Rubén Cortés Sevilla

OPTIMIZATION OF AN ULTRASONIC IONIZATION SOURCE USING A QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

TREBALL DE FI DE GRAU

Supervised by Dr. David Kilgour and Dra. Maria Elena Fernández Gutiérrez

Grau de Química

UNIVERSITAT ROVIRA i VIRGILI

NOTTINGHAM TRENT UNIVERSITY

Nottingham

2017

Acknowledgments

I would like to use this occasion to thank my supervisor Dr. David Kilgour for his excellent guidance, valuable support and constant encouragement to keep learning and working in this new field of work. Without him this project could not be possible.

Secondly, I must thank Nigel Mould and Cheryl Smith for helping me during all this time in the analytical laboratory, teaching me how to use the QTof-MS and giving me the advice to solve any kind of problem I could have during my stay.

I also take the opportunity to express my gratitude to my research group members Bijol Bakhai and Patrick Meier. We have been working in similar project during 4 months, sharing excellent moments and helping each other.

Finally, all my gratitude to my family, who encouraged me to live this experience. And how not, special thanks to Maribel, the person who has been supporting and cheering me the most during the toughest moments of this experience and the degree. Without her help, all of this would have not been possible.

Index

Acknowledgments

Abstract

Objective

1 Introduction 2

2 Fundamentals/Basis 5

2.1 QuadrupoleTime-of-flight Mass Spectrometry 5

2.2 Ambient ionization 6

3 Set up of the ionization source 9

4 Experimental part 11

4.1 Sample choice 11

4.2 Ionization source choice 14

4.2.1 Mist Generator 14

4.2.1 Acoustic Wave Nebulization (AWN) 15

4.2.2 Bottle tap 16

4.2.3 Conclusions 17

4.3 QTof modes of operation 18

4.4 Source temperature 20

4.5 Sample volume 21

4.6 Orientation effect 22

4.7 APCI needle optimization 25

4.8 Heated Bazooka Capillary effect 27

4.9 Continuous flow effect 31

5 Conclusions 35

6 Bibliography 37

Abstract

Quadrupole Time-of-Flight Mass Spectrometer is one of the best ways to identify and quantify different compounds in complex samples due to its high performance. Fast data acquisition, high resolution and high mass accuracy are few of its different advantages. However, this instrument normally has as an ionization source an Electrospray Ionization, one of the most important things that makes the QTof-MS a common choice in analytical laboratories.

The main advantage of the ESI is that it makes soft ionization, allowing to analyse big molecules without fragmenting them to little charged particles. Even though this advantage is the main factor to choose this ionization source, with ESI it cannot be optimized separately the nebulization and droplet charging.

It is wanted to find another ionization source less dependent of these parameters and easier in its working process, so all this problem can be solved with ambient ionization, specifically an ultrasonic ionization source.

All the optimization process is shown in this project, where different set ups are going to be tried in order to achieve the same value of highest peak intensity with the ESI, or as well-known in this project as sensitivity. The optimization of QTof-MS modes of operation, sample volume and source temperature is followed by trying different set ups as an APCI needle, a Heated Bazooka Capillary or different plastic tubes to see the effect of orientation.

Objective

The aim of this project is to develop and optimize an ultrasonic ionization source using a Quadrupole Time of Flight Mass Spectrometry (QTof-MS)

It is wanted to change the actual Electrospray Ionization (ESI) for the ultrasonic ionization source due to the several advantages this can provides, as for example the separate optimization of both nebulization and droplet charge, that it would be the most important.

1 Introduction

In recent years the complexity of samples and studies in analytical chemistry has been increasing, causing more difficulty obtaining high quality data for every component of interest while maintaining optimum performance throughout the analysis.[1]

Different obstacles can appear, complicating the whole analysis and forcing chemists to overtake all the possible issues in order to make meaningful measurements. A way to do it is the optimization of different parameters during the whole process, since taking the sample until the time of analysis.

Nevertheless, sometimes it can suppose an issue, because depending on the instruments used and the different method of analysis, not always they can meet the requirements for what it wanted to be analysed.

As a consequence, is necessary to assess the advantages and disadvantages of the different instruments before starting to analyse a sample, so after choosing the proper one, we can optimize its parameters.

For the scientist who needs to identify and quantify different compounds of a complex sample in the same analysis, a Quadrupole Time-of-Flight Mass Spectrometer (QTof-MS) is one of the best ways to do it. An instrument that combines the high performance of time-of-flight analysis in both mass spectroscopy (MS) and tandem MS (MS/MS) modes, with the effective ionization source electrospray ionization (ESI).[2]

In order to obtain the results for this project, it has been used the Xevo G2-XS QTof Mass Spectrometer from the company Waters with ESI as a standard ionization source. (Figure 1)[3]

Figure 1: Xevo G2-XS QTof from Waters.

The importance of the QTof-MS is given by its characteristics, because it is slightly better than the other instruments in different ways, as for example:

- Fast data acquisition rate (30 scans/sec in MS or MS/MS mode)
- Mass range up to m/z 20 to 100,000
- High resolution (>40,000)
- High mass accuracy (<1ppm RMS in infusion both in MS and MS/MS)
- Clean MS/MS spectra due to orthogonal pulsing into the Time-of-flight section

One of the most important parts of the QTof-MS that makes it a common choice in analytical departments is its ionization source, the electrospray ionization.[4]

ESI is a technique normally used to analyse biological macromolecules, such as proteins or peptides, due to its soft ionization because this process does not fragment the large molecules into smaller charged fragments.

Is for that the ESI has some advantages as an ionization source, allowing us to analyse samples with large molecular weights and biological samples with non-covalent interactions.[5]

Even though the soft ionization is the main strength, the ESI has a few more advantages that make it a well-adjusted ionization source. These are:

- Adaptable to liquid chromatography
- Adaptable to many different analysers such as an ion trap, triple quadrupole or time of flight
- No matrix interference
- Sensitive ion source
- Can work in atmospheric pressure

On the other hand, it has several disadvantages reducing the field of application or making the process more complicated:

- Presence of salts or complex mixtures can reduce sensitivity
- Simultaneous mixture analysis can be poor[6]
- Spraying into a very thin needle can produce blocking
- Inability to optimize the nebulization and the droplet charging separately

Despite in general terms the electrospray ionization is considered an acceptable ionization source, the last disadvantage can be an issue depending in the situation. Not always we want to optimize both nebulization and droplet charge, but only one of them. Also, it is wanted an ionization source less dependent in little parameters, making it simpler in its working process.

These problems can be largely avoided with one type of ambient ionization, specifically with the acoustic or ultrasonic one.

In general terms changing the ionization source into an ultrasonic one can result in same results while the working process is simpler and has cheaper costs.

In this project the ultrasonic ionization source optimization will be carried out, trying different set ups and parameters to see if the exchange of sources can be beneficial or not. The whole investigation will be made in Nottingham Trent University (Nottingham) in Rosalind Franklin Building and it will be supervised by Dr. David Kilgour.

2 Fundamentals/Basis

2.1 Quadrupole Time-of-flight Mass Spectrometer

The Quadrupole Time-of-flight Mass Spectrometer (Figure 2) has been a powerful and robust instrument for almost 25 years due to its unique capabilities that I introduced in the first point of this project. These advantages are a consequence of its functioning, as the QTof-MS is slightly more complex than the most common instruments in an analytical laboratory.[2]

The QTof-MS has a quadrupole operating as an ion guide in MS mode and as mass selector in MS/MS mode. Moreover, a time-of-flight (TOF) is orthogonally placed to the quadrupole as a mass resolving device for both MS and MS/MS modes. Between these 2 devices there is a collision cell to induce fragmentation in MS/MS experiments.

The final detector is a microchannel plate with high sensititivity.[7]

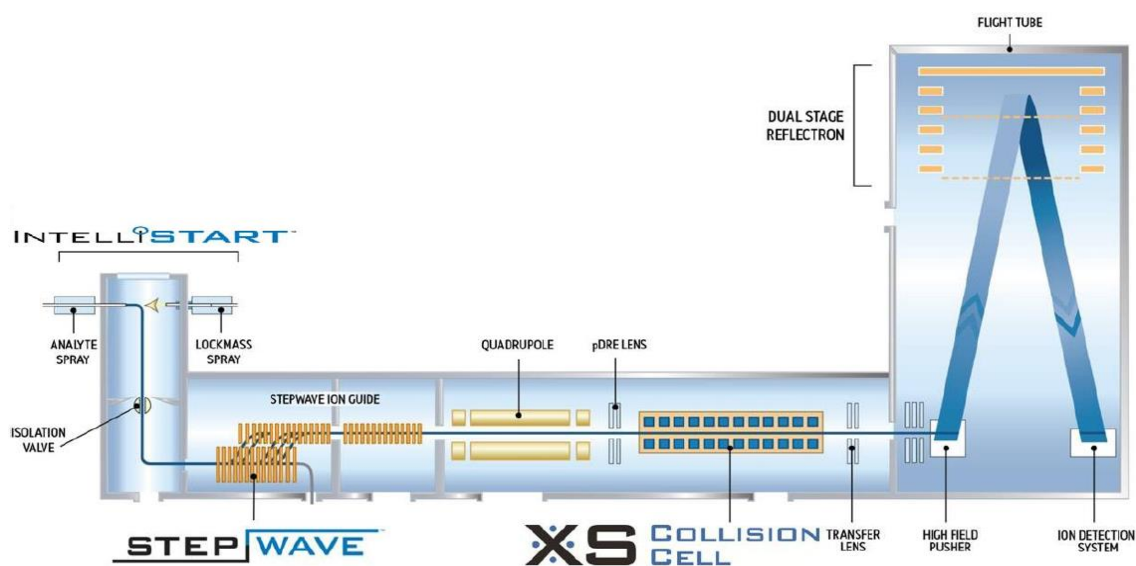

Figure 2: Xevo G2-XS QTof working process.

On the other hand, the QTof-MS mainly uses an Electrospray Ionization (ESI) (Figure 3)[8]. The ESI working process consists in pumping a dilute solution of analyte through a capillary at a very low flow rate while a high voltage is applied to it, that can be positive or negative depending on the analytes chosen.

This voltage provides the electric field gradient needed to produce charge separation in the sample while it is nebulized.[9]

As it is known, this ionization source can be changed into another one in order to solve many disadvantages, so this is why in this project the optimization of an ultrasonic ionization source is going to be tried.

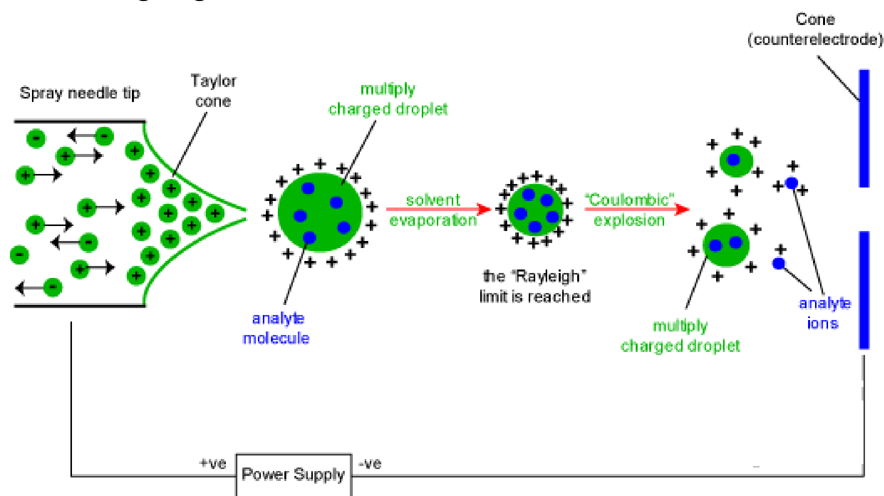

Figure 3: ESI working process.

