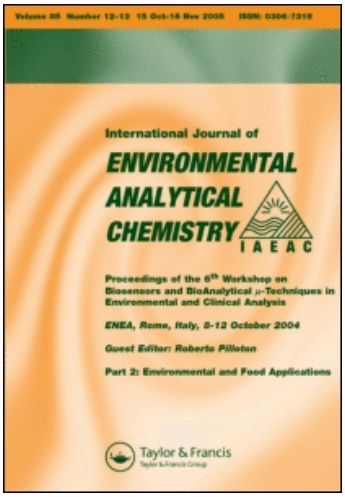

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Determination of thiophanate-methyl and chlorotoluron in water samples by improved single-drop microextraction coupled with high-performance liquid chromatography

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An improved single-drop microextraction method for the determination of thiophanate-methyl and chlorotoluron in water samples was developed, with analysis by high-performance liquid chromatography-ultraviolet detection (HPLC-UV). Several parameters such as solvent type, salt concentration, stirring rate, extraction time, pH and organic drop volume were investigated. The optimum experimental conditions found were: 20 μL 1-octanol, 10% (w/v) NaCl, 600 rpm stirring rate, 40 min extraction time, neutral sample pH and 5 mL water sample. Under the optimum conditions, the enrichment factors were 45.3 and 107.0 folds for thiophanate-methyl and chlorotoluron, respectively. The method exhibited a wide linear range (1–100 $\mu g\,L^{-1}$), reasonable detection limits (0.35 $\mu g\,L^{-1}$) and suitable repeatability (RSD < 9.6%) for both analytes. The proposed method was validated with three real water samples fortified at two levels, and reasonable spiked recoveries were achieved in the range of 84.0% ~ 110.3%. The experimental results indicated that the improved SDME was a simple, reliable and convenient technique and could easily be used for the enrichment of other pollutants.

Keywords: improved single-drop microextraction; HPLC-UV; thiophanate-methyl; chlorotoluron

1. Introduction

Thiophanate-methyl, one important member of the benzimidazolic family, is widely used in agriculture for pre- and post-harvest treatment for the control of a wide range of fruit and vegetable pathogens. Chlorotoluron is one of the popular herbicides for the control of broadleaf and grassy weeds in many agricultural crops. They are released into the environment from manufacturing, transportation and agricultural applications. Accordingly, their prolonged use may lead to pollution of surface and ground waters by themselves and their metabolites. Because of their possible toxic effects, widespread use

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and insufficient data, monitoring of their residues has become a priority in pesticide control and health care.

The analyses of pesticides in water samples are generally performed by gas chromatographic (GC) or liquid chromatographic (LC) techniques. High-performance liquid chromatography (HPLC) is the preferred technique for the determination of these pesticides due to their polar characteristics, low volatility or thermal instability [1–3]. These pesticides are present in the environment at $\mu g\,L^{-1}$ level or less. Therefore, an effective extraction/purification approach prior to final analysis is necessary. Currently, several sample preparation techniques can be used, e.g., liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) [4–6]. However, LLE is time consuming, generally labour intensive, and requires large volumes of expensive, toxic and environmentally unfriendly organic solvents. In off-line SPE, a small part of the final extract is used for analysis, while the major part remains unutilised. This results in reduction in sensitivity of the overall method. To overcome this problem, SPE is performed in on-line mini-columns and the whole extract is transferred to the analytical column. Such couplings are often cumbersome and require elaborate instrumentation. In contrast, SPME has been proved to be a rapid, simple and easy to automate extraction approach. However, the high cost, fibre fragility and possible sample carry-over between runs are the drawbacks of the technique [7,8].

