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# Preconcentration of acrylamide with dispersive liquid-liquid microextraction based on solidification of floating organic drop prior to determination by HPLC

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## Abstract

A novel, rapid, simple and sensitive dispersive liquid-liquid microextraction method based on the solidification of floating organic drop (DLLME-SFO) combined with high performance liquid chromatography-ultra violet detection (HPLC-UV) was used to determine acrylamide in potato chips. The derivation of the acrylamide happened in the presence of KBr, $KBrO_3$, $H_2SO_4$. Based on previous studies, 1-undecanol was selected as the extraction solvent. The factors affecting the extraction efficiency of DLLME-SFO such as the volume of the extraction solvent, kind and volume of the disperser solvent, effect of concentration of KBr, $KBrO_3$, $H_2SO_4$ and time of centrifuge, time of derivative and extraction time were investigated and the optimal extraction conditions were estimated. Under the optimum conditions (extraction solvent: 150µL1-undecanol; disperser solvent: 1000 µL acetone; concentration of KBr, $KBrO_3$:10 ppb; concentration of $H_2SO_4$: 0.001 mol/L; extraction time: 3min), calibration curve is linear in the range of 0.5-15 ppb and correlation of determination ($R^2$) is 0.993.The method was successfully applied for the determination of acrylamide in the actual potato chips.

**Keywords:** Acrylamide, DLLME-SFO, HPLC, 1-undecanol.

## Introduction

Acrylamide (2-Propenamide) is the monomer form used to synthesize polyacrylamide and a known neurotoxic compound [1]. Acrylamide was illustrated in Figure 1. Acrylamide is polarity, hydrophilic,and unsaturated amide, therefore; using classical analytical techniques is difficult to analyze it, individually in complex matrixes [2,3].

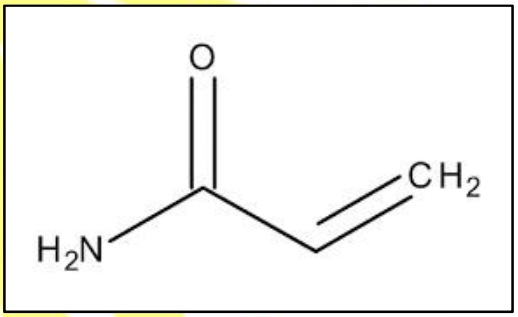


**Figure1.** The chemical structure of acrylamide

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Acrylamide naturally forms as a byproduct of cooking process in carbohydrate-rich foods at high temperature and low moist conditions [4]. The most possible path to acrylamide formation during the browning process is Millard reaction of reducing sugars with asparagine at temperature higher than 120 ºC [5]. Acrylamide is a neurotoxic compound identified as a probable human carcinogen (group2A) and genotoxicant [6,7]. International Agency for Research on cancer classified acrylamide as a suspected human carcinogen [8]. Since then, International Organization such as the World Health Organization, Food and Agriculture Organization of the United Nations (FAO/WHO) [9], Joint Institute for Food Safety and Technology (JIFSAN/NCFS) [10] have found acrylamide in various food stuff. Since 2002, high levels of acrylamide have been found in some foods, such as fried,baked, grilled or toasted foods [11,12].

Preparing sample is necessary in order to extract, isolate, and concentrate analytes from complex matrices to obtain sample suitable for instrumental analysis. Conventional liquid-liquid extraction (LLE), presenting high reproducibility and high sample capacity, is the most widely used sample preparing method for liquid sample [13]. LLE requires large amounts of expensive and toxic solvents, leads to the generation of hazardous laboratory waste [14]. In the last decade, many methods discovered in analytical chemistry which lead to miniaturize and minimize organic solvent were used in sample preparation. The dispersive liquid-liquid microextraction(DLLME) method was designed by Assadi *et al.* in 2006 [15].However, the extract solvent is limited in the solvents which have higher density than water, such as chlorobenzene, chloroform, carbon tetrachloride, and carbon disulfide, and all of them are toxic and environment-unfriendly.

