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Determination of Five Isothiazolinone Preservatives in Liquid Detergents by High Performance Liquid Chromatography

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Abstract. In this study, an associate analytical approach supported by high performance liquid chromatography (HPLC) was developed to analyze five isothiazolinones in liquid detergents in the meanwhile. The isothiazolinones were extracted from liquid detergents by ultrasound with methanol as the extraction solvent and analyzed by HPLC. The gradient elution procedure uses acetonitrile-water as the mobile phase. The isothiazolinones were effectively separated by Agilent ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 μm), and detected by diode array detector. The maximum absorption detection wavelengths of MIT, CMIT, OIT, DCOIT, and BIT were 275 nm, 275 nm, 282 nm, 285 nm, and 318 nm, severally. Quantitative analysis use of external standard method. The method was illustrated the excellent linearity with the standard curve correlation coefficients of determination (R^2, 0.9992-1.0), LOD ranged from 0.06-0.19 μg/g. The spiked recoveries were 92.73%-109.92% with the RSD (n=7) varied from 0.06%-2.26%. A total of 30 liquid detergents samples were inspected, the results demonstrated that MIT, CMIT, and BIT were contained with the concentration varied from 4.26 μg/g to 102.62 μg/g. And, it described the matrix effects of liquid detergents. Conclusion this method was recognized by excellent linearity, precision, accuracy, and was validated as a suitable and reliable technique for determination of isothiazolinone preservatives in liquid detergents.

Keywords. Isothiazolinone preservatives, high performance liquid chromatography, diode array detector, liquid detergents, matrix effect

1. Introduction

Liquid detergents are a class of substances composed of water, surfactant, preservative, and various additives. Liquid detergents are rich in a large amount of water and various surfactants, which providing conditions for the breeding and reproduction of microorganisms. In order to prolong the shelf life of the product, preservatives play a particularly role in protection of liquid detergents. Isothiazolinone preservatives are widely applied in liquid detergents with its remarkable antimicrobial characteristics. They are a class of sulfur-contaning heterocyclic compounds, including 2-Methyl-4-Isothiazoline-3-one (MIT, CAS No. 2682-20-4), 5-Chloro-2-methyl-4-choroisothiazolinone-3-one (CMIT, CAS No. 26172-55-4), 2-n-Octyl-4-isothiazolin-3-one (OIT, CAS No. 26530-20-1), 4,5-Dichloro-2-octyl-isothiazolone (DCOIT, CAS No.

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64359-81-5), and 1,2-Benzisothiazolin-3-One (BIT, CAS No. 2634-33-5). Owing to the broad-spectrum, high efficiency and environmental friendliness properties, isothiazolinone preservatives are widely used to control the growth of microorganisms in various fields, for instance cosmetics, paints, detergents, toys and other industries. However, isothiazolinone preservatives had been reported that they can cause allergic reactions or contact dermatitis. Several studies had reported that isothiazolinone preservatives had become one of the main allergens in EU, which had aroused wide concerns with its risk to human health [1-3]. In China, there are no laws and regulations for liquid detergents, which except for used in food-related liquid detergents shall comply with the limit of MIT is less than 0.01%, and 3:1 CMIT/MIT mixture is less than 0.0015%. Therefore, the relevant regulations are established to ensure the safe use of isothiazolinone preservatives in liquid detergents. More researches are needed to obtain more exposure data to provide a basis for further risk assessments.

It is reported that the detection methods of isothiazolinone preservatives include gas chromatography (GC) [4, 5], high performance liquid chromatography (HPLC) [6-10], high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [11, 12], and gas chromatography-mass spectrometry (GC-MS) [13, 14]. HPLC techniques are widely used in environmental, food, cosmetics and other detection areas. The ingredient of liquid detergents is complex, it's easy to lead to pollution and quantitative inaccuracy of mass spectrometry, if the sample pretreatment purification is not complete. In conclusion, HPLC is the optimum analysis technique for detection isothiazolinone preservatives in liquid detergents, and there are few reports about the simultaneous examination of five isothiazolinone preservatives in liquid detergent by High performance liquid chromatography at the seam time.

The main objective of this work is established an accurate and effective detection method which is appropriate and reliable for determination of five isothiazolinone preservatives (MIT, CMIT, BIT, OIT, and DCOIT) in liquid detergents by optimizing the extraction conditions of sample pretreatment and analytical conditions. To provide technical supports for testing the content of isothiazolinone preservatives in liquid detergents, in order to improve the quality of liquid detergents and ensure consumer safety.