2.2 Ambient ionization

Ambient ionization is a way of ionization which ions are formed out of the mass spectrometer in an ion source with little sample preparation, at ambient conditions and that does rapid analyses.[10 11]

The main advantage of this technique is it works in ambient conditions. Due to this, the ionization occurs at atmospheric pressure while in other sources occurs at vacuum.

Moreover, it can do analyses of a wide range of substances from various surfaces and complex matrices. These samples can be allowed to keep their chemical / physical / biological states before the ionization without external interference.[12]

Ions' production can happen by different techniques such as:

- Extraction
- Plasma
- Laser or Non-Laser
- Acoustic desorption.

The field of this project is focusing in the use of acoustic desorption. This is because this technique is simpler in its working process than the other ones obtaining the same results.

Acoustic desorption consists in desorbing the analyte generally by a piezoelectric element or through an induced laser acoustic wave generating an aerosol plume. The plume is in its majority neutral, however because of the nebulization it is slightly charged.

Following the spray, the neutral plume can be ionized by reactive ion species or charged solvent droplets from an external ionization source, as for example an Atomic Pressure Chemical Ionization (APCI).[10]

Acoustic desorption englobes different techniques that have variations in their working process between them. Few examples of these can be Surface Acoustic Wave Nebulization (SAWN), Ultrasonication-assisted spray ionization (UASI) and Radiofrequency Acoustic Desorption and Ionization (RADIO).

Regarding this project, we have 3 different devices to start the development and optimization of the source.

- Mist generator (Figure 4)
- Acoustic Wave Nebulizer (AWN) (Figure 5)
- Bottle top humidifier (Figure 6)

Figure 4: Mist generator.

Figure 5: Acoustic Wave Nebulizer (AWN)

Figure 6: Bottle top humidifier

Regarding the Acoustic Wave Nebulizer (AWN), a Mist Generation Transducer (2.8 MHz) was bought in STEMiNC's webpage (STEINER & MARTINS, INC.)[13]. To complete the device a support was made with a 3D-printer in Nottingham Trent University in Physics Building. Regarding the Mist generator[14] and the Bottle top humidifier[15], both were bought in eBay.

These 3 devices use piezoelectric technology even they have little variations.

This technology is understood as an electric charge that accumulates in solid materials as crystals or ceramics when a mechanical stress is applied to it.[16]

The 3 ultrasonic devices are nebulizers that use a bulk piezoelectric crystal that generates acoustic signal at high frequency. This signal travels through the liquid towards the liquid surface inducing capillary waves on it that result in producing aerosol. This way to produce nebulization and aerosol is very simple and efficient, however, it tends to overheat when it is in constant use during a long period resulting in a poor nebulization.

Talking about the AWN and the Bottle Top Humidifier, they consist of a mechanically vibrating plate perforated with micro apertures in both sides. The liquid is in contact with the top surface of the mesh which is assembled with a metallic holder and a piezoelectric ring actuator. This piezoelectric ring vibrates and excites the mesh pushing the liquid through the apertures of the top surface and expulsing aerosol droplets by the other side.[17] (Figure 5)[17]

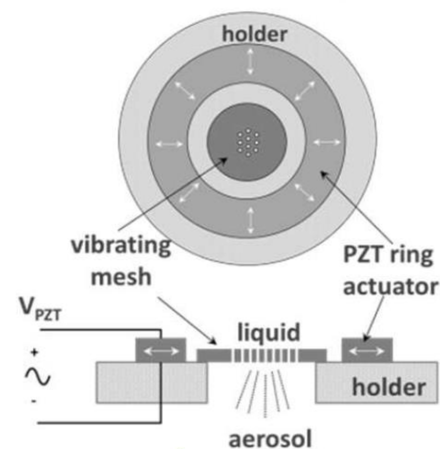

Figure 5: Mesh nebulizer

About the Mist generator, this device has the same piezoelectric technology as the other 2 mentioned before. However, it does not have the micro apertures in both sides but it only consists in forming the aerosol on the top surface due to the excitement of the mesh.

3 Set up of the ionization source

For the following optimization using the ultrasonic ionization sources, it was necessary to remove the ESI. Before this, we had to look that the instrument was on 'Standby'.

During the protocol to follow, the attached tubes of the ESI (ESI inlet tube and Lockspray tube) were removed. The cables inside the MS door were also disconnected before removing the whole source. (Figure 6 and 7)

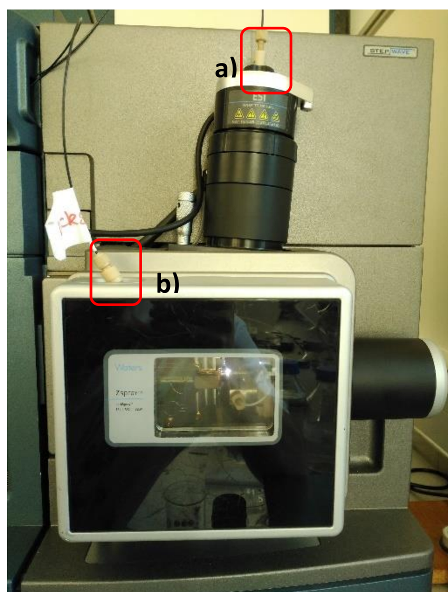

Figure 6: a) ESI inlet tube
b) Lockspray tube

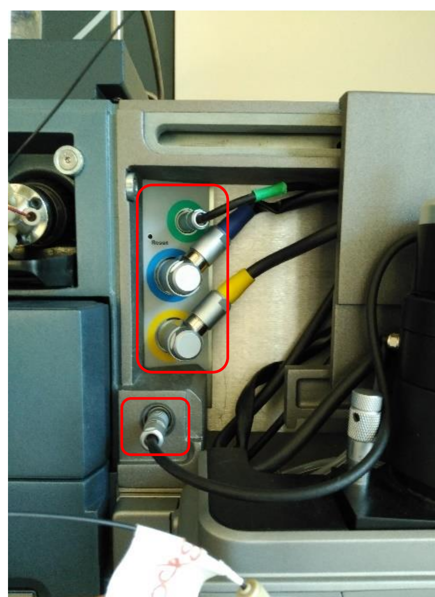

Figure 7: Cables inside the MS door.

Once they were removed, we could take out the ESI and connect a little metal piece in the place of the blue cable removed before, below the label 'Reset'. Another self-made metal plate was placed in the sensors shown in the next figure. (Figure 8 and 9)

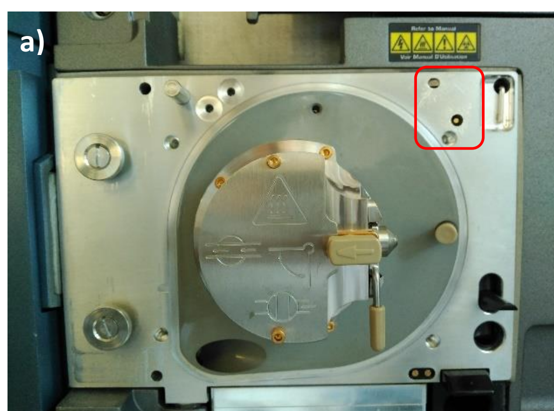

Figure 8: a) Source when the ESI is removed marking the sensors b) Sensors zoomed

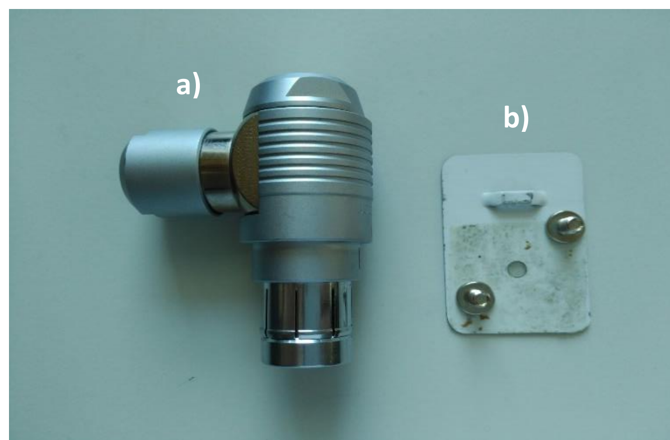

Figure 9: a) Metal piece to insert in 'Reset' place
b) Plate to cover the sensors

The final part of setting up the new ultrasonic ionization source was closing the door with the cables and putting some tape to the metal plate to make sure that sensors were completely sealed.

To make sure that all the process was correctly followed, we had to put the QTof-MS in 'Operate' and select 'Nanoflow source'.

If none of these steps were made wrong, a green light in the window MS Tune had to appear, letting us know that the set up for further experimentations with ultrasonic ionization sources was ready.

4 Experimental part

This part includes all the choices that have been carried out during the optimization process.

4.1 Sample choice

To start the development and subsequent optimization of the ultrasonic ionization source, it was necessary to choose a sample to use during the whole development process. This sample must have an easy spectrum with noticeable peaks to distinguish it from possible variations.

The sample chosen for the process was lager beer, specifically a 440 ml Fosters can, that it was a type of pale coloured beer.

The spectrum of Fosters beer was the following one. (Figure 10)[18]

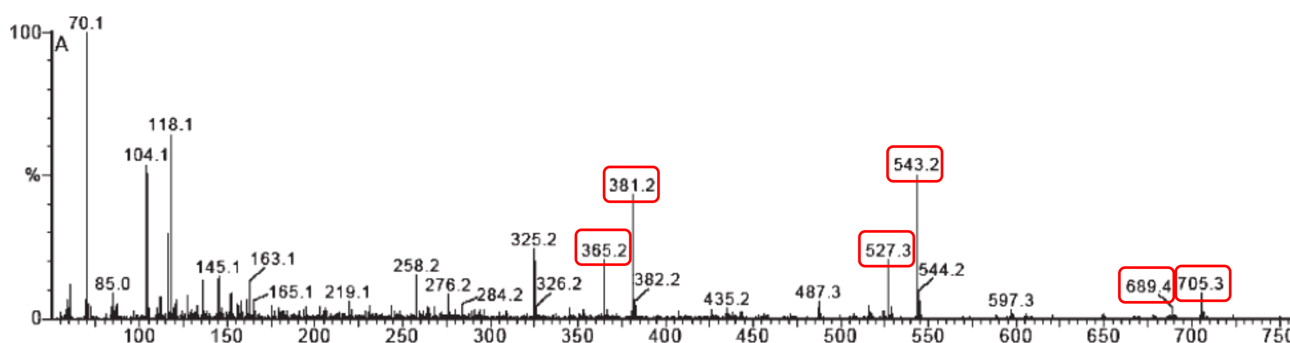

Figure 10: ESI pale coloured beer mass spectrum

This spectrum had some typical peaks in the m/z range of 70 to 750 corresponding to sodium $[M + Na]^+$ and potassium $[M + K]^+$ adducts. These adducts were quite resistant towards dissociation, so each ion was a representation of a single component of the mixture.

As it is known beer is a complex mixture with different compounds in it such as proteins, nucleic acids, lipids and carbohydrates. In this last group of compounds are included saccharides, important component for the flavour.[19]

In the spectrum, we see saccharides due to there are the major component in beer, being the others at low concentrations. Moreover, in this spectrum the m/z range is between 70 to 750, where possibly other components do not appear. Regarding the spectra obtained in the bibliography, we decided to focus only in these peaks, the saccharides.

Thanks to this, different typical peaks can be seen in the spectrum, as for example the adducts of maltose (m/z 365 and 381), maltotriose (m/z 527 and 543) and maltotetrose (m/z 689 and 705).

These peaks are a reliable reference to confirm if during the optimization process we are obtaining the same spectra.

ESI spectrum of Fosters was made to see if the typical peaks coincide with the ones in the bibliography. Moreover, it was also wanted as a reference spectrum to see the highest peak intensity, or as we will say from now on, sensitivity, so during the optimization we could compare the sensitivities obtained.