More recently, a new method of liquid-phase microextraction based on solidification of floating organic droplet (LPME-SFO) was introduced byLeong and Huang [16]in which, a small volume of the extractant with low density, low toxicity and proper melting point near room temperature was used. It is readily solidified at low temperatures and floated on the surface of aqueous solution, and thus can be collected easily. This method is based on the principle of DLLME and SFODME [17]. In this method, a convenient mixture of 1-undecanol (as extractant solvent) and disperser solvent is rapidly injected in to an aqueous sample by syringe, then; a cloudy solution is formed. The enormous contact area between the extractant droplets and sample solution is beneficial for rapid mass transfer from the aqueous phase to the organic phase, and the analysis time is significantly shorter. Moreover, the solidified phase can be easily transferred from the aqueous phase. The extraction solvent was collected on the top of the test tube, and then cooled by inserting it into an ice bath for some minutes. The solidified extraction solvent was then transferred in to a suitable vial and immediately melted at room temperature, and finally determined by a suitable instrument. The advantages of DLLME-SFO are simplicity, high efficiency, rapidity, high recovery and other advantages are low cost, simple extraction device and utilization of very small amounts of less-toxic organic solvents. DLLME-SFO is widely applied to the

preparation of environmental samples [18-21] and seldom applied to the analysis of drugs in complex biological fluids [22-24]. In this research, the ability of the combination of DLLME-SFO and HPLC-UV for the separation and determination of acrylamide in potato chips was studied.

**Experimental**

*Materials*

Acrylamide (99%) was available from Merck (Schuchardt,Germany), PotasiumBromat (99%), Potassium bromide (99%), Sulfuric acid (98%) and Aceton, Methanol, Ethanol, Acetonitril(HPLC grade) as disperser solvents and 1-undecanol as extraction solvent. The stock solution of acrylamide (100 mg/L) was prepared by dissolving the compound in distilled water, then appropriately diluted with water to prepare working standard with required concentration.

The stock solution of KBr, $KBrO_3$, $H_2SO_4$ were prepared by dissolving them in distilled water too and all stock solutions and working standards were stored in a fridge at 4 C° until further use.

**Instrumentation**

HPLC-UV analysis was done on Agilent technologies 1200 series HPLC instrument equipped with a vacuum degasser, a binary pump, a diod array detector and a temperature-controlled column oven.

The sample extracts were injected *via* a 20 µL sample loop onto ZORBAX-SB $C_{18}$column at room temperature. The detection wavelength was 265 nm. An isocratic elution program with the mobile phase comprising solvents A and B at a flow rate of 1 mL/min was used. Solvent A was 10% water and 90% (v/v) acetonitril. Under this chromatographic condition, acrylamide in the test samples was baseline separated and eluted.

**DLLME-SFO procedure**

In each experiment, 1.5 mL of the solution containing acrylamide was placed in a centrifuge tube. Next 3 mL of KBr and $KBrO_3$ solutions and 1.5 mL $H_2SO_4$ were added to the acrylamide solution**.** After 10 min (time of derivatization), the mixture of 1-undecanol (extraction solvent) and acetone (dispersive solvent) was immediately injected into the sample. Due to the dispersion of fine droplets of 1-undecanol in aqueous solution, a cloudy suspension was formed and acrylamide was extracted into the droplets in some seconds. After centrifuging the solution at 4500 rpm for 5 min, 1-undecanol was raised to the surface of the aqueous solution because the density of 1-undecanol is less than that of water. The test tube was placed in to an ice bath for some minutes and the droplet solidified due to the low melting point (11 °C) of 1-undecanol. The solidified droplet was removed with a spatula and placed into a vial at room temperature. Subsequently, 20 µL of the extractant was collected with a syringe and injected on to the HPLC.

**Results and discussion**

*Effect of extraction time*

To increase the precision and sensitivity of the DLLME - SFO method, it is necessary to select an exposure time that guarantees the equilibrium between the aqueous and organic phases. In DLLME-SFO, the time between the injection of the dispersed solvent containing the extraction solvent and the beginning of the centrifugation was called extraction time. A series of experiments were performed and the extraction time profile was obtained by plotting the relative peak area against the extraction time evaluated in the range of 1-10 min. As Figure 2 shows, the relative peak area was increased by

increasing time up to 3 min and then remained constant. Thus, the exposure time of 3 min was selected as the optimal extraction time.