2. Experiments

2.1. Instruments

Agilent 1260 HPLC system equipped with diode array detector (DAD) (Agilent 1260, Agilent Technologies, USA); Electronic analytical balance (MS105DU, Mettler Toledo, Switzerland); The vortex oscillator (Lab Dance, IKA, Germany), and ultrasonic cleaner (KH3200DE, Kunshan Hechuang, Jiangsu, China) were used to extract compounds from liquid detergents; The centrifuge (TGL-16, Sichuan Shuke, China) was used to centrifuge sample solutions; Purified water was produced by Milli-Q Q-POD® system (Merck Millipore, USA).

2.2. Reagents and Standards

HPLC grade methanol and HPLC grade acetonitrile were obtained from Merck (Darmstadt, Germany); Reference substances of MIT (purity: 98.8%), CMIT (purity: 99.2%), OIT (purity: 99.3%), DCOIT (purity: 99.8%), and BIT (purity: 99.7%) were purchased from Bepure®. Accurately weigh each reference substances to prepare the standard stock solution, and the mixed standard solutions constant volume with methanol. The concentration of the mixed standard stock solution of MIT, CMIT, OIT, DCOIT, and BIT was 1155.2 μg/mL, 817 μg/mL, 1218 μg/mL, 1222.8 μg/mL, and 1068 μg/mL. Accurately transfer standard solutions to the volumetric flsk, dilute with methanol to volume to prepare the operation solutions and make working curves.

2.3. Sample Preparation

Liquid detergents were obtained from the local market with different companies. Approximately weigh about 1.0 g (accurate to 0.1 mg) of liquid detergents to 10 mL tube, and dilute with methanol to volume. The solutions mixed by vortex oscillator and placed in ultrasonic cleaner for extraction 20 min at 30 °C. Next, the sample solutions were centrifuged for 5 min. Finally, the extraction solution was filtered by 0.45 μm organic filter membrane for HPLC detection.

2.4. Chromatographic Conditions

Isothiazolinone preservatives were analyzed by HPLC equipped with DAD detector. The chromatographic separation was made on Agiltent ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 μm). The elution procedure of acetonitrile (mobile phase A) and purified water (mobile phase B) is gradient elution. The flow rate, column temperature, and injection volume were set as 1.0 mL/min, 30°C, and 10 μL, respectively. The gradient elution procedure is shown in Table 1.

Table 1. Gradient elution procedure.

Time/min	Acetonitrile/%	Water/%
0.00	10	90
10.00	35	65
10.05	90	10
20.00	90	10
20.05	10	90
30.00	10	90

3. Results and Discussion

3.1. Detection Wavelength

The spectrograms of five isothiazolinones were determined by full-wavelength scanning. The absorption spectra of MIT, CMIT, OIT, DCOIT, and BIT showed a maximal at wavelength about 275 nm, 275 nm, 282 nm, 285 nm, and 318 nm, respectively. The wavelengths were selected to record spectrograms of five isothiazolinones, which combined with the absorption and baseline interference of each substance. The absorption spectrograms of standard solution were showed in Figure 1.

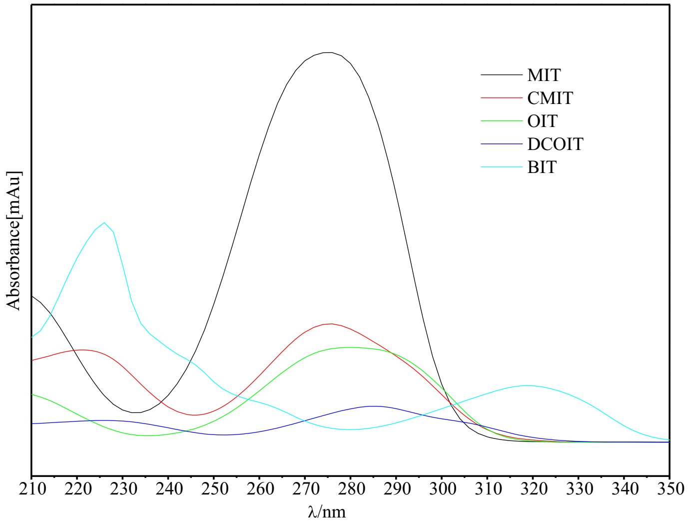

Figure 1. Absorption spectrograms of standard solution.

