JOURNAL OF SCIENCE AND TECHNOLOGY
ISSN: 2229-8460 e-ISSN: 2600-7924

JST

Vol. 17 No. 2 (2025) 142-154
https://publisher.uthm.edu.my/ojs/index.php/jst

Quantification of Perfluorooctanesulfonic Acid in Human Serum Using Triple Quadrupole Liquid Chromatography–Mass Spectrometry

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DOI: https://doi.org/10.30880/jst.2025.17.02.011

Article Info

Received: 16 July 2025
Accepted: 5 December 2025
Available online: 30 December 2025

Keywords

Liquid chromatography, perfluorooctanesulfonic acid, PFOS, metabolic syndrome, serum, cardiovascular disease, persistent organic pollutant

Abstract

Perfluorooctanesulfonic acid (PFOS), a persistent organic pollutant, has an unclear association with metabolic syndrome (MetS), particularly in Southeast Asian populations. Given the lack of PFOS detection in Malaysia, this study's rationale was to develop a simpler, faster, and cost-effective analytical method. We developed and validated a simple, sensitive LC-MS/MS method for PFOS quantification in human serum. The method employed an optimized Liquid-Liquid Extraction (LLE) using formic acid, acetonitrile, and n-hexane. PFOS and its internal standard were quantified using LC-MS/MS 6490 QQQ in multiple reaction monitoring (MRM) mode. The assay showed high specificity, acceptable precision, accuracy, and recovery, with a linear calibration range of 20–500 ng/mL. The validated method was used to determine PFOS level in serum from 40 healthy controls and 40 MetS patients. PFOS was detected in four MetS samples but not in any control samples. However, no significant difference was observed between the two groups (p-value = 0.1156). A sensitive and robust LC-MS/MS method for PFOS quantification was successfully developed. The preliminary detection of PFOS in a subset of MetS patients, even without statistical significance, highlights the need for larger cohort studies and further investigation into individual MetS components to fully elucidate any potential association.

1. Introduction

Organic fluorochemicals are utilised in several industrial uses, including fire-retardant foams, paints, lubricants, paint, polishes, and surfactants. According to recent research, numerous perfluorochemicals (PFCs) are common pollutants detected in humans and animals worldwide. Perfluorooctanesulfonic acid (PFOS) is one of the PFCs detected in the environment and living organisms. PFOS is a manmade chemical invented in the late 1950s by a 3 M company from the US. PFOS is known to have adverse effects on human health, such as reproductive toxicity [1], neurotoxicity [2], metabolic dysregulation [3], and cardiovascular toxicity [4].

The United States Environmental Protection Agency (EPA) has established a tolerable amount of PFOS in drinking water of up to 70 ng/L. However, certain states and research experts argue that the EPA rules are not adequately safe [5]. Similarly, various PFC limitations in Canada, as determined by drinking water advisory committees and other organizations, range from 10 ng/L to hundreds of ng/L [6]. Beyond regulatory limits, many epidemiological studies suggest that PFOS exposure may raise the risk of diseases such as cardiovascular diseases (CVDs) [5, 6].

PFOS and other PFCs can be absorbed into human tissues through consumer products and environmental contamination. Several studies have reported its presence in human blood samples, a detection that was almost impossible 30 years ago due to technological limitations [7-10]. PFOS accumulates in the human body at levels ranging from 13 to 30 ng/mL because of its high biliary reabsorption rate and low levels of excretion in the urine [7, 10]. Furthermore, due to its structural similarities to fatty acids, 96% of PFOS binds to B-lipoproteins, enabling its transport via the bloodstream and leading to increased concentration in human serum [11-14].While the concerning effects of PFOS are evident, a notable lack of institutions or centres is equipped to detect PFOS in human serum, particularly in Southeast Asia. For analysis, PFOS is typically tested in human serum or plasma because, compared to whole blood, the concentration in serum or plasma is approximately twofold higher due to the whole blood's cellular component displacement [11].

Several techniques have been established for the quantitative determination of PFOS utilising various analytical techniques based on prior investigations [7-10]. Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry with Triple Quadrupole configuration (UHPLC-MS/MS-QQQ) technique has been widely used to detect PFOS due to several factors. Its high sensitivity, specificity, and accuracy make it a suitable technique for detecting PFOS. Additionally, its ability to efficiently separate and quantify PFOS from complex sample matrices, along with its capability to minimise interference from other compounds, further supports its suitability for this purpose. Moreover, UHPLC-MS/MS-QQQ offers a relatively rapid analysis time and requires minimal sample preparation, making it a practical choice for detecting PFOS in human serum [15, 16].This setup also ensures high accuracy in quantification [16, 17].

