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**Research Article**

# Development of supramolecular-based dispersive liquid-liquid microextraction (SM-DLLME) for the determination of acrylamide in food samples via HPLC analysis

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## Abstract

The study presents a successfully developed supramolecular-based dispersive liquid-liquid microextraction (SM-DLLME) for the determination of acrylamide in food samples via HPLC analysis. The detection and quantification of acrylamide was performed at a wavelength of 210 nm. A thorough optimization was performed for seven extraction parameters via the one-variable-at-a-time (OVAT) method. Using the refined experimental parameters, this technique achieved good linearity ranging from 100 to 1000 µg kg$^{-1}$ accompanied by the coefficient of determination, $R^2$ value of 0.9967, high sensitivity with low limit of detection (LOD) value at 13 µg kg$^{-1}$, limit of quantification (LOQ) at 40 µg kg$^{-1}$ and excellent recovery of 91.4-105 %. The method provides a good precision value with a relative standard deviation (RSD) of less than 1.5 % for both intra and inter-day. The SM-DLLME technique managed to extract acrylamide in food samples by applying the green chemistry concept in a fast and efficient way. The developed and validated method for the determination of acrylamide was applied to real food samples with six different types of potato fries obtained from three different fast-food restaurants, frozen potato fries cooked via deep-fried and air-fried, along with homemade potato fries.

**Keywords:** SM-DLLME, acrylamide, 1-pentanol, HPLC, potato fries

## Introduction

Acrylamide is a natural compound comprised of three carbon atoms, five hydrogen atoms, a nitrogen and an oxygen atom in the formula of $CH_2$=$CHCNH_2$. Acrylamide is known as 2-propenamide and exists as a white odorless and colorless crystalline solid. About nineteen years ago, acrylamide was found in commercial processed foods and believed to be formed due to a high-temperature process which causes a reaction called Maillard Browning, through an interaction between free amino acid asparagine and a carbonyl group of glucose as the reducing sugar in carbohydrate-rich foods. Due to this reaction, acrylamide causes the production of toxic substances as well as reduces the nutritional value and safety of foods. This toxic substance is considered a plausible cause of carcinogenic substances in those selected foods yielding genotoxic and neurotoxic effects [1].

Based on regulation bodies such as the International Agency for Research on Cancer (IARC), United States Food and Drug

Administration (USFDA), National Toxicology Program (NTP), Environmental Protection Agency (EPA), Food and Agriculture Organization of the United Nations (FAO), and European Food Safety Protection (EFSA) acrylamide is classified as a carcinogenic substance to the human bodies. Numerous studies have been performed to understand the correlation between acrylamide to cancer in laboratory animals and concluded that it causes cancer at very high doses and is detected via acrylamide markers in blood. The evidence in humans is not confirmed yet but still, acrylamide is a human health concern, and its consumption should be monitored [2,3].

Numerous studies have been performed to understand the correlation between acrylamide to cancer in laboratory animals and concluded that it causes cancer when acrylamide is provided at a very high dose and detected via acrylamide markers in blood. The evidence in humans is not confirmed yet but still, acrylamide is a human health concern, and its consumption should be monitored. Several types of carbohydrate-rich foods hugely contribute to acrylamide formation, as listed in **Table 1** [4].

Among all these food categories, potatoes are seen with the highest content of acrylamide. Several studies showed differences in acrylamide content between dried potatoes and fresh potatoes. This is because the amount of reducing sugars is distinct in each type. Factors that influence the reducing sugar levels in these foods are climatic conditions, fertilizer application rates, cultivars, storage temperature and time. Fried potato products (french fries and potato chips) contribute about 49 % acrylamide exposure in adults and up to 51 % in adolescents and toddlers. Adults who consume coffee and soft bread are exposed to 34 % and 23 % of acrylamide exposure. Children, however, are exposed to up to 25 % of acrylamide from breakfast cereals and biscuits, their main important dietary sources [5,6]. Due to these factors, organizations such as the USFDA and FAO can identify ways to reduce the content of acrylamide in certain foods by controlling the level of reduced sugars. The World Health Organization (WHO) has come up with a guideline mentioning that the threshold limit for acrylamide in drinking water is 0.5 µg $L^{-1}$ but the limit of acrylamide in foods is not specified [2].

Several techniques have been developed to identify and quantify acrylamide content in certain foods using sensitive analytical instruments such as HPLC-DAD, GC-MS, LC-MS, HPLC-UV and UV/Vis spectrophotometer. Acrylamide has a limitation in terms of lacking a strong chromophore for UV detection and is not fluorescent [1]. It has low molecular weight with low volatility which also causes issues on some of the quantification techniques used. To identify this analyte of interest, a proper extraction method is required. Acrylamide is mostly found at trace level concentration and the sensitivity and accuracy are limited due to a large matrix interference coming from the food samples. So far, common methods such as SPE, LLE, ELISA, Soxhlet, and Bromination are used widely by analytical scientists for the determination of acrylamide in laboratories. However, due to the drawbacks of the conventional extraction method, which is time-consuming, complicated, and requires a large usage of organic solvents and sample volumes, in this study, a rapid and sensitive extraction technique dispersive liquid-liquid microextraction (DLLME) [7,8].