In recent years, a novel sample preparation technique termed liquid-phase microextraction (LPME) or single-drop microextraction (SDME) [9–16] has been developed on the basis of traditional LLE. SDME, as its name suggests, makes use of only microlitres of solvent for concentrating analytes from aqueous samples without an additional solvent evaporation step. Although traditional SDME proves to be a simple, inexpensive, fast and virtually solvent-free sample pre-treatment technique, problems with drop instability and low sensitivity are often encountered. This may be due to the small contact area between the tip of the needle and the suspended drop and relatively large drop volume could result in the high probability of drop detachment. In order to avoid these problems, Liu and his coworkers [17,18] proposed that the microsyringe needle tip was sheathed with a 3-mm-long silicon rubber tube or a 3-mm-long polytetrafluoroethylene tube. A funnelform single-drop microextraction was also developed [19]. In our previous work, a small bell-mouthed extraction device was adopted to assist the drop suspension. Thus, the drop volume increased and its stability was improved. This improvement was successfully applied for the enrichment of triazine herbicides [20]. However, very few applications were found in the literatures. Hence, the main objective of this paper was to enlarge the applicability of the improved SDME in the environmental field and with thiophanate-methyl and chlorotoluron as the model compounds.

2. Method

2.1 *Reagents and standards*

Thiophanate-methyl (purity 95.0%) was purchased from Institute of Environmental Protection and Monitoring, Department of Agriculture (Beijing, China). Stock standard solution was prepared in methanol at $400\,\mu g\,mL^{-1}$and stored at 4°C. Chlorotoluron standard solution at a concentration of $100\,\mu g\,mL^{-1}$ was obtained from Yingtianyi Standard Sample Company (Beijing, China). Mixtures of standard working solutions

for extraction were prepared by diluting with ultrapure water each day for the optimisation procedure. 1-Heptanol and 1-octanol (A.R.) were obtained from Tianjinbodi Chemical Cooperation (Tianjin, China). 1-Butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$) was purchased from Acros Organics (New Jersey, USA). LC-grade methanol was obtained from Scharlou Chemie SA (Barcelona, Spain). Ultrapure water was prepared in the lab using a water treatment device ''Ultra-Clear'' (SG Wasseraufbereitungsanlagen, Barsbüttel, Germany). All other solvents and reagents used were of analytical reagent grade unless otherwise stated.

2.2 *Instrumentation*

The extraction and injection were carried out using a 25 μL HPLC microsyringe (Shanghai, China). A S23-2 digital magnetic stirrer (Shanghai Sile Instrument Co., China) and a 5 mm stirring bar were used to stir the solution.

HPLC analysis was carried out on a LC-10AT liquid chromatography (Shimadzu, Japan) with two LC-10ATvp pumps and a SPD-10Avp UV/Vis detector. Chromatographic separations were performed on a VP-ODS C_{18} column (250 × 4.6 mm ID, particle size 5 μm) (Shimadzu, Japan). Data acquisition and process were accomplished with a Chromato-solution Light Workstation (Shimadzu, Japan). The mobile phase consisted of water and methanol in a ratio of 45 to 55 (v/v) flowing at 0.7 mL min^{-1}. UV detection at 230 nm was used for quantification. Under these chromatographic conditions, baseline separation can be obtained.

2.3 *Proposed SDME procedure*

The preparation process of the small bell-mouthed device is the same as that reported earlier [20]. 5 mL of sample solution spiked at a known concentration with all target analytes was filled into a 5 mL standard flask and then a teflon coated stir bar was placed into the vial. Before each extraction, a commercially available 25 μL microsyringe was washed at least 10 times with solvent in order to eliminate the bubbles in the barrel and the needle. After the uptake of 20 μL 1-octanol, the microsyringe needle was inserted through the septum and then tightly affixed with the small device, and was immersed into the 5 mL sample solution and kept at the same height. The plunger is pushed down to expose the drop to the sample solution. When the magnetic stirrer was switched on, extraction occurred. After extraction for a prescribed time, the drop was retracted into the microsyringe. Then, the microsyringe was removed from the vial and the small device was also removed from the tip of the needle by means of a forceps. The acceptor drop was immediately injected for analysis.

2.4 *Traditional SDME procedure*

Traditional SDME was performed in a 5 mL standard flask which was placed on a magnetic stirrer. 1.5 μL 1-octanol was suspended on the tip of a 25 μL LC microsyringe immersed in the 5 mL sample solution spiked at a known concentration of analytes. During the extraction, the sample solution was stirred at 600 rpm. After extracting for 40 min, the drop was retracted and injected into the HPLC for analysis.

