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Graphical Abstract 833x571mm (72 x 72 DPI)

Rapid analysis of trace volatile formaldehyde in aquatic products by a derivatization reaction-based surface enhanced Raman spectroscopy

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Abstract

As an illegal food preservative, it still remains a challenge for the on-site rapid analysis of trace formaldehyde in aquatic products. In this work, a simple on-site rapid quantification method for the trace volatile formaldehyde in aquatic products was developed by a derivative reaction-based surface enhanced Raman spectroscopy (SERS) technique coupled with a homemade portable purge-sampling device. Trace formaldehyde separated from complicated aquatic matrices via purge-sampling procedure was reacted with a derivative reagent to produce a Raman-active analyte for the consequent SERS analysis. Au/SiO₂ nanoparticles (NPs) were employed as the enhancement substrate to achieve the significant enhancement of Raman signal intensity. Conditions of derivative reaction and SERS detection were optimized in detail, and the selectivity of this analytical method was also evaluated based on related analogs. Under optimal conditions, an extremely low detection limit was achieved as $0.17 \mu g/L$. It was satisfactory that trace volatile formaldehyde can be actually found in fresh squid and shrimp samples without obvious matrix interference and quantified to be 0.13-0.21 mg/kg, when this method was applied for real on-site rapid analysis projects. The recoveries of spiked aquatic product samples were found to be 70.0-89.1% with RSDs of 2.3-7.2% (n=3). The results suggested that the proposed method was reliable and suitable for the on-site rapid analysis of trace formaldehyde in aquatic products.

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Keywords: SERS, rapid quantification, volatile formaldehyde, aquatic products.

1. Introduction

Formaldehyde is ubiquitous in environmental samples such as air, water, industrial products and food samples. Formaldehyde can be used for preservation of biological specimen. However, toxic formaldehyde will do great harm to human health, so it cannot be directly used as food preservative in our daily life [1]. A maximum daily reference dose (RfD) for formaldehyde set by United States Environment Protection Agency is 0.2 mg/kg [2]. Nowadays some unscrupulous traders added or sprayed formaldehyde on aquatic products as an illegal food preservative in order to maintain the freshness of aquatic products during the transport and storage process, which would cause a serious food safety problem [3]. For example, Italian Ministry of Health set the acceptable formaldehyde values of 60 and 10 mg/kg for Gadidae and crustaceans sample, respectively [4]. However, due to the strong volatility of formaldehyde and the complicated matrices of aquatic products, it is difficult to measure the trace original amount of formaldehyde in aquatic products without suitable on-site rapid quantification methods. Moreover, when food safety emergencies occur, suitable rapid methods should be applied for analysis of a large number of samples in order to obtain accurately and timely analysis results and minimize the harm and influence caused by food safety incidents as much as possible. Thus, it is essential to develop accurate on-site rapid quantification methods for the trace illegally added formaldehyde in aquatic products.

Nowadays, spectroscopy methods including spectrophotometry [5] and fluorescence method [6], chemiluminescence [7] and chromatography methods including gas chromatography [8] and liquid chromatography [9] are the most widely used analytical methods for analysis of trace formaldehyde. Noordiana et al. [10] found that the actual formaldehyde contents in squid and birdshrimp sample were 0.49 ± 0.04 and 1.08 ± 0.11

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mg/kg respectively by a UV-Vis spectrophotometric method. Bianchi et al [11] found that the existent formaldehyde content in different fish products ranged from 6.4 ± 1.2 to $293\pm$ 26 mg/kg by solid-phase microextraction coupling with gas chromatography-mass spectrometry detection. Ding et al. [12] established an analytical method for determination of formaldehyde in squid and shrimp samples by microwave-assisted extraction coupled with high performance liquid chromatography, and the contents were found as 15.1 ± 0.5 and 6.7 ± 0.1 mg/kg, respectively. These methods usually possess excellent analytical precision and sensitivity and are suitable for the quantification of trace formaldehyde in real food samples. However, since these methods usually depend on the large-scale and relatively expensive instruments, samples should be delivered to the lab for the consequent analysis, which requires long analytical cycle time and is not suitable for the on-site rapid quantification of formaldehyde in market aquatic products.

