Determination and Confirmation of Organophosphate Pesticides and Their Metabolites in Beef Tissue Using Thermospray/LC–MS†

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The ion formation characteristics of a group of organophosphate pesticides (OPs) and some of their primary and secondary metabolites were determined using a thermospray interface coupling a high-performance liquid chromatograph to a mass spectrometer (TSP/LC–MS). Full-scan mass spectra using a variety of ionization techniques, including filament-off, filament-on, and discharge-on, and two detection modes, positive ion (PI) and negative ion (NI), were collected for reference standards. A majority of the OPs and metabolites investigated produced ions in greatest abundance in the NI, discharge-on mode. Using a modification of a previously developed method, 17 OPs and metabolites were extracted from lean (4.1% fat) and fatty (29.3% fat) beef muscle. Recovery studies were performed at the 1 ppm fortification level in each sample type. Recoveries using negative ion TSP/LC–MS in the selected ion mode (SIM) ranged from 10 to 100+%. Response of the MS detector was linear over the 1–100 ppm concentration range for all detected compounds. Detection limits ranged between 1 and 5 ppm (20–100 ng injected). Five ions per compound were monitored in SIM, and their intensities were adequate for confirmation of compound identity.

Keywords: *Organophosphate pesticide; metabolites; thermospray mass spectrometry; beef*

INTRODUCTION

Potential exists for the presence of organophosphate pesticide (OP) residues as well as products of OP metabolism in animal tissues intended for food use. Formation of the primary metabolite (oxon) results in a compound exhibiting more potent cholinesterase-inhibiting activity than the parent OP and thus, is, considered a bioactivation (Sipes and Gandolfi, 1991; WHO, 1986).

The toxic potential of OP hydrolysis products (secondary metabolites) has not been established. However, regulatory limits for a number of these compounds are included within the published tolerance levels for food products (CFR, 1993). Conjugates of these metabolites, produced during normal phase II metabolism, can be hydrolyzed by intestinal microflora. The resulting hydrolysis product can be absorbed in the enterohepatic circulation, introducing the potential for recycling (Chambers and Chambers, 1991). Felsot and Pederson (1991) noted that, although acute toxicity is almost never associated with these transformation products, potential long-term effects have been a concern for some time.

High-performance liquid chromatography (HPLC) coupled with mass spectroscopy (MS) provides the analyst with a powerful tool for residue determination. Interfacing these devices is most commonly accomplished at present by thermospray (TSP) technique (Lindsay, 1992). The TSP/LC–MS configuration allows the molecular mass characterization of many members of the OP class of pesticides and metabolites. Numerous workers have investigated the application of TSP/LC–MS to OP analysis. Voyksner and Haney (1985) attempted to evaluate and optimize several parameters, including interface temperature, mobile phase buffer, and system stability, for pesticide determination. Exploring the use of nonpolar mobile phases for TSP/LC–MS of several pesticides, including OPs, was the focus of a study by Barcelo et al. (1990). In another study, Barcelo et al. (1987) investigated the effect of chloride addition to the HPLC mobile phase for detection enhancement using an on-line LC–MS method for OPs. Modern instrumentation allows the option of monitoring the formation of negative ions (NI) as well as positive ions (PI). Several recent studies of OPs comparing the relative advantages of this capability have been performed (Barcelo et al., 1990; Farran et al., 1988, 1990; Barcelo and Albaiges, 1989; Parker et al., 1982a; Barcelo, 1988a). Frequently, LC–MS produces very limited molecule fragmentation, thereby precluding accurate structural characterization. Betowski and Jones (1988) overcame this shortcoming by utilizing HPLC in conjunction with tandem MS.

The utility of HPLC with diode array detection for OP and metabolite determination in beef tissues was investigated in a previous study (Ioerger and Smith, 1993). The present study is a continuation of that work by application of TSP/LC–MS to the OP and metabolite analysis. To our knowledge, little work has been done to date on the application of TSP/LC–MS for the determination of OPs and their primary and secondary metabolites in beef tissue. The objectives of the present study were (1) to determine the ion formation characteristics of a group of OP parent compounds as well as their primary and secondary metabolites using TSP/LC–MS and (2) to evaluate the effectiveness of TSP/LC–MS for the determination and confirmation of OPs and their primary and secondary metabolites in beef muscle samples varying in fat content.

