Irish Mass Spectrometry Society

The Fragment

Jan 2016 **Volume 1 Issue 2**

On behalf of the committee of the IMSS I am very pleased to bring you the second issue of our newsletter 'The Fragment'. The objective of the IMSS is to provide a forum for practitioners of Mass Spectrometry. To do this, a meeting of the IMSS is held each year. This year's meeting will be held on 18th of May in the Moran Red Cow Hotel, Dublin.

We had an excellent meeting in May 2015 with a wide variety of Mass Spectrometry related presentations. In particular special mention goes to Caomhin Logue for his presentation on 'Sweetener Analysis using LC-MS-MS'. Caomhin is currently completing his Post Graduate Studies in UU Coleraine and was judged to have given the best student presentation by the IMSS judging panel. I hope you enjoy the newsletter and if you would like to contribute please contact us at contact@imss.ie .We look forward to seeing you all at the next IMSS meeting.

Richie Maguire - IMSS Chairperson

Dates for the Diary 2016

IMSS annual meeting 18th of May, 2016, Red Cow Moran's hotel, Dublin

Metabolomics 2016, 27th-30th June, 2016, Convention Centre, Dublin

ASMS annual conference, 5th-9th June 2016, San Antonio, TX

BSPR 2016 Meeting, 25th - 27th July 2016, University of Glasgow, UK

BMSS Annual meeting, 13th - 15th Sept 2016, Eastbourne Winter Gardens, UK

IMSS Committee 2016

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Newsletter Editors: Brian Flatley, Philip White and Richie Maguire

Publication Focus: Detection of Pyrrolizidine Alkaloids in Honey

Dr. Caroline T. Griffin

Pyrrolizidine alkaloids (PAs) are a large group of natural toxins produced by plants, several of which are known to be highly hepatotoxic and also have been shown to be carcinogenic. PAs have been associated with a number of livestock diseases and cases of human poisoning following contamination of staple foods, generally grain crops, or upon consumption of some herbal remedies. Other possible food sources of exposure include honey, milk and eggs. The European Food Safety Authority (EFSA) carried out a safety assessment of PAs in honey in 2011 and summarised that a health concern exists for high consumers of honey. A maximum daily PA intake limit of 0.007 µg per kilogram of body weight per day was recommended. However, there are no regulations for PAs in honey and as the European Union relies on imports for the majority of its honey, it is important that a testing protocol be implemented for PAs.

Research has been conducted at Cork Institute of Technology (CIT) under the 'Safe and Healthy Foods' project to investigate the presence of PAs in food using LC-MS.

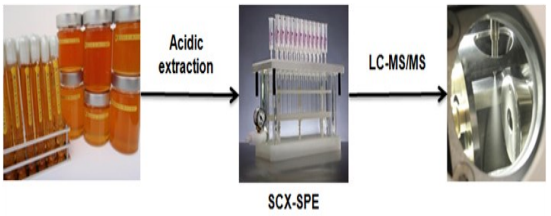

Figure 1: Extraction and determination of Pyrrolizidine Alkaloids in Honey

The focus of a recent publication in *Food Additives and Contaminants: Part A* was the development of a rapid protocol for the screening of honey involving minimal sample preparation via strong cation exchange (SCX) solid phase extraction (SPE) followed by an isocratic chromatographic method coupled to a triple quadrupole mass spectrometer.

The significance of this protocol is that it allows for shorter analysis times facilitating a high-throughput, having a 50% improvement over previously published methodologies, which is particularly advantageous for regulatory testing laboratories.

This research group has determined, from a large scale survey of honeys over a three year period, that approximately 24% of retail honeys contain one or more PAs. Thus, high consumers of honey in Ireland are at risk of exceeding the maximum daily PA intake limit as proposed by EFSA. Of the indigenous honeys tested no PA toxins were detected.

However, Ireland is heavily reliant on imported honey to meet consumer demand and although measures exist within the food supply chain to improve both food safety and traceability, there is still a distinct lack of same with regards to honey and PA toxins.

References: Griffin CT, Mitrovic SM, Danaher M, Furey A. (2015). Development of a fast isocratic LC-MS/MS method for the high-throughput analysis of pyrrolizidine alkaloids in Australian honey. *Food Additives and Contaminants: Part A*, 32(2), 214-228.

Griffin CT, O'Mahony J, Danaher M, Furey A. (2015). Liquid chromatography tandem mass spectrometry detection of targeted pyrrolizidine alkaloids in honeys purchased within Ireland. *Food Analytical Methods*, 8(1), 18-31.

