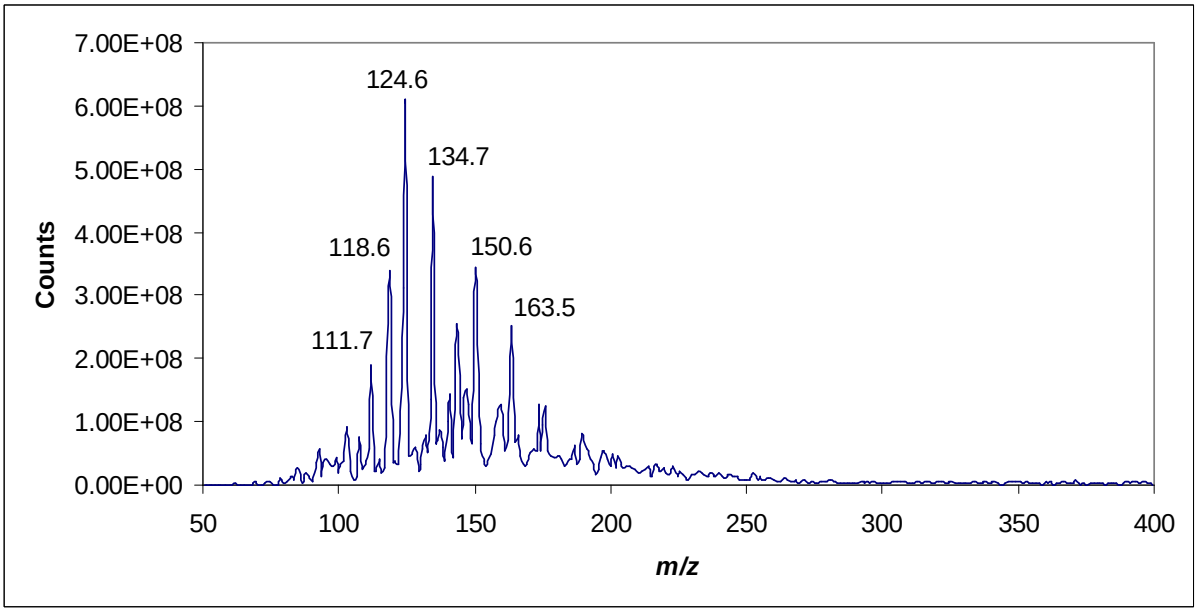
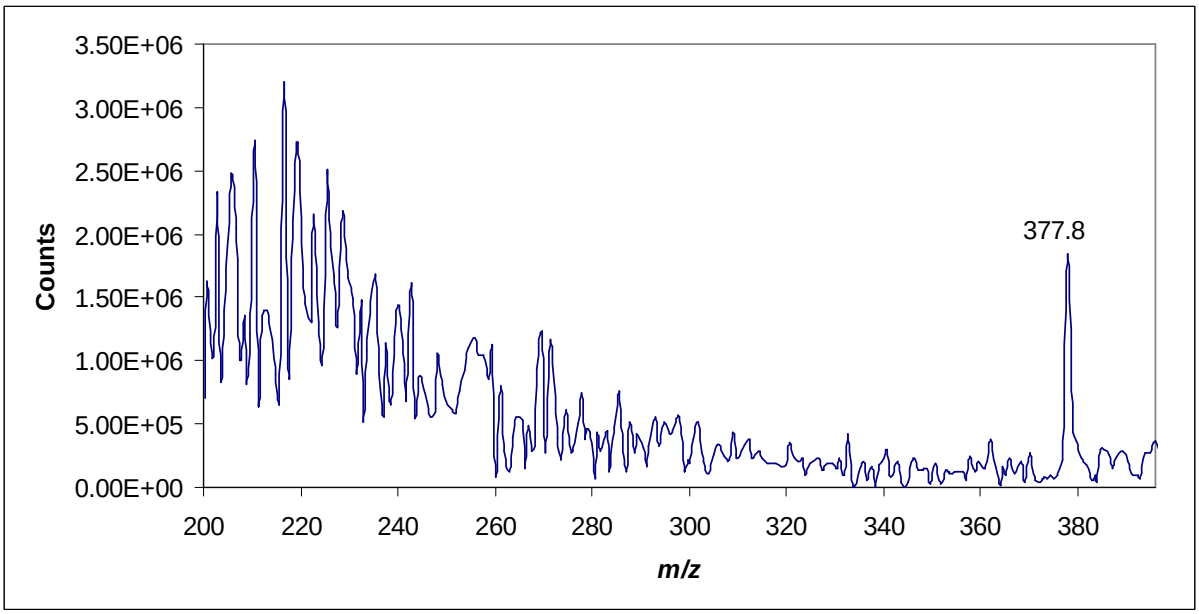


Figure 15. PSI spectrum with eluent containing NO_3^- and 10 ppm PENT. [PENT–NO_3^-] with *m/z* of 378 can be seen. Spectrum is measured with 3Q.



Summary

In the course of this master's thesis MS ionization technique – paperspray ionization – was studied for different applications.

First necessary equipment had to be developed and applied to work. Different methods of cutting the paper were tested. Significant differences between methods could not be found. Different filter papers were tested for use in PSI. Although significant differences were not found Whatman filter paper was chosen for use in PSI due to its stable spray.

FT ICR spectra with PSI were recorded in positive and negative ion mode. It was observed from these spectra that different papers give different MS spectra and therefore papers can be potentially distinguished. Also information about the papers can be extracted. As an excample ion with *m/z* of 185.1143 was fragmented in the FT ICR cell using a laser. The ion could be identified as diglycol ethyl ether, which is a possible solvent, used in paper industry.

Different methods were tested for identification of pesticides from orange peels. It was found that wiping method could be used to identify imazalil and thiabendazole. Possibility to identify different pesticides from other fruits and vegetables with wiping method was studied. Only imazalil and thiabendazole could be identified on orange, lemon and grapefruit and imazalil could be identified on lime.

The possibility of quantitative analysis of imazalil and thiabendazole from an orange with wiping method was studied. It was found that using an internal standard and MS/MS measurements RSD of less than 10% can be achieved with PSI. However as isotope labeled compounds were not available no suitable internal standard was found. Moreover the wiping efficiency was found to be poor. Therefore it was concluded that quantitative analysis by this method is not possible in the scope of this work. However it is shown that as a scanning method it is applicable.

Also the possibility of identifying different explosives with PSI was studied. The formation of eluent clusters in the negative ion mode creates a low *S/N* with PSI. Therefore the explosives could not be identified when solutions with concentration of 1 ppm of the analytes were measured.

In conclusion all the aims of the study were met. Further new developments in the filed of classification of papers based on PSI spectra as well as quantitative analysis of different analytes are possible and will be conducted in near future.

PABERPIHUSTUSIONISATSIOONI JA SELLE VÕIMALIKE RAKENDUSTE UURIMINE

Hanno Evard

Kokkuvõte

Käesoleva magistritöö käigus uuriti massispektromeetria ionisatsiooni meetodi paberpihustusionisatsiooni ja selle võimalikke rakendusi.

Arendati välja ning rakendati tööle paberpihustusionisatsiooni jaoks vajalikud seadmed. Katsetati erinevaid meetodeid pionisatsioonipinna - paberist kolmnurga - lõikamiseks, kuid olulisi erinevusi meetodite vahel ei täheldatud. Erinevaid filterpabereid võrreldi PSI mõõtmistel kasutamise seisukohast lähtuvalt. Kuigi olulisi erinevusi paberite vahel ei leitud valiti Whatman filterpaber edasiseks kasutamiseks stabiilse pihustuse tõttu. Analüütidena uuriti erinevaid amiine ja pestitsiide.

Kasutades FT ICR spektrid positiivses ja negatiivses töörežiimis tehti kindlaks, et erinevaid pabereid on võimalik karakterseerida ja eristada PSI kasutamisel. Näiteks õnnestus iooni *m/z*-ga 185.1143 fragmenteerimisel FT ICR rakus ioon identifitseerida kui diglükool etüül eeter, mida kasutatakse paberi tootmisel.

Uuriti erinevaid meetodeid pestitsiidide identifitseerimiseks apelsini koorelt. Leiti, et pühkimismeetodiga saab imasaliili ja tiabedasooli tuvastada. Samuti uuriti teiste pestitsiidide tuvastamise võimalusi erinevatelt puu- ja köögiviljadelt pühkimismeetodiga. Imasaliil ja tiabendasool suudeti tuvastada apelsinilt, sidrunilt ja greibilt ning imasaliil suudeti tuvastada laimilt. Teiste pestitsiidide olemasolu puu- ja köögiviljadelt ei tuvastatud.

Täiendavalt uuriti imasaliili ja tiabendasooli kvantitatiivse või poolkvantitatiivse analüüsi võimalust apelsinilt pühkimismeetodiga. Leiti, et sisestandardi ja MS/MS kasutamisel on võimalik saavutada korduvus all 10%. Seoses isotoopmärgistatud standardite puudumisega ei õnnestunud sobivaid sisestandardeid leida. Lisaks sellele näitavad katsete tulemused, et pühkimise efektiivsus on liiga madal kvantitatiivseks määramiseks. Siiski on see meetod sobilik nö „skriininguks“.

Lisaks uuriti lõhkeainete identifitseerimise võimalusi. Kasutatud eluendiga tekkisid PSI mõõtmistel negatiivses töörežiimis solvendi klastrid, seetõttu ei olnud võimalik saavutada piisavat signal-müra suhet. Seega 1 ppm konsentratsiooniga lõhkeainete lahustest ei tuvastatud vastavaid aineid.

Kokkuvõtlikult täideti kõik tööle püstitatud eesmärgid. Töö tulemusena selgitati välja mitmes perspektiivikad arengusuunad, millega on plaanis tulevikus edasi tegeleda.

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Acknowledgements

I would like to thank Anneli for being a great mentor and for leading me into the wonderful world of mass spectrometry.

Also I would like to thank Professor Ivo Leito for helping me with many difficult problems and giving me good advice.

I would also like to thank Tõiv Haljasorg for teaching me all about the mass spectrometer and the FT ICR and Karl Kaupmees for the COSMO calculations.

I am very thankful for everyone in the lab and in Chair of Analytical Chemistry for making working and studying so interesting and fun.

Appendix 1

Figure 16. Template.

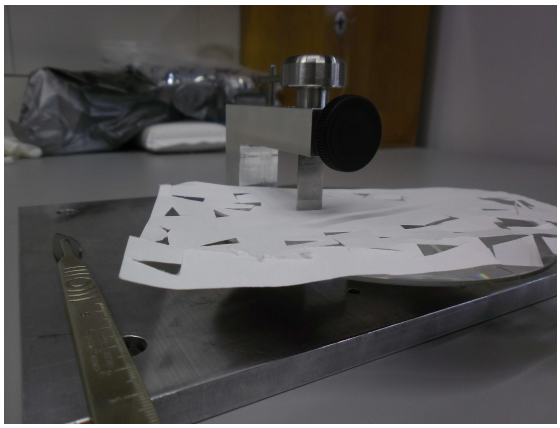


Figure 17. Paper placed in the holder.

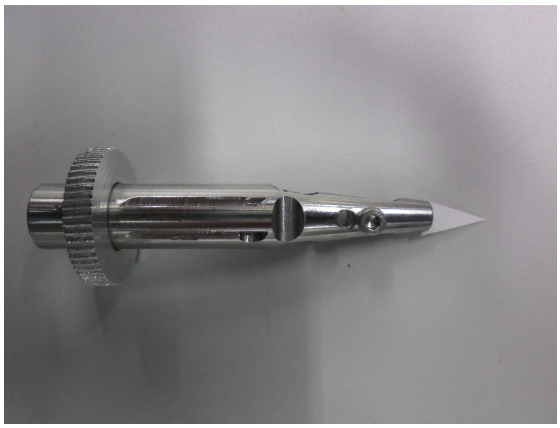
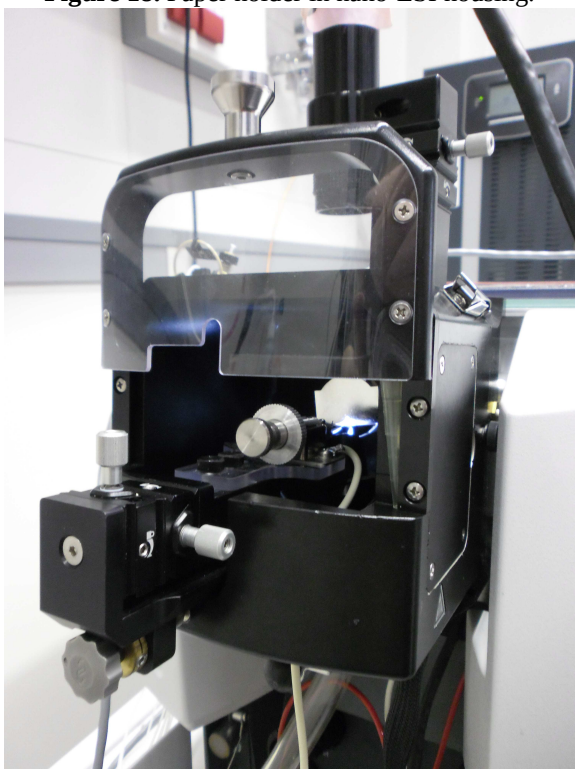


Figure 18. Paper holder in nano-ESI housing.



Appendix 2

Figure 19. Upper triangle is cut by the first and lower triangle with the second method.

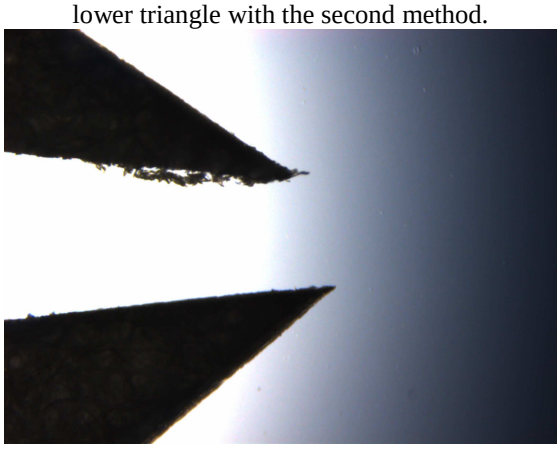


Figure 20. Triangle cut with the first method. No split end.

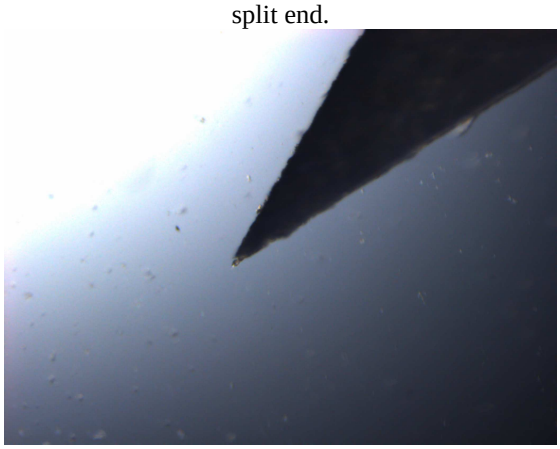


Figure 21. Triangle cut with the second method resulting in split tip.