To date, rapid detection techniques for formaldehyde mainly depend on gas sensor [13] and visual analysis technique [14]. Gas sensors possess small size and good portability coupled with integrated circuit devices [15]. Several formaldehyde sensors based on the sensoring materials such as carbon nanotubes (CNTs) [16], SnO₂-NiO nanometer polycrystalline composite [17] and ZnO [18] have been developed, but their poor anti-interference property usually hindered their actual quantification applications for real analytical projects. Visual analysis techniques for formaldehyde in previous reports were usually based on sharp color changes before and after corresponding derivatization reactions, which allowed the analytical results to be captured by our naked eyes [19]. Visual analysis techniques gain its attraction due to its effectiveness, simplicity, high selectivity and low cost. However, visual analysis techniques usually possess slow response and relatively low

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sensitivity, which make them prone to achieve false positive or negative results. Thus, visual analysis technique is only suitable for the qualitative or semi-quantitative detection of formaldehyde [20]. There are still few works focusing on the development of accurate on-site rapid quantification methods for trace formaldehyde in aquatic products.

Raman spectroscopy, depending on Raman scattering, is a spectroscopic technique to observe vibrational, rotational and other low-frequency modes in molecular structures. However, Raman scattering is very weak, so it is hard to be used in real analytical projects. Surface-enhanced Raman spectroscopy (SERS) is a new rapid analytical technique that enhances Raman scattering by molecules adsorbed on rough metal surfaces or by nanostructures [21]. The remarkable enhancement factor can be achieved 10¹¹ or more by SERS [22], which means the extremely high analytical sensitivity and makes the detection of single molecules possible in theory. Moreover, owing to its operation simplicity, high detection speed and small portable SERS spectrometer, SERS has been considered a potentially excellent on-site rapid analytical technique. However, during analytical projects for real samples, SERS signal is easily interfered by the co-existent matrix. Thus, nowadays SERS is mainly applied for the detection or semi-quantification for target compounds in some simple samples such as water [23], milk [24], fruit [25] and pure substance [26-28], instead of gas target compounds from complicated food samples.

Enhancement of the sensitivity and selectivity of SERS technique would greatly facilitate the application of SERS in real food rapid analysis projects. Firstly, the usage of suitable SERS substrates contributes most to enhance its analytical sensitivity. Many efforts have been gone for the development of novel SERS substrates such as Au nanoshell [29], Au nanorod [30], multibranched metal nanoparticle [31] and Au-Ag bimetallic nanoparticle

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(NPs) [32]. However, the reproducibility and stability of SERS substrates proposed still remains a challenge during real analytical projects. Shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) proposed by Tian and his co-workers [21] used an ultrathin yet continuous shell of silica isolates the Au core as SERS substrate, which could generate sensitive and reproducible SERS signals during analytical procedures. Thus, SHINERS possessed the possibility to be an effective and stable SERS-based analytical technique [33]. Secondly, via derivatization reaction some target compounds without direct SERS response would be changed to testing compounds with SERS respond, which would greatly expand SERS application scope in analytical chemistry [34-36]. Moreover, selection of suitable derivatization reagents would improve the derivatization selectivity towards target compound but not some other co-existent matrix components, which would further benefit the improvement of the selectivity of SERS analysis. Thirdly, for gas target compounds, it is necessary to combine with the appropriate pre-concentration technique such as purge-sampling which can separate target compounds from substrate effectively followed by an enrichment process in order to increase the selectivity and sensitivity during SERS analysis.

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In this work, a derivatization-based SERS method was developed for the on-site rapid quantification of trace volatile formaldehyde in aquatic products. Volatile formaldehyde from complex aquatic products was collected by a homemade purge-sampling device. Then, via a specific chemical derivatization reaction, SHINERS fingerprint characteristic of formaldehyde derivatization product was observed for the consequent qualification and quantification. Crucial conditions of chemical derivatization reaction and SHINERS analysis were optimized in order to achieve better analytical sensitivity and selectivity. The

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anti-interference experiment was conducted with the related discussion for the good selectivity. Finally, under optimal sampling conditions this new method was actually applied for the on-site rapid quantification of trace volatile formaldehyde in squid and shrimp samples.

