

DEVELOPMENT OF ANALYTICAL METHOD FOR DETERMINATION OF THIOSULTAP-DISODIUM RESIDUE IN PALM OIL MATRIX

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ABSTRACT

Thiosultap-disodium (thiosultap) has been identified to be the potential alternative to monocrotophos and methamidophos for controlling bagworms and leaf eating caterpillars infestation in oil palm plantations. This article reports a simple and fast method of determining thiosultap residue in palm oil matrix using liquid chromatography coupled to a triple quadrupole mass spectrometer. The proposed method has been in-house validated. Method recoveries were found to be satisfactory within the range of 72% to 103%, method precision was good with a relative standard deviation (RSD) of 4% to 6% for repeatability and RSD of less than 12% for intermediate precision. Method limit of detection and determination were estimated from matrix matched calibration curves, the values were 2.9 ng ml⁻¹ and 8.7 ng ml⁻¹, respectively. The fact that no maximum residue limit has been set for thiosultap in palm oil internationally, suggesting that the default value of 0.01 mg kg⁻¹ maximum allowable limit is to be enforced. An analytical method that will enable the determination of thiosultap residue at this default level therefore is required to facilitate the monitoring of potential thiosultap residue contamination in palm oil traded in Malaysia.

Keywords: thiosultap, methamidophos, monocrotophos, palm oil.

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INTRODUCTION

Oil palm is a major agricultural commodity with significant economic and social benefits to Malaysia. In year 2018 alone, the total of Malaysian palm oil and its products exported was close to 24.82 million tonnes with a total revenue of RM 65.12 billion (Kushairi *et al.*, 2019). Like any other agricultural crops, oil palm is also subjected to attack from various pests and insects. The use of chemical pesticides is justified only if the infestation level has reached the threshold value whereby economic

loss becomes significant. Otherwise, integrated pest management (IPM) strategy is the more preferable way of pests and diseases control among Malaysian oil palm planters (Ariffin and Basri, 2000; Wood, 2002; Hunt, 2018). Bagworm and nettle caterpillars are the two damaging pests commonly found in Malaysian oil palm plantations (Ariffin and Basri, 2000). The main problem with these two leaf eating pests is that they can spread over a huge area of plantations if not detected and treated in the early stages of an outbreak.

The top three insecticides used in Malaysian oil palm plantations for the control of bagworms and leaf eating caterpillars are acephate, methamidophos and monocrotophos (MPOA, unpublished data). Methamidophos and monocrotophos are classified as Class IB restricted pesticides whereas acephate, although categorised in a less toxic group (Class

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III) (WHO, 2010), due to its tendency to metabolise into methamidophos, its usage is also restricted. The use of these three insecticides is tightly controlled by the Malaysian Pesticide Board and they are only allowed for application in oil palm and coconut plantations by trunk injection.

Concern about the effect of pesticides usage is not limited to human health alone; effects of pesticide uses to birds as well as other beneficial insects are also on the rise (BBC, 2013; Carolina, 2013). Monocrotophos, methamidophos and acephate have been proven to be harmful to birds, bee and aquatic organisms (WHO, 2009; Pohanish, 2015) and both monocrotophos and methamidophos are listed in the list of WHO highly hazardous pesticides which their usage is recommended to be progressively reduced or eliminated (FAO, 2019). With all the concerns regarding the safe use of these three insecticides, sooner or later it is necessary for the industry to look for alternative insecticides to replace methamidophos, monocrotophos and acephate, to combat bagworm and nettle caterpillars.

Thiosultap has been identified as the suitable alternative to the said insecticides as reported by oil palm planters from Indonesia (Djoko *et al.*, 2012) and Papua New Guinea (NBPOL, 2016). From literatures, thiosultap is a nereistoxin analogue insecticide of stomach poison with some contact and systemic actions (University of Hertfordshire, 2018), it is moderately toxic to mammals with an acute oral toxicity of LD_{50} in male rat = 451 mg kg^{-1} of bodyweight and no observable effect level (NOEL) of 50 mg kg^{-1} per day (Wang *et al.*, 1997). No carcinogenicity, mutagenicity and teratogenicity have been observed for thiosultap and it was also reported to be harmless to the reproductive ability of mammals (Wang *et al.*, 1999). Despite its acceptable level of safety with regard to humans, animals and crops, no reported maximum residue limit (MRL) in any crop or commodity - pesticide combinations is found internationally. Any crop or commodity traded with potential contamination of thiosultap residue is therefore subjected to a default MRL of 0.01 mg kg^{-1} .

