

Study on rapid extraction and detection of chlorpyrifos poisoning from green tea

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Article

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Abstract

A simple, rapid, and environmentally friendly method has been established for the determination of chlorpyrifos residue in green tea by dispersive liquid-liquid microextraction (DLLME) and gas chromatography-flame photometric detection (GC-FPD). Some experimental parameters that influence extraction efficiency, such as the kind and volume of disperser solvents and extraction solvents, extraction time, addition of salt and pH, were investigated. And the optimal experimental conditions were obtained, quantitative analysis was carried out using external standard method. The correlation coefficient of the calibration curves was 0.999 with in 0.05mg/kg to 5 mg/kg. The results showed that under the optimum conditions the enrichment factors of the chlorpyrifos was about 484, the recoveries for standard addition fell in the range from 91.94% to 104.70% and the relative standard deviations (RSD) was 4.61%. The limit of quantification (LOQ) of chlorpyrifos in green tea was 0.02µg/ml at the signal/noise (S/N) ratio of 3.

Introduction

Chlorpyrifos is a kind of broad spectrum, efficient, moderate toxicity, and long residual effect period of organophosphorus pesticides, has a good stomach toxicity and contact action, it is mainly used for the prevention and treatment of cotton, vegetables, tea, fruit and crops on the harmful insects and mites [1–3]. For the toxicity of chlorpyrifos has already had a relevant research [4–7], with its wide application, is bound to cause pollution to the environment and food, threats to human health, the current many countries for chlorpyrifos at the maximum residue limits of agricultural products is more and more strict, but our country has not yet set environmental health standard of chlorpyrifos and maximum allowable residues in green tea. Therefore, research of chlorpyrifos pesticide residues analysis method on green tea, to ensure that food and environment safety is of great importance.

In 2006, Rezaee etc. [8] first reported the DLLME technology. This sample pretreatment technology is integrates sampling, extraction and concentration into an organic whole, with a series of advantages including operation is simple, fast, low cost, high enrichment efficiency and environmentally friendly [9–11]. At present, the method has been applied to the organophosphorus pesticide [12–18], carbamates pesticide [17–21], neonicotinoid [22], polycyclic aromatic hydrocarbons [23–26] and other organic pollutants analysis determination. At the present, the DLLME technology for the determination of chlorpyrifos residue in fruits, vegetables and herbal medicines has been reported [27–28], but the application of the technology for determination of chlorpyrifos residue in green tea less reported. This study establishing new method of DLLME and GC coupled techniques for the determination of chlorpyrifos residue in green tea.

Materials And Methods

Instruments and Reagents

Gas chromatography was an Shimadzu GC-17A (Shimadzu, Japan company), equipped with a FPD detector and an analytical column DB-1701 Ultra Inert capillary column (30 m length× 0.53 mm I.D.×1µm film thickness, Agilent Technologies, USA); Pipetting gun 10–100 µL (German Brand); 10 mL glass centrifuge tube plug pointed bottom; 1 µL injection needle (Hamilton, Switzerland company).

Chlorpyrifos (certified analytical standard, 98%) was purchased from Dr. Ehrenstorfer company (Germany); Carbon tetrachloride(CTC), trichloroethane(TCE), chlorobenzene[MCB], acetone (analysis of pure) was purchased from Shanghai chemical reagent co., LTD (Shanghai, China); Acetonitrile (chromatography, American Fesher company); Experiment with water for Milli-Q pure water (Milipore companies in the United States). Green tea to buy in a store.

Chlorpyrifos Solution

The confecting of chlorpyrifos standards: according to samples from 102.0 mg chlorpyrifos standards in 100 mL volumetric flask and dissolved in acetone and constant volume, the mixture of 1000 mg/L standard stock solution; Take the standard stock solution with acetone diluted 10 mg/l chlorpyrifos standard solution.

