SIMULTANEOUS EXTRACTION AND CLEAN-UP OF CHLORPYRIFOS FROM SPINACH (*Spinacia oleracea*) USING PRESSURISED LIQUID EXTRACTION (PLE)

(Pengekstrakan dan Pembersihan Serentak Klorfirifos Daripada Bayam, *Spinacia oleracea*, Menggunakan Kaedah Pengekstrakan Cecair Tekanan Tinggi)

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Abstract

A simple one-step extraction and clean-up using pressurised liquid extraction (PLE) of chlorpyrifos from spinach (*Spinacia oleracea*) was developed. In this study, the effect of Florisil placed inside the extraction cell on recovery of chlorpyrifos and percentage bleachability of chlorophyll-a was investigated. Extractions were carried out using *n*-hexane and performed using these PLE conditions: extraction temperature of 100 C, pressure of 1500 psi and static extraction time of 10 min. Increase in the amount of Florisil resulted in increase in the bleachability of chlorophyll a. It was found that 1.0 g Florisil was able to absorb 92.0% chlorophyll a without affecting the recovery of chlorpyrifos.

Keywords: spinach, pressurised liquid extraction, simultaneous extraction and clean-up

Abstrak

Satu langkah pengekstrakan dan pembersihan ekstrak menggunakan kaedah pengekstrakan cecair tekanan tinggi (PCTT) bagi klorfirifos daripada bayam (*Spinacia oleracea*) telah dibangunkan. Dalam kajian ini kesan Florisil yang diletakkan dalam sel pengekstrakan ke atas perolehan semula klorfirifos dan peratus pelunturan klorofil a telah dikaji. Pengekstrakan dijalankan menggunakan *n*-heksana dengan keadaan operasi PCTT: suhu pengekstrakan 100 °C, tekanan 1500 psi dan masa pengekstrakan 10 minit. Pertambahan amaun Florisil memberi kesan terhadap pelunturan klorofil a. Didapati 1.0 g Florisil berupaya menjerap 92.0 % klorofil a tanpa memberi kesan ke atas perolehan semula klorfirifos.

Kata kunci: bayam, pengekstrakan cecair tekanan tinggi, pengekstrakan dan pembersihan serentak

Introduction

Developing faster, cost-effective and eco-friendly procedures in the determination of pesticides in food matrices is a pressing demand to meet the challenges of modern food quality requirements. PLE is one of the latest techniques developed for extraction of solid samples and was introduced in 1995. PLE allows faster extraction than classical methods and requires only small volume of solvent [1]. Interest in selective PLE procedures where integrated clean-up strategies are utilized to combine extraction and clean-up is increasing as they dramatically cut time and costs in the sample preparation step [2]. Several types of sorbents have been used by researchers to achieve these objectives [2,3]. In this study, the possibility of adding sorbent into the extraction cell to remove chlorophyll a from spinach extract was investigated. The effect of different dosage of sorbent on the amount of chlorophyll removed (% bleachability of chlorophyll) and the percent recovery of chlorpyrifos was examined.

Experimental

Sample Preparation

Fresh spinach was obtained from local market in Shah Alam. The samples were washed under running tap water and air-dried until the moisture content reached 11.00 ± 0.67%. Prior to extraction, samples were further chopped to 3 mm diameter.

Chemicals and materials

Chlorpyrifos was obtained from Riedel-de Haën (Seelze, Germany), *n*-hexane was of pesticide grade purchased from Merck (Darmstadt, Germany). Diatomaceous earth non-washed, SiO_2 approximately 90 % purity (Sigma, USA) and anhydrous sodium sulphate (HmbG Chemicals) were used as drying agents for PLE

extraction. Florisil (Fisher Scientific, Loughborough, United Kingdom) used for clean-up was activated at 130 ^{o}C for 24 hours. Activated carbon, (REM Chemicals, U.K.) was soaked with methanol (MERCK, Germany) for 24 hours, pH maintained about 6-7, filtered with Whatman no 1 filter paper and dried at 50 ^{o}C. Both activated carbon and Florisil were cooled in desiccator prior to use.

