Accelerated Analysis of On-Site Pesticide Detection in Vegetables by Agilent 5975T LTM GC/MSD and TSP

Application Note

Food Diagnostic

Authors

Suli Zhao, Andy Zhai
Agilent Technologies (Shanghai) Co., Ltd.
412 Yinglun Road
Shanghai 200131
China

Abstract

Thermal separation probe (TSP) is a rapid, rugged, and inexpensive approach to gas chromatography (GC) or gas chromatography mass spectrometry (GCMS) for analysis of semi-volatiles such as pesticides. With TSP, no sample cleanup is required to achieve quantitative and confirmatory results for quick, on-site detection of pesticides. A fast method is established for the transportable 5975T LTM GC/MSD. With the help of DRS software, we provide a good solution for accelerated analysis of pesticides for on-site detection.

Agilent Technologies

Introduction

Current methods used in the analysis of pesticide residues in vegetables are time-consuming, labor intensive, costly, or do not detect a wide range of analytes. Extraction of fruit and vegetables with acetone, acetonitrile or ethyl acetate is often followed by a clean-up step to remove co-extractives before gas chromatographic analysis. In traditional method, clean-up is necessary to prevent the build-up of nonvolatile matrix components in the injection liner and capillary column, and reduce the rate of deterioration in chromatographic performance of the GC system. For example, the U.S. Food and Drug Administration (FDA) is responsible for the monitoring of vegetables in the United States, and although they achieve a wide analytical range, the methods require cleanup and solvent evaporation steps prior to analysis using selective GC detectors. QuEChERS has been demonstrated to quickly extract pesticide residues from vegetables and save time in pretreatment process, but is not very suitable for on-site analysis, which needs a quicker cycle and much less solvent cost. Clean-up techniques such as solid phase extraction (SPE), liquid–liquid partitioning and gel permeation chromatography (GPC) are often employed, but increase the overall sample preparation time, increase the cost of the method, and can result in the loss of pesticide recovery.

TSP for GC injection is a technology that minimizes sample preparation and still provides a rugged analytical approach for complex matrices. TSP involves the placement of a small amount of sample material or liquid extract into a 40 µL disposable micro-vial. The sample and micro-vial are manually placed into the GC/GCMS inlet and heated rapidly to thermally desorb semi-volatile components, such as pesticides, in the sample. A major benefit to the TSP approach is that nonvolatile matrix components, which normally contaminate the GC liner and column in traditional injection approaches, remain in the micro-vial, which can be disposed after every injection. With the TSP and its thermal extraction, only compounds that can be vaporized from the vial are introduced into the column. There were many applications on similar probes in the past with a programmed temperature injector.[1]

In this application note, an Agilent 5975T LTM GC/MSD was evaluated to detect pesticides in vegetables that were pretreated, without a cleanup step, with the TSP tool and an isothermal inlet. In complex extracts without the cleanup step, TSP requires a selective detection technique to determine the analytes among the many semi-volatile matrix components. Agilent's DRS software and RTL function are good tools to extract the targets from matrix semi-volatiles in a short time. A 5975T LTM GC/MSD with a quick ramp heating oven rate and fast cooling cycle provides an ultra-fast sample cycle for this application.

Highlights

- TSP (Thermal separation probe)
- Agilent's RTL pesticides library and DRS software
- Confirm the blind added multi-pesticides in 2–3 min after sample running
- Transportable 5975T LTM GC/MSD

Experimental

Software required

- G1701EA GC/MS ChemStation (latest version)
- G1716 MSD Deconvolution Reporting Software (Version A.04.00 or newer)
- G1033A NIST08 Mass Spec Library + AMDIS + NIST Library Search
- G1675AA Japan Positive List

Reagents and chemicals

All reagents were analytical or HPLC grade. The pesticides were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water was from a MilliQ system (Milford, Mass, USA).

Equipment and Materials

This experiment was performed on an Agilent 5975T LTM GC/MSD. Extraction was achieved with Agilent SampliQ QuEChERS AOAC Extraction kits (p/n 5982-5755, Agilent Technologies Inc., Wilmington, DE, USA).