DLLME is fast, inexpensive, and compatible with a lot of analytes as well as analytical instruments. The technique involves a process that requires a smaller volume of solvents, with a faster phase transfer time. This procedure involves the generation of microunits of acceptor phase volume within the donor phase to increase the contact surface. Two types of solvents are required as mixtures in this extraction procedure which are dispersive solvent and extraction solvent. Dispersive solvent must be miscible with the donor and acceptor phase while extractant solvent specifically has to be immiscible with the donor phase [9]. The DLLME technique uses an addition of supplementary reagents or energy to enhance the formation of the cloudy solution. To accelerate the formation of fine droplets of extraction solvent to enhance the transfer of analytes, additional reagents like surfactants (SA-) chelating and ion pairing (IP-), as well as effervescence agents (EA-) are used in this technique. Additional energies such as ultrasonication (UA-), magnetic stirring (MSA-), vortex mixing (VA-), and microwave irradiation (MW-) are commonly used [10].

A supramolecular solvent (SUPRAs) is a special type of solvent that has been widely used for many analytical extraction techniques due to its unique properties and features such as the presence of different polarity regions, amphiphiles formation, and excellent solvation properties for inorganic and organic compounds. The processes for the formation of SUPRAs can be seen in **Figure 1**. Since its introduction, the solvent has been consumed massively for the extraction of hydrophobic organic compounds that originate from environmental and biological liquid samples. The main purpose of developing an extraction technique with SUPRAs-based is to decrease the usage of organic solvents in laboratories. This supramolecular solvent is attractive as it uses the concept of green chemistry along with high extraction efficiency, rapidity, and is highly suitable for miniaturized extraction technique. SUPRAs have been successfully applied in large applications such as pesticides, foods, and biological samples with high recoveries in the range of 80 - 100% [11].

Thus, this study aims to design and assess an efficient, rapid, and eco-friendly extraction method for detecting acrylamide in fried potato products. This will be achieved using dispersive liquid-liquid microextraction (DLLME) enhanced by supramolecular solvents (SUPRAs). This technique aims to improve sensitivity and extraction efficiency while significantly reducing the use of organic solvents, making it both cost-effective and environmentally sustainable. By employing SUPRAs in this process, the study seeks to overcome common challenges in acrylamide detection, such as matrix interference, while promoting green chemistry practices in analytical testing.

## Materials and Methods

### Chemicals and reagents

Certified acrylamide standard was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade methanol 99.7% (MeOH), 1-pentanol, 1-hexanol, 1-heptanol, and 1-octanol were purchased from Sigma-Aldrich (St.Louis, MO, USA). Deionized water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). A range of micropipettes from 100 µL to 5000 µL obtained from Sartorius and Eppendorf were used throughout the experiments. Waters HPLC vials and 200 µL inserts were used as well.

### Preparation of acrylamide standard and working solutions

Standard acrylamide was dissolved in methanol at a concentration of 1000 µg kg$^{-1}$ and stored in the refrigerator at 4 °C as stock solution prior to use. Working standard solutions at different concentrations were prepared by diluting the standard stock solution in deionized water, whenever required.

**Table 1.** The content of acrylamide in several food categories

| Food Category | Food Category Content of Acrylamide (µg kg$^{-1}$) |
|---|---|
| Biscuits and crackers | 30-3200 |
| Potato chips | 170-3700 |
| Breakfast cereals | 30-1346 |
| Potato fries | 200-12000 |
| Coffee powder | 170-351 |
| Roasted sunflower seeds | 66 |

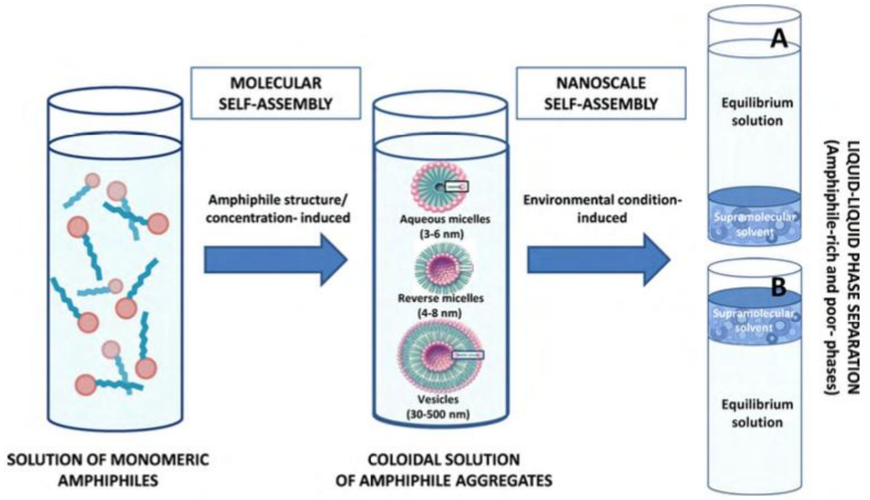

**Figure 1.** Two main processes of self-assembly that resulted in the formation of SUPRA

### Instrumentation

All analysis was performed using Waters ACQUITY UPLC/UHPLC Systems consisting of a quaternary pump with a vacuum degasser, a temperature-controlled column oven, an autosampler, and with photo-diode array (PDA) detector. A centrifuge (Thermofischer) was used for the liquid-liquid separation procedure, together with a vortex mixer. The HPLC separations were performed using a Kromasil C8 column (250 x 4.6 mm i.d., 5 µm) at 0.4 mL/min. The wavelength chosen was at 210 nm, the injection volume was 50 µL, and the oven temperature was at 30 °C. A gradient elution program with the mobile phase comprising of solvents A and B (A: 0.005 M heptanesulfonic acid in water with 0.1 % (v/v) acetonitrile [12]. B: pure acetonitrile). The elution program was applied as follows: 100 % A for 15 minutes, decreased to 20 % A from 15 to 17 minutes, and kept at 20 % A for 5 minutes. Increased A to 100% from 22 to 24 minutes and kept at 100 % A for 6 minutes. Under these chromatographic conditions, acrylamide and the food components in the test food samples were all baselines separated and eluted. All instrument control, analysis, and data processing were performed via Waters Empower 3 Chromatography Data Software (CDS).

