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Determination of Triton X-100 in water samples using dispersive liquid-liquid microextraction and HPLC with the aid of response surface methodology

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Abstract

An improved method for the determination of Triton X-100 in water samples has been developed using dispersive liquid-liquid micro extraction (DLLME) prior to HPLC analysis. The volume of extraction solvent (CHCl₃), disperser solvent (acetone) and sample solution, pH, ionic strength, and extraction time were selected as interested variables in DLLME process. Firstly, a screening experiment, Taghuchi two-level orthogonal array, was employed to identify the important factors. Then the elucidation and optimization relationship between the response and the important factors was investigated by response surface methodology (RSM). The central composite design as the most popular of the many classes of RSM designs was used. The optimum experimental conditions found from this statistical evaluation were included: sample volume: 10.0 mL, volume of extraction solvent (chloroform): 137.7 μ L, volume of disperser solvent (acetone): 0.6 mL, centrifugation time: 5.0 min, pH: 6.2 (natural pH), 2.5% (w/v) NaCl and extraction time: 6.0 min. Under the optimum conditions, the preconcentration factor of 120 was achieved. Calibration graph was linear in the range of 0.01-100.0 mg L⁻¹ with correlation coefficient of 0.9961. The Limit of detection (LOD) of 3.0 μ g L⁻¹ was obtained for Triton X-100 determination.

Keywords: DLLME, Triton X-100, Experimental design, HPLC.

1. Introduction

Detergents are employed in wide range in different industries. Among detergents, Triton X-100 is a commercial non-ionic surfactant in the aqueous solutions of detergent industry, agro chemistry, cosmetic, metallographic and textile industry. Detergents are employed in a myriad of uses ranging from the biochemical solubilization of membranes during cell lysis to viral inactivation [1] to bactericides in the formulation of creams or tablets [2]. With these widespread uses, a number of sensitive and high resolution techniques have been developed for the determination of low concentrations of these analytes in different matrices. Many of these techniques and some applications have been reviewed by Vogt and Heinig [3], in which a substantial number of these assays were directed at evaluating trace detergents

and their degradation products in environmental analyses. Morelli and Szajer [4, 5] also provided additional and rather comprehensive reviews on surfactant analyses and methodology. Other examples of assays to determine the presence of detergents in wastewater streams include that of Barco et al [6].

In many circumstances, more than one type of surfactant is used in various steps of the purification process. For example, cell or membrane lysis can be achieved by treatment with the non-ionic Triton X-100. This application of the surfactant also aids in the inactivation of some types of enveloped viruses such as HIV and hepatitis B and C viruses. This is the rationale employed for the production of human plasma where Triton and non-ionic surfactants are added to the plasma and incubated [1]. After the incubation period, the biologically active plasma

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proteins remain intact and are then purified chromatographically [7].

In the midst of these myriad applications, it is therefore essential to demonstrate process consistency and control by monitoring clearance of detergents such as the nonionic Triton and/or cationic detergents that have been introduced throughout the purification process. The residual concentrations of each of these surfactants may vary widely depending on the step of the process and the stage or extent of purification. Thus, any assay to determine the clearance of these detergents would necessarily need to employ a wide linear dynamic range. In addition, there are potential matrix effects because of the presence of the product (primarily protein acetous in nature) as well as residual host cell nucleic acids, carbohydrates and proteins. Furthermore, in a process where ionic, non-ionic and zwitterionic surfactants are used, mixed micelles of these surfactants can develop, complicating their separation prior to quantization of the individual detergents. To enable in-process decisions to be made in a timely fashion, it is essential to evaluate large numbers of samples quickly with conventional equipment [8].

Sample pre-treatment is often the bottleneck in a measurement process, as they tend to be slow and labor-intensive. In routine analysis, liquid-liquid extraction (LLE) is the most widely used sample preparation technique, whose goal is cleanup, enrichment and signal enhancement. However, some shortcomings like the use of extensive amounts of hazardous organic solvents and sample volumes, the generation of large amounts of pollutants make this procedure time consuming, expensive, environmentally unfriendly, tedious, and laborious and hence potentially prone to sample contamination when ultra-trace determinations are required [9-11].

Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation procedures as they lead inherently to a minimum solvent and reagent consumption and drastic reduction of laboratory wastes [12, 13]. Under this context, unconventional LLE methodologies have been arisen like: single drop micro extraction (SDME), wetting film extraction (WFE), cloud point extraction (CPE), homogeneous liquid-liquid extraction (HLL), dispersive liquid-liquid micro extraction (DLLME) and dispersive liquid-liquid micro extraction based on solidification of a floating organic drop (DLLME-SFO). Among them, dispersive liquid-liquid micro extraction (DLLME) firstly developed by Rezaee et al. in 2006 [14], presented a micro extraction technique, termed dispersive liquid-liquid micro extraction (DLLME) based on ternary component solvent systems. An appropriate mixture of an extraction solvent and a disperser solvent, with high miscibility in both aqueous and organic phase (extraction solvent) is rapidly injected into the

sample solution (aqueous phase). By this procedure a cloudy solution (high turbulence) is produced, which is consisted of fine droplets of the extraction solvent, dispersed throughout the aqueous solution. Consequently, the analyte is extracted into the fine droplets of extraction solvent usually through complex formation. After centrifugation, the fine droplets are settled down at the bottom of the conical test tube, removed by a syringe and analyzed by various analytical techniques like gas chromatography (GC) [15], gas chromatography-mass spectrometry (GC-MS) [16], high-performance liquid chromatography (HPLC) [17] □ electro thermal atomic absorption spectrometry (ETAAS) [18] and FAAS [19].

This method has been reported as a useful sample pretreatment procedure due to its main advantages: simplicity of operation, low time and cost, high recoveries and enrichment factors, low consumption of organic solvents, etc. Since its introduction, DLLME has been applied for the extraction of several organic and inorganic compounds mainly from water samples [20].

2. Experimental

2.1. Apparatus

The HPLC system equipped with K-1001 quaternary solvent delivery pump with an online degasser, a UVD K-2600 detector capable of detecting at four wavelengths, and a sampling valve with 20 μ L sample loop (Knauer, Germany) was employed. A reversed-phase Perfectsil Target C18 column (250 mm \times 4.6 mm I.D., with 5 μ m particle sizes) was used for separation at ambient temperature and ChromGate software package was employed to acquire and process chromatographic data. The mobile phase used for the analysis consisted of acetonitrile and water ratio of 60:40 □ (v/v) at a flow rate of 2 mL min⁻¹. The wavelength of detector was set at 280 □ nm. A BEL PHS-3BW pH-meter (BEL, Italy) with a combined glass-calomel electrode was used for pH adjustment. A Hettich centrifuge model EBA 20 (Hettich, Germany) was used for phase separation.

2.2. Chemicals and solutions

All chemicals such as Triton X-100, water, acetone, methanol, chloroform, carbon tetrachloride, dichloromethane and sodium chloride were highest-purity grade reagents from Merck Company (Darmstadt, Germany).

2.3. DLLME process

A 10.0 mL aqueous sample solution with the ionic strength of 2.5% (w/v) NaCl, containing 0.01 mg L⁻¹ of Triton X-100 compound was placed in a 12.0 mL screw cap glass test tube with conical bottom. A 0.5 mL acetone (disperser solvent), containing 130 μ L chloroform (extraction solvent), was injected rapidly into the sample solution by a

1.0 mL Hamilton syringe (USA) and the mixture was gently shaken. A cloudy mixture was formed in the test tube. The mixture was centrifuged for 4 min at 4000 rpm. The fine droplets of chloroform were sedimented at the bottom of the test tube. Volume of the sedimented phase was determined as 70 μL using a 100 μL micro syringe. The sediment phase is evaporated in room temperature. Then, 0.5 mL of methanol was added to the sediment phase and 20 μL injected into the HPLC system for analysis.