Instrument conditions

Table1. Instrumentation and Conditions of Analysis

Instrumentation	
GCMS system	Agilent 5975T LTM GC/MSD
Inlet	Split/splitless (liner: 5062-3587)
Column	HP-5 ms LTM 10 m × 0.18 mm × 0.18 µm
Guard column	1 m column with same phase as analytical column, connected to the injector.
Experimental conditions	
Inlet temperature	260 °C
Injection volume	1µL
Injection mode	splitless; purge after 1min: 100 mL/min
Carrier gas	helium
Head pressure	1.6 mL/min, constant flow mode
LTM oven temperature	50 °C (0.2 min), 125 °C /min, 125 °C (0 min), 50 °C/min, 300 °C (2 min)
Method	RTlocked to chlorpyrifos methyl at 2.70 min
Transfer line temperature	260 °C
MSD interface	270 °C
Ion source	230 °C
Quad. temperature	150 °C
Ionization mode	EI
Scan mode	full scan, 50–550 *m/z*
EMV mode	Gain factor
Gain factor	5.00
Resulting EM voltage	1129 V
Solvent delay	0.5 min

Sample preparation

Samples

Organically grown, pesticide-free cucumbers, tomatoes, and green peppers were purchased from a local grocery store. The samples were spiked to different concentration levels with a certain number of pesticides.

Sample preparation

Extraction/Partitioning

Approximately one pound of cucumbers or tomatoes were chopped into small, bean-sized cubes. Two ceramic homogenizers (p/n 5982-9313) were placed into a 50 mL centrifuge tube (from the SampliQ QuEChERS extraction kit) and a 15 g (± 0.1g) amount of previously homogenized sample was placed into the same tube. QC samples were added with 100 µL of appropriate QC spiking solution. A 100 µL amount of internal standard spiking solution was added to all samples except the control blank. Tubes were capped and vortexed for 1 min. A 15 mL amount of 1% HAc in ACN was added to each tube using the dispenser. An Agilent SampliQ QuEChERS extraction salt packet from the kit (p/n 5982-5755), containing 6 g of anhydrous $MgSO_4$, and 1.5 g of anhydrous NaOAc, was added directly to the tubes. The salt bag was massaged carefully to break up any salt clumps before pouring. The tubes were examined to ensure that no powder was left in the threads or rims of the tubes. Sample tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted with the entire sample and crystalline agglomerates were dispersed. Sample tubes were centrifuged at 4,000 rpm for 5 min. The liquid layer was taken for GCMS injection.

Results and discussion

There is increasing pressure to reduce costs in pesticide testing and increase productivity without sacrificing analytical quality. TSP has been tested as a tool for reducing sample pretreatment time. We used extractions of tomato, cucumber, and pepper to test the TSP's pesticide detection capabilities, including peak discrimination, repeatability, and so on. The LTM column rapidly heats up and cools down column temperature, further reducing run time and cycle time.

Fast methods established for on-site pesticide detection with TSP

An Agilent GC MXLATOR software tool was used to find operating conditions for the faster methods. The faster methods were scaled exactly as predicted, using a combination of Agilent's method translation (MTL) and RTL software. Because scaling was exact, these faster methods could be used with precisely scaled pesticide libraries, making the screening process even more powerful and adaptable to individual needs [2]. With this software, we obtained the fast method from a method with a 30 m, 0.25 mm, 0.25 µm column to a method with a 10 m, 0.18 mm, 0.18 µm column, to obtain a fivefold shortened running time. The original method was based on tha Japanese positive list method [3]. We also shortened the retention index of relative compounds in the library with a fivefold time reduction. The application of all the results in this paper was retention times locked to corresponding requirements.

With the fast method, we tested the TSP availability for the pesticides including organo-phosphorous, organo-chlorine and pyrethroid pesticides. The added concentration was 5.0 µg/µL and injected volume was 1 µL. Figure 1 shows that TSP injection can produce good peak shapes for all different types of pesticides with no peak discrimination.