Standard analysis of PFOS shows that human serum samples can be analysed using LC-MS/MS by modifying the Forsthuber method [7]. In the preliminary part (data not shown), we used the Oasis® HLB cartridge for SPE extraction using the method by Kärrman, van Bavel [8]; however, the recovery was poorer than that of LLE. Liquid-liquid extraction (LLE) was chosen since the method is comparable to solid-phase extraction (SPE) [18]. Many studies have demonstrated that LLE can be used to detect low concentrations of contaminants [10, 19]. Although the original method [7] demonstrated the removal of n-hexane, in our study, we collected n-hexane and ACN to extract the PFOS in lipoproteins and albumin in the samples. Whole blood was not used as an extraction sample, as previous work showed that serum or plasma PFOS concentrations are twofold higher [20].

The mobile phase from previous methods is summarised in **Table 1**. It was reported that bovine serum was used to replace human serum in preparing the calibration standard curve for matrix-matched calibration standard purposes [9]. In this study, we adapted and validated a previous quantitative method with a few modifications covering PFOS in a single analysis, minimising the needed volume of serum. Foetal bovine serum (FBS) was used as a replacement for human serum in developing the standard curve since this reagent was readily available in our lab, and others have shown no significant difference between human and bovine serum matrices [20]. Our objective is to measure PFOS using available equipment LC-MS/MS 6490 QQQ and to compare the PFOS concentration in normal and metabolic syndrome patients.

2. Objectives

This study aims to quantify PFOS levels utilising the LC-MS/MS 6490 QQQ equipment, validate the methodology employed, and assess the disparity in PFOS concentrations between healthy individuals and patients with metabolic syndrome in human serum samples.

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Table 1 *Previous method for PFOS detection using LC-MS/MS*

References	Type of Extraction	Reagent	
		Mobile Phase A	Mobile Phase B
[10]	LLE + SPE	0.1% Formic Acid in water	Methanol
[21]	SPE	20 mM ammonium acetate in water	0.3% ammonium hydroxide in Methanol
[20]	SPE	51% ACN	49% 2 mM Ammonium Acetate
[7]	LLE	20 mM ammonium acetate in water	Methanol
[9]	Precipitation	2 mM ammonium acetate in water	Methanol (9:1) and 2 mM NH4Ac in methanol

3. Materials and Methods

3.1 Equipment and Chemical

The equipment used in the study is UHPLC-MS/MS-QQQ (UHPLC model: Agilent 1290 infinity; QQQ Model: A6490) (series number: SG1410A203) (Agilent, USA). Perfluorooctane Sulphonate (PFOS or Heptadecafluorooctanesulfonic acid), was purchased from Sigma–Aldrich (Switzerland). Sodium Perfluoro-1-Octanesulfonate (PFOS-13C8) 99% purity) 50 µg/ml of methanol was purchased from Cambridge Isotope Laboratories, Inc. (USA). The Oasis® HLB Cartridge was purchased from Waters (Massachusetts, US). Methanol, acetonitrile, n-hexane and HPLC-grade formic acid were purchased from Supelco (Darmsdat, Germany). LC-MS quality water was purchased from Supelco (Darmsdat, Germany). Calf serum (fetal bovine serum, FBS) was purchased from GIBCO (USA).

3.2 Sample Size

The sample size was calculated based on https://wnarifin.github.io > Sample size calculator. The study is based on sensitivity/specificity estimation of sample size from independent control and experimental subjects. An expected sensitivity of 0.99 and an expected specificity of 0.99 were selected. The 4.7% prevalence of MetS was selected based on previous findings [22] With a 95% confidence interval and with a 10% dropout, the final sample size is at least 10.

3.3 Quantification of PFOS in Human Serum

The concentration of PFOS in the blood samples of 40 metabolic syndrome patients (MetS) and 40 healthy individuals (control group) was measured using the matrix-matched PFOS standard. Metabolic syndrome was defined as at least 3 factors observed in the patients with conditions, as shown in **Table 2** below. Ethics approval and informed consent were obtained prior to commencement. Ethics approval from the UiTM Research Ethics Committee was obtained from REC/03/2020 (FB/47) (5th May 2020). The committee operates in accordance with the ICH Good Clinical Practice Guidelines, Malaysia Good Clinical Practice Guidelines, and the Declaration of Helsinki. The criteria that define metabolic syndrome are referred to as Joint Interim Societies (JIS criteria) [23].

Table 2 *JIS criteria*

Risk Factors	Criteria
Waist	Male ≥ 90 cm; Female ≥ 80 cm (South Asian cut-points)
Circumference	Systolic BP ≥ 130 and/or Diastolic
Blood Pressure (BP)	BP ≥ 85 mmHg or on treatment for HPT
Fasting Serum Glucose	≥ 5.6 mmol/L or on treatment for elevated glucose
Triglycerides	≥ 1.7 mmol/L or on treatment for TG
HDL-C	Male < 1.0 mmol/L; Female < 1.3 mmol/L or on treatment for HDL-C

3.4 Determination of the Peak

LCMS/MS datasets are composed of time series of individual full scan spectra interspersed by one or more MS/MS spectra derived from the fragmentation of one or more species present in the precursor ion. Then, 100 ng/mL PFOS without matrices was injected into an LC-MS/MS Agilent A6490 Triple Quadrupole (QQQ). The selection of

precursor ions was determined using the 'optimiser tool' in the Agilent A6490 QQQ to determine the precursor ions in total ion chromatogram (TIC) analysis. The optimisation of collision energy was tested at 40, 50, 60 and 70. The acquisition method for the source parameters is as in Table 3. The procedure was carried out followed by multiple reaction monitoring (MRM) to identify PFOS based on precursor ions.

