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Density-adjusted liquid-phase microextraction with smartphone digital image colorimetry to determine parathion-methyl in water, fruit juice, vinegar, and fermented liquor†

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To achieve rapid and convenient on-site pretreatment and determination of parathion-methyl, a density-adjusted liquid-phase microextraction with smartphone digital image colorimetry was established to detect parathion-methyl in food samples. In this study, the environmentally friendly biomass-derived solvent guaiacol was used as the extractant. Salt and water, as density regulators, realized the two movements (floating–sinking) of the extractant and full contact between the extractant and the sample solution to establish an environmentally friendly, fast, and efficient pretreatment method. Under strong alkaline conditions, parathion-methyl generated a yellow product; then, a smartphone was used to obtain the image of the yellow product for intensity analysis. Parathion-methyl has a good linear relationship in the range of 0.01–1 mg L⁻¹, and the limits of detection and quantification are 0.003 and 0.01 mg L⁻¹, respectively. This method has been successfully applied to the determination of parathion-methyl in spiked water, fruit juice, vinegar, and fermented liquor with a recovery of 91.6–106.5% and a relative standard deviation of 0.6–6.0%. The established density-adjusted liquid phase microextraction with smartphone digital image colorimetry is rapid, convenient, and environmentally friendly for the determination of parathion-methyl in food samples.

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1. Introduction

Organophosphate pesticides have been widely applied in agricultural production since their discovery.¹ Parathion-methyl is a widely used organophosphate pesticide² that is related to acute toxicity through inhibiting the enzyme acetylcholinesterase in the nervous system.³ Parathion-methyl is also classified as a toxicity category I (extremely toxic) insecticide by the WHO and U.S. EPA with a median lethal dose (LD₅₀) value of <5 mg kg⁻¹.^{4,5} The excessive and inappropriate use of parathion-methyl causes the accumulation of pesticides in food products. Therefore, it is challenging and urgent to control parathion-methyl residues. The fast and convenient determination method of parathion-methyl in foods should be exploited to ensure food safety.

Liquid phase microextraction (LPME) is an emerging sample preparation technique with the advantages of versatility, adaptability, and low solvent usage.⁶ However, the extraction solvents used in LPME are usually volatile and flammable and may pose threats to human health. Another disadvantage of LPME is the use of dispersants and auxiliary extraction equipment (microwave,⁷ ultrasound,⁸ vortex,⁹ and stirring). The disadvantages of the use of acetone,¹⁰ acetonitrile,¹¹ and methanol¹² as dispersants are known to be hazardous to operators and the environment.¹³ The disadvantage of the use of microwave, ultrasound, vortex, and stirring as auxiliary equipment are known to be inconvenient and unsuitable for on-site detection. Therefore, it is worth paying attention to replacing traditional toxic organic solvents with environmentally friendly alternative solvents and establishing LPME without dispersants and auxiliary equipment.

Ionic liquids and deep eutectic solvents are considered environmentally friendly organic extraction solvents¹⁴ compared with carbon tetrachloride, toluene, and hexane in LPME.^{15,16} However, they require time-consuming synthesis and are easily destroyed during the LPME process. Biomass-derived solvents are liquids obtained from renewable resources,¹⁷ which bear the key advantages of being derived from renewable resources.¹⁸ They have the advantages of low toxicity,

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availability, renewability, biodegradability, and reasonable prices.¹⁷ Therefore, it is environmentally friendly to use biomass-derived solvents instead of toxic organic solvents.

Methanol, acetonitrile, and acetone are often used as dispersants, which are combined with microwave, ultrasonic, vortex, and stirring equipment to achieve full contact between the extractant and the samples and assist extraction. Emerging extraction methods use the principle of relative movement to achieve full contact between the extractant and the samples, which achieves environmentally friendly, fast, and efficient extraction without the use of dispersants and auxiliary equipment.

If an extractant with a similar density to the sample is designed, the movement of the extractant and full contact of the extractant with the sample solution can be achieved by adjusting the density of the extractant or sample. The biomass-derived solvent guaiacol has similar density to water, so guaiacol can be considered a good choice. There are two main methods for density adjustment: to adjust the density of the extractant and to adjust the density of the sample. The density of the extractant can be changed by adding heavy or light solvents that are miscible with the extractant, but it will increase the volume of organic solvents and decrease the sensitivity of the method. Meanwhile, the density of the sample can be easily adjusted only by adding common salts and water to realize the two movements (floating–sinking) of the extractant and LPME. Therefore, the proposed density-adjusted LPME method is fast and efficient compared to the traditional LPME method.

