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Journal of Chromatography A, 754 (1996) 455–462

JOURNAL OF CHROMATOGRAPHY A

Off-line high-performance liquid chromatography and solid-phase extraction clean-up for confirmation of pesticide residues in fresh produce by gas chromatography–mass spectrometry

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Abstract

GC–MS or GC–IT in conjunction with the GC–ECD, GC–FPD and HPLC Post–Column Derivatization for multiresidue scan analyses is a great complementary instrument for identification or confirmation purposes. However, MS in EI mode serves as a non-selective detector that is easily susceptible to interference by produce matrices in trace level residue analysis. The development of an effective clean-up method is essential. In this research, a combination of HPLC/C_{18} and SPE/Florisil clean-up methods were used to reduce matrices in 10 commodities. Using the PBM system, the reference spectra of the computerized Anaheim data base were fingerprint matched to the fortified residues at the 50 ppb level. For most commodities, the PBM quality value was above 90% with the exception of whole oranges. The study demonstrates that the produce matrix of individual commodities varies considerably. The method met difficulties in the analysis of citrus due to the citrus peels. Although the method is commodity dependent, the clean-up procedure has reduced matrix interference in most cases. Efficiency is approximately in a range of 10^5 to 10^6 times, thus the method is effective in confirming trace level pesticide residues in fresh produce.

Keywords: Environmental analysis; Sample preparation; Pesticides

1. Introduction

California spends more than $47 million each year on what is the nation's most comprehensive pesticide regulatory program. Under this program, fresh produce are collected and submitted to the State laboratory by California EPA, Department of Pesticide Regulation, Enforcement Branch [1]. The California Department of Food and Agriculture (CDFA), Anaheim Pesticide Residue Laboratory is one of the three State laboratories responsible for pesticide residue analysis in the Southern California area, including Mexican imports into California. Using CDFA's multiresidue extraction method [2], residues are analyzed in three groups based on the classification of pesticide molecular structure composition: (I); The halogen containing residues are scanned by GC–ECD, (II); Phosphorus containing residues are analyzed by GC–FPD in P-mode and (III); N-methylcarbamates are determined by HPLC with post-column derivative techniques [3].

Ever since aldicarb tainted California watermelons were identified as the cause of the poisoning epidemic in the Western States and Canada on July 4th of 1985 [4], pesticide residues in food have raised public concern and become a national topic in food

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0021-9673/96/$15.00
PII S0021-9673(96)00374-3

safety issues. Consequently, the recommendations of The US Congress, Office of Technology Assessment [5] are to increase the variety of pesticides, including metabolites and reduce the detection limits of pesticides in produce with no established tolerance [6].

Fortunately, our routine multiresidue scan method meets the recommendations due to the drastic improvement in sensitivity in late model GC and HPLC systems. However, the protocol from US EPA [7], US FDA [8] and our own agency [9] requires confirmation analyses on all violations in order to establish indisputable scientific evidence in case of enforcement action or litigation. As known GC–ECD, GC–FPD and HPLC results only provide quantitative, elemental and peak retention time data, but lack the specificity necessary for molecular structural identification.

Accordingly, mass spectrometry (MS) has been designated to bridge the gap. Because MS is a two-dimensional detection method, it provides peak retention time and mass spectrum [10]. The full spectrum profile in the computer library is a fingerprint identification for any uncertainty. In addition, the late model bench top mass selective detector and ion trap (IT) are user friendly and cost effective. The popularity of GC–MSD and GC–IT is evidenced by their extensive use in most analytical laboratories.

However, like flame ionization detection (FID), MS and IT in the electron impact (EI) mode are general detectors that respond well for pure standards, with little to no matrix interference. As for pesticide residues in produce, the non-selective nature of EI–MS makes analytical tasks very challenging and difficult. Because residues constitute only trace amounts (ppm to ppb) in the relatively massive plant tissue, the produce matrix always overshadows the wanted residue spectrum. It is almost impossible to obtain quality data without first minimizing the matrix interference. Therefore, an effective clean-up method is necessary before further advancement can occur.