EXPERIMENTAL PROCEDURES

Reagents. Ronnel, fenthion, parathion, paraoxon, chlorpyrifos, coumaphos, coumaphos oxon, famphur, famphur oxon, and chlorpyrifos-methyl reference standards were ob-

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tained from ChemService, Inc. (West Chester, PA). Ronnel oxon was donated by Dow Elanco (Midland, MI), and fenthoxon was a gift from Mobay Corp. (Kansas City, MO). Stirofos was purchased from Supelco, Inc. (Bellefonte, PA), and 3-methyl-4-(methylthio)phenol was from Sigma Chemical Co. (St. Louis, MO). Other standards used in the study included *p*-nitrophenol, 3-chloro-4-methyl-7-hydroxycoumarin, and 2,4,5-trichlorophenol obtained from Aldrich Chemical Co. (Milwaukee, WI).

Ammonium acetate for buffer preparation was also from Aldrich. Solvents used were OPTIMA grade acetonitrile, ethyl acetate, methanol, and ACS grade petroleum ether (Fisher Scientific, Pittsburgh, PA). Deionized water was obtained from a Sybron/Barnstead PCS unit (Barnstead/Thermolyne, Inc., Dubuque, IA).

Apparatus. A Hewlett-Packard Model 1090M Series II liquid chromatograph equipped with a DR5 (binary) solvent delivery system and a scanning UV–visible photodiode array detector (DAD) was used. A Hewlett-Packard ChemStation (Pascal series) with software HP79988A rev. 5.22 and HP79997A rev. 5.20 was used for system control, data storage, and processing. Separations were performed on a 250 × 4.6 mm i.d. Bio-Sil C_{18} HL 90 column (Bio-Rad Laboratories, Richmond, CA) having a particle size of 5 μm. A 10 mm x 4.6 mm cartridge packed with Econosil C_{18} 5 μm (Alltech Associates, Inc., Deerfield, IL) was used as a guard column.

A Hewlett-Packard Model 5989A MS Engine equipped with a thermospray interface was used for collection of mass spectral data. System control, data storage, and processing were achieved with a Hewlett-Packard 59940A MS ChemStation (HP-UX series) using software HP59944B rev. B.04.03.

Chromatographic Conditions. Organophosphate pesticides and their metabolites were separated using a step-gradient mobile phase described in a previous study (Ioerger and Smith, 1993). The initial mobile-phase composition was acetonitrile–0.1 M ammonium acetate (55:45 v/v), which was held constant from 0.00 to 11.00 min. At 11.10 min, acetonitrile was increased to 65%. From 11.10 min, the gradient was programmed linearly to a final composition of acetonitrile–0.1 M ammonium acetate (70:30 v/v) at 31.00 min.

MS Conditions. Full-scan mass spectra (*m/z* 83.00–750.00) of individual OP and metabolite reference standards were obtained by direct injection (no chromatographic column). The concentrations of all such injections were 100 ppm. The scan threshold was set at 90 with an a/d value of 2, resulting in 0.28 scan/s. Mass spectra of reference standards were generated using positive or negative ion detection with either filament-off, filament-on, or discharge-on ionization modes. Ion source temperature was maintained at 225 °C, and the quadrupole temperature was 100 °C. The thermospray probe interface temperature was set to produce 95% solvent vaporization as determined by thermospray probe surveys. For the mobile phase of acetonitrile–0.1 M ammonium acetate (55:45 v/v), this temperature was about 99 °C. Increasing the acetonitrile component of the mobile phase to 65% during the step-gradient elution required lowering the thermospray vaporization temperature to 95 °C to maintain 95% solvent vaporization. Mass spectrometer conditions during selected ion mode (SIM) analyses were the same as those for full-scan data. Specific ions monitored for each OP and metabolite and the relative intensities obtained from standard injections are presented in Table 1. Dwell time was set to 50 ms for each ion, and electron multiplier voltage was 1948–2255 V depending on results from instrument tuning.

