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Short communication

Use of the Keele injector for sample introduction for gas chromatographic analysis of vinclozolin in lettuces

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Abstract

We examined a Keele injector for sample introduction for gas chromatographic analysis of vinclozolin treated in lettuces. Samples in milligram quantity were introduced into a glass tube in a Keele injector at a gas chromatograph injection port. The glass tube was then crushed to allow the sample to carry onto a capillary column in a normal manner. The standard calibration curve for quantitative detection of vinclozolin was obtained by determining vinclozolin spiked in samples at variable concentrations. The calibration curve showed a linear response to vinclozolin ranging from 0.05 to 1.0 μg/g, giving a slope value of 174.8, the *y*-intercept value of −2.8146 and the mean r^2-value of 0.9994. Limit of quantification for vinclozolin was 0.05 μg/g by this method, comparable to 0.01 μg/g by a normal injector. When samples treated previously with vinclozolin were determined by the Keele injector, vinclozolin was found to be about 30% lower as compared to a normal method, suggesting about 70% recovery of the spiked vinclozolin by the Keele injector. From these results, the Keele injector was suggested to be potential for sample introduction in gas chromatographic analysis of vinclozolin in lettuce samples.

Keywords: Injection methods; Keele injector; Vinclozolin; Pesticides

1. Introduction

Traditional approach for determination of pesticides in agricultural samples requires solvent extraction and cleanup procedures. The procedures are critical steps in pesticide analysis. They are tedious, time consuming and expensive because of high cost of the solvent. Given that agricultural productivity have been increased and large number of samples should be examined routinely, so a simple method is required for rapid determination of pesticides.

In an effort to develop less time-consuming and more economical methods for pesticide analysis, solid-phase microextraction has been introduced as an alternative method for sample preparation [1–3]. In solid-phase microextraction, analytes are sorbed

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into a liquid matrix coated onto a fused-silica fiber. The sorbed analytes are then thermally desorbed in a gas–liquid chromatograph (GC) injector port to carry them into GC column. Another approach is to use a solid injector for direct sample introduction instead of using a conventional syringe-based sample injector. Amirav and Dagan [4] and Jing and Amirav [5] demonstrated a new type of direct sample introduction (DSI) device allowing extract-free dirty sample introduction for gas chromatographic analysis of pesticides. This type of DSI device has become commercially available from Varian as the ChromatoProbe.

Vinclozolin has been used widely for the control of fungal diseases in a variety of vegetables and fruits in Korea since 1980. Vinclozolin is currently of toxicological concern due to its endocrine disrupter effect [6,7]. The growing toxicological concern over viclozolin has prompted studies on analytical techniques for better monitoring this pesticide in agricultural products. A high-performance liquid chromatography (HPLC) and gas–liquid chromatograph (GC) methods were reported elsewhere [8–11]. In these studies, samples were usually optimized into set-up conditions to give clean extracts. To the best of our knowledge, injecting samples directly into GC would be more rapid method for vinclozolin analysis providing samples can be determined without any interference in chromatogram analysis.

In this study, we examined a Keele injector for direct sample introduction in gas chromatographic analysis of vinclozolin in vegetable sample. Lettuces were used as a typical sample, since vinclozolin has been used extensively in lettuces in Korea. Without solvent extraction, the samples in a small size were directly introduced into GC by using the Keele injector. Using the Keele injector allowed quantitative determination of vinclozolin in the lettuces samples.

2. Experimental

Vinclozolin-free lettuces were treated with the formulated vinclozolin, Nolan®. For the control, the lettuces had no vinclozolin. The lettuce samples were removed from the formulated solution and allowed to stand at room temperature until the solution ceased dropping from the samples. The samples were dried in a drying oven at 60 °C for 1 h and cut into a small size. The dried samples were carefully weighed in milligram quantity and introduced into a soft glass capillary tube (1.2 mm i.d. × 30 mm in length). Both ends of the tube were sealed briefly in a flame. The tube was then placed in a Keele injector. A Keele injector was purchased from GSG Analytical Instruments (Warrington, Cheshire, UK) and modified for a gas chromatograph injector in our laboratory (Fig. 1). After heating at injection port for about 5 min, the tube was crushed by lowering injector plunger to carry sample analytes onto GC column by carrier gas.