By using a GC–MS in EI mode for residue identification purposes, this research study targets two objectives: (1); to reduce the fresh produce background interference to a minimum through a combination of HPLC and SPE clean-up systems and (2); to examine the profile of various background noises caused by 10 commodities in 5 classes of fresh produce.

2. Experimental

2.1. Chemicals

The solvents used in the experiment were residue analysis grade reagents from EM Science. The sodium sulfate (Na_2SO_4) anhydrous granular was purchased from EM Science. The reagent grade florisil (60–100 mesh) was purchased from Aldrich and put in an oven at 130°C overnight for activation. The dacthal, diazinon and atrazine standards (100%) were supplied and purity certified by the California Department of Food and Agriculture (CDFA) standards repository.

2.2. Instrumentation

A Hewlett–Packard (HP) 5890A GC system equipped with a HP 5970 mass selective detector was used for the experiment. The temperature program was: initial temperature 70°C held for 2 min, ramp rate 10°C/min, final temperature 280°C held for 2 min. The injection mode was splitless, held for 1 min and the injection volume was 2 ul. For the MS system, the parameters were: mass range 50 to 400 u, A/D sample 4, acquire time 22 min, fil/mul delay 3 min, peak threshold 150 counts. A HP-1 column (methylsilicone) was used. The column size was 12 m×0.2 mm I.D. and coated film thickness was 0.33 μm.

2.3. Preparation of fortified produce samples

Produce was grouped into 5 classes consisting of leafy vegetables, roots, fruits, citrus and spices. Each group contained 2 commodities in the experiment. Each commodity was chopped and mixed in a food chopper. A mass of 200 g of well chopped sample was placed in a 1 qt (1000 ml) Mason jar, and each jar was then fortified with 10 μg each of dacthal, diazinon and atrizine pesticides.

2.4. Sample extraction and clean-up procedure

A volume of 200 ml of acetonitrile was poured into each fortified sample jar. The contents were blended by an Omni–Mixer for 2 min. Approximately 20 g Na_2SO_4 was added into the sample to facilitate solids and liquids separation. The mixture

was filtered through a sharkskin filter paper and the filtrate was collected in a 8 oz glass bottle. Ca. 50 g NaCl was added to the bottle, which was then shaken vigorously for 1 min and centrifuged for 2 min at 201 *g* (1500 rpm). The top 100 ml acetonitrile layer was transferred into a 250 ml beaker and evaporated to near dryness on a 100°C water bath with gentle air stream.

2.4.1. HPLC/C_{18} clean-up step

The dried sample was dissolved by adding 2 ml methanol, and then transferred into a 1.5 ml vial for HPLC/C_{18} run. A volume of 1 ml was injected into a Varian 5000 HPLC with C18 column (size 5 μm, 15 cm×4.6 mm I.D.). With the 1.5 ml/min flow-rate, the gradient program was:

Time (min)	H_2O%	Acetonitrile%	Event
0.00	90	10	
4.00			Discarded fraction
5.00	40	60	
10.00	40	60	
13.00			Collected fraction
14.00	10	90	Column wash
18.00	90	10	Synchronize cycle
20.00	90	10	Equilibrium

The fraction of 15 ml was collected from 4 to 13 min into a 20 ml bottle. Salt (ca. 0.5 g) was added into the bottle, shaken vigorously, and then centrifuged for 2 min at 201 g. The top ca 6 ml acetonitrile layer was transferred into a 100 ml beaker with an adjustable pipet and evaporated to near dryness on a 100°C water bath with a gentle air stream.

For standard (10 μg/ml) testing purposes, a Spectroflow 757 UV detector with the wavelength at 250 nm was used in the preliminary trial. The chromatogram is presented in Fig. 1.