Extraction Procedure. Lean (4.1% fat) and fatty (29.3% fat) beef muscles were obtained from a local supermarket and analyzed for moisture and fat content (AOAC, 1990). For OP residue analysis, a modification of a previously developed OP and metabolite extraction procedure was used (Ioerger and Smith, 1993). The 10 g samples of ground muscle were extracted as described in that method (i.e., extraction by blending with 2.7 g of NaCl plus 20 mL of ethyl acetate–methanol (90:10 v/v), up to the point of first filtrate collection. This filtrate was collected into a 50 mL volumetric flask for 10 min. At the end of 10 min, the spiked muscle residue was pressed with a spatula to express most of the solvent. The muscle residue and filter paper were re-extracted by blending for 2 min at low speed after addition of 25 mL of extraction solvent. The mixture was allowed to set for 10 min, briefly remixed, and added to another filter (Whatman No. 2). The blender was washed with an additional 5 mL of extraction solvent, which was added to the filter. Collection of this filtrate was continued into the same 50 mL volumetric flask for 10 min. The spiked muscle residue was pressed again to express retained solvent. The collected filtrate was brought to volume with extraction solvent. Filtrate was transferred to a screw-cap sample bottle and allowed to chill in a freezer for at least 6 h at −13 °C. Liquid filtrate was separated from precipitate by cold filtering through Whatman No. 2 filter paper for 5 min. Precipitate was discarded, and 25 mL of collected filtrate was taken to near dryness on a rotary evaporator at 40 °C under reduced pressure. The residue remaining after rotary evaporation was resuspended in 1 mL of methanol and treated as in the previous method (i.e., C_{18} SPE cleanup and 0.45 μm filtration; Ioerger and Smith, 1993).

Table 1. Negative Ions Monitored and Approximate Relative Ion Intensities Used for Confirmation of OPs and Metabolites in the Study

compound	ions monitored/relative ion intensities				
p-nitrophenol	138/100	139/63	261/36	277/36	278/36
3-chloro-4-methyl-7-hydroxycoumarin	175/65	209/100	210/67	211/89	419/39
3-methyl-4-(methylthio)-phenol	153/90	213/100	214/96	215/88	367/66
paraoxon	138/100	258/40	274/41	275/53	276/41
coumaphos oxon	153/91	209/93	346/100	347/76	348/80
famphur	141/100	200/66	216/95	281/60	310/55
2,4,5-trichlorophenol	195/65	197/100	199/81	201/57	233/53
ronnel oxon	125/85	197/100	199/85	218/79	254/78
stirofos	125/100	126/62	166/62	185/83	186/67
parathion	154/96	169/100	291/99	292/81	331/78
coumaphos	169/100	211/90	225/91	362/87	364/76
chlorpyrifos-methyl	141/94	212/94	214/100	216/76	285/67
ronnel	141/98	211/96	213/100	215/89	270/73
chlorpyrifos	169/100	212/98	313/84	315/82	351/72

Compound Response Linearity and Limit of Detection. Pure reference standards of the pesticides and metabolites were used to make stock solutions. From these, serial dilutions were made to give concentrations in the range of interest. Mass spectrometer detector response linearity was examined for reference standards of the OPs and metabolites over the 1–100 ppm concentration range. Analyte peaks obtained in NI discharge-on SIM were integrated and plotted as functions of concentration.

Recovery Analysis. Recovery studies were performed at the 1.0 ppm fortification level of each pesticide for three extractions in each muscle type (lean and fatty). These samples were prepared by adding 100 μL of a 100 ppm standard of each OP and metabolite to 10 g of ground muscle before extraction. The extractions were performed as described, and duplicate injections of 20 μL from each extraction were made. Injections of standard at a concentration of 5 ppm containing all of the OPs and OP metabolites were made before and at the end of a day's sample runs. These peak areas were averaged and used to calculate that day's compound recoveries according to

$$\% \text{ recovery} = \frac{(5 \text{ ppm})(\text{DF})(\text{extracted cpd area})(100)}{(5 \text{ ppm cpd area})} \quad (1)$$

where DF is the dilution factor derived as 0.20 from (50 mL of collected filtrate/10 g of tissue)/25 mL aliquot of filtrate. Recovered compound identification was by retention time, presence or absence of ions monitored in SIM, and relative abundance ratios of the five ions monitored per OP compound. Abundance ratios for ions of a given recovered OP compound were compared to abundance ratios of the same ions monitored for reference standards.

