



## Summer studentship report

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*Project title:* The investigation of sulfluramid's transformations, and their behaviour in soil and plants

### Abstract



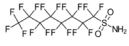
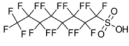
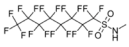
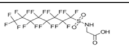
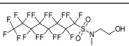
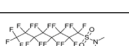
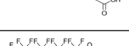
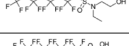
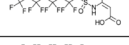

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In this work the transformation behaviour of sulfluramid (N-EtFOSA) in two soil-leafy green systems is investigated. Detection and quantitation were performed using a high performance-liquid chromatography coupled with high-resolution tandem mass spectrometry (HPLC-HRMS/MS) instrument using Electrospray ionisation (ESI) in negative polarity mode. Sulfluramid and transformation products were determined and quantified in the samples. Determined in all 3 matrices were: PFOA; FOSA; PFOS; and FOSAA. Furthermore, a novel transformation product has been found and its structure proposed. The novel product was detected in only the basil leaves, suggesting an interesting species-specific transformation. This report also outlines the capabilities of a high-resolution mass spectrometer.

## Introduction

Per- and polyfluoroalkyl substances (PFAS), refer to a complex group of (>9000) (1) related organofluorine chemicals that were fabricated in the 1940s (2) and have been extensively used since. Their extreme durability and physicochemical properties have resulted in their ubiquitous use in industry and consumerism- spanning from fire-fighting foams, and non-stick pans, to the perhaps lesser known- textiles, electronic devices, dental floss to all forms of cosmetics such as lip balms, and in some cases being an undisclosed ingredient (3–5). They bioaccumulate and biomagnify through protein binding (rather than lipid partitioning) including to those found in the blood and liver of animals (6). Hence our inescapable exposure, from food and drinking water consumption, and even inhalation of the dust and air indoors (7,8). PFAS exposure is unavoidable, they are transported over long-range distances, and are capable of migration through the 5 environmental spheres (9,10), consequently, they are found at significant levels in places far from anthropogenic sources such as in polar bears in the arctic regions, to penguin eggs in antarctica (11,12). They are referred to as ‘Forever chemicals’- as only the non-fluorinated functional moieties of the chain are unstable and prone to biodegradation and transformation to other active PFAS, that remain highly persistent in the environment and the food chain, for an extremely long and still yet to be determined length of time (13).

Table 1: Sulfluramid and transformation products, with corresponding structures, molecular formula, calculated mass error values, and HPLC-HRMS/MS ES<sup>-</sup> data for those detected.

Proposed Structure	Name	Acronym	Molecular Formula	Exact Mass [M-H] <sup>-</sup> (Da)	Accurate Mass [M-H] <sup>-</sup> (m/z)	Mass Error (ppm)	Retention Time (min)
	N-Ethylperfluorooctane sulphonamide	N-EtFOSA (Sulfluramid)	C <sub>10</sub> H <sub>6</sub> F <sub>17</sub> NO <sub>2</sub> S	525.97750	525.9783	1.5210	14.02
	Perfluorooctanoic acid	PFOA	C <sub>8</sub> HF <sub>15</sub> O <sub>2</sub>	412.96643	412.9677	3.0753	13.05
	Perfluorooctane sulphonamide	FOSA	C <sub>8</sub> H <sub>2</sub> F <sub>17</sub> NO <sub>2</sub> S	497.94620	497.9474	2.4099	13.67
	Perfluorooctanesulphonic acid	PFOS	C <sub>8</sub> HF <sub>17</sub> O <sub>3</sub> S	498.93022	498.9314	2.3651	13.34
	N-Methylperfluorooctane sulphonamide	N-MeFOSA	C <sub>9</sub> H <sub>4</sub> F <sub>17</sub> NO <sub>2</sub> S	511.96185	Not detected	—	—
	Perfluorooctane sulphonamidoacetic acid	FOSAA	C <sub>10</sub> H <sub>3</sub> F <sub>17</sub> NO <sub>4</sub> S	555.95168	555.9525	1.4749	13.55
	N-Methylperfluorooctane sulphonamidoethanol	N-MeFOSE	C <sub>11</sub> H <sub>8</sub> F <sub>17</sub> NO <sub>3</sub> S	555.98807	555.9525	-63.9762	13.3 and 13.5
	N-Methylperfluorooctane sulphonamidoacetic acid	N-MeFOSAA	C <sub>11</sub> H <sub>6</sub> F <sub>17</sub> NO <sub>4</sub> S	569.96733	Not detected	—	—
	N-Ethylperfluorooctane sulphonamidoethanol	N-EtFOSE	C <sub>12</sub> H <sub>10</sub> F <sub>17</sub> NO <sub>3</sub> S	570.00372	Not detected	—	—
	(a)	Novel**	C <sub>11</sub> H <sub>4</sub> F <sub>17</sub> NO <sub>2</sub> S	583.94660	583.9462	-0.6850	13.34
	(b)	Novel**	C <sub>11</sub> H <sub>4</sub> F <sub>17</sub> NO <sub>2</sub> S	583.94660	583.9462	-0.6850	13.34
	N-Ethylperfluorooctane sulphonamidoacetic acid	N-EtFOSAA	C <sub>12</sub> H <sub>8</sub> F <sub>17</sub> NO <sub>4</sub> S	583.98298	583.9462	-62.9813	13.76

