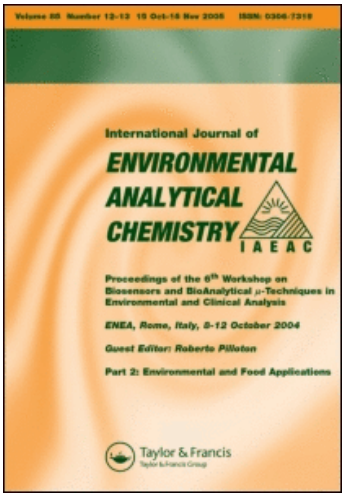

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Quantitative LC-ESI-MS Analysis for Pesticides in a Complex Environmental Matrix Using External and Internal Standards

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QUANTITATIVE LC-ESI-MS ANALYSIS FOR PESTICIDES IN A COMPLEX ENVIRONMENTAL MATRIX USING EXTERNAL AND INTERNAL STANDARDS

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Quantitative liquid chromatography electrospray mass spectrometry (LC-MS) analysis for pesticides in a complex environmental matrix using external and internal standard calibration was investigated. Various approaches to introducing different internal standard compounds to address quantitative errors associated with signal suppression were also examined. The study involved the analysis of pesticides in wheat hay matrix samples using three kinds of internal standard compounds: deuterium labeled (D_3), carbon-13 (singly labeled), and structural analogs (derivatives) of the target analytes. Introduction of the internal standard by volumetric addition and direct post-column infusion were also studied and compared.

Isotopically labeled internal standards (i.e. D_3- ^{13}C-) were found to be effective in correcting quantitative errors associated with signal suppression. The application of singly labeled ^{13}C compounds may result in nonlinear calibration due to mass interference with the target analyte species. The interference may be compensated by using quadratic curve-fitting or subtraction of the interfering component. Although ineffective as volumetric internal standards, structural analogs can be effective in compensating for signal suppression when introduced into the LC effluent by continuous post-column infusion. Furthermore, the post-column introduction method allows the application of a single internal standard compound for the quantification of each analyte in a multi-component mixture.

The use of internal standards can be effectively incorporated into residue analysis development methods for pesticides in environmental matrices. High accuracy and reproducibility can be achieved while improving method efficiency by reducing the need for comprehensive sample clean-up.

Keywords: LC-MS; pesticides; internal standards; quantitative analysis

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INTRODUCTION

Over the last ten years, liquid chromatography-mass spectrometry (LC-MS) has developed into a powerful analytical technique, combining the separation capabilities of liquid chromatography (LC) with the sensitivity and selectivity of mass spectrometry (MS) [1,2]. The development of atmospheric pressure ionization techniques has facilitated online interface between LC instrumentation and the mass spectrometer. One such technique, electrospray ionization (ESI), revolutionized LC-MS by allowing efficient ionization of nonvolatile organic analytes with minimum fragmentation. As a result of these developments, LC-MS has become a standard technique for the detection and quantification of pesticides, herbicides and other compounds which are of environmental interest [3–5].

Current residue analysis methods developed for the quantification of pesticides from environmental matrices typically consists of an analyte extraction step, followed by a series of purification or clean-up procedures (Figure 1). The clean-up procedures tend to be the most labor-intensive portion of the method. For example, the clean-up procedures developed for tebufenozide and hydroxy-tebufenozide (Figure 2) in rotational crops involve liquid-liquid extractions, followed by open column and solid phase extraction (SPE) separations [6]. The method is also complicated by solvent-stationary phase compatibility, requiring solvent evaporation and redissolution during the transition between different procedures. Although complex and labor intensive, this type of residue analysis method allows reliable quantification of pesticides to parts-per-billion concentration levels by LC-MS with external standard calibration.

The extensive clean-up serves to address two general problems caused by the matrix: chemical interference and matrix signal effects (i.e. signal suppression or enhancement). Problems associated with mass interference may be reduced or eliminated by open column chromatography. However, sufficient matrix components may still exist in the sample to induce significant signal suppression/enhancement and prevent reliable external calibration (calibration curves prepared by using absolute signal response). For this reason, matrix samples generally require a comprehensive set of clean-up procedures to produce accurate and reproducible quantitative information.

Internal standards have been used to resolve a number of different issues encountered in quantitative mass spectrometry [7]. Volumetric internal standards, where the internal standard is introduced into the sample after clean-up, but prior to instrumental analysis, is often used to address variations in instrumental conditions (e.g. injection volume). Surrogate internal standards, where the internal standard is added prior to any sample preparation procedure, are routinely applied to compensate for analyte losses attributed to the extraction procedures,

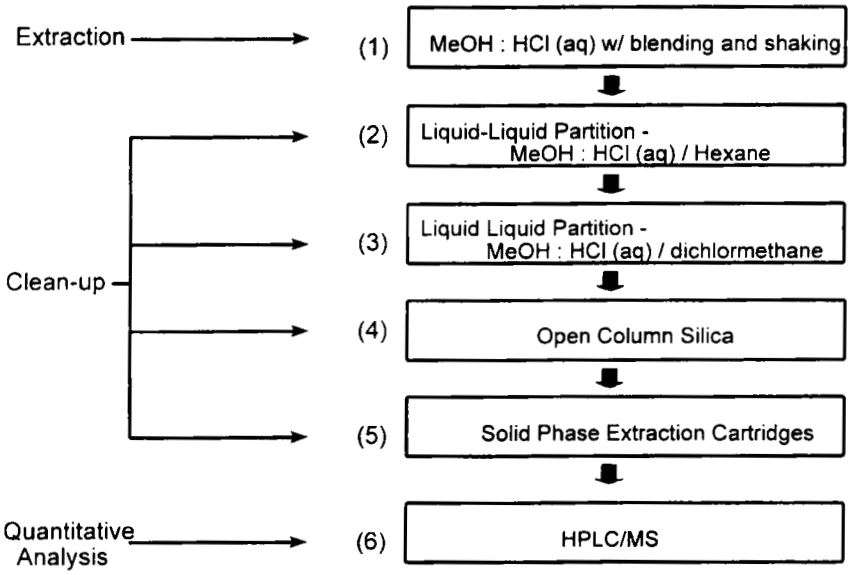

FIGURE 1 Example of a standard recovery method applied for the extraction, clean-up and quantification of pesticides from a plant matrix