Despite its large production capacity, Malaysia imports palm oil from its neighbouring countries (mainly Indonesia) and processes it locally either for local usages or re-export purposes (MPOB, 2019). Potential risk of thiosultap residue contamination in the imported palm oil products and our commitment in supporting the safety aspect of palm oil throughout its supply chain, triggered us for the development of a simple and fast analytical method of thiosultap residue determination in palm oil matrix, to fulfil the need for monitoring as well as enforcement purposes.

From literatures, both gas chromatography (GC) and liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques were

effective in determining thiosultap residue in crops and foods. Workflow in the determination of thiosultap using GC usually involved extraction step under acidic condition followed by conversion of thiosultap to nereistoxin in an alkaline medium with the aid of sodium sulphide as a catalyst. Nereistoxin was then extracted using an organic solvent before the final sample was analysed (Standardisation Administration of China, 2003; Zhang, 2005; Li *et al.*, 2012; AQSIG, 2014), quantification of thiosultap was then calculated from the amount of nereistoxin found. The workflow is laborious and involves the use of toxic sodium sulphide that is difficult to acquire.

On the other hand, determination of thiosultap residue via LC-MS/MS technique was much simpler compared to GC. No conversion of thiosultap to nereistoxin was required, the extraction and clean-up of thiosultap residue from its matrices were performed through quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Ferrer *et al.*, 2010) or direct sample injection without further clean-up after the extraction of thiosultap residue using mix methanol/water (1:1, v/v) as the solvent (Lu *et al.*, 2008; Huang *et al.*, 2015).

To the best of our knowledge, there is no reported analytical method for determination of thiosultap residues in palm oil matrix currently available, and herein this article reports a simple and fast analytical method for the determination of thiosultap in palm oil matrix using triple-quadrupole LC-MS/MS.

MATERIALS AND METHODS

Reagents and Chemicals

All solvents used were of at least analytical reagent grade, thiosultap standard material (97.4% purity) was obtained from Sigma-Aldrich (USA). Ultrapure water from a Merck Millipore Direct Q® 8-UV-R water purification system (Darmstadt, Germany) was used throughout the experiment. All standard solutions prepared in this study were stored in refrigerator at temperature of 4°C-8°C prior to use. Maximum storage period for stock standard solution was 14 days and other working standard solutions used in this study were freshly prepared daily.

Crude Palm Oil (CPO)

Blank CPO was obtained from a nearby palm oil mill and was filtered through 10% oil weight of anhydrous sodium sulphate (MPOB, 2005), to ensure the sample used in method validation studies is dry and free from insoluble materials. This blank CPO was pre-analysed prior to method validation study to ensure it was free from thiosultap residue.

Standard Solutions

A thiosultap stock solution was prepared by dissolving 10.3 mg of the standard material in 100 ml methanol to give a final concentration of 100 $\mu\text{g ml}^{-1}$. This stock solution was used for the preparation of working stock solutions (10 $\mu\text{g ml}^{-1}$) in sample spiking and in the preparation of calibration standard solutions. Matrix matched calibration standard solutions were prepared through serial dilution of thiosultap working stock solutions in blank CPO extract to give matrix matched calibration working standard solutions required.

Sample Extraction Procedures

Extraction procedures by Fariq Fitri *et al.* (2017) with slight modification was followed in this study. Five grams portion of melted blank CPO was first weighted into a 50 ml polyethylene self-standing centrifuge tube. Then, 5 ml of dichloromethane was added and mixed well, this was then followed by the addition of 5 ml of water prior to the extraction process.

The mixture was shaken (vortex mixer) for 2 min \times 3 times, with a 2 min intermittence heating step (60°C, water bath) in between (Figure 1). After the completion of the extraction process, the mixture was centrifuged at 4000 rpm (2147 \times g) for 10 min to allow phase separation. Aliquot of 1 ml from the aqueous phase was then taken for LC-MS/MS analysis.

Matrix Matched Calibration Curve

A six concentration levels matrix matched calibration curve of thiosultap was constructed by the injection of matrix matched working standard solutions at 5, 10, 20, 30, 50, and 70 ng ml^{-1} , respectively in three replicates. Individual matrix matched working standard was placed randomly in the injection sequence to minimise the effect of instrumental drift.