### Sample preparation and pre-treatment

Potato fries from three different fast-food restaurants were purchased in Shah Alam, Selangor and the selected fast-food restaurants were Brand A, Brand B, and Brand C. Frozen potato fries were purchased from a local supermarket; some amount was deep-fried while some other amount was air-fried using Russell Taylor’s Air Fryer at 200 °C for 10 minutes. Homemade potato fries were prepared at home by cutting potatoes into small sizes and deep-fried until golden. Each representative sample (100 g) was chopped and homogenized using a homogenizer. Then, the sample solution was weighed at 20 g into a 500 mL flask. 200 mL of deionized water was added and left at room temperature for 20 minutes for swelling. Then, the sample was shaken for 1 hour at 60 °C to enhance the extraction of acrylamide and centrifuged for 10 minutes at 5000 RPM. The supernatant solution was transferred into a 250 mL flask and defatted using 1 mL n-hexane. The fat layer was removed using a plastic *Pasteur* pipette and the solution was filtered using a 0.45 µm membrane filter. The filtered solution was stored at 4 °C before the SM-DLLME analysis [12].

### SM-DLLME procedure

About 4.2 mL sample solution containing the analyte was dissolved in deionized water and pipetted into a 15 mL centrifuge tube. Then, 800 µL SUPRAs solvent (extraction solvent) and 400 µL THF (dispersive solvent) were added into the same centrifuge tube. Water was used in this procedure to allow an efficient formation of amphiphilic structures such as micelles. Both solutions were then mixed via vortex for 20 seconds and followed by centrifugation for 5 minutes at 6000 rpm to enhance the phase separation. Afterwards, 100µL of the extractant-rich phase (which was the top layer) was transferred into a 200µL insert placed into an HPLC vial. The solution was injected into HPLC and run according to the chromatographic conditions set. **Figure 2** shows a schematic diagram for the optimized SM-DLLME procedure [13].

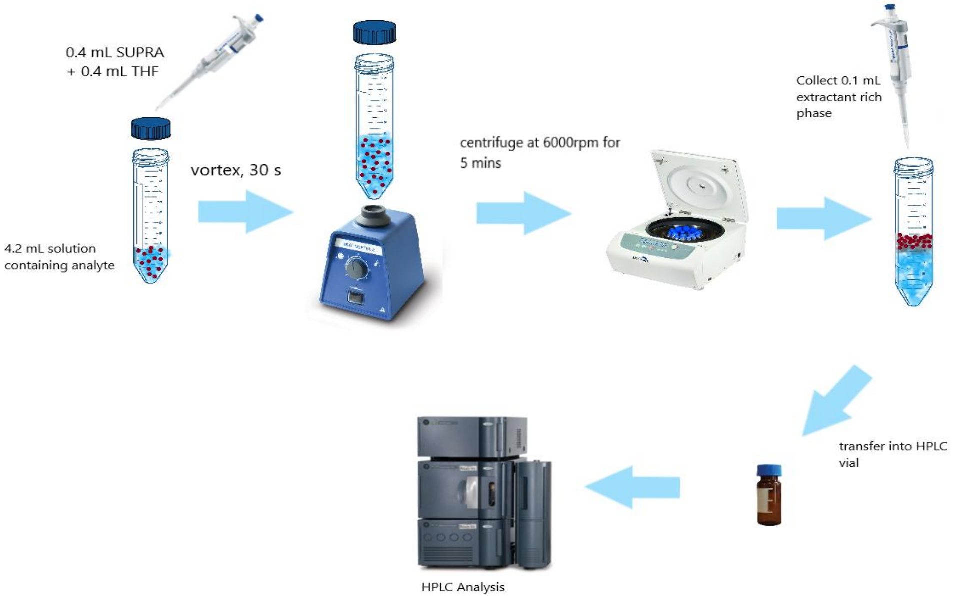

**Figure 2.** Schematic diagram for SM-DLLME procedure.

### Optimization of extraction variables

Factors that could affect the extraction efficiency such as type of SUPRAs, sample pH, the volume of SUPRAs, type of dispersive solvent, addition of salt concentration, extraction time, and volume of dispersive solvent were investigated in this study. The approach for optimization variables was one-variable-at-a-time (OVAT).

### OVAT optimization

Using the OVAT approach, each run was prepared in triplicate to observe the reproducibility of the experiment data. Seven important variables that could give significant effects were optimized using this method such as types of SUPRA, sample pH, volume of sample, volume of SUPRAs, types of dispersive solvent, addition of salt concentration, extraction time, and volume of dispersive solvent.

