Determination of Phenols in Wastewater by Dispersive Liquid-Liquid Microextraction Coupled to Capillary Gas Chromatography

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Abstract—A simple and reliable method combining dispersive liquid-liquid microextracion (DLLME) with capillary gas chromatography (GC) using flame ionization detection was developed for simultaneous determination of phenol (PN), *p*-methyl phenol (*p*-MPN), *o*-nitrophenol (*o*-NPN), and 2, 4-dichlorophenol (DCPN) in wastewater. To achieve this goal, dispersive liquid-liquid microextraction (DLLME) was applied as a sample preparation technique. The DLLME conditions such as the types and volume of extraction solvent, the types and volume of disperser solvent, pH value and salt addition were studied and optimized. The method was linear in the ranges from 6.0×10^{-3} to 100.0 $\mu g\cdot mL^{-1}$ for aforementioned phenols with R^2 (correlation coefficients) $\geqslant$ 0.9955. The DLLME procedure allowed efficient recovery of the investigated phenols ranging between 85 % and 96 % with a relative standard deviation (RSD) $\leqslant$ 3.2 for actual wastewater samples spiked with 10, 40 and 80 $\mu g\cdot mL^{-1}$ of phenols, respectively. These results show the potential of this technique for phenols monitoring in wastewater samples. Furthermore, the investigated methods are simple, reproducible, and inexpensive.

Keywords-phenols; capillary gas chromatography; flame ionization detection; dispersive liquid-liquid microextraction; wastewater

I. INTRODUCTION

Phenols, cresols, and nitrophenols are widely applied in the pharmacological and chemical industries. They are involved in the production of polymers, drugs, dyes, explosives, pesticides, disinfectants, antiseptics, medicinal preparations, and antioxidants [1, 2]. Phenol is also a toxic compound, which very often appears in the industrial and municipal wastes. That is a very serious problem for the environment. Therefore, monitoring the phenols in environment is very important and urgent. As far as we known, the researches of detecting the phenols in environment have been active. Presently, a number of analytical approaches have been reported for the determination of various phenolic compounds, especially for some aqueous matrixes such as wastewater, beverages, and potable water etc. [3-8]

Dispersive liquid-liquid microextraction (DLLME) has emerged recently as a new and very efficient alternative technique to concentrate target analytes dissolved in organic extracts, allowing a high enrichment factor in one simple and quick manner [9]. It is generally known that DLLME has been applied to the analysis of water, wine, fruit and juice, honey, pharmaceutical and biological samples [10-11], and so on.

In this paper, DLLME-GC was established for the determination of phenols in wastewater, and the features of the proposed method were discussed in detail in the following sections.

II. EXPERIMENTAL

A. Apparatus and reagents

Measurements were carried out with Aglient 6890 N gas chromatograph fitted with a flame ionization detector (FID) (Agilent Corporation, America). Separation was performed on a HP-5 capillary column (30m × 0.32mm I. D. × 0.25 μm film thickness). DLLME procedure was conducted in a 10 mL glass centrifugal tube.

Analytical grade standards of the phenols used in this work were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), which included PN, *p*-MPN, *o*-NPN, and DCPN. Dichloromethane, trichloromethane, carbon tetrachloride, aceton, acetonitrile and isopropanol (Concord, Tianjin, China), were all HPLC grade solvents, unless otherwise specified. The deionized water was purified with an Aquapro series water purification system (Chongqing, China).

Stock solutions (1000 $\mu g\cdot mL^{-1}$) were prepared in deionized water and stored in the fridge at 4 °C. Working standard solutions of each compound were prepared by appropriate dilution of the stock solution using deionized water. The wastewater samples were taken from a dye house (Jiujiang, China). All real water samples were filtered further by using a 0.45 μm membrane, and stored in amber bottles at 4 °C until analysis.

B. Chromatographic condition

The oven temperature program was as follows: the initial temperature 50 °C increased to 70 °C (hold for 2 min) at a rate of 20 $°C\cdot min^{-1}$, and then to 230 °C at 15 $°C\cdot min^{-1}$ and finally held for 2 min. The injector temperature was 260 °C and the detector temperatures were 290 °C. Nitrogen (99.999 %) was used as carrier and make-up gas at flow rates of 5.0 and 30 $mL\cdot min^{-1}$, respectively. The flow of air for FID was 400 $mL\cdot min^{-1}$

and flow rate of hydrogen was 40 mL·min^{-1}. One microliter was manually injected using the splitless mode.