2. Experimental

2.1 Chemical reagents

3-Methyl-2-benzothiazolinone hydrazone (MBTH) hydrochloride monohydrate (98%) was purchased from Acros (New Jersey, USA). Acetaldehyde, propionaldehyde, butyraldehyde, n-hexanal and 1-octylaldehyde were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). Methanol, ethanol, acetone n-propanol, triethylamine and $NH_4Fe(SO_4)_2 \cdot 12H_2O$ were all analytical reagent-grade and obtained from Tianjin Chemical Reagent Plant (Tianjin, China). Au/SiO₂ colloids (2.94×10^{-4} mol/L) having pinhole which were consisted of the Au NPs cores with the diameter of 55 nm around and the silica shell thickness of 1-2 nm, was supplied friendly by Prof. Tian from Xiamen University (Xiamen, China). Stock solution of formaldehyde was prepared by diluting standard formaldehyde solutions with desired concentrations were prepared by appropriate dilution of the stock solution.

2.2 Instruments

A portable DeltaNu (Laramie, WY) battery-powered Raman spectrometer (model Inspector Raman, diode laser excitation wavelength λ_{ex} =785 nm) with a liquid-N₂-cooled CCD detector (Model Spec-10:400B, Roper Scientific, Trenton, NJ), and a data-acquisition

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system (Photometrics, Tucson, AZ) was used to demonstrate the feasibility of a field-portable device for the detection. Liquid Chromatography-Mass Spectrometry (LC-MS) 2010A (Shimadzu, Japan) was used to qualitatively. To acquire SHINERS spectra, 500 μ L of the standard solution or sample extract solution of aquatic products was added to a glass tube followed by the addition of 10 μ L of Au/SiO₂ colloid NPs. After the solution was completely mixed, corresponding spectra were recorded. Laser intensity was set at the 'High' level. Each spectrum was the average of ten scans with exposure time of 3 s per scan.

In order to collect volatile formaldehyde from aquatic products, a portable purgesampling system was developed based on the combination of a sample collection cell, a sampling pump and gas flow circuit (**Fig. 1**). Sampling pump provides a negative pressure to drive volatile formaldehyde separated from complex aquatic product matrices by nitrogen followed by being absorbed and enriched in a solution containing the derivatization reagent of MBTH. Combined with the portable SERS instrument, it can be applied for the on-site rapid quantification of trace formaldehyde in aquatic products.

2.3 Derivatization

Formaldehyde has no obvious SERS response due to its simple structure, in our work a derivative method was generated for the SHINERS analysis of formaldehyde. The purged formaldehyde was reacted with the derivatization reagent of MBTH to form its formaldehyde azine [37,38] which could generate the strong SHINERS signal after the addition of Au/SiO₂ NPs (Fig. 2). The structure of formaldehyde azine was determined by liquid chromatography-mass spectrometry (LC-MS) (see the supplementary material, Fig. 1s). The samples were dissolved in ethanol and the data were recorded by positive ion mode. The molecular ion peak of formaldehyde-MBTH derivative was observed at m/z 367.1,

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which was consistent to the molecular weight of the formaldehyde-MBTH derivatization product. Mobile phase was methanol. MS spectrum was acquired across the mass range of 10–2000. The Raman shift of 1275 cm⁻¹ was directly proportional to the quantity of formaldehyde in the samples.

2.4 Aquatic products

Aquatic products including fresh squids, fresh shrimps, dried squids and dried shrimps were purchased from a seafood market in Guangzhou (Guangdong, China). Fresh aquatic products were selected for uniform color and freshness. Dried aquatic products were selected for uniform size and similar dryness. After being purchased, fresh aquatic products were stored in a foam box with ice, and dried aquatic products were stored in a foam box in a-dry environment prior to the consequent analysis.

2.5 Study of SHINERS selectivity

Selectivity of this SHINERS method was evaluated based on the Raman shifts of the mixed standard solution containing formaldehyde analogs such as methanol, ethanol, trimethylamine, triethylamine, acetone, butanal and hexanal. Five mililiters of 50 μ g/mL MBTH and 0.3 mL of 10 mg/mL NH₄Fe(SO₄)₂ were consequently added into the mixed standard solution. After the derivatization reaction was completed, SHINERS detection was conducted to record corresponding responds for the evaluation of selectivity.

