Based on Deep Eutectic Solvents Combined with Gas Chromatography Determination of γ-nonolactone in Coconut Juice

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Research Article

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Abstract

Determination of γ-nonolactone in coconut juice by deep eutectic solvents (DES) combined with gas chromatography was developed. Acetonitrile added deep eutectic solvents was used to extract γ-nonolactone from coconut juice with ultrasonic assist. The effects of different proportions of DES on the extraction efficiency of γ-nonolactone in coconut juice were investigated. The results showed that the extraction efficiency of γ-nonolactone in coconut juice with acetonitrile solution added with deep eutectic solvents was the highest, and the samples could be directly injected through membrane after high-speed refrigerated centrifugation. γ-nonolactone has a good linear relationship in the range of 0.1 ~ 20.0 μg/mL, the detection limit of the method is 0.2 mg/kg, the limit of quantification is 0.5 mg/kg, and the addition level is 0.2, 0.5, 10.0 mg/kg recovery the range is 81.5 ~ 105.0%,and the relative standard deviation (n = 6) is in the range of 0.6 ~ 5.8%. The method is simple to operate, has high sensitivity, and good precision. It can provide a reference for the analysis and detection of whether coconut water is added with γ-nonolactone .

Introduction

Coconut juice is made from fresh coconut pulp. Processed by saturated coconut pulp, saturated lactone compounds are a very important food flavour. They are naturally found in fruits. They are widely used in essence of various flavors such as beverages and baked foods (Li, et al., 2021). γ-nonolactone is a kind of lactone compounds obtained from the lactonation of hydroxyl carboxylic acids. They are rich in juice and fruit aroma, and have the characteristics of long fragrance retention time. γ-nonolactone is a typical representative of γ-lactone, which has coconut flavor and grease, can be used in with essence and flavoring (e.g. coconut and almond essence) with oil essence (Sun, et al., 2019). γ-nonactone is considered as a self limiting additive in National standard for food safety standard for use of food additives in China (GB 2760 – 2014). Its dosage is often controlled by the degree of consumer acceptance. With the development of food industry, the use of essence and fragrance is becoming more and more popular. The threshold value of taste buds for consumers is also increasing year by year, which may cause food flavor and essence to increase gradually during the use, which is harmful to health (Huang, et al., 2013; Sun, et al., 2017). However, there is no national standard method for the determination of γ-nonolactone in coconut juice in China. Therefore, it is necessary to establish determination of γ-nonolactone in coconut juice.

Deep eutectic solvents (DES) as a new green solvent has the characteristics of low volatility, biodegradable and environment friendly, low cost and flexible combination compared with traditional organic solvents. It can be widely used in sample pretreatment by combining various extraction technologies, such as ultrasonic assisted extraction (Liu, et al., 2018; Liu, et al., 2017), microwave-assisted extraction (Chandra and Girirajsinh, 2019; Torbatim, *et al.*, 2019), hollow fiber extraction (Mogaddam, et al., 2020; Khataei, et al., 2018) and solid-phase extraction (Yang, et al., 2020; Li and Row, 2019). This technology can not only improve the extraction efficiency, but also reduce the cost of routine analysis and reduce the impact on human health and environmental pollution. DES has been widely

applied to the analyses of various compounds in the environmental (Tang, Dai, and Row, 2018; Qu, et al., 2019), food real samples (Ge, et al., 2021; Shamsipur, Mafakheri, Babajani, 2022) and has attracted great attention due to its simplicity of operation, rapidity, low price, high recovery rate and environment-friendly (Abolghasemi, Piryaei and Imani, 2020; Hsu and Ding, 2022; Li and Ding, 2022; Wang, *et al.*, 2021).