2.4.2. SPE/Florisil clean-up step

The SPE/Florisil column was made with a Whatman Air Displacement Tip (size 0.5–5 ml). A small piece of glass wool was inserted into the tube in order to cover the tip. Approximately 0.8 g of activated florisil was put into the tube, and ca 0.5 g of Na_2SO_4 was added on the top. Prior to use, the SPE/Florisil column was rinsed with 5 ml of 15% acetone in hexane and 5 ml of hexane.

Ca. 2 ml hexane was added to the sample beaker, and then poured into a just rinsed column. The column was twice eluted with 5 ml of 15% acetone in hexane. The eluate was collected in a 15 ml graduated conical tube and concentrated to 1 ml with a gentle air concentrator (N–EVAP) in preparation GC–MS analysis.

3. Results

Three pesticides, dacthal, diazinon and artrazine, were selected to represent halogen, phosphorus and nitrogen containing compounds, respectively. Each pesticide was fortified with 50 ppb into 10 commodities or 5 classes of fresh produce. Prior to the experiment, the GC–MS was tuned to meet the DFTPP (decafluorotriphenylphosphine) criteria as established by the US EPA requirement for pesticide analyses [11]. Using an early model MS system (HP-5870), a concentration of 5 ng in 2 ul for each injection was found to adequately attain the full MS spectrum.

A set of data, including a total ion chromatogram, peak retention times, area counts, area %s and ratio %s, was reported by the ChemStation through an existing computerized data analysis program. Subsequently, the compiled pesticide reference spectra by the Anaheim laboratory were sequentially searched and matched to each integrated peak in the total ion chromatogram based on the probability based matching system (PBM) [12]. By using this laboratory's custom made MS macro program, a list of possible pesticides corresponding to retention times, area %s, and matching qualities was generated.

Fig. 2 demonstrates the computer generated total ion chromatogram (TIC) of the potato sample and the library matched spectrum for 3 fortified pesticide residues. The results of identification or confirmation are presented in Table 1. With the clean-up method, all the PBM values, except for whole oranges, were above 90% and considered a very good qualitative matching figure. These results show that the clean-up method works well for most fresh produce at the 50 ppb level.

Table 2 and Fig. 2 present signal and noise ratios. The residue signal level and the corresponding produce matrix interference in total ion chromatogram (TIC) are expressed as an area abundance (%).