Pressurised Liquid Extraction (PLE)

Extractions were done using ASE 200 accelerated solvent extractor (Dionex Ltd. Camberly, Surrey, UK). Samples (2 g air-dried spinach leaves), 1 diatomaceous earth and 1 g anhydrous sodium sulphate were accurately weighed into 22 mL cells with cellulose filter at the bottom end as shown in Figure 1. The sample cells were then closed to finger tightness and placed into the carousel of the ASE 200 system. Extractions were done using *n*-hexane. Chlorpyrifos was extracted using the following conditions: temperature of 100 ^{o}C, pressure of 1500 psi and static time of 10 minutes. Other conditions were; preheat time: 2 min, flush volume: 80 %, purge time: 60 seconds and static cycle: 1. The extracted analytes were purged from the sample cell using pressurised nitrogen (125-150 psi; 1 psi = 6894.76 Pa). The extract was evaporated to dryness (rotary evaporator and nitrogen purge) and redissolved with 1 mL *n*-hexane prior to GC analysis.

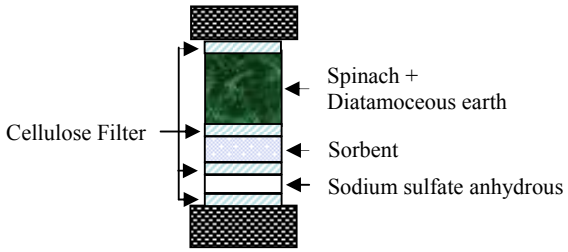

Figure 1: Schematic diagram of the packing of ASE 200 extraction cell (22 ml)

UV/Vis Analysis

The absorbance of chlorophyll-a was measured at 662nm using Perkin Elmer Lamda 25. The % bleachability of chlorophyll-a due to the presence of sorbent was calculated using the formula given below [4]:

$$\% \text{ Bleachability} = \frac{\text{Absorbance (662nm) of crude extract - Absorbance (662nm) of bleached extract}}{\text{Absorbance (662nm) of crude extract}} \times 100$$

Gas Chromatographic analysis

Chlorpyrifos was analyzed using Agilent Technologies 7683B Series GC system equipped with electron capture detector. A HP-5 MS capillary column (Agilent 19091S-433, 0.25mm x 30m 0.25µm) was used. Column temperature was gradually increased from 180 ºC (1 min) to 230 °C at 5 °C min^{-1}. Nitrogen was the carrier gas, at a flow rate of 2.0 mL/min in split mode (20:1).

Results and Discussion

Effect of sorbents on the recovery of chlorpyrifos

Florisil has been widely employed in a clean-up step in pesticides analysis [5]. As shown in Table 1, good recoveries (92.2 -102.2 %) of chlorpyrifos were obtained using various amount of Florisil. However, low recovery of chlorpyrifos (53.3 %) was obtained using 0.8 g activated carbon.

Effect of sorbents on the bleachability of chlorophyll

Florisil has been used as the adsorbent to clean up xanthophylls, carotene oxidation products, and chlorophylls to quantify carotene in plant according to AOAC Method [6]. The effect of sorbent on the bleachability of chlorophyll-a was quantified based the absorbance at 662 nm. As shown in Figure 1, the absorbance at 662 nm decreases with an increase amount of Florisil added to the extraction cell. Increase in adsorption can be

attributed to increased surface area and the availability of more binding sites for adsorption [7]. The % bleachability of chlorophyll-a is shown in Table 1. As the amount of the Florisil increases, the green colour of the extract faded, finally giving a pale green extract with an addition of 1.0 g Florisil. Further increase in the amount of Florisil produced a yellow extract revealing the presence of yellow β-carotene. Activated carbon was able to absorb chlorophyll-a, β-carotene and other coloured pigments as the extract obtained was colourless.