γ-nonolactone is usually analyzed by Gas chromatography (GC (Tong, Nie and Zhou, 2016; Lu,et al., 2021), Gas chromatography-mass spectrometry (Nie, 2017), Liquid chromatography mass spectrometry (LC-MS/MS) (Wang, et al., 2016), GC-MS and LC-MS have high sensitivity and are suitable for the analysis of low residual components in food and fruit juice,The content of γ-nonolactone is generally high in real sample, and mass spectrometry has high requirements for the analysis of sample matrix, resulting in high sample pretreatment cost. GC has been widely applied to the analyses target compound and low application cost.

In the present work, we used ultrasound assisted DES combined with the GC to determine γ-nonolactone in different coconut juice sample. The key parameters affecting the efficiency of DES were researched.

Experiment

MATERIALS

7890A Gas chromatograph were purchased from Agilent Technology Co., Ltd (Agilent ,USA)，which is equipped with hydrogen flame ionization detector, 3-18K high speed centrifuge were purchased from Sigma Technology Co., Ltd，Sigma, Germany，IKA MS3 high-speed tissue homogenizer (Germany Aika Instrument Equipment Co., LTD.) ，Mili-q type pure water meter (Merck Millebo Company)，SK8200H ultrasonic cleaning machine (Shanghai Keguide Ultrasonic Instrument Co., LTD.);X205DU Electronic analytical balance (Mettler Toledo, Switzerland).

γ-nonolactone (CAS:104-61-0, purity≥98%)were purchased from Sigma(USA)，Chromatographic pure acetonitrile purchased from Merck (Germany)，Choline chloride, sodium chloride, ethylene glycol, glycerol, urea were purchased from Kemio Chemical Reagent Co., Ltd (AR, Tianjin，China). Coconut juice were purchased from supermarket.

Preparation of standard solution and DES

Standard stock solution: accurately weigh 50 mg (accurate to 0.01 mg) of γ-nonolactone , and the stint by 50 mL with acetonitrile, and store it away light-free at - 20 ℃.

Standard working solution: accurately transfer 0.1 mL of the standard solution from the γ-nonolactone standard stock solution, added it into a 10 mL brown volumetric flask, this solution was diluted to 10 μg/L with acetonitrile.

Preparation of DES1: accurately weigh 13.96 g of choline chloride (accurate to 0.01 g) and 18.62 g of ethylene glycol (accurate to 0.01 g), which were added into a 50 mL round bottom flask, and magnetically

stir at 80 ℃ until the solution becomes clear and transparent.

Preparation of DES1: accurately weigh 13.96 g of choline chloride (accurate to 0.01 g) and 18.42 g of glycerol (accurate to 0.01 g), which were added into a 50 mL round bottom flask, and magnetically stir at 80 ℃ until the solution becomes clear and transparent.

Preparation of DES1: accurately weigh 13.96 g of choline chloride (accurate to 0.01 g) and 12.01 g of urea (accurate to 0.01 g), which were added into a 50 mL round bottom flask, and magnetically stir at 80 ℃ until the solution becomes clear and transparent.

1% DES acetonitrile solution: transfer 0.5 mL of DES (1, 2 or 3), which was added into 50 mL of acetonitrile and mixed evenly.

Sample pretreatment

Accurately weigh 5.0 g of coconut juice, then 5.0 mL acetonitrile (containing 0.1% DES1) was added for vortex mixing for 1 min, ultrasonic for 10 min, 0.3 g sodium chloride was added for vortex mixing for 1 min, 10000 r/min centrifuge for 5 min at 4 ℃, take the supernatant and filter it through 0.22 μm filter membrane, for the next test.

Analysis conditions

Chromatographic separations were performed on a DB-23 (Agilent) capillary column(60 m×0.25 mmi.d., 0.25 μm film thickness, cyanopropyl Polysiloxane Stationary Phase) with the following conditions: Helium (99.999 %) was used as the carrier gas, gas flow 30 mL/min, assisted gas flow 330 mL/min and injector temperature 250 ℃. The detector temperature is 270 ℃. The oven temperature was programmed as follows: initial temperature 60 ℃ (3 min hold) , then heated to 160 ℃ at a rate of 15 ℃/min (0 min hold), then heated to 210 ℃ at a rate of 8 ℃/min (0 min hold), then heated to 230 ℃ at a rate of 3 ℃/min (0 min hold). Asplitless injection mode was used, and the injection volume was 1 μL.