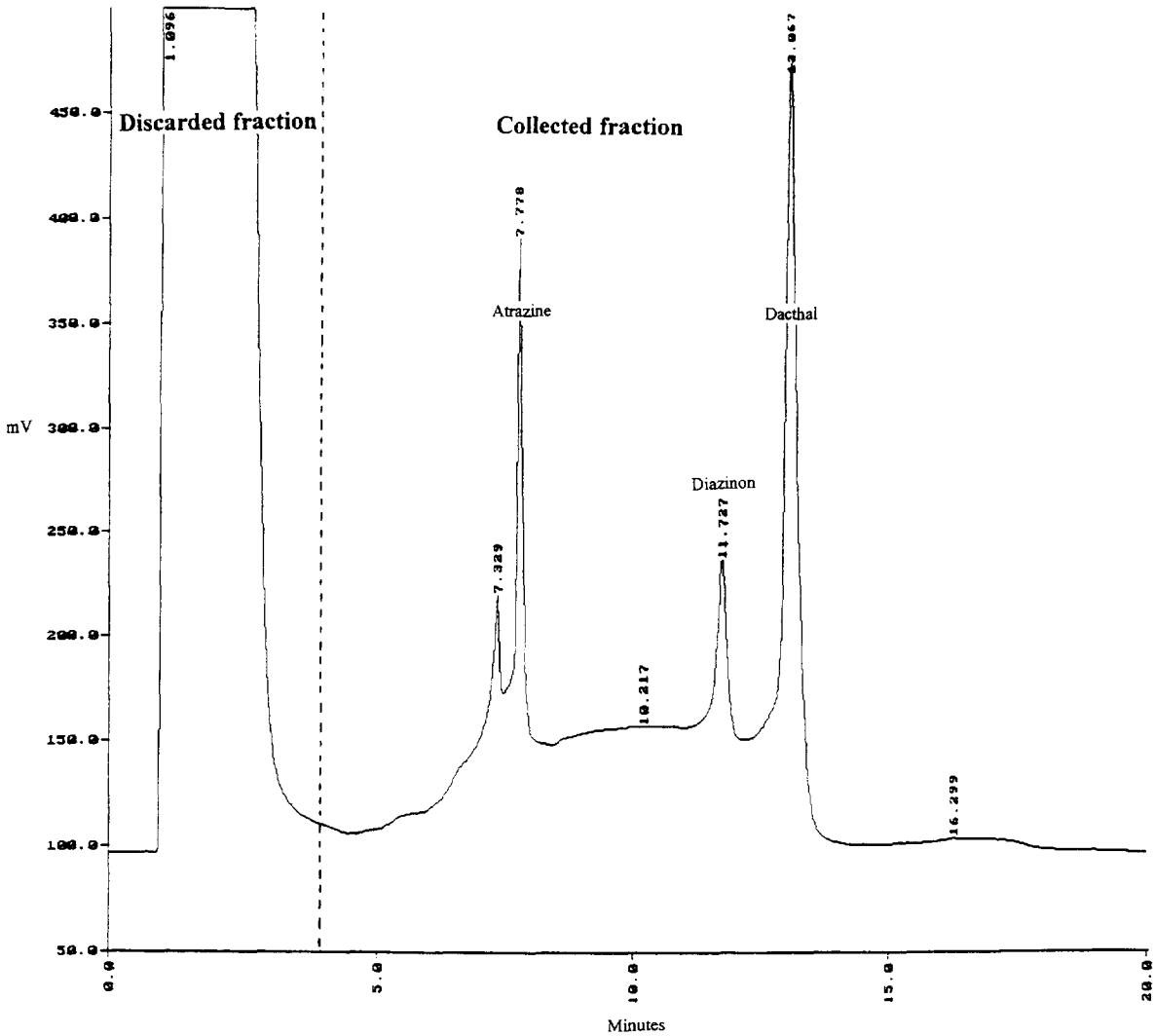

Fig. 1. The chromatogram of the discarded fraction and the collected fraction.

In reviewing the average matrix interference in Table 2, the background noise generated from leafy vegetables and fruits was reduced more effectively than in citrus and spices.

In the case of whole oranges, dacthal was the only residue detected. This indicates that the method does not work well with citrus.

4. Discussion

In the past decade, bench top MS has played a major role for trace toxic substance and pesticide analyses in the environmental field. The achievement is astonishingly successful in the area of air, water and soil due to their simple matrices and the drastic improvement in MS sensitivity. However, analyzing pesticide residues in fresh produce is very difficult as one of the major concerns has been instrumental background noise contributed by coeluting substances from complex matrices of various fruits and vegetables. MS in EI mode is a general detector that detects residues as well as matrix coelutes without selectivity. If the matrix interference are not reduced or removed, the wanted analyte signals are always overshadowed by the predominate noise background