2.5 *Sample collection*

Reservoir water sample was collected from Shimen Reservoir in the region of Xinxiang, Henan Province. Tap water sample was taken from a water tap after flowing for 10 min in our laboratory. Well water was obtained from Xinxiang, Henan Province. Before use, all the environmental water samples were filtered through 0.45 μm micropore membranes and stored in brown glass bottles at 4°C until analysis, respectively.

3. Results and discussion

Generally, an acceptor drop suspended on the tip of a microsyringe in a sample solution suffers from three forces: a downward gravity; an upward floating force; and an adhesion force. It is the adhesion force that makes the drop adhere to the needle tip. A large drop can be more easily detached because the adhesion force is constant under experimental conditions while the upward floating force increases with increasing the volume of the drop in the case of $\rho_w > \rho_o$ (ρ_w is the density of the aqueous solution and ρ_o is the density of extractant). By using the small bell-mouthed device, the drop is protected by a spring force of the device wall and prevented from floating upward from the needle tip. Therefore, the suspension of a larger volume of drop becomes easier and more reliable than those in the traditional SDME. This study explored the applicability of the improved SDME for the analysis of thiophanate-methyl and chlorotoluron in water samples. The parameters related to the improved SDME were assessed using mixed working solutions at 20 μg L^{-1} for each analyte.

3.1 *Selection of extraction solvent*

The selection of an appropriate extraction solvent is an essential consideration for a successful SDME. Extraction solvents are selected based on the primary principles such as a low solubility in aqueous solution, high extraction capability of interested compounds and good LC chromatography behaviour. Three solvents of 1-heptanol, 1-octanol and $[C_4MIM][PF_6]$ were tested to select the best one for the extraction of thiophanate-methyl and chlorotoluron in water samples. Extraction was performed by using the three solvents with the solvent volume of 10 μL, no salt addition, extraction time of 30 min, stirring rate of 600 rpm. The experimental results revealed that $[C_4MIM][PF_6]$ extracted the target compounds poorly due to its quickly dissolution in aqueous sample solution. 1-Octanol extracted both pesticides better than 1-heptanol, with an acceptable reproducibility, since it has less solubility in water and higher boiling point. So, 1-octanol was chosen as the extraction solvent in further experiments.

3.2 *Effect of salt concentration*

Sodium chloride (NaCl) or other salts are usually added to aqueous sample to improve the extraction efficiencies of analytes because of the salting-out effect. However, previous studies indicated that, depending on the target analytes, an increase in the salt concentration of the aqueous sample may have various effects upon extraction: it may enhance [21], not influence [22], or even limit [23] extraction. In this study, the effect of salt concentration on extraction efficiency was investigated in the range of 0–20% (w/v). As can be seen in Figure 1, the extraction efficiency increased with NaCl