C. Implementation of experiment

A 5.00 mL aqueous sample with its pH value adjusted to 8-11 in the presence of 5% Na_2CO_3, was placed into a 10 mL glass centrifugal tube. The mixture of 1.0 mL isopropanol (as a disperser solvent) and 0.2 mL dimechloromethane (as an extraction solvent) was rapidly added into the above-mentioned aqueous sample. After gently shaking for 10 min, the tube was centrifuged for 5 min at 4,000 rpm, then 1.0 µL of the sedimented organic phase was withdrawn using a 10.0 µL microsyringe (Agilent, America) and injected into the GC.

The linearity and linear range of the method were established using calibration curves obtained via the sextuplicate analysis of 4 phenols at six concentration levels—1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg·mL^{-1} in the standard working solutions. The limits of detection (LOD) were obtained from the analytical curves and calculated from the following expressions: LOD = 3σ/s, where σ is the standard deviation of the blank sample response (n = 20) and s is the slope of the analytical curve.

The analysis of wastewater sample was complete in 8 h. The concentrations of phenols in wastewater were determined. Then three different concentration levels were individually spiked to the wastewater samples and the recovery and precision of the method were obtained by assessing six replicates at each concentration.

III. Results and Discussion

A. The optimization of DLLME conditions

There are several factors affecting the DLLME extraction process, including extraction solvent, disperser solvent, volume of the extraction solvent and disperser solvent, pH value of the aqueous solution, and the salting effect. The major task on the optimization of DLLME conditions is to determine disperser solvent and extraction solvent. Among of the selected solvents, dichloromethane exhibits perfect property. The extraction rates of all phenols detected are greater than 75% for dichloromethane. And the extraction rates of phenols are greater than those of acetone and acetonitrile using dichloromethane as disperser solvent. The orthogonal tests show the optimized DLLME conditions: 0.2 mL dichloromethane (extraction solvent), 1.0 mL isopropanol (disperser solvent), pH = 8-11, and 6% Na_2CO_3 (salt).

B. Identification of the optimal chromatographic conditions

To separate these phenols as soon as possible and obtain normal peaks shapes, a lot of tentative experiments were performed to determine the appropriate injector temperature, column temperature and flow rate of carrier gas. We find it is hard to separate *o*-NPN and DCPN completely due to similarity of their chemical and physical property during experimental process. As shown in Fig. 1a, under the optimized chromatographic conditions, PN, *p*-MPN, *o*-NPN, and DCPN were separated completely.

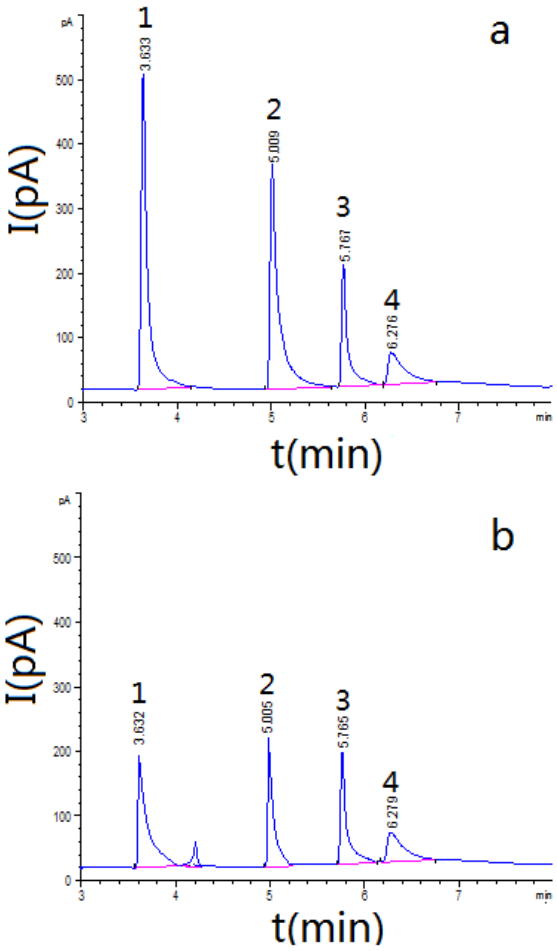

Figure 1. Chromatograms of phenols in a standard solution (a) and an actual wastewater sample solution after extraction by DLLME (b) under the optimized GC-FID conditions. PN (1), *p*-MPN (2), *o*-NPN (3), and DCPN (4)

C. Linear range, calibration curve and limits of detection

Under the optimized GC-FID conditions, calibration curve, linear range and detection limits of phenols were listed in Table 1. Seen from Table 1, obviously, the detection limits of all four phenols were very low, and the lowest one was 3.2×10^{-4} µg•mL^{-1} (PN). The wide linear range (span four orders of magnitude) and good linearity ($R^2 \geqslant 0.9955$) showed high sensitivity and accuracy of this method.

