Summer studentship report 2026

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Project title: Using volatile perfluorooctanoic acid (PFOA) to create a novel micellar liquid chromatography couple mass spectrometry (MLC-MS) system to predict blood brain barrier (BBB) permeability

Abstract

This study explores potential models to mimic the blood-brain barrier (BBB) to assess BBB permeability This proof-of-concept project employs the novel use of perfluorooctanoic acid (PFOA) as a volatile surfactant in a novel micellar liquid chromatography (MLC) system with an aim to couple with mass spectrometry (MS) to detect therapeutic analytes. The data was used as a BBB permeability predictive model using literature logBB values and experimental logk values. The use of PFOA in MLC had proven capability to screen neutral and acidic analytes, albeit with the use of an organic additive, but was unable to elute and detect basic analytes, a characteristic of a large proportion of neuro-therapeutic drugs, suggesting that biomimetic MLC with PFOA may be an unsuitable technique at present to be used in a BBB mimicking context. This suggests further investigation is needed to explore the application of MLC-MS for permeability prediction.

Report

1. Introduction

Micellar liquid chromatography (MLC) is liquid chromatography that has a surfactant within the mobile phase which when exceeding critical micelle concentration, forms a micellar phase within the column which interacts with analytes causing retention [1]. This technique has been used in multiple applications but has particular therapeutic significance in its biomimetic capabilities as an *in vitro* model for biological membranes.

The blood-brain barrier (BBB) has been simulated using MLC [2] to model the permeability of neuro-therapeutics that target the brain. MLC has previously been incompatible with mass spectrometry

(MS) due to the micellar agents used being non-volatile and likely to damage and contaminate the MS system [1,3]. The surfactant perfluorooctanoic Acid (PFOA) has been used successfully in LC-MS [4] and

electrokinetic chromatography [5] exploiting its volatility but currently not in an MLC workflow that can be coupled with MS.

An MLC-MS system would have the ability to screen for therapeutics in the field of drug design and pharmaceutical analysis, and could offer high-throughput screening of large datasets in mixtures thanks to the higher selectivity over UV offered by m/z ratio. This, in turn, could be used for creating predictive permeability models without quantification. By using an MLC system, the reduced need for organic solvents could also lead to a decrease in solvent waste and a more sustainable analysis overall [6].

The aim of this project is to explore the use of PFOA in a novel MLC system that can successfully identify and produce high resolution chromatograms. The system compatibility with MS and the creation of a predictive model of the passage of therapeutics across the BBB will then be assessed.

2. Experimental

2.1. Reagents

Chemicals used in the study are listed below with the supplier used;
Aminopyrine (Arcos Organics; CAS:58-15-1), Antipyrine (Thermo Scientific; CAS:60-80-0), Caffeine (Sigma Aldrich; CAS:58-08-2), Carbamazepine (Sigma Aldrich; CAS:298-46-4), Cimetidine (Arcos Organics; CAS:51481-61-9), Citalopram (APExBIO; CAS:59729-32-7), Diphenhydramine (Thermo Scientific; CAS:147-24-0), Ethosuximide (Sigma Aldrich; CAS:77-67-8), Indomethacin (Molekula; CAS:53-86-1), Ketorolac (Cayman Chemical Company; CAS:74103-07-4), Metoprolol (Sigma Aldrich; CAS:56392-17-7), Nicotine (Thermo Scientific; CAS:54-11-5), Paroxetine (Thermo Scientific; CAS:110429-35-1), Phenylbutazone (Thermo Scientific; CAS:50-33-9), Promethazine (Acros Organics; CAS:58-33-3), Quinidine (Thermo Scientific; CAS:56-54-2), Ranitidine (Medchemxpress; CAS:66357-59-2), Salicylic acid (Sigma Aldrich; CAS:69-72-7), Valproic acid (Sigma Aldrich; CAS:1069-66-5), Venlafaxine (Thermo Scientific; CAS:99300-78-4), Uracil (Thermo Scientific; CAS:66-22-5) , Ammoniumn acetate (Alfa Aesar; CAS:631-61-8), Perfluorooctanoic acid (Sigma Aldrich; CAS:335-67-1), Methanol HPLC Grade (Honeywell; CAS:67-56-1).