The use of a suitable sorbent in PLE extraction led to efficient extraction and clean-up of chlorpyrifos from spinach extract. Addition of 1.0 g of Florisil was sufficient to clean the extract without compromising on the efficiency of extraction. Eventhough activated carbon was able to completely absorb chlorophyll and other pigments, some of the chlorpyrifos was all also retained resulting in low recovery (53.3 %).

Table 1. Effect of sorbent on the PLE extraction of chlorpyrifos from spinach

Sorbent added	Recovery (%)	Bleachability (%)
Without sorbent	104.5	0
0.2 g Florisil	92.2	37.6
0.4 g Florisil	101.6	49.4
0.6 g Florisil	102.2	81.6
0.8 g Forisil	99.9	89.2
1.0 g Florisil	102.0	92.0
1.5 g Florisil	96.5	99.8
0.8 g activated carbon	53.3	100

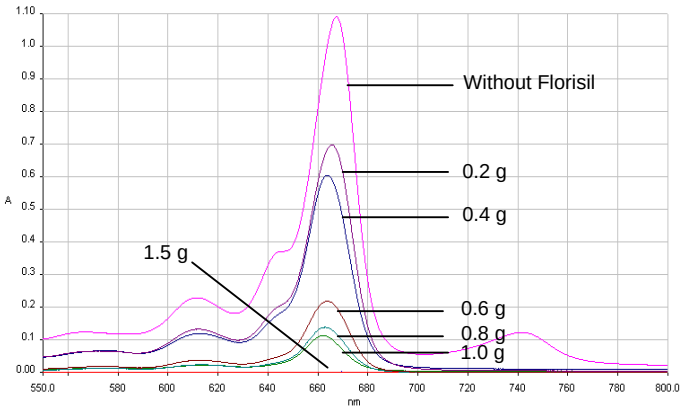

Figure 1. UV/Vis spectrum of spinach extract using different amount of Florisil

Conclusion

Simultaneous extraction and clean-up for the analysis of chlorpyrifos in spinach can be achieved by the addition of Florisil (1:2; g Florisil:g sample) in the extraction cell. This approach is able to simplify the analysis and reduce solvent usage.

Acknowledgements

The authors would like to acknowledge the financial support of Universiti Teknologi MARA and FRGS grant (5/3/1318).

References

1. Hussen, R. Westbom, N. Megersa, L. Mathiasson , Bjorklund, E. 2006. Development of a pressurised liquid extraction and clean-up procedure for the determination of α-endosulfan, β-endosulfan and endosulfan sulphate in aged contaminated Ethiopian soils. Journal of Chromatography A. 1103, 202-210.
2. Björklund, E., Sporring, S., Wiberg, K., Haglund, P. and Holst, C. 2006. New strategies for extraction and clean-up of persistent organic pollutants from food and feed samples using selective pressurised liquid extraction. Trends in Analytical Chemistry 25 (4), 318-325.
3. Rodil, R. and Moeder, M. 2008. Development of a simultaneous pressurised-liquid extraction and clean-up procedure for the determination of UV filters in sediments. Analytica Chemica Acta 612 (2), 152-159.
4. Omar, S., Girgis, B. & Taha, F. 2003. Carbonaceous materials from seed hulls for bleaching of vegetable oils. Food Research International 36 (1): 11-17.
5. Hussen, R. Westbom, N. Megersa, L. Mathiasson , Bjorklund, E. 2007. Selective pressurised liquid extraction for multi-residue analysis of organochlorine pesticides in soil. Journal of Chromatography A. 1160, 64-70.
6. Official Methods of Analysis of AOAC International 2000. 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.
7. Mor, S., Ravindra, K., Bishnoi, N.R. 2007. Adsorption of chromium from aqueous solution by activated alumina and activated charcoal. Bioresource Technology 98: 954-957.