3.2. Optimization of Extraction

In this study, it compared the extraction efficiency of water, acetonitrile, methanol and ethanol. The results showed that the solubility of the sample in acetonitrile solvent was poor, and a large number of insoluble substances were precipitated. The MIT chromatographic peak appeared front phenomenon by ethanol extraction, which could result in inaccurate peak area quantification. Although the samples can be dissolved completely in water, the extraction efficiency is poor. As reported, OIT, COIT, and DCOIT are slightly soluble in water [15]. The sample extracted by methanol solvent had optimal solubility and extraction efficiency, the chromatographic peak is perfect. Therefore, methanol had been adopted as the sample extraction solvent. Then, this study investigated the influence of ultrasonic extraction temperature and extraction time, it showed that the influence of these two parameters on the sample extraction efficiency was not significant. Combined with the laboratory conditions and sufficient extraction, it is determined that liquid detergents were ultrasonic extraction for 20 min at 30°C.

3.3. Optimization of Chromatographic Method

In chromatographic technology, the column stationary phase plays an important role in separation of analytes. In this study, Agilent ZORBAX ODS (5 µm, 4.6 mm × 250 mm), Agilent ZORBAX SB-C8 (5 µm, 4.6 mm × 250 mm) and Agilent ZORBAX SB-C18 (5 µm, 4.6 mm × 250 mm) chromatographic columns were examined under the identical mobile phase gradient elution program. The separation effects of different columns on target substances and sample matrix interference were evaluated to select appropriate chromatographic columns. It is showed that the separation effect of ZORBAX ODS column was poor. The compounds can be separated well in ZORBAX SB-C8 column, however the chromatographic peak shape is not ideal, and the interference of sample effect was obvious. Moreover, it is showed that the MIT chromatographic peak appeared front phenomenon. Isothiazolinone preservatives are a class of polar compounds, which ZORBAX SB-C18 can be used in wide pH range, and is especially suitable for polar compounds with difficult to separate. Therefore, Agilent ZORBAX SB-C18 was selected as the object for further study owing to its better chromatographic performance.

In this study, it investigated the performance of the different mobile phase, flow rate and elution methods. The mobile phase of methanol-water (0.1% formic acid), and acetonitrile-water were examined. The results showed that the mobile phase of acetonitrile-water can separate the target substance completely and effectively. The optimum mobile phase conditions were using gradient elution with the flow rate of 1.0 mL/min.

The influence of the column temperature on target compounds separation of 25°C, 30°C, 35°C, and 40°C were explored. It indicated that with increase of column temperature, the retention time of five analytes moved forward. And the compounds can be separated well at the above of examined column temperatures. The peak area almost keep unchanged, and the column temperature had little effects on the five isothiazolinone preservatives. Generally, the column temperature affects the gradient elution ability to the target analytes by change the density and viscosity of the mobile phase. By reported, the column temperature is normally controlled lower than 35°C. Therefore, the column temperature was set at 30°C.

The chromatogram of isothiazolinone preservatives under the optimization of chromatographic conditions is shown in Figure 2.

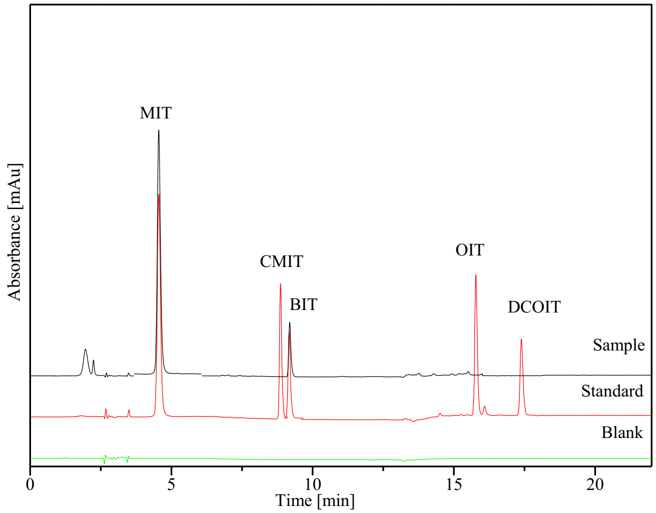

Figure 2. Chromatograms of isothiazolinone preservatives under the optimization of chromatographic conditions.