2.1. Mobile Phase Preparation

A mobile phase of 10 mM ammonium acetate and 30 mM perfluorooctanoic acid was made at pH 7.4 to match biological conditions. The appropriate mass of each compound was weighed out and dissolved with Milli-Q water using a magnetic stirrer overnight due to the micellar nature of PFOA. This was then transferred to a volumetric flask and pH was then adjusted dropwise using concentrated ammonium solution and read using a calibrated benchtop pH meter, before being made up with Milli-Q water to the correct volume. The solution was filtered using Nalgene CA MF75 0.2 µm Duran filters (Thermo Scientific).

2.2. Preparation of Drug stock Solutions

3 mg/ml stock solutions of all 20 drug compounds were prepared by accurately weighing out 9.0 mg of

each drug (Brash Weighing Scales, Serial no:21000106) in 4 ml amber vials (Agilent) and dissolving them in 3 ml of HPLC grade methanol and ensured thorough mixing with a benchtop vortex mixer. These stocks were stored in a -20 ºC freezer. Working analytes of each compound were prepared by transferring appropriate amounts of each stock to a new amber vial and dissolved in mobile phase before being filtered into 2 ml Agilent HPLC vials with disposable 1 ml syringes and nylon 0.2 µm membrane filters.

2.3. MLC Conditions

MLC analysis was performed on an Agilent 1100 HPLC system using an Agilent Zorbax SB-C8, 2.1 x 100 mm, 3.5 µm column at 37 ºC. Initially a 100% 30 mM PFOA and 10 mM AA mobile phase isocratic system at 0.2 ml/min. Later this was increased to an isocratic 0.4 ml/min flow rate and a 90:10 (v/v) MP/HPLC grade methanol for analysis. A gradient method was developed for compounds which were not detected during the isocratic runs, this started 10% methanol and increased to 40% after an hour where it was held for 10 more minutes before being allowed time to re-equilibrate the micellar conditions in the column. Each sample was 125 µg/ml with a 3 µl injection.

2.3. Dynamic Light Scattering Conditions

To confirm the micellar nature of the 10% methanol mobile phase, dynamic light scattering (DLS) was performed on the mobile phase. A Malvern Zetasizer (Nano Series) Model ZEN3600 machine by adding 10% methanol to the 30 mM PFOA and 10 mM AA mobile phase and allowing time to see if collapse takes place before being added to a DTS1070 disposable folded capillary cell. The same was done with the mobile phase without any methanol added. A backscatter angle of 175 º was used and Dispersant RI of 1.330 with a repeat of 4 runs.

3. Results and Discussion

3.1. MLC Chromatograms

The therapeutic analytes chosen for the project were selected from available compounds that had known logBB values from [2]. Chromatographic analysis was carried out at 220, 230, 250, 280 and 300 nm to ensure detection of all compounds. The retention times of the 20 drugs (**Table 1**) were collected initially during an isocratic mobile phase method. Methanol was introduced at 10% (v/v) isocratic, which eluted a number of compounds.