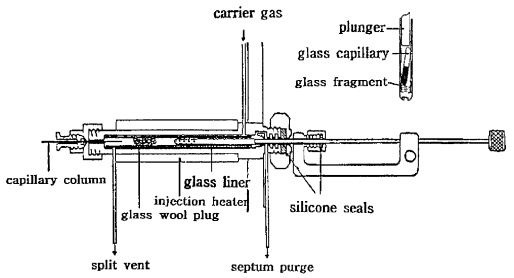

Fig. 1. Drawing of a Keele injector used in this study. This was reprinted from [18] with permission from the author.

In some experiments, the dried samples obtained above were used to determine the concentration of vinclozolin by a traditional method. For this, 5 g of samples were extracted with six times their volume of acetone–dichloromethane (1:1 (v/v)) as described earlier [10]. The extracts were centrifuged at 8000 × *g* and the supernatant was collected. The extract was concentrated to dryness by a rotary vacuum evaporator at 40 °C. The dry extract was dissolved in *n*-hexane and a 1 μl of aliquot was injected in a normal GC injector. The concentration of vinclozolin were calculated from its standard calibration curve and compared with the concentration of vinclozolin found by the Keele injector.

A Hewlett-Packard 5890 gas chromatograph equipped with electron capture detector (ECD) was used for determination of vinclozolin. The column was Hewlett-Packard HP-5 (15 m × 0.53 mm i.d., 1.5 μm thickness) capillary column. Operation conditions are as follows: injector temperature, 200 °C; oven program temperature, 100 °C for 2 min, increased from 10 to 220 °C; carrier gas N_2 at a constant flow, 1.0 ml/min; split ratio, 10:1.

The stock solutions of the vinclozolin standard were prepared by dissolving 10 mg of the standard in 100 ml of *n*-hexane to give final concentration of 100 μg/ml. The working solutions for GC injections were prepared by serially diluting the stock solutions in the same solvent. Standard calibration curves were obtained by injecting five-level concentrations of the working solutions and measuring the peak areas of their chromatograms. For the Keele injector method, an aliquot (1 μl) of the working solutions was added to control samples prepared in a glass capillary tube. The tube was dried at room temperature to allow solvent evaporation, sealed briefly in a flame and introduced into the Keele injector as described above. For a normal injector method, a 1 μl of aliquot of the working solutions was injected by using a syringe. The ratio of signal to noise (S/N) was 3.

3. Results and discussion

Fig. 2 shows the calibration curves of vinclozolin injected by the Keele injector and a normal syringe. The data given are means with maximum 5% error in triplicate measurements. The standard calibration curve obtained by the Keele injector showed a linear response to vinclozolin ranged from 0.05 to 1.0 μg/ml, giving a slope value of 174.8, the *y*-intercept value of −2.8146 and the mean r^2-value of 0.9994. When vinclozolin was injected by a syringe in a normal manner, approximately 34% higher response than the Keele injector was observed. The difference in response between the Keele and normal injector methods was similar at all injections for each working solution. The Keele injector method showed a temperature-dependent response, giving higher loss of vinclozolin at higher temperature. This suggests that the loss would be much more significant for more labile pesticides. Therefore, optimum temperature should be tested with the Keele injector for such pesticides. As considered the temperature-dependent loss of vinclozolin with the Keele injector, the samples should be injected immediately without the extended 5 min to have better detection. The 5 min were needed to have a constant pressure of carrier gas before the injection, which allowed a constant baseline for ECD signals after the injection. When samples were determined without the extended time, a slightly higher concentration of vinclozolin was observed as compared to that determined with the time, suggesting the involvement of the extended stay for the loss. The exact reason is not still clear if the 34% loss, as compared to a normal injection method, was due to the sample capillary tube hot sealing, glass wool plugged or sample matrix effect. Therefore, further study is required to address this.

