

Determination of thiram in tobacco by surface-enhanced differential Raman spectroscopy

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Abstract. This study had developed a rapid, simple, accurate, and efficient method for the qualitative and quantitative determination of thiram residues tobacco and tobacco products based on surface-enhanced Raman spectroscopy and differential Raman spectroscopy. Acetonitrile was used as the extractant and PSA and GCB as the scavengers to effectively reduce the matrix effect. At the same time, sodium chloride and hydrochloric acid were used to induce the agglomeration of the target molecule and the nano particles of enhanced colloid substrates. With this approach to improve the sensitivity of analysis, our results showed that the fine linear equations of thiram with the correlation coefficients equaled 0.9986. The detection of limits was 0.0084 mg/L and the recovery was within 85–117% and the RSD was less than 10%. The detection method of thiram had a good accuracy and precision, which was potentially suitable for other dithiocarbamate pesticide residues in tobacco and tobacco products.

Keywords: surface enhanced differential Raman spectroscopy (SERDS), tobacco, Dithiocarbamate, Thiram, internal standard method

1. Introduction

Thiram is a typical dimethyl dithiocarbamate (DTCs) pesticides, which are a kind of organic sulfur protective fungicide [1]. In tobacco, thiram is mainly used to control anthrax, damping-off, root rot, scab or black shank. Due to its relatively stable surface on tobacco, it is easy to cause residues. Thus thiram and other DTCs pesticides are of high detection rate in tobacco and tobacco products [2,3].

At present, the commonly used detection methods for DTCs are liquid chromatography [4,5], gas chromatography [6,7] and chromatography-mass spectrometry [8]. The standard detection method for such fungicides in tobacco and tobacco products, YC/T 405.4-2011, is also liquid gas chromatography-mass spectrometry [9]. Compared with the early spectrophotometry, these methods have great improved sensitivity. However, the detection principle was all fundamentally based on the dithiocarbamate acid hydrolysis method proposed by Keppel in 1969, which was complex for the pretreatment operation and time consuming, and only provided the total amount of DTCs residues [10]. In recent years, method based on the negative ion scanning mode of liquid chromatography-tandem mass spectrometry has established [11], however, this method still relied on large-scale laboratory equipment with great limitation. Therefore, it is very important to establish a simple, rapid and suitable method for rapid analysis for on-site detection.

With the development of surface-enhanced Raman spectroscopy (SERS), it has been used for rapid on-site analysis because of its simple operation, high sensitivity and no need of complex pretreatment of samples. In recent years, the application of SERS in the detection of pesticide residues in fruits and vegetables has become more and more mature [12]. Differential Raman spectroscopy is based on differential and Raman derivatization technology, which can effectively

remove the fluorescence interference and improve the detection sensitivity [13]. However, no research has been conducted on the combined application of surface enhancement technology and differential technology to the detection of pesticide residues. In this paper, the surface enhanced and differential Raman technology was first used for the rapid detection of thiram in tobacco without sample derivatization. The operation was simple and the detection time was short. The effective binding of surface enhanced and differential Raman technology was greatly improve the sensitivity and stability of the detection of thiram, which would reduce measurement errors caused by unstable factors such as light source, detection background noise, and operation. This method has important practical significance in actual detection.

2. Materials and Methods

2.1 Main instruments and reagents

Portable differential Raman spectrometer: SERDS Portable-Base, Nanjing Jianzhi Instruments Co., Ltd.; Centrifuge: DM0412S, Dalong Xingchuang Experimental Instrument (Beijing) Co., Ltd.; Vortex mixer: MIX-30, Jinchang Experimental Instrument Factory, Baita, Jintan District.

Gold nano-sol: SSN-4, Nanjing Jianzhi Instruments Co., Ltd.; Thiram standard: purity 98.0%, Beijing Tanmo Quality Inspection Technology Co., Ltd.; N- propylethylenediamine (PSA): 40-63um, Tianjin Bona Ajar Technology Co., Ltd.; Graphite Carbide Black (GCB): 120/400mesh, Tianjin Bona Ajar Technology Co., Ltd.; Acetonitrile, methanol, ethyl acetate, hydrochloric acid, sodium chloride, sodium iodide, sodium hydroxide, and sodium bromide: analytically pure, Shanghai Guoyao Group Chemical Reagent Co., Ltd.; Experimental water was ultra-pure water.

2.2 Sample Preparation

0.30g of tobacco powder sample was weighed and put into a 10mL stoppered centrifuge tube. Added 3mL of acetonitrile into the tube. After being shaken up and down, the sample was extracted by ultrasound for 3 min and centrifuged at 10000 r/min for 2min. Then 1mL of clear liquid was added into a 2mL stoppered centrifuge tube. 20 mg of PSA and 20 mg of GCB scavenger were added. After fully shaking and mixing, the sample was centrifuged at 10000 r/min for 1min and the clear liquid was finally collected to be detected.