Table 3 *Source parameters*

Parameters	Capability Values
Gas Tempt (°C)	270
Gas Glow (l/min)	11
Nebulizer (psi)	59
Sheath Gas Heater	300
Sheath Gas Flow (GHz)	11
Capillary (V)	3000
V Charging	1500

3.5 Optimisation of the Standard Curve

Four extraction methods were selected to determine the extraction method of PFOS from serum compounds in human samples prior to the development of the standard curve. From three extraction methods (data not shown) [7, 8, 10], an extraction method with modification was selected as the method to develop the standard curve. Known concentrations of PFOS (10, 20, 40, 100, 300 and 500 ng/mL) were spiked in fetal bovine serum (FBS) in 15 ml polypropylene tubes. Then, 2 µl of PFOS-13C8 (50 ng/mL) was added to each sample of the standard PFOS curve, making the value of each sample 500 µl. Then, 150 µl of 0.5 M formic acid, 5 ml acetonitrile and 2 ml n-hexane were loaded. The tubes were vortexed and placed in an ultrasonic bath (Sonicator Branson, 5800) for 20 minutes, 40°C. Centrifuge and collect supernatant. Similar concentrations were prepared as pure calibration standard spikes in water as the solvent. The volume was dried under nitrogen flow. The dried sample was reconstituted with 1 ml acetonitrile. The sample was filtered using an Agilent Econo Filter (PTFE 0.2 µm) and injected into an LC-MS/MS 6490 QQQ, operating in negative ion mode. Ten microliters of sample were injected into a UHPLC Guard ZORBAX Eclipse Plus C18 (Agilent, USA) and ZORBAX RRHD 2.1 × 50, 1.8 µm column (Agilent). The eluent system comprised water (A) and acetonitrile (B), following the running conditions adapted from [24], with modifications made to the flow rate and retention time tuning, as indicated in **Table 4** below. All the steps involved for samples optimisation were summarised in supplement 1. These mobile phases offer comparable separation with fewer steps compared to others. Alternative methods employing an SPE kit may require the addition of ammonium acetate to disrupt weak ion-pair interactions with the kit (such as Oasis®) [20], necessitating unnecessary extra steps for sample preparation. Subsequently, a similar concentration of ammonium acetate may need to be added to the mobile phase. However, in our study, we found it unnecessary to incorporate ammonium acetate into the mobile phase.

Table 4 *Mobile phase composition changes as follows*

Time	Water	Acetonitrile	Flow Rate
2 min	90%	10%	0.6 ml/min
5 min	3%	97%	0.6 ml/min
11.75 min	3%	97%	0.6 ml/min
2.5 min	90%	10%	0.6 ml/min
15 min	90%	10%	0.6 ml/min

3.6 Method Validation

The LC-MS/MS method was validated with respect to the specificity, linearity and sensitivity, precision and accuracy, matrix effects and recovery.

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3.6.1 Specificity

The specificity test was conducted by comparing chromatograms of samples of PFOS in water, samples of serum extracts spiked with PFOS and samples of serum extracts in control human serum.

3.6.2 Linearity and Sensitivity

To evaluate the method's linearity and sensitivity, two distinct PFOS concentrations in water were utilised as calibration standards, while the same concentrations of PFOS in a matrix solution (FBS) served as matrix standards. Three replicates for each calibration point were included in the calibration curves, and linear regression was used to determine their linearity.

Based on previous research showing that the chemical can be detected at 33 ng/mL in serum [21] , the calibration range was reduced to 10 ng/mL by visual evaluation of 10 analytical runs [25]. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use recommendations were followed in calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ).

$$\text{LOD} = 3.3 \text{ x } \frac{\text{residual SD}}{\text{slope of the calibration curve}} \quad (1)$$

$$\text{LOQ} = 10 \text{ x } \frac{\text{residual SD}}{\text{slope of the calibration curve}} \quad (2)$$

where SD is the standard deviation of the signal at the lowest point of the calibration curve.

3.6.3 Precision and Accuracy Study

The intraday accuracy and precision measurements were conducted using measurements of six concentrations of PFOS (within the calibration range: 10 ng/mL, 20 ng/mL, 40 ng/mL, 100 ng/mL, 300 ng/mL, and 500 ng/mL) dissolved in water on a single assay, repeated three times within a day.