In default of optimization the spectrum was made in Sensitivity mode, Ion Positive Mode, MS mode and the following parameters (Figure 11):

ES+ parameters	
Capillary (Kv)	3,00
Sampling Cone	200
Source Offset	150
Source Temperature (ºC)	150
Desolvation Temperature (ºC)	250
Cone Gas (L/h)	50
Desolvation Gas (L/h)	600

Fluidics parameters	
Infusion Flow Rate (µl/min)	10,0
Flow State	Infusion
Fill Volume (µl)	50
Reservoir	A

Figure 11: a) ES+ parameters
b) Fluidics parameters

The spectrum was made in the m/z range of 100 to 2000 to see if there were important peaks that were not in the reference spectrum of the bibliography.

The spectrum obtained was the following one (Figure 12):

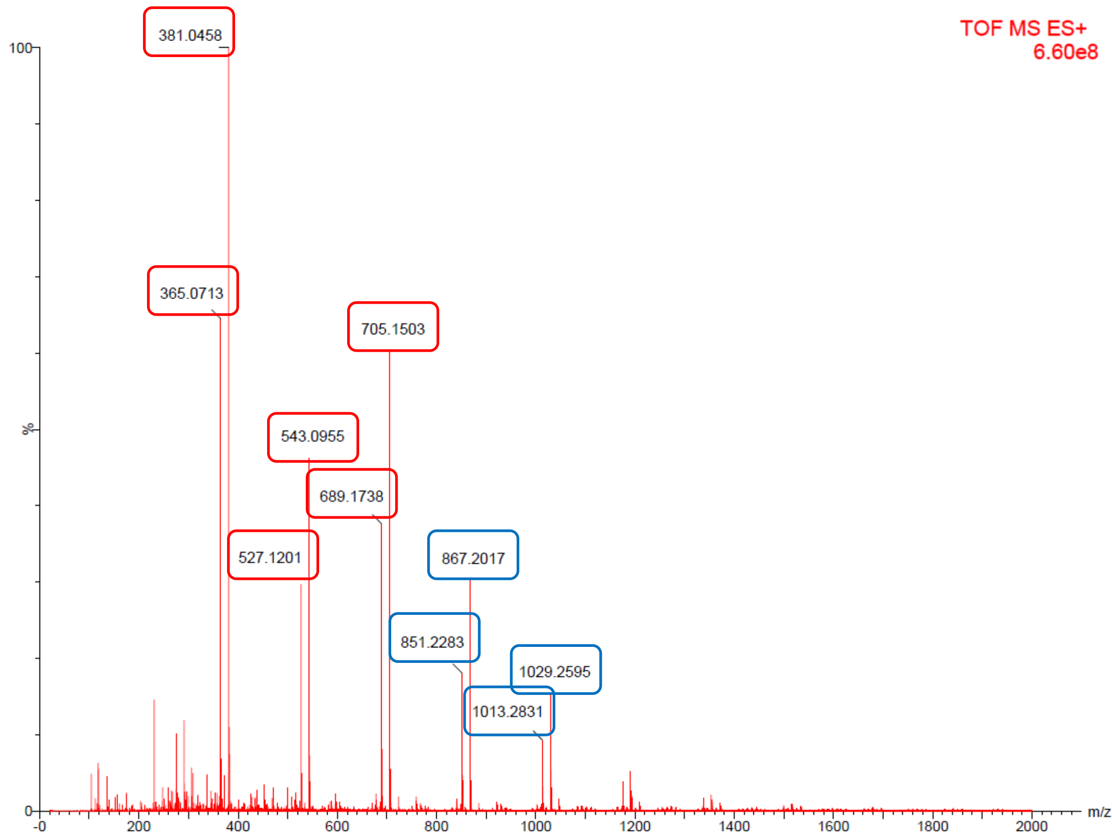

Figure 12: ESI Fosters mass spectrum

As it can be seen, in this spectrum are the 6 typical peaks that we could see in the ESI pale coloured beer spectrum (Figure 6) as well as 4 different peaks corresponding to the adducts of maltopentose (m/z 851 and 867) and maltohexaose (m/z 1013 and 1029).

Moreover, a sensitivity of $6{,}60 \cdot 10^8$ was obtained. To see how the optimization was going we had to compare the subsequent spectra with this one in order to check if a sensitivity of 10^8 or similar can be obtained with an ultrasonic ionization source.

Even though the first thing to achieve was the highest possible sensitivity, we also had to check the peaks of the spectra. A different shape of the spectra could indicate that we made a mistake during the optimization or the sample was not clean.

4.2 Ionization source choice

Before starting the optimization, choosing the best ultrasonic ionization source between the 3 we had at our disposal was needed.

To made this decision the 3 sources were tried in the same conditions in order to see the sensitivity comparison and other factors could affect the decision.

The experiments were made focusing the 3 devices directly to the inlet. Also, they were made with 10µl of sample and the following parameters (Figure 13):

Nanoflow+ parameters	
Capillary (Kv)	3,00
Sampling Cone	200
Source Offset	150
Source Temperature (ºC)	150
Cone Gas (L/h)	50
Nano Flow Gas (Bar)	0,30
Purge Gas (L/h)	350

Figure 13: Nanoflow+ parameters for the 3 experiments

Due to the sample was injected directly in the inlet, we did not obtain a chromatogram with different compounds shown by retention time, otherwise we had a chromatogram showing different peaks that were the single injections we made. No matter the experiment, the spectra obtained was the correspondent to the highest peak in the chromatogram.

4.2.1 Mist Generator

As it is already known, the Mist Generator does not have micro apertures in both sides, so it was difficult to orient the spray to the inlet. Due to his lack of micro apertures, the liquid in the surface sprayed in every direction and did not focus in one point as it was required.

Moreover, because of the shape of the device, the orientation to the inlet was difficult, and consequently, reproducibility for following experiments would not be good. The spectrum obtained was the following one (Figure 14):

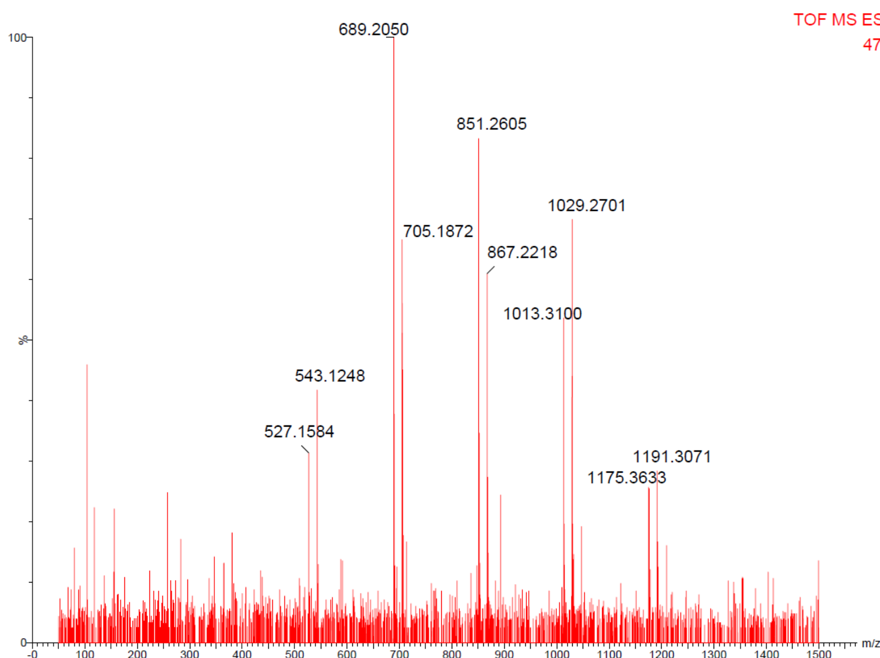

Figure 14: Mist Generator mass spectrum

Although the typical peaks could be seen, the sensitivity was way too low (479) from the one we wanted to obtain. This could be due to the problems of the device mentioned before.

4.2.1 Acoustic Wave Nebulization (AWN)

In contraposition to Mist Generator, the AWN has micro apertures in both sides, so the spray had better orientation to the inlet. To help the spray, the strength of spray could be changed with a little screwdriver, however, there were not numbers to know the strength value and make it reproducible.

In addition, this device had a support made by a 3D-printer, so it was easier to make the experiment and orient the spray.

The spectrum obtained was the following one (Figure 15):

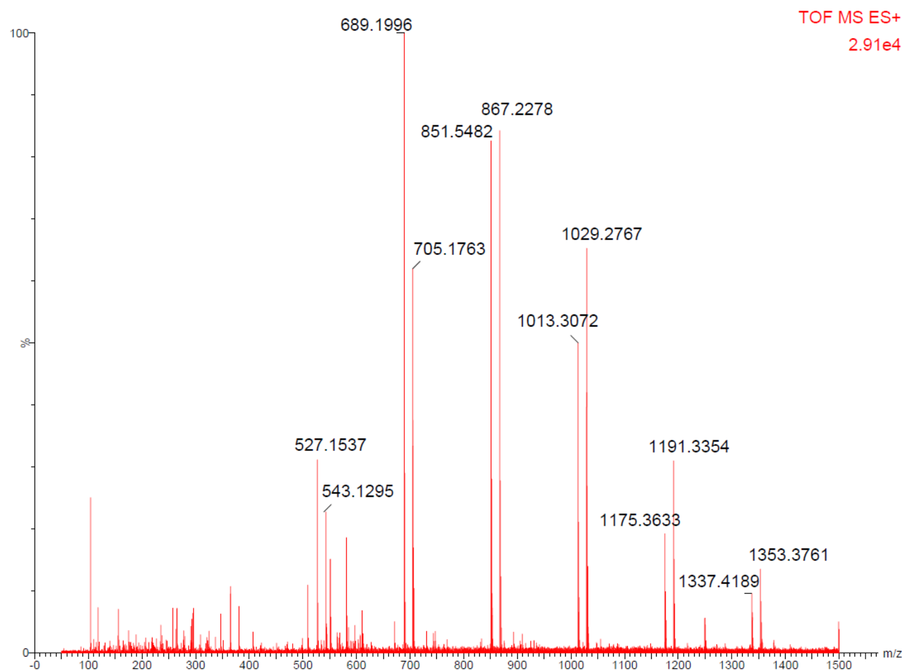

Figure 15: AWN mass spectrum

In this case, the spectrum obtained was good. The typical peaks could be seen while the sensitivity was high ($2{,}91 \cdot 10^4$).

4.2.2 Bottle top humidifier

The bottle top humidifier used the same technology as the AWN with slightly variations in its functioning. The device we used for the experiment was a modification of the original we bought in internet, as we removed the plastic support from the piezoelectric plate to put it in a new plastic device to make the set up easier.

However, it was more difficult to produce a proper spray and make it reproducible because the micro apertures on the bottom side needed a humid piece of paper on the surface to work. With this little piece of paper, the sample was not in direct contact with the plate, being maybe a factor of good spray.

If there was not the piece of paper, the nebulization did not occur, or if it did it, the spray spread in every direction.