2.3 SERS detection methods and conditions

0.15 mL of SSN-4 gold nano collosol, 0.05mL of the tested solution, and 0.05mL of procoagulant were sequentially added into a 1.5mL liquid phase detection bottle. After being mixed and absorbed, the sample was tested on the SERDS Portable-Base.

The scanning range was 150-3500 cm^{-1} . A dual-frequency light source was equipped with the wavelength of $784.5\text{nm} \pm 0.5\text{nm}$, $785.5\text{nm} \pm 0.5\text{nm}$. The laser power was 450mW. The integration time was 10 s.

3. Results and discussion

3.1 Thiram urface enhanced-differential Raman spectroscopy

Thiram was diluted step by step with ultra-pure water to the corresponding concentrations and used as the solutions to be tested, and the surface enhanced differential Raman spectra was obtained by detecting according to Method 1.4 (Figure. 1). It can be seen that the characteristic peaks of thiram mainly lie in 546cm^{-1} , 924cm^{-1} , 1140cm^{-1} , 1375cm^{-1} , 1441cm^{-1} and 1513cm^{-1} , which belong to S-S telescopic vibration, C-S telescopic vibration, CH_3 swing vibration and C-N telescopic vibration, CH_3 symmetrical bending vibration, CH_3 antisymmetric bending vibration and C=N telescopic vibration [10,14].

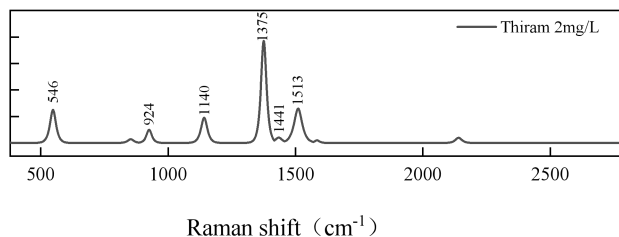
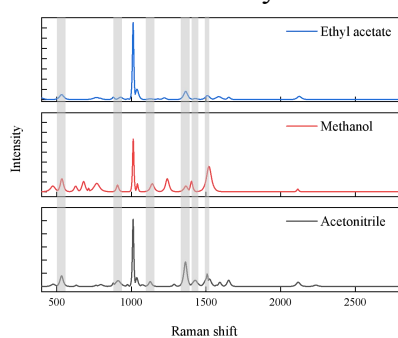


Figure 1. Surface-enhanced differential Raman spectrum and characteristic peaks of thiram

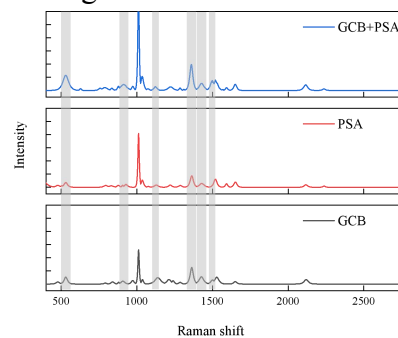
3.2 Optimization of sample preparation method

3.2.1 Selection of extractant

The extraction effects of acetonitrile, ethyl acetate, and methanol, which of three different polar extractants, on thiram in tobacco were comparatively studied in the experiment. The results are shown in Figure 2. When the spiked concentrations of thiram were the same, the effects of the three extractants were acetonitrile > methanol > ethyl acetate. It was believed that since the gold nano Raman enhanced agent was an aqueous collosol, the adsorption of samples on the surface of the gold nano particles would be affected if the samples were in the non-polar or weak-polar organic reagent, thus affecting the enhanced effect. However, the stronger polarity of the solution the more impurities were extracted, thus the stronger the matrix effect would be. Aetonitrile had strong versatility, less oil soluble, and good extraction effect for most pesticides. At the same time, its polarity was relatively moderate, so the effect of extraction solvent on the adsorption effect of gold nano Raman enhance was minimized while the extraction effect was ensured. As shown in Figure 2 (a), six characteristic peaks of thiram in the sample were clearly visible after extraction with acetonitrile, while only some characteristic peaks were detected after extraction with methanol and ethyl acetate, especially the signal strength at the peak of 1375cm-1 became significantly smaller. Therefore, acetonitrile was finally selected as the extraction agent.



(a) Comparison of extraction effects



(b) Comparison of purification effects

Figure 2. Optimazation of sample preparation (The shaded area represents the characteristic peak)

3.2.2 Selection of Purifying Agent

The purification step is particularly important in the detection of samples with complex matrix. The removal of interfering substances is conducive to reducing the limit of detection and increasing the accuracy of the results. The purification effect is mainly determined by the choice of adsorbent. The main interfering factors in tobacco and tobacco products are nitrogen compounds, carbohydrates, organic acids, dyes, etc. PSA can effectively adsorb carbohydrates, fatty acids, organic acids, phenols and a small amount of dyes in the matrix. GCB can remove dyes, sterols and non-polar interfering substances. So two adsorbents may remove most of the interfering substances in tobacco. Controlling for other conditions, the purification effects of the two adsorbents on the samples were compared in the experiment, as shown in Figure 2 (b). The experimental results showed that PSA or GCB alone could not achieve the best purification effect. When PSA and GCB were used in combination, the removal effect of matrix interference substances in samples was the best.