2.6 Application for the formaldehyde from aquatic products

Aquatic products were minced by a commercial meat grinder. Then, five grams of minced sample was placed in a 250-mL sampling vial sealed with a rubber mat quickly. After that ultra-pure N_2 was used to purge the sampling vial for 25 min and carry the volatile formaldehyde to be absorbed by 5.0 mL of 50.0 µg/mL MBTH solution followed by the

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addition of 0.3 mL of 10 mg/mL $NH_4Fe(SO_4)_2$. After the derivatization reaction was completed, 10 μ L Au/SiO₂ NPs was added into the solution followed by SHINERS analysis.

For the spiked experiments, corresponding standard solutions with different formaldehyde concentrations of 95, 180 and 360 mg/L were sprayed on the surface of aquatic products. After 30 min, spiked samples were analyzed by use of the above SHINERS method for the recovery evaluation.

3. Results and discussion

3.1 Qualification for formaldehyde by the derivatization-SHINERS method

Obvious Raman signals cannot be acquired, if formaldehyde was mixed with Au/SiO₂ NPs directly. Via a suitable derivatization reaction, formaldehyde can be changed to a SHINERS-active compound for consequent analysis. Herein, MBTH was used as the derivatization reagent to react with target formaldehyde. This derivatization reaction would generate a formaldehyde azine compound containing abundant Raman-active bonds which could generate strong characteristic SHINERS signals when mixed with Au/SiO₂ NPs. The fingerprint SHINERS spectra of this derivative were used for the identification of formaldehyde as **Fig. 3** shown. It was seen from **Fig. 3** that there were four stable characteristic peaks observed at 873, 1275, 1401 and 1511 cm⁻¹. The vibration frequencies of the external plane of C-H ring and the bending vibration were attributed to 873 cm⁻¹. Sharp and strong peaks for =C-N and =N-N stretching modes were attributed to 1275 and 1401 cm⁻¹, respectively. The typical absorption band at 1511 cm⁻¹ was attributed to the vibration of benzene ring skeleton.

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3.2 Optimization of derivatization and analysis conditions

First of all, a suitable derivatization reagent should be selected in order to efficiently

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react with formaldehyde and generate sensitive SHINERS signals, which was the precondition for this derivatization-SHINERS method for formaldehyde. In the study, dinitrophenylhydrazone, MBTH and 2,4-pentanedione were taken into consideration for the selection of optimal derivatization reagent. The peak heights of the derivative of MBTH and formaldehyde at 1275 and 1401 cm⁻¹ were higher than those of the other formaldehyde derivatives caused by dinitrophenylhydrazone and 2,4-pentanedione (see the supplementary material, Fig. 2s). Moreover, when MBTH was used as the derivatization reagent, the interference caused by alcohol, ketone and amine compounds would be effectively avoided. Thus, MBTH was selected as the optimal derivatization reagent.

Apart from derivatization reagents, derivatization reaction conditions such as acidity, reaction time, temperature and concentration of reaction reagent and Au/SiO₂ NPs were optimized, which would directly affect the derivatization reaction equilibrium and further the rapid analysis sensitivity of formaldehyde by SHINERS.

3.2.1 Optimization of reaction conditions

The derivatization reaction was affected by the acidity of reaction solution, so the effect of HCl concentration on SHINERS sensitivity was optimized. It could be seen from **Fig. 4A** that SHINERS intensity increased dramatically by increasing HCl concentration up to 0.10 mol/L, and then slowly decreased with continuously increasing HCl concentration. When the acidity was higher than the optimized acidity, excessive amount of HCl would hinder the nucleophilic addition between the protonated MBTH and formaldehyde. On the other hand, when the acidity was lower than the optimized acidity, the oxidation intermediate of MBTH was unstable, therefore affecting the formation of the product as well. Finally, 0.10 mol/L of HCl in NH₄Fe(SO₄)₂·12H₂O was selected as the optimal acidity for the consequent

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experiment. $NH_4Fe(SO_4)_2 \cdot 12H_2O$ acted as a crucial catalyst during derivatization which would greatly affect the reaction rate. As shown in Fig. 4B, the dosage of NH₄Fe(SO₄)₂·12H₂O was optimized ranging from 0.1 to 0.8 mL. It could be seen that SHINERS intensity increased dramatically with the increasing volume of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ up to 0.3 mL and then was kept stable at higher concentrations. Low concentration of Fe(III) had a positive correlation with the reaction rate as a catalyst, but excessive catalyst could not make a contribution to reaction. Finally, 0.3 mL $NH_4Fe(SO_4)_2$ ·12H₂O was selected as the optimal dosage.