GC-FPD Analytical Condition

Injector temperature, 220 °C, splitless; detector temperature, 250 °C; oven temperature program starting at 170 °C, and ramp 20 °C min⁻¹ to 210 °C, 0.5 min at 210 °C, and then ramp 10 °C min⁻¹ to 230 °C, 3 min at 230°C, a total of 7.5 min. Under these conditions chlorpyrifos retention times were approximately 4.4 min (Fig. 1).

Sample preparation

Take green tea sample 20 ml into 100 ml measuring cylinder, then add 4 g Nacl and 50 ml of acetone solution. thermal agitation after 3 min, room temperature let stand for 30 min, take 1 ml solution (dispersant), to be the next step.

DLLME Procedure

Take 1 mL dispersant and 22 µl extraction solvent, in turn, add to 10 ml Sharp bottom plug centrifuge tube, gently shake. Then add 5 ml ultrapure water, gently oscillation, extracting agent evenly dispersed in the water phase, the formation of water/dispersant/extraction agent emulsion system, place 2 min at room temperature. Then to 3500 r/min 2 min, the centrifugal extraction agent deposit in the bottom of the centrifuge tube, trace sampler has absorbed 1µl sedimentary facies, the GC analysis.

Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Effect of the Type and Volume of Extraction Solvent

This experiment select 3 kinds of organic solvent is chlorobenzene (density of 1.10 g/ml), carbon tetrachloride (density of 1.59 mg/l) and trichloroethane (density of 1.35 g/ml), according to section 1.5 steps, the extraction effect of 3 kinds of extraction solvent on of chlorpyrifos has tested. The results show that (as shown in Fig. 2), trichloroethane has the highest concentration coefficient and good recovery, extraction effect was better than the other two. so choose trichloroethane as extracting agent in this project.

The extraction solvent volume affect the enrichment ratio of DLLME directly, thus affecting recovery, 5 extraction volume have selected in this experiment: 20, 22, 25, 30 and 35 μ l. Results shows, that with the increase of the extractant volume, recoveries first increases then decreases by chlorpyrifos, 22, 25 μ l are can achieve ideal recovery, but with increase of the extractant volume, the enrichment factor decreases obviously. As 22 μ l of the extractant volume, the recoveries and enrichment factor are better (Fig. 3, Fig. 4).

Effect of the Type and Volume of Dispersant Solvent

This experiment choose acetonitrile and acetone as dispersant, mix with 22 μ l trichloroethane respectively, and add 5 ml water. The acetone extraction efficiency highest, the acetonitrile extraction rate is lower than acetone, and the peak has interference (Fig. 5, 6). So the experiment choose acetone as dispersant.

Experiment with different volume (700, 800, 900 and 1000 μ L) of acetone and 22 μ l trichloroethane as extraction system, after extraction of centrifugal, sample injection 1 μ l sedimentation volume, of the peak area increased with the increase of dispersing agent volume. The result is due to the volume of acetone increased, makes a certain amount of trichloroethane more dispersed in water, the the extraction efficiency relative higher. When acetone volume is 1000 μ l, peak area reached the highest. So select volume of acetone is 1000 μ l (Fig. 7, 8).

Effect of Extraction Time and Eentrifugal Time

For this study, chose 3500 r/min, and the centrifugal time is 2, 5, and 10 min, with the increase of the centrifugal time, the peak area of chlorpyrifos keep the same level, centrifugal time had no significant effect on the extraction efficiency. extraction agent of scattered in the mixture as long as through the short time of centrifugal can deposit to the bottom of the tube, it is one of the great advantages in this DLLME method, so choose 2 min is more timesaving (Fig. 9).

Effect of Concentration of Salt

In this experiment, add NaCl change salt concentration in the water phase, concentration of 0%, 2% and 5%. Results (Fig. 10), a mixture of the increase of the extraction solvent solubility in the aqueous phase with the salt concentration increased, but volume of precipitated phase increased in the end, affecting the extraction efficiency of method, so the experiment without salt.