Current detection methods of parathion-methyl mainly involve gas chromatography,¹⁹ high-performance liquid chromatography,²⁰ mass spectrometry,²¹ and electrochemical methods.²² However, expensive equipment, long analysis times, and well-trained operators are required. Colorimetry has the advantages of simple operation, fast quantification, *etc.*²³ Under strong alkaline conditions, parathion-methyl produces a yellow substance, which is suitable for colorimetry. Emerging digital image colorimetry involves two steps: image acquisition and color readout.²⁴ In recent years, digital image colorimetry has gained attention due to its low cost in rapid real-time quantitative determination. Digital image colorimetry has been used to determine pesticides,²⁵ veterinary drugs,²⁴ and heavy metals.²⁶ Digital image colorimetry can be performed by transferring the captured images into the computer for further

analysis by software (*e.g.*, Image J).²⁷ Alternatively, it can be performed by in-built or open-source applications in smartphones (*e.g.*, Color Grab, Photometrix *etc.*) to directly pick the color data.²⁸ Because smartphone cameras can distinguish small changes in colors with small measurement errors and more accurate results, smartphones are considered an excellent image acquisition tool.²⁹ Smartphone digital image colorimetry (SDIC) often utilizes RGB color space (red, green, and blue), where any color can be divided into the three basic colors and read out by smartphone software.²⁸ It is convenient, fast, low cost, portable, and suitable for on-site analysis³⁰ and has a wide range of application prospects in food analysis.

The purpose of the established method is to realize fast, convenient, and environmentally friendly on-site pretreatment and visual determination of parathion-methyl in food samples. The density-adjusted LPME method was first established and combined with SDIC to quantitatively analyze parathion-methyl in spiked water, fruit juice, vinegar, and fermented liquor.

2. Experimental

2.1 Reagents and materials

Parathion-methyl (98.2%) was obtained from ANPEL-TRACE Standard Technical Services Co., Ltd. (Shanghai, China). Guaiacol, methanol, KCl, NaCl, Na₂SO₄, NaNO₃, trimethylhexylammonium bromide (C₉H₂₂NBr), and NaOH were obtained from Aladdin Industrial Co., Ltd. (Shanghai, China). Water, cherry juice, vinegar, and fermented liquor were purchased from local markets (Jinzhong, Shanxi).

2.2 Apparatus

The Xiaomi 10S smartphone was purchased from Xiaomi Technologies Co., Ltd. (Beijing, China). A free application Color Grab (Loomatix, version 3.6.1, 2017), which was available for Android systems, was used to convert the images to RGB values.

2.3 Microextraction and detection procedure

The extraction and detection procedure are shown in Fig. 1. Then, 150 μL of colorless extraction solvent guaiacol was added into 5 mL of the sample solution in a 10 mL centrifuge tube. After 1500 mg of KCl was added, guaiacol floated from the bottom to

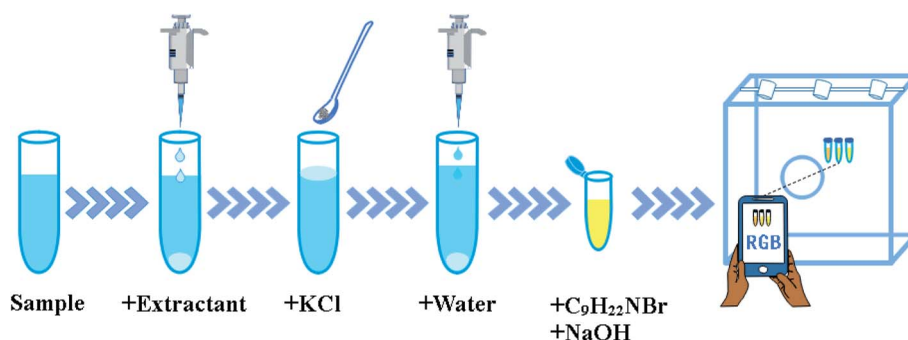


Fig. 1 Schematic representation of the density-adjusted LPME-SDIC.