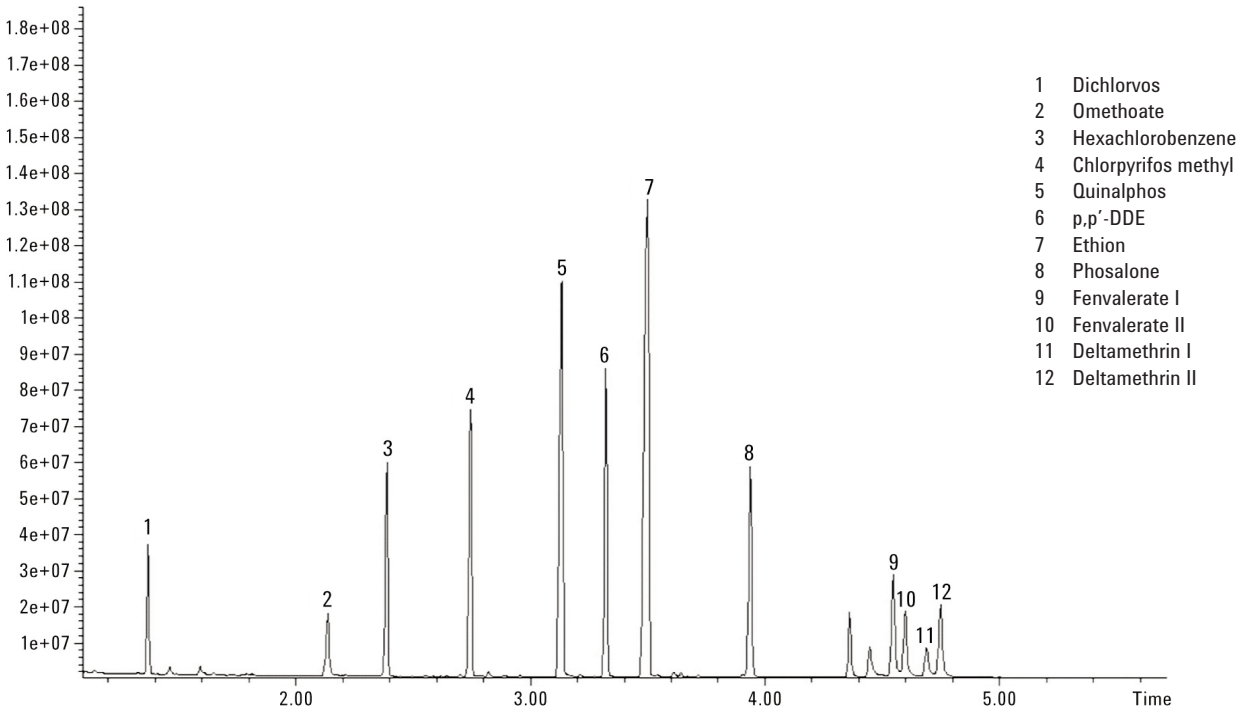

Figure1. TIC of 12 pesticides in cucumber extraction.

Repeatability test with TSP

In order to evaluate the stability of the system we tested 17 organo-chloro pesticide solutions in acetone at a 1.0 µg/mL concentration. We continuously injected eight times and the RSD% of all the compounds was 2.3 ~7.8% with 1 µL injection volume. The RSD results may have been affected by the manual syringe volume. The results could satisfy the requirements of on-site analysis.

Keeping clean capability test with TSP

We tested the baseline of 50 continuous injections of tomato extractions without a clean-up step. The main compositions of extraction were some sugars, vitamins, and pigments. Most of them remained in the vial and only volatile compounds that can be vaporized from the vial were introduced into the system. At 20 injections, the vial became dark, but the liner remained clean; this shows that the TSP has the capability to trap the heavy matrix, which would be removed with a clean-up step. In this test, the running time was extended for higher boiling compounds. We tested one baseline every 10 injections. Figure 2 compares five baselines. It shows that the system could stay clean after the rich interference sample was injected. The sample was extracted without a clean-up step, showing TSP could be used for the no-clean-up sample test and save more sample preparation time.