Method Validation

The linear range of chlorpyrifos was 0.1–10 mg/l. Results show, in the optimized conditions, the peak area of chlorpyrifos had good linear relationship within a certain range, the Regression equation was $Y = 16086x - 1448$, the regression coefficients were greater than 0.999. The LOD was 0.02 µg/ml. The enrichment factor was 554.51. The recoveries for standard addition was 98.07%, the intra-day relative standard deviations (RSD, n = 4) was 4.61%, the veracity and accuracy of the method can meet the requirement of pesticide residue analysis (Table 1).

Table 1

Recovery assay, Recovery assay, precision (repeatability) and trueness of target compounds in green tea

	Spiked Sample					RSD%(N = 4)
	1	2	3	4	Average	
Volum(µl)	9.50	8.20	8.50	9.40	8.90	6.31
Sampling concentration(ng/ml)	9.68	12.77	11.51	10.41	11.09	10.52
Enrichment factor	483.90	638.39	575.25	520.51	554.51	10.52
Recovery(%)	91.94	104.70	97.79	97.86	98.07	4.61

Discussion

In the DLLME method, extracting agent is one of the important factors affecting the extraction efficiency, then the main principle of choose it is: the extraction ability of extracting agent on the target have higher; Density is greater than the water and insoluble in water; To target without interference, the qualitative and quantitative analysis of the target will not affected.

Dispersing agent should be able to dissolved extraction agent completely, and soluble in water. Effect of dispersant is maximize for extraction agent with the contact area of the sample solution, its solubility in water, the greater the formation of droplets will be smaller, and the bigger with the target contact area, and the extraction efficiency higher. Volume of dispersant will affect the dispersion degree of extraction agent in water, which affects the sedimentation volume, which influence the extraction efficiency.

The extraction time in this method, refers to after the dispersant, extraction agent and ultrapure water mixture to before the centrifugal a period of time. This experiment select extraction time is 1, 3, and 5 min. The results showed extraction time had no significant effect on the extraction efficiency, and extraction time 1 min enough to form emulsion to the water phase of target transfer to the organic phase to two phase equilibrium.

Salt effect is often in the process of microextraction evaluation of a parameter, the change will change the concentration of salt in the water solution of ionic strength, is different of the extraction effect. The

increase of ionic strength makes increase solubility of extractant in the aqueous phase, to improve recovery, but increase the volum of the sedimentary facies after the centrifugal, the concentration decreases of the target in sedimentary facies, enrichment coefficient dropped significantly.

Conclusions

A simplified and rapid method was developed for the determination of chlorpyrifos residue in green tea by DLLME and GC-FPD in this experiment. In this method, the acetone was used not only as solvent to extract but also dispersant, the flame photometric detector is highly selective to organophosphorus compounds, so the organic solvent can directly into the chromatographic instrument analysis after DLLME operation, the interference of the impurity on the analysis is quite small, and a better extraction effect was obtained under the optimized extraction condition. The results showed that all kinds of analysis parameters meet the requirements of the pesticide residues analysis for agricultural products and the sensitivity was high when use the DLLME-GC-FPD detection of chlorpyrifos pesticide residues in green tea, this method is suitable for chlorpyrifos residues detection in green tea, it to broaden the application range of the DLLME techniques also is of great significance.

Declarations

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Author contributions: Conceptualization, H.T.; methodology, YJ.F.; validation, H.T.; formal analysis, C.M.; investigation, H.T.; resources, YJ.F.; data curation, H.T.; writing—original draft preparation, XF. Y.; writing—review and editing, H.T., CH. P., XF. Y., amd SH. L.; visualization, H.T.; supervision, H.T.; project administration, H.T. and YJ. F.; funding acquisition, H.T. All au-thors have read and agreed to the published version of the manuscript.

Competing interests: The authors declare no competing interests.

Additional information: The datasets generated and/or analysed during the current study are available in this published article [and in supplementary information].