Reaction time and temperature were important factors influencing the equilibrium and degree of derivatization reaction. As shown in **Fig. 4C**, the reaction time was optimized ranging from 5 to 60 min. It could be seen that SHINERS intensity increased with the prolonging reaction time from 5 to 25 min. When the reaction time was over 25 min, SHINERS intensity remained unchanged at nearly the same level. As shown in **Fig. 4D**, the reaction temperature was optimized ranging from 5 to 40 °C. It could be seen that SHINERS intensity increased with the increasing temperature from 5 to 15 °C. When the reaction temperature was over 15 °C, SHINERS intensity remained unchanged at nearly the same level. The result suggested that the reaction equilibrium was achieved when the reaction time reached 25 min at the temperature of 15 °C. Comprehensively considering the operation simplicity and speed, the reaction time of 25 min under the room temperature was used as optimal reaction conditions for the consequent experiment.

3.2.2 Optimization of SHINERS analysis conditions

SHINERS analysis conditions such as Raman laser intensity, integration time and Au/SiO₂ NPs quantity will greatly affect the analytical sensitivity and should be optimized in

 detail. Prior to SHINERS detection, 500 μ L of formaldehyde derivative was mixed with unconcentrated Au/SiO₂ NPs. Then, SHINERS intensity at 1275 and 1401 cm⁻¹ were measured for the optimization of SHINERS analysis conditions (see the supplementary material, Fig. 3s).

The laser intensity is of positive correlation with Raman scattering within a certain range. Integration time is the sample exposure time under laser, during which the number of photons are accumulated by a photoelectric counter. Both laser intensity and integration time will directly affect the intensity of scattering and further the analytical sensitivity. The laser intensity from 'Low' to 'High' was studied during SHINERS analysis. SHINERS intensity of formaldehyde derivative increased with the increasing laser intensity. The maximum SHINERS intensity was achieved when laser intensity was set at 'high' level selected for the consequent SHINERS analysis. On the other hand, with prolonging integration time more photons would be captured by the photoelectric counter, which would result in the improvement of SHINERS signals. The different integration time ranging from 1 to 15 s was investigated in the study. SHINERS intensity of formaldehyde derivative increased with the prolonging integration time up to 7 s and then decreased on the contrary when the integration time was over 7s. The main reason was that the saturation of SHINERS signals would occur with the integration time over 7 s and overlong integration time which would also cause peak broadening and lower the resolution of SHINERS analysis. Thus, the optimal integration time was selected as 7 s.

The amount of Au/SiO_2 NPs added into the testing solution would directly affect the enhancement of SHINERS signals. The different volume of Au/SiO_2 NPs solution ranging from 1 to 25 µL was optimized during SHINERS analysis. SHINERS intensity increased

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with the increasing volumes of Au/SiO_2 NPs. However, the overflow phenomenon occurred when the volume of Au/SiO_2 NPs was over 10 µL. The SHINERS intensity can be greatly enhanced when two or more NPs are brought closely together. Within the suitable concentration range, the more Au NPs in the testing solution would result in the greater enhancement effect. Comprehensively considering the SHINERS resolution and sensitivity, 10 µL of unconcentrated Au/SiO₂ NPs solution was used in the consequent experiment.

3.3 Study of the selectivity of SHINERS analysis

Apart from target formaldehyde, there are other volatile analogs released from aquatic products including methanol, ethanol, acetone, butanal, hexanal, trimethylamine and triethylamine, which may be originated from the environment or aquatic product metabolism and be potential interferences for the on-site rapid quantification of trace formaldehyde by SHINERS. In our work, some typical potential volatile interference compounds were chosen for the selectivity study of this SHINERS method for trace formaldehyde in aquatic products. It can be seen from Fig. 5 that methanol and ethanol can not generate the characteristic SHINERS response at 1275 and 1401 cm⁻¹ which are two typical peaks for the quantification of formaldehyde after derivatization. Since methanol, ethanol, trimethylamine and triethylamine can not react with the derivative reagent of MBTH, the SHINERS rapid analysis of formaldehyde would not be interfered by these compounds. Acetone, butanal and hexanal have carbonyl groups, so the derivatization reaction between MBTH and carbonyl groups would occur which is similar as the one between MBTH and formaldehyde. Thus, it can be seen that a significant peak at 1401 cm⁻¹ is generated at SHINERS spectra of butanal and acetone. However, due to the steric effect, potential interference compounds of acetone, butanal and hexanal with long carbon chains are more difficult to form the same derivative