3.3 Preliminary exploration of SERS detection conditions

The coagulant can induce the agglomeration of target molecules and nano enhanced reagent, which is the key to improve the sensitivity of SERS analysis. The salt solution of halogen ion was one of the most common coagulant. The effects of halogen ion and the acidity and basicity of the solution on the enhancement were studied in this experiment. 1375cm⁻¹ was taken as the contrast peak of thiram. The results showed that the enhancement effect of Cl⁻ was greater than Br⁻ and I⁻. In addition, the acidity and alkaline of the solution had a greater effect on the enhancement effect. Thus hydrochloric acid and sodium hydroxide were used to control the pH of the test solution with sodium chloride as coagulant. The results showed that the best enhancement effect appeared in the slightly acidic solution.

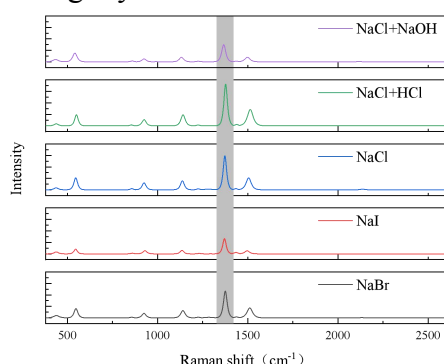


Figure 4. Surface-enhanced differential Raman spectra of Thiram in different solvents

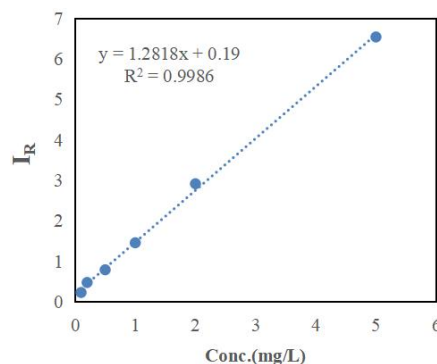


Figure 5. The standard curve of thiram

3.4 Linear range and limit of detection

In the experiment, surface-enhanced Raman detection was performed on standard solutions of thiram with different concentration gradients. The standard working curve was drawn as Figure 6. The results showed that thiram exhibited a good linear relationship within the range of 0.1–5 mg/L, with the linear equation of $y = 1.2818x + 0.19$ and the correlation coefficient of 0.9986. The LOD of thiram was 0.0084 mg/L based on the calculation of 3 times standard deviation of 10 parallel experiments.

3.5 Accuracy and precision of the method

Three simulated positive samples with different spiked concentrations were prepared, and the specific spiked concentrations were shown in Table 1. The recoveries of the thiram were in the range of 85–117% with RSD within 10%, demonstrating that the method has good accuracy and precision.

Table 1. Accuracy and precision of the method

Add level (mg/kg)	10	5	2
Recovery (%)	88.4	110.5	105
	90.1	109.7	110.2
	85.3	117.2	94.7
RSD (%)	2.4	4.1	7.9

4. Conclusion

In summary, we have successfully combined surface-enhanced Raman spectroscopy with differential Raman spectroscopy, and developed the rapid, simple, accurate, and efficient qualitative and quantitative detection of thiram in tobacco and tobacco products. The specific results are as follows: (1) Surface-enhanced differential Raman spectra of thiram was analyzed, and the Raman characteristic peaks were obtained and their attribution was analyzed. (2) The extraction and

purification method of thiram in tobacco and tobacco products were optimized, and a rapid, simple and efficient pretreatment method using acetonitrile as the extractant and PSA and GCB as the scavenger was obtained. (3) A simple exploration of the pro-solvent in the SERS detection has revealed that Cl^- enhanced the target substance more than Br^- , and I^- and its effect would be optimized when the pH of the solution was less than 7. The linear equations of thiram were $y=1.2818x+0.19$ (correlation coefficient $r = 0.9986$). According to the calculation of three times standard deviation of ten parallel experiments, the detection limits was found to be 0.0084mg/L of thiram. For the determination of the spiked samples, the recoveries were 85–117% of thiram with RSD less than 10%. The developed method of thiram was with good accuracy and precision, which was potentially suitable for other dithiocarbamate pesticide residues in tobacco and tobacco products.

Acknowledgments

This work was financially supported by projects grant from China National Tobacco Corporation Yunnan Company Science and Technology Plan Project (Grand No. 2020530000241037, 2020530000241034). The authors wish to acknowledge Professor Anyuan Cao, Peking University, for his help in this study.

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