The spectrum obtained was the following one (Figure 16):

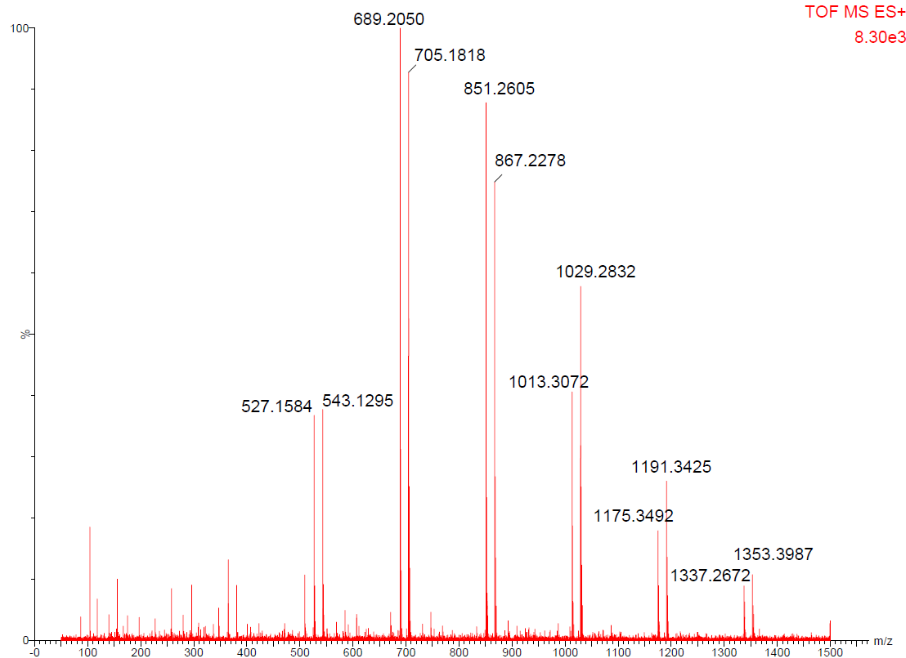

Figure 16: Bottle top humidifier mass spectrum

In this spectrum, all the typical peaks appeared and the sensitivity was not so bad ($8,30 \cdot 10^3$).

4.2.3 Conclusions

To choose the ultrasonic ionization source for the subsequent optimization we had to compare the spectra obtained and the different problems we had during the experiments.

First of all, we had to look the sensitivity. Comparing the 3 spectra, the one with the highest sensitivity was with the Acoustic Wave Nebulization ($2,91 \cdot 10^4$), followed by the Bottle top humidifier ($8,30 \cdot 10^3$) and the Mist Generator (479). As we could see, the AWN was the best ionization source to start the optimization.

On the other hand, if we focused on the different problems of the different devices, we could see that experiments with AWN were more reproducible than the Bottle Top as we could change the spray strength.

To conclude, the subsequent optimization would be done with the AWN, as it was the best choice to start.

4.3 QTof-MS modes of operation

Once we had the ultrasonic ionization source to start the process, there were certain parameters of the instrument that we had to optimize such as:

- Sensitivity or Resolution mode
- MS or MSMS mode
- Ion positive mode or Ion negative mode

The QTof-MS could work in different modes of operation that could change the shape or the sensitivity of the spectra, so it was necessary to stablish the optimum modes.

Firstly, there were 2 principal modes of operation that had to be try. These were Sensitivity and Resolution mode. (Figure 17 and 18)

The experiments were made in MS mode, Ion Positive mode, 150 Cº source temperature and 5µl of sample volume.

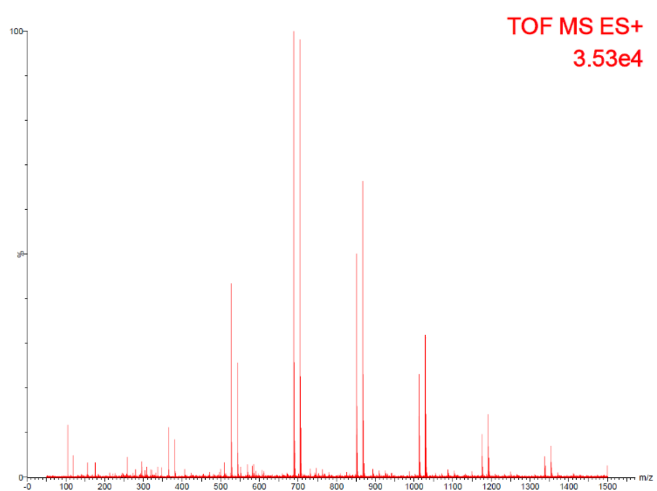

Figure 17: Sensitivity mode mass spectrum

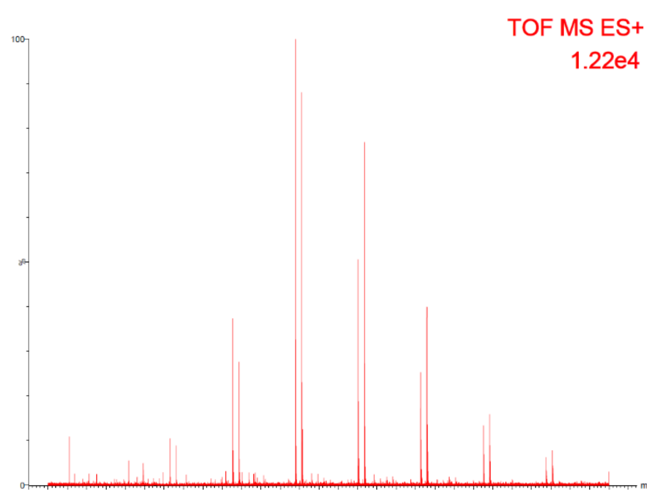

Figure 18: Resolution mode mass spectrum

As it was expected, with Sensitivity mode we obtained more sensitivity ($3,53 \cdot 10^4$) than with the Resolution mode ($1,22 \cdot 10^4$). Moreover, the difference between the 2 spectra was not as big as we thought, however, in future experiments Sensitivity mode was used because this mode focused more in having better sensitivity rather than resolution.

Also, QTof-MS could work in MS and MSMS mode, allowing with this last one more fragmentation to check the structure of the molecules. Although we did not want this, we compared the sensitivities between modes. (Figure 19 and 20)

The experiments were made in Sensitivity mode, Ion Positive mode, 150 Cº source temperature and 5µl of sample volume.

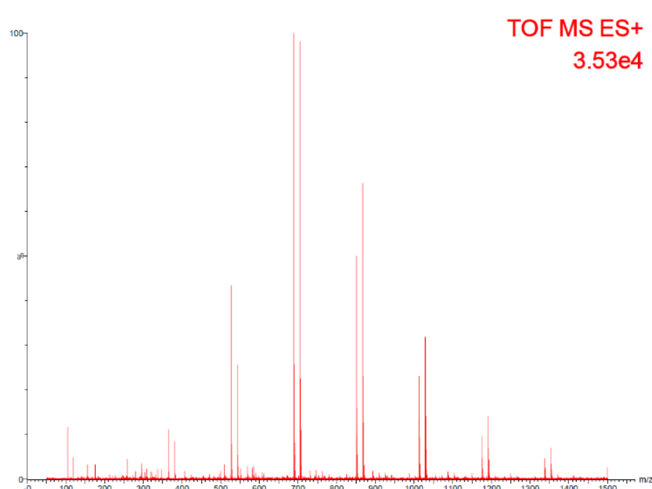

Figure 19: MS mode mass spectrum

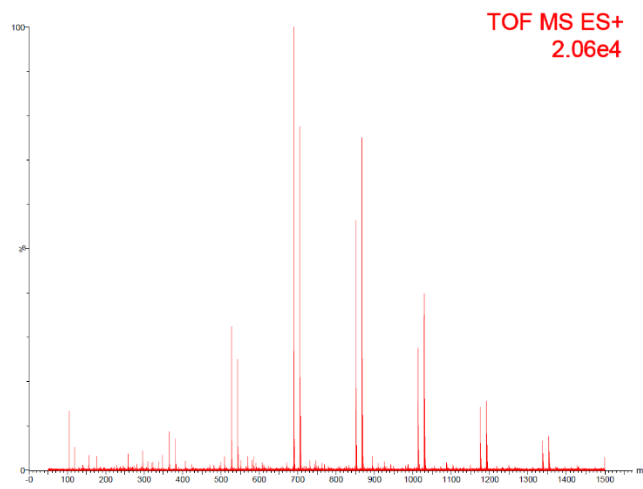

Figure 20: MSMS mode mass spectrum

As we could see, we obtained more sensitivity in MS mode ($3,53 \cdot 10^4$) than in MSMS mode ($2,06 \cdot 10^4$). For this reason and because we did not want the more fragmentation, MS mode was chosen for the optimization.

Another parameter to optimize was the Ion positive or Ion negative mode. The ion positive mode is for molecules with more affinity to gain a proton than lose it. A lot of organic compounds are like this, so this was why ion positive mode was chosen at first. However, to be sure, Ion Negative mode was tried too. (Figure 18 and 19)

The experiment was made in Sensitivity mode, MS mode, 150 Cº source temperature and 5µl of sample volume. (Figure 21 and 22)

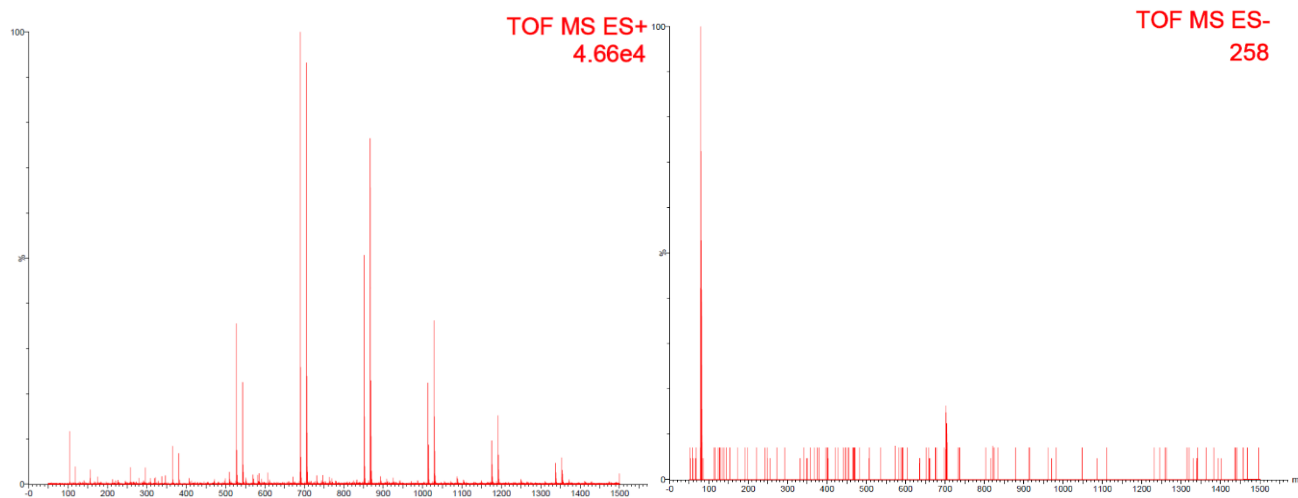

Figure 21: Ion Positive mode mass spectrum

Figure 22: Ion Negative mode mass spectrum

In the Ion Negative mode mass spectrum, we could see that there was not almost signal and the spectrum did not have the shape of the reference spectrum. This happened as we expected, so finally the chosen mode was the Ion Positive.

For further experimentation and due to this optimization, Sensitivity mode, MS mode and Ion Positive mode would be the parameters used.

4.4 Source temperature

Regarding the source temperature, the standard temperature of the instrument was 150 Cº. This parameter helps the desolvation, so theoretically, the hotter the better. To run the experiment, we tried a temperature 50 Cº lower than the one we had. (Figure 23 and 24).

This parameter was the source temperature, so the sample was nebulized and directly entering the inlet. The result of this effect could be different if the sample was heated before entering the inlet. This experiment would be made in a following set up.

The experiment was made in Sensitivity mode, MS mode, Ion positive mode and 5µl of sample volume.