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of azine with MBTH than formaldehyde which has the obvious SHINERS response at 1275 cm⁻¹. Therefore, the rapid quantification of formaldehyde in aquatic products by this derivative SHINERS method can be precisely performed at 1275 cm⁻¹. From all the mention above, it can be concluded that this rapid SHINERS analysis method is not interfered by commonly co-existed interference compounds and possesses excellent analytical selectivity for the quantification of trace formaldehyde in aquatic products.

3.4 Rapid quantification of formaldehyde in aquatic products by SHINERS

3.4.1 Development of SHINERS method

After the excellent potential of SHINERS method was validated, this method was applied for the rapid quantification of trace volatile formaldehyde in aquatic products. Firstly, crucial sampling conditions based on real aquatic products including sampling time and flow speed which would greatly influence the enrichment efficiency and analysis sensitivity were optimized in this study, prior to the development of this new SHINERS method (see the supplementary material, Fig. 4s). Flow speed was optimized ranging from 0.05 to 0.4 L/min in sufficient time. The adsorption amount of formaldehyde increased with the increasing flow speed up to 0.2 L/min and then inversely decreased when the flow rate was over 0.2L/min. Sampling time was optimized ranging from 5 to 50 min. SHINERS intensity increased with the prolonging reaction time from 5 to 25 min. When the sampling time was over 25 min, SHINERS intensity remained unchanged at nearly the same level. Low flow rate and short sampling time would result in incomplete adsorption of target formaldehyde by MBTH solution. With the prolonging sampling time the adsorption equilibrium was achieved, and finally SHINERS intensity would remain at a high and stable level. However, overquick flow speed would cause the incomplete attachment of formaldehyde and

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derivative reagent in the solution and result in the inversely decreasing adsorption of formaldehyde. Therefore, we selected the optimal sampling time at 25 min with an optimal flow speed of 0.2 L/min.

Under optimal sampling conditions, a new SHINRES rapid quantification method for trace volatile formaldehyde in aquatic products was successfully established based on the SHINERS spectra intensity at Raman shift of 1275 cm⁻¹. A good linear response was found within the concentration range from 0.4 to 4.8 μ g/L with the linear equation of H=1270C+1475 (R^2 =0.9839, see the supplementary material, Fig. 5s). Detection limit was determined to be 0.17 μ g/L based on a signal-to-noise ratio of 3 (S/N=3) with relative standard deviations (RSDs) of 0.19% (n=11). All the characteristic parameters of this novel SHINERS method validated its good reliability and sensitivity for the rapid quantification of trace volatile formaldehyde in aquatic products.

3.4.2 On-site rapid quantification of trace formaldehyde in aquatic products

After the development and validation of this SHINERS method, it was actually applied for the on-site rapid quantification of trace volatile formaldehyde in aquatic products including fresh squid, fresh shrimp, dried squid and dried shrimp samples. Corresponding SHINERS spectra for the on-site rapid quantification of trace volatile formaldehyde in these aquatic products are shown in **Fig. 6**. Formaldehyde contents in aquatic products (*C*) were quantified according to the external calibration equation mentioned above and calculated by the formula as $C=(H-b) \cdot V_0/(a \cdot m_s)$. Here, *H* was the peak height of corresponding SERS spectrum of aquatic samples; *a* and *b* were the slope and pitch of the external calibration equation respectively. V_0 was volume of sampling containers, and m_s was the mass of aquatic samples. It was satisfactory that trace formaldehyde was actually found and quantified in all

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aquatic products without any obvious matrix interference by the proposal method and quantified to be 0.13-0.21 mg/kg. Also, the spiked experiment was conducted for the method validation (**Table 1**). The spiked aquatic product samples were prepared with the formaldehyde concentration set at 0.10-0.26 mg/kg. The recoveries were found to be 70.0-89.1% with RSDs of 2.3-7.2% (n=3), respectively. The whole analytical procedure involving sample preparation and SHINERS detection would be finished within 50 min. From all the results mentioned above, it is clear that the proposed method is applicable for the on-site rapid analysis of trace volatile formaldehyde in aquatic products.