Results And Discussion

Pretreatment conditions

Effect of kinds of DES on recovery

Three kinds of DES were prepared by choline chloride with ethylene glycol (DES1), glycerol (DES2) and urea (DES3), respectively. The effects of three kinds of DES as extractant on the recovery of γ-nonolactone were compared.The extraction recoveries of γ-nonolactone by DES1, DES2 and DES3 were 85%, 63% and 52% respectively. The extraction efficiency of γ-nonolactone by DES1 was significantly higher than the other kinds of DES. At the same time, the viscosity of EDS1 in ethylene glycol system was significantly lower than the other kinds of DES, Therefore, ethylene glycol system is selected for subsequent experiments.

Effect of solvent ratio on recovery

According to the literature and the material characteristics of eutectic solvents, the molar ratio of choline chloride and ethylene glycol was selected as 1:3 and stirred at 60 ℃ for 30 min, to make uniform transparent liquid. 0, 20, 50 and 100 μL of eutectic solvents were added respectively to investigate the effects of different proportions of eutectic solvents on recovery. The results are shown in Figure 1.

It can be seen from the experimental results that without adding eutectic solvent, the recovery of γ-nonolactone is only 0.04%, adding 20 μL is 45.2%, adding 50 μL is 95.4%, and the recovery of adding 100 μL is 91.8%. The experimental results are shown in Fig.1. With the increase of EDS volume, the recovery increases gradually, reaches the maximum at 50 μL, continue to increase the volume and the recovery rate begins to decline. In the extraction process, after extracting γ-nonolactone fully, increasing the volume of DES1 has no obvious effect on the recovery of γ-nonolactone. Instead, the adsorbed γ-nonolactone is dislodged from the surface and dispersed into coconut juice, this leads to a decrease in recovery. Therefore, the volume of DES1 added in this experiment is 50 μL.

<Fig.1>

Effect of salt dosage on recovery

The influence of inorganic salt used for salting out on **recovery** and adding 0.2, 0.5 and 1.0 g of sodium chloride on the test results were investigated. After 10 min of ultrasound, clear two-phase distribution can be seen after centrifugation at 4℃, 10000 r/min for 5 min. The results are shown in Fig.2.

<Fig.2>

Matrix effect (ME) is one of the key factors affecting the quantitative accuracy, which will have a great impact on the accurate quantification of the target compound. Before established method, the matrix effect of the tested substance must be evaluated, and effective measures must be taken to eliminate or compensate, so as to improve the reliability of the analytical method. At present, the evaluation method of ME is often evaluated by the ratio of the slope of the matrix standard curve to the slope of the solvent standard curve. The ME the following formula can be calculated.

$ME = K_a / K_b$ ---------- -(1)

-Ka refers to the slope of the calibration curve of the sample matrix,

-KB refers to the slope of the solvent calibration curve.

The established method, K_b is 8.25, Ka is 2.16(0.2 g NaCl), Ka is 7.97 (0.5 g NaCl), and Ka is 8.99 (1.0 g NaCl). When the matrix effect is closer to 1, it indicates that the matrix has less obvious influence on the results. Inorganic salt addition and recovery are satisfactory at this time. Considering the influence of ME, 0.5 g sodium chloride is selected as the inorganic salt used in this experiment (See Fig. 2).