Griffin CT, Gosetto F, Danaher M, Sabatini S, Furey A. (2014). Investigation of targeted pyrrolizidine alkaloids in traditional Chinese medicines and selected herbal teas sourced in Ireland using LC-ESI-MS/MS. *Food Additives and Contaminants: Part A*, 31(5), 940-961.

Griffin CT, Danaher M, Elliott CT, Kennedy DG, Furey A. (2013). Detection of pyrrolizidine alkaloids in commercial honey using liquid chromatography-ion trap mass spectrometry. *Food Chemistry*, 136(3-4), 1577-1583.

Method development: The use of Large Volume Injection (LVI) to improve sensitivity in the analysis of ultra trace compounds using GC-MS/MS

Dr. *Philip White*

Instrument model	**Agilent 7000A GC-MSMS**
Inlet settings	
Inlet gas	Helium
Inlet mode	PTV solvent vent
Septum purge flow	on 3mL/min
Purge flow mode	Switched
Temperature program	60°C for 0.29 min then 600°C/min to 325
Gas saver	On 20 mL/min after 3 min
Purge flow to split vent	60mL/min at 2.79 min
Vent flow	100 mL/min
Vent pressure	5 psi until 0.29 min
Cryo	On
Injector settings	
syringe size	50µL
Injection volume	20µL
Injection Dispense Speed	69 µL/min
Viscosity delay	3 sec
Oven temperature settings	50°C, 15°C/min to 160°C 10°C/min to 310°C

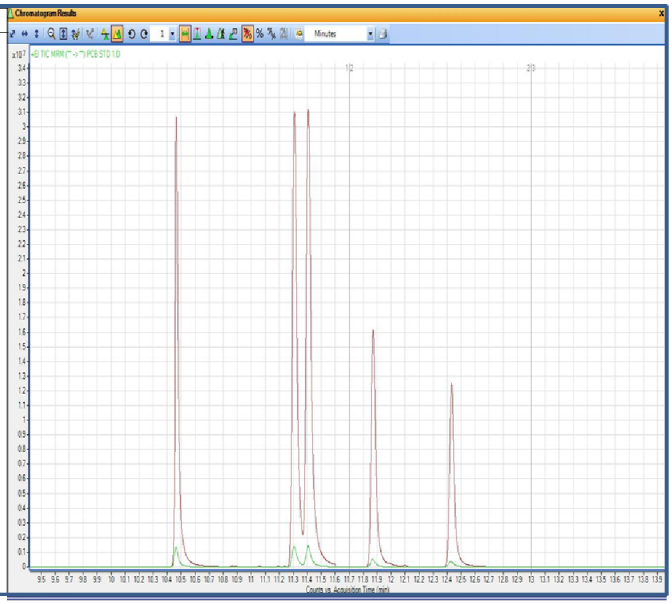

Figure 2: Typical GC-MS inlet and Injector parameters used in LVI (left) 2 µl (green) vs 20 µl (Red) Injection of the same polychlorinated biphenyl (PCB) sample (40 ppb) using LVI

With the increased need for improved sensitivity the analytical chemist is constantly challenged to provide robust, fit for purpose analysis in a variety of different matrices. Coupled with the inherent difficulties encountered in the analysis of ultra trace compounds found in food safety testing samples, concurrent legislation seems to require lower and lower LOQs.

Many of these issues can be overcome by increasing sensitivity of the instrument through different technical setups. Large volume injection (LVI) is one of the methods available to the analytical chemist. LVI techniques in GC mass spectrometry have received much attention in the last 10 years because of the reduced LOQs and with the improvements in HRGC-MS/MS SIM and MRM methods in relation to baseline noise levels means that the amount of analyte reaching the detector can vastly increase sensitivity. Figure 2 above shows the instrumental set up, including the inlet temperature ramp, used on an Agilent 7000A GC-MS/MS instrument.

The chromatogram to the right in figure 2 shows an injection of a 40 ppb PCB standard, first using a regular 2 µL injection analysed in a splitless GC-MS/MS set up (in green) overlaid with a 20 µL LVI injection (Red). It is clearly obvious that the LVI injection offers far better sensitivity in relation to the regular splitless set up. This indicates that, once a sample is clean in relation to matrix interference, LVI can offer the analytical chemist the opportunity to increase sensitivity and accuracy of analysis at a level far below what is currently possible using regular splitless GC injections.