3.4. Matrix Effects

The matrix effects of liquid detergents are very complex due to the presence of various of surfactants and additives. The matrix effects may cause interference to the analysis. Therefore, it is necessary to evaluate the matrix effects of the sample to ensure the accuracy and precision of the quantitative analysis. The blank matrix solution was prepared according to the sample preparation in Section 2.3. The working curves of solvent standard solution and matrix standard solution were prepared according to the method in Section 2.2. And the linear equations of the corresponding standard curves are established respectively. The matrix effects of the sample were evaluated by the formula Matrix Effects (ME)= [(matrix standard curve slope/solvent standard curve slope-1)] × 100%. When |ME|<20%, 20%≤|ME|≤50%, and |ME|>50%, it indicates that the matrix effects is weak, medium and strong, respectively. At the same time, the medium and strong matric effects should take measures to eliminate the matric effects [16, 17]. As showed in Table 2, the matrix effects of MIT, CMIT, OIT, DCOIT, and BIT are 0.079%, -0.946%, 0.504%, -0.974%, and 1.606%, respectively. It is indicated that the liquid detergents almost had no matrix effects, and the solvent standard curve can be directly applied to quantitative analysis. The ME data of liquid detergents are shown in Table 2.

Table 2. Solvent standard curve, Matrix standard curve, and ME.

Compounds	Solvent standard curve	Matrix standard curve	ME/%
MIT	$y = 39.08x-0.521$; $R^2 = 0.9997$	$y = 39.111x-6.996$; $R^2= 0.9997$	0.079
CMIT	$y = 24.73x-19.410$; $R^2 = 0.9992$	$y = 24.496x+4.101$; $R^2= 0.9999$	-0.946
OIT	$y = 15.688x-8.364$; $R^2= 0.9999$	$y = 15.767x+7.912$; $R^2 = 1.0$	0.504
DCOIT	$y = 15.598x-4.208$; $R^2=1.0$	$y = 15.446x+22.756$; $R^2= 0.9998$	-0.974
BIT	$y = 17.997x+1.965$; $R^2 = 0.9997$	$y = 18.286x-2.289$; $R^2 = 0.9998$	1.606

3.5. Limit of Detection (LOD), Calibration Curves, Accuracy, Precision, and Recovery

In the optimum experimental conditions, the standard curves of analytes were drawn with the peak area (Y) and the concentration (X) prepared by dilution of the standard working fluid. Within the linear range, the linearity of MIT, CMIT, OIT, DCOIT, and BIT were satisfactory. It observed that the correlation coefficients for five isothiazolinone preservatives were more than 0.999. The limit of detection (LOD) and limit of quantification (LOQ) are determined by signal-to-noise rate of 3 (S/N=3) and 10 (S/N=10), respectively. The LOD and LOQ of MIT, CMIT, OIT, DCOIT, and BIT were 0.06 μg/g to 0.19 μg/g and 0.21 μg/g to 0.62 μg/g, respectively. The data are shown in Table 3. It is suggested that the generating method is well suited for quantitative test of five isothiazolinones preservatives in liquid detergents.

Table 3. Standard Linear equation, correlation coefficients (R^2), LOD and LOQ.

Compounds	Linearity range (μg/mL)	Standard linear equation	Correlation coefficients	LOD (μg/g)	LOQ (μg/g)
MIT	5.8-115.5	$y = 39.08x-0.521$	0.9997	0.06	0.21
CMIT	4.1-81.7	$y = 24.73x-19.410$	0.9992	0.08	0.25
OIT	6.1-121.8	$y = 15.688x-8.364$	0.9999	0.19	0.62
DCOIT	6.1-244.56	$y = 15.598x-4.208$	1.0	0.17	0.56
BIT	5.3-106.8	$y = 17.997x+1.965$	0.9997	0.18	0.59

The validity of the method was demonstrated by measure the recovery with addition of three-level isothiazolinones. In this study, the average recoveries between 92.73% and 109.92% for the five analytes. The precision of method is normally expressed as the relative standard deviations (RSD), which obtained from multiple testing of the same sample under the prescribed conditions [18]. In this study, the precision of method was demonstrated by testing each standard spiked sample for repeating 7 times. At each level of concentration recovery rate was done, the relative standard deviations (RSD) varied from 0.06%-2.26%, as shown in Table 4.