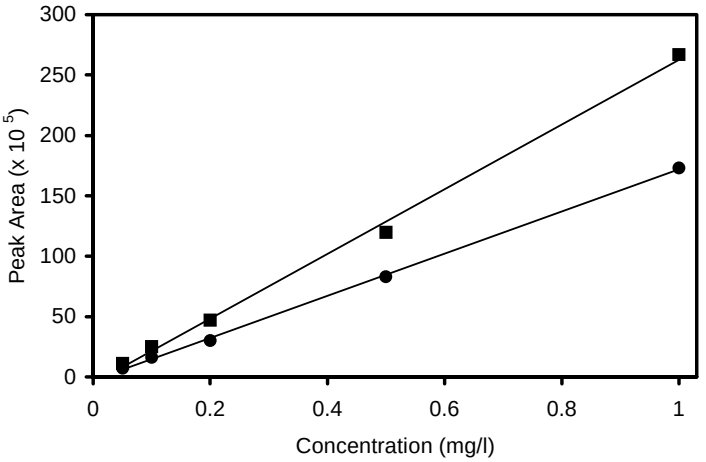

Fig. 2. Standard calibration curve of vinclozolin injected by: a normal (■) and the Keele injectors (●). In the Keele injector, samples in a milligram quantity were placed into a glass tube and spiked with a variable concentration of vinclozolin before the injection. The data given are means with maximum 5% error in triplicate measurements.

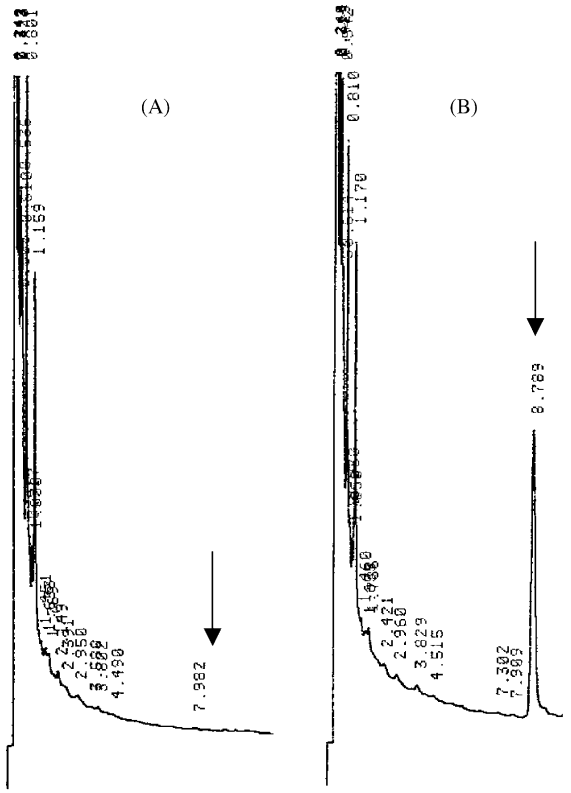

Fig. 3. Typical chromatograms of: (A) control and (B) vinclozolin-treated samples injected by the Keele injector. The arrow symbols represent the time where vinclozolin was detected on chromatogram by GC-ECD.

Fig. 3 shows GC chromatograms of control and vinclozolin-treated samples injected by the Keele injector. No detectable chromatogram peak at a retention time of vinclozolin was observed in control samples (Fig. 3A), suggesting no interferences in the area of interest of the chromatogram. In contrast, a chromatogram peak that was not observed in control samples was observed at 8.79 min in lettuce samples treated with the Nolan® (Fig. 3B). This peak had the same retention time as that of the vinclozolin standards. These observations suggested that this peak was vinclozolin applied in the samples. The concentration of vinclozolin in the samples was about 0.72 μg/g from the standard calibration curve of vinclozolin injected by the Keele injector. When lettuce samples were extracted with a solvent and injected by a normal manner, vinclozolin in the samples was found to be 1.04 μg/g, which was approximately 1.4 times higher than the concentration determined by the Keele injector. This difference in concentration of vinclozolin was similar to the difference found in calibration curve profiles (Fig. 2). In calibration curve assay, a normal injection method showed about 34% higher response than the Keele injector method. This suggests that the loss of vinclozolin in standard calibration assay and sample analysis was probably from an unidentified same reason.