Precision was expressed as the coefficient of variation (CV), whereas accuracy was expressed as the relative error (RE). The formula to obtain CV% and RE are as follows:

$$\text{CV} = \frac{\text{standard deviation}}{\text{mean}} \text{ x } 100 \quad (3)$$

$$\text{RE} = \frac{\text{measured value}}{\text{actual value}} \text{ x } 100 \quad (4)$$

3.6.4 Matrix Effect

There are 3 approaches to evaluate the matrix effect. The approaches include signal-based methods, concentration-based methods [26], or calibration-graph methods (accessed on 11 April 2022 https://sisu.ut.ee/lcms_method_validation/54-quantitative-estimation-matrix-effect-recovery-process-efficiency, University of Tartu, Estonia). In this study, two calibration graphs are prepared. One calibration graph is where 6 different PFOS concentration levels were prepared in solvent (water), and the other calibration graph with similar concentrations was prepared in FBS (known as a matrix-matched calibration graph). For both graphs, the slope was calculated and compared to the obtained % matrix effect (ME) using the formula below:

$$\% \text{ ME} = \frac{\text{Slope matrix matched}}{\text{Slope solvent}} \text{ x } 100\% \quad (5)$$

3.6.5 Recovery

The recovery was determined by comparing the MS response level of spiked samples pre- and post-extraction, according to the SANCO guide, using the average result of 3 replicates. The recovery percentage was calculated by dividing the value for the MS response of the measured amount by that of the spiked amount. Six concentration levels of PFOS ranging from 10 ng/mL to 500 ng/mL were tested within the linearity range. The formula is as below:

$$\% \text{ Recovery} = \frac{\text{Measured amoun}}{\text{Spiked amount}} \text{ x } 100\% \quad (6)$$

4. Results and Discussion

Negative electrospray ionization mass spectrometry was previously employed for the analysis [7, 10], producing a main ion at m/z 499.94 and a recognized fragmentation pattern. Our experiments revealed the same pattern (**Figure 1**). LC-MS/MS protocol optimisation aimed to produce separate symmetrical peaks with high resolution. The C18 LC column with a 1.80 µm particle size, 97% acetonitrile as a mobile phase, and a speed of no more than 0.60 ml/min gave the greatest results for LC separation and offered the most hospitable circumstances for MS/MS observations.

4.1 Peak Optimisation and Identification

Figures below show the MS/MS scan of the PFOS compound within 15 minutes of analysis. Collision energy (CID) was determined to be optimum at 60 with precursor ions at 81.90 m/z, 130.00 m/z, 168.70 m/z and 230.00 m/z in TIC multiple reaction monitoring (MRM) analysis to identify PFOS based on the precursor ion compound. The 130 m/z was automatically detected and selected as the quantifier, while the rest were selected as qualifiers (81.90 m/z, 168.70 m/z and 230.00 m/z) by Agilent Mass Hunter QQQ Quantitative because 130 m/z is the most abundant ion fragmentation to parent ion 499.94 m/z PFOS. For PFOS-13C8 (522.9 m/z), this compound, under the fragmentation of 80.10 m/z as a quantifier and 99.10 m/z as a qualifier, was chosen to distinguish the MRM between the internal standard and the target compound. The fragmentation at this m/z for the internal standard has been previously reported [27].

4.2 Specificity

A comparison of a blank reference sample (water) with spiked blanks (PFOS added to water or serum) demonstrated a high specificity of the method. The PFOS-13C8 as an internal standard is on the sharp peak at 522.90 detected at 4.894 RT Figure 1 (a) with optimum fragmentation Figure 1 (b).

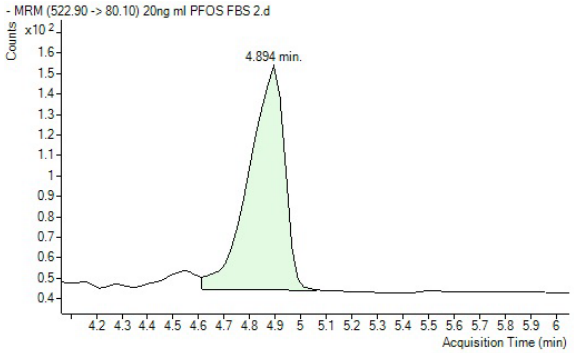

Fig. 1 (a)

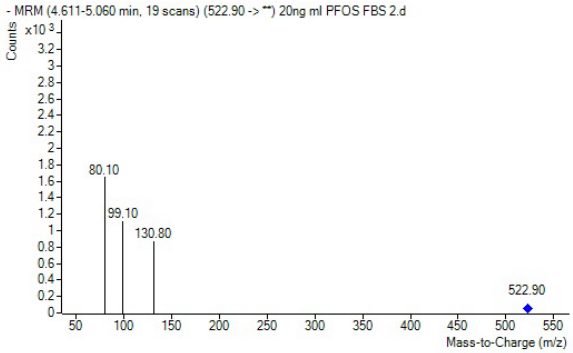

Fig. 1 (b)

Fig. 1 *(a) Chromatogram of PFOS-13C8 detected under 80.10 m/z product ions; and (b) MS Spectrum of PFOS-13C8 with optimal breakdown indicated by no peak remaining at 522.9 m/z*

The specificity is determined based on the sharp peak at m/z 499.94 observed in water where no other compound is present. Neither pure water Figure 2 (a) nor pure serum chromatograms Figure 2 (b) showed any peaks with PFOS. Then, the peak can be detected again with a coefficient of variation (CV) of less than 1% after PFOS is spiked and extracted from FBS. Peaks with PFOS-specific MS fragmentation were observed when spiked in water Figure 2 (c) and FBS at low (20 ng/mL) concentrations Figure 2 (d).