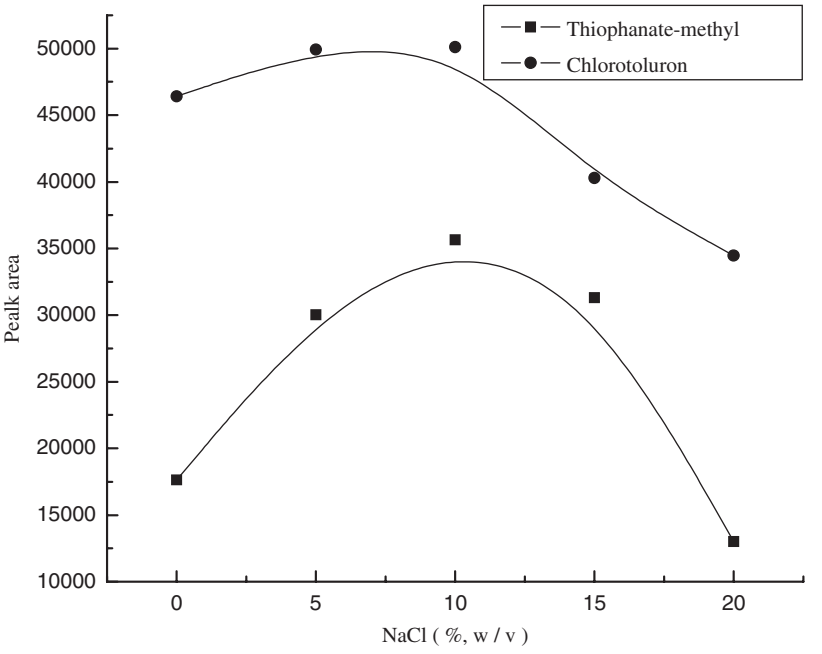

Figure 1. Effect of salt concentration on extraction efficiency. At neutral pH, 5 mL sample solution was enriched for 30 min with 20 μL of 1-octanol at a stirring rate of 600 rpm.

concentration up to 10% (w/v). With the further increase of NaCl concentration, the responsive peak area was reduced. This behaviour can be explained by considering two simultaneously occurring processes. Initially, increasing salt concentration, the salting-out effect enhanced the analytes recoveries. However, in competition with this process, the presence of salt caused a second effect. As the salt concentration increased further, the second effect began to predominate, adverse for the extraction, whereby the physical properties of the Nernst diffusion film were changed, reducing the diffusion rate of the analytes into the solvent drop. Based on these observations, it was decided to maintain the salt content at 10% (w/v) NaCl for all subsequent experiments.

3.3 *Effect of stirring rate*

In SDME, a change of stirring rate is expected to affect extraction dynamics. The extraction can be accelerated by stirring the aqueous sample because of the decreased thickness of the interfacial layer surrounding the solvent drop as well as the continuous exposure of the extraction surface to fresh aqueous sample [24]. In the proposed SDME, with the help of the small extraction device, 20 μL 1-octanol drop was stable due to the increased contact area and the roughness of the contact surface. So a relatively fast stirring rate could be used. The effect of stirring rate on the extraction efficiency was examined in the range of 0–800 rpm. As expected, extraction efficiency increases with stirring rate up to 800 rpm. However, with extraction at 800 rpm, air

bubbles were occasionally generated that adhered to the surface of the solvent drop, thus leading to poor reproducibility. Therefore, 600 rpm was deemed to be the optimum stirring rate.

3.4 *Effect of extraction time*

Mass transfer between the donor and acceptor phase is a time dependent process. Figure 2 shows the extraction time profile for the analytes. The extraction time profile indicated an initial rapid partitioning between these two phases, followed by a slower uptake profile until the partitioning has reached equilibrium. It was clear that the time required for full equilibrium was 40 min for thiophanate-methyl and 50 min for chlorotoluron. This behaviour of thiophanate-methyl could be explained by the fact that it is more hydrophobic than chlorotoluron and might diffuse from the aqueous sample to the solvent drop more quickly. An extraction time of 40 min was selected since the reasonable extracted amounts of the analytes were achieved in this analysis time.

3.5 *Effect of sample pH*

The pH value of sample solution is a critical parameter in SDME. The variation of the pH will change the ionization form of certain analytes and will thereby affect their water solubility and extractability. The effect of sample pH in the range of 3 to 11 was evaluated by adjusting the sample pH using dilute HCl or NaOH solutions. The results are shown in