2.4. Calculation of enrichment factor

The enrichment factor (EF) was defined as the ratio between the analytes concentration in the sedimented phase (C_{sed}) and the initial concentration of analytes (C_0) within the sample:

$$EF = \frac{C_{\text{sed}}}{C_0} \quad (1)$$

The C_{sed} was obtained from calibration graph of direct injection of Triton X-100 standard solution in the chloroform at the range of 0.01-10.0 mg L^{-1} .

2.5. Data analysis and statistical methods

For multi factor linear regression, statistical analysis, and the polynomial equation, response surface curve Minitab Trial Version 15 software was used (Minitab, Inc.).

3. Results and discussion

The type of dispersive and extraction solvents used in DLLME is an essential consideration for efficient extraction. The extraction solvent should be higher density than water, high extraction capability of the interested compounds and low solubility in water and dispersive solvent should be miscible with both water and the extraction solvent. Type of the extraction and disperser solvent will be figured out by the elementary examination. Therefore, acetonitrile, acetone and methanol were tested as the dispersive solvents and chloroform, chlorobenzene and 1, 2 dichloromethane were studied as the extraction solvents. For determination

of type of extraction and disperser solvent, 9 experiments were performed that have been shown in Table 1. Because sediment phase was not formed in experiments 4, 5 and 6, therefore the results have not achieved for these experiments. Through the experiment the most suitable dispersive and extraction solvent pair was selected as acetone–chloroform solvent pair. Two levels of designing was done to investigate effective factors on extraction efficiency and to optimize them.

3.1. Screening design

Different variables can affect the extraction yield in the DLLME process such as the types of extraction solvent and disperser solvent and volume of them, salt effect, volume of sample, extraction time and initial pH of solution. An experimental 2-level factorial was built for the determination of the main factors affecting the extraction efficiency (Table 2). This design is useful as with few experiments it is possible to detect the most important factors that affect significantly the process [21]. The effects of the studied factors in the screening experiment are portrayed in Figure 1 in the form of a Pareto chart. Considering the positive effect, the volume of extraction solvent (chloroform) was the most significant factor and was evaluated in the central composite design for further assessment. The volume of dispersive solvent (acetone) seems to be the next most significant factor, with a positive effect on the extraction efficiency. On the other hand, volume of sample, addition of salt and sample pH showed low positive effect and therefore, volume of sample 10.0 mL, amount of salt (NaCl) 2.5% (w/v) and natural pH (pH = 6.2) was chosen for further experiments.

Finally, the factors that were considered in the next optimization step were volume of disperser (acetone) and extraction solvent (chloroform), time of extraction respectively, was chosen for next step experimental design

Table 1. Design experiment for determination of type of extraction and disperser solvent.

Number of experiment	Type of extraction solvents	Type of disperser solvents	Peak area
1	Chloroform	Acetone	267658
2	Chloroform	Methanol	207654
3	Chloroform	Acetonitrile	45027
4	1, 2 dichloromethane	Acetone	0
5	1, 2 dichloromethane	Methanol	0
6	1, 2 dichloromethane	Acetonitrile	0
7	Chlorobenzene	Acetone	128442
8	Chlorobenzene	Methanol	227818
9	Chlorobenzene	Acetonitrile	99859

Table 2. Assignment of factors and levels of the orthogonal array design.