Table 2. Chemical Formulas and Molecular Weights of OPs and Metabolites Used in the Study

compound	MW	formula
famphur oxon	309.3	$(CH_3O)_2PO_2C_6H_4SO_2N(CH_3)_2$
p-nitrophenol	139.1	$C_6H_4NO_2OH$
3-chloro-4-methyl-7-hydroxycoumarin	210.6	$C_{10}H_6O_2ClOH$
3-methyl-4-(methylthio)-phenol	154.2	C_8H_9SOH
paraoxon	275.2	$(C_2H_5O)_2PO_2C_6H_4NO_2$
fenthoxon	262.3	$(CH_3O)_2PO_2C_7H_6SCH_3$
coumaphos oxon	346.7	$(C_2H_5O)_2PO_2C_{10}H_6O_2Cl$
famphur	325.4	$(CH_3O)_2PSOC_6H_4SO_2N(CH_3)_2$
2,4,5-trichlorophenol	197.5	$C_6H_2Cl_3OH$
ronnel oxon	305.5	$(CH_3O)_2PO_2C_6H_2Cl_3$
stirofos	366.0	$(CH_3O)_2PO_2C_2HClC_6H_2Cl_3$
parathion	291.3	$(C_2H_5O)_2PSOC_6H_4NO_2$
fenthion	278.3	$(CH_3O)_2PSOC_7H_6SCH_3$
coumaphos	362.8	$(C_2H_5O)_2PSOC_{10}H_6O_2Cl$
chlorpyrifos-methyl	322.5	$(CH_3O)_2PSOC_5HNCl_3$
ronnel	321.6	$(CH_3O)_2PSOC_6H_2Cl_3$
chlorpyrifos	350.6	$(C_2H_5O)_2PSOC_5HNCl_3$

RESULTS AND DISCUSSION

Preliminary analysis of the OPs and metabolites by thermospray LC–MS (TSP/LC–MS) focused on the investigation of characteristic ion formation under various ionization conditions. Table 2 provides the structural formulas and molecular weights of the OPs and metabolites used in the study. Work in this portion of the study involved the injection of pure reference standards to acquire full-scan mass spectra of individual OPs and metabolites. The ionization methods used included filament-off, filament-on, or discharge-on in order of mildest to more energetic ionizing conditions. With the HPLC mobile phase of acetonitrile–0.1 M ammonium acetate, solvent cluster ions were apparent in the mass spectra of blank mobile-phase injections. The most apparent solvent cluster ions appeared at *m/z* 100 in the PI detection mode and at *m/z* 119 in the NI detection mode. The signal at *m/z* 100 was identified tentatively as the $[CH_3COO + CH_3CN]^+$ or $[(CH_3CN)_2 + NH_4]^+$ ion, and the proposed identification for the 119 mass was the $[CH_3COO + CH_3COOH]^-$ ion. Similar solvent cluster ions have been reported in an earlier investigation (Barcelo, 1988b).

Base peaks for the OPs and metabolites using PI monitoring mode were most frequently the $[M]^+$ ion and the $[M + H]^+$ and $[M + NH_4]^+$ adduct ions. Such ions have been observed previously for the OP class using PI TSP/LC–MS (Voyksner and Haney, 1985; Betowski and Jones, 1988; Barcelo and Albaiges, 1989; Farran et al., 1988). Adduct ions predominated in the PI mass spectra for filament-on and discharge-on ionization modes. Fragment ions were observed as base peaks for paraoxon and parathion in the form of $[M - C_2H_5]^+$ and for ronnel as the $[(CH_3O)_2PSO]^+$ ion. Unidentified fragment ions also appeared at *m/z* 300 (base peak), 212, and 168 for famphur oxon and *m/z* 141 and 113 for 2,4,5-trichlorophenol, and an unidentified adduct ion base peak appeared at *m/z* 151 for *p*-nitrophenol. No positive ions were detected without use of the filament or discharge electrode.