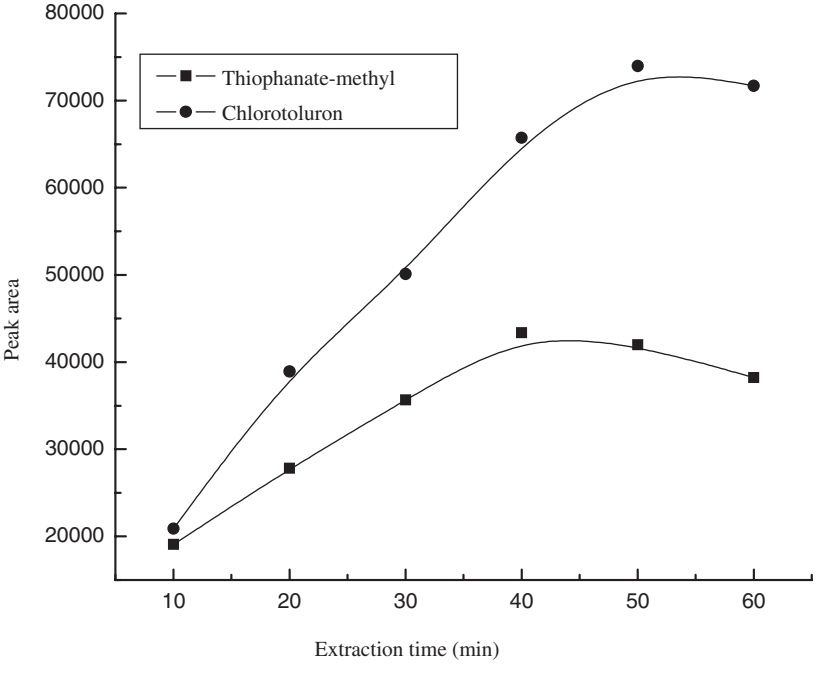

Figure 2. Effect of extraction time on extraction efficiency. At neutral pH, 5 mL sample solution containing 10% NaCl was enriched with 20 μL of 1-octanol at a stirring rate of 600 rpm.

Figure 3. It was observed that the peak areas of thiophanate-methyl and chlorotoluron increased continuously when the pH value of sample solution was increased from 3.0 to 7.0 and decreased dramatically when the pH was changed from 7.0 to 11.0. This observation may be explained by the fact that the two compounds are easy to be decomposed at alkaline conditions [25,26] therefore, 7.0 was selected as the optimum pH value of the sample solution.

3.6 *Effect of drop volume*

In SDME, the volume of the solvent drop does not remain constant during the extraction because no solvent is completely immiscible with water. However, a slight decrease in drop volume is acceptable because it will occur to the same extent for standard and the sample. Generally, the enrichment factors of analytes can be increased by using a small acceptor drop. However, the absolute chromatographic peak area was increased with the increase of the volume of the acceptor drop. The reason is that a larger amount of analytes is extracted into the larger drop in a given time. With the bell-mouthed extraction device, a 20 μL 1-octanol drop could be manipulated easily and reliably under the stirring rate no more than 600 rpm. The effect of drop volume on extraction efficiencies is shown in Figure 4, which indicated that the use of a large solvent drop resulted in an increased analytical response of the instrument. Consequently a 20 μL 1-octanol was chosen in the following experiments.