### Method validation

Method validation (linearity, limits of detection (LOD) and quantitation (LOQ), matrix effect and recovery were performed using the proposed in-syringe SM-DLLME method. Linearity was investigated over the range of 100 to 1000 µg kg$^{-1}$. The LOD and LOQ values were calculated based on the standard deviation of the response of the standard blank and the slope of the calibration curve. The pre-concentration factor was calculated from the spiked peak. While recovery studies were carried out using spikes at three concentration levels (200, 500, and 1000 µg kg$^{-1}$). All spiked samples were extracted in triplicates. All the formula is shown below.

$$\text{RSD} = \frac{\text{standard deviation}}{\text{mean}} \times 100\% \qquad (1)$$

$$\text{LOD} = \frac{\text{standard deviation}}{\text{slope}} \times 3.3 \qquad (2)$$

$$\text{LOQ} = \frac{\text{standard deviation}}{\text{slope}} \times 10 \qquad (3)$$

$$\%\ \text{Recovery} = \frac{C_{ext}}{C_{int}} \times 100 \qquad (4)$$

$$\text{ME} = \frac{\text{slope of calibration curve for acrylamide in sample matrix}}{\text{slope of calibration curve for acrylamide in water}} \qquad (5)$$

Where $C_{ext}$ is the concentration of analyte in the extractant phase, while $C_{\text{int}}$ is the initial concentration of acrylamide.

## Results and Discussion

### Identification of acrylamide through peak assignment

The real sample solution was injected into the HPLC-DAD system after the optimized SM-DLLME conditions. Acrylamide peak was observed in the sample solution, assigned based on the retention time (Rt = 12.007) and compared with the standard solution of 500 µg kg$^{-1}$. Since DAD was used as the detector, both UV spectra were compared to confirm the identification of acrylamide. The identified peaks in both standard and sample solutions were compared (**Figure 3**).

### Optimization of extraction conditions

Seven parameters contributing to the SM-DLLME performance were chosen and studied. The parameters were the type of SUPRAs, volume of SUPRAs, type of dispersive solvent, volume of dispersive solvent, sample pH, and extraction time. All parameters were optimized using the OVAT technique, and at each condition, the preparation was triplicated. Acrylamide standard solution of 500 µg kg$^{-1}$ in deionized water was used for the whole SM-DLLME optimization process.

### Type of SUPRAs

Four supramolecular solvents, 1-pentanol, 1-hexanol, 1-heptanol, and 1-octanol were used to study the extraction effects of acrylamide in food samples. Different types of SUPRAs will provide different effects due to their distinct solvent properties of self-assembly and interaction with the analyte of interest. The extraction of acrylamide with 1-hexanol, 1-pentanol, 1-heptanol, and 1-octanol was evaluated and summarized in **Figure 4(a)**. 1-pentanol was chosen as the extraction solvent as it obtained the highest peak area compared to other SUPRAs. The size of aggregation depends highly on the hydrocarbon chain length and the width of the head group in terms of inter-molecular forces and 1-pentanol is the smallest alkanol with only five carbons, eleven hydrogens and one -OH bond. Due to its shortest alkyl group, it provides the highest polarity compared to the other alkanols studied and is advantageous for self-aggregation and rapid formation of micelles [11]. 1-pentanol also has the lowest molecular weight with the lowest density which allows it to be easily formed at the top layer while reducing the possibility of contamination while extracting the upper phase.

### Volume of SUPRAs

The volume and composition of SUPRAs solvent are key factors that greatly affect the extraction capability [14]. To study the volume of selected SUPRAs solvent, 1-pentanol was examined in the range of 600 µL to 1200 µL and added into the SM-DLLME procedure. The total volume was fixed at 5 mL which constituted of sample containing 500 µg kg$^{-1}$ acrylamide solution in deionized water and the volume of SUPRA studied. As shown in **Figure 4 (b)**, the volume of 1-pentanol influenced the extraction of acrylamide as the peak area decreased when the volume of SUPRAs increased (≥ 800 µL). This is because as the volume of extraction solvent increases, the viscosity of the extraction-rich phase increases as well resulting in a higher dilution effect. Below the volume of 800 µL, the extractant-rich phase formed was reduced. A decrease in solvent volume has been related to the formation of a more concentrated extractant-rich phase. When the volume of 1-pentanol was decreased to 500 µL, there was no separation observed at all. This was due to the insufficient amount of SUPRAs solvent that was not able to allow the formation of amphiphile and coacervation process to take place. The amphiphile could not aggregate thus trapping the analyte of interest into the micelle's formation. Therefore, the amount of 1-pentanol was selected at 800 µL and applied to the SMDLLME extraction procedure. The volume was kept constant for further optimization studies.