**Effect of centrifuging time**

If the centrifugation time is not enough, the organic phase cannot be completely collected on top of the vial. Different centrifugation times (3,5,10,15) were used and the results (Figure 3) show that the optimal time was obtained 5 min at 4500 rpm.

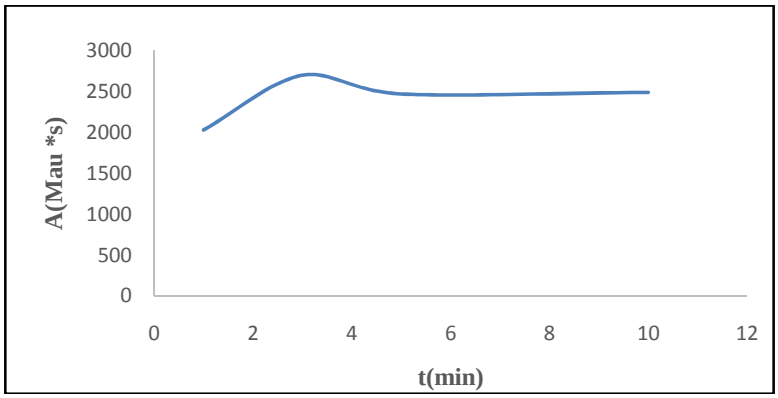


**Figure 2.** Effect of extraction time. Conditions: acrylamide: 10ng/mL; centrifuging time:10 min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; $KBrO_3$ concentration: 30ng/mL; volume of 1-undecanol: 100µL; dispersive solvent volume:1 mL

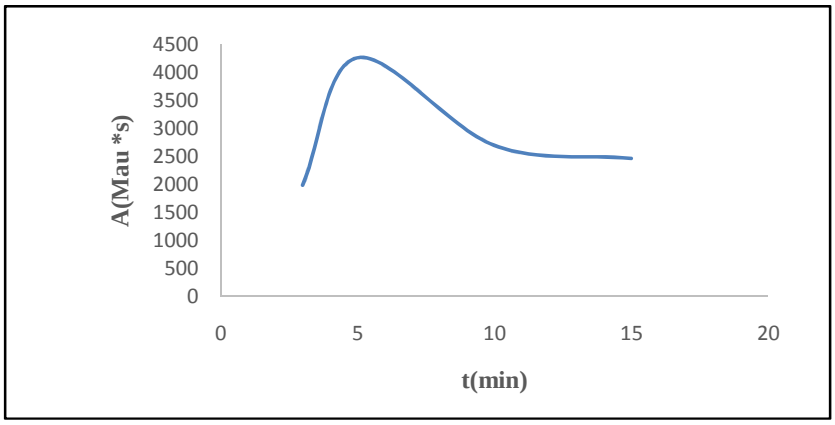


**Figure 3.** Effect of centrifuging time. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; $KBrO_3$ concentration: 30ng/mL; volume of 1-undecanol: 100µL; dispersive solvent volume: 1 mL

**Effect of $H_2SO_4$, KBr, $KBrO_3$ concentrations**

To investigate the effect of $H_2SO_4$, KBr, and $KBrO_3$ concentrations is enough to know that HPLC separation demands derivatization of acrylamide, which is well done in most laboratories with hydrobromicacid (HBr) and saturated $Br_2$solution [25-30]. The overplus bromine is then removed by

addition of sodium thiosulfate until the solution becomes colorless, then the derivative reaction is finished. In the present study, according to Zhang, Y. *et al.* [31], the derivatization was applied with KBr, $KBrO_3$ and $H_2SO_4$ on the basis of the following reaction:

$KBrO_3 + KBr + H_2SO_4 \longrightarrow Br_2 + K_2SO_4 + H_2O$

$CH_2=CH–CO–NH_2 + Br_2 \longrightarrow CH_2Br–CHBr–CO–NH_2 + CH_2=CBr–CO–NH_2$

The results (Figures 4,5,6) show that optional concentration for KBr, $KBrO_3$ and $H_2SO_4$ are respectively10 ppm, 10ppm, and 0.001 M.