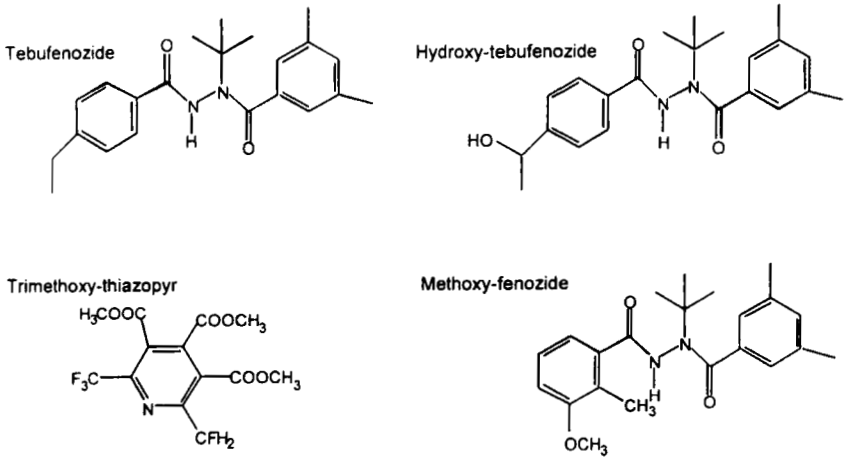

FIGURE 2 Structures of pesticides studied

as well as variations in instrumental conditions. Although volumetric and surrogate internal standards are generally used to address different issues, both are capable of correcting signal suppression.

The best internal standards are isotopically labeled compounds which elute simultaneously with the target analyte during LC separation while being completely resolved by the mass spectrometer (e.g. D_3, $^{13}C_3$ labeled compounds). However, these compounds may be difficult to obtain due to expensive synthesis and/or purification procedures. For certain cases, singly labeled compounds (e.g. ^{13}C), although not completely resolved from the target analyte by the mass spectrometer, may be more conveniently available.

Compounds which are structurally different, but similar in chemical and physical characteristics (i.e. structural analogs or target analyte derivatives) have been applied as internal standards for mass spectrometry analysis [8]. However, the primary disadvantage of this type of internal standard for LC-MS is that the target analyte and the internal standard will elute from the LC at different times. As a result, the matrix suppression experienced by the internal standard may be completely different from that of the target analyte.

In this report, various approaches to the introduction and application of internal standards for LC-MS analysis of environmental samples were studied. Analytes used in this study are triester-thiazopyr, tebufenozide, hydroxy-tebufenozide and methoxy-fenozide (Figure 2). Matrix signal effects were induced by omitting certain clean-up steps for the preparation of wheat hay matrix samples. Internal standards were introduced into the sample volumetrically prior to LC-MS analysis or by post-column infusion (i.e. direct introduction into the LC-effluent). Quantification results using external standard calibration were compared to those obtained by D_3-, and ^{13}C- isotopically labeled and structural analog internal standards. Furthermore, the potential of using internal standards to significantly improve the efficiency of the residue analysis methods and method development process was explored.

EXPERIMENTAL

Materials

Tebufenozide (active ingredient in Confirm™, and Mimic™), hydroxy-tebufenozide, triester-thiazopyr (active ingredient in Visor™), and methoxy-fenozide are Rohm and Haas Company products (Philadelphia, PA). Wheat-hay matrix were obtained from the Rohm and Haas Company. Solvents used in the extraction and clean-up procedure were of HPLC grade and obtained from Fisher Scientific (Pittsburgh, PA).

General Extraction and Clean-up Method

The extraction and clean-up procedures were performed using a blank wheat hay matrix sample. Target analytes were added to the sample after completion of the clean-up procedures. This was done to focus only on signal loss attributed to matrix suppression, not sample loss associated with the extraction and clean-up.

The residue analysis method used in this experiment is illustrated in Figure 1. The analysis method consists of the following procedures: (1) extraction by shaking/blending, (2) liquid-liquid partition with hexane, (3) liquid-liquid partition with dichloromethane, (4) silica open column separation, (5) clean-up by solid phase extraction cartridges, (6) analysis by LC-MS. A summary of the method extraction and clean up procedures is described below.

Extraction

Two grams of wheat hay were mixed with 150 mL of extraction solvent (90% methanol / 10% 0.10 N HCl) and shaken for approximately 30 minutes. The extract was separated by vacuum filtration; the filter cake was rinsed with 50 mL of extraction solvent.

Partition Clean-Up. The filtrate was transferred to a 500 mL separatory funnel and liquid-liquid extraction was accomplished with hexane and dichloromethane (MDC). Hexane partition involved the addition of 20 mL of 20% aqueous NaCl solution followed by 50 mL of 100% n-hexane. The aqueous phase was saved for MDC partition. 100 mL of 20% aqueous NaCl was added followed by 100 mL of MDC. The organic phase was collected, followed by a second 100 mL MDC partition. Both fractions of the organic phase were combined, collected and dried with a rotary evaporator.