Effects of centrifugal temperature on test results

The effects of different centrifugation temperatures on the test results were investigated. Under the conditions of 0, 4 and 20 ℃, 10000 r/min centrifugation for 5 min, the two-phase distribution was not obvious under the condition of 20 ℃, the acetonitrile phase had white flake precipitation, the two-phase stratification was obvious under the condition of 4 ℃, the acetonitrile phase was clear and transparent, and the results at 0 ℃ were similar to those at 4 ℃. Considering the test cost, 4 ℃ was selected as the centrifugation temperature in this experiment

Verification of the Methodology

Standard curve, linear range and detection limit

In this study, external standard method was used for quantification, concentration of 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/mL γ-nonolactone standard solution were prepared, respectively. According to peak area (Y) and concentration of standard solution (X, μg/mL) drawing standard curve the linear relation between peak area and concentration of γ-nonolactone was good in the range of 0.1~20.0 μg/mL with the correlation coefficient r is 0.99999. The limits of detection (LOD) and the limits of quantification (LOQ) were obtained by the linear equations LOD=3 s/N and LOQ=10 s/N. See Fig. 3 and Fig. 4. The results show that LOD of γ-nonolactone is 0.2 mg/kg and LOQ is 0.5 mg/kg. The developed method has good sensitivity.

<Table 1>

<Fig.3>

<Fig.4>

Accuracy and precision

To determine the accuracy, recovery experiments were performed using three different concentrations of real coconut juice samples spiked with known amounts of γ-nonolactone standard. γ-nonolactone spiked levels were 0.2, 0.5, and 10 mg/kg, respectively. The samples were measured using the optimized procedures established above. The experiment was performed in six repeated tests at each concentration level. The results showed that the recoveries of the γ-nonolactone are 89.4% ~ 105.0% within the range of spiked concentration. The relative standard deviation (RSD) is 0.6% ~ 5.8% (Table 2), It shows that the method has good stability and accuracy, and can meet the detection requirements of γ-nonolactone compounds in coconut juice.

Real samples testing

The quantitative analysis of γ-nonolactone in 10 purchased coconut juice samples from supermarkets was carried out by using the established method. The results show that, 3 purchased coconut juice samples were detected to contain γ-nonolactone , with the contents of 15.4 mg/kg, 35.8 mg/kg and 41.6

mg/kg respectively. γ-nonolactone was not detected in the samples of the other seven brands. Explain that γ-nonolactone is used in some coconut juice.

Conclusion

Using ultrasonic assisted acetonitrile solution with DES extraction of γ-nonolactone in coconut juice by gas chromatography hydrogen flame ionization detector. The developed method has the advantages of simple sample pretreatment and accurate results. It is suitable for the rapid detection of γ-nonolactone in coconut juice. The established method not only provides a sensitive, accurate and quick method for the quality and safety of food in China, but also provides technical support for identifying the coconut juice products with no flavors and labels on the products. It has important practical significance for the protection of consumers' rights and interests and human health.

Declarations

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Compliance with Ethical Standards

Conflict of Interest Yuling Gao declares that she has no conflict of interest. Xiaolin Zhang declares that he has no conflict of interest. Yongxiao Guo declares that she has no conflict of interest.

Author Contribution Yuling Gao and Yongxia Guo conceived and designed this study. Yuling Gao and Xiaolin Zhang conducted these experiments. Yuling Gao and Xiaolin Zhang analyzed the data and prepared the figures and illustrations. Yuling Gao wrote the manuscript. All authors read and approved the submission of the manuscript.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Tabel 1 Linear range、linear equation、r、LOD and LOQ of γ-nonolactone

Compound	Linear range (μg/mL)	Linear equation	r	LOD (mg/kg)	LOQ (mg/kg)
γ-nonolactone	0.1~20.0	y=8.252x-0.176	0.99999	0.2	0.5

Tabel 2 Recovery and RSD of γ-nonolactone in spiked coconut juice

Compound	spiked (mg/kg)	Recovery (%)	RSD (%)
γ-nonolactone	0.2	89.4	5.8
	0.5	100.9	1.1
	10.0	105.0	0.6