Sulfluramid (Table 1), a commonly used name for a commercial insect bait, with the active ingredient N-ethyl perfluorooctane sulphonamide (EtFOSA) is classified by the WHO as a Class II pesticide - moderately hazardous (14). However, it is known to transform to various PFAS, a subject area poorly understood, and of great scientific focus recently. Amongst the sulfluramid transformation products, two of the most studied PFAS has been found. Namely: perfluorooctane sulphonic acid (PFOS) which is a priority hazardous substance under the Water Framework Directive (15); and perfluorooctanoic acid (PFOA). They are subject to restrictions according to The Stockholm Convention of Persistent Organic Pollutants (POPs) 2009. PFOS, its salts and perfluorooctanesulphonyl fluoride (PFOSF) – a precursor to many PFOS based compounds – are pursuant to Annex B of the convention (Restriction of production and use). PFOA and its related substances, are pursuant to Annex A (Elimination). They comply with the Stockholm Conventions toxicity criteria for bioaccumulation, mutagenicity, and endocrine disruption. Despite the well documented health and environmental hazards, and restrictions of PFAS, specific exemptions for the production and uses are

still in place (16). Brazil was granted a licence for ongoing use of sulfluramid as it is used extensively for the control of leaf cutting ants of the genera *Atta.spp* and *Acromymex*, where their mutualistic symbiotic relationship with fungus is necessary for their survival. Their management is necessary to protect the economy as one of the most important contributors to the global market for a variety of crops.

Using only the declared data it has been estimated that 76-616 tonnes of PFOS could have been emitted from the use of sulfluramid alone in Brazil between 2010-2018 (18,19). The estimations were derived from the yields of PFOS determined from a study by Zabaleta, et al. Where PFOS yields using technical sulfluramid were 34 % and commercial 277 %. Significant amounts of PFOS and related compounds are found with uncertain sources due to the transformation of precursors and undeclared uses. Studies have demonstrated that metabolite transformation products in various plants differs to that by soil and microbes and the transformation pathways are species specific (19,20). The study outlined in this report aimed to investigate the sulfluramid transformation and uptake behaviour in soil and two widely used leafy greens.

## **Materials and Methods**

The reagents were purchased from Sigma Aldrich ( $\geq 98$  % purity, Darmstadt, Germany)- technical sulfluramid, perfluorooctane sulphonamide (FOSA) and perfluorooctane sulphonic acid (PFOS). Acetonitrile (ACN) & formic acid ( $\geq 98\%$ , Optima LCMS grade, Fisher Scientific, Loughborough, UK). Sodium Chloride ( $\geq 99$  % Sigma Aldrich). Deionised (DI) water (Purelab, Elga, UK) with a resistance of 18 M $\Omega$ .

### ***Experimental***

Plant pots (10) were filled with store bought compost. 10 mL of solution of sulfluramid ((0.5 mg mL<sup>-1</sup>) in methanol/DI water 50/50) was spiked into 3 lettuce plants & 2 basil plants, leaving 5 controls (3 lettuce and 2 basil). All experiments were performed in lab environments and watered from below. Samples were cropped after 35 days and 41 days for the basil and lettuce respectively. The plants were placed into 10 mL centrifuge tubes (Eppendorf, polypropylene). Soil samples (10) collected from each pot were stored in a 10 mL centrifuge tube and placed in the freezer. The plant material was homogenised in a food processor prior to extraction.