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Figures

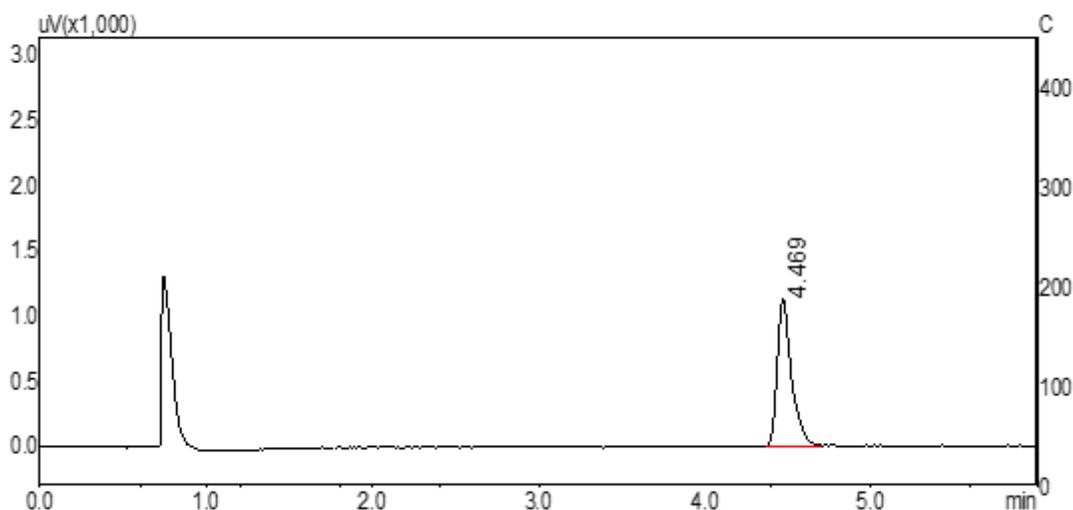
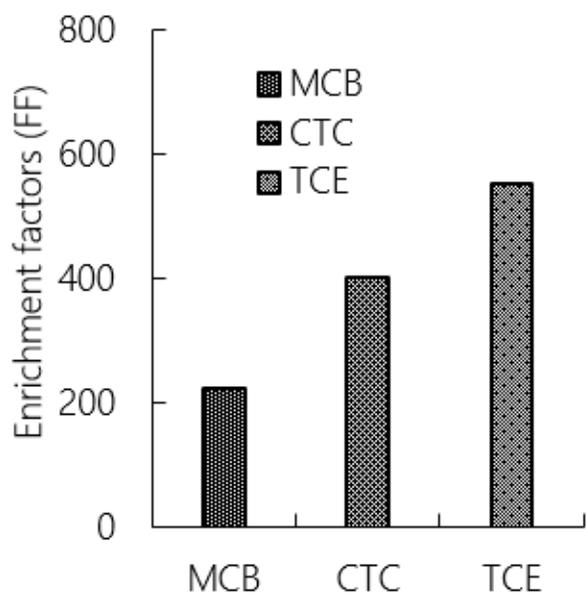
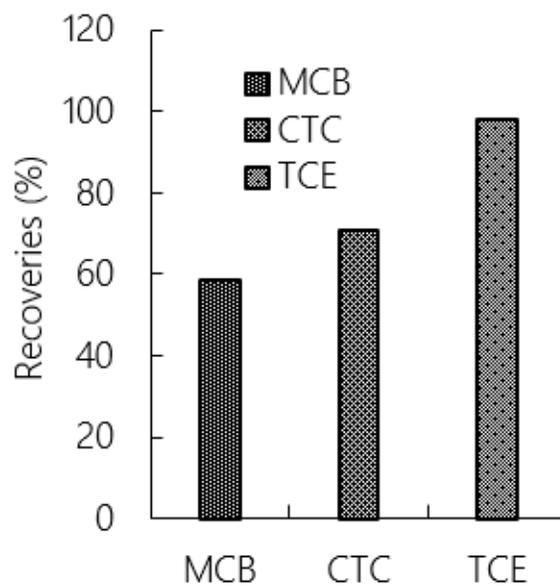


Figure 1

Chromatogram of chlorpyrifos



(a)



(b)

Figure 2

Different type of extraction solvent , (a)Enrichment factors of different type of extraction solvent, (b)Recoveries of different type of extraction solvent