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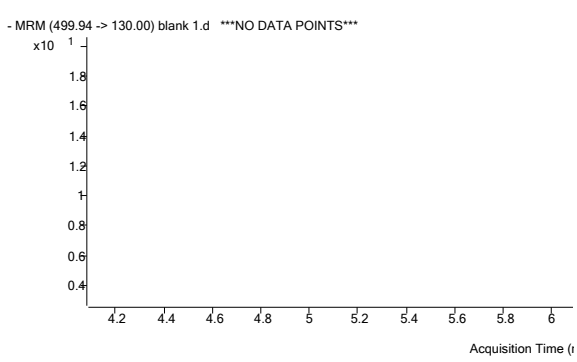
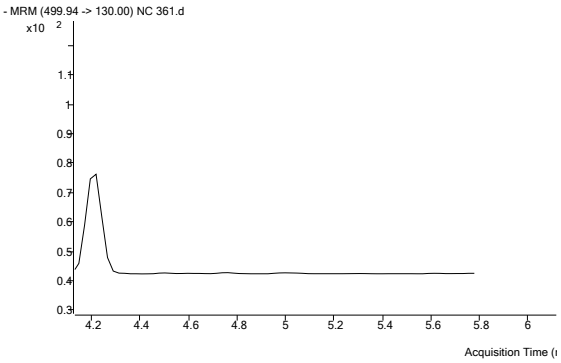
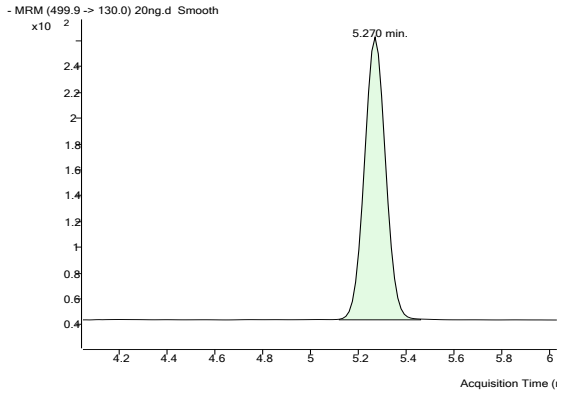


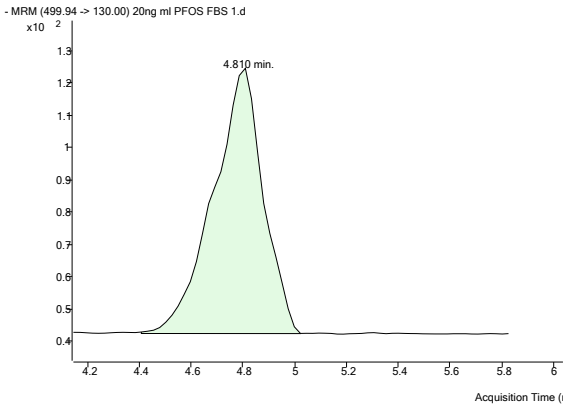
2 (a)



2 (b)



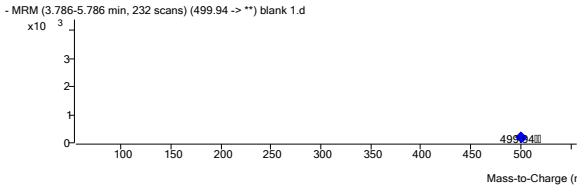
2 (c)



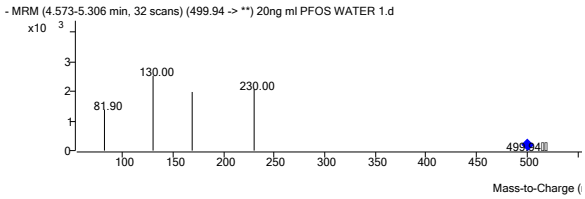
2 (d)

Fig. 2 *(a) Chromatogram in water (Blank); figure 2 (b) control human serum; figure 2 (c) 20 ng/mL of PFOS spiked in water; and figure 2 (d) 20 ng/mL of PFOS spiked in FBS (matrix effects) at 130 m/z quantifier condition*