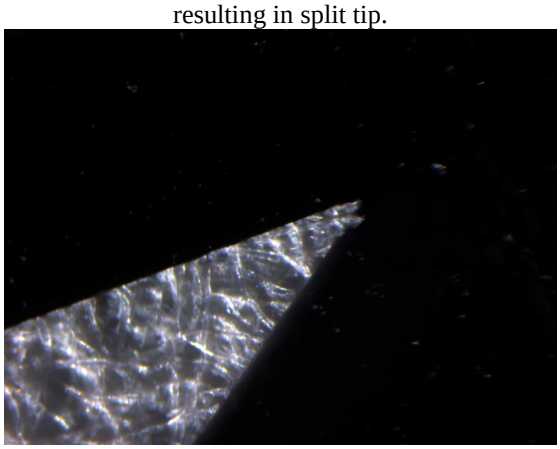


Figure 22. Stable spray can not be achieved with triangle tip with many fibers.

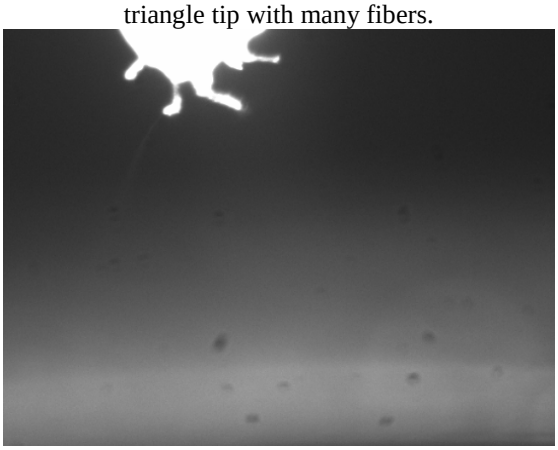


Figure 23. Paper with blunt tip.

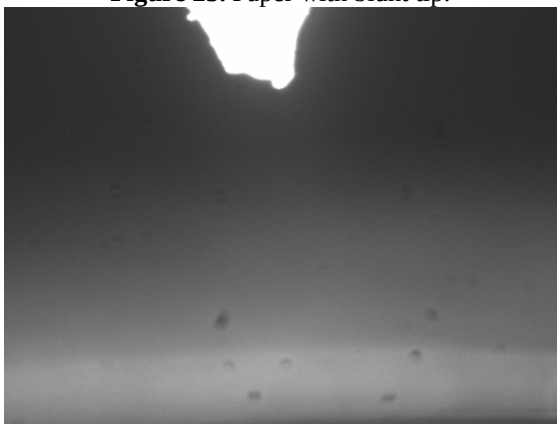
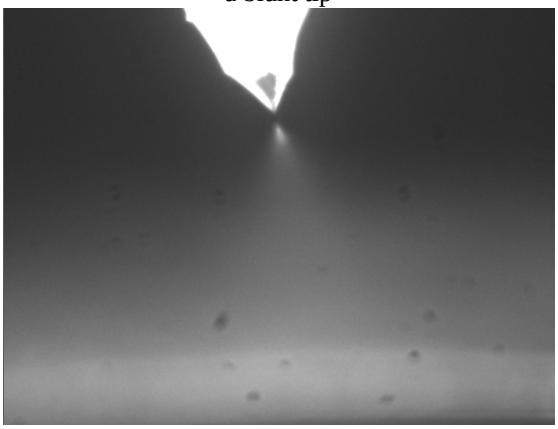


Figure 24. Spray formed with a paper triangle with a blunt tip



Appendix 3

Figure 25. 3Q spectrum of 88 filter paper.

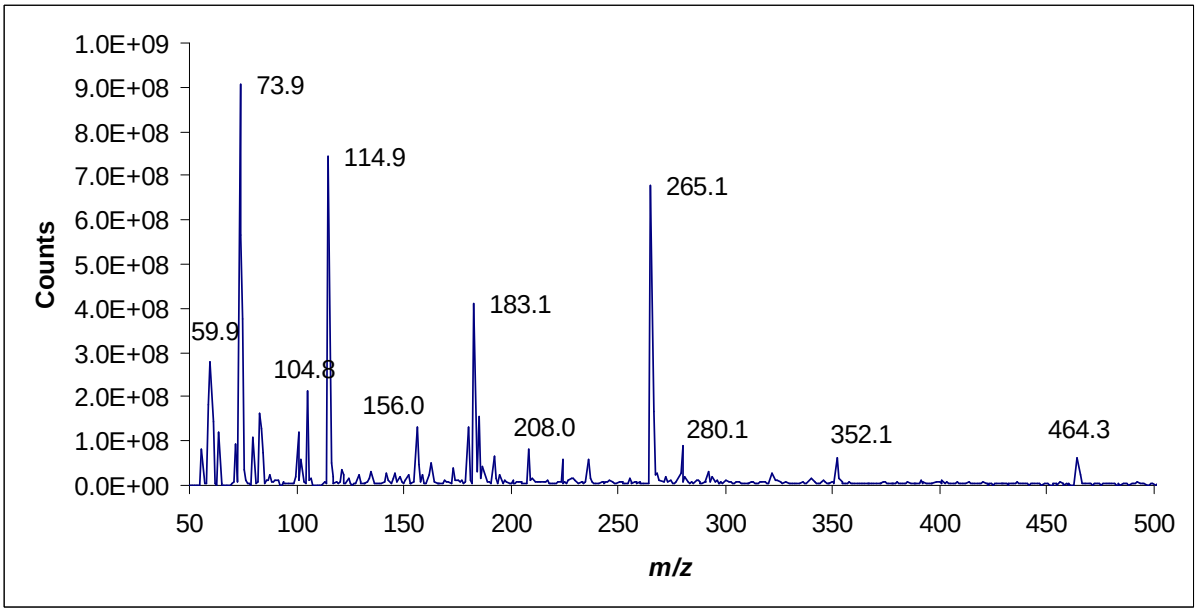


Figure 26. 3Q spectrum of 91 filter paper.

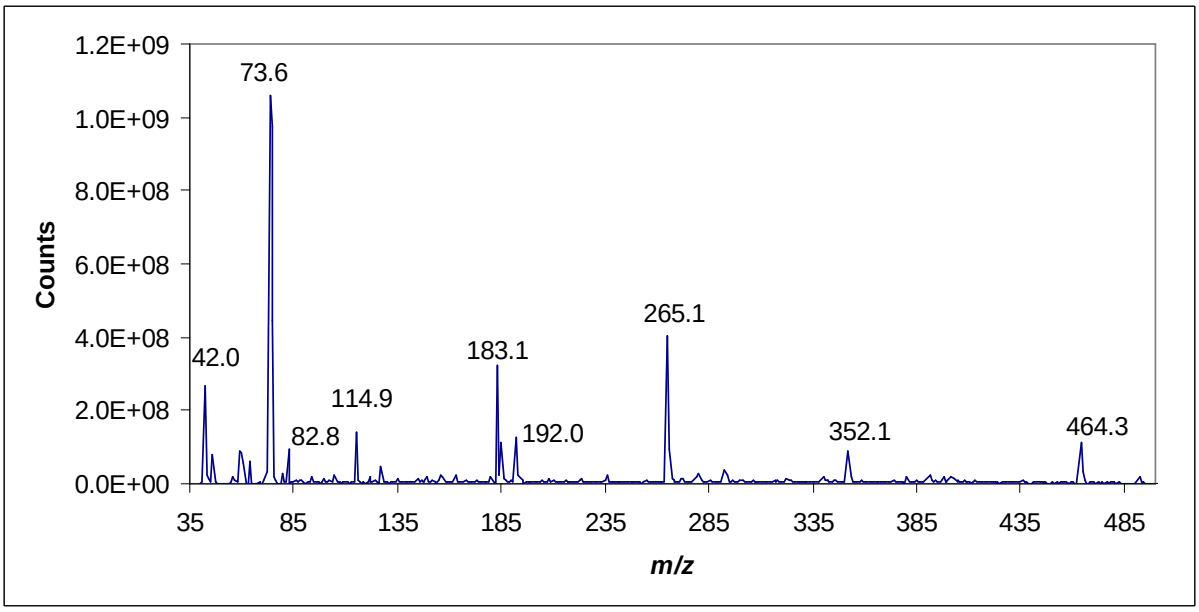
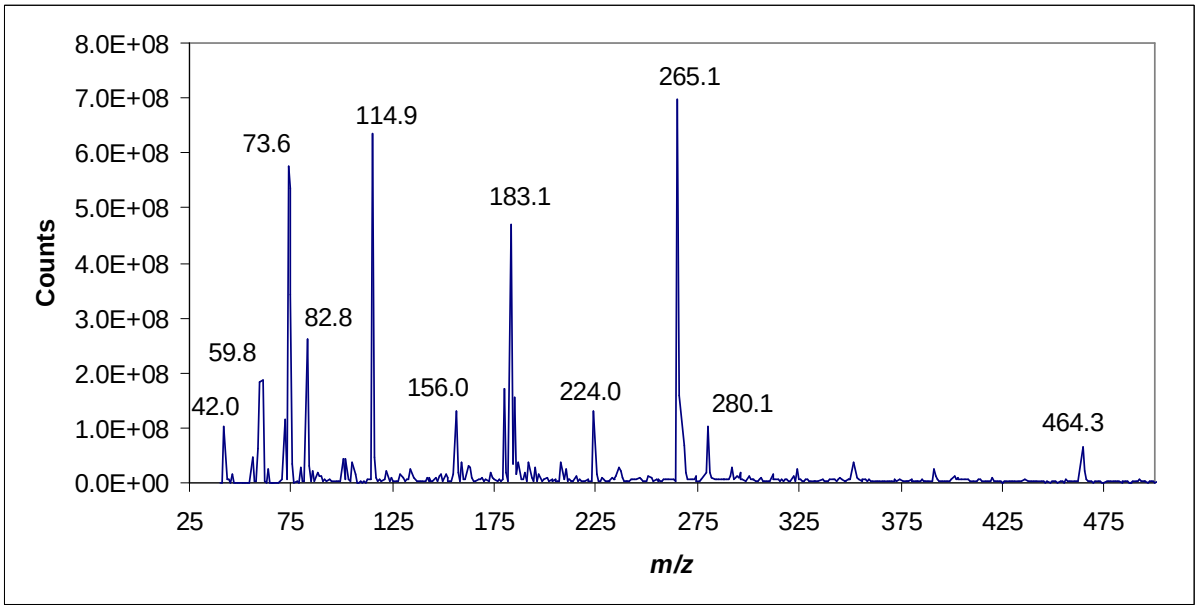


Figure 27. 3Q spectrum of 390 filter paper.



Appendix 4

Figure 28. Mass spectrum of imazalil (297 *m/z*). Spectrum is measured with 3Q.

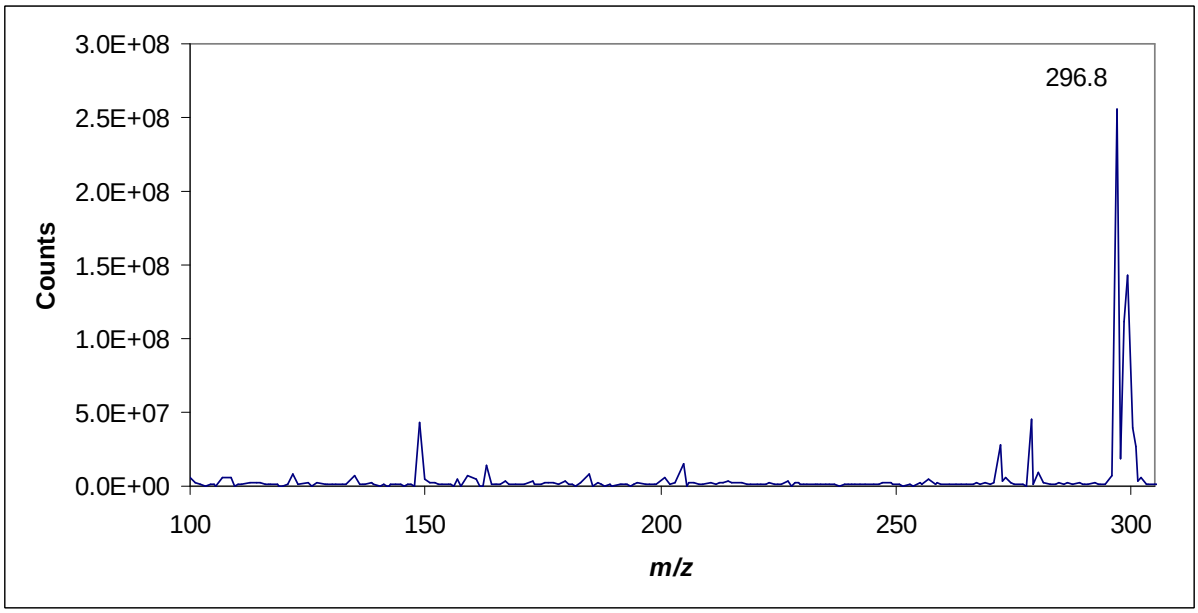


Figure 29. Mass spectrum of thiabendazole (202 *m/z*). Spectrum is measured with 3Q.

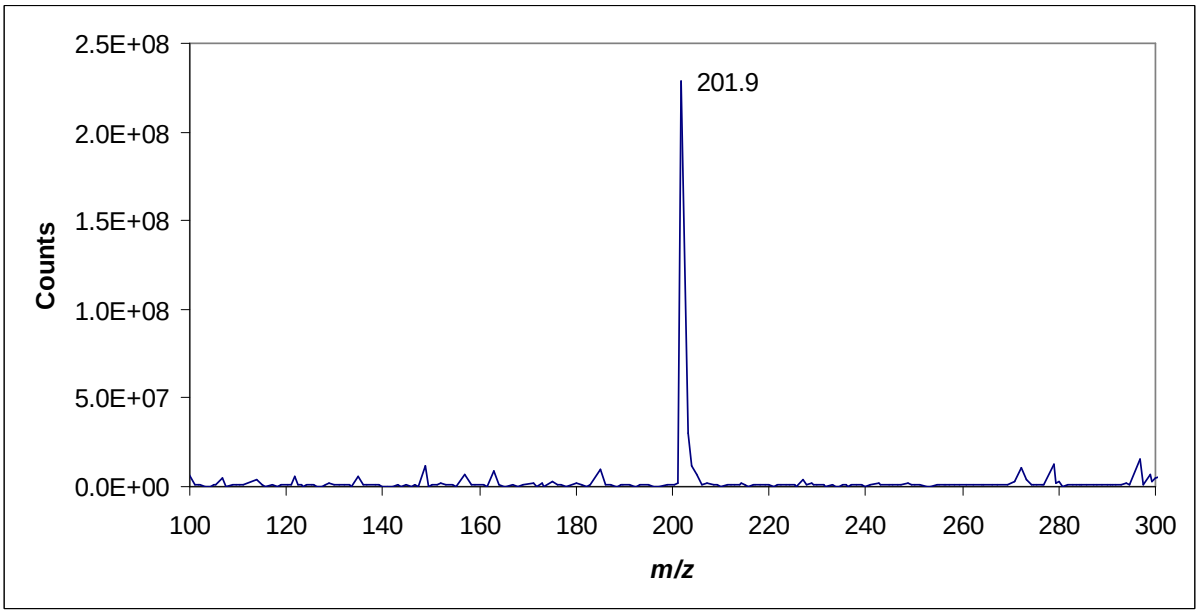


Figure 30. Mass spectrum of methiocarb (226 *m/z*). Spectrum is measured with 3Q.