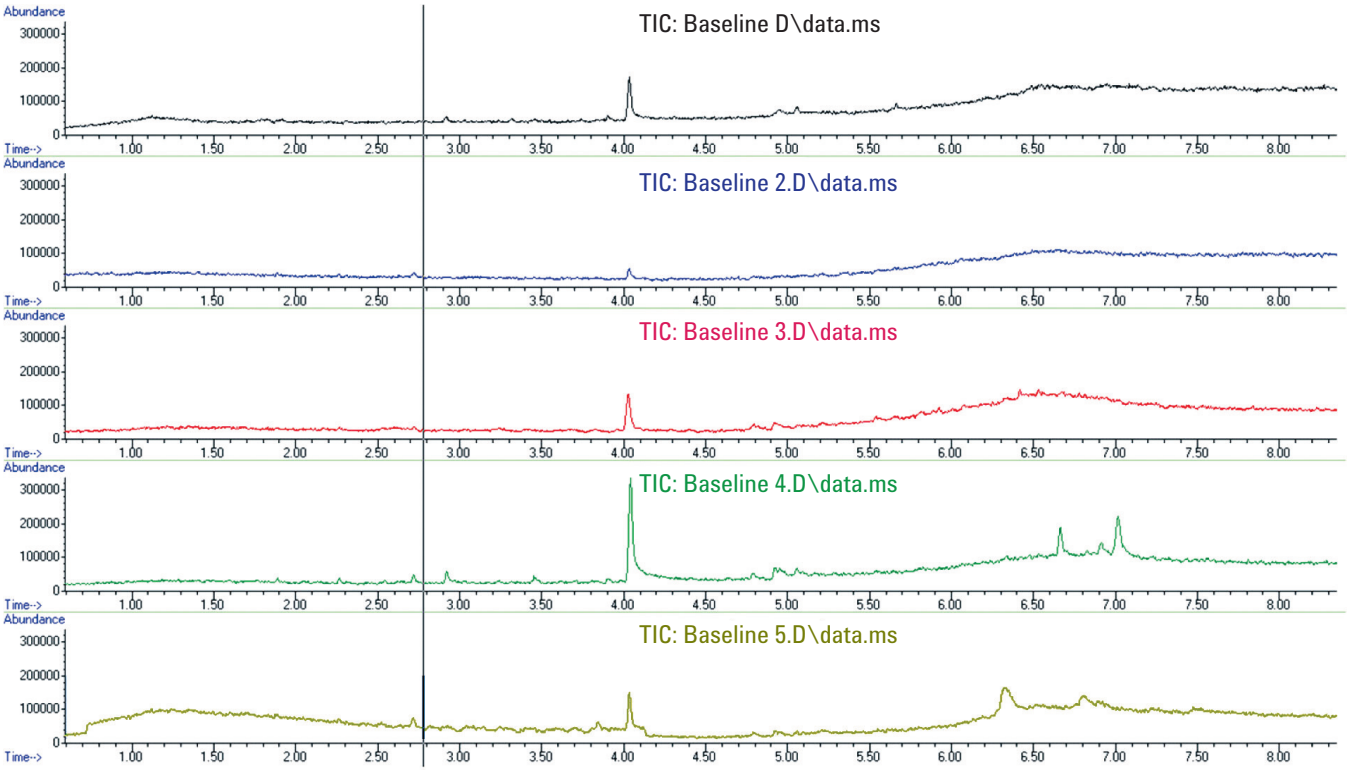

Figure 2. Baselines of the 50 injections test.

Blind pesticide spiked samples and Real sample test with TSP

Three samples of vegetable extracts were spiked with different pesticides at different concentration levels. Eighty spiked pesticides were tested in three samples. With the TSP injection and AGILENT DRS software, most of the tested pesticides could be confirmed when the concentration level was higher than 100 ppb except acephate and methamidophos. When the concentration was increased to 500 ppb, all of the tested pesticides could be confirmed by DRS. Figure 3 shows a chromatogram of a mixture of three samples that was spiked with nine organo-phosphorous pesticides (100 ng/mL each). Table 2 is the DRS report of the results.

Three pesticides were identified and confirmed by DRS in a tomato extract that was not spiked. They were Pyrimethanil, Procymidone and Dimethomorph. Figure 4 shows a spectra report of procymidone from AMDIS software. It shows that it would not identify the compounds by using only a library search, without deconvolution. All the results show that with the fast method and TSP, we can identify unknowns with the help of DRS library.