Figures

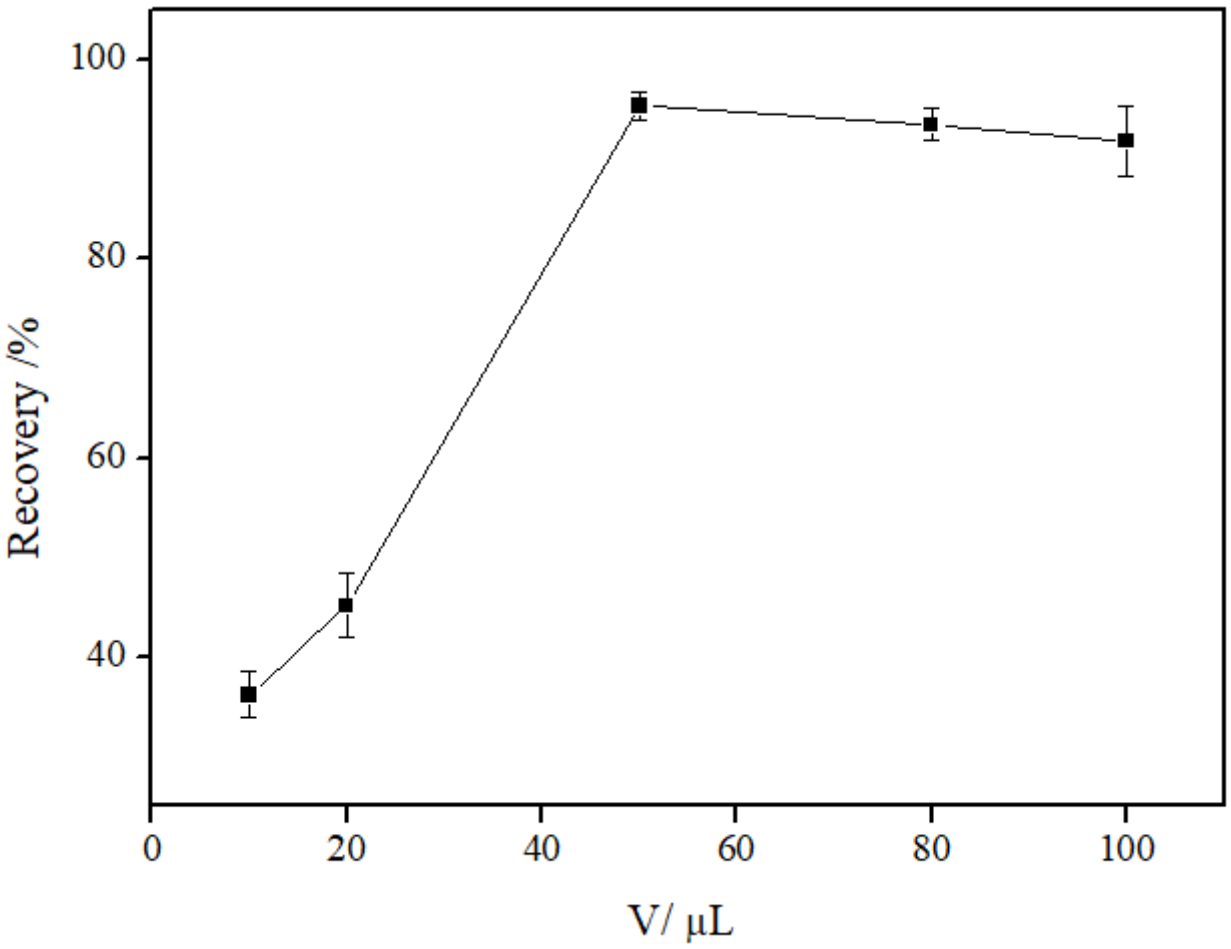

Figure 1

Effect of volume of DES on extraction efficiency