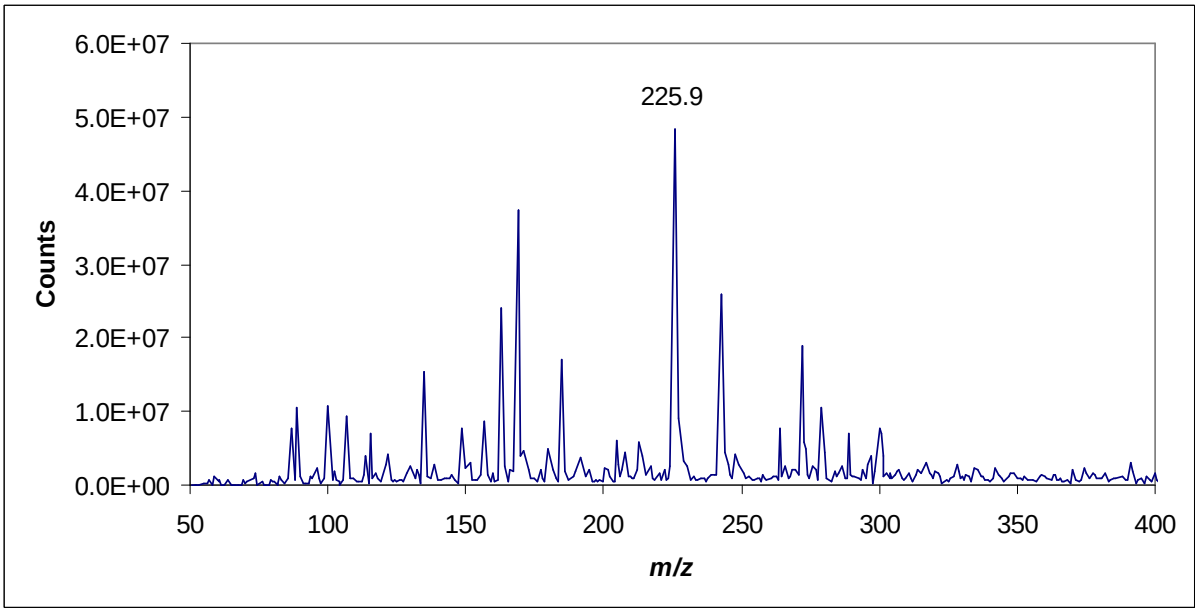


Figure 31. Mass spectrum of aldicarb (213 *m/z*). Spectrum is measured with 3Q.

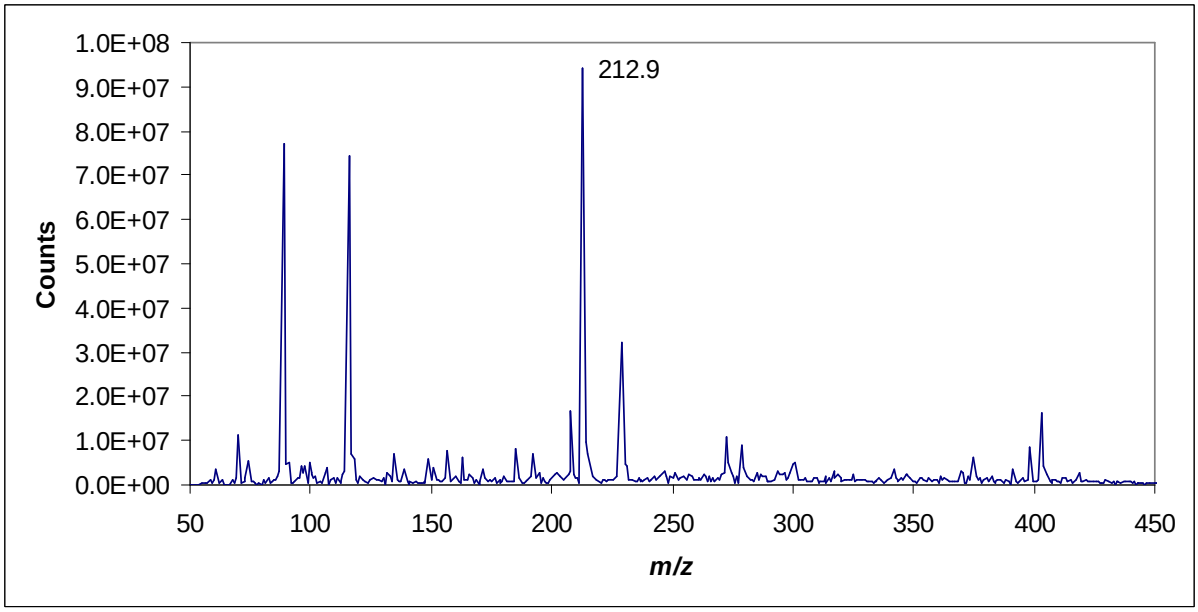


Figure 32. Mass spectrum of methamyl (163 *m/z*). Spectrum is measured with 3Q.

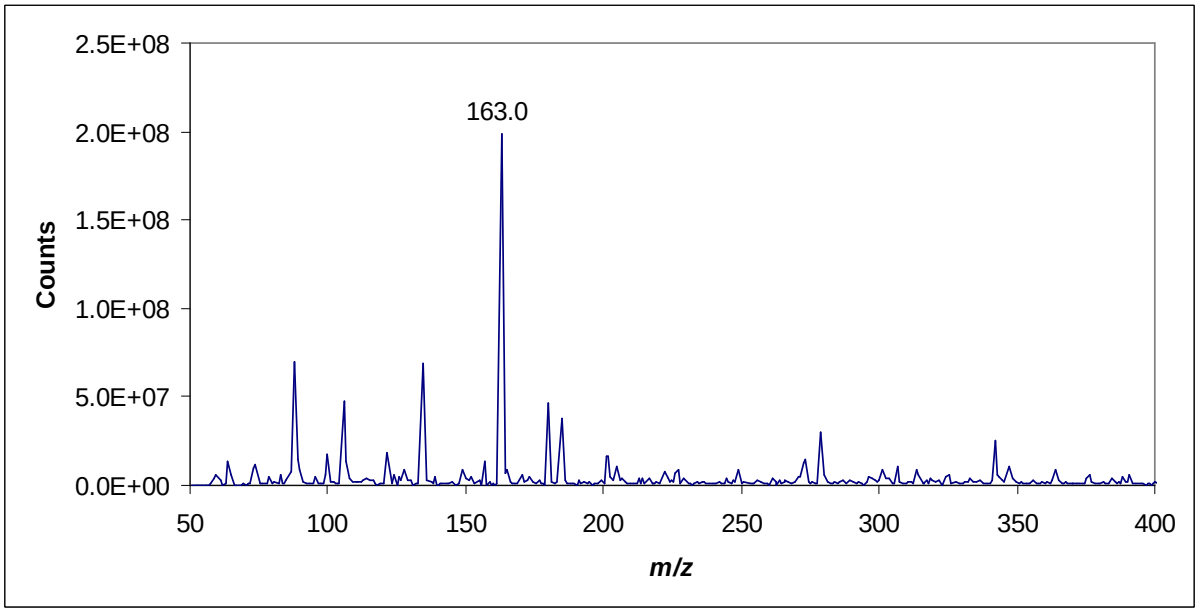


Figure 33. Mass spectrum of diethyl amine (74 *m/z*). Spectrum is measured with 3Q.

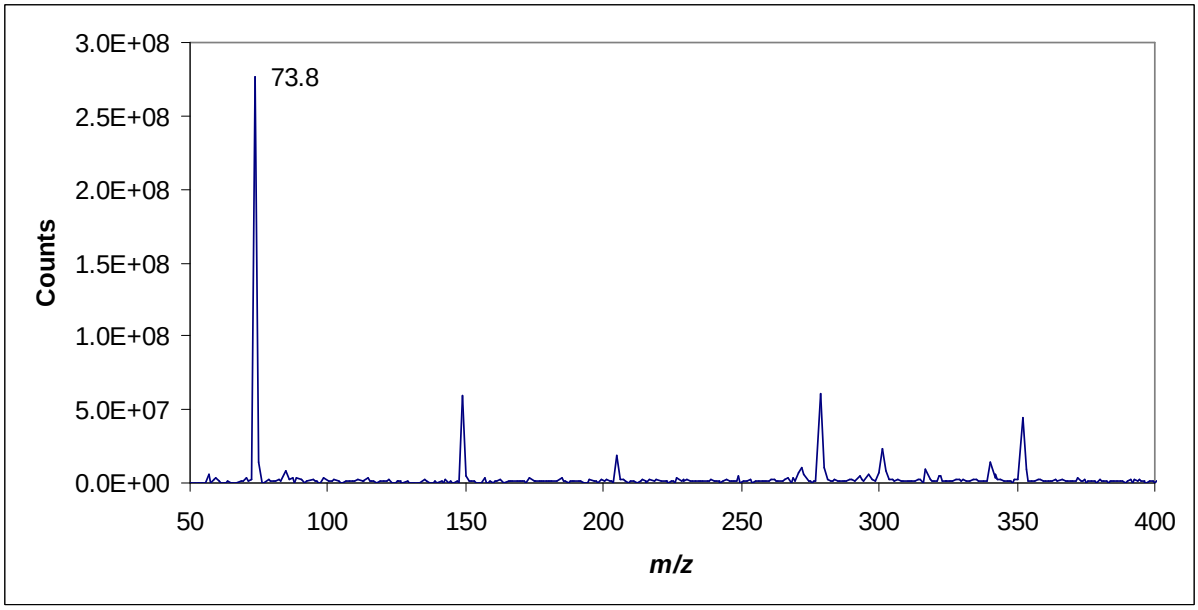


Figure 34. Mass spectrum of triethyl amine (112 *m/z*). Spectrum is measured with 3Q.

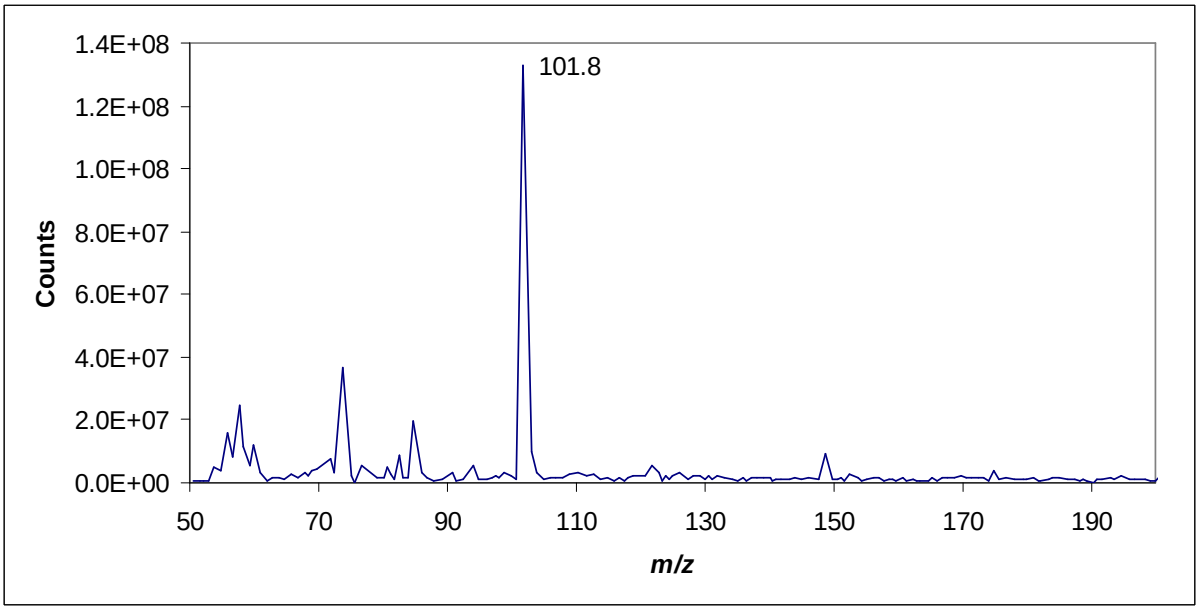


Figure 35. Mass spectrum of anilin (94 *m/z*). Spectrum is measured with 3Q.

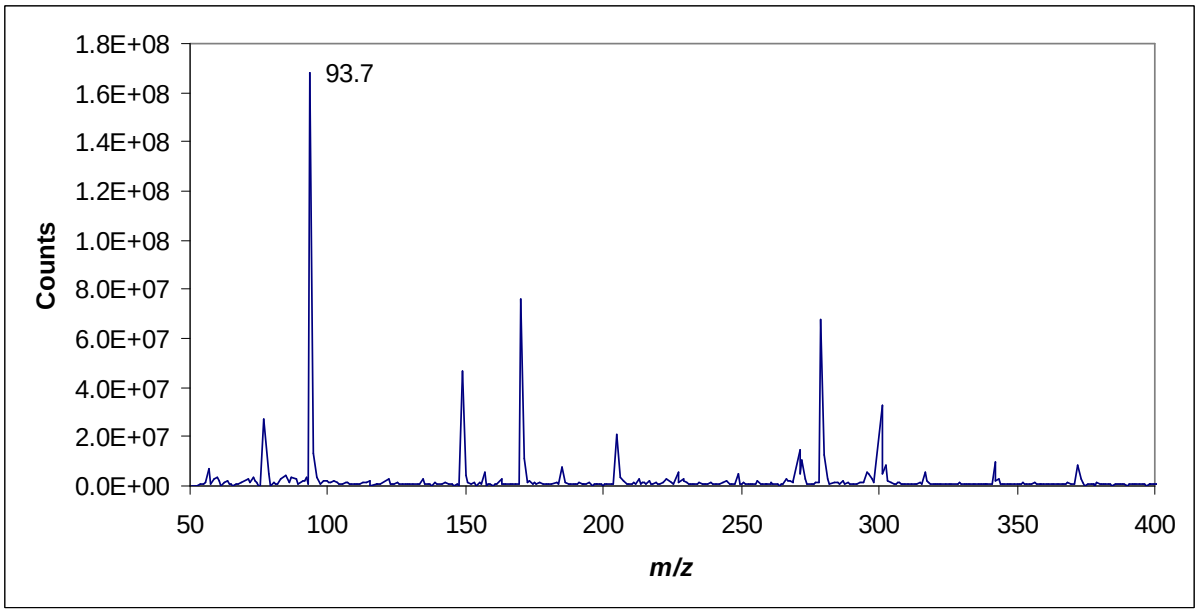


Figure 36. Mass spectrum of diphenylamine (170 *m/z*). Spectrum is measured with 3Q.