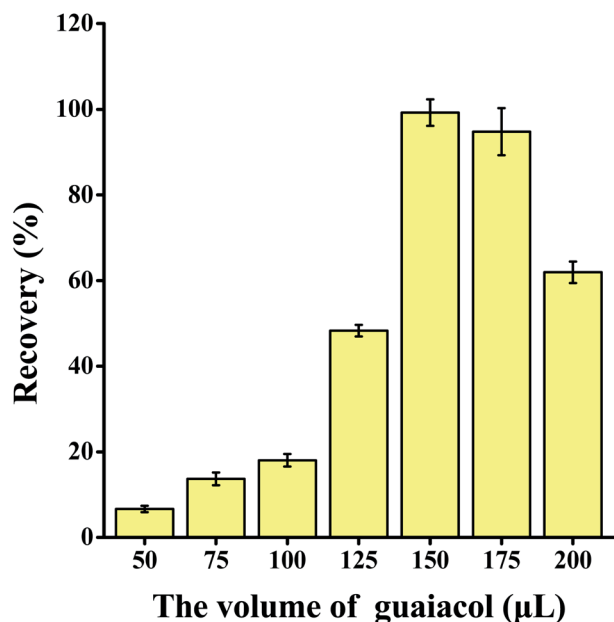


Fig. 2 Optimization of the volume of extractant.

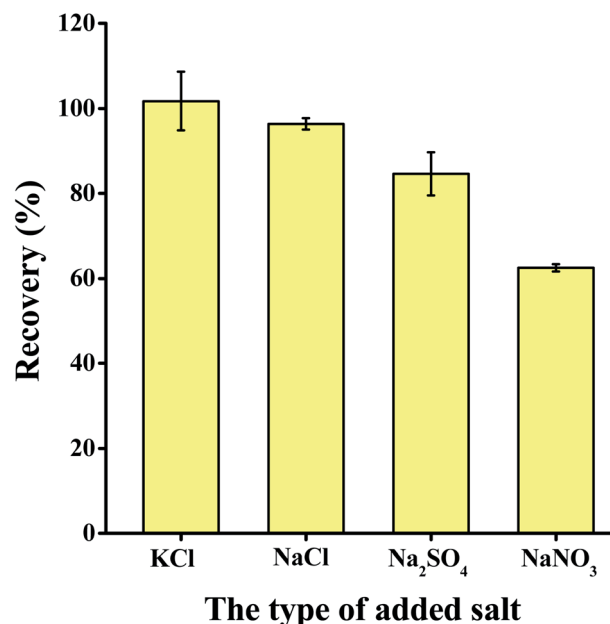


Fig. 3 Optimization of the type of added salt.

the top of the sample solution. After 4000 µL of water was added, the extractant sunk to the bottom of the centrifuge tube again.

The extractant was collected into another 1.5 mL centrifuge tube, which contained 100 µL of C₉H₂₂NBr (10 mol L⁻¹) and 100 µL of sodium hydroxide solution (10 mol L⁻¹) to generate yellow product *in situ* (Fig. S1†). The yellow organic phase was placed in the middle of an opaque white box (30 cm × 30 cm × 30 cm). The top of the box was surrounded by LED light panels to provide an isotropic lighting. Moreover, a small hole (diameter, 5 cm) was formed in the centre of the top to allow photography. The change in colour was captured by Xiaomi 10S smartphone with 108 Megapixel, then the red (R), green (G), and blue (B) channel values of the middle pixel region were read in RGB mode with Color Grab application. The RGB intensity (*I*) was calculated by the formula: $I = 1/B$.

2.4. Calculation of extraction recovery

The recovery was calculated to evaluate the proposed method as follows.

$$\text{Recovery}(\%) = \frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{added}}} \times 100\%$$

C_{Found} is the total concentration of parathion-methyl after the addition of a known amount of parathion-methyl to the sample; C_{Real} is the original concentration of parathion-methyl in the sample; C_{Added} is the concentration of parathion-methyl added to the real samples.

3. Results and discussion

3.1 Optimization of extraction conditions

The volume of the extractant, type and volume of salt added, volume of water added, and sample volume were optimized to

ascertain the best extraction parameters to use. Each experiment was repeated three times.

3.1.1 Effect of the volume of extractant. The volume of the extractant has a critical role in the recovery of analytes.³¹ The experiment investigated the effect of different volumes of guaiacol (50, 75, 100, 125, 150, 175, and 200 µL) on the extraction efficiency (Fig. 2). The extraction efficiency significantly increased with increasing extractant volume from 50 to 150 µL. When the volume of extractant was more than 150 µL, the extraction efficiency decreased. The reason may be that inadequate extraction solvent leads to insufficient extraction. An excess volume of extraction solvent may cause small droplets to not be properly formed.³² The increase in the volume of extraction solvent results in the dilution effect. Therefore, 150 µL of guaiacol was used in subsequent experiments.

