

## **Determination of 2, 4-D in Water Samples by Salting-Out Assisted Liquid-Liquid Extraction-UV-Vis**

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**Abstract:** A novel method for determination of 2, 4-dichlorophenoxyacetic acid (2,4-D) was developed by using salting-out assisted liquid-liquid extraction (SALLE) via water-miscible acetonitrile as the extractant coupled with ultraviolet visible spectrophotometry. SALLE conditions, including volume of salting-out solvent, the pH of sample solution and salting-out solvent as variable factors. The optimal salting-out parameters were obtained as follows: 2 mL of acetonitrile was added to 2 mL of sample solution with pH = 2 and then 6 mL salting-out solvent containing 5 mol/L sodium chloride at a pH of 2 was added to the solution for extraction. This procedure afforded a convenient and cost-saving operation for 2,4-D, such as good linear relationships ( $R = 0.9979$ ) between absorbance and concentration from 2.5  $\mu\text{g/mL}$  to 25  $\mu\text{g/mL}$ , low limits of detection (0.75  $\mu\text{g/mL}$ ). The method recoveries obtained at fortified three concentrations for two water samples ranged from 83% to 100%.

**Keywords:** Salting-out assisted liquid-liquid extraction, 2, 4-D, Ultraviolet visible spectrophotometry.

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### **1. INTRODUCTION**

Phenoxyacid herbicides are used to control the growth of different undesired vegetable species in agriculture, gardening and forestry. They are widely used due to their relative low cost, effectiveness and high selectivity. Hydrophilic and low volatility properties of these compounds promote their entry into the aqueous environment [1]. Many of them can cause damage to human health and wildlife. The maximum contaminant level of 2, 4-dichlorophenoxyacetic acid (2,4-D) is set as 0.07 mg/L in drinking water by EPA of United States [2]. For these reasons, a rapid, selective, and sensitive method is desirable for determining the trace levels of 2,4-D directly from aqueous samples.

To date, several methods have been developed for the determination of 2,4-D including liquid chromatography (LC) [3,4], gas chromatography (GC) [5], electrochemical sensor method [6,7] and capillary electrophoresis [8]. However, the concentration of 2,4-D in samples is very low and the matrix of some samples are complex. In all the above work, many extraction methods such as dispersive liquid-liquid micro extraction (DLLME) [3], molecularly imprinted solid phase microextraction (MISPE) [4], dispersive solid phase extraction [2,5], molecularly imprinted membrane extraction [6,7] and liquid liquid liquid micro extraction (LLLME) [8] have been used. Most of these methods are often complicated and time spending, e.g. the synthesis of the solid sorbent such as molecular imprinted polymer or fibre and the need of special instrument.

Fortunately, salting-out assisted liquid-liquid extraction (SALLE) provides a simple, rapid alternative which is a technique based on LLE in which an appropriate concentration of salt is

added to achieve the separation of aqueous phase from the partially miscible organic phase [9] and simultaneously the target analytes are extracted into the separated upper organic phase. This method coupled sample clean-up (e.g. acetonitrile deproteinization) with enrichment (via salting-out extraction) has been reported for water [10], biological [11, 12], and drug [13] sample extraction processes.

In this study, we propose the use of SALLE for extraction of 2, 4-D from two water samples, and ultraviolet visible spectrophotometry (UV-Vis) for determination of 2, 4-D. To the best of our knowledge, this is the first demonstration for SALLE-UV-Vis for 2, 4-D analysis.

## 2. EXPERIMENTAL

### 2.1. Chemicals and Materials

2,4-D were purchased from Sigma-Aldrich (Steinheim, Germany), HPLC-grade methanol (MeOH) and acetonitrile (ACN) were provided by J&K Chemical (Beijing, China). NaCl, NaH<sub>2</sub>PO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, NaOH, and other affiliated chemicals were all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solvents and chemicals were of analytical grade and used without further purification unless otherwise specified. A salting-out solution of 5 mol/L NaCl was prepared by dissolving appropriate amounts of NaCl in 100 mmol/L phosphate buffer, and the pH was adjusted by 1 mol/L H<sub>3</sub>PO<sub>4</sub> and NaOH.

HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA), and was used throughout the work.

### 2.2. Apparatus

An Hitachi U-2910 UV-Vis spectrometry was provided by Hitachi Instrument Inc. (Hitachi, Japan). All the samples were passed through microporous nylon filters of 0.45 μm pore sizes in diameter (Pall Corporation, USA).