### ***QuEChERS Extraction***

A wet weight (~5 g) of each sample was transferred to a clean polypropylene centrifuge tube, topped with DI water (1 mL) and sodium chloride (1 g) shaken vigorously for ~30 seconds then vortexed for 5 minutes. ACN (~5 mL) (accurate sample to ACN 1:1 ratio) added then centrifuged @ 3000 rpm for 10 minutes. The supernatant was collected using a pipette and transferred to a glass test tube and fully evaporated at 40°C in an electric heater. The samples were reconstituted with (1 mL) ACN/DI water (20/80 v/v) and passed through a 0.2  $\mu$ m nylon syringe filter into polypropylene LCMS screw top vials.

### ***Systems Parameters***

The LCMS/MS system consisted of an Ultimate 3000 RS pump, Ultimate 3000 RS autosampler and Ultimate 3000 RS column oven all from Dionex) attached to a Q-exactive Orbitrap mass spectrometer detector (Thermo Scientific) with Electrospray ionisation (ESI) source. The column oven was set at 30°C and the autosampler at 10°C.

A reversed phase C18 column (Thermo Scientific, Accucore, 2.6  $\mu$ m chromatography column (100  $\times$  2.1 mm)) and C18 guard cartridge and a delay column was used to separate the analytes with a gradient elution method. A constant flow rate of 0.2 mL.min<sup>-1</sup> was set. Acetonitrile (A) was used as the organic solvent, and the aqueous (B) 10 mmol ammonium formate in DI water (18 M $\Omega$ ), adjusted to pH 3.5 with formic acid. Starting with 2 % of (B) over 1 min and maintained there for 10 mins, then increased to 35 % (B) and held for 5 mins, then decreased back to 2 % (B) and equilibrated there for 6 minutes. The full ms scan range was set at 80-900 m/z with a mass resolution of 17,500. Trace finder and Chromeleon software was used for the LC-MS data acquisition and peak processing.

## Results and Discussion

High resolution mass spectrometry (HRMS) has the potential to provide elemental composition of organic molecules. This is measured by the mass accuracy which is a calculation used in HRMS to determine how close the experimental mass (accurate mass) is to the theoretical mass (exact mass). The calculation is shown in Equation.1. The mass accuracy acceptance criteria for an ion is ( $\pm 5$  ppm) .

$$Mass\ Accuracy = \frac{Accurate\ mass - Exact\ mass}{Exact\ mass} * 1 \times 10^6 (ppm) \quad \dots 1$$

A summary of sulfluramid and its suspected and detected transformation products is listed and can be seen in Table 1. A [M-H]<sup>-</sup> precursor ion was found at m/z 555.9 which has potential to be either FOSAA or N-MeFOSE. Their respective mass accuracies are ~1.5 ppm, and -63.9 (see Table 1.). FOSAA can be assigned with certainty by calculating and comparing the mass errors, which can only be distinguished on a high-resolution mass spectrometer rather than low resolution. Similarly, the ion at m/z 583.9 has several possibilities and the correct ion determined structures are proposed and shown on Table 1 at Novel\*\* (a & b). On examination of the product ion spectrum, there was no ion consistent with the loss of a methyl group, therefore it is less likely to be the N-methyl version Novel (b). There was a product ion with m/z 497.9469 consistent with the loss of C(OH)CHCOOH group in Novel structure (a), therefore this is more likely.

Multiple transformation products were determined across all matrices. N-EtFOSE, N-MeFOSAA, and N-MeFOSA were not detected in any of the samples. FOSAA and PFOS were by far the most abundant products. FOSA and PFOS have only one mass unit difference which would be problematic if they co-eluted given their structure similarity. However, a chromatographic separation was afforded between the two, and this can be seen in Figure 2 (c & d)

Table 2: Estimated mass and mole balance data for the transformation products detected in total soil and plant material resulting from soil fortified with sulfluramid. A 20 % moisture content in the soil samples has been assumed and accounted for.