Spiked Samples Preparation

Melted blank CPO (300 g) was stirred in a dry and clean 500 ml beaker (tall form) placed in a water

bath set-up controlled at 60°C on top of a hot plate. The 30 μl of thiosultap stock solution (100 $\mu\text{g ml}^{-1}$) was then added. The whole mixture was allowed to continue stirring for another 40 min prior to the sub-sampling of spiked control samples.

While keeping the stirring on, 5 g each of spiked sample (spiking level of 10 ng g^{-1}) was weighed. A total of 50 spiked samples were obtained and were stored in a deep freezer controlled at -18°C. These samples were used in method validation study. Same procedures were repeated for the preparation of samples spiked with thiosultap at 50 ng g^{-1} level.

LC-MS/MS Analysis

LC-MS/MS analysis was performed with an AB Sciex QTRAP® 4500 (Foster City, CA; USA) triple quadrupole MS/MS with ESI in the negative ion mode at 5500 V, 550°C, coupled to an Eksigent Ekspert™ UltraLC 100 UHPLC system (Eksigent, Redwood City, CA, USA). Instrument control and data acquisition/processing were performed using Applied Biosystems Analyst 1.6 software supplied. The analytical column was a Phenomenex Kinetex® C18 100 Å 50 \times 2.1 mm i.d., 2.6 μm particle size, coupled to a 2 \times 2.1 mm i.d. Security Guard C18 column. The column temperature was 40°C, injection volume was 10 μl and flow rate was 200 $\mu\text{l min}^{-1}$. The mobile phase was composed of methanol as the organic phase and an aqueous solution of ammonium acetate (10 mM, adjusted to pH 5.0). The following gradient elution profile was used: the initial conditions (95% aqueous phase) were maintained for 0.5 min and then the organic eluent was increased to 95% in 5 min. This composition was kept constant during 5 min, before being returned to the initial conditions after 0.5 min, keeping this composition during 5 min prior to the next analysis, giving a total runtimes of 16 min. Determination of thiosultap residue was performed via multiple reaction monitoring (MRM) experiment with three precursor-daughter ion transitions monitoring. Detail setting of the mass spectrometer such as declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) can be found in Table 1. Examples of blank CPO extract and thiosultap standard chromatograms are showed in Figure 2.

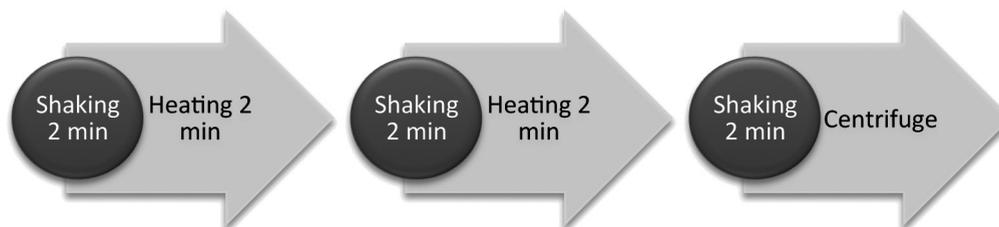


Figure 1. Sample extraction process with intermittence heating (total shaking period = 6 min).

TABLE 1. MULTIPLE REACTION MONITORING EXPERIMENT PARAMETERS SETTING IN THIOSULTAP DETECTION

Ion transition	DP (volt)	EP (volt)	CE (volt)	CXP (volt)	Remark
310 → 185	-27	-10	-29	-6	Quantifying ion
310 → 230	-27	-10	-23	-7	Qualifying ion
310 → 246	-33	-10	-18	-5	Qualifying ion (optional)

Note: DP - declustering potential, EP - entrance potential, CE - collision energy, CXP - collision cell exit potential.