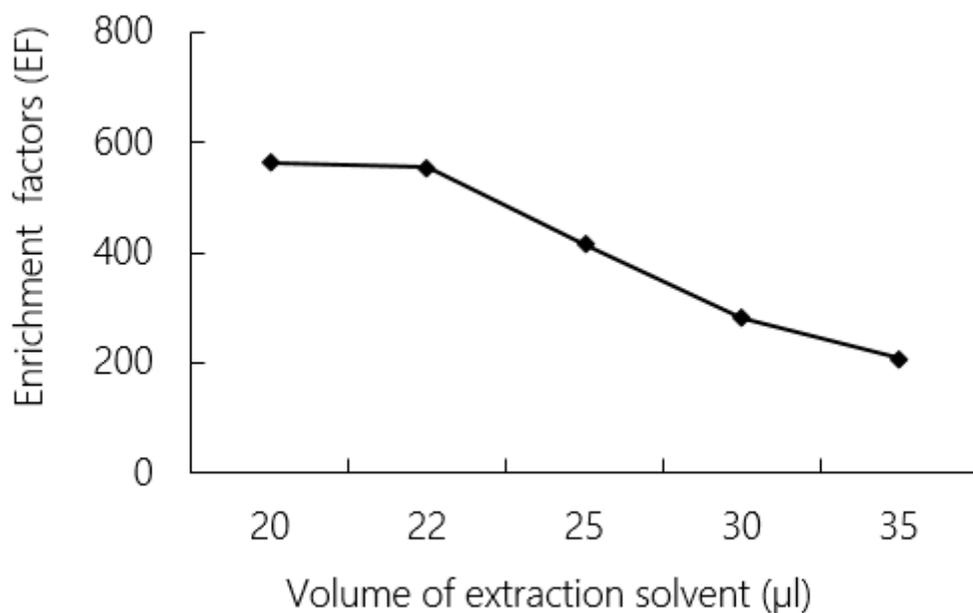


Figure 3

Enrichment factors of different volume of extraction solvent

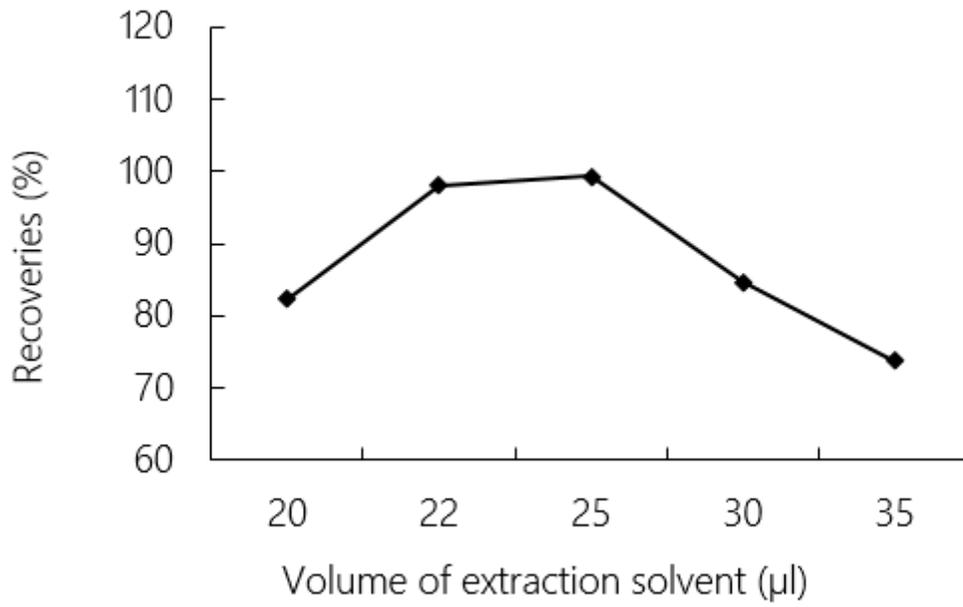


Figure 4

Recoveries of different volume of extraction solvent