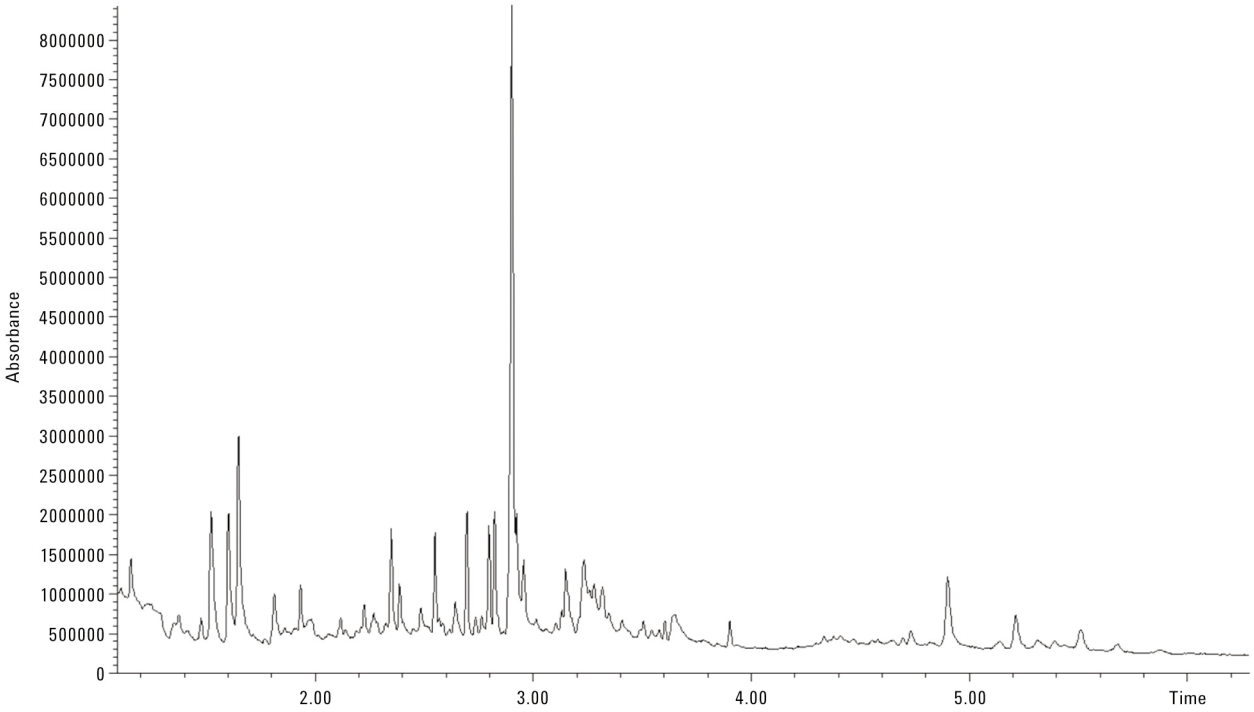

Figure 3. TIC of 100 ppb pesticides spiked mixture of three samples.

Table 2. DRS for the Mixture of Three Samples Whose Chromatogram is Shown in Figure 3

			Amount (PPB)		AMDIS		NIST	
R.T.	**Cas number**	**Compound name**	**Chemstation**	**AMDIS**	**Match**	**R.T. Diff sec.**	**Reverse match**	**Hit num.**
1.3748	62737	Dichlorvos	98		79	–0.1	74	1
2.2261	13194484	Ethoprophos	98		96	–0.2	89	1
2.3503	298022	Phorate	100		97	–0.6	92	1
2.5501	333415	Diazinon	100		78	–0.2	74	1
2.7639	298000	Methyl parathion	100		92	–0.4	81	1
2.8992	121755	Malathion	100		77	–0.1	81	1
2.9245	2921882	Chlorpyrifos	98		93	–0.3	87	1
2.9565	56382	Parathion	100		79	–0.3	77	1
3.1993	961115	Tetrachlorvinphos	98		83	–0.1	82	1