Analyte	Retention time (min)	Methanol needed for elution (%)	Charge at pH 7.4
Aminopyrine	20.573	10	0
Antipyrine	20.316	10	0
Caffeine	4.011	0	0
Carbamazepine	19.452	40	0
Cimetidine	n/a	n/a	0

Citalopram	n/a	n/a	+1
Diphenhydramine	n/a	n/a	+1
Ethosuximide	2.673	0	0
Indomethacin	5.750	0	-1
Ketorolac	0.715	0	-1
Metoprolol	n/a	n/a	+1
Nicotine	n/a	n/a	+1
Paroxetine	n/a	n/a	+1
Phenylbutazone	3.842	0	-1
Promethazine	n/a	n/a	+1
Quinidine	n/a	n/a	+1
Ranitidine	20.192	40	+1
Salicylic acid	0.583	0	-1
Valproic acid	n/a	n/a	-1
Venlafaxine	n/a	n/a	+1

Table 1: *Chromatograph data of PFOA MLC system on therapeutics, retention time was taken in triplicates. The relationship between charge of analyte was included as well as if the analyte was detectable with the presence of methanol*

From the 20 drugs screened through the MLC system only 10 could be eluted and detected, and all of which were all neutral or acidic compounds except for ranitidine.

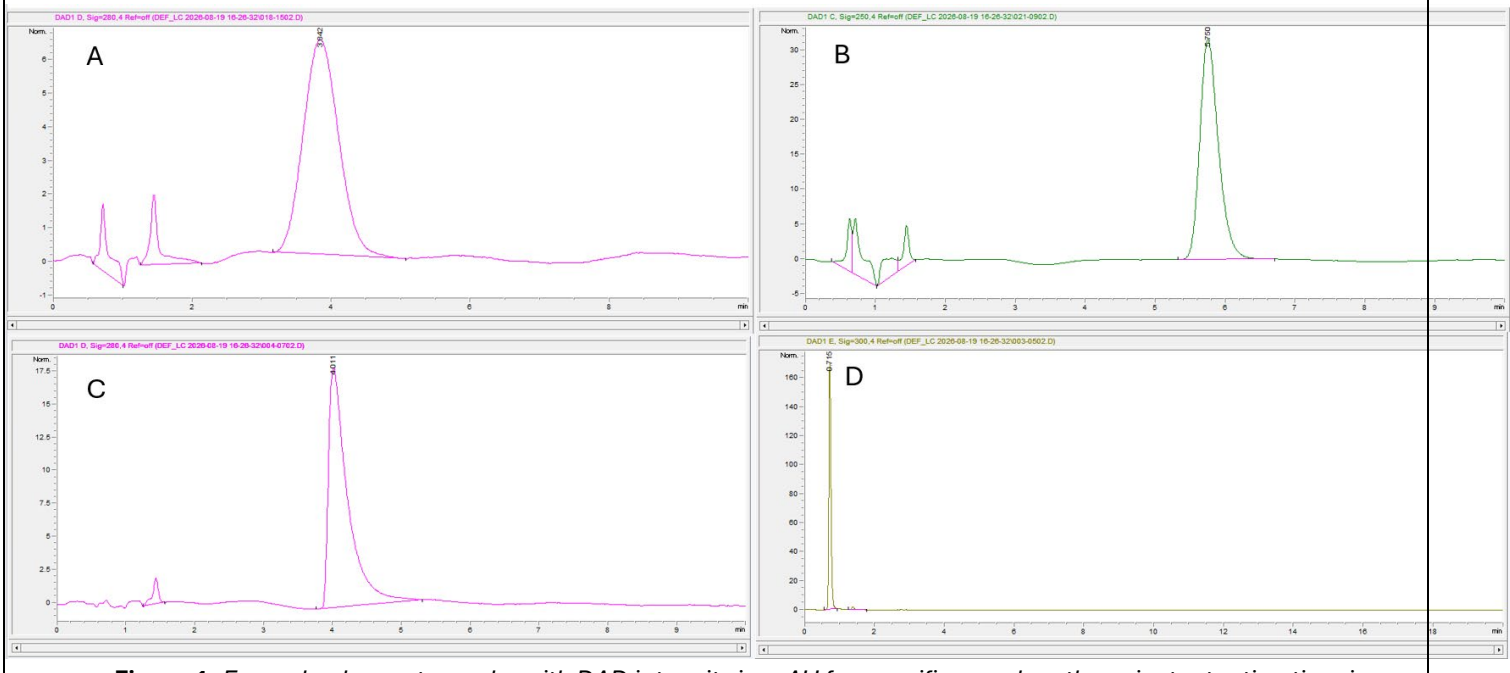

Figure 1: *Example chromatographs with DAD intensity in mAU for specific wavelength against retention time in min of integrated peak. Analytes included are: Phenylbutazone A, Indomethacin B, Caffeine C, Ketorolac D.*

