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Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection

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ABSTRACT

The observed chromatographic response for organophosphorus pesticides in extracts from milk and butterfat is shown to be matrix dependent. The matrix protects the organophosphorus compounds from adsorption and/or decomposition in hot vaporizing injectors ensuring a more complete transfer from injector to column compared to the results observed when standards dissolved in matrix-free solvent are used. This results in recoveries in excess of 100% for residue-free extracts spiked with organophosphorus pesticides when standards prepared in residue-free solvents are used for calibration. The chromatographic response enhancement is minimized by using hot on-column injection at an optimized injection temperature, but not completely eliminated. The preferred method of calibration is to use matrix standard solutions prepared by adding known amounts of organophosphorus pesticides to residue-free sample matrix of the same character and in similar concentration to the samples to be analyzed.

INTRODUCTION

Organophosphorus pesticides are widely used in agriculture and are known to accumulate in the fatty receptacles of plant and animal tissues that form a substantial portion of the dietary intake of the world's human population [1]. Organophosphorus pesticides encompass a vast number of chemical species dictating the use of multiresidue methods for the economical screening of foods for contamination. Current methods employ a wide range of sample isolation procedures, reviewed in refs. 2 and 3, followed by gas chromatography and phosphorus element-selective detection, in general, for the separation and detection of the pesticides of typical trace residue levels.

In spite of wide application only a few reports have appeared concerning the influence of the sample matrix on the gas chromatographic properties of the organophosphorus pesticide residues. Using packed columns Carson [4] reported recovery data from 70 to 180% for

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organophosphorus pesticides in seven non-fatty foods fortified at the 2–10-ppb (w/w) level. Gillespie and Walters [5] noted recoveries in the range of 110–130% for organophosphorus pesticides in fortified samples of vegetable oils and butterfat after cleanup by solid-phase extraction and chromatography on a packed column with flame photometric detection. It was noted that compounds with P=O bonds, such as acephate, methamidophos, azodrin, etc., gave particularly high recoveries. Since degradation of these compounds by gas chromatography has been reported [6], it was speculated that co-injection of residual sample matrix presumably protects the analytes from thermal degradation and/or prevents analyte adsorption by covering active sites in the gas chromatographic system, hence giving a higher response when compared with standards prepared in a matrix-free solvent. Preparing standards in a solution of a residue-free, processed sample, to create a "matrix standard solution" provided a practical means of correcting recovery values to the normal range (80–103%) as reported [5].

The use of modern open tubular columns and injection techniques has not fully resolved the question concerning the matrix-enhanced chromatographic response of the organophosphorus pesticides. Mallet and Mallet [7] obtained response enhancement ratios from 1.09–3.00 relative to standards prepared in a matrix-free solvent for organophosphorus pesticides isolated by solid-phase extraction. Stan and Goebel [8,9] and Stan and Muller [10] have investigated the influence of different sample introduction techniques on the recovery of organophosphorus pesticides by open tubular column gas chromatography. Losses of organophosphorus pesticides in vaporizing injectors was attributed to the thermal stress imposed on the sample and the possibility of adsorption by the liner. These factors vary with the chemical structure of the pesticide and affect individual pesticides differently. They also concluded that substance losses were less using cold on-column injection compared to temperature programmed vaporization, which in turn were less than when hot-splitless injection was used. Hernandez *et al.* [3] studied a number of sample cleanup procedures for fortified peach extracts and obtained recoveries of 72–140% for organophosphorus pesticides using split injection and open tubular column gas chromatography.

The purpose of the present study was to determine the influence of matrix-induced changes in the chromatographic response of organophosphorus pesticides using open tubular column gas chromatography and to explore possible solutions for its irradication or control under conditions suitable for the determination of pesticide residues in fatty foods.

EXPERIMENTAL

All solvents were Omnisolv grade from EM Science (Gibbstown, NJ, USA). The organophosphorus pesticides methamidophos, acephate, omethoate, diazinon, dimethoate and chlorpyrifos were obtained from the US Environmental Protection Agency Repository (Research Triangle Park, NC, USA). Adsorbex RP-18, 400 mg, solid-phase extraction cartridges were obtained from Bodman (Aston, PA, USA) and used for the milk analysis. Extrelut QE disposable columns, 3 ml capacity, from EM Science and Sep-Pak C_{18}, 5 g, cartridges from Millipore (Marlborough, MA, USA) were used for the butterfat analysis. A Visiprep SPE vacuum manifold (Supelco, Bellefonte, PA, USA) was used for sample processing.

A Hewlett-Packard 5880A gas chromatograph with flame photometric detector and Hewlett-Packard 5880A Level Four data station was used for gas chromatography. The standard split/splitless injector was used for splitless injection and hot on-column injection by changing injection liners (J & W Scientific, Folsom, CA, USA). For separations a 2–3 m × 0.53 mm I.D., deactivated retention gap and a 15 m × 0.53 mm I.D., 1.0 μm film thickness, DB-17 fused-silica open tubular column (J & W Scientific) were used. Operating conditions were varied widely in different studies and the relevant details are given below in the text.