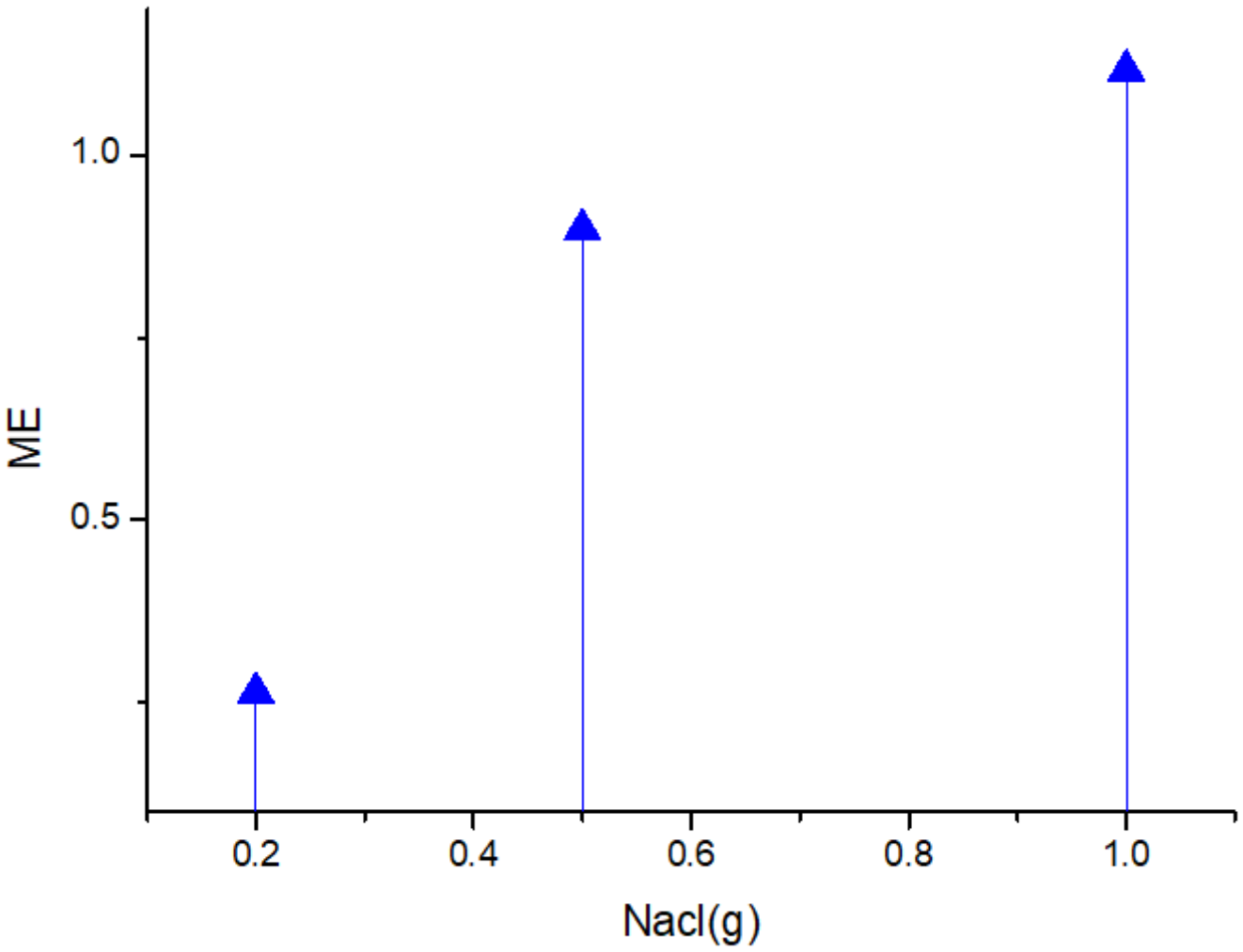

Figure 2

The influence of different salting out on