Open Silica Column Clean-up

30 mL of activated silica (63–200 mm particle size) was slurry packed with 100% n-hexane into a 19 mm ID × 25 cm chromatographic column. The sample, dissolved in 25 mL of 5% ethyl acetate / hexane, was added to the column. The sample container was rinsed with 10 mL of the 5% ethyl actetate solution, followed by 50 mL of 10% ethyl acetate solution. The sample was eluted with 100 mL of 50% ethylacetate and dried. Solution fractions were dried by rotary evaporation.

Solid Phase Extraction Clean-up

50 mg phenyl cartridges (Supelco, Bellefonte, PA) were preconditioned with 5mL of 5% acetonitrile/water (ACN/W) solution. Samples were dissolved in 5% ACN/W and added to the column. Columns were rinsed with 5 mL of the 5%

ACN/W solution and subsequently washed with 5 mL of 10% ACN/W. The sample was eluted with 15 mL 35% ACN/W, and the extract was collected and dried by rotary evaporation. The sample was redissolved in 4 mL 1:1 ACN/W, fortified with analyte and internal standard, and analyzed by LC-MS.

Application of Internal Standards

Internal standards were introduced volumetrically after completing clean-up procedures, but prior to LC-MS analysis. Samples were fortified with the internal standard at a concentration of approximately 0.1 to 0.2 μg/mL. Three sets of samples were prepared and run in sequence: standard solution samples, fortified matrix samples and standard solution samples. Peaks characteristic of the analyte and internal standards were integrated from LC-MS single-ion chromatographs. Calibration curves were generated by plotting analyte concentration *vs.* analyte signal area (without internal standard calibration) or analyte to internal standard signal ratio (internal standard calibration). Correlation of the calibration curves generated from the standard and fortified matrix sample sets were used as criteria to determine and evaluate signal suppression.

LC-MS Analysis

Liquid chromatography was carried out with a HPLC-1100 (Hewlett Packard, Wilmington, DE); injection volume was 50 or 100 μL. Analytes were chromatographed with a Supelco (Supelco, Bellefonte, PA) Supelcosil LC-18-DB (15cm × 3mm × 5μm) column. The solvent gradient was composed of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The flow rate of the mobile phase was 0.8 mL/min. The initial gradient was 60% A and was decreased to 40% at 1 minute. Phase A was decreased to 10% at 6 minutes. Phase A was returned back to 60% at 8 minutes.

LC-MS analysis was performed on an API-365 triple quadrupole ESI mass spectrometer (Perkin Elmer, Foster City, CA) and Mariner time-of-flight ESI mass spectrometer (Perseptive Biosystems, Framingham, MA). The API-365 was operated under positive mode with the following settings: *ca.* 3500V needle potential, 30V orifice, 200V ring voltage. For MS/MS analysis of methoxy-fenozide, the daughter ion at 149.2 m/z was monitored. The Mariner was operated under positive mode with spray tip, nozzle, and skimmer potential applied at *ca.* 3200V, 110V and 12V respectively.

Effluent from the LC was split to allow a flow rate of 10 to 25 μL/min to the ion source. The API-365 was used for single-ion monitoring of analytes under

MS and tandem MS/MS modes. The Mariner was used to acquire mass spectra in the mass range of 340 to 380m/z.

A schematic for post-column introduction of the internal standard is shown in Figure 3. A syringe pump (Cole-Parmer Instrument Co., Vernon Hills, IL), was used to mix a 1.0 ppm standard solution into the LC effluent, immediately prior to the ion source. The flow rate of the syringe pump was set ca. 1 μL/min.

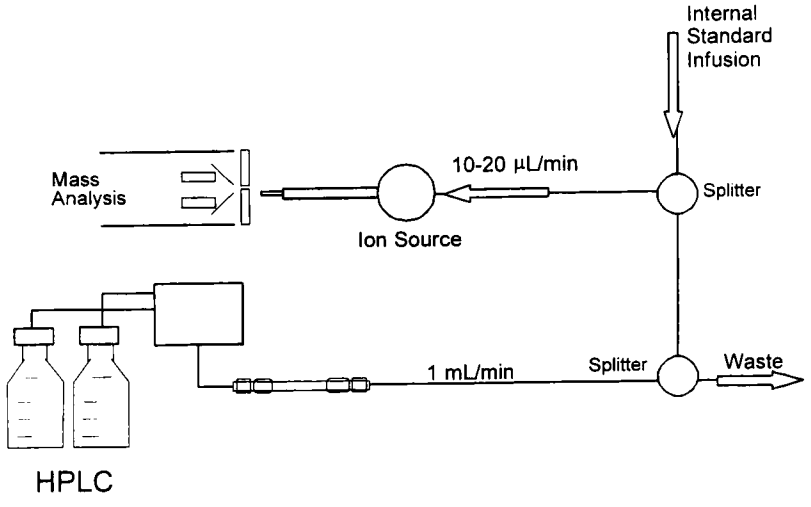

FIGURE 3 Schematic of the post-column infusion technique for internal standards

RESULTS AND DISCUSSION

A. Signal Suppression

Matrix induced signal suppression effects are routinely addressed when performing LC-MS analysis of compounds in physiological matrices [9, 10]. Although the actual mechanism is still unclear, reports suggest that signal suppression in electrospray occurs when excess electrolytes promotes charge transfer reactions in the gas phase [11–13]. The charge transfer reactions inhibit efficient ionization of the target anlayte, thereby producing lower signal. Presumably, the excess electrolytes responsible for signal suppression are extracted from the matrix with the analyte.