Plant	PFAS	Spiked Sulfluramid (µg)	Mass in Soil (µg)	Mass in Plant (µg)	Total (µg)	Moles in Soil (nmol)	Moles in Plant (nmol)	Total (nmol)	Spiked Sulfluramid (nmol)
Lettuce	Sulfluramid	5000	2.6191	5.3137	7.9328	4.9679	10.0791	15.0470	9484
	PFOA		4.7857	0.4481	5.2338	18.7912	5.1785	23.9697	
	FOSA		1.8078	4.9045	6.7123	3.6217	9.8257	13.4474	
	PFOS		9.3980	2.5899	11.9880	11.5577	1.0823	12.6400	
	FOSAA		29.4056	0.1268	29.5324	52.7758	0.2276	53.0034	
	Sum Total		48.0162	13.3831	61.3992	91.7142	26.3932	118.1074	
Basil	Sulfluramid	0.4870	0.2489	0.7359	0.9237	0.4721	1.3958		
	PFOA	0.2431	0.0902	0.3333	7.9611	0.5998	8.5609		
	FOSA	1.0556	0.1571	1.2127	2.1148	0.3147	2.4296		
	PFOS	3.9816	0.3000	4.2816	0.5870	0.2179	0.8050		
	FOSAA	7.8910	0.0271	7.9180	14.1623	0.0486	14.2109		
	**Novel	ND	0.5011	0.5011	ND	0.8562	0.8562		
	Sum Total	13.6582	1.3244	14.9826	25.7490	2.5094	28.2584		

Table 2. Displays the mass (µg) and mole (nmol) balance data of the detected transformation products in the total plant and soil. The values shown in the table have been corrected for concentration factors, and an assumed 20 % moisture content in the soil samples accounted for. The results present an incomplete material balance. The sum of the compounds detected in both the soil and lettuce samples equates to ~1 % of the mass of spiked sulfluramid, and for soil + basil ~ 0.3 %. These discrepancies could arise from several factors and more work is required. Measures to prevent volatilisation from surface of soil and testing the water in the tray underneath the plant pots is worth consideration for future work.