3.1.2 Effect of the type of salt added. The addition of salt helps increase the density of the sample solution. The experiment investigated the effect of different types of salt (KCl, NaCl, Na₂SO₄, and NaNO₃) on the extraction efficiency (Fig. 3). KCl had the best extraction efficiency, possibly because the salting-out effect and density affect the extraction efficiency. Therefore, KCl was used in subsequent experiments.

3.1.3 Effect of the amount of salt added. The experiment investigated the effect of different amounts of KCl added (1300, 1400, 1500, 1600, 1700, and 1800 mg) on the extraction efficiency (Fig. 4). The extraction efficiency increased with increasing amounts of KCl added from 1300 to 1500 mg. The reason may be that the salt addition improves the extractant movement and the contact between sample and extractant. When more than 1500 mg KCl was added, the extraction efficiency decreased, possibly because the increased viscosity of the solution decreases the efficiency of the mass-transfer process due to the viscous resistance effect.³³ Therefore, 1500 mg of KCl was used in subsequent experiments.

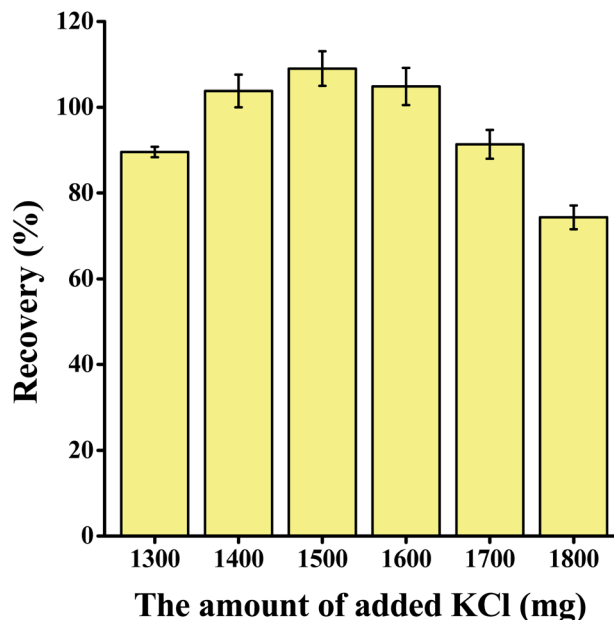


Fig. 4 Optimization of the amount of added salt.

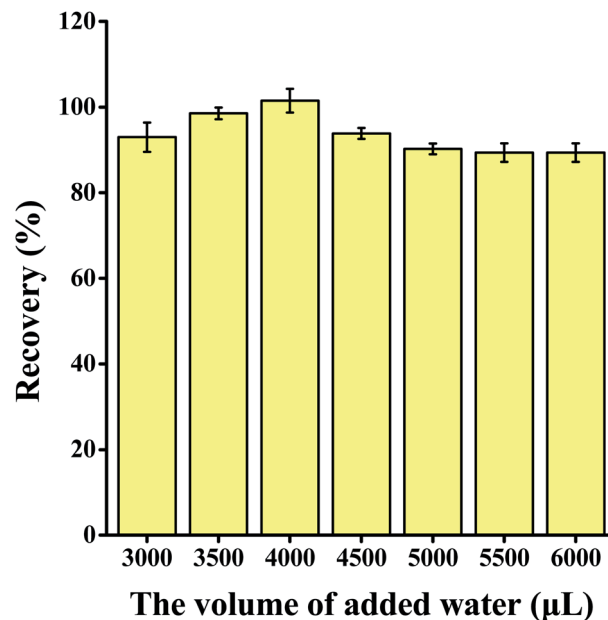


Fig. 5 Optimization of the volume of added water.

3.1.4 Effect of added water. In the density adjustment process, the volume of added water affects the sinking of the extractant and distribution of the analyte. The experiment investigated the effect of different volumes of added water (3000, 3500, 4000, 4500, 5000, 5500, and 6000 μL) on the extraction efficiency (Fig. 5). The extraction efficiency reaches the maximum with increasing volume of added water to 4000 μL. Subsequently, when the volume of added water continues to increase, the extraction efficiency decreases. The reason may be that the added water increases the sinking speed of the extractant and the contact between sample and extractant. Excess water can reduce the extraction efficiency due to the redistribution of parathion-methyl into the sample. Therefore, 4000 μL of water was added for subsequent experiments.