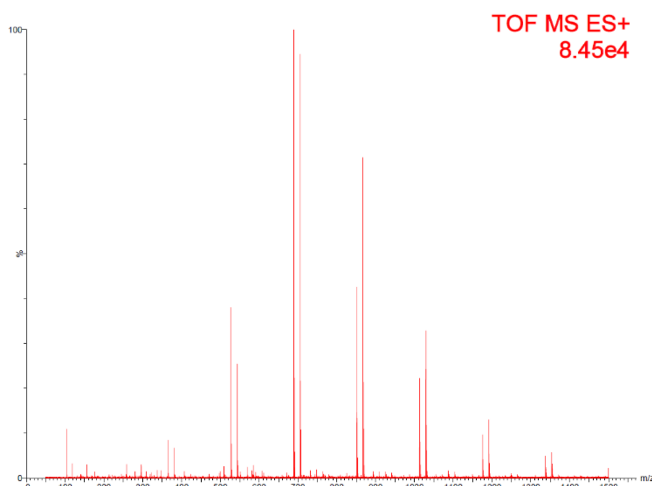

Figure 23: 150 Cº source temperature mass spectrum

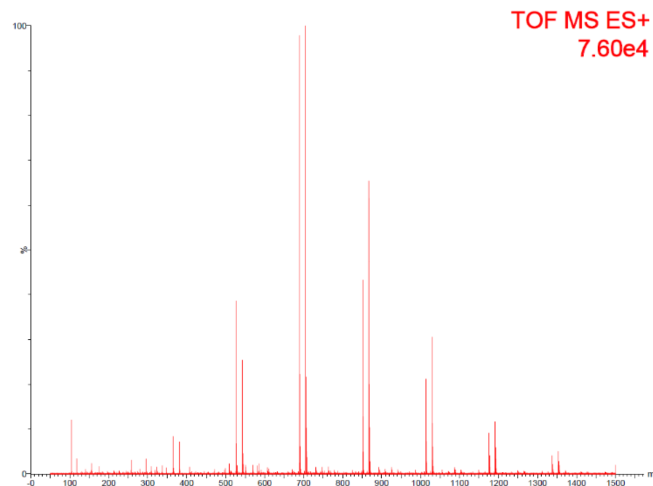

Figure 24: 100 Cº source temperature mass spectrum

This change of temperatures did not make a big difference between spectra, as with 150 Cº we obtained a sensitivity of $8,45 \cdot 10^4$ and with 100 Cº $7,60 \cdot 10^4$. Anyway, the following experiments would be made in 150 Cº as we obtained good results. Nevertheless, in future experiments when the ultrasonic source working process would be optimized the temperature parameter could be a factor to optimize again.

4.5 Sample volume

Finally, to optimize the basic parameters to start, we had to look the difference between sample volumes. (Figure 25 and 26)

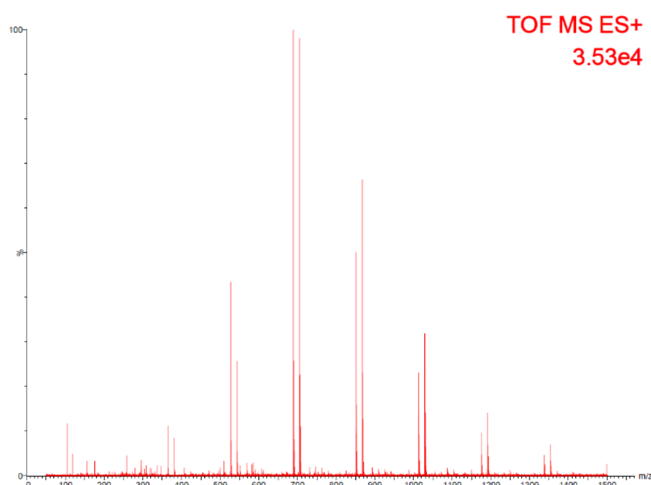

Figure 25: 5µl sample volume mass spectrum

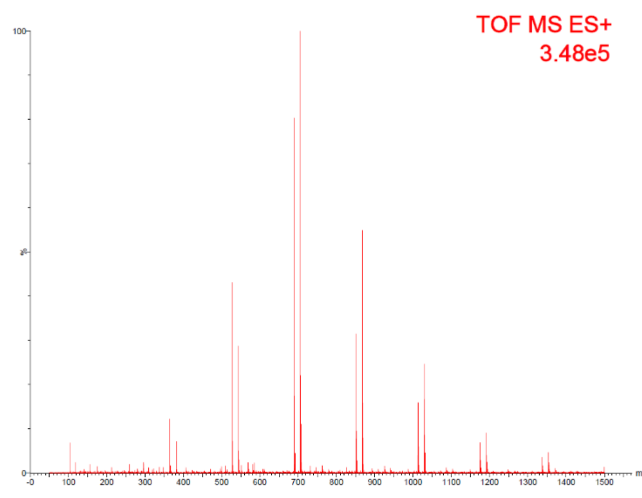

Figure 26: 10µl sample volume mass spectrum

Firstly, we made the experiments with 5 µl as we obtained good results. However, we wanted to double the volume (10µl) to see if the sensitivity or the spray was better.

The experiment was made in Sensitivity mode, MS mode, Ion positive mode and 150 Cº source temperature.

In this spectra we could see a big increase in the sensitivity. With 10 µl we obtained 10 times more sensitivity ($3{,}48 \cdot 10^5$) than with 5 µl ($3{,}53 \cdot 10^4$). This could be in part for the volume we injected, so more sample got into the inlet producing higher peaks. Moreover, with 10 µl the spray was quite better. For these reasons the final sample volume was 10 µl.

Higher sample volumes were not tried at first because we wanted to optimize the process with the smallest amount of sample volume possible, so we considered that more than 10 µl was too much. Higher volumes could be tried in the future, but for following experiments 10 µl would be the sample volume used.

4.6 Orientation effect

In the previous optimization experiments, the AWN was positioned in front of the inlet as it was the easiest and most direct way to do the injection. Nevertheless, the direction of the source could affect the sensitivity, so this was why a different orientation was tried.

A problem of having the ultrasonic ionization source just in front of the inlet was that the whole spray comes into the instrument, having the big droplets that did not make signal and contaminated the spectrum.

Instead of this lineal orientation, we made a plastic tubes with a bend of 90º that could be used to inject the sample. This could help the experiment because the biggest droplets would stop on the curvature and only the smallest ones would enter through the inlet, having better sensitivity and less contamination

Also, these tubes could make the spray focusing better as the sample was travelling through longer distance. To help the focusing, we made little holes at the end of the tube to let some air pass to push the sample into the inlet.

Thanks to these tubes, we could expect an increase of the desolvation, signal stability and droplet focusing.

To run the experiment, we made 3 plastic tubes with different height to see if there was a correlation between sensitivity and the distance the spray travels. (Figure 27, 28 and 29)

Figure 27: 1 cm entry into the tube

Figure 28: 4 cm entry into the tube

Figure 29: 8 cm entry into the tube

All the tubes were tried with the same conditions, using the AWN with 10 µl of beer and the same power of spray. The spectra obtained was the correspondent to 8 injections during 5 minutes. (Figure30)

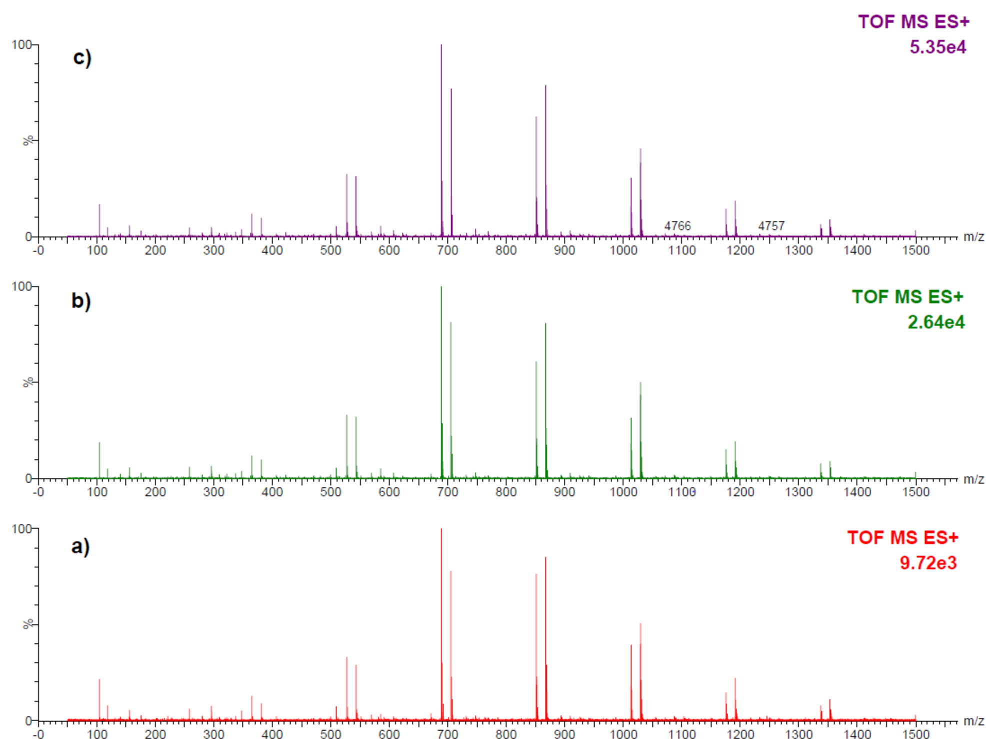

Figure 30: a) 1 cm tube spectra b) 4 cm tube spectra c) 8 cm tube spectra

Between these 3 spectra there was not a big difference between sensitivities. This result was not expected, as when we optimized the sample volume, 10 µl gave us a sensitivity of 10^5, 10 or even 100 times more than with the tubes. Even though the results are quite worse, the spray was better, both the focusing and the strength.

Due to this unexpected result, we made the same experiment with the same conditions 2 more times to observe any solution.

With the first new experiment, we obtained sensitivities of $2{,}58 \cdot 10^3$ (1 cm tube), $3{,}54 \cdot 10^5$ (4 cm tube) and $5{,}17 \cdot 10^5$ (8 cm tube). While in the second experiment we obtained sensitivities of $7{,}46 \cdot 10^4$ (1 cm tube), $3{,}27 \cdot 10^4$ (4 cm tube) and $6{,}12 \cdot 10^5$ (8 cm tube).

As we could see, there was an increase of the sensitivity being the highest with the 8 cm tube. Nevertheless, in the 3 experiments there was not a correlation between sensitivities, observing an important issue of the ultrasonic ionization source, specifically the AWN. Bad reproducibility.

This problem could be a consequence of 2 different factors:

- Distance between the inlet and the source
- Spray strength

We tried to reproduce the same parameters in these 2 factors during the whole experiments, but regarding the distance between the inlet and the source, it was difficult to measure exactly, as a little variation could affect in a big way.

On the other hand, the spray strength could be changed poorly, as the method to do it was turning a screw without any indication to know what amount of strength we had.

Despite the bad reproducibility of the ultrasonic ionization source, we opted to use the 8 cm tube for further experiments as it was the one with highest sensitivity.

4.7 APCI needle optimization

Up to this moment, all the experiments were carried out without external ionization, just with the ultrasonic ionization source nebulization. For this reason, external sample ionization was wanted to see in the case there were any advantages.