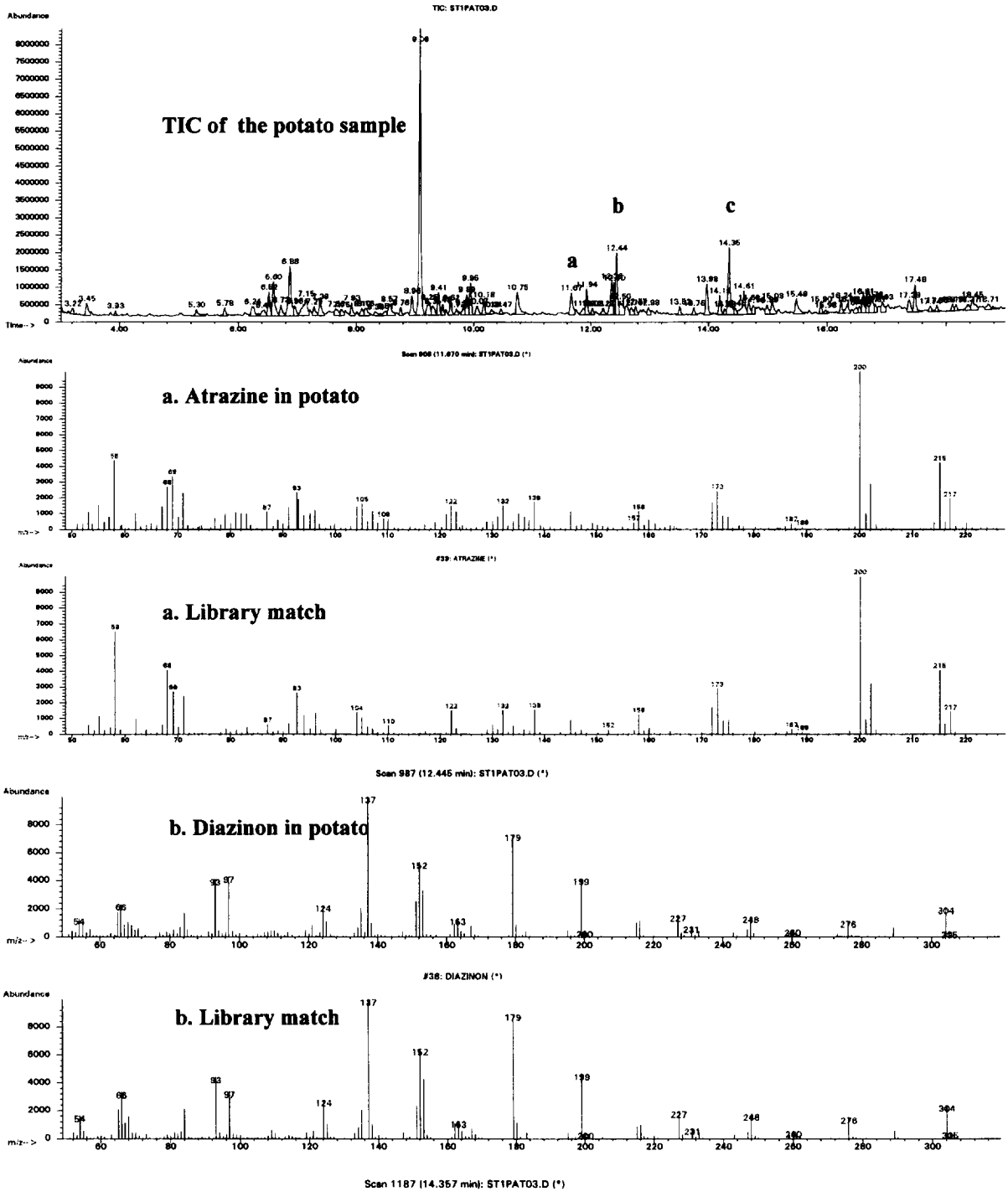

Fig. 2. (*Continued on p. 460*)