3.1.5 Effect of the sample volume. In the density adjustment process, the different sample volumes may affect the extraction efficiency and method sensitivity. The experiment investigated the effect of different sample volume (3, 4, 5, and 6

mL) on the extraction efficiency (Fig. S2†). There were no significant differences for the extraction recovery of parathion-methyl with sample volume from 3 to 6 mL. Therefore, in order to make the experimental extraction process more convenient, 5 mL sample volume was selected for further experiments.

3.2 Method validation

To validate the performance of the proposed method, the linear range (LR), correlation coefficients (R), limits of detection (LOD), limits of quantification (LOQ), and intraday and interday relative standard deviation (RSDs) of the established method were evaluated, and the results are shown in Table 1 under the optimal extraction conditions (Table 1).

In this experiment, parathion-methyl had good linearity in the linear range of 0.01–1 mg L⁻¹, with RGB intensity (I) as the ordinate and concentration as the abscissa (Fig. 6). The slope of

Table 1 Analytical performance of the density-adjusted LPME-SDIC^a

Sample	Regression equation $A = (a \pm SD_a)c + (b \pm SD_b)$	Enrichment factor	Extraction efficiency (%)	R^2	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	Intra-day RSD (%) ($n = 3$)	Inter-day RSD (%) ($n = 3$)
Water	$A = (0.002612 \pm 0.000231)c + (0.005537 \pm 0.000578)$	47	94.9	0.995	0.003	0.01	1.0	1.4
Fruit juice	$A = (0.002285 \pm 0.000165)c + (0.011845 \pm 0.000462)$	47	93.1	0.993	0.003	0.01	1.7	2.5
Vinegar	$A = (0.000620 \pm 0.000080)c + (0.007099 \pm 0.000121)$	50	100.4	0.993	0.003	0.01	0.9	2.4
Fermented liquor	$A = (0.000390 \pm 0.000041)c + (0.007555 \pm 0.000076)$	49	97.6	0.992	0.003	0.01	1.4	2.4

^a A, intensity; c, parathion-methyl concentration in real samples (mg L⁻¹); a, slope; b, intercept; SD_a and SD_b, standard deviations of slope and intercept, respectively.



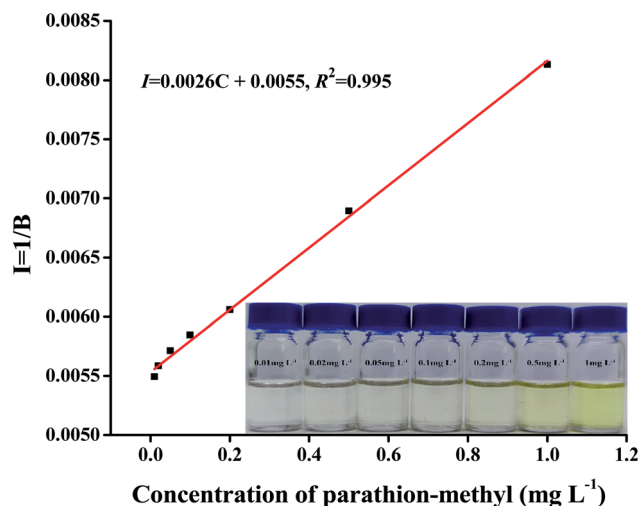


Fig. 6 Linear curve of parathion-methyl.

water, fruit juice, vinegar, and fermented wine are 0.002612, 0.002285, 0.000620, and 0.000390, respectively. The intercept of water, fruit juice, vinegar, and fermented wine are 0.005537, 0.011845, 0.007099, and 0.007555, respectively. The enrichment factor of water, fruit juice, vinegar, and fermented wine is 47, 47, 50, and 49, respectively. The R^2 values were 0.998, 0.993, 0.993,

and 0.992. The limits of detection and quantification were determined to be 0.003 mg L⁻¹ and 0.01 mg L⁻¹ based on signal-to-noise ratios of 3 and 10, respectively. The intraday and interday RSDs were 0.9–1.7% and 1.4–2.5%, respectively.

3.3 Anti-interference test

To validate the anti-interference performance of the proposed method, the specificity of the proposed method was investigated by preparing and analyzing samples spiked with different insecticides (parathion-methyl, bifenthrin, deltamethrin, clothianidin, dinotefuran, thiacloprid, acetamiprid, fipronil). The result showed that other insecticides did not cause interference with the determination of parathion-methyl (Fig. S3†).