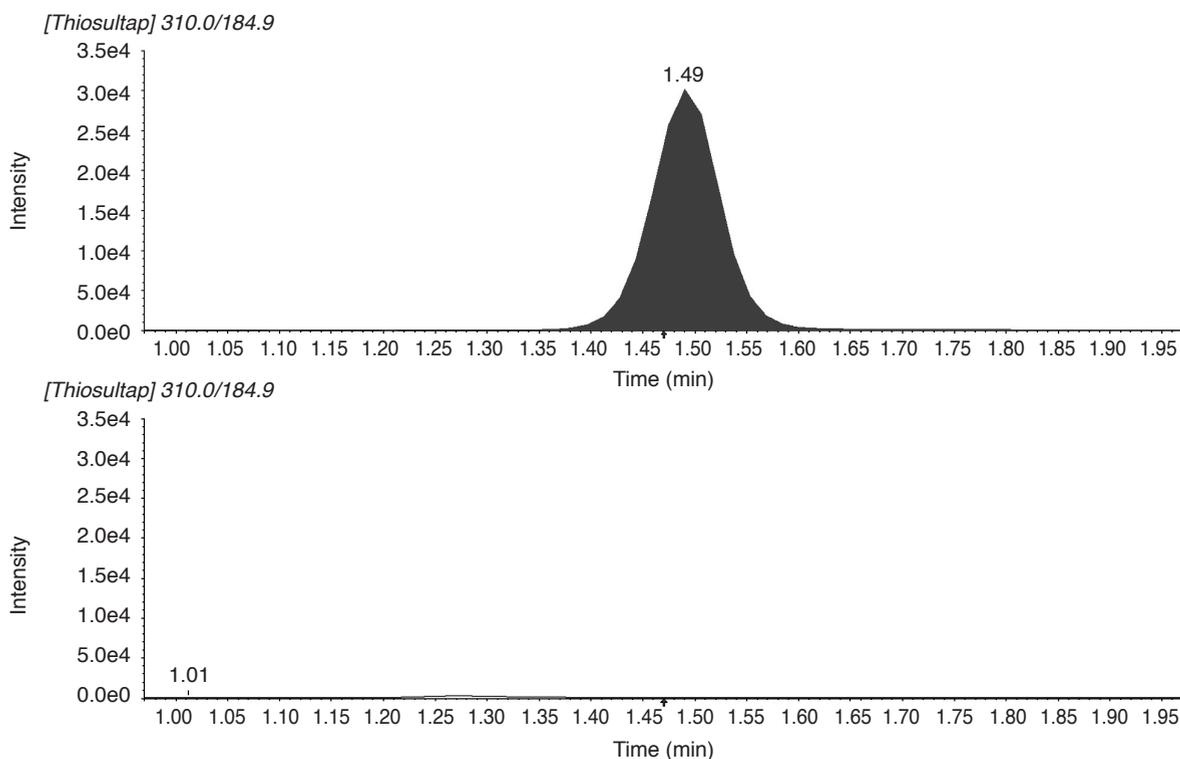


Figure 2. Chromatograms of thiosultap at 5 ng ml⁻¹ (top) and blank crude palm oil extract (bottom).

Method Validation

All method validation studies were conducted using blank CPO following the requirements set in SANTE guidance document (European Commission, 2017). Method performance parameters such as linearity, selectivity, limit of detection (LOD), limit of quantification (LOQ) and method precision were particularly emphasised.

RESULTS AND DISCUSSION

Linearity, Matrix Effect and Selectivity

To study the matrix effect (ME) in the quantification of thiosultap residue, two calibration curves (5-70 ng ml⁻¹), one prepared in ultrapure water and another one prepared in blank matrix extract were plotted (Figure 3), ME was then quantified by using Equation (1) (Pano-Farias *et al.*, 2017). From Figure 3, it can be seen that thiosultap signal suffered from ion enhancement effect caused

by the matrix (> 30% of ME). Therefore, matrix matched calibration curve was used in this method.

$$ME = \left(1 - \frac{\text{Solvent slope}}{\text{Matrix matched slope}}\right) \times 100\% \quad \text{Equation (1)}$$

Calibration curves obtained were linear through visual evaluation. From the residual plotting, no discernible distribution pattern was found confirming that linear regression model was an appropriate function. As for the method selectivity, comparison of chromatogram for blank CPO extract and thiosultap standard at 5 ng ml⁻¹ (Figure 2) showed that no significant interference signal was observed at the time window where the thiosultap signal appeared (retention time = 1.49 min), suggesting that the method selectivity was acceptable.