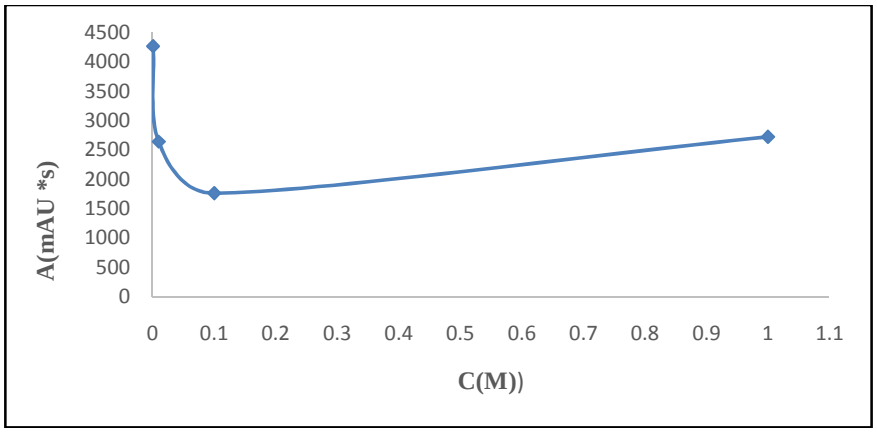


**Figure 4.** Effect of $H_2SO_4$ concentration. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5 min; KBr: 10 ng/mL; KBrO3 concentration: 30ng/mL; volume of 1-undecanol: 100µL; dispersive solvent volume: 1 mL

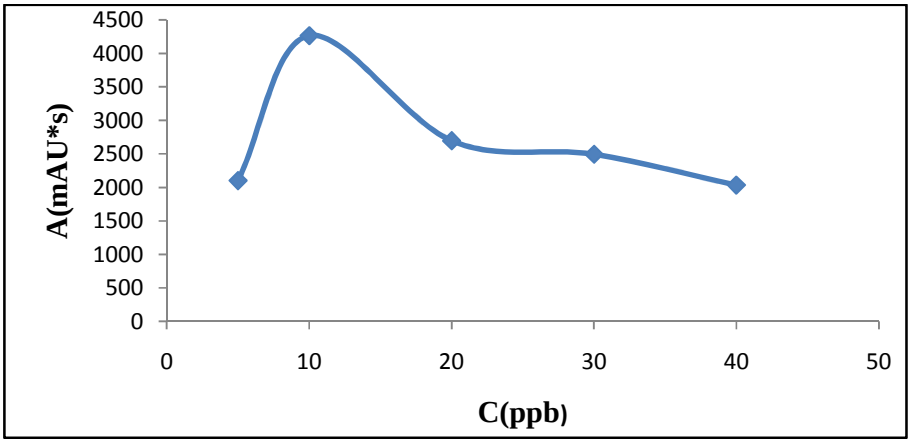


**Figure 5.** Effect of KBr concentration. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5 min; $H_2SO_4$ concentration: 0.001 M; $KBrO_3$ concentration: 30ng/mL; volume of 1-undecanol: 100µL; dispersive solvent volume: 1 mL

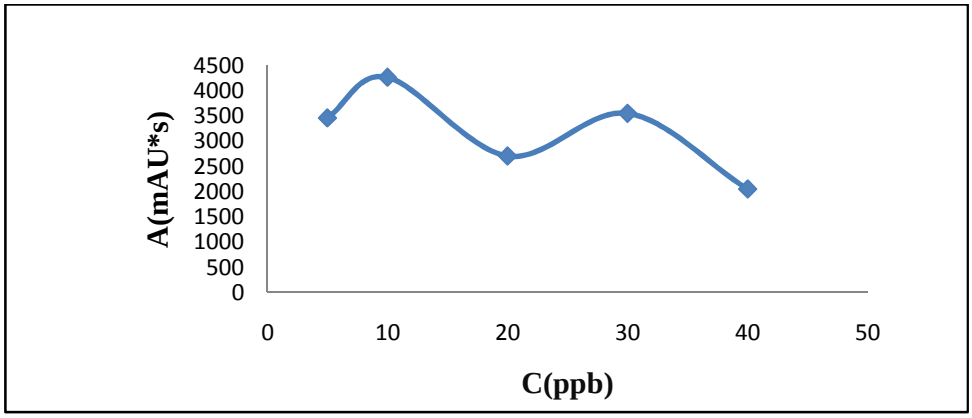


**Figure 6.** Effect of $KBrO_3$ concentration. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; volume of 1-undecanol: 100µL; dispersive solvent volume: 1 mL