Factors	Levels	
	1	2
V ₁ : Volume of extraction solvent (μL)	50.0	138.0
V ₂ : Volume of disperser solvent (mL)	0.15	1.5
V ₃ : Volume of sample (mL)	5.0	9.0
t: Time of extraction (min)	0.9	6.0
S: Salt effect (%w/v)	0.0	5.0
p: pH	4.0	9.0

Experimental Design						
Trials no.	V ₁	V ₂	V ₃	t	S	P
1	-1	-1	1	1	-1	-1
2	1	1	1	1	1	1
3	1	-1	-1	-1	-1	1
4	-1	1	1	-1	-1	1
5	0	0	0	0	0	0
6	-1	1	-1	-1	1	-1
7	1	-1	1	-1	1	-1

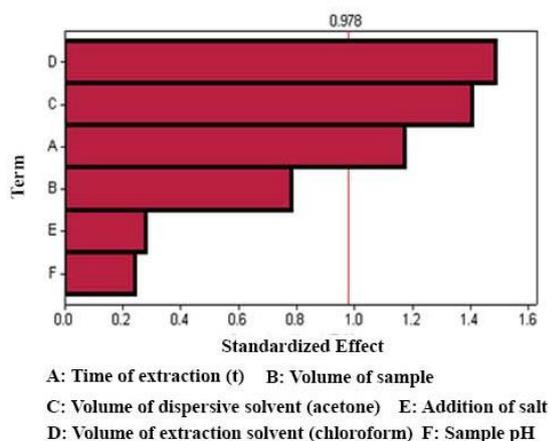


Figure 1. Pareto chart of the main effects obtained from the 2-level factorial design for Triton X-100.

3.2. Optimization design

This step is concerned with optimizing the values of the significant variables in order to obtain the best response. The second-order model correlating the response function with the independent factors could be established using the data provided by the central composite design. There were three factors chosen from the first screening experiments, so the model takes the following form [22]:

$$y = a_0 + \sum_{i=1}^3 a_i x_i + \sum_{i=1}^3 \sum_{j=1}^3 \frac{a_{ij} x_i x_j}{2} + \sum_{i=1}^3 a_{ii} x_{ii}^2 \quad (2)$$

Where y is the dependent variables EF; x_i is the independent variable. The terms represent the regression coefficient of the models. n is the number of design evaluations. A_0 is the deviation between the observed and the predicted response in the design point i and n . This equation following the main effects, interaction effects, and quadratic effects were optimized and evaluated in this design. A3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. In this study, the three variables considered were volume of chloroform (V_1) and acetone (V_2) and time of extraction (t). The low, central, and high levels of these variables, as well as the location of their star points, are also given in Table3.

The five-level factorial design allows for the fitting of a quadratic model to the data. The mathematical models were obtained by applying an edited Minitab program to perform the multivariate regression analysis on the chromatographic data for each design point. The second-order equation can quantitatively describe the relationship between the responses and independent variables, so that the EF can be predicted at any point within the factor domain, even though that point has not been included in the design.

After fitting Eq. (2) by a least-squares regression, the mathematical model obtained for the responses is listed in Table 3. The experimental data shows a good accordance with the second-order polynomial equations. The coefficient of determination, R^2 were

Table 3. Experimental variables, levels and matrix of the circumscribed central composite design.

Variables	Level			Star point(α)	
	Low	Central	High	- α	+ α
Volume of Chloroform(V_1)	68.0 μ L	94.0 μ L	120.0 μ L	50.273	137.727
Volume of acetone(V_2)	0.5 mL	1.0 mL	1.5 mL	0.159	1.84
Time of extraction (t)	2.0 min	3.5 min	5.0 min	0.977	6.023

Experiment number	$V_1(\mu$ L)	$V_2(\text{mL})$	t (min)
1	94.000	1.000	3.500
2	68.000	1.500	2.000
3	120.000	0.500	2.000
4	68.000	0.500	2.000
5	94.000	1.000	3.500
6	94.000	1.000	0.977
7	120.000	1.500	5.000
8	120.000	0.500	5.000
9	50.273	1.000	3.500
10	68.000	0.500	5.000
11	94.000	0.159	3.500
12	94.000	1.000	6.023
13	94.000	1.841	3.500
14	94.000	1.000	3.500
15	94.000	1.000	3.500
16	120.000	1.500	2.000
17	137.727	1.000	3.500
18	68.000	1.500	5.000
19	94.000	1.000	3.500
20	94.000	1.000	3.500

more than 0.95 (0.9611) for EF. The adjusted R^2 is equal 0.99471 which indicated a good accordance with the experimental data and a good fitting ability for the model. Thus, the procedure demonstrates the ability of the model to work as a predictive tool.