One of the factors affecting sensitivity of a GC-MS/MS instrument is the amount of solvent that can be injected before poor chromatography (i.e. bandbroadening, peak tailing etc) can compromise your analysis. Typical splitless GC-MS/MS trace analysis entails 1 – 2 µl of your standards and samples being injected into an inlet (Figure 3) where the temperature (typically 250 – 300°C) vaporises the semi-volatile analytes facilitating transfer to the GC column under the influence of the mobile fluid. LVI injections typically transfer anything up to 100 µl of the sample into the inlet.

Typically, the temperature in the inlet during the injection cycle is kept low, approximately 20°C below the lowest vaporisation point of the analytes in question. The split valve (Figure 3) of the inlet is open to waste removing the evaporated solvent from the sample analytes. Once the solvent is removed, the split valve is closed and the inlet temperature is ramped quickly to a temperature of 250 – 350°C vaporising the analytes and allowing them to enter the column. The analytes are now free of the affects of the solvent meaning that they can be easily separated on the column with little of the adverse affects found when injecting excess solvent .

Is LVI for me?

LVI, if used correctly can dramatically increase the sensitivity of the instrument. The potential of LVI to increase confidence in structural identification and sensitivity/specificity means that if trace analysis (sub PPB) is a requirement in your laboratory then you should consider using LVI.

What do I need to perform LVI?

LVI can be performed on any GC instrument with pretty much any detector as long as you have an inlet capable of changing between split and splitless modes. Many of the more modern instruments have multi-mode inlets capable of performing a variety of injection types (cold on column, pulsed split/splitless) so solvent vent modes are also quiet common. The graphite/vespel types of ferrules may also require changing to a different type e.g graphite/stainless steel) because of the increased pressure placed on them during the inlet temperature ramp. You will need to change the regular 1 – 10 µl syringe for a larger one (e.g. 50 µl).

Consider also changing the liner from the typical straight type to the internally dimpled/baffled type. These liner types work by spreading the larger volume injected, over more surface area enhancing the solvent vent and analyte transfer portions of the inlet cycle. The larger injection volume may also mean more matrix being sent onto your column which can cause blockades and hence a reduction in sensitivity. With LVI therefore, it may be prudent to ensure good sample clean up in your extraction methodology or perhaps invest in a back-flush type system to prevent this from occurring.

Lastly, with the acceptance of this method, many of the major manufacturers provide LVI software in their instrument operating systems which can make the method development of such techniques very easy and also reduce the reluctance involved in attempting to try something different. With a little method development the powerful LVI technique can rightly take its place amongst the arsenal of the analytical chemist.

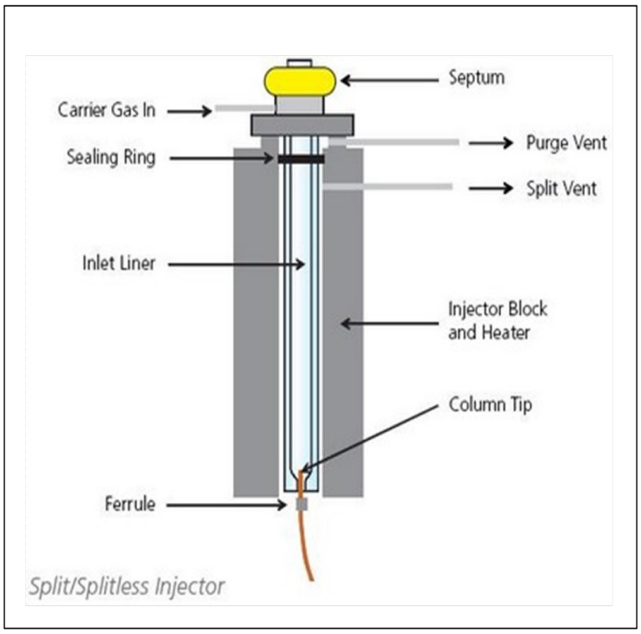

Figure 3: Typical GC-MS inlet

Get Involved

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Connect with us on **Linkedin**:

https://www.linkedin.com/groups/8435142

Call for speakers: If you are interested in speaking at this years IMSS meeting please get in touch at contact@imss.ie before 31st of March 2016

Poster Session: For the first we will be having a poster session, so if you have a mass spec related poster you want to present, send details to contact@imss.ie before the 31st of March, 2016

See www.imss.ie/meetings for guidance on speaking and presenting posters at IMSS meetings