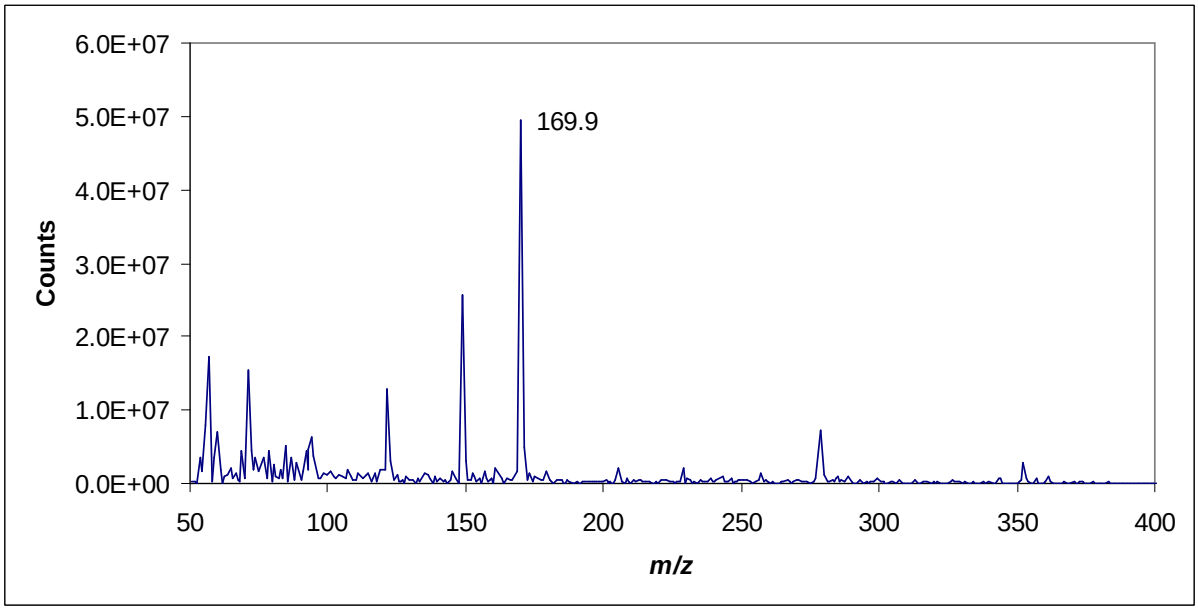
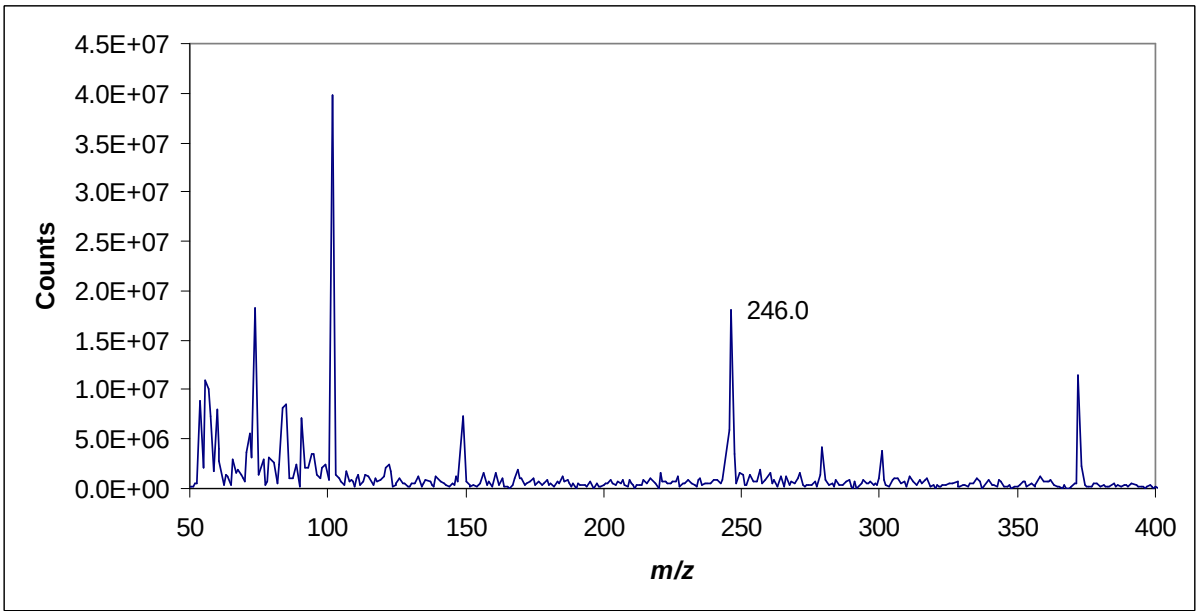


Figure 37. Mass spectrum of triphenylamine (246 *m/z*). Spectrum is measured with 3Q.



Appendix 5

Figure 38. 3Q mass spectrum of grapefruit measure with PSI wiping method.

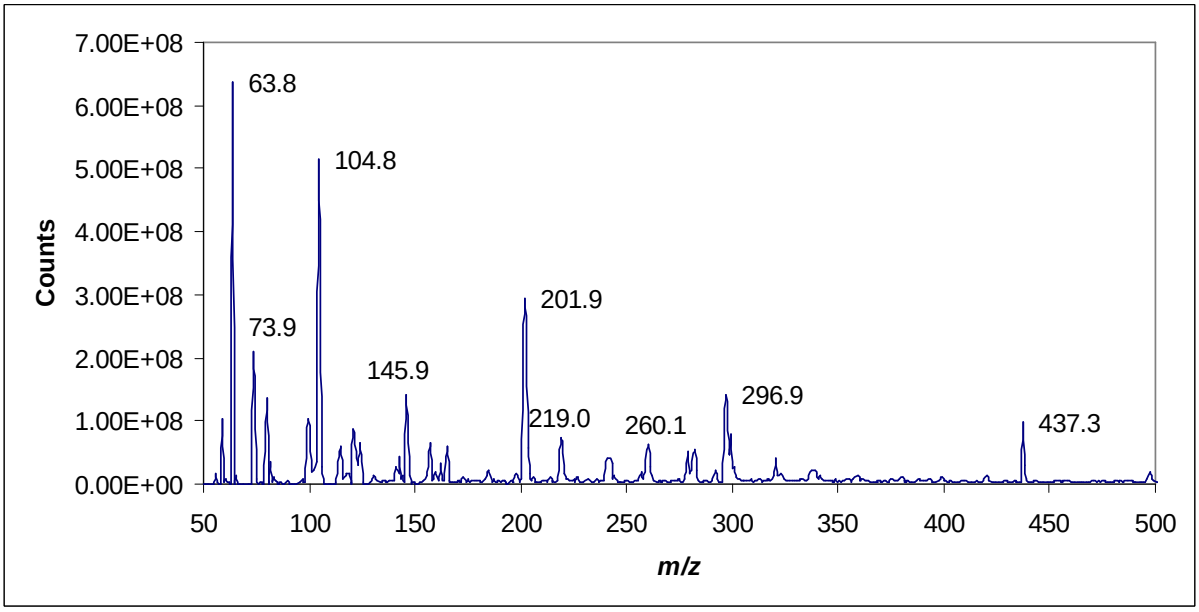


Figure 39. 3Q mass spectrum of orange measure with PSI wiping method.

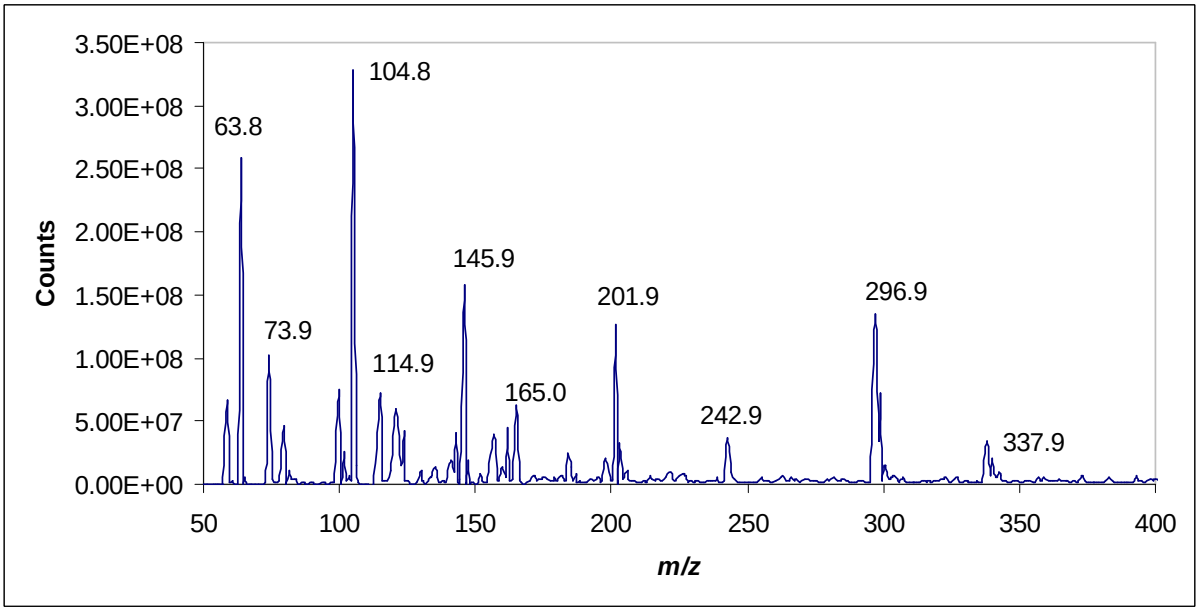


Figure 40. 3Q mass spectrum of mandarin measure with PSI wiping method.

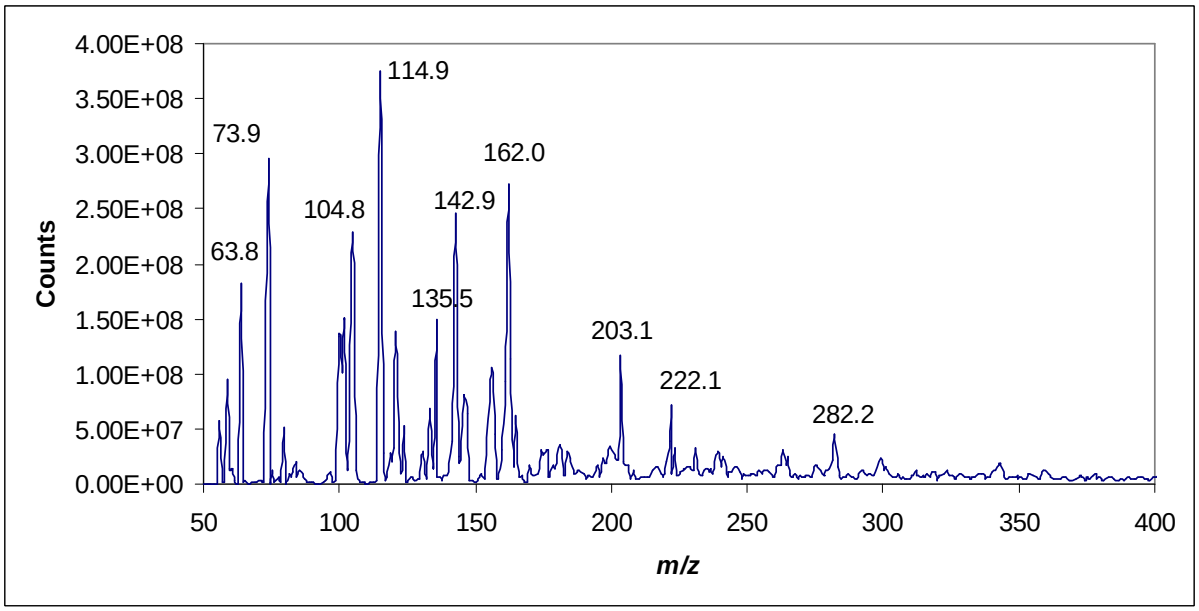


Figure 41. 3Q mass spectrum of lemon measure with PSI wiping method.

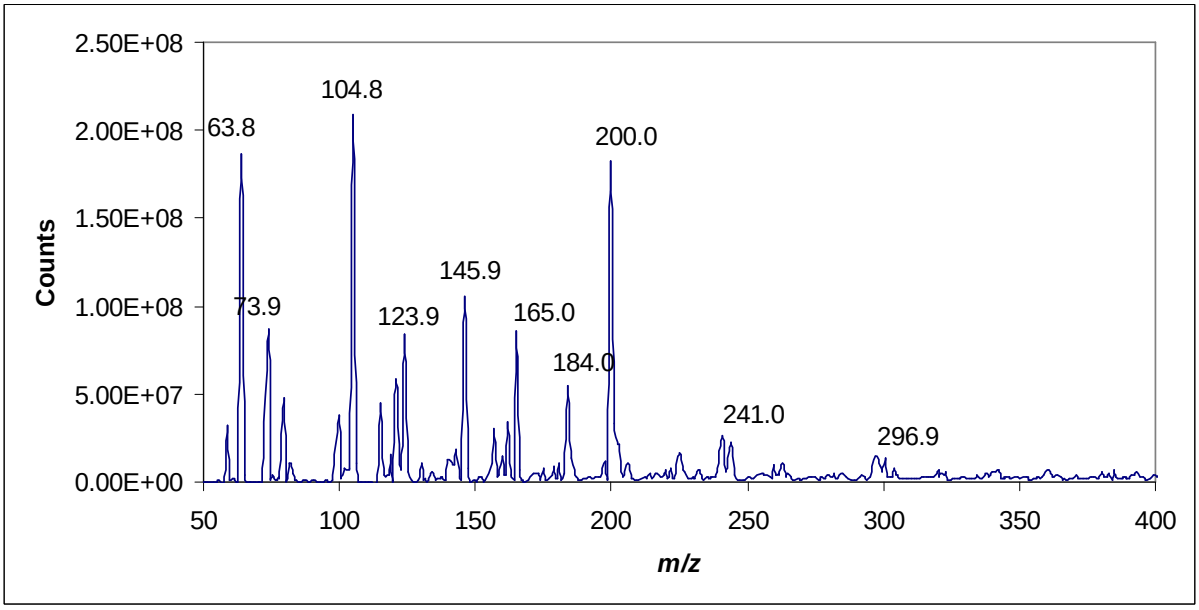


Figure 42. 3Q mass spectrum of lime measure with PSI wiping method.