Ion base peaks in the NI detection mode exhibited greater variety among compounds in terms of proposed structures than those in the PI detection mode. In contrast to the PI mode, fragment ions predominated in the NI mass spectra of OPs and their metabolites. Base peaks most frequently took the form of $[M]^-$, $[M - H]^-$, or $[FG]^-$ [where FG is the functional group represented as $(RO)_2PSO^-$ or $(RO)_2PO_2^-$ with R equal to CH_3 or C_2H_5]. Other base peak ions formed included $[M - FG]^-$ (the so-called thiophenolate or phenolate ion), $[M - R]^-$, $[M + CH_3COO]^-$, $[M - HCl]^-$, $[M - 3Cl]^-$, and the dimerization product $[2M - H]^-$. The few base peak adduct ions present in the NI mode included the $[M + 20]^-$ (filament-on) and $[M + CH_3COO]^-$ (discharge-on and filament-off) ions for 3-methyl-4-(methylthio)phenol and the $[M + 2H]^-$ base peak for paraoxon (filament-off).

Many of the ions for OPs seen in this work have been noted in other studies involving NCI (Stan and Kellner, 1982), pulsed positive negative CI (Stan and Kellner, 1989), and TSP/LC–MS in the negative mode (Durand et al., 1991). Discussions of the mechanisms for the structure and formation of these ion fragments are found in these references.

The most apparent trend in terms of the effect of ionization method on ion abundances was that, with three exceptions, NI detection after discharge-on ionization provided the greatest ion intensities. Monitoring the positive ions occurring after filament-on ionization showed that fenthion, fenthoxon, and famphur oxon produced ions of highest intensities in that mode. Higher ion intensity translates into greater compound sensitivity in relation to the detection of extracted analyte residues via the TSP/LC–MS system.

Enhanced sensitivity in the NI mode for OP compounds has been noted previously (Parker et al., 1982a,b; Barcelo et al., 1987) and has been attributed to the formation of more stable negative ions by the thermal electrons in the MS source. In contrast, however, other studies have found increased sensitivity using the PI mode (Barcelo, 1988b; Barcelo and Albaiges, 1989), thus precluding the recommendation of one ion monitoring mode over the other for OP analysis based simply on compound classification.

Because a majority of the OPs and metabolites investigated produced ions of highest intensities in the NI, discharge-on mode, this detection and ionization mode was selected for use in the tissue recovery study. For each compound, ions were selected to maximize sensitivity and specificity for use in the SIM. Specific *m/z* values of ions monitored, as well as approximate relative ion intensities used for confirmation of OPs and metabolites, are included in Table 1.

Regression analysis results for serial dilutions of OP and metabolite reference standards obtained in the NI, discharge-on SIM indicated that MS detector response was adequate for quantitative analysis. Two exceptions were noted in the study. The compounds fenthion and fenthoxon were not detected in the NI mode at the concentrations used. For fenthion and fenthoxon, ion production intensities were greater in the PI, filament-on mode by factors of 2.3 and 19 times, respectively.

Detection limits were at least 1 ppm (20 ng injected) for all but three of the compounds studied. In addition to fenthion and fenthoxon being undetectable below 100 ppm (2 μg injected), famphur oxon could not be detected below the 5 ppm (100 ng injected) concentration level.

Examples of SIM chromatograms obtained for reference standards and an extract of spiked lean beef muscle are provided in Figure 1. Table 3 indicates the recoveries obtained by the application of the modified extraction method and subsequent TSP/LC–MS determination for the spiked lean muscle samples. Recoveries from these samples (4.1% fat) ranged from 65 to 126% at the 1 ppm