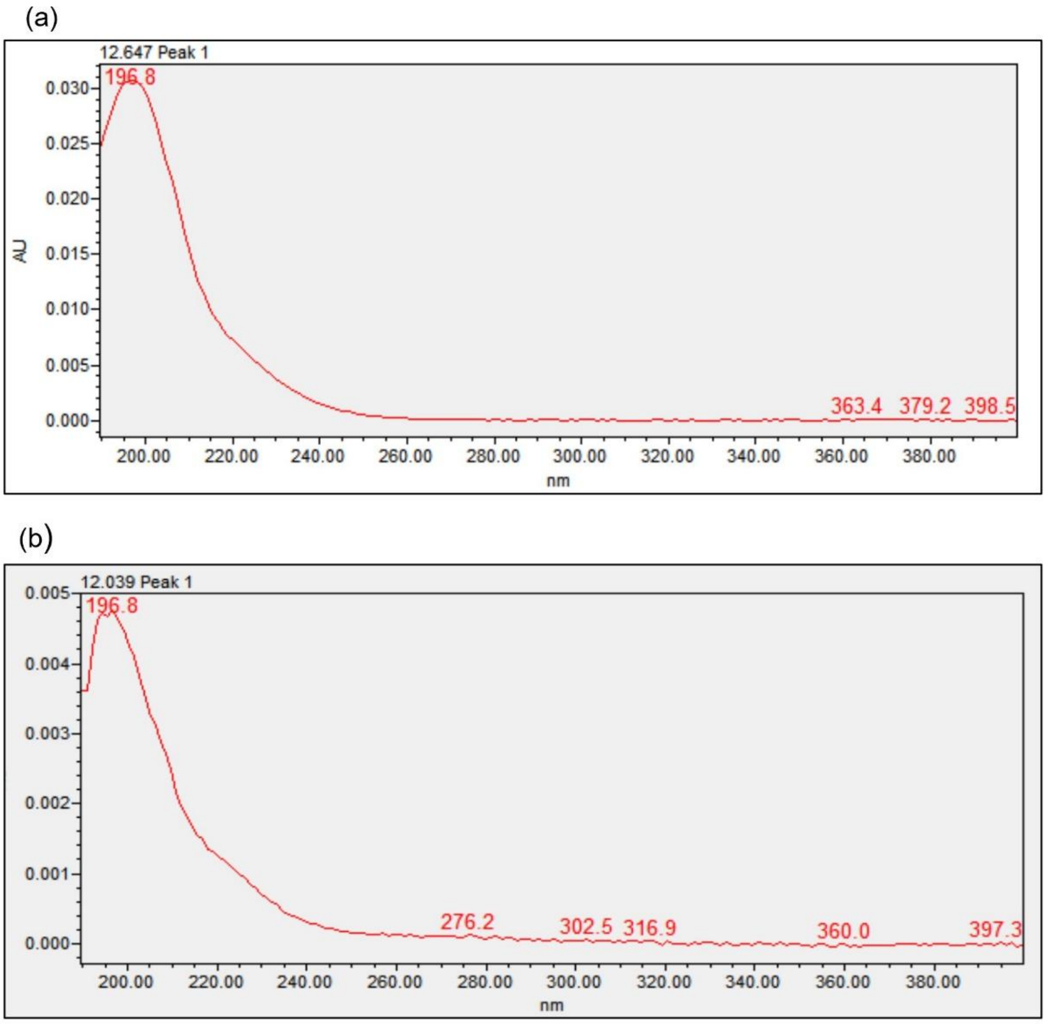

**Figure 3.** The spectrum for identified acrylamide peak (a)standard and (b) in potato fries sample

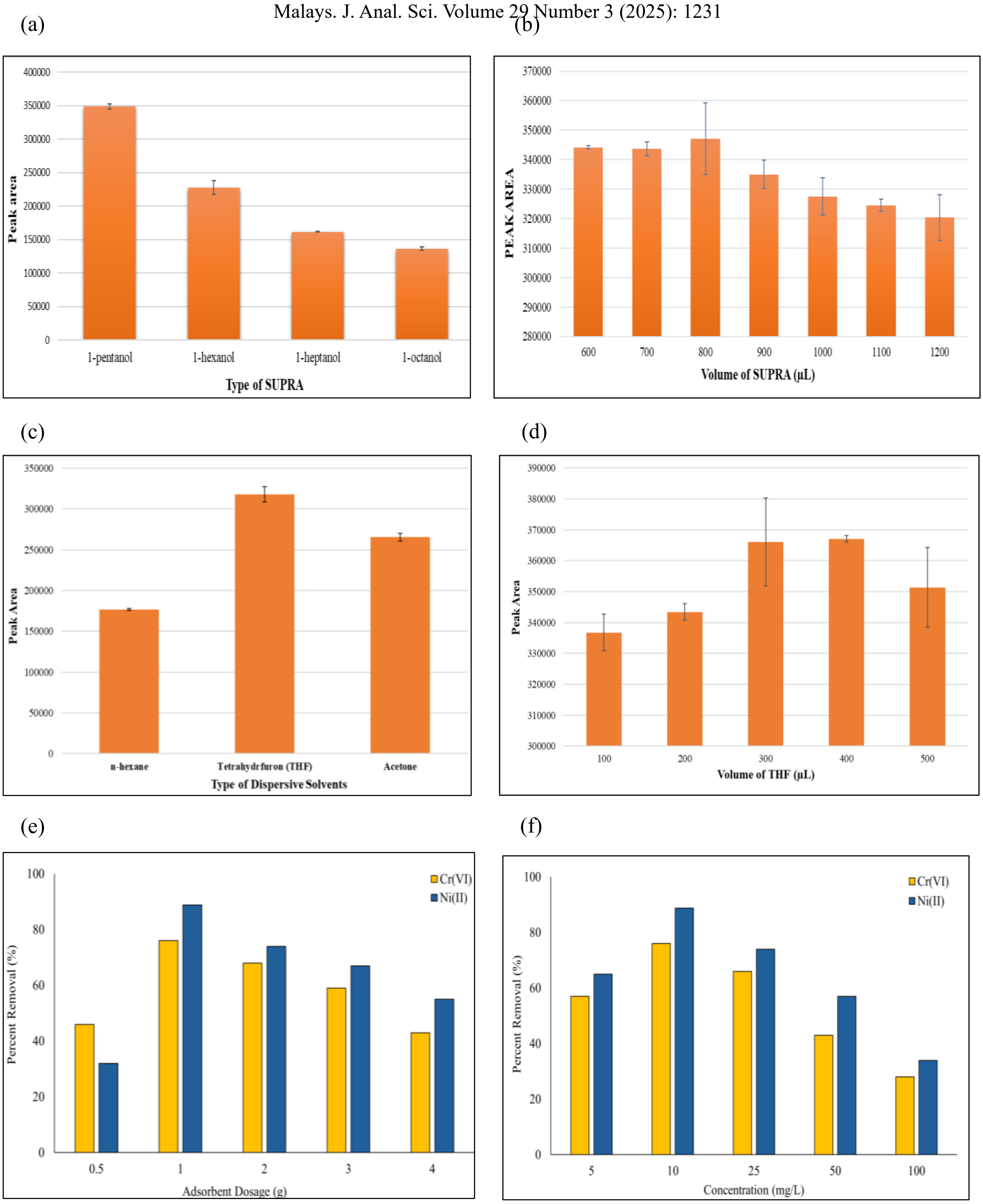

**Figure 4.** (a) Type of SUPRA (Condition: SUPRA volume, 4.2 mL; dispersive solvent, THF; volume of dispersive solvent, 400 µL; extraction time, 30s); (b) SUPRA volume (Condition: type of SUPRA, 1-pentanol; dispersive solvent, THF; volume of dispersive solvent, 400 µL; extraction time, 30s); (c) type of dispersive solvent (Condition: type of SUPRA, 1-pentanol; SUPRA volume, 800 µL; volume of dispersive solvent, 400 µL; extraction time, 30s); (d) volume of dispersive solvent (Condition: type of SUPRA, 1-pentanol; SUPRA volume, 800 µL; type of dispersive solvent, THF; extraction time, 30s); (e) extraction time (Condition: type of SUPRA, 1-pentanol; SUPRA volume, 800 µL; type of dispersive solvent, THF; volume of dispersive solvent, 400 µL); (f) sample pH (Condition: type of SUPRA, 1-pentanol; SUPRA volume, 800 µL; type of dispersive solvent, THF; volume of dispersive solvent, 4000 µL and extraction time, 20s).

### Type of dispersive solvent

Solvents such as carbon tetrachloride, tetrahydrofuran (THF), acetone, n-hexane, isopropanol, dimethylformamide, dichloromethane, chloroform, and water have been used as the dispersive solvents in most DLLME technique. Solvents such as n-hexane, tetrahydrofuran, and acetone are miscible with both water and extraction solvents. Dispersive solvent has improved some of the LLME extraction techniques by directly affecting the formation of the cloudy solution. When added to the sample solution and mixed with the extraction solvent, it forms tiny droplets that promote a rapid transfer of analyte of interest and dispersion until a cloudy solution is formed. In this experiment, three dispersive solvents were analyzed, and observation was made to understand the improvement provided in the extraction efficiency of acrylamide including n-hexane, THF, and acetone. About 400 