Environmental matrixes (e.g. water, soil, plant) can also contribute to significant suppression of the analyte signal. Signal suppression attributed to the wheat

hay matrix is illustrated in Figure 4. A tebufenozide and hydroxy-tebufenozide (1:1) solution was infused continuously into the LC effluent, as shown in Figure 3. In a blank LC run (injection of 1:1 acetonitrile:water) under isocratic conditions (60% ACN/W), the selected-ion chromatograph trace of tebufenozide and hydroxy-tebufenozide was flat, indicating consistent signal response with no matrix signal effects (Figure 4.1A and 4.2A). However, when the wheat extract (bypassing steps 2–5 of Figure 1) was injected, a significant decrease of both analyte signal intensities was observed (Figure 4.1B and 4.2B). Signal suppression ranged from 0% to 70%, depending on the elution time.

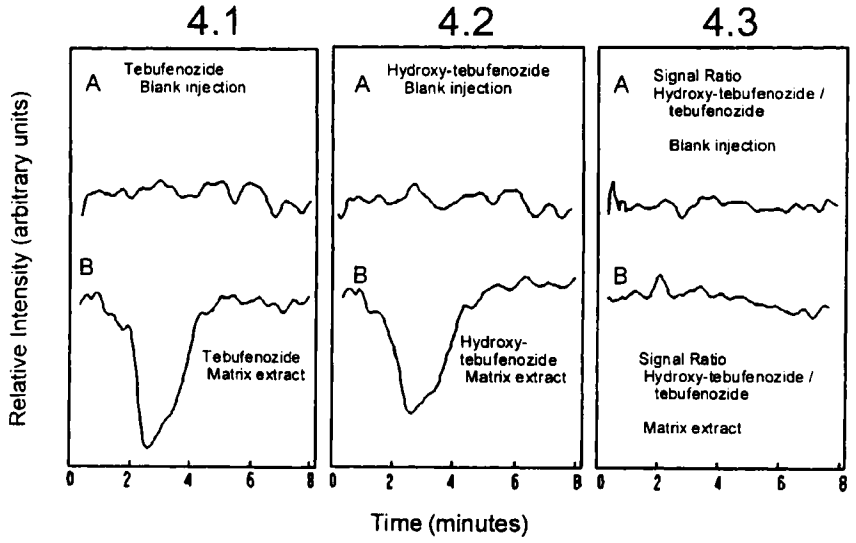

FIGURE 4 LC-MS single-ion chromatograms of tebufenozide and hydroxy-tebufenozide under isocratic conditions (60% ACN:W) using continuous post-column infusion. In each frame, (A) is a blank HPLC run and (B) is for an injection of a wheat hay matrix extract after liquid-liquid partition. Frame 4.3 shows the signal ratio of hydroxy-tebufenozide to tebufenozide for the corresponding chromatograph traces

One strategy which is often used to obtain accurate and reproducible quantification data from matrix samples is to incorporate clean-up or purification procedures into the analysis method [10]. An alternative strategy is the application of internal standards. The introduction of a compound sharing similar chemical properties with the target analyte assures both compounds will experience similar signal suppression or enhancement effects. As a result, the signal ratio of the analyte and internal standard will always be constant. This is illustrated by Figure 4.3A and 4.3B. The ratio of the signal traces of tebufenozide and

hydroxy-tebufenozide from the matrix run restores the consistent baseline, thus demonstrating the internal standard signal compensation effect.

B. D_3 Labeled Internal Standards

The ideal internal standard is an isotopically labeled analog of the target analyte. It also desirable that the internal standard is sufficiently labeled so that its mass will not overlap with the target analyte's naturally occurring isotopic species. Such mass separations can be achieved by multiple substitution of deuterium in of the target (e.g. D_3 labeled compounds).

Figure 5 shows the calibration curves for triester-thiazopyr obtained from standard solutions and from fortified wheat hay matrix samples after undergoing the complete extraction and clean-up procedures (all steps outlined in Figure 1). A strong correlation between the fortified matrix and standard solution calibration curves was observed for the external calibration method (Figure 5A). A similar correlation was also found for curves using D_3- labeled triester-thiazopyr as an internal standard (Figure 5B). The average recovery data (calculated quantity of pesticide found in the fortified matrix sample compared to the actual analyte quantity) were found to be similar: external calibration resulted in 100% ± 10% while internal calibration resulted in 101% ± 10% recovery. The comparable recoveries show that accurate quantification after extensive clean-up is possible both with and without internal standards. However, it should be emphasized that this would not be the case should the clean-up procedures be omitted.

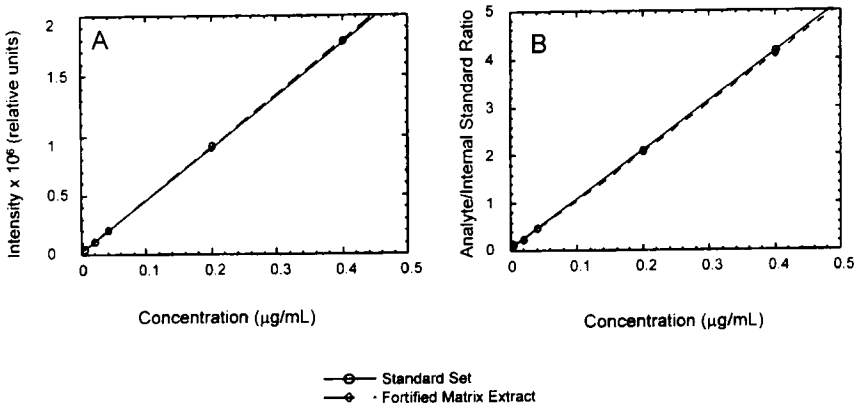

FIGURE 5 Calibration curves for triester-thiazopyr obtained with (A) external calibration, and (B) D_3-triester-thiazopyr internal calibration. The curves were obtained for a standard sample set (1:1 ACN:W) and a matrix fortified sample set (complete comprehensive clean-up) Concentration of internal standard was 0.1 µg/mL

When certain clean-up procedures for matrix samples are bypassed from the analysis method, signal suppression becomes readily apparent. Figure 6 shows the calibration curves obtained from the standard triester-thiazopyr solutions and fortified matrix samples when the SPE procedure is bypassed (step 6 of Figure 1). Three sets of samples were run in sequence: standard solutions, fortified matrix, and a repeat of the standard solution set.