**Selection of extractant solvent volume**

To achieve optimal results, a suitable extraction solvent must be selected. Specifically, the extraction solvent must have low solubility in water, low toxicity, an appropriate melting point close to or below room temperature and a density less than that of water. Moreover, it must be able to extract the target analytes and be well separated from the analyte in the resulting chromatograph. 1-Undecanol was used as the extraction solvent in subsequent experiments. In this study, the optimal volume of the extraction solvent was also investigated. To this end, acrylamide was extracted with different volumes of 1-undecanol (100,150,200,250 µL) at constant volume of acetone (1000 µL). Atthe plot show (Figure 7), 150 µL is the optimal volume of extraction solvent.

Selection of the type and volume of dispersive solvent

In DLLME-SFO, the dispersive solvent must be miscible with both the extraction solvent and the aqueous sample. In this study, acetonitril, methanol, ethanol and acetone were evaluated as dispersive solvents. The results (Figure 8) revealed that acetone is the best disperser solvent.to evaluate the effect of the dispersive solvent on the enrichment factor (EF).The volume of acetone was varied between 250-1250 µL. As shown in Figure 9, a cloudy state was not sufficiently formed when low volumes of acetone were employed, and a low EF was obtained. Moreover, the EF increased as the volume of acetone increased from 250 µL to 1000 µL and decreased as the volume of acetone increased from 1000 µL to 1250 µL. This result may be attributed to the increased solubility of the extraction solvent in water as the volume of acetone increased. Thus, to obtain a high EF, 1000 µL of acetone was selected as the volume of dispersive solvent in subsequent experiments.

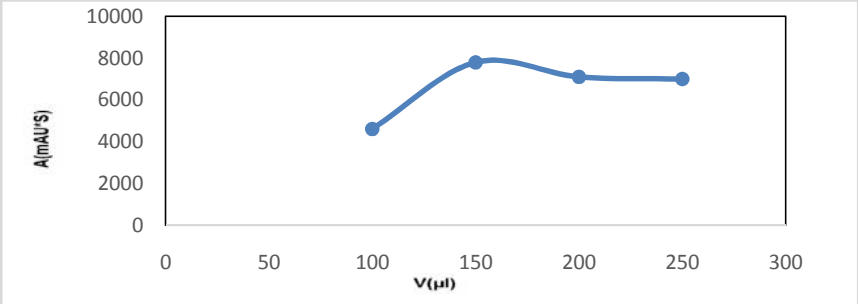


**Figure 7.** Selection of extractant solvent volume. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5 min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; $KBrO_3$ concentration: 10ng/mL; extracant solvent: 1-undecanol; dispersive solvent volume: 1 mL