A direct sample introduction (DSI) device has been well demonstrated in an effort to develop more economical method for pesticide analysis [12,13]. With the DSI device, a disposable vial containing a small amount of sample previously blended with acetone was placed into a temperature-programmable GC injector port and heated gently at first to evaporate solvent in the samples. The vial was then heated gradually to desorb thermally pesticides in the samples. Using the DSI device coupled with a selective GC detector such as sulfur or phosphorus detector and GC mass spectrometry have been reported for selective detection of many pesticides in complex sample extracts [14,15]. Taken from these studies, the direct sample introduction method is suggested as a new approach for better monitoring pesticides in samples.

In this study, we examined a Keele injector as a method for direct sample introduction. This injector was developed initially for analysis of the biological constitutes of insect glands [16–18], but little work has been reported for pesticide analysis. With the Keele injector, samples in milligram quantity were directly determined without solvent extraction procedure. It could be argued that such samples would not be a representative for entire samples. The experimental error due to weighing the samples would be reduced once a Keele injector device for introduction of samples of more than a milligram size is available. It must be emphasized that careful sample handling is required in weighing the sample in milligram quantity and delivering it into a glass capillary tube to avoid an experimental error. When the samples were determined by the Keele injector, the glass particles remained in the injector at the end of analysis could be disposed simply after every injection by removing the Keele injector from injection port. Waiting for the detector signal to be constant after the first analysis allowed a new

sample preparation for the second injection. Although the Keele injector did not use solvent for sample preparation, it needed a small quantity of solvent for other purposes. For example, solvent washing by a normal injection manner was required to have long-term stability of GC column and to keep the column and injector clean.

Currently, agricultural business is growing by aid of world-trade market, giving large population of the products. However, no information on what pesticides would be found in the products is available, before they are examined. This knowledge informs us about an important issue that more rapid screening method is required for determination of pesticides in the products. By using a Keele injector coupled with a gas chromatograph–mass spectrometry, screening rapidly the samples and then knowing the contamination history of the samples by the pesticides would allow pesticide examiners to perform further analysis of the samples in more detail, if necessary.

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References

[1] M. Natangelo, S. Tavazzi, R. Fanelli, E. Benfenati, J. Chromatogr. A 859 (1999) 193.
[2] S.-F. Chen, Y.-S. Su, J.-F. Jen, J. Chromatogr. A 896 (2000) 105.
[3] M.C. Sampedro, O. Martín, C.L. de Armentia, M.A. Goicolea, E. Rodriguez, Z.G. de Balugera, J. Costa-Moreira, R.J. Barrio, J. Chromatogr. A 893 (2000) 347.
[4] A. Amirav, S. Dagan, Eur. Mass Spectrom. 3 (1997) 105.
[5] H. Jing, A. Amirav, Anal. Chem. 69 (1997) 1426.
[6] Overview of the vinclozolin risk assessment, US Environmental Protection Agency, Office of Pesticide Programs Registration Document, 2 February 2001.
[7] L.E. Gray, J. Ostby, E. Monosson, W.R. Kelce, Toxicol. Ind. Health 15 (1999) 48.
[8] M. Sandahl, E. Ulfsson, L. Mathiasson, Anal. Chim. Acta 424 (2000) 1.
[9] E. Viana, J.C. Molto, G. Font, J. Chromatogr. A 754 (1996) 437.
[10] J. Oliva, S. Navarro, A. Barba, G. Navarro, J. Chromatogr. A 833 (1999) 43.
[11] P. Sandra, B. Tienpont, J. Vercammen, A. Tredoux, T. Sandra, F. David, J. Chromatogr. A 928 (2001) 117.
[12] S.J. Lehotay, A.R. Lightfield, J.A. Harman-Fetcho, D.J. Donoghue, J. Agric. Food Chem. 49 (2001) 4589.
[13] P. Jüngel, S. de Koning, U.A.Th. Brinkman, E. Melcher, J. Chromatogr. A 953 (2002) 199.
[14] A. Amirav, H. Jing, J. Chromatogr. A 814 (1998) 133.
[15] S.J. Lehotay, J. AOAC Int. 83 (2000) 680.
[16] E.D. Morgan, L.J. Wadhams, J. Chromatogr. Sci. 10 (1972) 528.
[17] A.G. Bagneres, E.D. Morgan, J. Chem. Ecol. 16 (1990) 3263.
[18] E.D. Morgan, Anal. Chim. Acta 236 (1990) 227.