Atomic Pressure Chemical Ionization (APCI) is a soft ionization method which uses gas-phase ion-molecule reactions at atmospheric pressure. The analyte is nebulized in a heated nebulizer that collides with a N_2 flow producing a gas. The gas, at atmospheric pressures, passes through a corona discharge charging the droplets. [20]

In our case, the nebulization occurred in the AWN at atmospheric pressure, so we needed the corona discharged needle to ionize externally the sample. (Figure 29)[21]

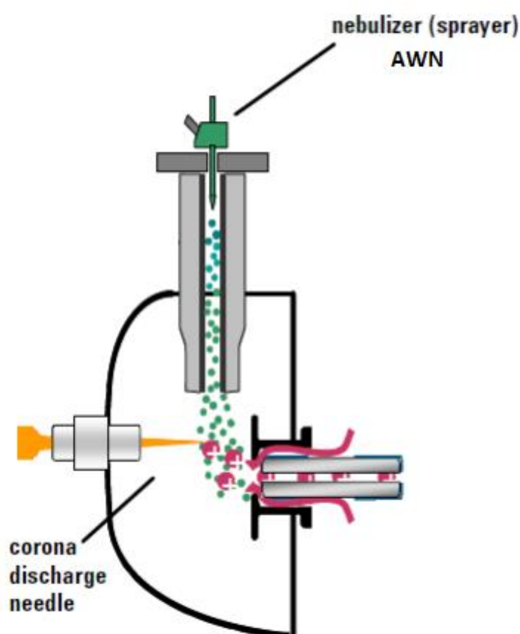

Figure 31: APCI working process

With the APCI needle, we could apply voltage to it and see how the sensitivity variated. The experiment was tried with the 8 cm tube mentioned before, but to set it up, we had to make a little hole in the tube in order to put the needle and connect it to the power supply. (Figure 30 and 31)

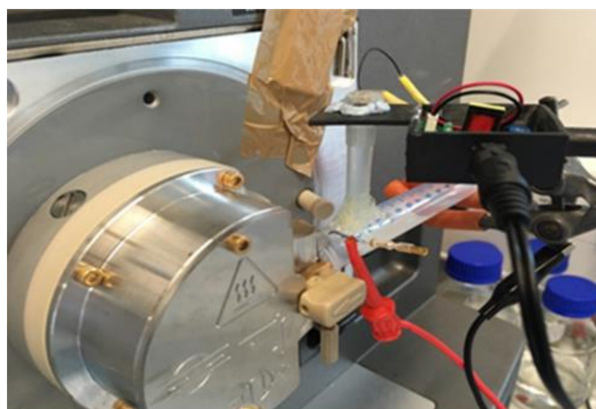

Figure 32: Set up of APCI experimentation

Figure 33: Power supply

Once the set up was ready, we had to apply voltage to the needle and carry out the experiment. The power supply had a voltage range of 0-2000 volts, so we analysed the beer sample with the voltage increasing every 200 volts, from 200 to 2000.

To obtain the best reproducibility possible, each voltage applied was measure injecting 2 times 10 µl of sample and maintaining the spray strength.

The results are shown in a graphic comparing the voltage applied with the highest peak intensity. (Figure 34)

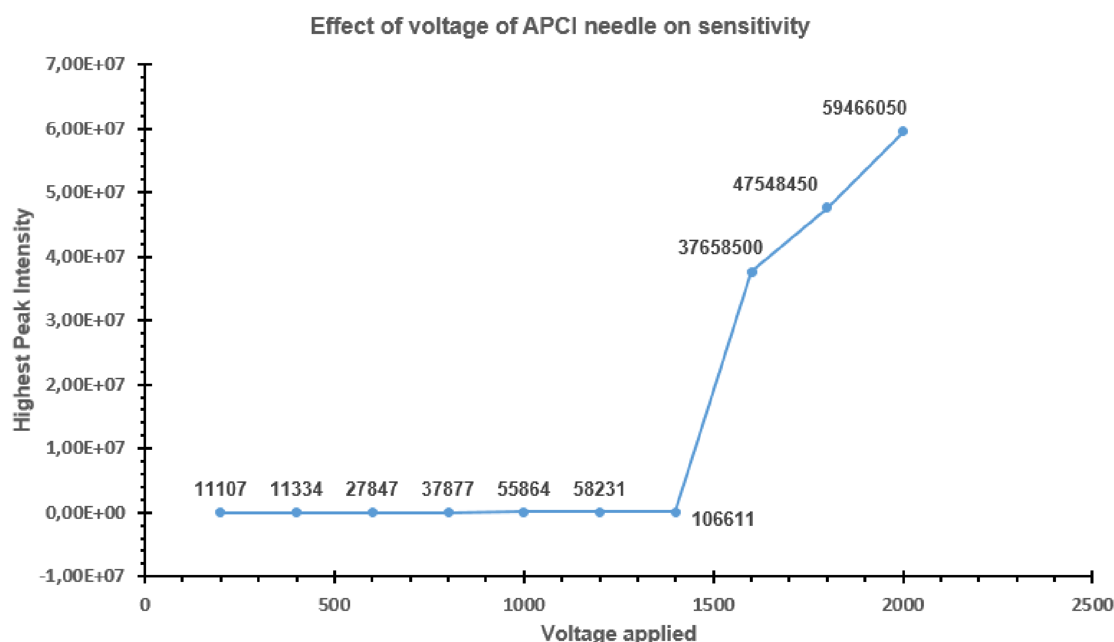

Figure 34: Effect of voltage applied in front of highest peak intensity

We could see that the graphic showed a positive correlation between the applied voltage and the highest peak intensity. From 200 to 1400 the peak intensity increased gradually, however, between 1400 and 1600 volts the peak intensity increased heavily. After 1600 volts, the peak intensity returned to increase in a gradual way.

Evaluating the results, we saw that between 1400 and 1600 volts the peak intensity turned from 10^4 to 10^7. In these high voltages, we achieved sensitivities of 10^7, 100 times higher than the one we had with previous optimizations and methods.

With external ionization, specifically with the APCI needle, we reached higher sensitivities when high voltages (+1400 volts) were applied.

4.8 Heated Bazooka Capillary effect

The Heated Bazooka Capilllary (HBC) was a device that could be installed replacing the previous inlet and be heated up to 550 ºC. Also, the device had a bend of 90º that could also help the spray focusing as we mentioned before in the orientation effect in this project. (Figure 35)

The interest of using this device was that the temperature of the Heated Bazooka Capillary could be controlled by an external controller. In addition, it could be a difference between heating the sample before entering the inlet and heating it once it was in inside the source.

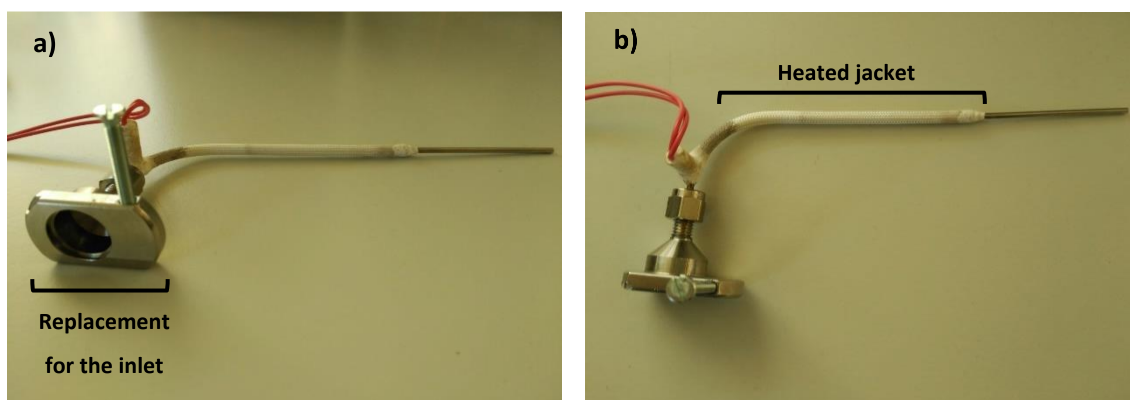

Figure 35: a) Front view of HBC b) Top view of HBC

The method of installing the Heated Bazooka Capillary was followed according the manual provided by the manufacture.

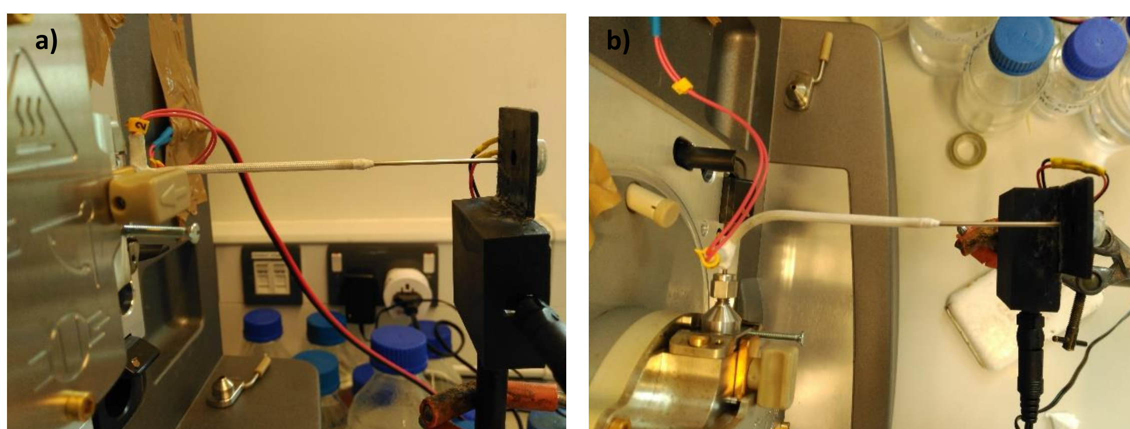

Figure 35: a) Front view of HBC set up
b) Top view of the HBC set up

With the Heated Bazooka Capillary, we received a table where the relationship between the temperature of the heated jacket and various parameters was shown in order to reproduce the experimentation and have an approximate idea of the temperature would have during the optimization. (Figure 36)

Voltage (V)	Intensity (A)	Resistance (Ohm)	Temperature (ºC)
1	0,69	1,45	61
2	1,02	1,96	114
3	1,28	2,34	184
4	1,42	2,82	250
5	1,5	3,33	314
6	1,56	3,85	367
7	1,66	4,22	408
8	1,75	4,57	466
9	1,86	4,84	514
10	1,97	5,08	563

Figure 36: Correlation between various parameters and temperature given by the manufacture.

To estimate the temperature, we had to make a graphic showing the Power and Temperature. The power value is the result of multiply the intensity per voltage, and it is given in Watts. Once we had the 2 values, we made a graphic of the power effect on the heated jacket temperature. (Figure 3)

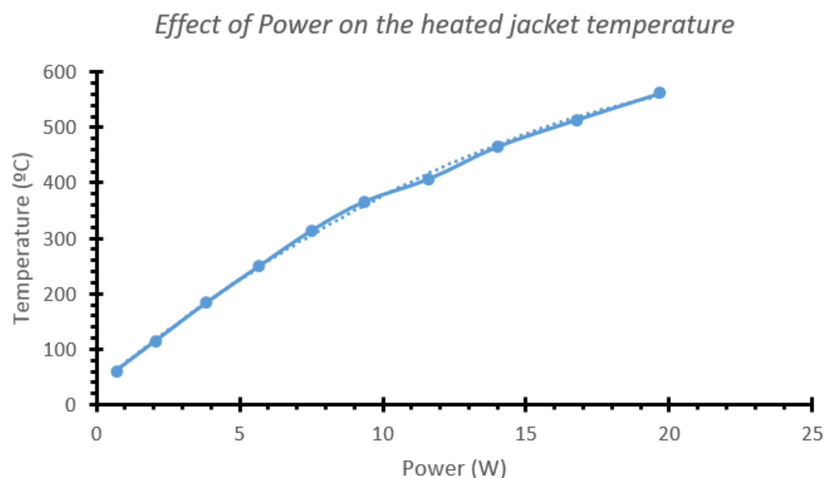

Figure 37: Effect of power on heated jacket temperature

Once we had the graphic, we had to look to the polynomial equation to obtain an estimate temperature interpolating the power.

$$y = -0{,}798x^2 + 42{,}311x + 33{,}701$$

With the external controller, we could set the intensity or the voltage. Setting one of these parameters made the other change and gave us both values.

To start the experiment, we put the first allowed value of intensity in the external controller and estimate the temperature. (Figure 38)

Intensity (A)	Voltage (V)	Power (W)	Temperature (ºC)
0,75	0,92	0,69	62,52

Figure 38: First parameters to set and estimate temperature of the heated jacket

When we set in the external controller 0,75 A of intensity the expected temperature would be 62,52 ºC, but to measure if the actual temperature of the heated jacket was this one, we used a Non-Contact Infrared Thermometer.