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good accordance with the second-order polynomial equations. The coefficient of determination, R^2 were more than 0.95 (0.9611) for EF. The adjusted R^2 is equal 0.99471 which indicated a good accordance with the experimental data and a good fitting ability for the model. Thus, the procedure demonstrates the ability of the model to work as a predictive tool.

ANOVA and regression analysis is used to assess the significance of the variables. Statistical significance were evaluated on the basis of the magnitudes of the coefficients in the regression equation, which could also illustrate the relative effects of linear, quadratic and cross-product interactions between the variables (Table 4). The lack of fit (0.174) was not significant ($p > 0.05$). The three-dimensional (3D) response surfaces plots relating EF with independent variables were generated by Minitab software (Figure 2).

Table 4. Coefficients of the regression equation for target analyte

	A
A_0	80.681
X_0	18.854
X_1	-4.963
X_2	5.438
X_1^2	-6.710
X_2^2	-4.502
R^2	0.9961

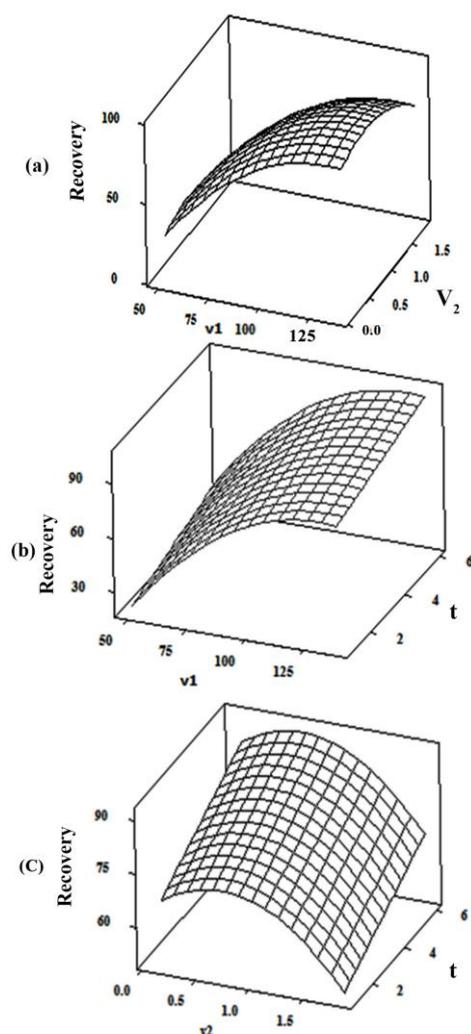


Figure 2. Response surface plots when optimizing the following pair of factors, while maintaining constant the remaining one at its mean values: (a) volume of extraction solvent- volume of disperser solvent (extraction time 3.5 min), (b) volume of extraction solvent-extraction time (volume of disperser solvent 1.0 mL), (c) volume of disperser solvent-extraction time (extraction solvent 94.0 μ L).

Also shows from result at high volume of acetone, the solubility of Triton X-100 in water increases, which will result in the decrease of the extraction efficiency and at low volume, acetone cannot disperse extraction solvent properly and cloudy solution is not formed completely. As could

be seen, extraction efficiency decreased with the increase of the volume of acetone. Mass transfer is a time-dependent process, and thus it is important to set up the extraction time profile of the target analytes so as to optimize the time parameter. In DLLME, extraction time is defined as an interval

between the injection of the mixture of disperser solvent (acetone) and extraction solvent (chloroform) and starting to centrifuge. After formation of cloudy solution, the surface area between the extraction solvent and the aqueous phase is infinitely large. As could be seen, extraction efficiency increased with the increase of time of extraction. By increasing the volume of chloroform, the volume of the sedimented phase increases, hence the effect of sedimented phase causes enrichment factor to increase slowly.