the test result

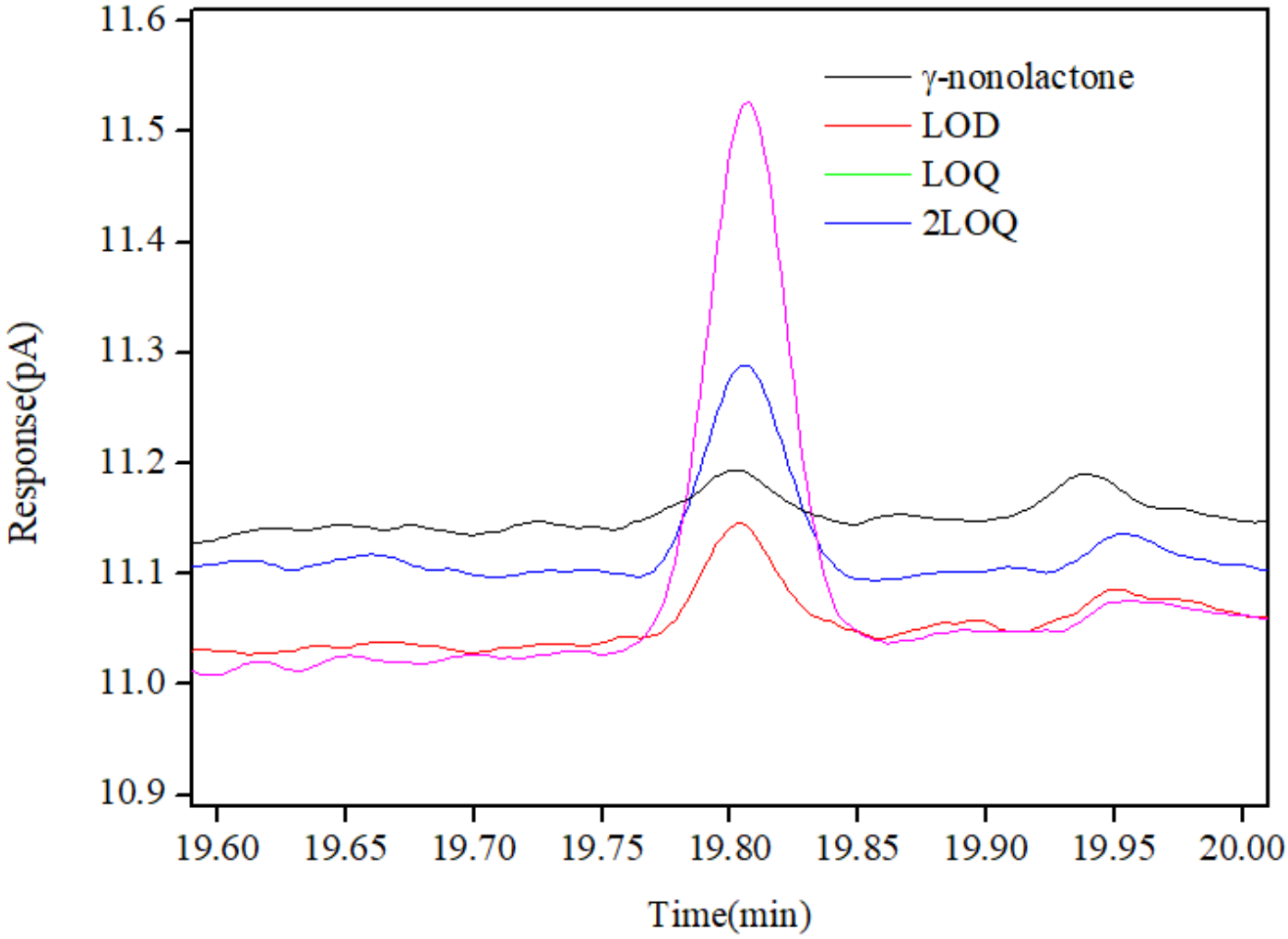

Figure 3

Chromatogram of LOD and LOQ of γ-nonolactone