When we try to make the first experiment we had 2 different issues affecting the whole experimentation:

- The spray did not pass well into the Heated Bazooka Capillary. Consequently, if the sample entered the inlet, it entered at low concentration.
- The actual temperature was not the expected one. The temperature measured with the Non-Contact Infrared Thermometer was 24 ºC while the estimated was 62,52 ºC.

The combination of both problems did not let us to make proper spectra, observing low sensitivity and bad reproducibility. (Figure 39)

We made 4 experiments trying to see if there was any correlation between them. All the spectra were made injecting 2 times 10 µl of sample.

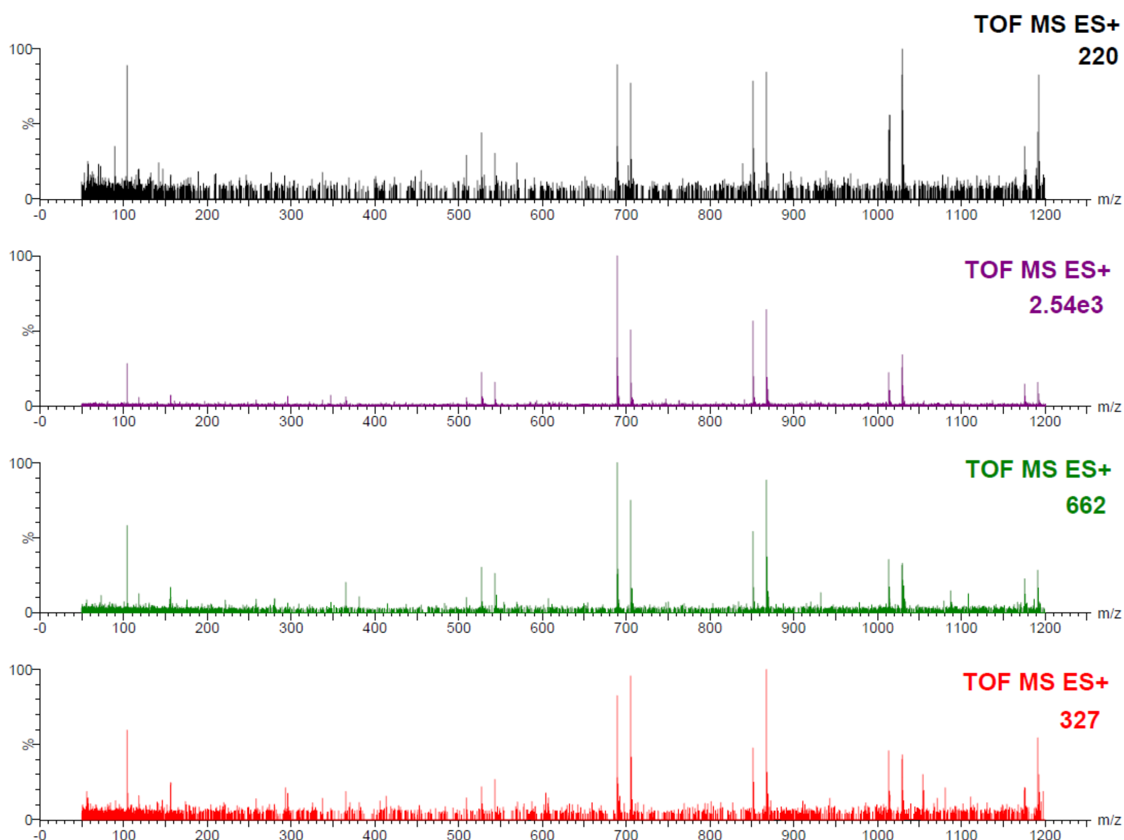

Figure 38: 4 different experimentations using the same conditions

As we saw the problem in the first experiment and its spectrum, we made 3 more spectra using the same conditions to check if it was a one-time problem or not.

In figure 38 we could see that the sensitivities of 3 spectra were very low (327, 662 and 220) and one spectrum showed a sensitivity quite higher ($2{,}54 \cdot 10^3$), observing and confirming the problems we talked about.

As a solution for the problem that almost no sample entered the inlet we tried another experiment with the 8 cm tube. However, we did not solve the issue, seeing that no sample entered the Bazooka.

About the difference between the expected temperature and the real one, we did not find any solution. We did not achieve to heat the Heated Bazooka Capillary even though we tried many different parameters.

To solve these problems, we needed to do more research and investigation, but for the moment we did not use the Heated Bazooka Capillary for following optimizations.

4.9 Continuous flow effect

All the previous optimization was made injecting 5 or 10 µl of beer sample in the ultrasonic ionization source using a micropipette. This way of injecting the sample was useful for our purpose and easy to handle. However, it could be an important factor that affected the reproducibility, being one of the most important issues we found in the use of ultrasonic ionization sources.

Using the micropipette to inject every time any value of sample, we produced a spray at intervals. The spray only lasted for a few seconds, showing in the chromatogram as many single peaks as injections made. (Figure 39)

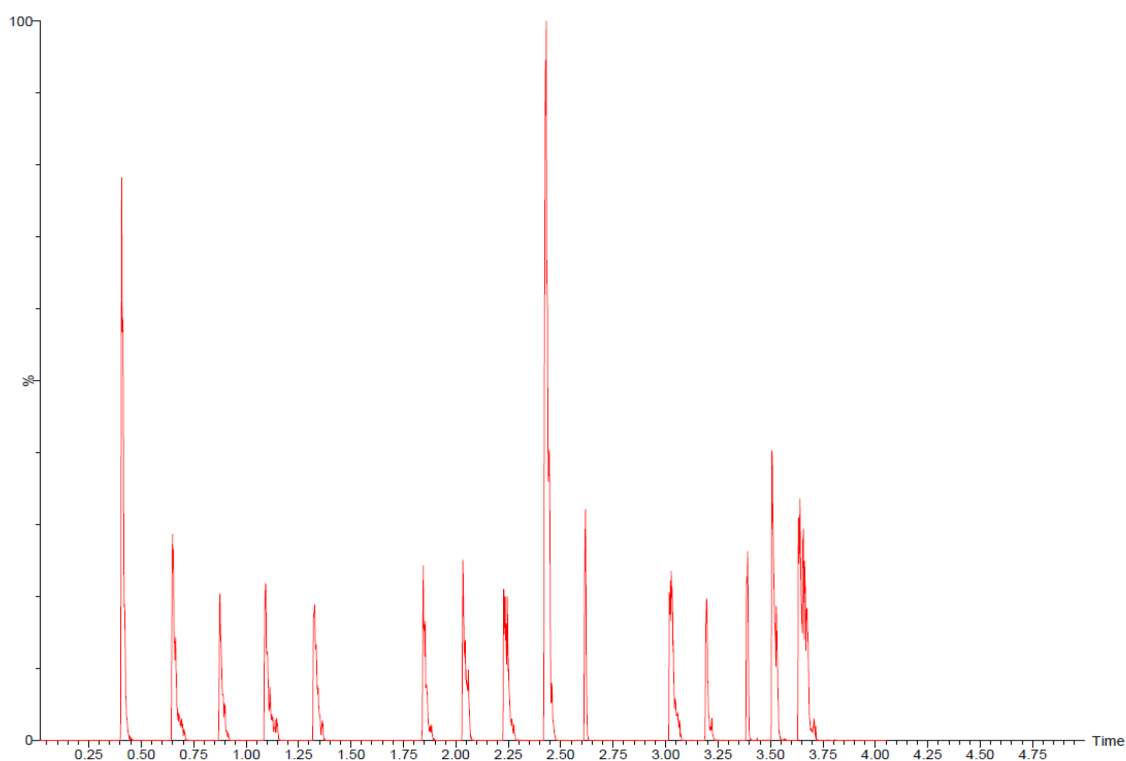

Figure 39: Chromatogram of 200, 400 and 600 volts in APCI optimization

This chromatogram is given as an example to see the impact of single sample injections. Each peak that can be seen was a single injection, and in the case of this chromatogram, there are 3 different groups of 5 peaks each that after we used to obtain the spectra.

As it could be seen, even we injected the same volume of sample every time, the peak height was not the same, confirming the reproducibility problem affecting the following spectra.

If we had a continuous flow of sample, maybe we would not have the reproducibility problem of the injections. However, the spray strength would persist as it was not something that could be solved with a constant flow.

Until this moment we only used the mass spectrometer even though a liquid chromatograph was connected. As it can be seen in figure 39 the chromatogram does not show retention times, because we did not want to identify compounds and we did not inject the sample into the liquid chromatograph, otherwise we injected it directly in the mass spectrometer.

To make a steady and continuous flow, we made a combined infusion of the sample using ultrapure water as mobile phase and beer as sample. To produce the combined flow, we set 0,250 ml/min of phase mobile and put in Fluidics parameters 10 µl/min of infusion flow rate and *Combined* Flow State. This combined flow came out of the lockspray cable.

Moreover, trying to obtain the best sensitivity possible, we set up the source with a 90º bend tube. (Figure 40)

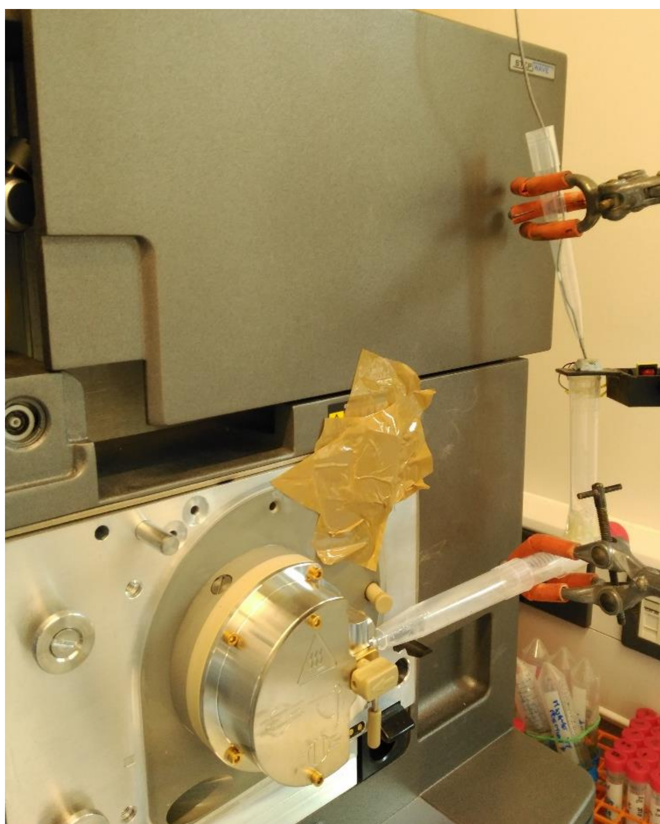

Figure 40: Set up for continuous flow effect

Once we had all the set up ready, we made the experiment during at least 5 minutes to see in the chromatogram if we had a steady line instead of single peaks. (Figure 41)