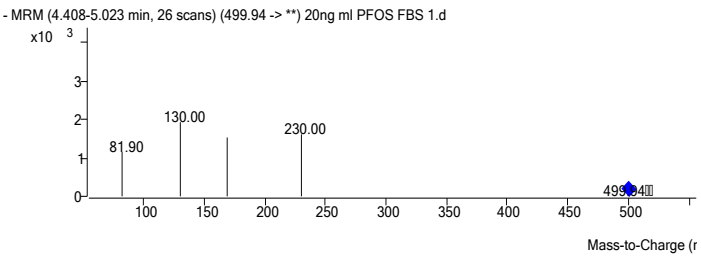
Figure 3 (a) confirmed that no contamination occurred during the fragmentation of water (blank) in comparison to PFOS spiked in water **Figure 3 (b).** The fragmentation of PFOS spiked in water is comparable to PFOS spiked in FBS, where all quantifiers and qualifiers are still recovered prior to extraction **Figure 3 (c).**



3 (a)



3 (b)



3 (c)

Fig. 3 *(a) No peak indicates no compound detected and fragmented in the blank; figure 3 (b) No peak effect remaining at 499.9 m/z indicates optimal breakdown of PFOS spiked in water; figure 3 (c) No peak effect remaining at 499.9 m/z indicates optimal breakdown of PFOS spiked in FBS*

Although MRM presented that the product ions of PFOS can be detected in extracted control human serum samples when compared to the blank (Figure 4), the relative response value is too low to be quantified for all product ions. This demonstrated that there is a possibility of PFOS existing in the control human serum, but it is too low to be detected. PFOS was found to accumulate in the human body at approximately 13 to 30 ng/mL due to a high biliary reabsorption rate and low levels of excretion in the urine [28, 29]. The concentration of PFOS accumulated in tissues varies, and the highest accumulation is in the liver [30]. Furthermore, the PFOS compound was previously reported to be detected in human serum from an urban population in Malaysia (n=23), with concentrations ranging from 6.2 to 18.8 ng/mL [31]. Therefore, human serum is not suitable for use as a matrix-matched calibration, which may later cause bias.

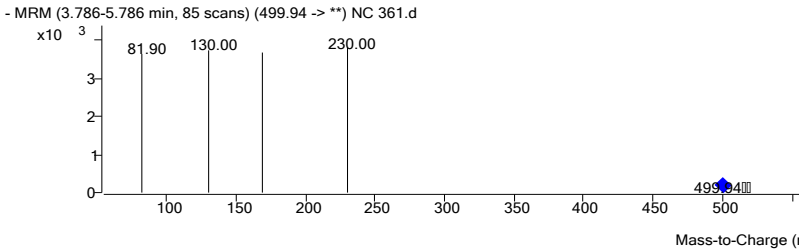


Fig. 4 *The Multiple Reaction Monitoring (MRM) fragmentation of perfluorooctane sulfonate (PFOS) in extracted control human serum samples was performed at a precursor mass-to-charge ratio (m/z) of 499.9*

4.3 Linearity and Sensitivity

While PFOS indeed could be detected at very low concentrations (20 ng/mL), the linearity range was more restrictive: after a series of adjustments, the reliable measuring interval ranged from 20 to 500 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the calibration curve obtained for serum extract and were 13 ng/mL (LOD) and 38 (LOQ), respectively, based on the formula given. A comparison of blank and spiked PFOS (added to water or FBS) demonstrated a high specificity of the method.

4.4 Precision and Accuracy

Levels of concentrations ranging from 10 ng/mL to 500 ng/mL were evaluated for precision and accuracy. However, the LOD was determined at 13 ng/mL after the calibration standard was plotted using concentration points ranging from 20 ng/mL to 500 ng/mL. The resulting fluctuations from 20 ng/mL to 500 ng/mL did not exceed 2% for intra-assays, with CV ranging from 0.01 to 1.732%. Furthermore, the RE for each concentration also did not exceed 18%. Although deviations for accuracy and precision were within limits recommended by most of the guidelines (IUPAC, FDA and SANCO, where 15-20% is given as an acceptable level of variation), it is recommended that the calibration samples be included in every sequence in future experiments with re-evaluation of calibration graphs to negate the natural instability of signal MS.

Table 5 *Precision and accuracy studies for 5 levels of PFOS concentrations in water*

Expected Concentration	Average Measured Mean Concentration (ng/mL)	CV (%)	Relative Error (%)
20.00	21.00	0.67	5.00
40.00	47.00	0.03	10.00
100.00	119.00	0.01	7.50
300.00	302.00	0.13	0.70
500.00	496.00	0.02	0.80

4.5 Matrix Effect

This matrix effect is assessed by the calibration graph method to evaluate the matrix effects (ME) over a wide concentration range. By using the calibration graph method, when the value is within the linear range, the slope is not influenced by the small amount of analyte originally present in the extract. The relative response versus relative concentration was plotted to obtain both graphs after normalisation with an internal standard. The slope obtained from the calibration curve of PFOS in water is 0.018751 Figure 5 (a), whereas the slope obtained from