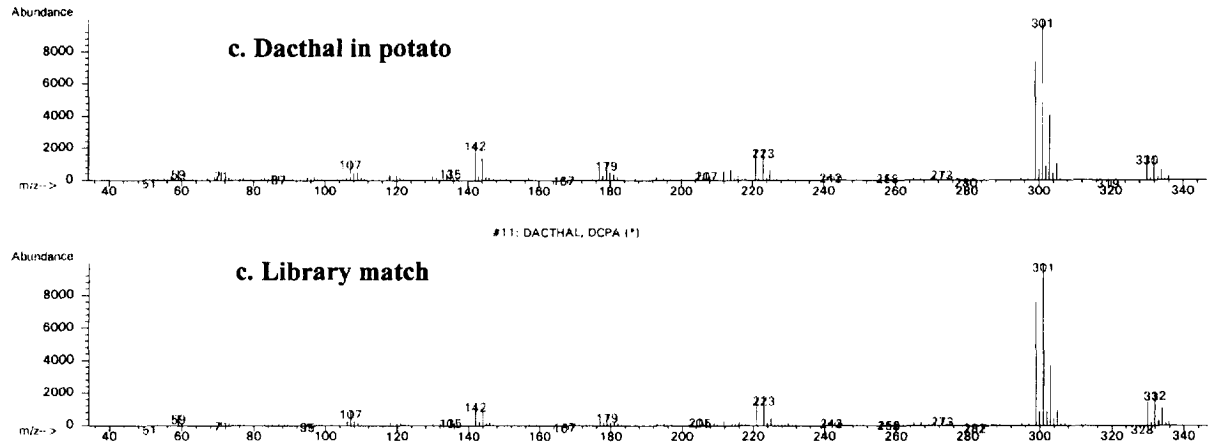

Fig. 2. Total ion chromatogram (TIC) of the potato sample and the library matched 3 residue spectra.

Table 1
Library retrieval results for 10 commodities at the 50 ppbfortified level by Probability Based Matching (PBM) system

Commodities		PBM (%)		
		Dacthal	Diazinon	Atrazine
Leafy	Lettuce	97	99	97
Vegetables	Spinach	96	99	95
Roots	Carrot	95	99	96
	Potato	99	99	96
Fruits	Apple	97	99	97
	Banana	94	98	98
Citrus	Orange (edible)	98	94	94
	Orange (whole)	95	[a]	[a]
Spices	Green Onion	97	99	98
	Chili Pepper	98	99	98

[a] None detected.

interference and as a result, not detected. Therefore, it is essential to focus on the produce clean-up method in order to further detect trace or ppb levels of pesticide residues.

Due to this reason, a combined HPLC/C_{18} and SPE/Florisil system was used to reduce the matrix interference in this study. As we know, the structure of C_{18} or octadecylsilane is a non-polar porous layer bead with extended long hydrocarbon chains when in an organic solvent, like acetonitrile or methanol [13]. However, the hydrophobic property of C_{18} would fold like a collapsed bottle brush in a polar solvent such as water. The non-polar nature of C_{18} in a polar solvent (H_2O) during separation is called reverse phase chromatography. By the reverse phase parti-

Table 2
Residue TIC Area Abundence (%) in Matrix Interference

Commodities		Dacthal (%)	Diazinon (%)	Atrazine (%)	Matrix[a] Interferences (%)	Average
Leafy	Lettuce	3.68	4.30	2.01	90.01	
Vegetables	Spinach	4.97	5.23	2.11	87.69	88.85
Roots	Carrot	0.31	0.37	0.33	98.99	
	Potato	5.37	4.52	2.12	87.99	93.49
Fruits	Apple	4.06	3.85	3.10	88.99	
	Banana	5.73	5.06	2.21	87.00	88.00
Citrus	Orange (edible)	3.20	2.90	2.36	91.54	
	Orange (whole)	0.51	0.00	0.00	99.49	95.52
Spices	Green Onion	0.72	0.90	0.59	97.79	
	Chilli Pepper	0.85	1.45	0.74	96.96	97.38

[a] Matrix Interference (%) = 100 − Dacthal (%) − Diazinon (%) − Atrazine (%).

tion chromatographic principle, extremely polar or water soluble components, such as plant pigment, sugars, peptides, free amino acids etc., were separated and discarded from the fortified residues during the front fraction, 0 to 4 min. HPLC run. The latter fraction, 4 to 13 min., was collected for further clean-up process by SPE/Florisil. Fig. 1 shows the discarded front fraction and the collected latter fraction in the chromatogram.