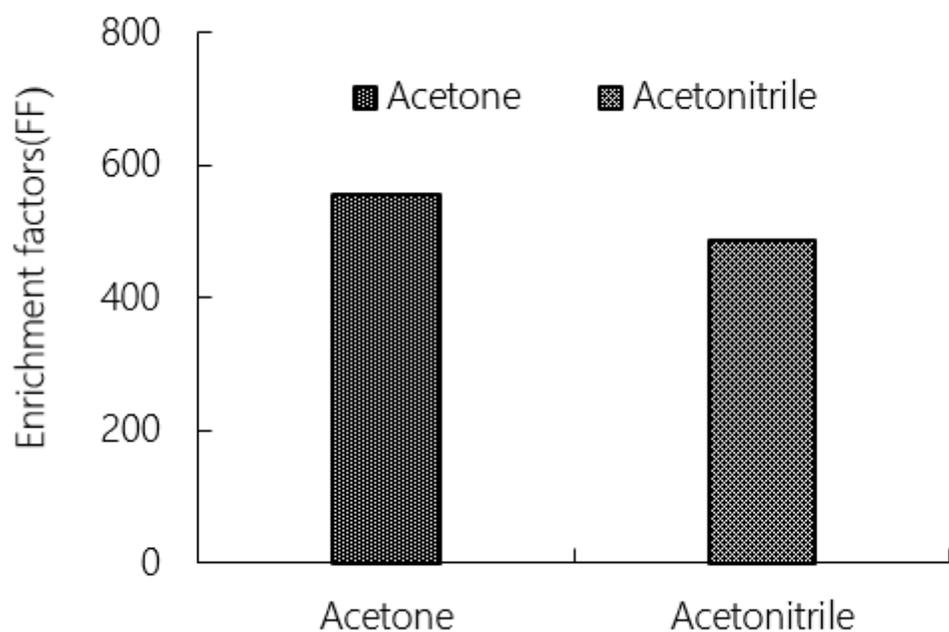


Figure 5

Enrichment factors of different type of dispersant

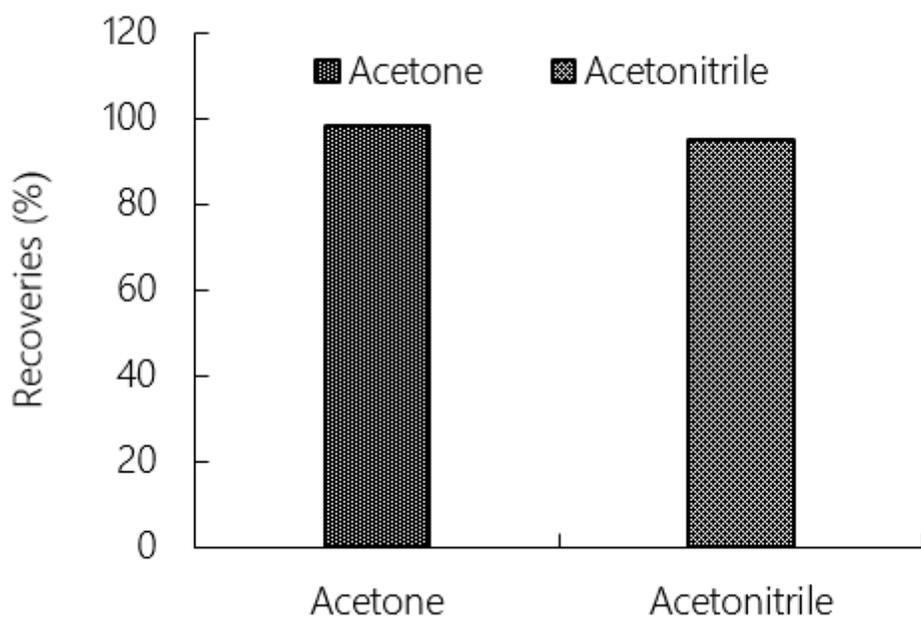


Figure 6

Recoveries of different type of dispersant