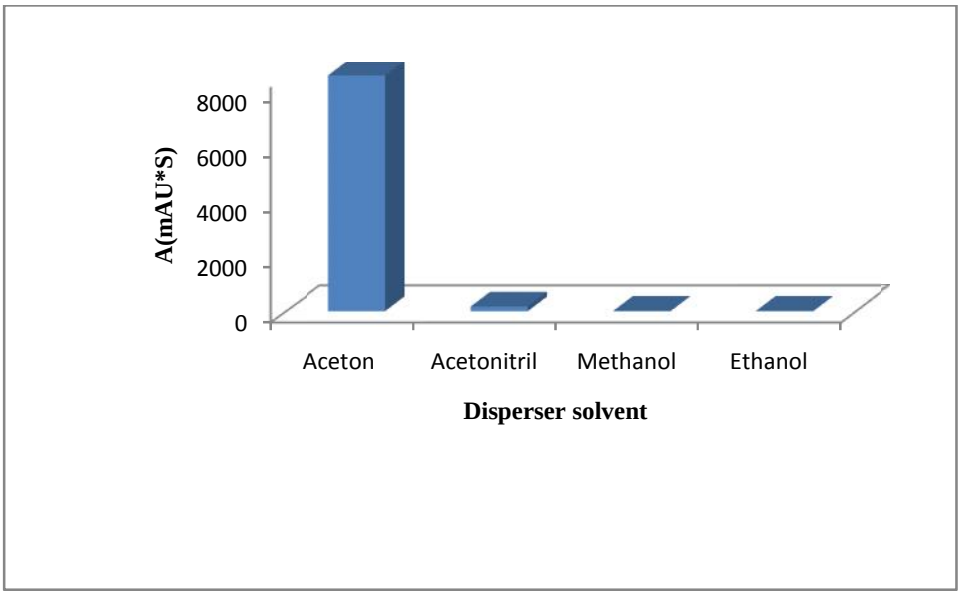


**Figure 8.** Selection of dispersive solvent type. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5 min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; $KBrO_3$ concentration: 10ng/mL; volume of 1-undecanol: 150µL; dispersive solvent volume: 1 mL

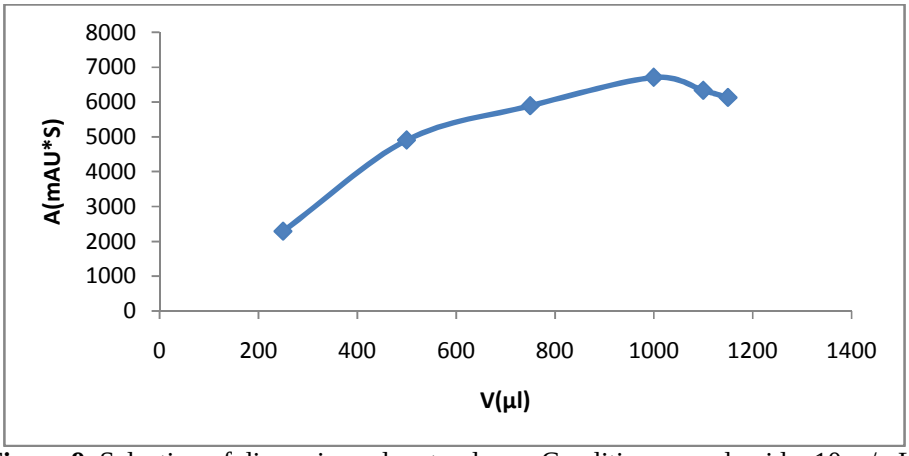


**Figure 9.** Selection of dispersive solvent volume. Conditions: acrylamide: 10ng/mL; extraction time: 3 min; centrifuging time: 5 min; $H_2SO_4$ concentration: 0.001 M; KBr: 10 ng/mL; $KBrO_3$ concentration: 10ng/mL; volume of 1-undecanol: 150µL

**Calibration, analysis, recovery and method performance**

Calibration curve (Figure 10) was constructed by plotting peak area against concentration of acrylamide (0.5-15ng/mL).The regression equation was determined as y = 342.6 x + 3362.3 ($R^2$ = 0.993), where x is the concentration of acrylamide (ng/ mL) and y is the peak area. The acrylamide content in potato chips was determined by our proposed HPLC-UV method. In addition, the recovery tests were conducted by adding known amounts of acrylamide (10 ng/ mL) to the potato chips sample followed by the same sample preparation procedure and HPLC analysis. The Recovery was 91.48%, demonstrating that our sample preparation methodology and HPLC analysis are satisfactory. To access the analytical performance of the proposed HPLC acrylamide method, parameters such as limit of detection (LOD) and limit of quantification (LOQ) were studied. The Relative Standard Deviation (RSD) of 1.46% was obtained. The LOD and LOQ were 0.1 ng/ mL and 0.33 ng/ mL and preconcentration factor was 10.