µL of dispersive solvent was added and the volume of 1-pentanol was reduced from 800 µL to 400 µL making the ratio of dispersive solvent to 1-pentanol to be 1:1. As shown in **Figure 4 (c)**, THF provided the highest peak area compared to n-hexane and acetone. THF also increased the peak response compared to the extraction procedure without the usage of the dispersive solvent. Consequently, THF showed a good improvement in the optimized SM-DLLME technique to extract acrylamide in food samples. Thus, THF was added to this technique and the volume of THF was studied for the next optimization procedure.

### Volume of dispersive solvent

The previous study has shown that the extraction efficiency of acrylamide is affected by the addition of a dispersive solvent. THF as the selected dispersive solvent affects the formation of a cloud solution that changes the degree of dispersion of the extractant solvent in the aqueous phase. Hence, another optimization procedure was carried out to study the volume of THF to be added. The volume of THF was investigated from 100 µL to 500 µL based on its ability to form a desirable amount of separation layer. At above 500 µL of THF, the solution cannot produce a separation layer due to an insufficient amount of 1-pentanol as the main extraction solvent in the SM-DLLME procedure. From the experimental data provided in **Figure 4 (d)**, the peak response increased first and then decreased upon the increase in the volume of THF. At 300 µL and 400 µL, THF showed closely similar peak responses but when comparing the values, 400 µL slightly provided a higher peak response. When the volume of dispersive solvent was increased further, the sedimented phase volume tended to decrease which resulted in lesser recovery [15]. At lower volumes, the dispersive solvent added was not enough to have a significant effect on the formation of the cloud solution. Hence, 400 µL THF was selected as the optimum volume for dispersive solvent in the optimized SMDLLME technique.