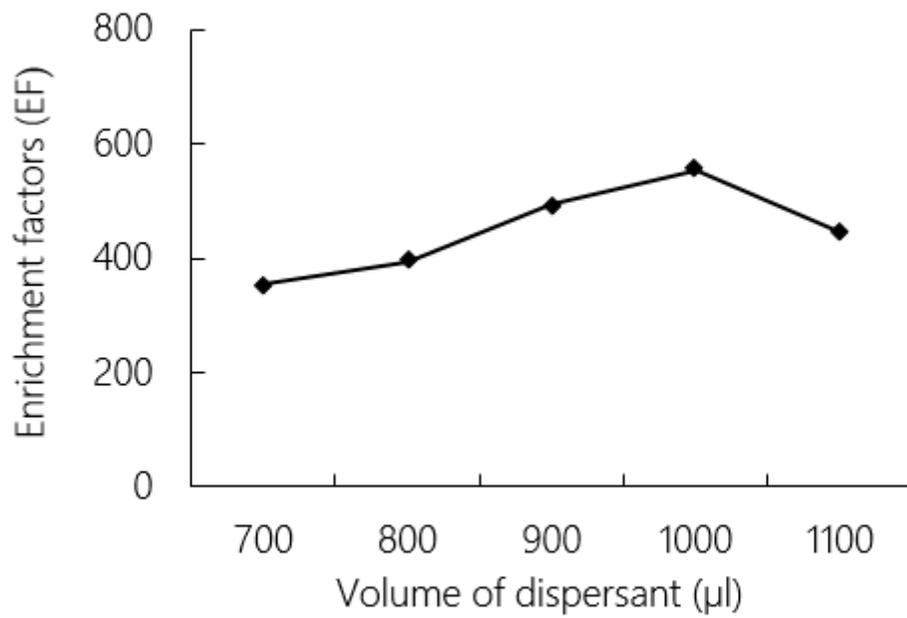


Figure 7

Enrichment factors of different volume of dispersant

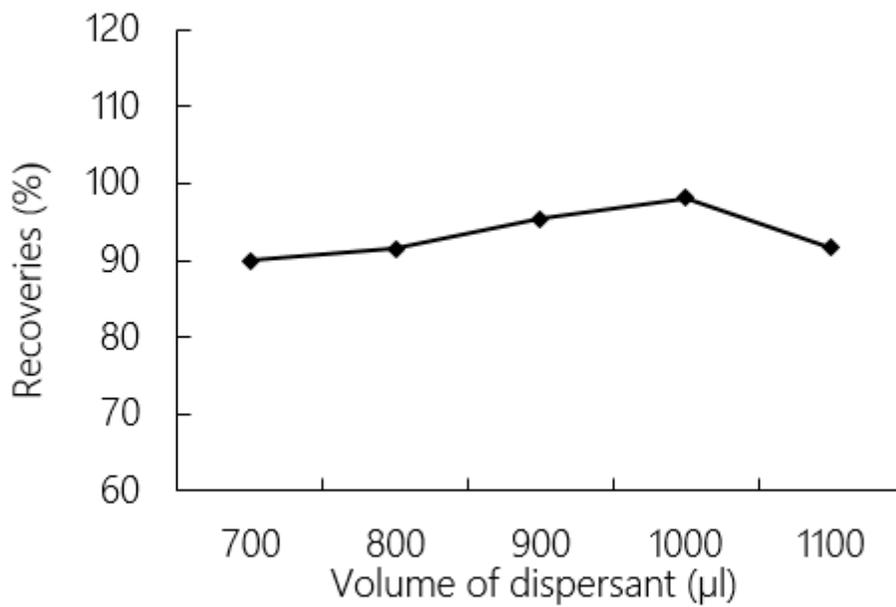


Figure 8

Recoveries of different volume of dispersant