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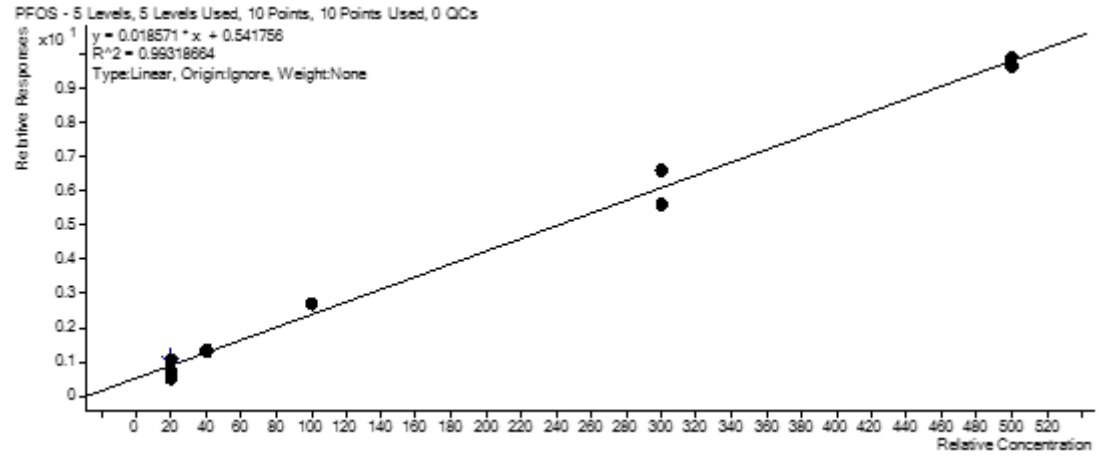
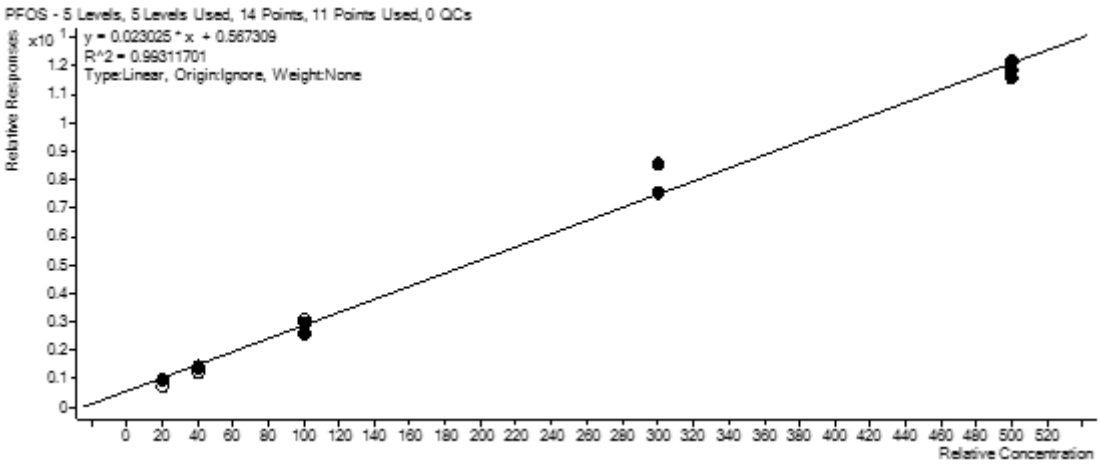
the calibration curve of PFOS in FBS is 0.023025 Figure 5 (b). The % ME obtained is 123%. This indicates that is slight ionization enhancement occurred during the analysis, that is 23%. However, it is considered acceptable since the increments is ≤ 85 % [32, 33].



5 (a)



5 (b)

Fig. 5 *(a) Standard addition curve of PFOS in water; and figure 5 (b) Standard addition curve of PFOS spike in FBS and extraction method*

4.6 Recovery

The extraction recoveries of PFOS were evaluated in triplicate for relative intensity by comparing the peak responses of pre-extracted samples to those of post-extracted samples at all levels. The average recoveries of PFOS extraction at all levels are shown in **Table 5.** The % recovery precision of PFOS was below 20% CV, indicating that the extraction efficiency of this method was adequate and reproducible. This demonstrates that the developed method has an acceptable extraction recovery.

Table 5 *Recovery study on PFOS extracted using the Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7]Forsthuber, Kaiser [7] method*

Spiked Concentration (ng/mL)	Average Extracted Mean Concentration (ng/mL)	Recovery (%)
20	21	105
40	47	118
100	119	119
300	302	104
500	496	99

4.7 PFOS Quantification in Control and MetS Serum

The analysis of 40 samples of the metabolic syndrome group (MetS) and 40 samples of the control group (healthy individuals) was carried out using the PFOS matrix-matched calibration curve. Profiles in the serum showed that PFOS was not detected in the control sample, while PFOS was detected in 4 MetS samples. No significant difference was observed in PFOS concentrations between the MetS and control groups (p-value = 0.1156, **Figure 6**). Our method is comparable to Solid phase extraction coupled to High-Performance Liquid Chromatography Turbo Ion Spray ionization-tandem Mass spectrometry (online SPE-HPLC-TIS-MS/MS). The instrument exhibits 118 $\pm$ 23 %, 93 $\pm$ 10 % and 102 $\pm$ 3 % at 3 different concentrations (0.2, 1.25 and 12.5 ng/mL) comparable to our method where we manage to measure down to 20 ng/mL without the need of using SPE [34].