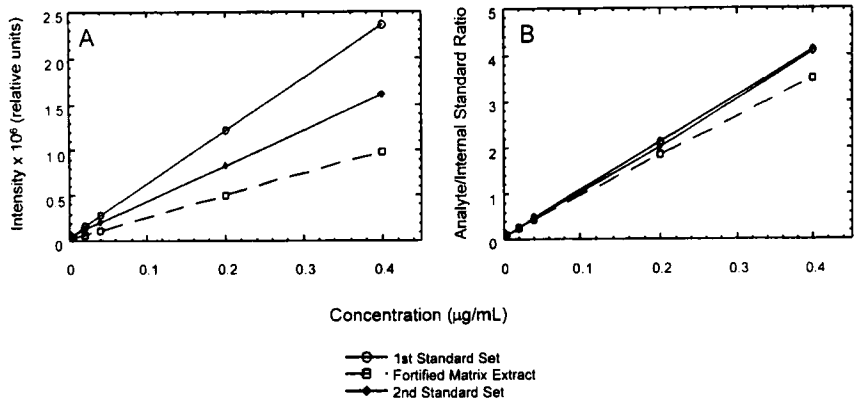

FIGURE 6 Analysis of triester-thiazopyr (A) with external calibration and (B) internal calibration using D_3-triester-thiazopyr. The curves were obtained from a standard sample set (1:1 ACN:W) and a matrix fortified sample set (bypassing SPE clean-up). Concentration of internal standard was 0.1 µg/mL

The signal response from matrix samples was nearly a factor of two lower compared to that obtained from the first set of standards (Figure 6A). Furthermore, the lower signal response obtained during the second standard set run indicates that matrix suppression is carrying over between sample runs (i.e. a memory effect). Based on the external calibrated data, the average percent of analyte recovered was 36% ± 34%. Figure 6B shows that application of a D_3 analog as a volumetric internal standard provides the necessary correction of signal suppression and memory effect to generate more accurate and precise quantification data (average recovery was 87% ± 5%). This confirms that extensive clean-up procedures are necessary to produce accurate and precise recovery data with external standards. However, the results also demonstrate that a less comprehensive clean-up may be used with the aid of internal standards.

C. Singly Isotopically Labeled ^{13}C Internal Standard

Isotopically labeled internal standards which are different from the target analyte by one mass unit (e.g. singly ^{13}C labeled compounds) may be used to address

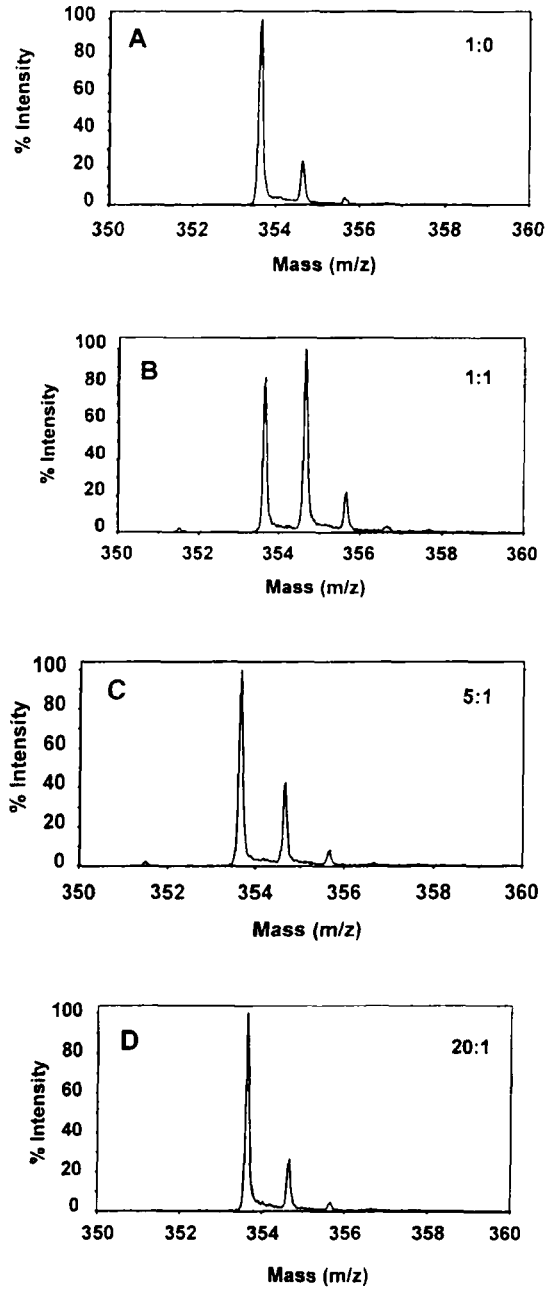

FIGURE 7 Mass spectra of (A) tebufenozide only, and (B) 1:1, (C) 5:1, (D) 20:1. tebufenozide: ^{13}C-tebufenozide mixture (v/v)

signal suppression. However, there can be some difficulties associated with the application of this type of internal standard. By comparison, a D_3 labeled compound is sufficiently different in mass so that the target analyte and internal standards are completely resolved by the mass spectrometer. But this is not the case for a ^{13}C labeled internal standard. Figure 7 shows the mass spectra of tebufenozide mixed with various concentrations of ^{13}C labeled tebufenozide. Interference readily occurs between the ^{13}C labeled internal standard and the naturally occurring ^{13}C isotope of analyte (Figure 7A-B). Furthermore, it should be noted that as the analyte concentration increases, the relative intensity of the ^{13}C internal standard peak and the ^{13}C analyte becomes virtually indistinguishable (Figure 7A and 7D). The significance of this effect becomes apparent in Figure 8.