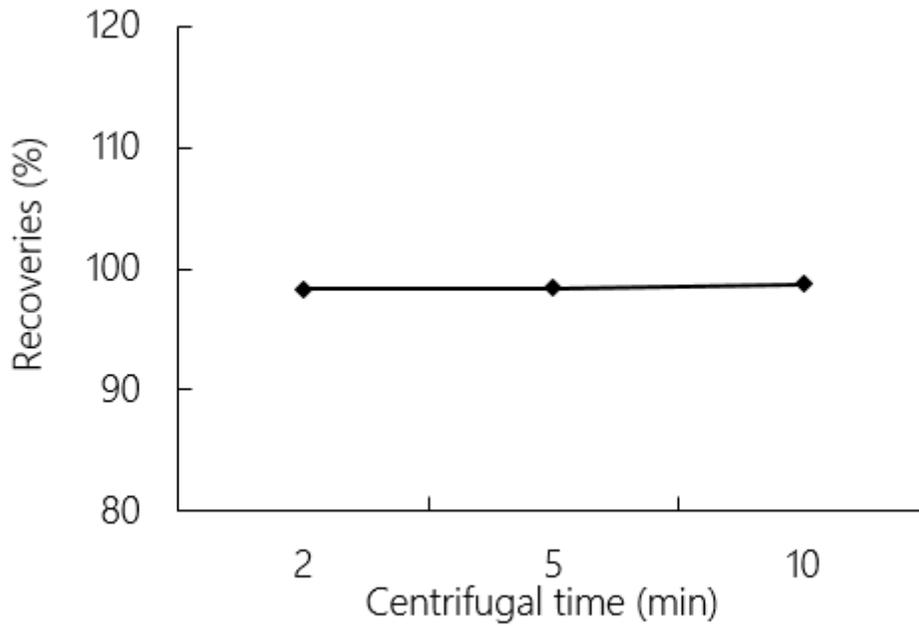


Figure 9

Recoveries of different centrifugal time

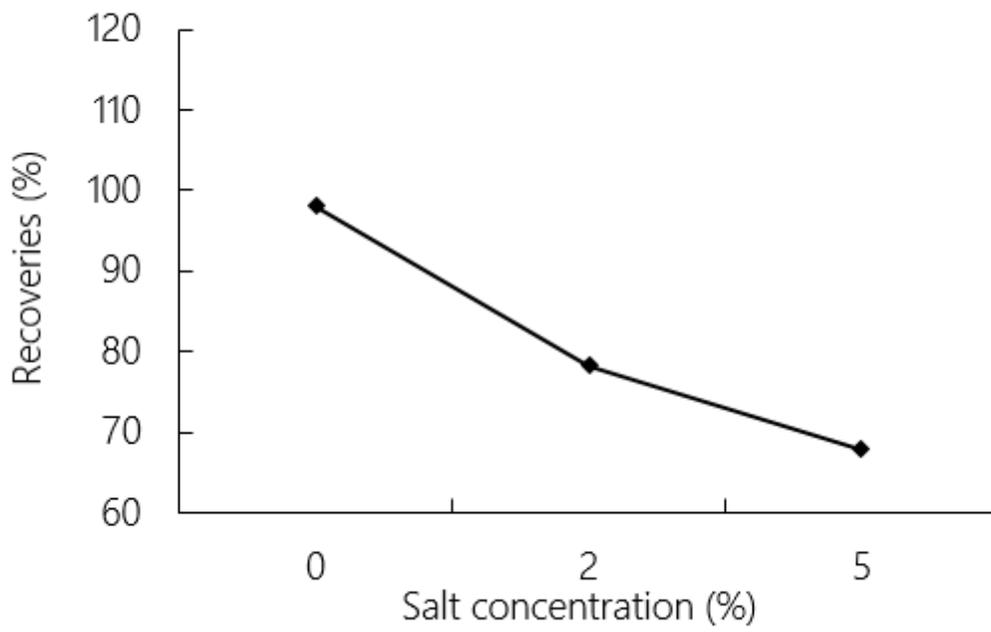


Figure 10

Recoveries of different salinity