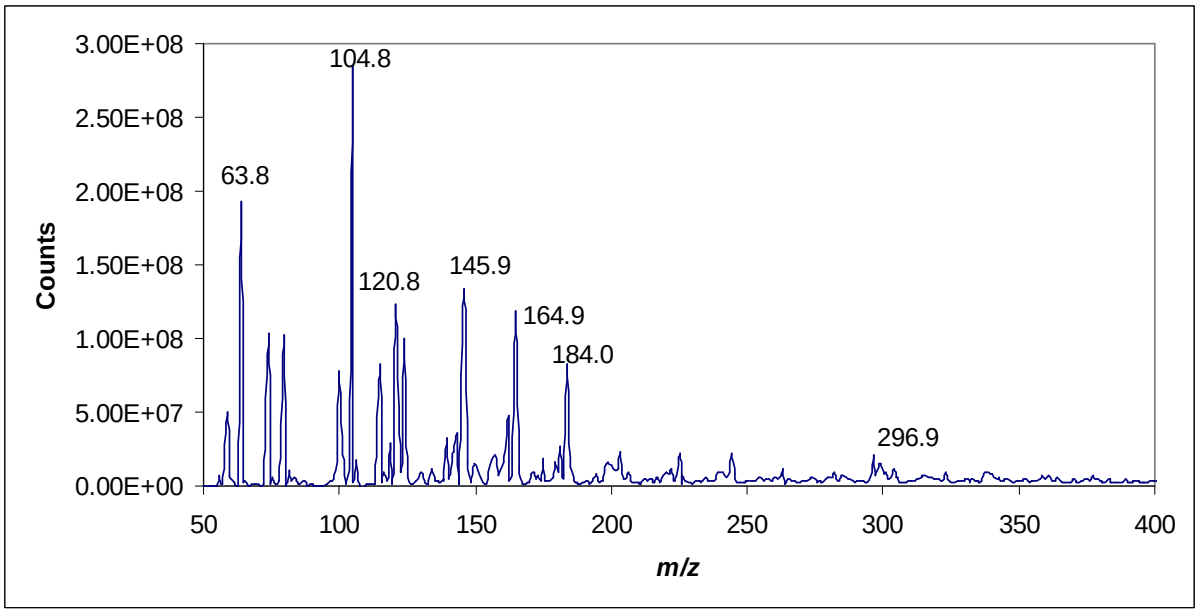


Figure 43. 3Q mass spectrum of apple measure with PSI wiping method.

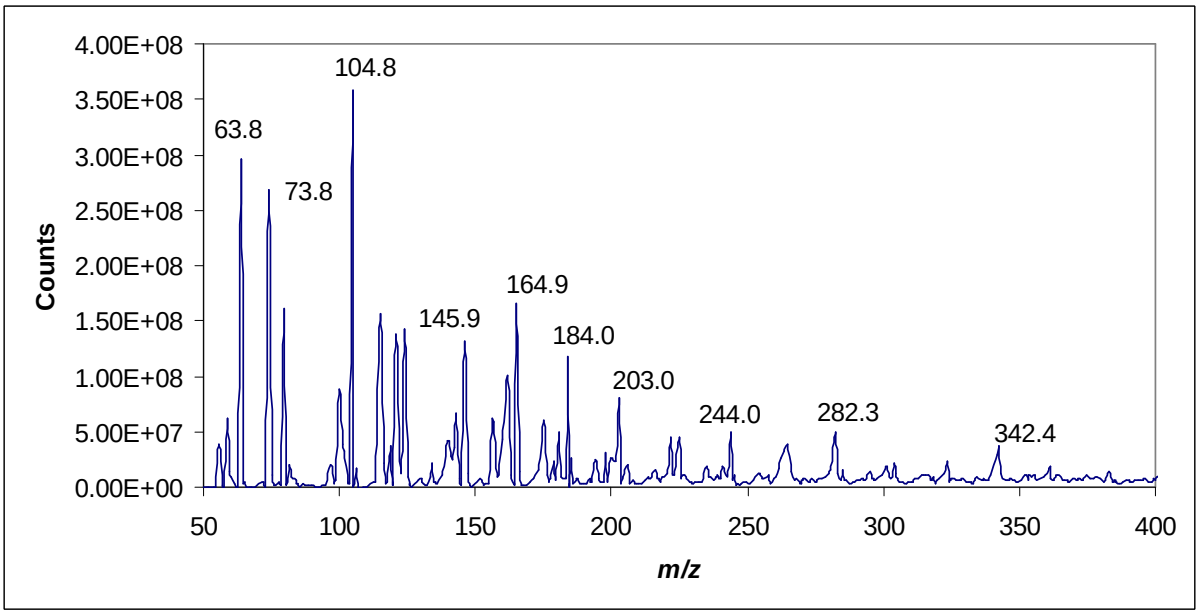


Figure 44. 3Q mass spectrum of pear measure with PSI wiping method.

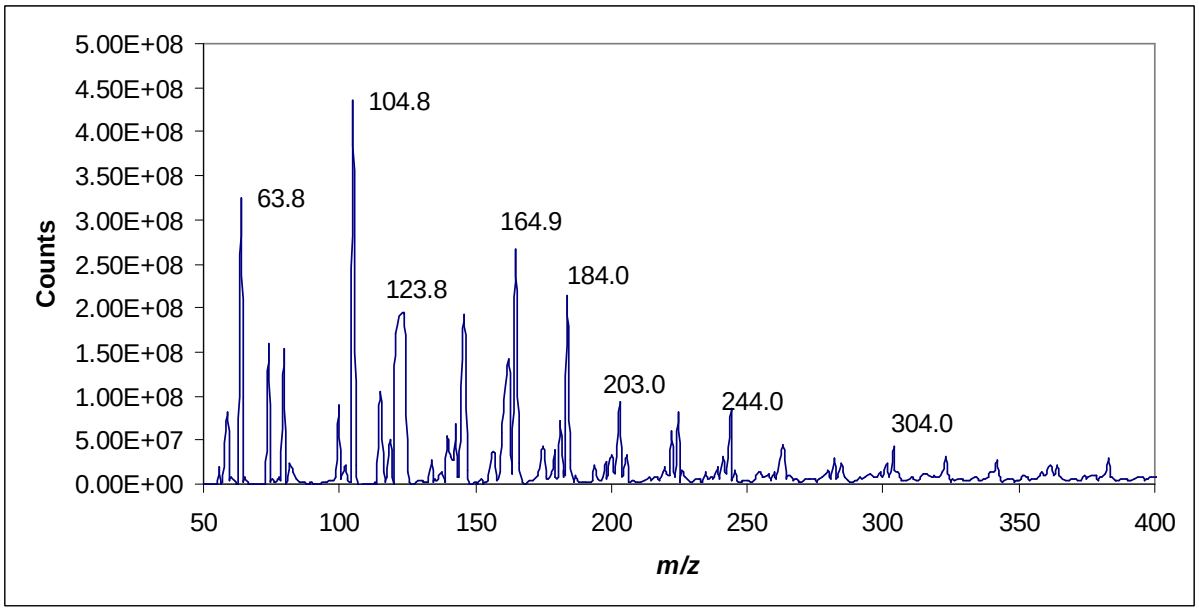


Figure 45. 3Q mass spectrum of tomato measure with PSI wiping method.

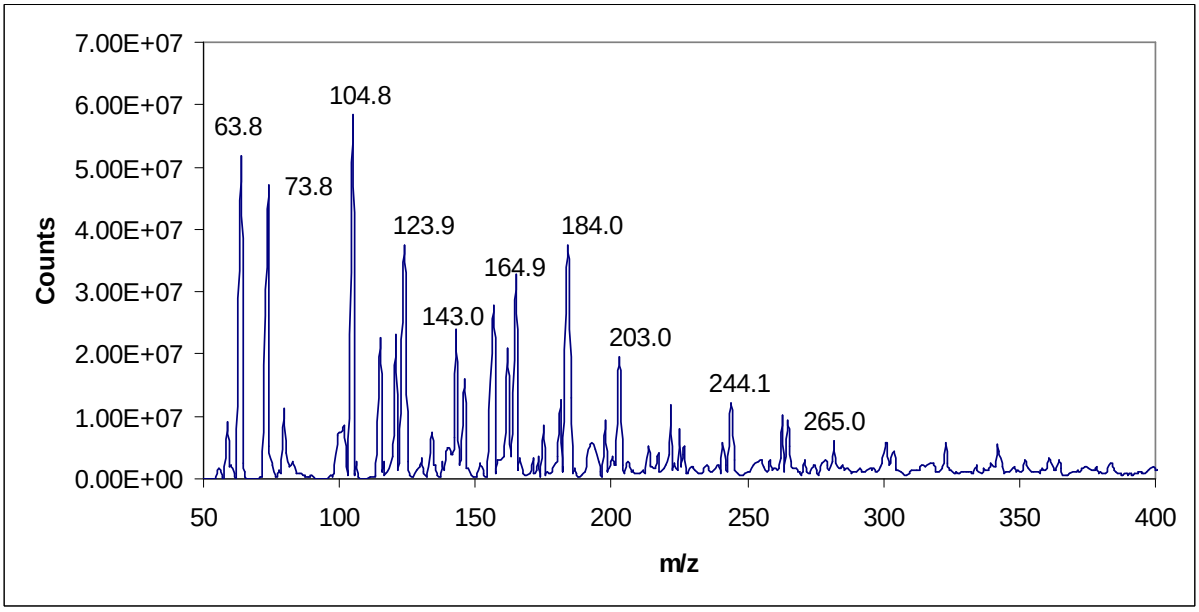


Figure 46. 3Q mass spectrum of strawberry measure with PSI wiping method.

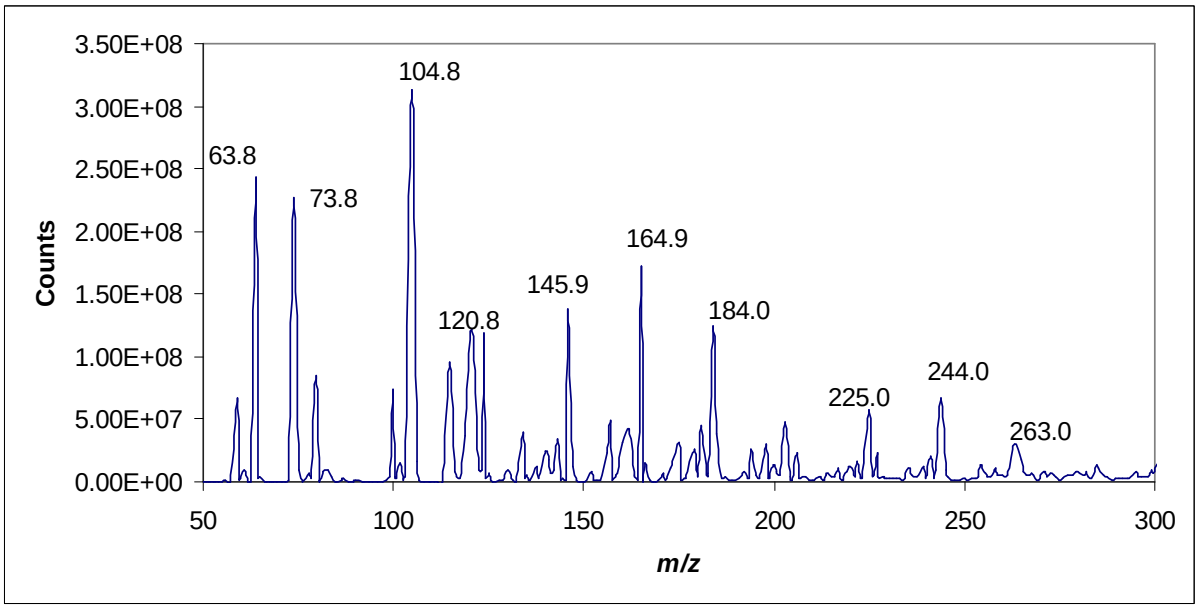
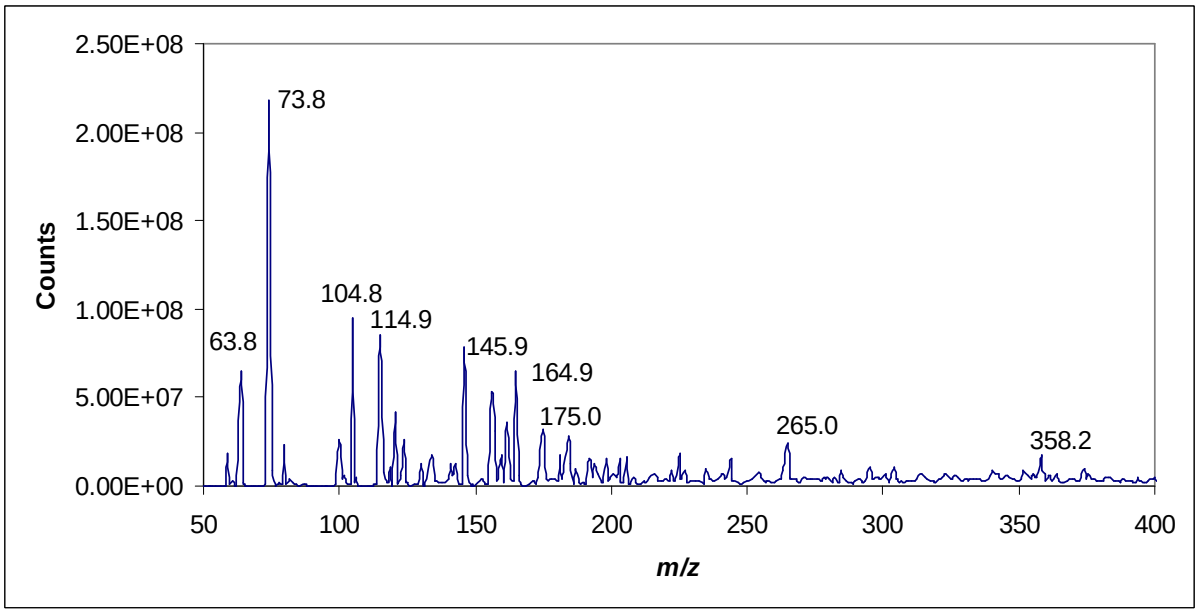


Figure 47. 3Q mass spectrum of sweet pepper measure with PSI wiping method.



Appendix 6

Figure 48. MS/MS measurements of thiabendazole (upper signal)and imazalil (lower signal) from orange by wiping method.