### Extraction time

Extraction time is one of the key factors in the DLLME technique as in most extraction methods. It is defined as the time interval between the addition of the mixture of extraction solvent (1-pentanol) and dispersive solvent (THF) to the sample before the centrifugation step. Vortex is added in the developed SM-DLLME technique to enhance the agitation of extraction solvent and dispersive solvent into the aqueous solution. When using a vortex, the generated fine droplets will increase the interfacial area available for mass transfer, decrease the diffusion distance, and increase the extraction rate at which the analyte could reach partition equilibrium in less than a minute [16]. In this study, vortex time from 10 to 30 seconds was analyzed to study the effect on the extraction time of acrylamide. The experimental data shown in **Figure 4 (e)** indicated that there was almost no impact of extraction time on the extraction efficiency of acrylamide. The peak response from 10 seconds to 30 seconds were pretty much alike and 20 seconds and 30 seconds of vortex provided similar peak response. It is understood that the surface area between the extraction solvent and aqueous sample phase is infinitely large after the formation of a cloudy state of the solution [17]. Due to this reason, the equilibrium state can be achieved very rapidly with the shortest extraction time. A shorter extraction time in the DLLME procedure gives a remarkable advantage when compared to any extraction procedure. In this optimized SM-DLLME technique, 20 seconds was selected as the ideal vortex timing to obtain the highest extraction efficiency of acrylamide in food samples.

### Effect of pH

The pH of the sample solution is one of the crucial factors in the microextraction procedure that involves the assistance of SUPRAs. It is because of sample pH that eventually influences the rate of transferring target analytes into the organic phase. Acrylamide is an extremely weak basic compound (essentially neutral), and a water-soluble compound that is frequently extracted at neutral pH during its analysis. In fact, at a lower pH than 3.0, the structure will be protonated and induce a positive charge to its structure. The amide group of acrylamides easily protonate at pH below 3, decreasing the extraction efficiency. At any other pH above 3, acrylamide will be in its natural form hence no pH dependency can be observed. This is summarized in Figure 4, extraction of acrylamide at alkaline conditions was 10 – 40 % higher when compared to extraction at neutral conditions [6]. The study has provided crucial information by revealing that acrylamide extraction at the alkali and neutral conditions was mostly similar. It was mentioned that extra acrylamide formed during the baking or cooking process was bound or entrapped in the sample matrix and thus was not extractable using water but could be released via the alkali extraction method. However, the extraction at alkali conditions can only apply to certain types of foods and is not representative of all. Other studies have also proved that acrylamide extraction in acidic conditions is possible provided with good recoveries. In this study, the effect of sample pH on the extraction efficiency was studied ranging from pH 3 to pH 9 by adjusting the pH of sample solution using appropriate amounts of 0.1M HCl and NaOH solutions. From the data shown in **Figure 4 (f)**, there was no significant change in peak response when pH was increased from 3 to 9. At neutral pH (pH 7), the peak response was slightly improved. Since the acrylamide compound is very stable at any pH and not influenced by pH, this makes water extraction at pH 7 to be the most favourable extraction technique. Hence, no pH adjustment was needed prior to the extraction procedure and neutral pH was chosen as the optimized condition of the SM-DLLME technique to extract acrylamide in food samples.

### Method validation

A series of spiked samples that ranged from 100 to 1000 µg kg$^{-1}$ were applied to plot the matrix match calibration curves (n=3). The method validation was done under optimized conditions (4.0 mL sample volume containing 500 µg kg$^{-1}$ acrylamide solution, 400 µL of 1-pentanol and 400 µL of THF, vortex for 20 seconds). The linearity shows the correlation of determination ($R^2$) ranging from 0.998 to 0.9967. The optimized method was determined by measuring the (repeatability) and inter-day (intermediate) at a spiking level of 500 µg kg$^{-1}$ in potato fries samples. Precision was determined on the same day with six duplicates while inter-day precision was calculated with seven replicates on three different days with relative standard deviation (RSD) ranging between 0.70 % to 1.5 %. **Table 2** displays the linear performance, LOD, LOQ, ME, and inter-day results. SM-DLLME can detect and quantify the acrylamide at concentrations as low as 13 µg kg$^{-1}$. Matrix effect (ME) was calculated based on equation 5 and the value was 1.02. The value showed that there was no matrix effect in the study as all samples were prepared in similar water matrices. Hence, ME was assumed to be insignificant.

### Recovery

Recovery was conducted at three concentration levels (200, 500, and 1000 µg kg$^{-1}$) into a negative potato fries sample, using homemade potato fries. The acrylamide standard stock solution was spiked into potato fries from low to high levels before the extraction procedure was conducted. The recovery result was tabulated in **Table 3**. It summarized the relative recovery data calculated from the experiment in which the recovery for acrylamide using the developed SM-DLLME technique ranged from 91.0 % – 105 % with acceptable RSD values at 0.5 % to 3.6 %.