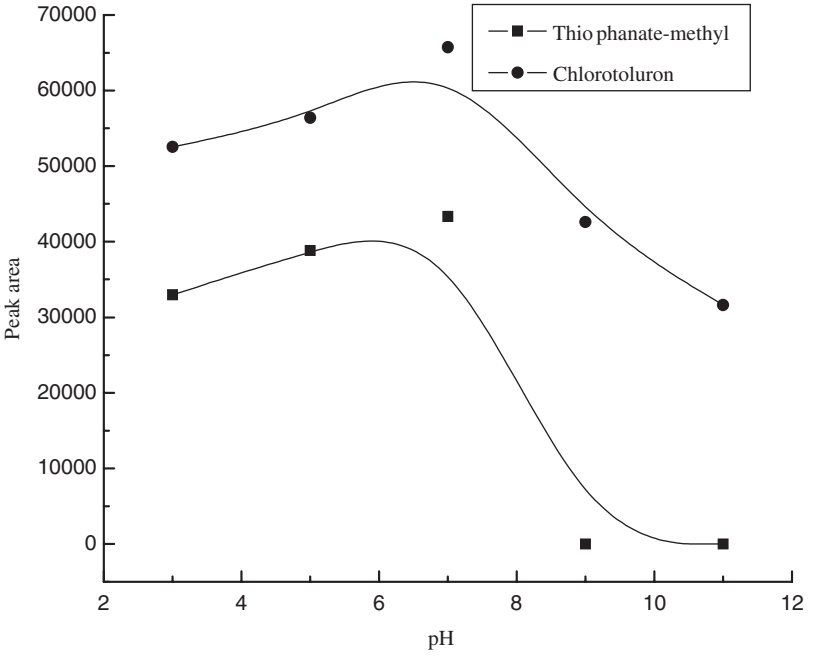

Figure 3. Effect of sample pH on extraction efficiency. 5 mL sample solution containing 10% NaCl was enriched for 40 min with 20 μL of 1-octanol at a stirring rate of 600 rpm.

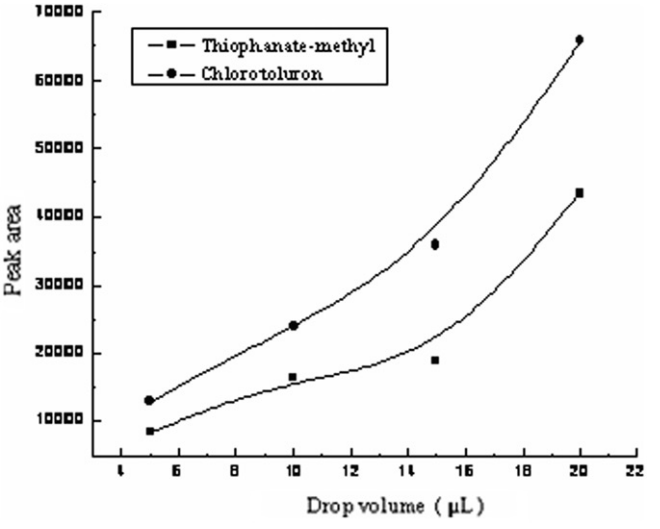

Figure 4. Effect of drop volume on extraction efficiency. At neutral pH, 5 mL sample solution containing 10% NaCl was enriched for 40 min at a stirring rate of 600 rpm.

3.7 *Method evaluation*

The performance of the proposed SDME was investigated under the optimal conditions. As shown in Table 1, the enrichment factor was 45.3-fold for thiophanate-methyl and 107.0-fold for chlorotoluron, respectively. The repeatability was carried out by six replicate extractions of spiked ultrapure water solution at 4 $\mu g\,L^{-1}$ for each analyte and the relative standard deviations (RSD%) were calculated to be 9.6% for thiophanate-methyl, and 7.7% for chlorotoluron. Under the same conditions, six bell-mouthed devices were also investigated to extract the analytes, and the relative standard deviations were 8.9 and 6.4% for thiophanate-methyl and chlorotoluron, respectively. For spiked aqueous standards, each analyte exhibited good linearity in the range of 1–100 $\mu g\,L^{-1}$ with the correlation coefficient (R) >0.9979. The limits of detection (LOD, $S/N = 3$) were 0.35 $\mu g\,L^{-1}$ for both analytes.

3.8 *Real water sample analysis*

The proposed SDME method was applied for determination of the two target compounds in tap water, well water and reservoir water. A general procedure was followed to extract the target analytes. No target analytes could be detected in the three samples. To assess matrix effects, the three samples were spiked with two concentration levels and the relative recoveries (defined as the ratio of the peak areas of analytes in real samples and the peak areas of analytes in pure water sample spiked with same amount of anlytes) are listed in Table 2. As indicated in Table 2, acceptable recoveries (84.0–110.3%) were obtained. The typical chromatograms of the two target compounds in blank, spiked reservoir water sample at two concentration levels are shown in Figure 5.

Table 1. Summary of the performance of the proposed SDME.

Target compounds	Enrichment factor[a]	Linear range ($\mu g\,L^{-1}$)	Correlation coefficient (R)	LOD ($\mu g\,L^{-1}$)	RSD (%)
Thiophanate-methyl	45.3 ± 2.2	1.0–100	0.9976	0.35	9.6
Chlorotoluron	107.0 ± 1.1	1.0–100	0.9979	0.35	7.7

Note: [a] The results presented are mean ± standard deviation ($n = 3$).