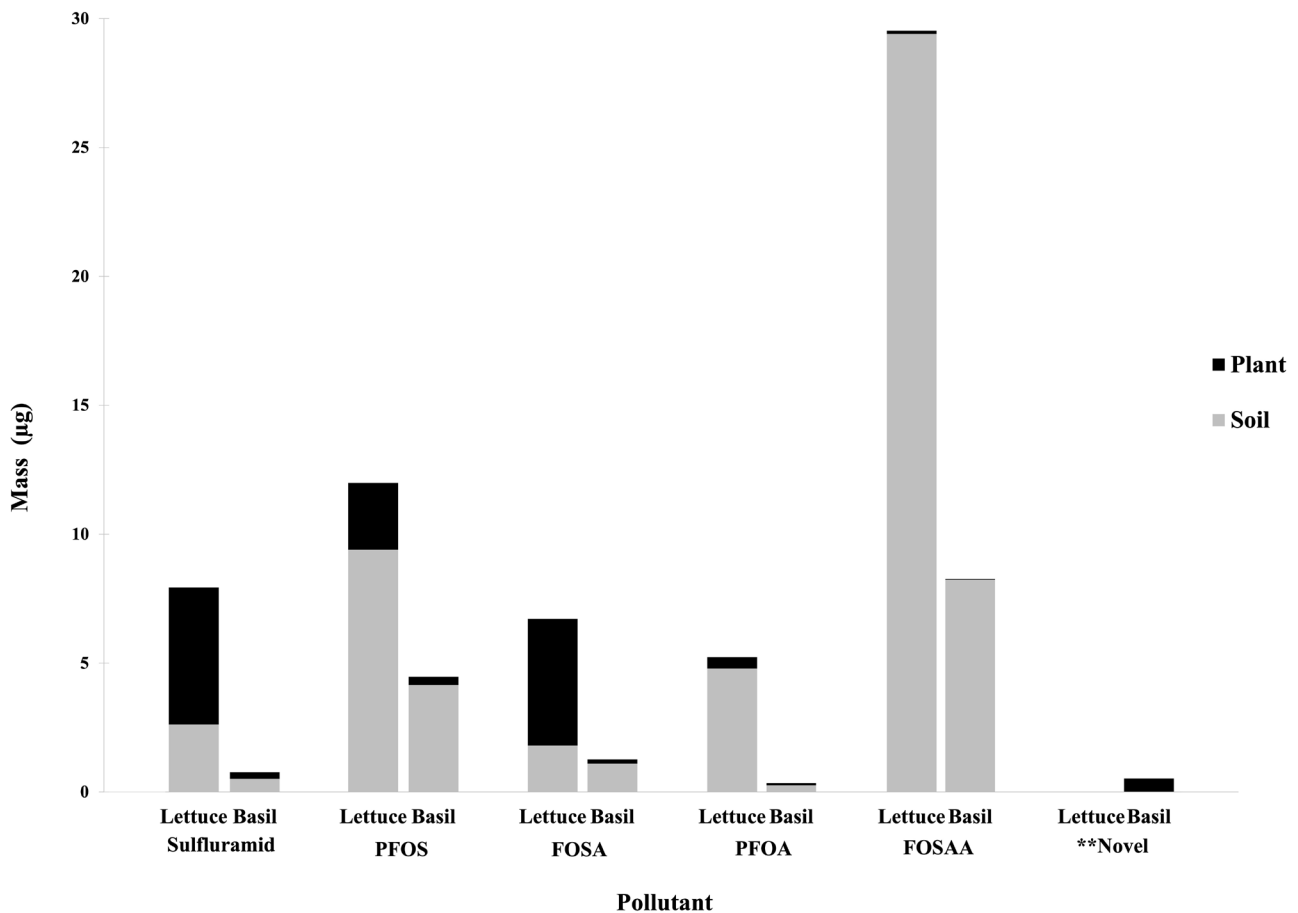
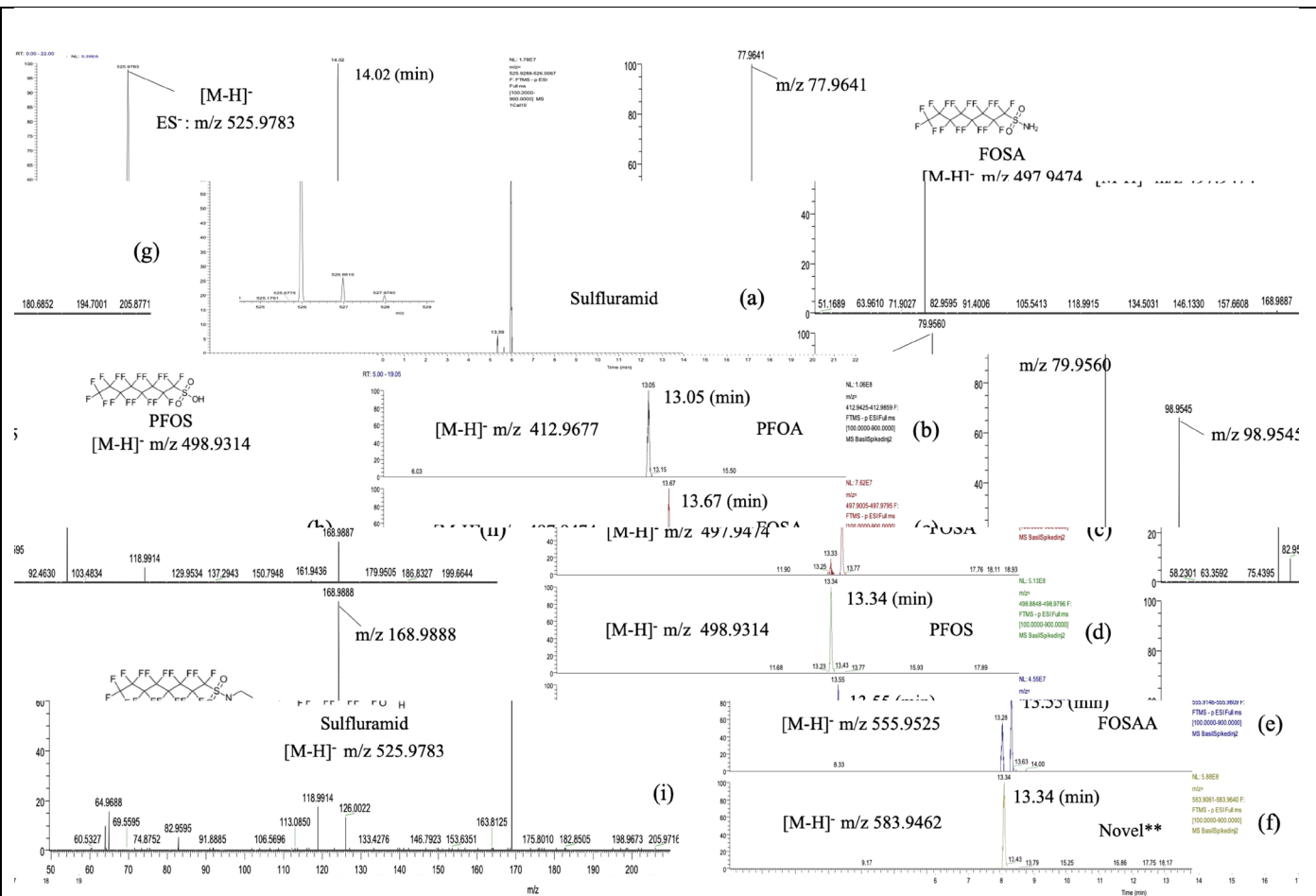


Figure 1: Clustered stacked column chart of the data from Table 2. The columns are clustered by plant type (lettuce & basil) and stacked based on the soil in which the plant was grown. It shows the mass ( $\mu\text{g}$ ) of pollutant found experimentally.