### Real sample analysis

With the acquired validation data, the developed SM-DLLME technique was applied to real samples to determine the acrylamide content in each type of food. Since acrylamide is highly observed in potato fries, six different potato fries were studied. Three potato fries were purchased from different fast-food restaurant brands in Shah Alam, Selangor which were Brand A, Brand B, and Brand C; one frozen potato fries were cooked using deep-frying and air-frying methods, along with home-made potato fries that were freshly prepared by cutting into smaller

**Table 2.** Analytical performance parameter of the developed SM-DLLME technique covering linearity, LOD and LOQ, precision, and matrix

| Analyte of interest | Regression Equation | Linear Range (µg kg$^{-1}$) | Detection Limit (µg kg$^{-1}$) | | % RSD at 500 µg kg$^{-1}$ (n=6) | | Matrix Effect (ME) |
|---|---|---|---|---|---|---|---|
| | | | **LOD** | **LOQ** | **Intra-day** | **Inter-day** | |
| Acylamide | y = 930.64x - 57163 | 100-1000 | 13 | 40 | 0.7 | 1.5 | 1.02 |

**Table 3.** Relative recovery obtained for acrylamide extracted using SM-DLLME

| Analyte of Interest | Spiked Concentration (µg kg$^{-1}$) | Amount Found (µg) | Relative Recovery ± RSD (n=3)(%) |
|---|---|---|---|
| Acrylamide | 200 | 189 | 94.7 ± 3.0 |
| | 500 | 525 | 105.0 ± 0.5 |
| | 1000 | 914 | 91.4 ± 3.6 |

fragments and cooked via deep-frying. The samples then were pre-treated before applying the optimized SM-DLLME conditions. **Table 4** summarizes the acrylamide content found in potato fries samples in µg kg$^{-1}$ together with the calculated RSD. In those six samples, sample B contained the highest concentration of acrylamide which was at 897 µg kg$^{-1}$ while the lowest concentration of acrylamide was observed in sample D with 71 µg kg$^{-1}$. The amount of acrylamide found in sample A and sample C were 388 µg kg$^{-1}$ and 206 µg kg$^{-1}$ while acrylamide content in deep-fried frozen fries was detected at 96 µg kg$^{-1}$. In the homemade fries sample, the acrylamide peak was not observed at all. **Table 5** shows the development of the supramolecular-based dispersive liquid-liquid microextraction (SM-DLLME) method compared to the present and other reported methods.

**Table 4.** Result for the real sample analysis in selected potato fries sample

| Type of Potato Fries | Sample ID | Concentration of Acrylamide Found (µg kg-1) | Average ± % RSD (n=3) |
|---|---|---|---|
| Brand A | A1 | 395 | 388 ±2.99 |
| | A2 | 374 | |
| | A3 | 394 | |
| Brand B | B 1 | 860 | 897 ±3.79 |
| | B 2 | 920 | |
| | B 3 | 911 | |
| Brand C | C 1 | 210 | 206 ±3.79 |
| | C 2 | 212 | |
| | C 3 | 197 | |
| Frozen Fries (deep-fried) | D1 | 71 | 71 ±1.15 |
| | D2 | 72 | |
| | D3 | 71 | |
| Frozen Fries (air fried) | E1 | 99 | 96 ±2.16 |
| | E2 | 95 | |
| | E3 | 95 | |
| Home-made fries | F1 | ND | ND* |
| | F2 | ND | |
| | F3 | ND | |

*ND-not detected

**Table 5.** Comparisons of analytical methods for acrylamide determination in food samples

| Analysis Method | Sample | Extraction Method | Linear Range (µg L$^{-1}$) | LOD (µg L$^{-1}$)/ µg/kg | Recovery (%) | Reference |
|---|---|---|---|---|---|---|
| LC-MS/MS | Baby foods | SPE | 10-1500 µg L$^{-1}$ | N/A | 93.9-102 | [18] |
| HPLC-DAD | Chinese food | SPE | N/A | 8.0 | 92-103 | [19] |
| GC-MS | Potato chips | DLLME | 2-500 ng mL$^{-1}$ | 0.6 | 97 | [20] |
| HPLC | Food | SPE | N/A | 21 | 83 | [21] |
| HPLC-UV | Bread and potato chips | ILUAE | 80-1800 | 25 | 90.6-109.8 | [22] |
| HPLC | Cookies, potato chips, rice crust and fried steam bun | SPE | 4-4000 | 5 | 73.13-98 | [23] |
| HPLC-DAD | Food | Co-extraction | 25-5000 | 6.90 | 87.72-96.7 | [24] |
| UPLC-MS/MS | Brewed coffee | DLLME | 3-100 | 0.9 | 97-106 | [25] |
| LC-DAD | Potato based food | SPE | 250-1000 | 2 | 92.8-96.2 | [26] |
| HPLC-UV | Potato fries | SM-DLLME | 100-1000 | 13 | 91.4-105 | Current work |

## Conclusion

A simple and rapid DLLME technique with the assistance of supramolecular solvent coupled with HPLC-DAD was completely and successfully developed to determine acrylamide in selected food samples. Method performance was accessed by performing validation parameters that include a linear range of 100–1000 µg kg$^{-1}$ with good coefficient of determination ($R^2$: 0.9967), LOD and LOQ values at 13 µg kg$^{-1}$ and 40 µg kg$^{-1}$, percentage recovery ranging from 91.4 %–105 %. Thus, conducted validation parameters have embodied that the developed method was able to produce reliable data with acceptable sensitivity and good reproducibility (RSD: 0.7–1.5 %) under the selected optimized conditions. The method was applied to six potato fries samples obtained from a variety of fast-food restaurants and homemade. The analyzed potato fries samples provided acrylamide content ranging from 692–3812 µg kg$^{-1}$. In future recommendations, some areas that can be improved in the method development such as the optimization of extraction solvent, type of salt concentration, and type of extraction mode including ultrasonication and shaking should be studied to fully understand the effects that they are contributing.

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