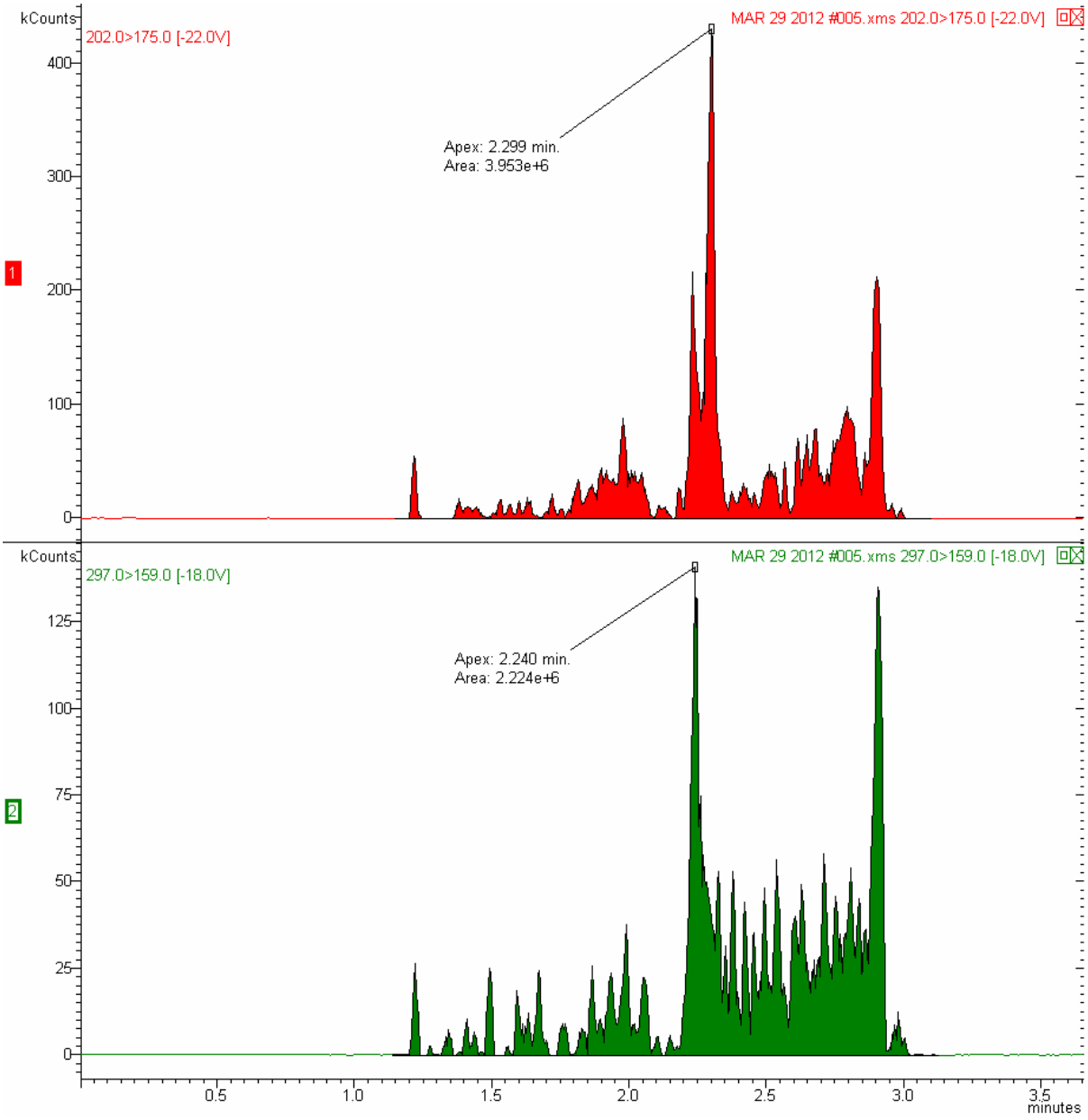


Figure 49. MS/MS measurements of thiabendazole (upper signal)and imazalil (lower signal) from grapefruit by wiping method.

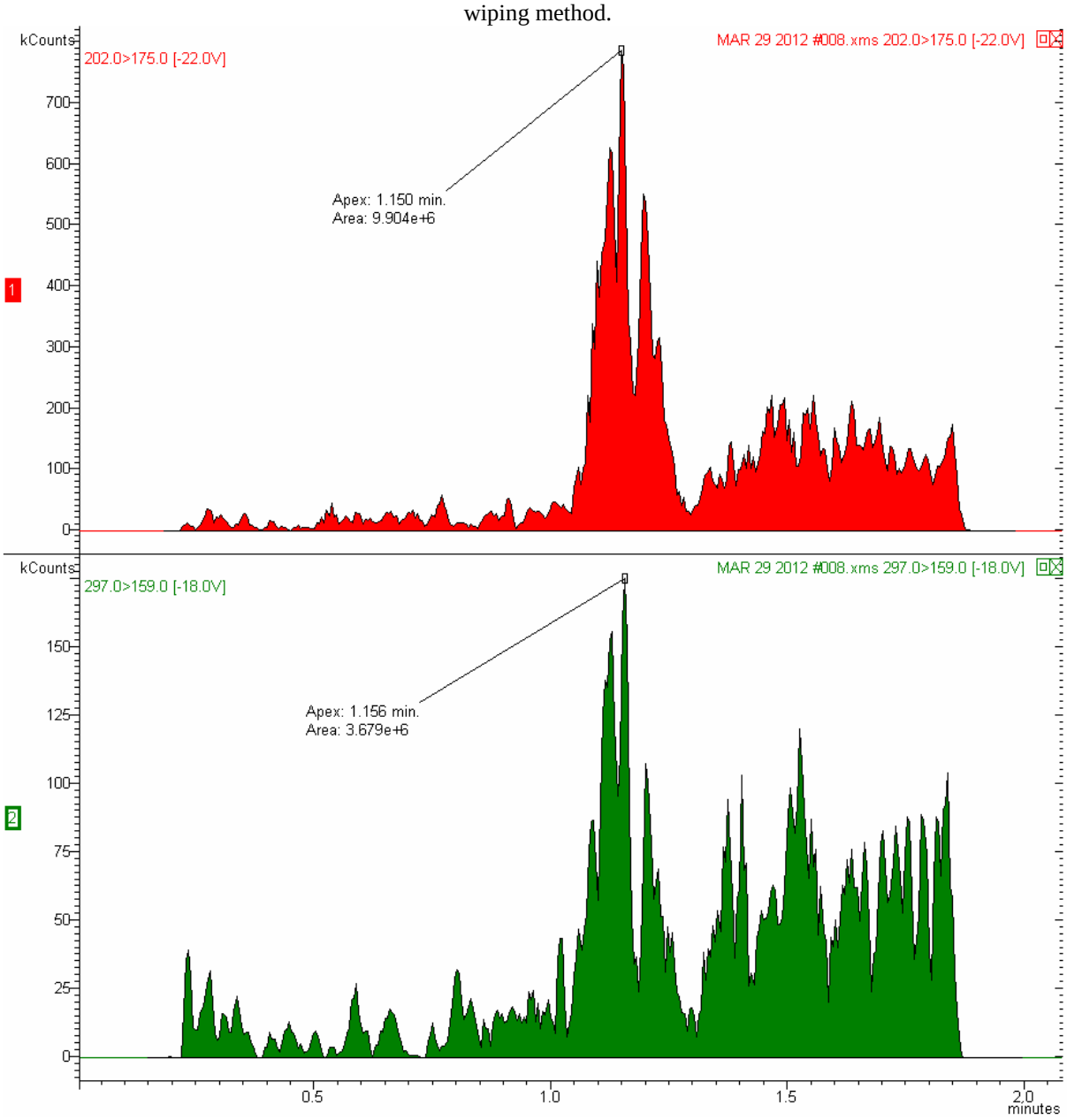


Figure 50. MS/MS measurements of thiabendazole (upper signal)and imazalil (lower signal) from lemon by wiping method.

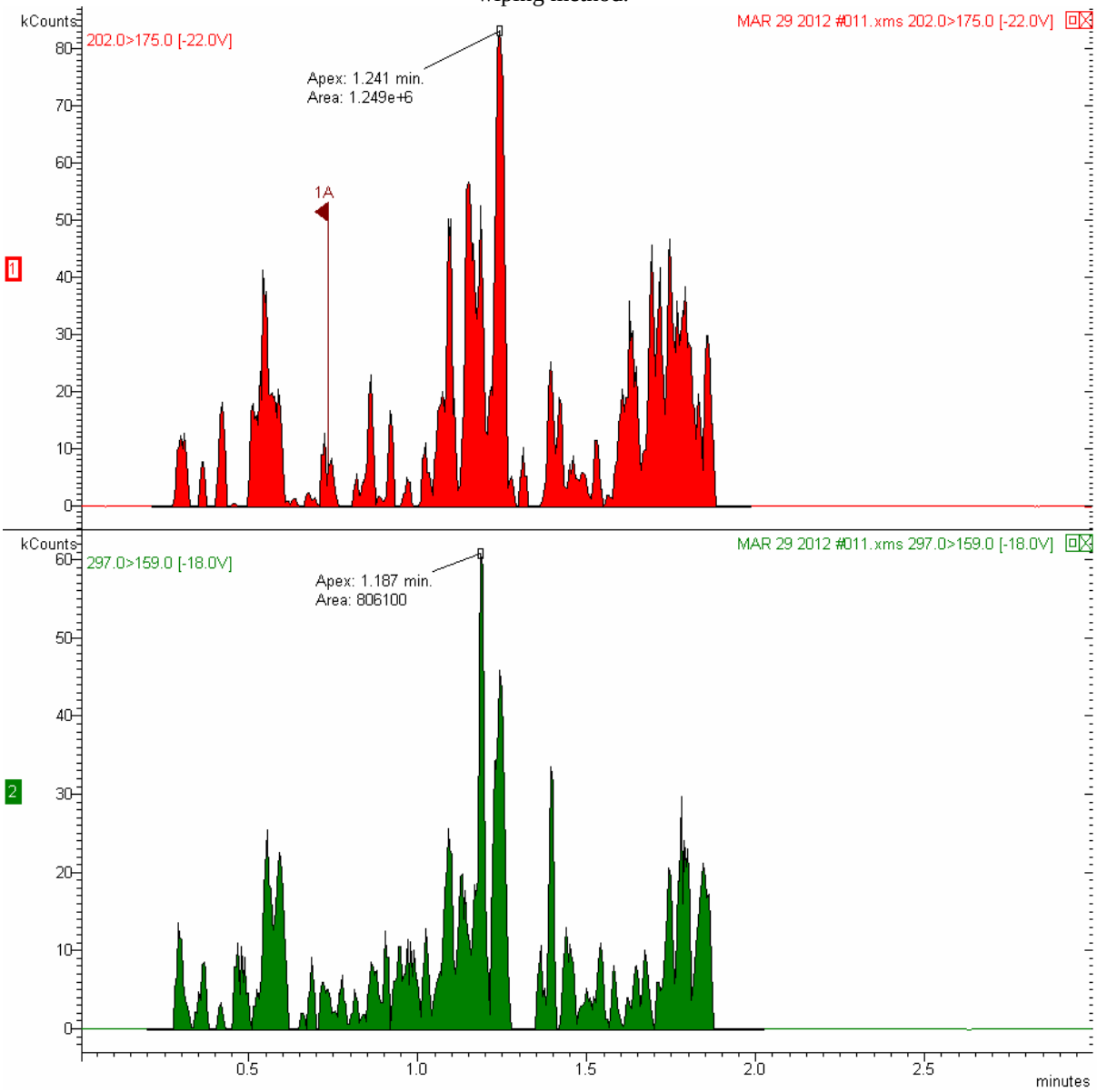
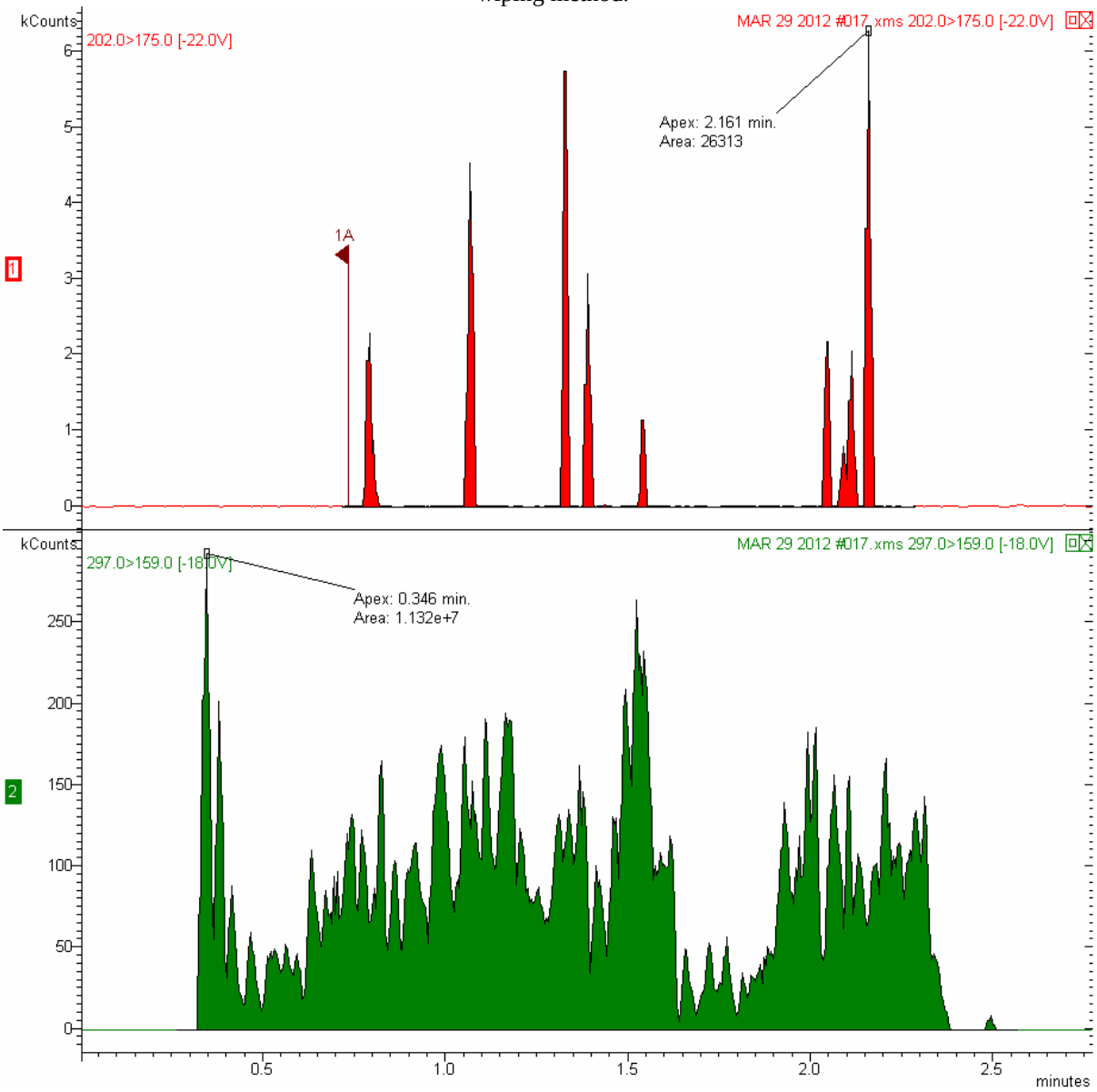


Figure 51. MS/MS measurements of thiabendazole (upper signal)and imazalil (lower signal) from lemon by wiping method.



Appendix 7

Figure 52. [tetryl–NO_3^-] MS^2 spectrum with nano-ESI. Parent ion is 349 *m/z*.