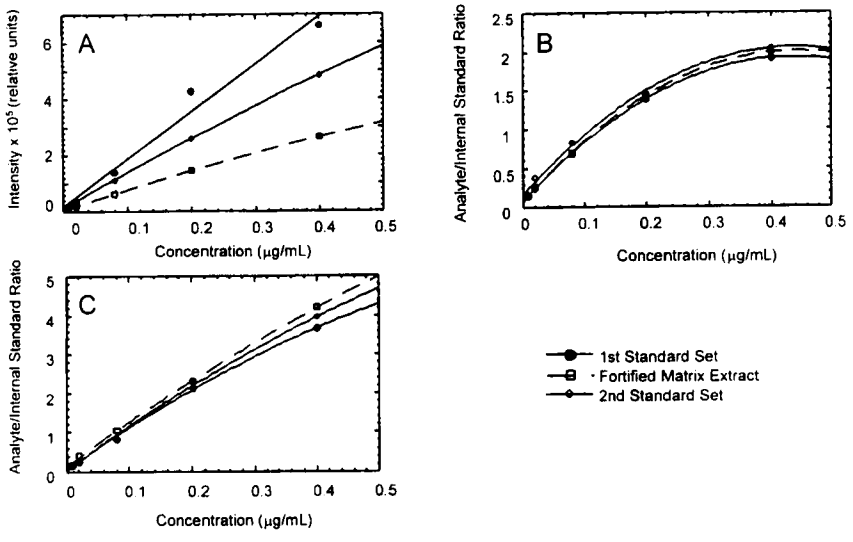

FIGURE 8 Analysis of tebufenozide (A) with external calibration and (B) ^{13}C-tebufenozide internal calibration, and (C) ^{13}C-tebufenozide internal calibration with background subtraction. The curves were obtained from a standard sample set (1:1 ACN:W) and a matrix fortified sample set (bypassing SPE clean-up). Concentration of internal standard was 0.1 µg/mL

Fortified wheat hay matrix samples with the SPE procedure omitted from the clean-up (step 5, Figure 1) were analyzed by LC-MS. Figure 8 shows tebufenozide calibration curves obtained from the first standard solution run, matrix samples, and a repeat of the standard solution run in sequence. Matrix signal suppression and memory effects are evident from Figure 8A. Figure 8B shows a calibration curve obtained by using ^{13}C-tebufenozide as an internal standard.

Despite the interference, the ^{13}C internal standard is capable of providing sufficient correction of signal suppression and memory effects. However, a significant slope decrease appears in the high concentration range. As the analyte concentration increases, the relative intensity of the actual ^{13}C internal standard becomes less significant relative to the target analyte's naturally occurring ^{13}C contribution, as shown in Figure 7.

The non-linear calibration may contribute to lower accuracy when quantifying at the higher concentration range. One method to correct for this effect is to increase the concentration of the ^{13}C internal standard. But this may not be desirable if the internal standard's isotopic purity is not sufficiently high. At higher concentrations, isotopic impurities (^{12}C) in the internal standard can become readily apparent, and result in a higher y-intercept on the calibration curve. Another method to correct for nonlinear-calibration involves subtracting the analyte ^{13}C contribution from the absolute ^{13}C signal, prior to generating the standard curve. The background subtraction strategy results in a more linear calibration. (Figure 8C). This demonstrates that a singly labeled internal standard can be used to address quantification errors associated with matrix signal effects, although it should be used with caution.

D. Application of Non-Isotopically Labeled Internal Standards

Although it is generally desirable to use isotopically labeled internal standards, non-isotopically labeled internal standards (structural analogs or target analyte derivatives) have been used to correct for signal suppression and instrumental errors in mass spectrometry [8]. However, there are some difficulty associated with using this type of internal standard for LC-MS. Structurally different compounds tend to be readily separated during LC separation. As a result, the different elution times for the analyte and structurally different internal standard can pose a significant problem when analyzing samples rich with matrix components. It was shown in Figure 4 that signal suppression can range from 0–70% depending on the time of elution. Therefore, if the internal standard is sufficiently separated from the target analyte, the two compounds may experience drastically different signal suppression.

Figure 9 shows calibration curves obtained by using ^{13}C-tebufenozide as a volumetric internal standard for the analysis of tebufenozide and hydroxy-tebufenozide mixture. Suppression in the wheat hay extract was induced by omitting the SPE procedure (step 5 of Figure 1). The retention times of the two compounds differ by approximately 3 minutes. As anticipated, a close correlation between the curves derived from the matrix and standard samples show that ^{13}C-tebufenozide was effective in correcting signal suppression for

tebufenozide (Figure 9A). However, correlation of calibration curves from the standard and matrix hydroxy-tebufenozide samples were generally poor when using the ^{13}C-tebufenozide internal standard (Figure 9B). The poor correlation indicates that ^{13}C-tebufenozide is not effective in correcting signal suppression for hydroxy-tebufenozide when introduced volumetrically prior to LC separation.