Figure 1 displays a graph of the mass distribution of the detected analytes (data in Table 2). All the columns show that a larger mass of compounds was detected in both the soil and plant material of the lettuce than the basil and soil. This could be for a number of reasons, including: water content; lipid and protein content; and growth time (lettuce was grown for 6 days longer). However, it can be seen that the novel transformation product was detected only in the leaves of the basil plants, this presents an interesting result that would need further investigation. There could be a species dependant transformation that is present in basil but not lettuce. Interestingly, a significant one-sided soil to plant ratio for FOSAA has been observed. FOSAA could be taken up by the plant more slowly or formed in the roots of the plants. It may be that FOSAA and PFOS (the most abundant lettuce and basil transformation products) could be end stage transformations, suggesting that the FOSAA product may remain longer prior to PFOS transformation. More PFOA is detected for the lettuce soil system than basil, but more work is required. Future work to develop a concentration vs time curve for transformation products could provide deeper insight into the transformation behaviour.



S<sup>-</sup> extracted ion chromatogram (EIC) and precursor ion for sulfluramid is shown in (a). Transformation products are shown in (b to f). The product ion patterns for FOSA, PFOS and sulfluramid are shown in (g, h, and i) respectively,

Figure 2: The HPLC-MS/MS EIC and precursor ion for sulfluramid and its transformation products. The EIC's for the sulfluramid transformation products are shown in (b to f).

It can be seen in Figure 2 (a-f) the retention times are fairly close. Now that some of the transformation products are known, the chromatography can be developed further to improve the chromatographic resolution. The product ions for sulfluramid and transformation products FOSA and PFOS are shown in Figure 2.

## Conclusion

In this work, the transformations and behaviour of sulfluramid was investigated in soil, lettuce and basil. A high-resolution mass spectrometry instrumental method was used to detect and quantify the transformation products and precursor sulfluramid. The lettuce was harvested after 41 days, and basil after 35 days, those detected in all three matrices were: Sulfluramid; PFOA; FOSA; PFOS; and FOSAA - detected in only the basil plant was a novel transformation product. Two proposed structures can be seen for this in Table 1 and the structure (b) has been determined as most likely based on product ion patterns. The presence in basil leaves alone suggest a species-specific interaction worth future investigation. The results present an incomplete mass balance, and repeated tests need to be carried out on the samples to gain better representation of the distribution of sulfluramid and transformation products in the matrices. Also, worth consideration would be to test the water underneath pots for analytes, and to apply measures to avoid any potential volatilisation. As well as spiking standards (where available) of the individual metabolites into pots to study their uptake/transformation behaviour and comparing it with the sulfluramid transformations. More results would be needed for insight into the transformation mechanisms. For example, plotting a concentration vs time curve for metabolites could provide insight into the order and location of transformations, aiding in the investigation of whether sulfluramid is breaking down in the soil or the plant material, or both, and the proportions.

## Skills and Reflection

Without the funding from the British Mass Spectrometry Society (BMSS), and the Chromatographic Society (ChromSoc), I would not have been able to work on this project. The project was investigating the pesticide sulfluramid, its transformations, and their behaviour in soil and plants. It was exciting to be able to work in a research lab alongside knowledgeable and passionate experts in the separation sciences and whose enthusiasm was contagious. I gained lots of practice with manual data processing and with using software such as Chromeleon, TraceFinder, and XCalibur. I got practical experience with evaluating a prototype column, and the mental warm up that comes with switching from a reversed phase to normal phase column, and with optimising the solvents and elution gradients - which until then I had only theoretical knowledge. Working with both high-resolution mass spectrometry and high-performance liquid chromatography, and with using PRM and ddMS2 is not something that can be done anywhere and from this opportunity I have been able to hone my experimental skills using top of the line equipment. I am due to start a PhD and this experience will be hugely beneficial, not only for that, but for my future career. The BMSS and ChromSoc are extremely valuable assets for research and as such, for society as a whole. I am excited to see where the toolset I have learnt thanks these societies can lead me in the future and aim to give back by my own contributions.

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