According to results obtained from screening and optimization study, the optimal experimental conditions were: the volume of extraction solvent 137.7 μL , the volume of disperser solvent 0.6 mL and extraction time 5.0 min.

3.3. Analytical performance

Quality variables including the limit of detection (LOD and linear range were investigated to evaluate the analytical performance of proposed method. The LOD was obtained based on a signal-to-noise ratio (S/N) of 3 using distilled water spiked with the Triton X-100 at very low level. The LOD value obtained in this study was 3.0 $\mu\text{g L}^{-1}$. The employed working range was set from 0.01-100.0 mg L^{-1} (number of calibration points, N=7). The experimental results showed good linear relationship between the peak area and the concentration with a correlation coefficient of 0.9961. These results indicated the proposed method had high sensitivity and stability and high potential to be a powerful and suitable preconcentration tool for trace analysis.

4. Conclusions

In this study, a new method based on microextraction technique described as dispersive liquid-liquid microextraction (DLLME) has been developed to determine the Triton X-100 in water samples. The optimization of the variables of DLLME was carried out with the aid of response surface methodology and experimental design. The mathematical model developed for enrichment factor to the main variables of DLLME proved to be an efficient strategy for optimization of the DLLME process. The proposed method greatly simplified the optimization procedure of DLLME on contrast with previous related studies. The resulting optimized procedure allowed quantification of trace levels of Triton X-100 in water samples whilst using DLLME coupled to HPLC.

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اندازه گیری تریتون X-100 در نمونه های آب با استفاده از روش میکرو استخراج مایع-مایع پخشی و HPLC، با کمک روش پاسخ سطح

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چکیده:

روشی برای اندازه گیری تریتون X-100 در نمونه های آب با استفاده از میکرو استخراج مایع-مایع پخشی و HPLC توسعه یافت. متغیرهای مختلف فرایند DLLME مثل حجم حلال استخراج (کلروفرم)، حلال پخش کننده (استون) و محلول نمونه، pH، قدرت یونی و زمان استخراج جهت بهینه سازی انتخاب شدند. ابتدا آزمایش غربالگری برای تعیین فاکتورهای مهم با استفاده از روش ناگوچی دو سطحی آرایه عمودی انجام شد. سپس با استفاده از روش پاسخ سطح ارتباط بین فاکتورهای مهم و پاسخ برقرار شد. روش طراحی ترکیبی مرکزی به عنوان مرسوم ترین روش پاسخ سطح استفاده گردید. شرایط آزمایشگاهی بهینه بدست آمده از بررسی های آماری عبارت بودند از: حجم نمونه: ۱۰ میلی لیتر، حجم حلال استخراج (کلروفرم): ۱۳۷/۷ میکرولیتر، حجم حلال پخش کننده (استون): ۰/۶ میلی لیتر، زمان سانتریفیوژ: ۵/۰ دقیقه، pH: ۶/۲ (pH طبیعی محلول نمونه)، ۲/۵٪ وزنی-حجمی سدیم کلرید، و زمان استخراج: ۶/۰ دقیقه. تحت شرایط بهینه فاکتور تغلیظ برابر با ۱۲۰ بدست آمد. منحنی کالیبراسیون در محدوده ۰/۰۱ تا ۱۰۰/۰ میلی گرم بر لیتر با ضریب همبستگی برابر با ۰/۹۹۶۱ خطی بود. حد تشخیص روش برای اندازه گیری تریتون X-100 برابر با ۳/۰ میکرو گرم بر لیتر بود.

کلمات کلیدی: DLLME، تریتون X-100، طراحی آزمایش، HPLC