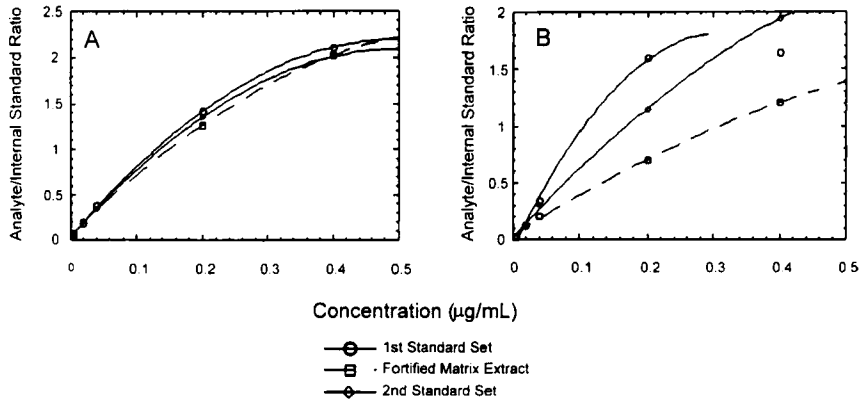

FIGURE 9 Calibration curves for (A) tebufenozide and (B) hydroxy-tebufenozide using ^{13}C-tebufenozide internal standard. The curves were obtained from a standard sample set (1:1 ACN:W) and matrix fortified sample set (bypassing SPE clean-up). Concentration of internal standard was 0.1 µg/mL

Failure to correct the signal suppression of hydroxy-tebufenozide may be caused by either chemical dissimilarities between the standard and analyte, or variations of suppression with respect to different elution times. However, Figure 4 shows that the signal ratio of the post-column infused hydroxy-tebufenozide and tebufenozide are constant throughout an LC run of wheat hay extract. This suggests that that variations in the extent of signal suppression is the primary contributor to failure in this case.

E. Continuous Post Column Infusion of Internal Standards

To compensate for variations in signal suppression with respect to elution time, the internal standard can be introduced continuously into the LC effluent. This post-column introduction method insures that the target analyte and internal standard ionize simultaneously under identical solution conditions (schematic shown in Figure 3). The setup allows the internal standard to mix in volumetric proportions with all components eluting from the LC column.

Tebufenozide and hydroxy-tebufenozide were analyzed in a wheat hay matrix (SPE procedures bypassed in clean-up procedures) using the volumetric internal standard and post-column introduction methods (Figure 10). ^{13}C-tebufenozide was used as the internal standard. Figure 10A-B shows the calibration curves for tebufenozide and hydroxy-tebufenozide, using external standard calibration (i.e. calibration based on absolute signal response). The matrix signal effect is apparent by the lower signal response obtained for the matrix sample; the memory effect is also evident for both analyte samples. Figure 10C-D shows the calibration curves obtained using ^{13}C-tebufenozide as a volumetric internal standard. As previously noted, tebufenozide signal suppression was compensated while hydroxy-tebufenozide was not. Figure 10E-F shows the calibration curves obtained by using the internal standard introduced by post-column infusion. Introducing the internal standards in this manner was found to be effective in correcting matrix effects for both tebufenozide and hydroxy-tebufenozide. Recovery of hydroxy-tebufenozide was improved to 94% ± 15%, compared to 67% ± 20% obtained by using a volumetric internal standard.

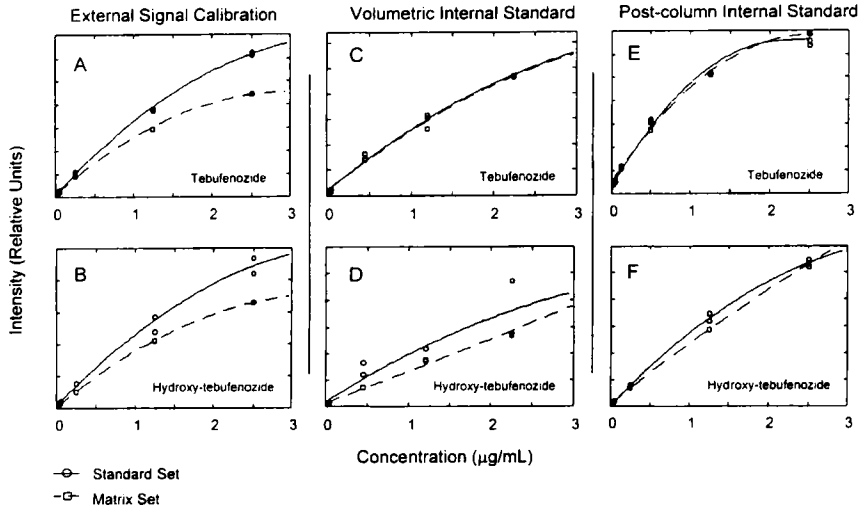

FIGURE 10 Calibration curves for tebufenozide and hydroxy-tebufenozide using external standard signal calibration (A,B), a volumetric internal standard (C,D), and a ^{13}C-tebufenozide post-column internal standard (E,F)

The application of the post-column internal standard introduction offers other advantages over surrogate or volumetric methods. Surrogate or volumetric internal standards generally require a single isotopically labeled internal standard for

each analyte to be quantified. In contrast, the post-column introduction method does not require isotopically labeled internal standards to provide effective compensation for signal suppression. Derivatives or structural analogs, which are more readily available, can be effective internal standards using this method. Furthermore, the results demonstrate that the post-column introduction method allows the application of a single internal standard to compensate signal suppression for multiple analytes in an individual sample.

It is important to note that the post-column introduction method cannot compensate for signal loss associated with sample preparation. However, this method may be of use in other applications where analyte loss attributed to extraction/clean-up procedures is determined (e.g. evaluation of method efficiency). In many analytical methods, signal loss associated with sample preparation may be nominal. For such cases, application of the post-column method may prove to be especially convenient.