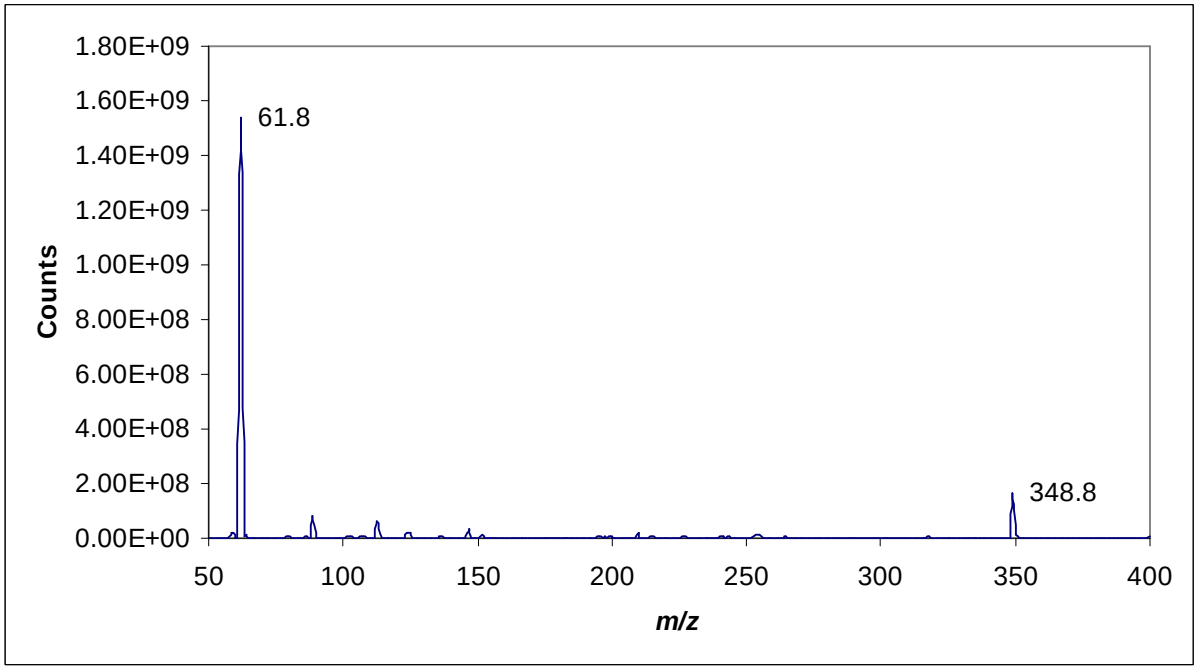


Figure 53. [PENT–NO_3^-] MS^2 spectrum with nano-ESI. Parent ion is 378 *m/z*.

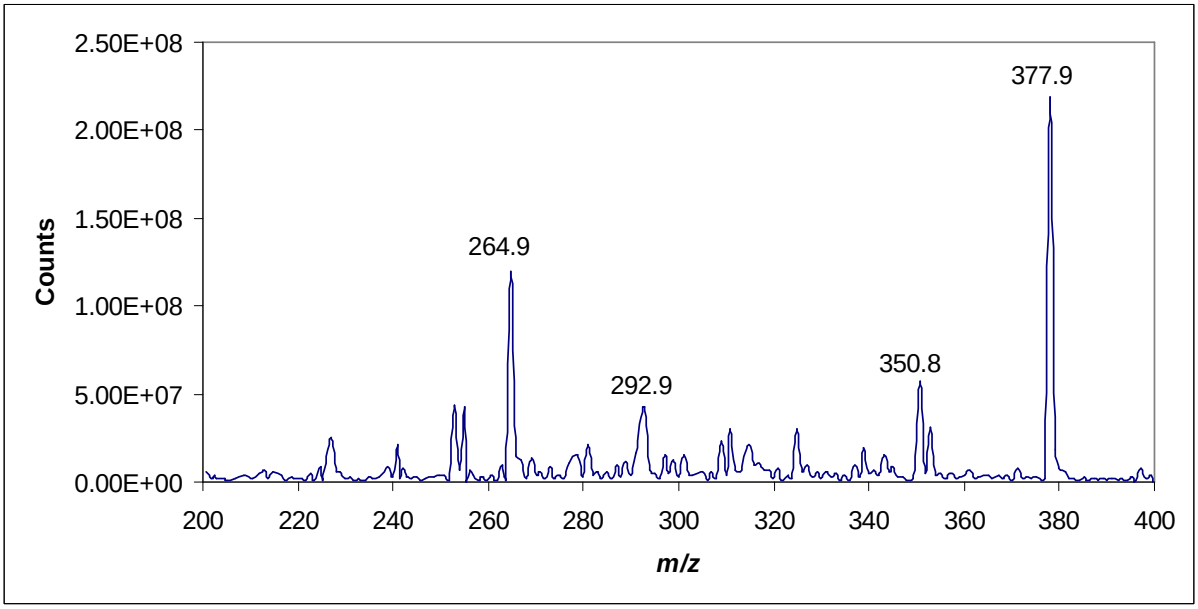


Figure 54. [RDX–Cl^-] MS^2 spectrum with nano-ESI. Parent ion is 257 *m/z*.

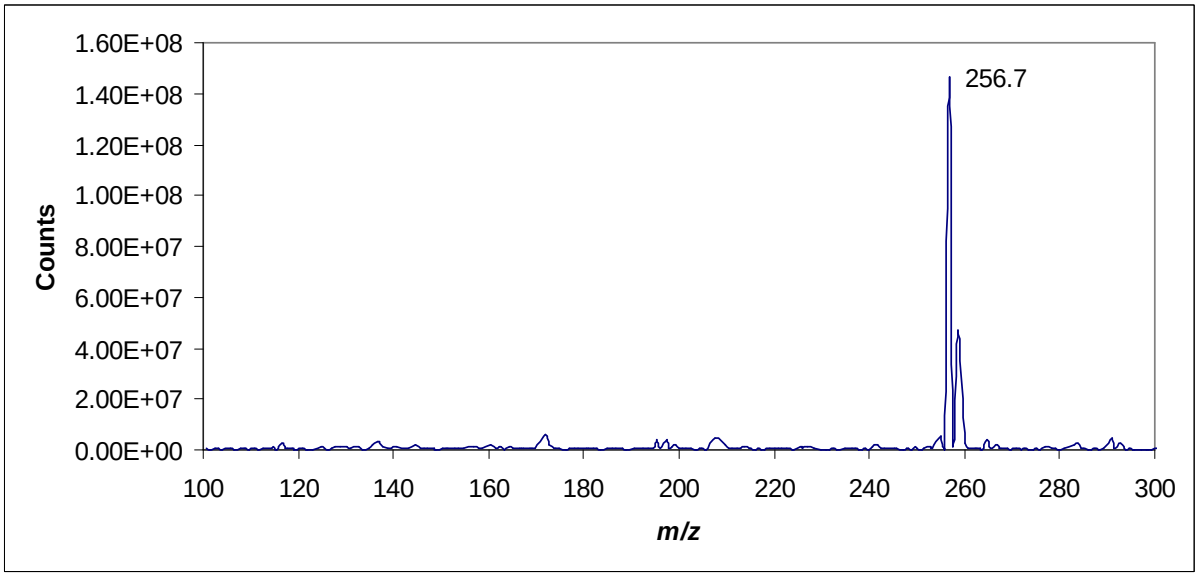


Figure 55. [tetryl–Cl^-] MS^2 spectrum with nano-ESI. Parent ion is 322 *m/z*.

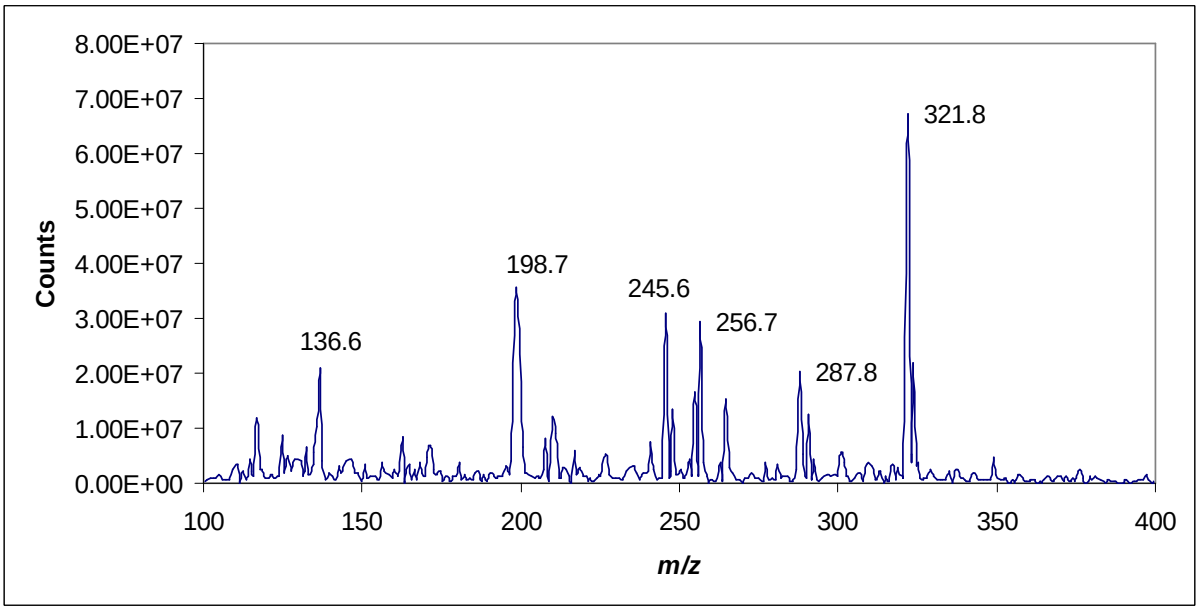
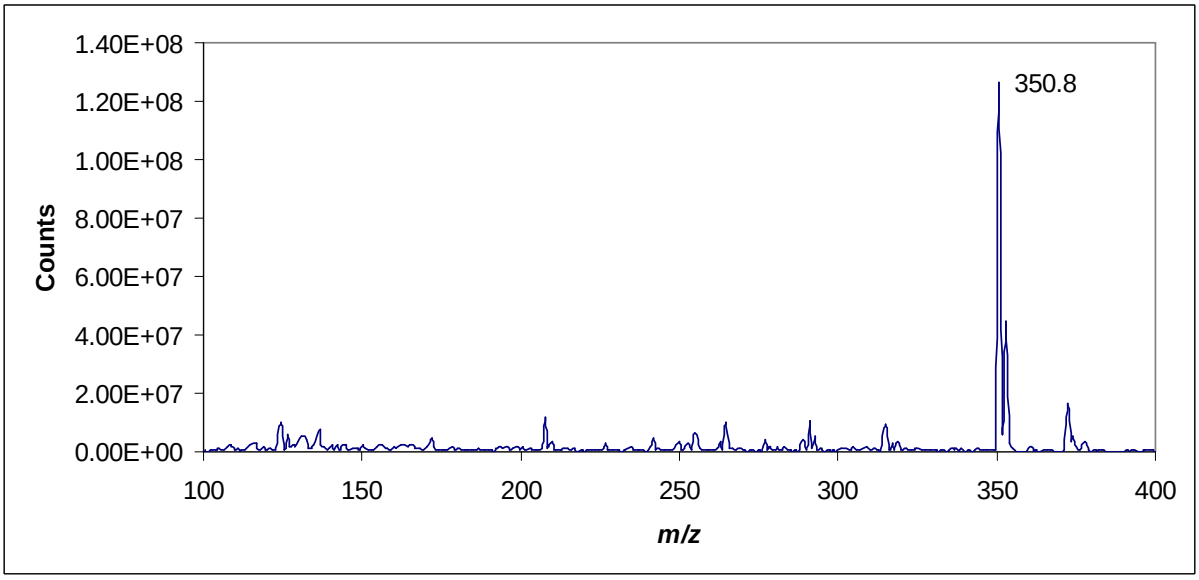


Figure 56. [PENT−Cl^-] MS^2 spectrum with nano-ESI. Parent ion is 351 *m/z*.



Appendix 8

Figure 57. Positive ion mode FT ICR spectrum of Whatman filter paper. 40-200 *m/z*.

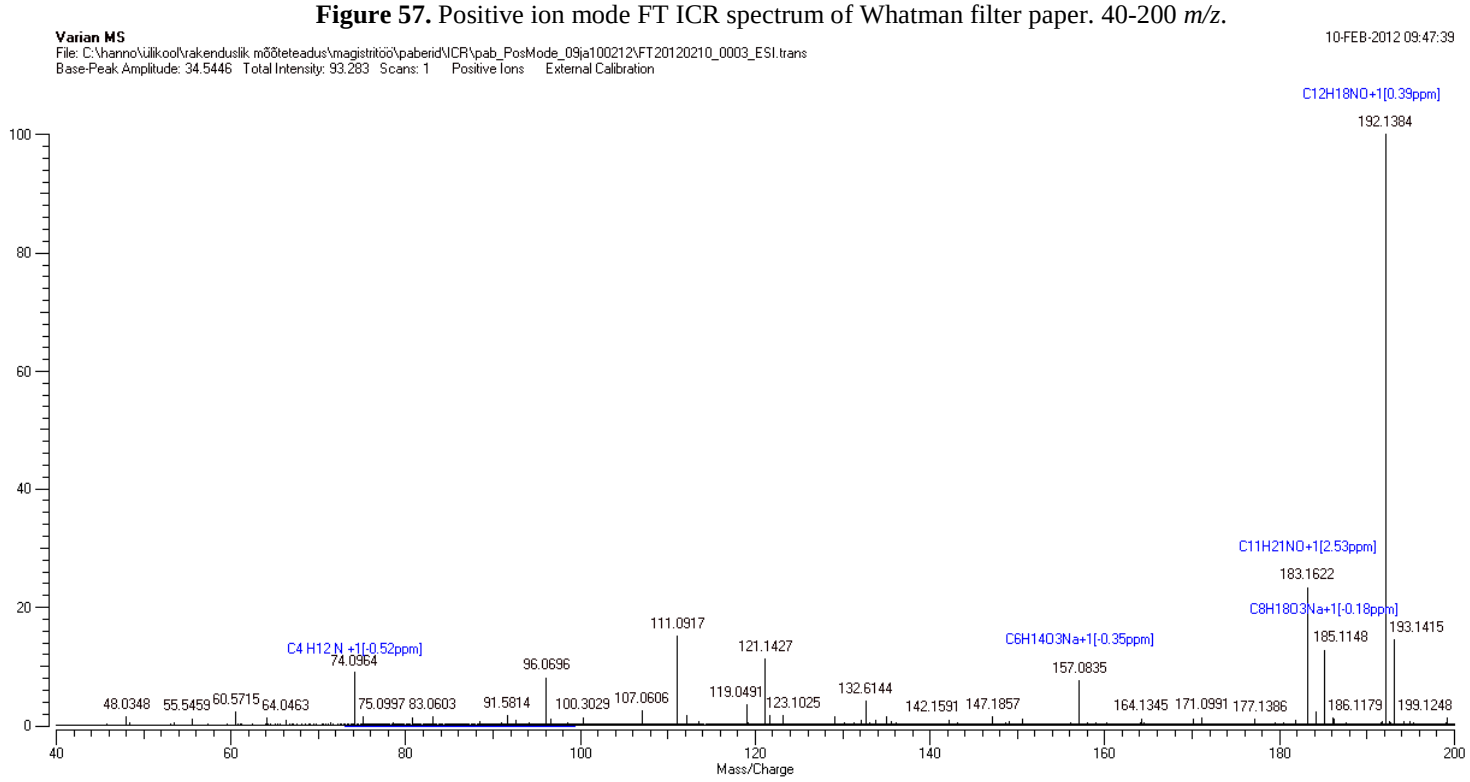


Figure 58. Positive ion mode FT ICR spectrum of Whatman filter paper. 150-400 *m/z*.