4. Conclusion

It still remains a challenge for the on-site rapid quantification of trace formaldehyde in aquatic products with complicated matrices. In this work, a new on-site rapid SHINERS quantification method for trace volatile formaldehyde in aquatic products was developed with relatively high selectivity based on a specific derivatization reaction between formaldehyde and MBTH and a portable homemade headspace purge-sampling device. This SHINERS method was not interfered by commonly co-existed interference compounds and possessed excellent analytical selectivity for the quantification of trace formaldehyde in aquatic products. A rapid SHINRES quantification method for trace volatile formaldehyde in aquatic products was successfully established with a linear range from 0.40-4.8 μ g/L (R²=0.9839) and a detection limit of 0.17 μ g/L (S/N=3). Finally, the proposal analytical method was actually applied for the on-site rapid analysis of trace volatile formaldehyde in aquatic products. It was satisfactory that trace formaldehyde in all aquatic products were actually found and quantified without any obvious matrix interference and quantified to be

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0.13-0.21 mg/kg. The recoveries of spiked aquatic product samples ranged from 70.0 to 89.1% with RSDs of 2.3-7.2%. The time cost for the whole analytical procedure involving sample preparation and SHINERS detection was less than 50 min. The that proposed SHINERS method was reliable for the on-site rapid quantification of trace volatile formaldehyde in aquatic products.

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Table 1 Recoveries of volatile formal	dehyde in aquati	c products by SHINERS
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Sample	Original content	Added	Found	Recovery	RSD
	(mg/kg)	(mg/kg)	(mg/kg)	(%)	(%, n=3)
Fresh squid	0.14	0.18	0.29	83.3	2.3
Fresh shrimp	0.21	0.10	0.28	70.0	7.2
Dry squid	0.19	0.21	0.35	76.2	3.0
Dry shrimp	0.13	0.26	0.36	89.1	4.5

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Figure captions

Fig. 1 Schematic of the rapid on-site formaldehyde detection from aquatic products

Fig. 2 Chemical derivatization reaction between MBTH hydrochloride and formaldehyde azine.

Fig. 3 Raman spectra of the derivative product of formaldehyde before and after the addition of Au/SiO₂ NPs at 785 nm excitation wavelength. The lines represent SERS spectra of MBTH with Au/SiO₂ NPs(1), the derivative product before(2) and after(3) the addition of Au/SiO₂ NPs, respectively.

Fig. 4 Optimization of derivatization reaction conditions including (A) the concentration of HCl with 0.3 mL NH₄Fe(SO₄)₂ and reaction time of 25 min under room temperature, (B) the dosage of NH₄Fe(SO₄)₂·12H₂O with 0.1 mol/L HCl and reaction time of 25 min under room temperature, (C) the reaction time with 0.3 mL NH₄Fe(SO₄)₂ and 0.1 mol/L HCl under room temperature, and (D) reaction temperature with 0.3 mL NH₄Fe(SO₄)₂ and 0.1 mol/L HCl and reaction time of 25 min. SHINERS conditions: Laser intensity, 48 mW; Integration time, 7 s; Au/SiO₂ NPs, 10 μ L. Formaldehyde concentration was set at 1.2 μ g/L. The black and red dots represent the Raman intensity at 1275cm⁻¹ and 1401 cm⁻¹, respectively.

Fig. 5 Selectivity evaluation of this SHINERS method based on comparison of SHINERS spectra of mixed standard solutions of volatile alcohols, aldehydes, ketone and amines. Concentrations of formaldehyde and interference compounds were set at $1.2 \mu g/L$.

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SHINERS conditions were as follows: laser intensity, 48 mW; integration time, 7 s; Au/SiO₂ NPs, 10 μ L.

Fig. 6 On-site SHINERS spectra of trace volatile formaldehyde in fresh squid, fresh shrimp,

dry squid and dry shrimp samples. Formaldehyde concentration was set at $1.2 \ \mu g/L$.



Fig.1 210x137mm (300 x 300 DPI) Analyst Accepted Manuscript

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Fig 4A 297x210mm (300 x 300 DPI)





Fig 4B 297x210mm (300 x 300 DPI)



Fig 4C 297x210mm (300 x 300 DPI)



Fig 4D 297x210mm (300 x 300 DPI)

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Fig 6 297x210mm (300 x 300 DPI)