F. Application of Internal Standard to Method Development

Residue methods for the analysis of pesticides can be developed so that internal standards are not required for accurate quantification. As a consequence of developing methods to purify analytes in complex matrices, these methods tend to be complex and time consuming. Depending on the number of samples to be quantified, the clean-up procedures alone can take several hours per sample. The time associated with performing this type of method not only adds considerable expense to the actual analysis, but complicates the method development process as well. In addition, relatively large quantities of waste are generated, which are often toxic and possibly radioactive (i.e. ^{14}C labeled tracers). The purpose of extensive clean-up is to eliminate effects contributed by the matrix: mass interference and signal suppression. By developing methods incorporating internal standards, errors associated with signal suppression can be addressed. As a result, several procedures from the standard clean-up method can be bypassed completely. Application of volumetric and post-column introduction of internal standards was shown to correct suppression effects for methods bypassing the SPE procedure.

It would be ideal if the clean-up procedure could be omitted entirely; however, then mass interference becomes an issue. This is apparent for the analysis of methoxy-fenozide when the open-column and SPE procedures are bypassed. Figure 11A shows the LC-MS single-ion chromatogram of methoxy-fenozide. Omission of the open-column separation procedure limits reliable quantification to approximately 0.02 μg/mL, due to the relatively large background. One technique which can be used compensate the mass interference effect is tandem mass

spectrometry, LC-MS/MS, where fragments characteristic of the target analyte and internal standards are monitored. Applying this technique with internal standards, background interference can be eliminated in addition to correction of suppression effects (Figure 11B). Using this technique, the limit of quantification was improved to 0.004 μg/mL. Although the application of LC-MS/MS may be used to address the mass interference issues, some form of clean-up will always be required. Minimum clean-up would prevent the physical accumulation of non-volatile matrix components in the electrospray interface, saturation of the LC column, and insure that the extent of signal suppression does not compromise the desired limit of quantification.

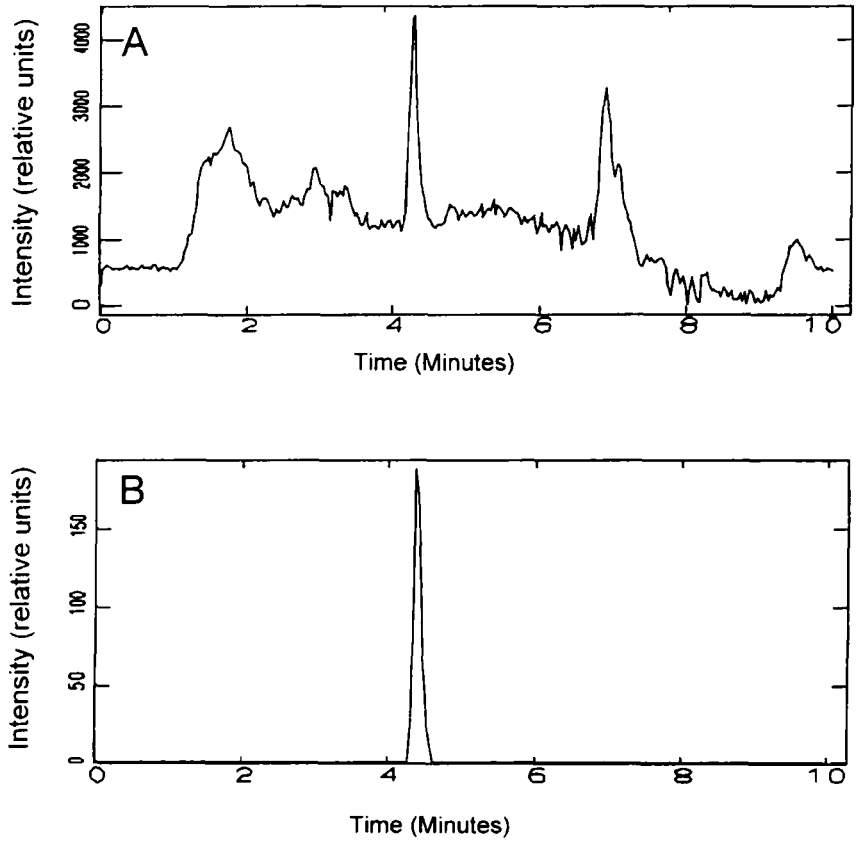

FIGURE 11 Single-ion chromatography of methoxy-fenozide (0.2 μg/mL) obtained by (A) LC-MS and (B) LC-MS/MS analysis (by-passing open column and SPE clean-up procedures)

CONCLUSION

In this report, various approaches to introducing and applying internal standard compounds for liquid chromatography mass spectrometry (LC-MS) analysis of pesticides was studied. Isotopically labeled internal standards were found to be highly effective in correcting matrix signal suppression when introduced as surrogates or volumetrically. Although D_3 labeled internal standards may be preferred, singly labeled ^{13}C compounds can be viable alternatives. Application of structural analogs of analytes as internal standards can be limited as surrogate/volumetric internal standards by issues associated with different LC elution times. However, both structural analogs and isotopically labeled compounds can be effective in correcting signal suppression for multi-component mixtures in a single LC run when introduced by continuous post-column infusion.

The application of internal standards to correct matrix signal effects allows a less comprehensive clean-up procedure than is necessary for LC-MS analysis. Clean-up can be further minimized by combining the application of internal standards with tandem MS (i.e. LC-MS/MS). Thus, the application of internal standards can significantly simplify residue analysis method development and clean-up while allowing the methods to be more robust, reproducible and generate less waste.

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