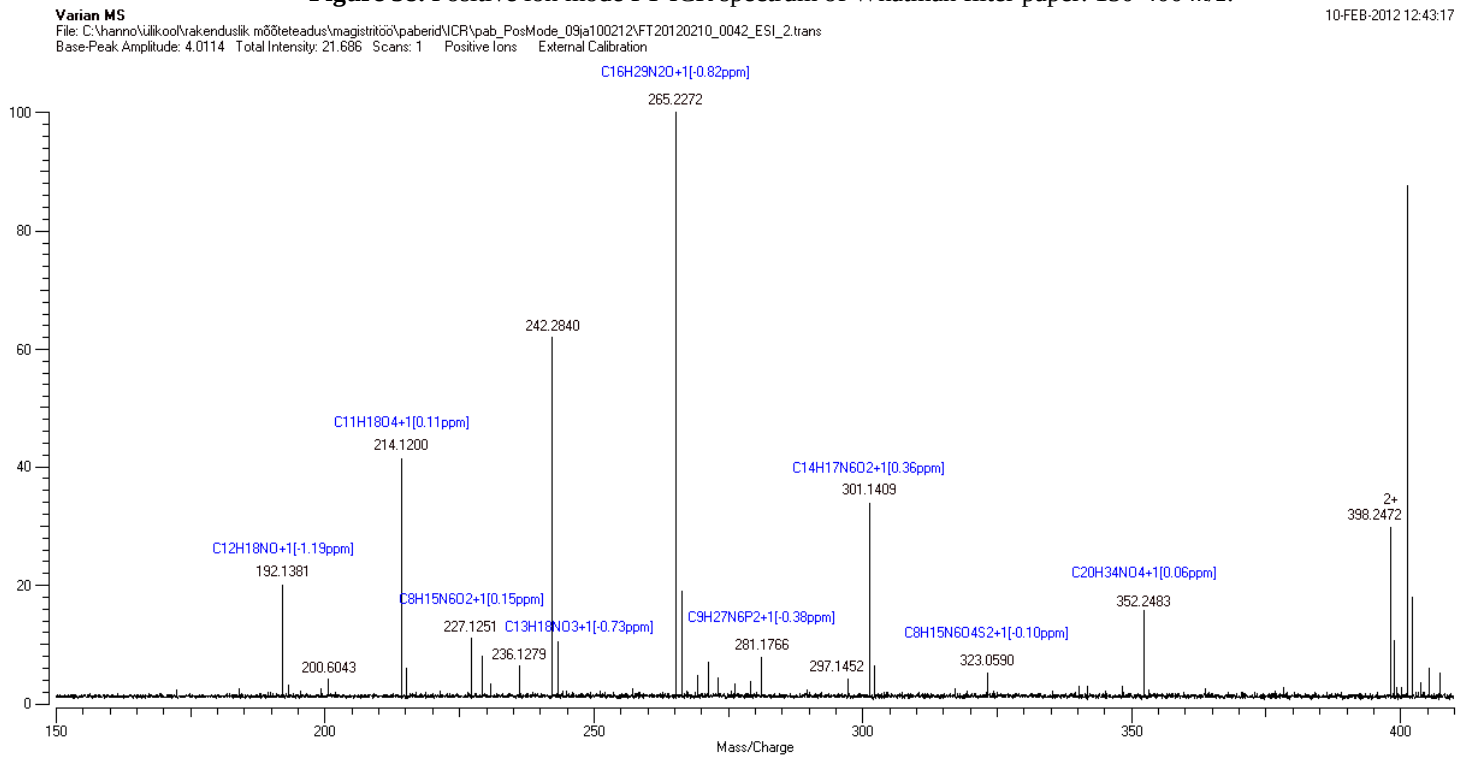


Figure 59. Positive ion mode FT ICR spectrum of Whatman filter paper. 100-600 *m/z*.

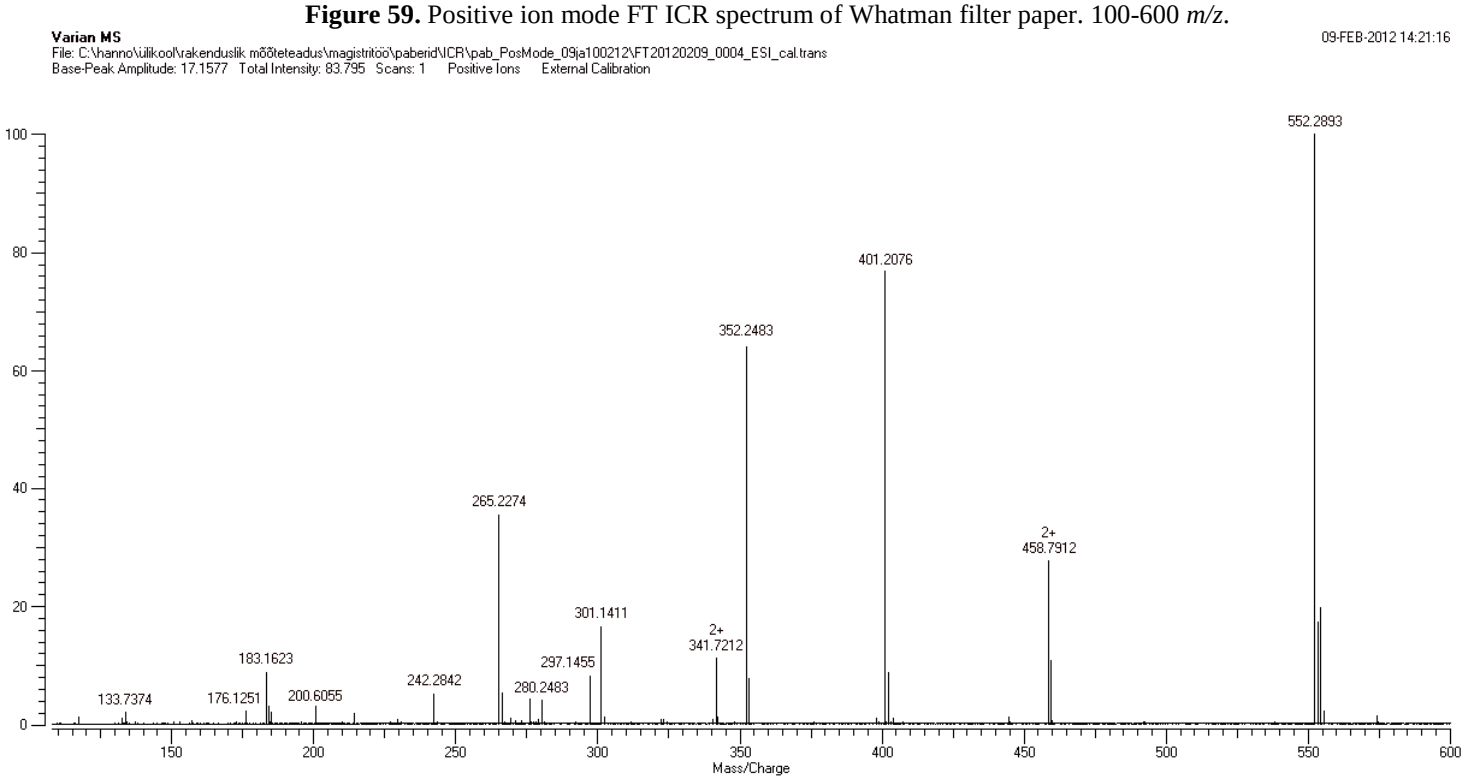


Figure 59. Negative ion mode FT ICR spectrum of Whatman filter paper. 40-200 *m/z*.

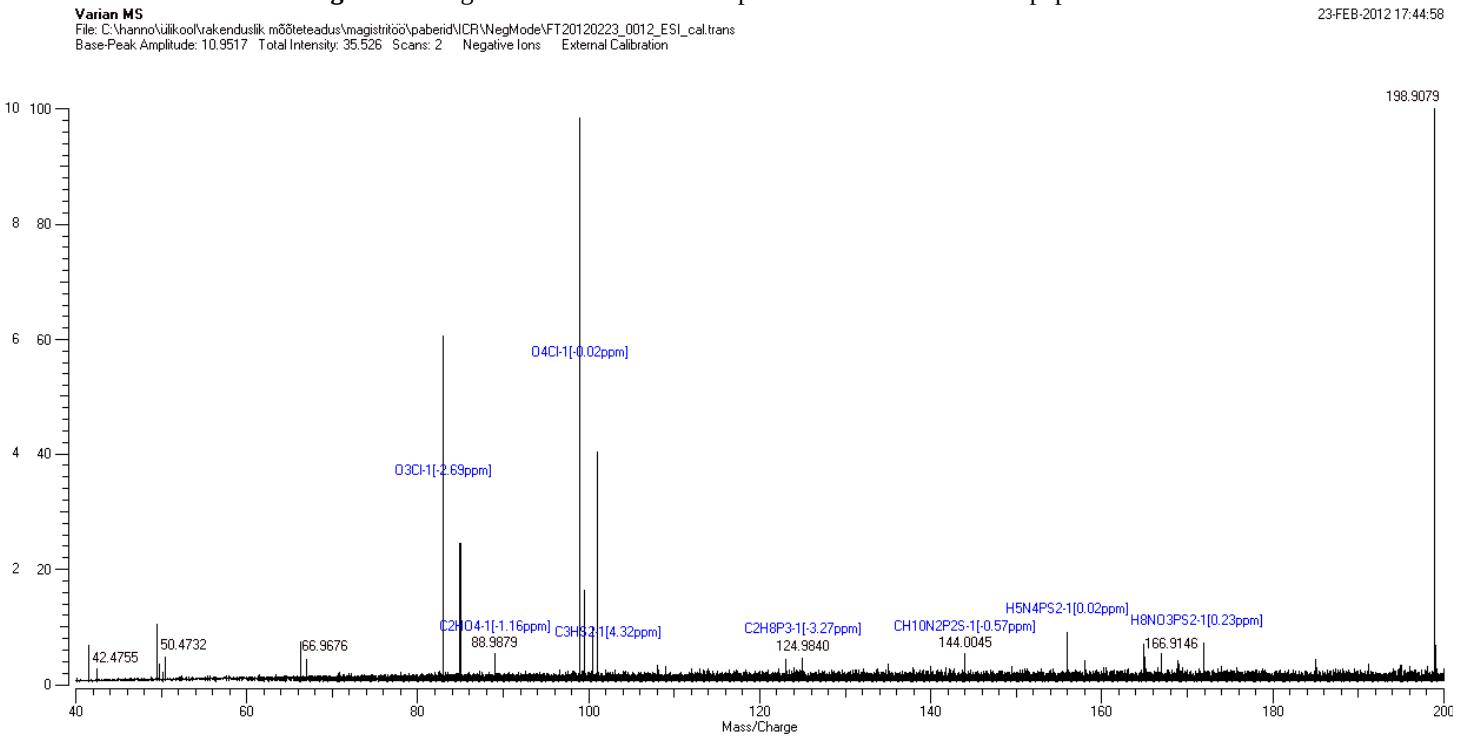


Figure 59. Positive ion mode FT ICR spectrum of clean Whatman filter paper. 40-200 *m/z*.

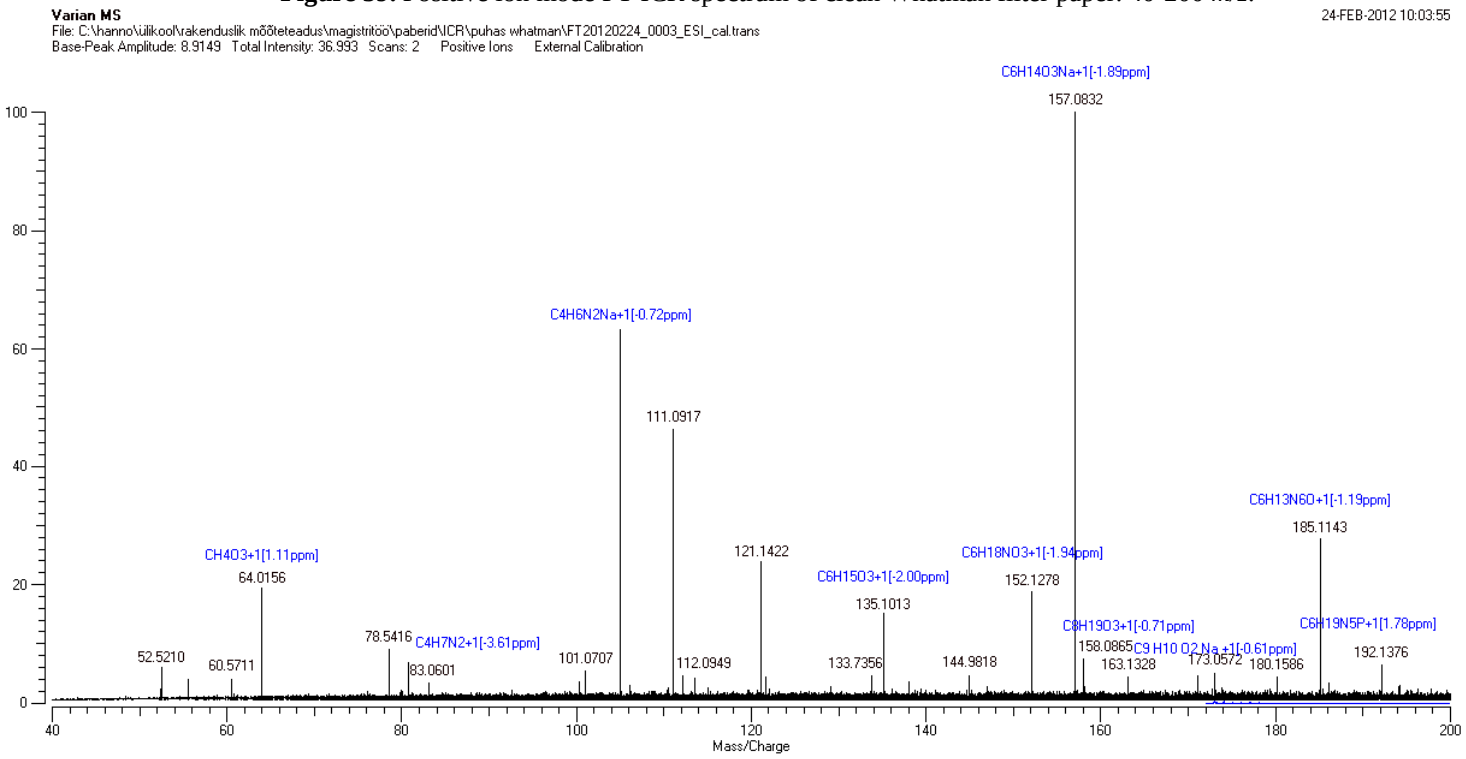


Figure 59. FT ICR fragmentation spectrum of 185 m/z ion.