Few studies have specifically examined the potential effect of PFOS on metabolic syndrome using nationally representative surveys. Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35]Fisher, Arbuckle [35] examined PFOS using the Canadian Health Measures Survey and also found no association with metabolic syndrome, glucose-related outcomes, or triglycerides [35]. Lin, Chen [36]Lin, Chen [36]Lin, Chen [36]Lin, Chen [36]Lin, Chen [36]Lin, Chen [36]Lin, Chen [36]Lin, Chen [36] examined earlier cycles of NHANES data and found that PFOS was not associated with the prevalence of metabolic syndrome in adults, but PFOS was associated with lower HDL cholesterol [36]. The median of 8.4 ng/mL PFOS was associated with MetS in the 2007-2014 survey with total samples of 2975 by using Solid phase extraction coupled to High-Performance Liquid Chromatography Turbo Ion Spray ionization-tandem Mass spectrometry (online SPE-HPLC-TIS-MS/MS) [34].

A recent investigation revealed a statistically significant positive correlation between PFOS serum levels and the individual components of Metabolic Syndrome (MetS) [22]. However, our findings diverge from most previous studies, as we observed no significant difference. While PFOS was detected in MetS cases, the results did not attain significance. One potential explanation is that further analysis incorporating other parameters, such as individual components like blood pressure or triglyceride levels, as confounding factors, may elucidate any association. Employing different statistical methodologies, such as multivariate analysis, may be necessary to confirm the association between PFOS in serum and MetS. Different statistical tools might need to be applied such as multivariate analysis to confirm the PFOS in serum associated with MetS.

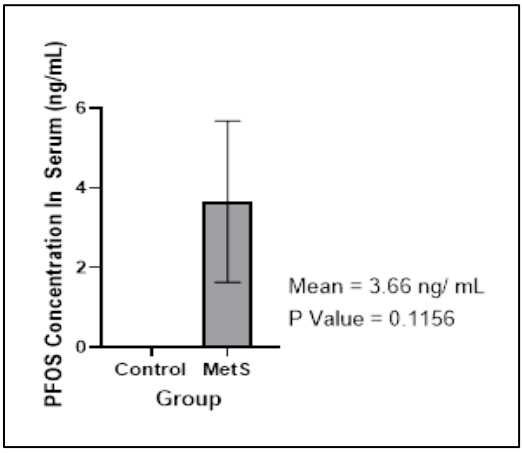


Fig. 6 *The graph shows the difference in the concentration of PFOS (ng/mL) detected in the control group (n = 40 not detected) and metabolic syndrome (MetS, n = 4 detected, n = 36 not detected). Statistical tests showed that the difference between the groups was insignificant (p-value> 0.05)*

5. Conclusion

The method described here is suitable for rapid, automated analysis of PFOS in human serum. Sample volumes up to 500 µl can be used, with some loss of sensitivity at 10 ng/mL. Our validation of the method established its robustness and accuracy. The method is comparable to previous studies and able to differentiate PFOS levels in diseased and healthy individuals, although larger sample sizes are required to achieve statistical significance. Instead of comparing the serum or plasma levels with confirmed MetS individuals, the approach needs to be further investigated with MetS components such as blood pressure, weight and cholesterol levels.

Acknowledgement

The authors gratefully acknowledge the financial support granted by Malaysian Ministry of Higher Education via Higher Institution Centre of Excellence (HICoE) research grant 600-RMC/MOHE HICoE CARE-I 5/3 (01/2025).

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***writing – original draft****: Siti Suhana Abdullah Soheimi, Normala Ab Latip, Siti Hamimah Sheikh Abdul Kadir;* ***writing – review & editing:*** *Normala Ab Latip, Effendi Ibrahim, Amirah Abdul Rahman, Siti Hamimah Sheikh Abdul Kadir;* ***writing – methodology:*** *Siti Nur Faridatul Asyikin Said, Normala Ab Latip;* ***visualization:*** *Siti Suhana Abdullah Soheimi;* ***software:*** *Siti Suhana Abdullah Soheimi;* ***investigation:*** *Siti Suhana Abdullah Soheimi;* ***formal analysis:*** *Siti Suhana Abdullah Soheimi, Siti Nur Faridatul Asyikin Said, Normala Ab Latip;* ***data curation:*** *Siti Suhana Abdullah Soheimi dan Siti Nur Faridatul Asyikin Said;* ***supervision:*** *Normala Ab Latip, Effendi Ibrahim, Amirah Abdul Rahman, Siti Hamimah Sheikh Abdul Kadir;* ***resources:*** *Normala Ab Latip, Effendi Ibrahim, Amirah Abdul Rahman, Siti Hamimah Sheikh Abdul Kadir;* ***conceptualization:*** *Siti Hamimah Sheikh Abdul Kadir. All authors reviewed the results and approved the final version of the manuscript.*

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