Table 2. Relative recoveries in spiked real water samples with two fortification levels for each analyte[a].

	Thiophanate-methyl		Chlorotoluron	
Sample	$4\,\mu g\,L^{-1}$	$16\,\mu g\,L^{-1}$	$4\,\mu g\,L^{-1}$	$16\,\mu g\,L^{-1}$
Tap water	84	106	108	96
Well water	102	110	103	97
Reservoir water	108	106	94	86

Note: [a] The results presented are mean for three determinations.

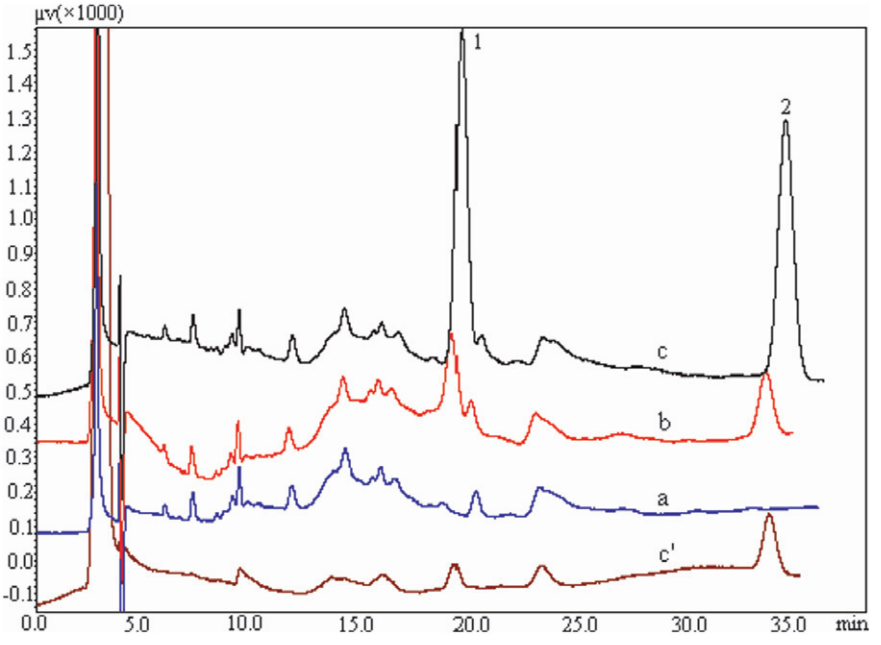

Figure 5. The chromatograms of reservoir water sample. (a) blank; (b) spiked with $4\,\mu g\,L^{-1}$ and (c) spiked with $16\,\mu g\,L^{-1}$ for each analyte after the proposed SDME; (c') spiked with $16\,\mu g\,L^{-1}$ for each analyte after the traditional SDME. Peaks: (1) thiophanate-methyl; (2) chlorotoluron.

3.9 *Comparison of the improved SDME with the traditional SDME*

The traditional SDME was also applied for the analysis of the target compounds in tap water, well water and reservoir water. Extractions were performed with the fortified concentration of $16\,\mu g\,L^{-1}$ for each analyte. As can be seen in Figure 4, the experimental results showed that the analytical response of the proposed SDME was greater than that of the traditional SDME.

4. Conclusions

In the present study, an improved SDME technique for enrichment of thiophanate-methyl and chlorotoluron in environmental waters has been developed. A comparison between the improved SDME and the traditional SDME was made for the extraction of the two analytes from real water samples. The results revealed that the traditional SDME had lower analytical sensitivity and higher probability of the solvent drop detachment. Using a small bell-mouthed device to perform SDME, the difficulty of suspending the relatively large volume of drop was overcome in the stirred solution and the improved SDME technique was very convenient in the manipulation for non-experienced operators. All these results demonstrated that the improved SDME have an excellent prospect in the future.

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