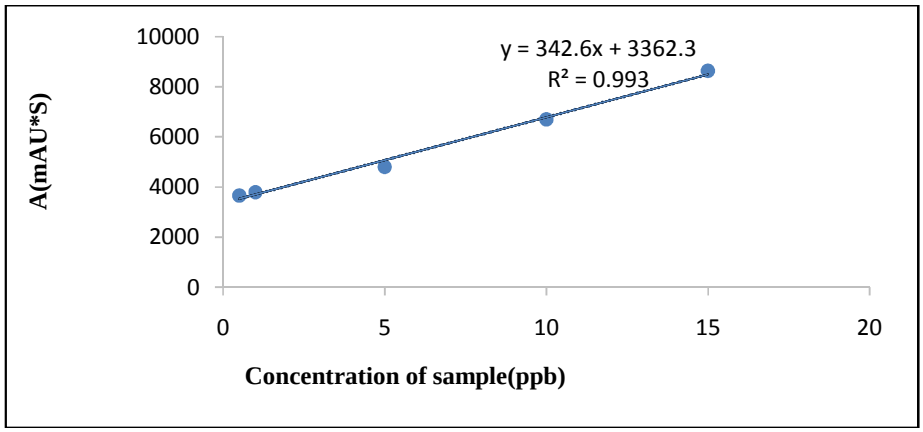


**Figure 10.** Calibration Curve

**Application of DLLME-SFO in real samples**

To evaluate the performance of the presented method, the extraction and determination of acrylamide in real samples, i.e., potato chips were performed under the optimum conditions established above. In this project, we provided 3 samples of potato chips and studied them in two steps. About 1 gr of potato chips after erode was added to the water, then we filtrated it several times with filter paper to reach clear solution. In the first step, the acrylamide content in real samples solutions (potato chips) was determined by our proposed HPLC-UV method. In the second step, the recovery tests were conducted by adding known amounts of acrylamide (10 ng/mL) to the real samples solutions (potato chips) and followed by the same sample preparation procedure and HPLC analysis. The results were shown in Table 1.

**Table 1.** Analytical results of acrylamide determination in potato chips and spiked real samples with the DLLME-SFO method(n=3)

| sample | Added(ng $mL^{-1}$) | Found(ng $mL^{-1}$) | R%(RSD%) |
|---|---|---|---|
| 1 | 0.00 | 8.90 | |
| | 10 | 19.45 | 105.50(0.65) |
| 2 | 0.00 | 7.34 | |
| | 10 | 17.13 | 97.90(0.43) |
| 3 | 0.00 | 7.41 | |
| | 10 | 17.21 | 98.00(0.40) |

**Comparison of the proposed method with other reported methods**

DLLME-SFO has the advantages of short extraction time, high recovery, and lower solvent consumption. The efficiency of the presented method was compared with other reported methods such as SPE, LLE, DLLME and SPME from the viewpoint of LOD, RSD, linear dynamic range (LDR) and extraction time. As shown in Table 2, DLLME-SFO has lower RSD in comparison with other methods and lower LODs in comparison with SPE and IEC methods. Additionally, the extraction time for the DLLME-SFO is very short and does not require special approach and instruments in the pretreatment step. Therefore, DLLME-SFO is indeed a simple, rapid, easy-to-use and environmentally friendly extraction procedure.

**Table 2.** Comparison of performance of present work with other reported methods applied for extraction and determination of acrylamide

| Method | LOD ($\mu gL^{-1}$) | Analysis time (min) | LR ($\mu gL^{-1}$) | RSD% | REF. |
|---|---|---|---|---|---|
| SPME-GC/PCI/MS | 0.1 | 20 | 1-1000 | 10.64 | 32 |
| SPE-GC-MS | 2 | - | 2-30 | 7 | 33 |
| IEC-MS | 0.2 | - | 0.5-5 | 12 | 34 |
| LLE-GC/ECD | 0.032 | - | - | 20 | EPA8032A |
| DLLME-GC/ECD | 0.001 | 5 | 0.05-6 | 3.6 | 1 |
| DLLME-SFO/HPLC | 0.1 | 3 | 0.5-15 | 1.46 | Present work |

**Conclusion**

This study has demonstrated the successful application of the DLLME - SFO method combined with HPLC-UV for determination of acrylamide in potato chips. DLLME-SFO used extraction solvent with lower toxicity instead of highly toxic solvent in conventional DLLME and it provides high extraction recovery within a short time. The experiment results revealed that this method provides good enrichment factor and good linearity over the investigated concentration range. In addition, simplicity, short time, low cost and minimum extraction solvent consumption are the advantages of the method.

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