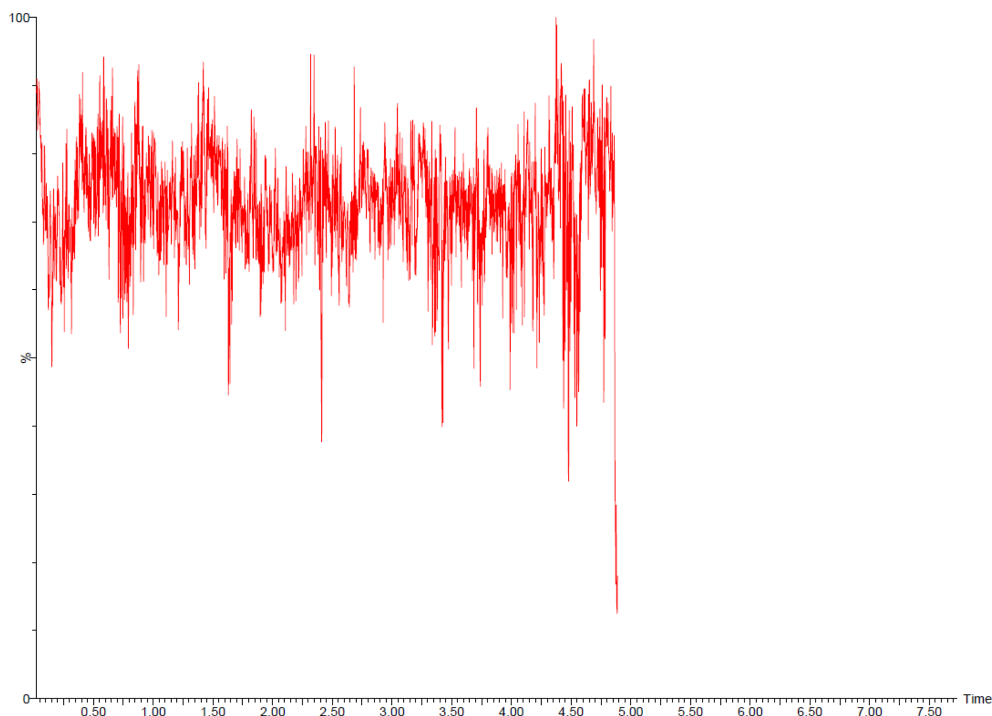

Figure 41: Chromatogram of continuous flow effect

As we expected, we obtained a stable chromatogram as far as could be expected. There were no single peaks, indication that continuous flow was produced. To see the sensitivity, we made the spectra of the whole chromatogram. (Figure 42)

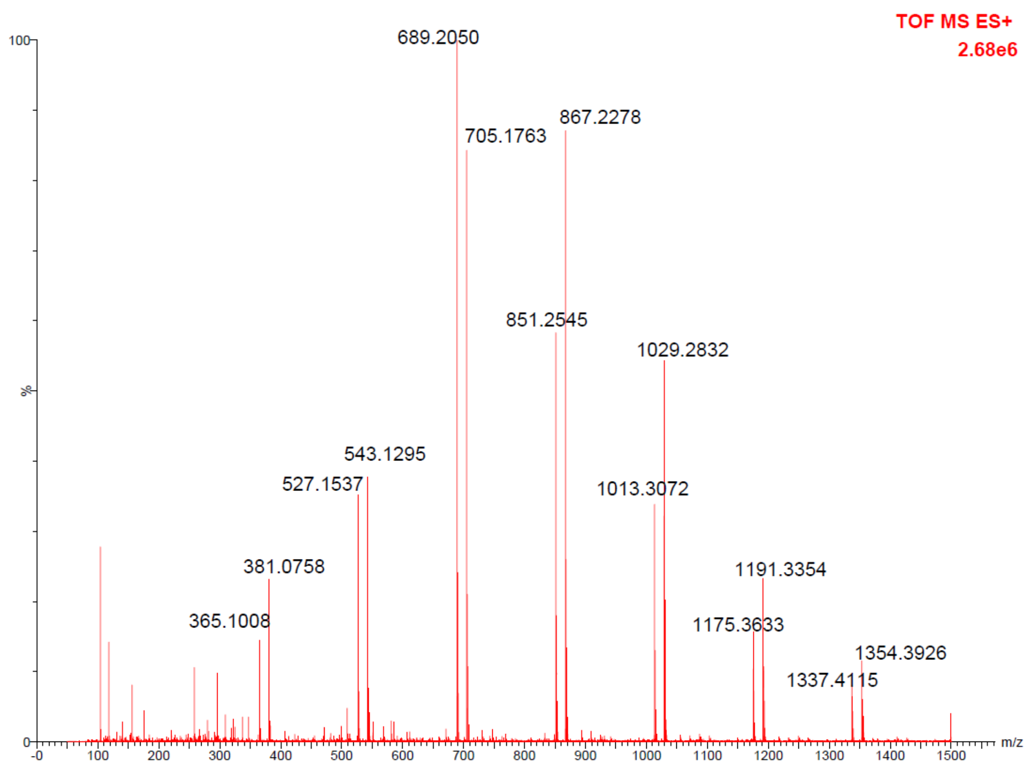

Figure 42: Spectrum of continuous flow effect

This spectrum showed all the typical peaks of beer and a sensitivity of $2,68 \cdot 10^6$. The sensitivity was higher than other optimizations with set ups using manual injection. The increase could be due to the continuous flow, avoiding the error of spraying at intervals and doing it constantly.

Furthermore, with this set up we could get rid of the reproducibility problem, obtaining a steady and constant flow.

5 Conclusions

The aim of this project was trying to check if an ultrasonic ionization source could be compared to electrospray ionization. The whole optimization process has been made trying different set ups for the new source and different parameters, seeing fundamentally the highest peak intensity of beer sample, or as we have been saying in this project, sensitivity.

We took as a reference the mass spectrum of a pale coloured beer (Fosters) made with Electrospray Ionization. The sensitivity we achieved in this experiment was $6{,}60 \cdot 10^8$, so in subsequent optimizations we tried to reach this sensitivity as much as possible.

During the whole project, we have been making the different experimentations with the Acoustic Wave Nebulizer, being the one with the best results compared with the Bottle Top Humidifier and the Mist Generator. Using this ultrasonic ionization source for the whole optimization, different parameters and set ups have been tried.

We stablished a method with all the optimized parameters such as the different modes of operation (Sensitivity, MS and Ion Positive Mode), sample volume (10 µl) and source temperature (150ºC). All the following optimizations and set ups have been made with this method.

We have seen that the source orientation has a big impact in the sensitivity value, being higher with this device than the without it. It helped the signal stability, droplet focusing and desolvation.

Regarding the Heated Bazooka Capillary, we did not obtain good results, having 2 different problem that we could not solve until now, like no sample entering the Bazooka and error between estimated and actual temperature.

On the other hand, we have confirmed that with external ionization such as Atmospheric Pressure Chemical Ionization (APCI) needle and the use of a continuous flow we obtained better results in sensitivity.

With the APCI needle we reached sensitivities of 10^7 at higher voltage applied (+1400), while with continuous flow we obtained sensitivities of 10^6 at the same time we solved

principally the reproducibility problem. In future studies, one set up that will be carried out, will be the use of Acoustic Wave Nebulizer with continuous flow and external ionization with an APCI needle. With this set up we could reach high sensitivity.

After all the different research and optimization, we have concluded that with an ultrasonic ionization source, in this case the Acoustic Wave Nebulizer, we have not been able to reach sensitivities as high as with the Electrospray Ionization. However, we have obtained good results with sensitivities up to 10^7.

This project will be continued in a PhD to keep developing different set ups and trying other ultrasonic ionization sources to achieve the best results possible. Also, it has been a base for further studies and research, so in a near future it is expected that we will be able to replace the Electrospray Ionization for an ultrasonic ionization source obtaining the same results.

6 Bibliography

(1) Waters. Xevo G2-XS QTof : Waters http://www.waters.com/waters/es_ES/Xevo-G2-XS-QTof-Quadrupole-Time-of-Flight-Mass-Spectrometer/nav.htm?locale=es_ES&cid=134798222 (accessed Mar 15, 2017).

(2) Chernushevich, I. V.; Loboda, A. V.; Thomson, B. A. An Introduction to Quadrupole-Time-of-Flight Mass Spectrometry. *J. Mass Spectrom.* **2001**, *36* (8), 849–865.

(3) Transform Your Lab with the Xevo G2-XS QTof : Waters http://www.waters.com/waters/promotionDetail.htm?id=134888007&locale=en_US (accessed May 2, 2017).

(4) Waters. *Xevo G2-XS Customer Training*; 2013.

(5) Murphy, J. Electrospray Ionization Mass Spectrometry https://chem.libretexts.org/Core/Analytical_Chemistry/Instrumental_Analysis/Mass_Spectrometry/Mass_Spectrometers_(Instrumentation)/Electrospray_Ionization_Mass_Spectrometry (accessed Mar 14, 2017).

(6) Skoog, D.; Holler, F. J.; Crouch, S. R. Principios de Análisis Instrumental; Santa Fe, 2008; pp 560–562.

(7) Waters Q-TOF Ultima ESI | Mass Spectrometry Lab | School of Chemical Sciences | University of Illinois, UC http://scs.illinois.edu/massSpec/instrum/qtof.php (accessed Apr 19, 2017).

(8) Bristol University. High Performance Liquid Chromatography Mass Spectrometry (HPLC/MS) http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html (accessed May 4, 2017).

(9) Cech, N. B.; Enke, C. G. Practical Implications of Some Recent Studies in Electrospray Ionization Fundamentals. *Mass Spectrom. Rev.* **2002**, *20* (6), 362–387.

(10) Monge, M. E.; Fernández, F. M. An Introduction to Ambient Ionization Mass Spectrometry. In *Ambient Ionization Mass Spectrometry*; 2014; pp 1–22.

(11) Chen, H.; Gamez, G.; Zenobi, R. What Can We Learn from Ambient Ionization Techniques? *J. Am. Soc. Mass Spectrom.* **2009**, *20* (11), 1947–1963.

(12) Huang, M. Z.; Cheng, S. C.; Cho, Y. T.; Shiea, J. Ambient Ionization Mass Spectrometry: A Tutorial. *Anal. Chim. Acta* **2011**, *702* (1), 1–15.

(13) STEMINC. Ultrasonic atomizing transducer - SMIST15F28RR111 https://www.steminc.com/PZT/en/ultrasonic-atomizing-transducer (accessed May 10, 2017).

(14) eBay. Ultrasonic Mist Maker Fogger Water Fountain Pond 12 LED BF https://www.ebay.co.uk/p/?iid=262342869629&&&adgroupid=33676502101&rlsatarget=pla-271883338035&abcId=893836&adtype=pla&merchantid=109712317&poi=&googleloc=9046402&device=c&campaignid=707291931&crdt=0&chn=ps (accessed May 10, 2017).

(15) eBay. Mini Portable Bottle Cap Air Humidifier with USB Cable https://www.ebay.co.uk/p/?iid=232181577118&&&adgroupid=39910336457&rlsatarget=pla-300977879461&abcId=1088886&adtype=pla&merchantid=101722709&poi=&googleloc=9046402&device=c&campaignid=805661516&crdt=0&chn=ps (accessed May 10, 2017).

(16) Gautschi, G. *Piezoelectric Sensorics*, 1st ed.; Springer-Verlag Berlin Heidelberg: New York, 2002.

(17) Olszewski, O. Z.; Macloughlin, R.; Blake, A.; Neill, M. O.; Jackson, N. ScienceDirect A Silicon-Based MEMS Vibrating Mesh Nebulizer for Inhaled Drug Delivery. *Procedia Eng.* **2016**, *0*, 1–4.

(18) Araújo, A. S.; da Rocha, L. L.; Tomazela, D. M.; Sawaya, A. C. H. F.; Almeida, R. R.; Catharino, R. R.; Eberlin, M. N. Electrospray Ionization Mass Spectrometry Fingerprinting of Beer. *Analyst* **2005**, *130* (6), 884–889.

(19) Cortacero-Ramírez, S.; Hernáinz-Bermúdez.De Castro, M.; Segura-Carretero, A.; Cruces-Blanco, C.; Fernández-Gutiérrez, A. Analysis of Beer Components by Capillary Electrophoretic Methods. *TrAC - Trends Anal. Chem.* **2003**, *22* (7–8), 440–445.

(20) Agilent. Atmospheric Pressure Chemical Ionization Source (APCI) http://www.agilent.com/en-us/products/mass-spectrometry/lc-ms-ion-sources/atmospheric-pressure-chemical-ionization-source-(apci) (accessed May 25, 2017).

(21) Borrull, F. CROMATOGRAFIA DE LÍQUIDS – ESPECTROMETRIA DE MASSES, 2016, 8.