### 2.3. Preparation of Standard And Sample

Standard stock solutions containing 1000 μg/mL of 2,4-D was prepared by dissolving the required amounts of the standard in MeOH. They were stored in a refrigerator at 4 °C. Working solutions were prepared from the stock solutions by dilution with appropriate amounts of Milli-Q water.

Lake water was collected from an artificial lake located in Longhua District of Haikou City (China). Tap water was obtained in the laboratory when needed. All the water samples were passed through microporous nylon filters with the pore sizes of 0.45 μm in diameter. The samples were kept under refrigeration at 4 °C in the dark. Several aliquots from 2 mL filtered water samples were spiked with 2,4-D standard with different concentrations and followed by the SALLE procedure.

### 2.4. SALLE Procedure

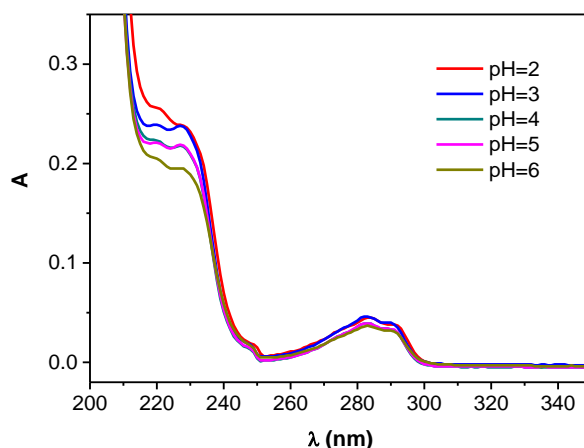
Briefly, 2.00 mL of water sample was placed in a 15 mL of screw-cap glass test tube and spiked with 2,4-D at 10 μg/mL. ACN (2 mL) was added into the sample solution. After the mixture was ultrasonicated for 1 min, 6 mL salting-out solution was added into the mixture and vortexed for 5 min. In this step, a two-phase solution was formed and the 2,4-D in the water samples were extracted into the upper organic phase (ACN phase). Then, the supernatant was collected for UV-Vis analysis which  $\lambda_{\max}$  was set at 220 nm.

## 3. EXPERIMENTAL

### 3.1. SALLE Condition Optimization

#### 3.1.1. Effect of sample pH

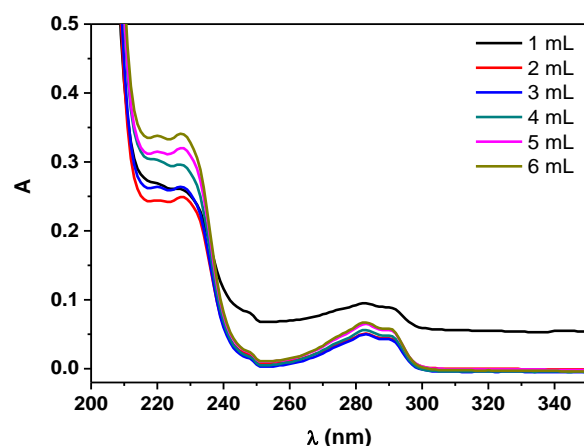
As shown in **Fig. 1**, the absorbance (A) of 2,4-D decreased with pH increase in the range of 2–6. This was because the extraction solution pH has great impact on ionization degree of 2,4-D. When pH was low, the neutral species of 2,4-D was so nonpolar that their solubility in water is extremely low, the 2,4-D was easy to be extracted into the ACN phase, then the value of A increased. So pH 2 was selected as the optimum pH value of sample solution.



**Fig1.** Effect of sample pH on extraction efficiency. SALLE conditions: sample volume, 2 mL; salting-out solution, 5 mol/L NaCl, 6 mL and pH 2; extraction solvent, ACN, 2 mL.