LOD and LOQ

LOD and LOQ were estimated from the calibration curves plotted during intermediate precision study following Equations (2) and (3)

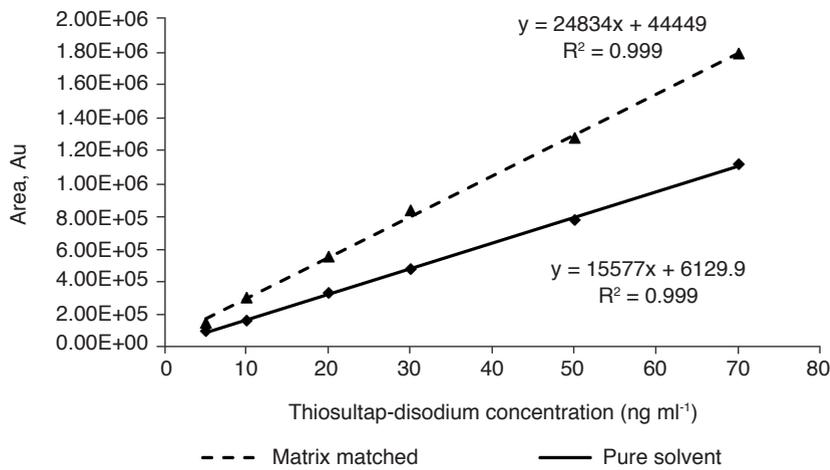


Figure 3. Thiosultap-disodium calibration curves (5 to 70 ng ml⁻¹) prepared in pure solvent and matrix matched.

(ICH, 2005), respectively. Most conservative values were selected as the estimated method LOD and LOQ. Method LOD and LOQ were 2.9 ng ml⁻¹ and 8.7 ng ml⁻¹, respectively.

$$\text{LOD} = 3.3 \cdot \frac{\sigma}{S} \quad \text{Equation (2)}$$

$$\text{LOQ} = 10 \cdot \frac{\sigma}{S} \quad \text{Equation (3)}$$

where, σ is the standard deviation of the regression line and S is the slope.

Repeatability and Intermediate Precision

Repeatability and intermediate precision data were obtained from the analysis of randomly picked spiked control samples at random interval throughout the method development period. Six batches of spiked control samples were analysed in four months period and the results are showed in Table 2. During the third and sixth batch of analysis, a minor tweak on the extraction procedures was performed whereby the intermittence heating steps had been omitted. The purpose of this minor tweak was to evaluate the effect of intermittence heating step to the extraction efficiency.

From the results, it can be seen that individual repeatability for a particular batch of analysis at different spiking levels was excellent (European

Commission, 2017; Huber, 2007) with a relative standard deviation (RSD) below 6% and 4%, respectively (far lower than maximum allowable repeatability RSD of 20% set in guideline).

Method precision may be affected by variations in experimental conditions such as different batches of reagents used, operator skill, column efficiency, different time in which the analysis was conducted and so on over a period of time. Impacts from those experimental variations to the method precision can be evaluated through intermediate precision. By using the same set of precision data as shown in Table 2, the method intermediate precision was found to have RSD values lower than the maximum allowable RSD set in the SANTE guideline (European Commission, 2017) with an RSD of 9.30% and 11.13% for samples spiked at 10 ng g⁻¹ and 50 ng g⁻¹, respectively.

Recovery Study

No separate experiment was carried out to evaluate the recovery performance of the method developed as the information required can be extracted from method precision data. In general, it can be said that the method recovery was well within general accepted ranges of 70% to 120% (European Commission, 2017; Huber, 2007), for both 10 ng g⁻¹ and 50 ng g⁻¹ spiking levels.

Interestingly, it was noted that when intermittent heating was omitted during the analyte

TABLE 2. RESULTS OF PRECISION STUDY

Sample No.	10 ng g ⁻¹ Spiking level			50 ng g ⁻¹ Spiking level		
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Average recovery (%)	102.5 (n=7)	100.7 (n=5)	83.5 (n=5)	80.6 (n=6)	93.0 (n=8)	72.2 (n=6)
RSD (%)	0.96	1.79	5.47	0.55	3.91	1.33

Note: RSD - relative standard deviation.

extraction (batches 3 and 6 of precision study), the recoveries of thiosultap residue dropped nearly ~20%, this showed that intermittent heating was crucial to the method. The possible explanation was that during the extraction process, possible formation of micro crystals in palm oil matrix may have prevented the partitioning of the analyte of interest into the aqueous phase and intermittent heating step has helped to melt the micro crystals formed and thus increased the extraction efficiency.

CONCLUSION

A short and simple method for thiosultap residue determination in palm oil matrix was developed. This method was validated for its linearity, selectivity, recovery, repeatability and intermediate precision. Scores obtained by all these method performance parameters were better or meet with the performance criteria set in the reference guideline. LOD and LOQ for the method were also estimated from its calibration curve and were found to be 2.9 ng ml⁻¹ and 8.7 ng ml⁻¹, respectively.

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