The chromatographs shown are representative to show the proof of concept of PFOA in an MLC system. Ketorolac (Figure 1D) was an example of an unretained compound in the system that eluted before the void in other compounds. Phenylbutazone (Figure 1A) and Indomethacin (Figure 1B) were both visibly identifiable during the MLC run and distinctive from the void peak, they had good uniformity, were symmetrical and had high resolution, which is usually not the case in a standard MLC system. Caffeine (Figure 1C) had a tailing effect but was still identifiable as a unique peak present in the system. This proves that the use of PFOA as a mobile phase to elute and detect analytes in a MLC system, however the incompatibility with basic therapeutic compounds is problematic for the current application as most therapeutics which target the CNS are basic in nature, such as antidepressants and those which target neurodegenerative disorders. An increase in the concentration of PFOA present or optimisation of the methanol percentage in the mobile phase could aid with detection as peaks were present when washing the column, suggesting that the compounds were being highly retained. The change of column to a CN or shorter column would also be prudent future work for screening bases. Future work includes further coupling of the MLC a QDa mass spectrometer.

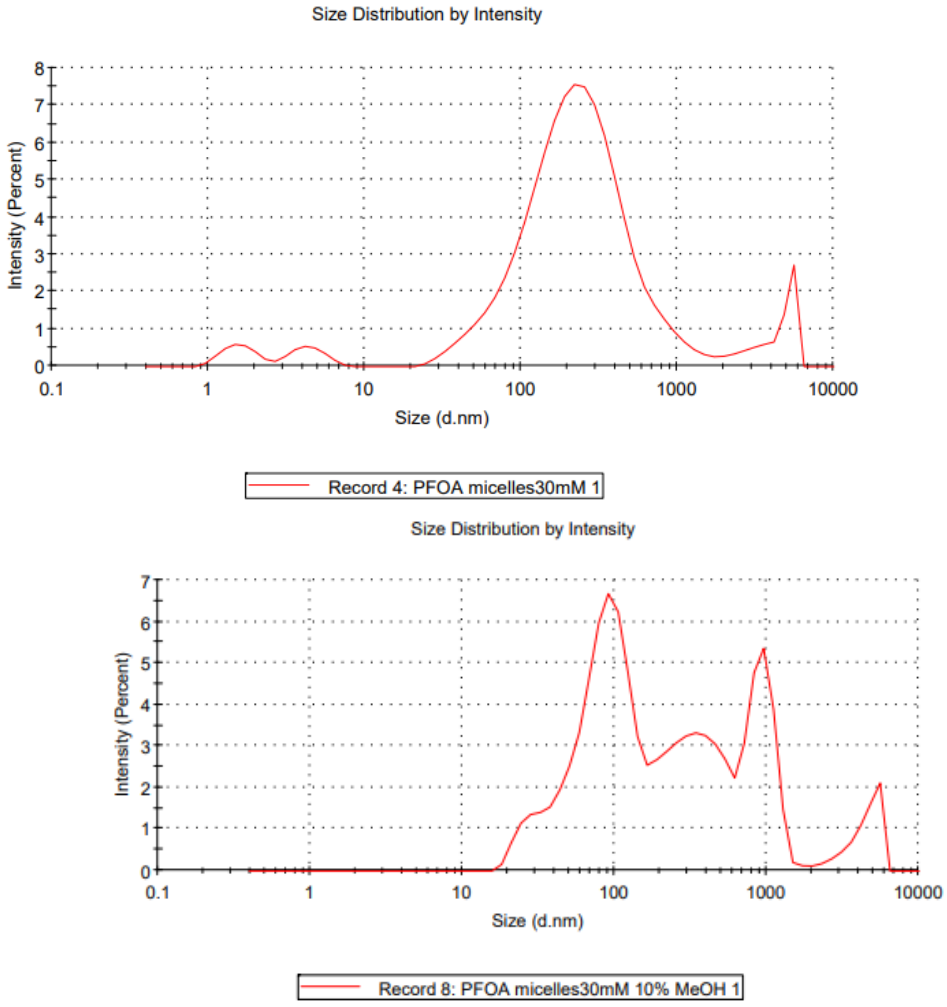

Figure 2: *DLS data of mobile phase nanoparticle size distribution between mobile phase with and without 10% methanol. The Z-Average of unmodified mobile phase was 213.0 d.nm and 10% methanol mobile phase was 325.3 d.nm.*

Micellar collapse does not occur in PFOA with 10% methanol (**Figure 2**), so the data collected was still achieved within a fully micellar system.

3.1. Predictive Modelling

Using the retention times obtained from the MLC experiments, logk values were calculated using the formula: $logk = (\frac{t_r - t_0}{t_0})$, where t_r is the retention time of the analyte and t_0 is the void time of the most unretained analyte through the MLC system which was salicylic acid. Uracil was used as an unretained analyte but had a retention time longer than both ketorolac and salicylic acid at 0.737 minutes. Using alvaDesc [7] software over 5000 molecular descriptors were generated for the 20 drugs used in the study, using these descriptors and logk values as a constant variable, an alvaModel [8] regression predictive model was created to predict logBB values which were externally imported from [1]. Multiple attempts of 10,000 iterations were performed. Unfortunately, due to the small size of the dataset (n=9), we could not perform an external validation of the model by splitting in training and testing set, which limits the reliability of our models. However, a positive trend between MLC retention, along with other descriptors, was observable (**Figure 3**).