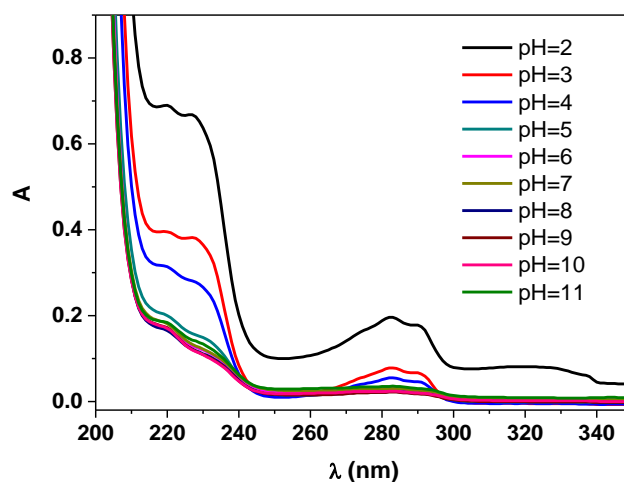
### 3.1.2. Effect of Salting-Out Solution Volume

Increasing the volume of salting-out solution decreased the volume of the ACN phase and therefore resulted in higher concentrations of 2, 4-D in the organic phase, then the value of A is bigger. When the salting-out solution volume was 6 mL, the A was the biggest, as shown in Fig. 2. So, 6 mL was selected in the following studies.



**Fig2.** Effect of sample pH on extraction efficiency. SALLE conditions: sample volume, 2 mL pH = 2; salting-out solution, 5 mol/L NaCl, and pH 2; extraction solvent, ACN, 2 mL.

### 3.1.3. Effect of salting-out solution pH



**Fig3.** Effect of salting-out solution pH on extraction efficiency. SALLE conditions: sample volume, 2 mL pH = 2; salting-out solution, 5 mol/L NaCl, 6 mL; extraction solvent, ACN, 2 mL.

Like sample pH, the salting-out solution pH also has great impact on ionization degree of 2,4-D. As shown in **Fig. 3**, the A of 2,4-D decreased with the pH increasing in the range of 2–5, and was more or less with the pH increasing in the range of 6–11. Consequently, the optimum salting-out solution pH was set at pH 2.

So, the optimized conditions for extraction of 2,4-D and ideal SALLE efficiency were attained as follows: salting-out solution, 5 mol/L NaCl in 100 mmol/L phosphate buffer, 6 mL and pH 2; extraction solvent, ACN, 2 mL; and sample, pH 2.

### 3.2. Analytical Figures of Merit of the SALLE-HPLC-UV Method

The above optimal extraction conditions for SALLE were employed in subsequent work. Standard curves were obtained from the analysis at various concentrations. Table 1 lists the linear range, slope, intercept, correlation coefficients (r), and limit of detection (LOD) for 2, 4-D. As seen, good linearity assessed at six different concentration levels was obtained between A and the corresponding concentrations of 2,4-D in the range of 2.5–25 µg/mL. LOD for the 2,4-D, calculated as the analyte concentration for which the value of A was three times the background noise (3S/N), was 0.75 µg/mL for the UV-Vis analysis.

**Table1.** Linear range, slope, intercept, correlation coefficients, and LOD for 2,4-D

Analyte	Linear range(µg/mL)	Linear equation	Correlation coefficient (r)	LOD (µg/mL)
2,4-D	2.5-25	A = 0.0287c + 0.1687	0.9979	0.75

The method was validated for reproducibility of the A of the analyses. The relative standard deviation (RSD) values of A for Intra-day (n=6) and Inter-day (n=6) are shown in Table.2.

**Table2.** Reproducibility of SALLE-UV-Vis method

Standard	Intra-day (n=5) (RSD,%)	Inter-day (n=5) (RSD,%)
	A	A
2,4-D	0.30	0.45

### 3.3. Determination of 2,4-D in Water Samples

The developed SALLE-UV-Vis method was further applied to tap and lake water samples in order to check its practicality. The 2,4-D were not detected in the water samples.

Recoveries were calculated for the spiked water samples with 2.5, 5 and 10 µg/mL standards, respectively. The results are listed in Table 3. Satisfactory recoveries were obtained, ranged from 83% to 100%. This validated the SALLE-UV-Vis greatly applicable for the selective extraction, and accurate quantitation of trace 2, 4-D in water samples.

**Table3.** Recovery of 2, 4-D from tap and lake water samples

Analyte	Added (µg/mL)	Recovery (%)	
		Tap water	Lake water
2,4-D	2.5	100	88
	5	85	93
	10	83	98

## 4. CONCLUSIONS

A simple, fast and sensitive SALLE-UV-Vis method was developed for the determination of 2,4-D in two water samples. Also, compared with the classic extraction methods based on LLE and SPE, which often required large volume of sample and organic solvent, this method is an excellent cost-effective alternative for sample preparation. The developed SALLE-UV-Vis offered wide linear range, good quantitative ability, and high precision.

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