Table 4. Recovery rate and RSD of each standard spiked concentrations (n=7).

Compounds	Spiked concentration (μg/mL)	Average Recovery (%)	RSD (%)
MIT	11.55	100.85	0.44
	46.21	92.73	0.06
	115.52	101.52	0.25
CMIT	8.17	109.92	2.26
	32.68	100.64	0.06
	81.70	100.16	0.41
BIT	10.68	101.18	0.53
	42.72	100.89	0.10
	106.80	102.61	0.23
OIT	12.18	109.87	0.69
	48.72	103.22	0.21
	121.80	101.13	0.25
DCOIT	12.23	109.13	0.76
	48.91	97.53	0.21
	122.28	98.94	0.31

3.6. Test Results of Liquid Detergents

The optimized analytical procedure was used to inspect the concentration of isothiazolinone preservatives in 30 commercial liquid detergents, which included 15 hand dishwashing detergents and 15 fabric detergents. The results are shown in Table 5. The results showed that none of the products contained OIT and DCOIT. Within the 15 hand dishwashing detergents, MIT were detected in 10 products with the concentration varied from 5.12 μg/g to 86.07 μg/g. CMIT were detected in 8 products with the concentration varied from 10.14 μg/g to 16.56 μg/g. Within the 15 fabric detergents, MIT were detected in 11 products with the concentration varied from 4.26μg/g to 102.62 μg/g. CMIT were detected in 6 products with the concentration varied from 9.07 μg/g to 20.69 μg/g. And BIT was detected in 2 products with the concentration of 62.95 μg/g and 89.16 μg/g. The results showed that usage of isothiazolinone preservatives in hand dishwashing detergents is significantly lower than fabric detergents, and BIT was found in fabric detergents. This is mainly due to the Chinese regulation clearly defined that the limits of MIT and 3:1 CMIT/MIT mixture in the liquid detergents for food-related products shall not exceed 0.01% and 0.0015%. However, there are not organized by Chinese regulation to be used for other types of liquid detergents, which cannot be belong to food-related or cosmetics fields. Combined with current Chinese regulations about liquid detergents, BIT preservatives may present safety risks. Therefore, it is necessary to realize its potential health risks, and more researches will be needed to further explore more accurate and rapid analysis methods.

Table 5. Analytical results of isothiazolinone preservatives in positive samples (μg/g).

Sample No	MIT	CMIT	OIT	DCOIT	BIT
1#	86.07	16.37	ND	ND	ND
2#	48.93	13.02	ND	ND	ND
3#	44.55	10.43	ND	ND	ND
4#	12.50	ND	ND	ND	ND
5#	64.81	13.64	ND	ND	ND
6#	45.92	11.82	ND	ND	ND
7#	62.12	ND	ND	ND	ND
8#	45.65	10.14	ND	ND	ND
9#	5.12	16.56	ND	ND	ND
10#	48.19	12.62	ND	ND	ND
11#	57.59	ND	ND	ND	62.95
12#	73.17	ND	ND	ND	ND
13#	4.74	17.78	ND	ND	ND
14#	64.73	9.07	ND	ND	89.16
15#	69.46	9.24	ND	ND	ND
16#	97.34	ND	ND	ND	ND
17#	102.62	ND	ND	ND	ND
18#	99.53	ND	ND	ND	ND
19#	80.65	10.44	ND	ND	ND
20#	89.68	20.69	ND	ND	ND
21#	4.26	17.96	ND	ND	ND

Note: 1#-10# are hand dishwashing detergents; 11#-21# are fabric detergents.
ND refers to non-detectable.

4. Conclusions

In this study, a method for simultaneous determination of isothiazolinone preservatives in liquid detergents by HPLC was established. The isothiazolinones were extracted from liquid detergents by ultrasound with methanol as the solvent and analyzed by HPLC-DAD. Under the optimized conditions, the compounds were separated completely and effectively. The matrix effects are at a low level. This method was simple, not time-consuming, and had a satisfactory linearity, accuracy, and precision. The method was validated to be an effective and reliable analysis, which was suitable for simultaneously detection multiple target analytes. The results of liquid detergents indicated that these studied samples containing a combination of isothiazolinone preservatives (MIT, CMIT, and BIT), which the maximum concentration of the MIT was 102.62 μg/g. BIT is not a regulated isothiazolinones preservatives used in liquid detergents and requires further researches to assess its potential risks to human health.

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