The collected fraction was concentrated and then cleaned up with an activated florisil column. Florisil, a synthetic magnesium silicate granular [14], is a common adsorbent used to remove wax, fatty acids and lipid related plant organics for pesticide residue analyses. Table 2 demonstrates the effectiveness of the combined HPLC/C_{18} and SPE/Florisil clean-up method for GC–MS analysis. The total ion signals of the three residues were enhance from an undetected ppb level to a detected per cent level for most commodities, except whole oranges. In other words, the efficiency of the clean-up process was enhanced about 10^5 to 10^6 times depending on the commodity. For example, in Table 2, the residue TIC area abundance % of carrot and green onion were enhanced 10^5 times and the rest of the commodities were in the range of 10^5 to 10^6 times. As expected, the method had problem with whole oranges. The volatile organics in citrus peel may be the most notorious contributor to matrix interference and has yet to be reduced or satisfactorily removed by any known clean-up method at the present time.

After the clean-up procedure, the samples were analyzed by GC–MSD. As stated in the introduction, the objective of this study is to focus on the qualitative confirmation of the existence of the trace (50 ppb) residues. As known, the probability based matching (PBM) system is the most commonly used method for molecular identification. This system uses stored reference spectrum in the library as a measuring stick to match the targeted compounds by the appearance of masses and abundances, called "reverse searching". The reverse search, in effect, ignores interference or unrecognized peaks generated by matrices. PBM performance has also been statistically evaluated to determine the actual reliability of the indications based on the degree of match found between the targeted compounds and the reference spectra. Thus, a reported 90% of the reliability value indicates that the identification was correct in 90% of the evaluation cases in which PBM retrieved a reference spectrum with this degree of similarity [12]. Therefore, a quantitative report of 90% matching was adapted as the parameter for this research.

GC–MS quantitative or recovery studies of residues in fresh produce have been of interest to chemists [15,16]. Prior publications dealt with limited commodities, such as fruits, roots etc., which had a lesser degree of matrix interference than spices and citrus with peels. Our experience from the recovery studies showed that the data were erratic and hard to reproduce. The reason for this is because MS in EI mode is a general detector that is able to concurrently detect residues as well as produce matrix. Therefore, if the produce matrix are not eliminated, the ions generated from the matrix appear everywhere and are distributed throughout the entire spectrum. It is impossible to obtain an accurate quantitative result at trace level with interference by unwanted ions. Furthermore, the profile of those matrix ions change from one commodity to another. Therefore, the unpredictable nature makes the selection of the wanted residue ions for quantitative calculation free from interference by matrix ions almost impossible.

Regardless of the encountered quantitative difficulties, GC–MS and GC–IT are powerful tools for residue identification or confirmation purposes. The sensitivity and selectivity of GC–ECD, GC–FPD and HPLC for a rapid and reliable quantitative result makes the MS system a logical complementary instrument in trace residues confirmation. This is particularly advantageous for a regulatory agency such as ours which often encounters enforcement action or even occasional litigation.

5. Conclusion

Taking advantages of the powerful PBM system, MS is a great instrument for pesticide residue confirmation analysis. However, because MS in EI mode is a non-selective detector, the trace residue ions are overshadowed by the massive interference ions in produce matrices. It is almost impossible to obtain high quality data if produce matrices are not reduced prior to MS determination.

This research has used a combined clean-up system which is comprised HPLC/C_{18} and SPE/Florisil steps for removing produce polar interference (water soluble substance) and non-polar interference (lipids), respectively.

The study has demonstrated that the produce matrix of individual commodities vary considerably; thus, the efficiency of the clean-up power is also varies within a range of 10^5 to 10^6 times. Furthermore, the method encountered difficulties in cleaning up the citrus matrix because of the complex nature of the peels.

For most commodities, the PBM quality values were reported above 90% and were as such considered to be high quality figures for fingerprint identification. Therefore, this is an effective confirmation method for trace residue analysis in fresh produce.

Acknowledgments

The author wishes to thank Bill Cusick (Chief of Chemistry, CDFA) and Ton Joe (Program Supervisor of Chemistry, CDFA) for their permission and encouragement of this research work.

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