3.4 Applicability

The analytical applicability of the developed method to food samples (water, fruit juice, vinegar, and fermented liquor) was evaluated. The parathion-methyl in the real samples was below detectable levels. Thus, to verify the accuracy and precision of the method, different known concentrations of parathion-methyl were spiked in real samples. The analysis results in Table 2 show that the spiked recovery in real samples was 91.6–106.5%, and the RSDs were 0.6–6.0%. Therefore, the proposed method has good reproducibility and precision. This

Table 2 Analysis of parathion-methyl in real samples

Matrix	Added (mg L ⁻¹)	Proposed method			Reference method		
		Found (mg L ⁻¹)	Recovery (%)	RSD (<i>n</i> = 3, %)	Found (mg L ⁻¹)	Recovery (%)	RSD (<i>n</i> = 3, %)
Water	0.01	0.009	96.8	1.2	0.009	92.4	3.0
	0.1	0.095	94.9	2.0	0.104	104.3	2.0
	1	0.929	92.9	3.2	1.059	105.9	2.3
Fruit juice	0.01	0.009	91.6	3.7	0.008	81.9	2.2
	0.1	0.094	93.5	3.2	0.104	103.7	1.1
	1	0.942	94.2	6.0	1.047	104.7	0.4
Vinegar	0.01	0.011	106.5	1.7	0.009	85.4	1.7
	0.1	0.101	100.6	3.1	0.093	92.9	0.8
	1	0.940	94.0	1.5	0.962	96.2	0.5
Fermented liquor	0.01	0.009	98.4	2.7	0.009	94.2	2.2
	0.1	0.101	100.5	0.6	0.097	96.8	1.2
	1	0.940	94.0	3.6	0.975	97.5	0.8

Table 3 Comparison of the density-adjusted LPME-SDIC with other determination methods of parathion-methyl

Method	Solvent (μL)	Extraction equipment	Auxiliary extraction time (min)	LOD (μg L ⁻¹)	Recovery (%)	Instrument	Ref.
DLSE	Dichloromethane (5000)	Stirrer, ultrasonic bath	25	0.025	94–99	GC-MS	19
VA-DLLME	Chloroform (290), acetone (280)	Vortexer	0.5	0.67	95–119	LC-MS/MS	20
VLLME-SID	Hexane (15), 1-dodecanol (15)	Vortexer	1	0.3	80–104	HPLC-DAD	34
VALLME	1-Bromobutane (80)	Vortexer	2	0.38	90–104	HPLC-UV	35
UTILDLPME	[C ₄ MIM]PF ₆ (75)	Ultrasonic bath	3	0.01	96–103	RPLC-UV	36
Density-adjusted LPME	Guaiacol (150)	—	—	3	92–107	SDIC	Current study



experiment was validated using the GB23200.113 method. The proposed method was accurate and reliable, and was deemed trustworthy for the detection and monitoring the level of parathion-methyl concentration in food samples.

3.5 Comparison with other methods

The method, solvent, extraction equipment, auxiliary extraction time, LOD, recovery, and instrument were compared to other determination methods of parathion-methyl^{19,20,34–36} (Table 3). The proposed method uses a small volume of environmentally friendly extraction solvent. No extraction equipment is involved, which makes the method faster and more convenient. A favorable LOD and recovery were achieved without chromatography or mass spectrometry. The proposed method is fast, convenient, and environmentally friendly.

4. Conclusions

In this study, density-adjusted LPME with the SDIC method was established to quickly determine parathion-methyl in food samples. In the pretreatment process, the biomass-derived solvent guaiacol was used as the green extractant, and salt and water were used as the density regulators to exclude the use of toxic dispersants and auxiliary equipment. In the detection process, parathion-methyl generated a yellow product under strongly alkaline conditions; then, a smartphone was used to obtain the image of the yellow organic phase for quantitative analysis. This method is fast, convenient, and environmentally friendly and has been successfully used to determine parathion-methyl in spiked water, fruit juice, vinegar, and fermented liquor. Therefore, this method can be applied to rapidly determine and screen parathion-methyl on-site in different food samples.

Author contributions

Xinyuan Bi: investigation, writing – original draft. Haijuan Jiang: investigation, writing – original draft. Xingle Guo: validation, formal analysis. Min Wang: investigation, data curation. Yu Niu: writing – review & editing. Liyan Jia: project administration, supervision. Xu Jing: conceptualization, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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