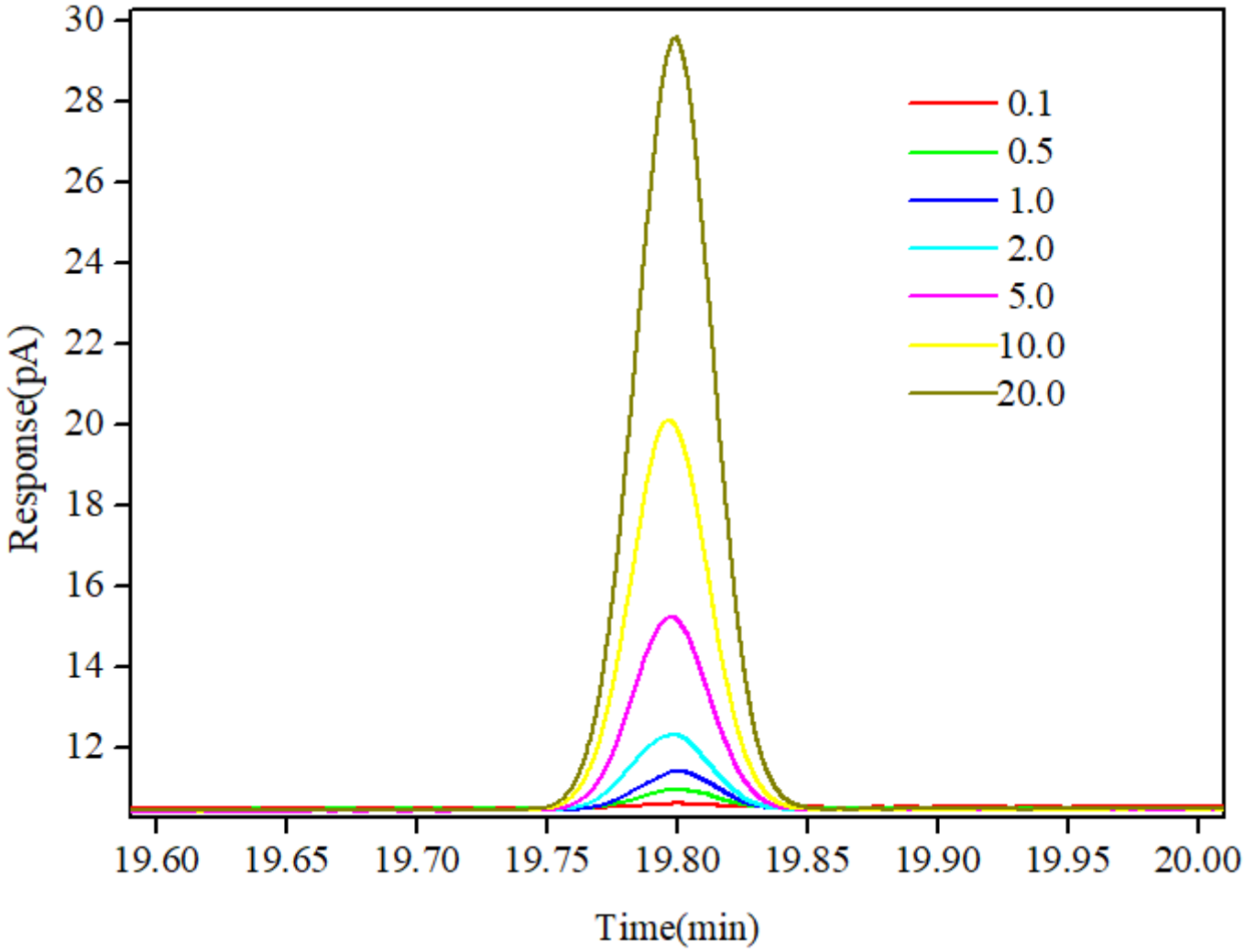

Figure 4

Chromatogram of different spiked samples