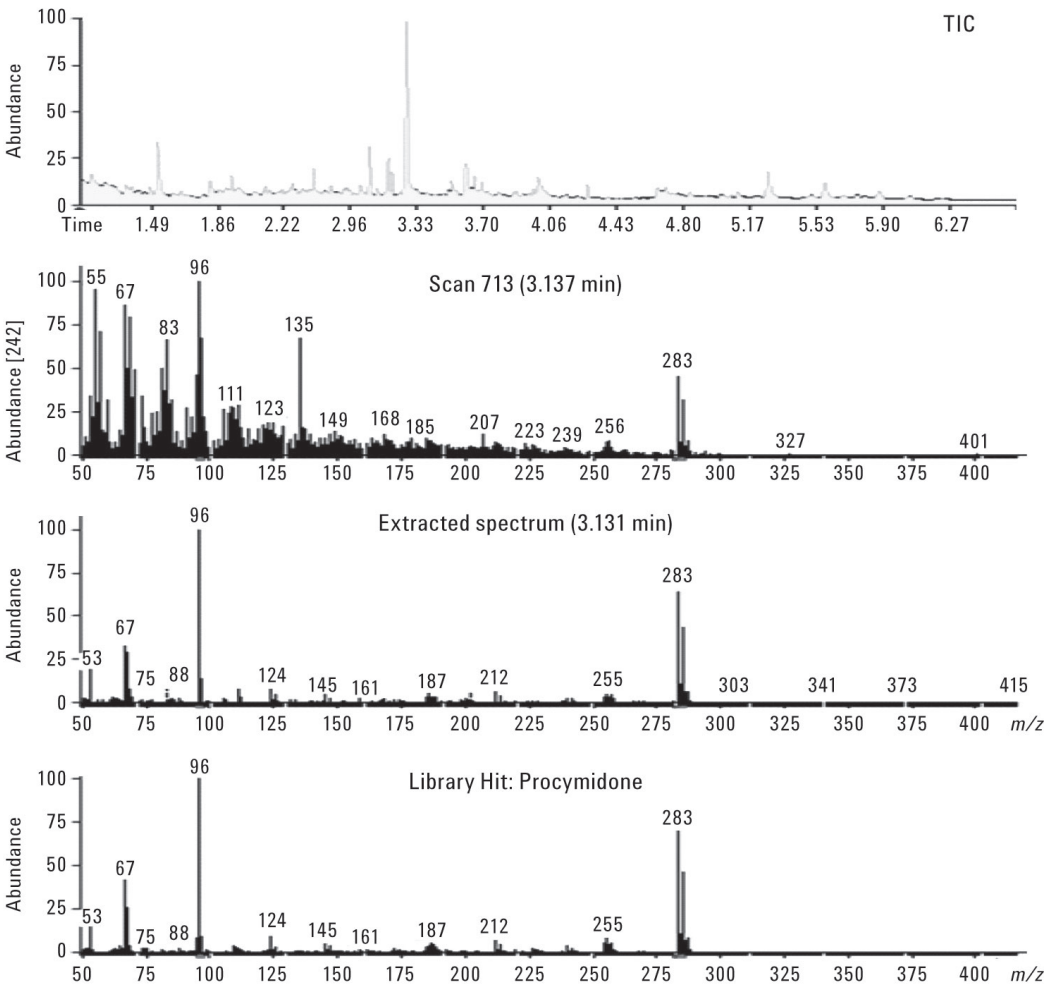

Figure 4. Deconvolution spectrum of procymidone in unspiked tomato extraction.

Conclusion

An Agilent 5975T LTM GC/MSD with TSP solution produced good results for on-site detections, meeting the requirements for on-site applications, such as quick response and fast identification. TSP can save sample pretreatment time and keep the system clean. Agilent DRS software helped extract the target compound's spectrum from the matrix interface and create a fast running method. This three-way combination provides a good solution for accelerated analysis of pesticides, especially for on-site detection.

Reference

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2. C. Kai Meng; "Fast Screening of Pesticides and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part II, Agilent Technologies publication 5980-1057EN.
3. Philip L. Wylie; Screening for Pesticides in Food Using the Japanese Positive List Pesticide Method: Benefits of Using GCMS with Deconvolution Reporting Software and a Retention Time Locked Mass Spectral Database, Agilent Technologies publication 5989-7436EN

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