TABLE I. Linear Range, Calibration Curve and Detection Limits

Compounds	Linear range ρ (µg•mL^{-1})	Working curve	LOD ρ (µg•mL^{-1})
PN	1.1×10^{-3}~ 100	y = 28.430 x - 96.400 (R^2 = 0.9987)	3.2×10^{-4}
p-MPN	1.1×10^{-3}~ 100	y = 26.065 x - 89.393 (R^2 = 0.9993)	3.4×10^{-4}
o-NPN	1.9×10^{-3}~ 100	y = 9.5014 x - 53.398 (R^2 = 0.9955)	5.6×10^{-4}
DCPN	6.0×10^{-3}~ 100	y = 10.578 x - 192.02 (R^2 = 0.9968)	1.8×10^{-3}

D. Determination of an actual wastewater samples

An actual wastewater sample gathered from the dye house was manipulated at room temperature as described in the *'implementation of experiment' section*. Fig. 1b shows the separation of phenols in an actual sample after

extraction by DLLME using the optimized GC-FID conditions. As shown, all of phenols in the actual wastewater samples have separated from each other completely and the separation of phenols in the actual sample agreed with that of standard working solution well, which can be seen from the comparison between Fig. 1a and Fig. 1b, indicating the validity of this method. It is noted that there is still a subtle difference between the chromatogram of phenols in the standard solutions and that of actual wastewater sample due to the difference of their injection time, however, it doesn't have an enough effect on accurate qualitative or quantitative analysis of these phenols in actual wastewater samples because the whole retention time of these phenols will change in the same regular forms such as shortening almost the same interval of time, as shown in Fig. 1b. The phenols in actual wastewater samples pretreated rightly were determined by external standard method, a method fairly easy to conduct, the average concentrations of PN, *p*-MPN, *o*-NPN, and DCPN in the actual sample was 7.68, 11.25, 10.80, and 15.34 $\mu g \cdot mL^{-1}$, respectively.

E. Recovery and precision for the method

The recovery was determined by comparing the analytical response of the corresponding analytes in the spiked samples before and after the extraction step, for three concentration levels (10, 40 and 80 $\mu g \cdot mL^{-1}$), being each level performed six times. In the same time, the precision of this method is estimated by the relative standard deviations of six replicate parallel experiments at three different concentration levels. To validate the accuracy of the proposed method more reasonably, we select high, mediate, and low three different concentrations in the linear range for all phenols in the wastewater sample according to the global standards. And to guarantee the rationality of the precision of the proposed method, we perform six replicate parallel experiments at each concentration level. As shown in Table 2, the mean recovery values ranged from 90.0 to 96 % for PN, from 88 to 95 % for *p*-MPN, from 87 to 94 % for *o*-NPN, and from 85 to 90 % for DCPN. The precision expressed as relative standard deviation (%RSD) was calculated from six replicates of spiked actual wastewater samples at three concentration levels. The precision obtained ranged from 1.1 to 2.3 % for PN, from 1.2 to 1.6 % for *p*-MPN, from 1.2 to 1.5 % for *o*-NPN, and from 1.9 to 3.2 % for DCPN, as can be seen in Table 2. The high recoveries and precision show the validity of this method. So this method is reliable and can be used to determine the phenols in wastewater and extended to other fields.

TABLE II. THE AVERAGE RECOVERIES (PERCENTAGE) OF PHENOLS AND PRECISION (% RSD) OF THIS METHOD ON SPIKED WASTEWATER SAMPLES USING DLLME PROCEDURES AND GC-FID ANALYSIS ($N = 6$)

Compounds	Added ρ ($\mu g \cdot mL^{-1}$)	Recovered ρ ($\mu g \cdot mL^{-1}$)	Recovery (%)	RSD (%)
PN	10	9.0	90	1.3
	40	38.4	96	1.1
	80	74.4	93	1.2
p-MPN	10	8.8	88	1.4
	40	36.0	90	1.6
	80	76.0	95	1.2
o-NPN	10	8.7	87	2.3
	40	37.2	93	1.8
	80	75.2	94	1.1
DCPN	10	8.5	85	3.2
	40	35.6	89	2.1
	80	72.0	90	1.9

IV. CONCLUSIONS

In this study, DLLME combined with GC-FID has been successfully applied to the determination of phenols in actual wastewater samples. The DLLME technique demonstrated good analytical performance for the extraction of phenols from the wastewater samples and proved to be time-saving, cheap and easy to perform.

Furthermore, the method presented good linearity, precision, recovery and accuracy. Therefore, it is clearly useful for monitoring phenols in wastewater samples.

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