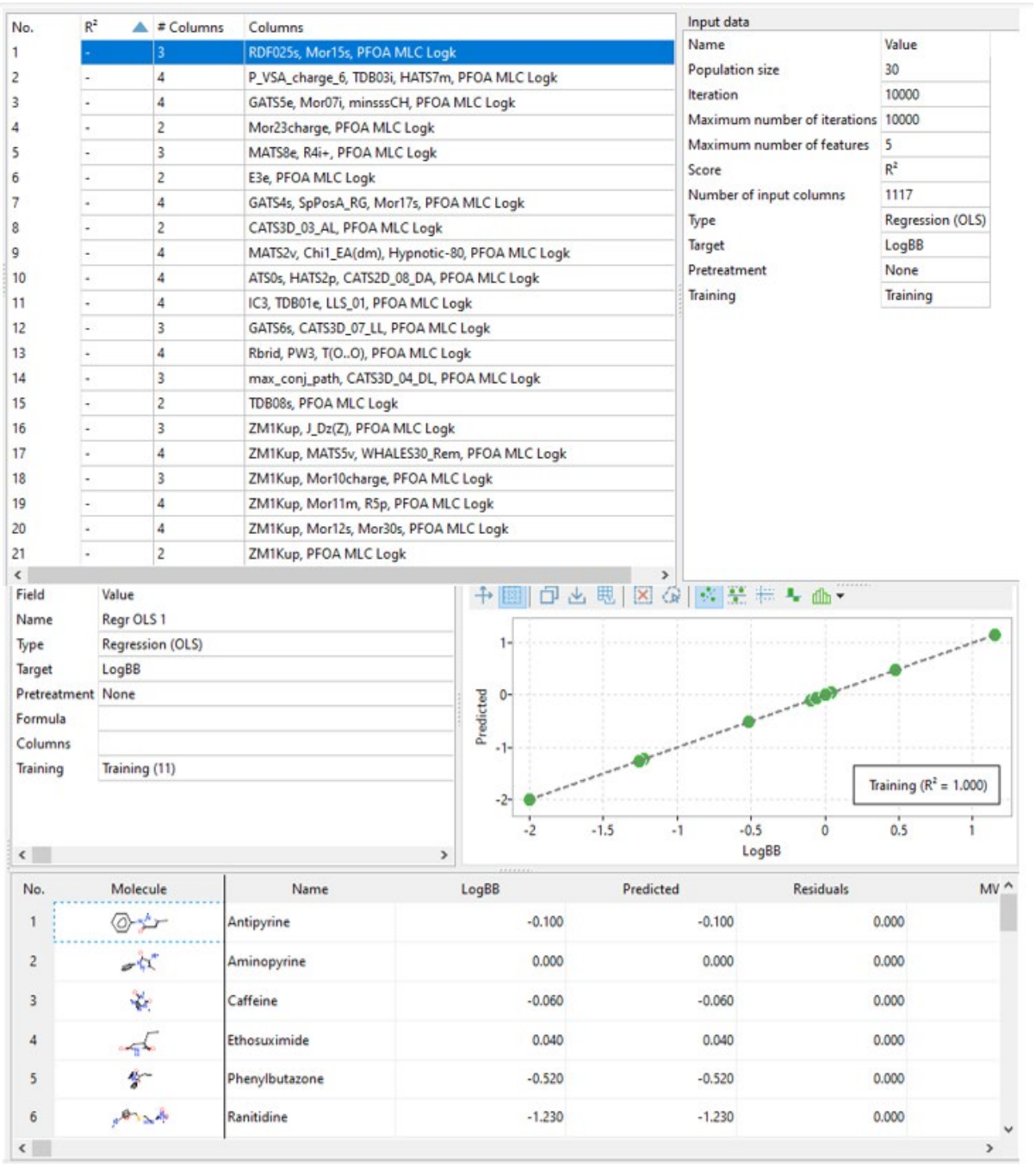

Figure 3: *Attempted LogBB prediction model using PFOA MLC logk as a fixed descriptor. Model was created using alvaDesc and alvaModel.*

It is most likely that there is not enough data to form a prediction with formula. More compounds with log k values would be needed to confirm this and so optimisation of the mobile phase composition and MLC system would increase the likelihood of creating a useful experimental prediction model.

4. Conclusion

This study proved that PFOA can be used as a mobile phase in a novel MLC system to detect therapeutic analytes. The inability to elute and detect basic analytes using the current methodology may render its use for blood brain barrier permeability prediction unsuitable, but further method optimisation is necessary to say for certain. More log k values of other compounds would help prove a predictive model useful as external validation was not viable.

5. References

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[8] Alvascience, alvaModel (software to model QSAR data) version 3.0.12, 2026, https://www.alvascience.com

…More on Oliver's experience:

Why did you want to do this research project?

- I wanted to get some experience doing research at a high academic level to see if it was something I enjoyed and was passionate for, I thoroughly enjoyed both the experience being in a drug design lab and learning about what kind of research happens there and it has made me want to do more research in my career.

What skills have you gained within the summer studentship?

- I have become fairly proficient in HPLC, a technique I had not done prior, learning and getting comfortable with the machine and analysis was both rewarding and interesting and doing so in a research setting is something I can take further and with finishing my current studies. I also do not have much work in labs doing research so it was beneficial to get more experience in that side of lab work.

How will these new skills impact on your career or CV?

- I have been considering moving away from my current job after finishing my part-time MSc into industry or a PhD and the experience working in a research lab and with a new technique will help my development and make me better equipped for both paths.

Any other relevant comments about your experience?

- Being able to work in the lab and chat with both supervisors and the PhD/ M-Res students about what they do was a greatly beneficial opportunity that I would not have gotten otherwise, I am very grateful for being offered the project and the pathways it will have opened for me.