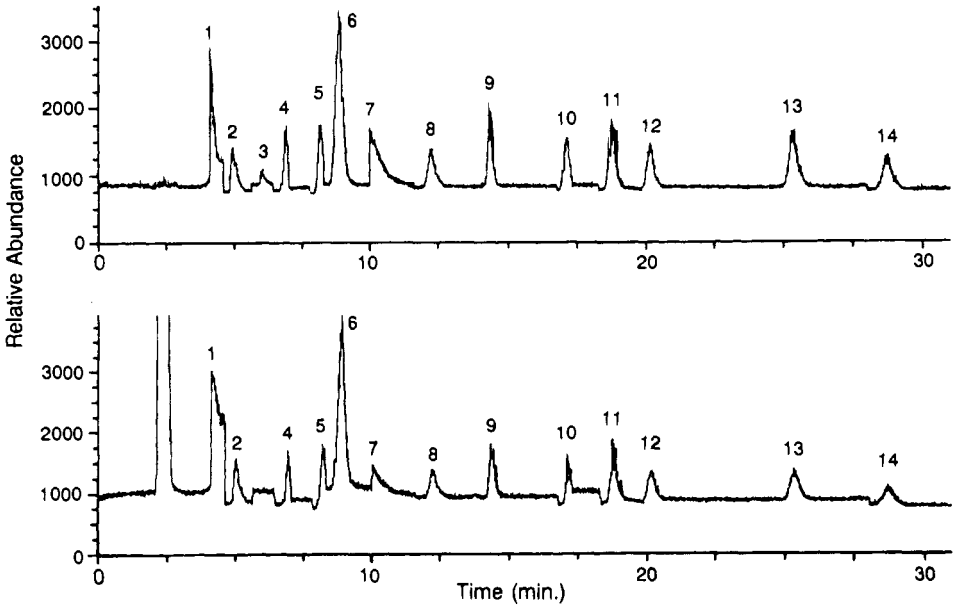

Figure 1. SIM chromatograms obtained in NI discharge-on TSP/LC–MS of mixed reference standards (5 ppm) (top) and of a spiked (1 ppm) lean beef muscle sample (bottom). Peak identities: (1) *p*-nitrophenol, (2) 3-chloro-4-methyl-7-hydroxycoumarin, (3) 3-methyl-4-(methylthio)phenol, (4) paraoxon, (5) coumaphos oxon, (6) famphur, (7) 2,4,5-trichlorophenol, (8) ronnel oxon, (9) stirofos, (10) parathion, (11) coumaphos, (12) chlorpyrifos-methyl, (13) ronnel, (14) chlorpyrifos.

Table 3. Recoveries of OPs and Metabolites from Lean (4.1% Fat) and Fatty (29.3% Fat) Beef Muscle Containing 1 ppm (Determination by Negative Ion Discharge-On TSP/LC–MS; Listed in Order of Elution from C_{18} Analytical Column)

compound	recovery[a] (%) lean	fatty
famphur oxon	ND	NA
p-nitrophenol	128 (35)[b]	109 (12)
3-chloro-4-methyl-7-hydroxycoumarin	112 (25)	92 (6)
3-methyl-4-(methylthio)phenol	ND	10
paraoxon	98 (41)	54 (9)
fenthoxon	ND	ND
coumaphos oxon	85 (55)	NA
famphur	126 (30)	82 (6)
2,4,5-trichlorophenol	65 (25)	68 (18)
ronnel oxon	101 (26)	75 (9)
stirofos	97 (30)	69 (17)
parathion	97 (25)	52 (6)
fenthion	ND	ND
coumaphos	114 (44)	64 (2)
chlorpyrifos-methyl	101 (32)	66 (3)
ronnel	80 (27)	46 (13)
chlorpyrifos	82 (41)	45 (9)

[a] Averages of duplicate injections of three extraction trials for each tissue type. ND, not detected; NA, not added to tissue sample. [b] Number in parentheses is the coefficient of variation.

level. Coefficients of variation were between 25 and 55%, with an average of 32%.

Four compounds were not detected in the spiked lean muscle: famphur oxon, 3-methyl-4-(methylthio)phenol, fenthoxon, and fenthion. Nonrecovery of 3-methyl-4-(methylthio)phenol correlates with a similar result obtained during an earlier phase of the study (Ioerger and Smith, 1993). Famphur oxon, fenthoxon, and fenthion are discussed above. Adequate quantitation of these three compounds possibly would require additional analyses in the PI mode.

Variability in the recovery results for lean muscle appeared somewhat high for all compounds. Wilkins (1990) observed a greater possibility for variability in CI because of compound sensitivities to differences in instrument design and performance, source temperature, and reagent gas purity. Thermospray LC–MS in principle operates similarly to CI-MS in that the volatilized mobile phase becomes the reagent gas for ion generation. Therefore, we would expect similar functional attributes from TSP/LC–MS. Yergey et al. (1990) stated that when using a filament or discharge to initiate ionization, ion formation processes in the TSP source appear to be identical to those occurring in a CI source. Bellar and Budde (1988), in studies of pesticides using TSP/LC–MS, noted that large variabilities in ion abundances were caused particularly by slight temperature variations in the TSP interface. These workers noted that temperature changes on the order of 0.3 °C would produce changes in ion abundances of as much as 30%.

Table 3 shows the results of the quantitative analyses to determine the recoveries obtained by application of the modified extraction method and TSP/LC–MS determination to the spiked fatty beef muscle samples. Recoveries from the fatty beef muscle (29.3% fat) ranged from 10 to 109% at the 1 ppm spike level. Coefficients of variation were between 2 and 18%, with an average of 8%.

The OP residue recovered from spiked fatty muscle in the greatest amount as from lean tissue was *p*-nitrophenol (109%), followed by 3-chloro-4-methyl-7-hydroxycoumarin (92%) and famphur (82%). The relative recoveries of the OPs and metabolites within the fatty muscle samples closely paralleled the relative recoveries obtained for the same compounds from the lean tissue samples. Ronnel (48%) and chlorpyrifos (45%) displayed the lowest recoveries in both tissue samples, with the exception of 3-methyl-4-(methylthio)-phenol (10%), which was recovered from only one extraction trial involving the fatty muscle.

The recovery of that compound from a fatty muscle sample and from none of the lean muscle samples suggests some kind of protein and 3-methyl-4-(methylthio)phenol interaction. This may take the form of the thio functionality on the 3-methyl-4-(methylthio)-phenol interacting with the sulfur in the tissue proteins. The lower protein content in fatty samples compared

to lean samples provides less opportunity for disulfide interaction.

Recovery variabilities for the fatty muscle samples were lower than corresponding CVs from lean muscle samples. These differences may reflect variation in the distribution of intramuscular fat (i.e., marbling) in the lean tissue of both (lean and fatty) sample types.

To identify analyte peaks obtained by TSP/LC–MS, a number of factors were considered. These included retention times within ±5 s (±0.3% of retention time) of corresponding standards and the presence of five characteristic SIM ions monitored per analyte. At least three ion ratios, relative to the most intense ion, needed to be within 20% of SIM ions monitored for reference standards of each compound (USDA, 1991). Figure 1 demonstrates the comparative retention times obtained for the OPs and metabolites from a mixed reference standard solution (5 ppm) and an extract from spiked (1 ppm) lean muscle. Blank (unspiked) muscle extracts also were analyzed using the same TSP/LC–MS conditions and were found to be free of the compounds tested for in the study. Retention times for all recovered compounds from both tissue types corresponded to retention times of reference standards, thereby allowing preliminary identification.

All recovered OPs and metabolites met confirmation criteria for relative ion intensities for all five of the ions monitored for each compound. Average ion abundance ratios for OPs and metabolites recovered from spiked tissue showed differences from corresponding standards ranging from 0 to 18%, with an average difference of 6%. Considering the variability in the production of ion abundances inherent in the CI-like environment of TSP/LC–MS, the observed abundance ratios for the recovered compounds proved to be sufficient for use as aids in the confirmation of recovered analytes.

A shortcoming of soft ionization processes like TSP/LC–MS has been the lack of appreciable numbers of ions produced for analyte identification purposes. The use of the discharge electrode (800 V) during analyses of OPs and OP metabolites appeared to enhance the production of ion fragments and adducts. The presence of a number of *m/z* values at appreciable intensities for each compound allowed for more meaningful comparisons to standards for identity confirmation.

CONCLUSION

Thermospray high-performance liquid chromatography–mass spectrometry was used successfully for determination of extracted pesticide and metabolite residues. A modification of a previously developed beef tissue extraction method was required for adequate TSP/LC–MS detection. Regression analysis showed that TSP/LC–MS response to OP and metabolite compounds was linear over the range of interest for all compounds detected in the NI mode. Coefficient of determination (R^2) values ranged from 0.967 to 0.999. Tissue recovery studies indicated good recoveries for most compounds, especially from lean muscle. A few recoveries in excess of 100% may reflect some type of sample matrix/detector effect. Some recoveries analyzed by TSP/LC–MS showed high variability, which may reflect fluctuating instrumental operating conditions (particularly interface temperatures) intrinsic to the TSP/LC–MS operating environment. This variability may be compensated for in practice by the selectivity and improved nature of analyte identification using an MS detection system. The study found that NI discharge-on TSP/LC–MS produced at least five ions for each compound at intensities adequate for confirmation purposes. Identities of all recovered compounds could be confirmed in terms of retention time and the presence and relative intensities of monitored ions.

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