The method used to process the milk extracts [11] and butterfat extracts [5,12,13] are described in detail elsewhere. Briefly, milk solids were precipitated with acetone–acetonitrile, the or-

ganophosphorus pesticides extracted from the supernatant with dichloromethane, and the residue after removal of the solvent taken up in acetonitrile and passed through a C_{18} solid-phase extraction cartridge. After solvent removal final residue containing pesticides was taken up in acetone (1 ml for an original sample size of 10 g of milk).

The butterfat dissolved in hexane was passed through Extrelut QE column which was mounted in series with a C_{18} cartridge. Both columns were eluted with a mixture of methanol–acetonitrile (1:1) saturated with hexane. The C_{18} column was eluted with additional methanol, the eluent evaporated to dryness and reconstituted in acetone to give a final concentration of about 180 mg/ml based on the original mass of butterfat.

RESULTS AND DISCUSSION

The analysis of fats and oils for residues of pesticides by gas chromatography requires that the contaminants are effectively isolated from the bulk of the fats to prevent contamination of the injector and columns with non-volatile and late eluting material. Solid-phase extraction using short columns (cartridges) packed with octadecylsilanized silica packings have proven particularly useful for isolating organophosphorus pesticides from fats [5,11–15]. The reduced solvent consumption, high sample throughput, low equipment costs, and suitability for multiresidue determinations have quickly promoted this approach to the forefront of pesticide analysis in foods. The milk extracts and butterfat extracts used in this report typically contain less than 2 mg/ml and 4 mg/ml, respectively, of matrix residues, and are suitable for routine analysis, producing little observable contamination of the chromatographic system.

There are features of the general gas chromatographic properties of organophosphorus pesticides which are not well documented in the literature. It has been our experience that several injections of matrix standard solution, are required at the beginning of the day to obtain reproducible peak area responses for the organophosphorus pesticides. During the course of a series of analyses in which standard solutions prepared in an organic solvent are injected interspersed between sample extracts there is generally a gradual but significant increase in the area response for the standards over time. For residue-free extracts spiked with organophosphorus pesticides recoveries between 100–300% have been observed when standards prepared in an organic solvent were used as the basis for the recovery calculation. Both the amount and the type of the matrix can effect the perceived observed recovery, and although the general trends remain true, individual compounds may show different increases in recovery with the same experimental conditions.

Fig. 1 shows comparative chromatograms obtained with the same chromatographic system with the splitless and on-column injection liners installed for the separation of a mixture of organophosphorus pesticides spiked into a residue free milk matrix extract. The peak shapes for on-column injection and the observed detec-

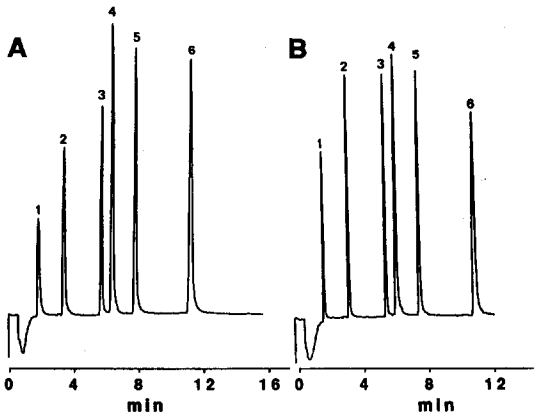

Fig. 1. Comparison of splitless injection (A) and hot on-column injection (B) for the separation and recovery of organophosphorus pesticides spiked into a response free milk extract. A 3 m × 0.53 mm I.D. deactivated retention gap coupled to a 15 m × 0.53 mm I.D. DB-17, 1 μm film thickness, open tubular column (connected to the detector by a 20 cm × 0.32 mm I.D. deactivated fused-silica capillary column threaded through the detector tube and terminated just below the flame) was used for the separation. Hydrogen was used as the carrier gas at 4 ml/min and the column temperature programmed from 80°C (2 min) at 30°C/min to 200°C (10 min). Peaks: 1 = methamidophos (0.27 ng); 2 = acephate (0.69 ng); 3 = omethoate (1.09 ng); 4 = diazinon (1.16 ng); 5 = dimethoate (1.21 ng); 6 = chlorpyrifos (1.82 ng).

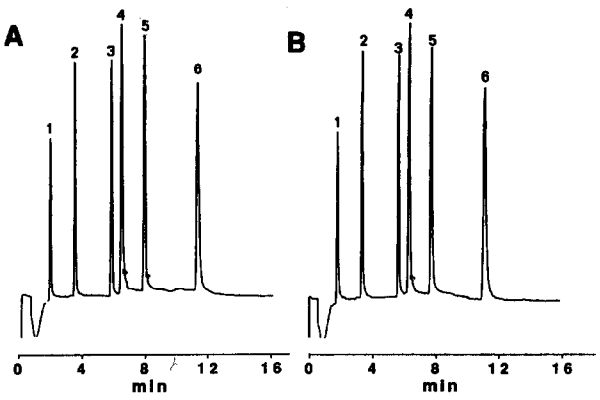

Fig. 2. Comparison of the separation and recovery of organophosphorus pesticides in acetone (A) and spiked into a residue-free butterfat extract (B). Both samples were introduced by hot on-column injection (230°C). Other conditions as for Fig. 1. The chromatographic response enhancement (peak area in chromatogram B/peak area in chromatogram A) is methamidophos (1.01), acephate (1.09), omethoate (1.10), diazinon (1.08), dimethoate (1.02) and chlorpyrifos (1.07). The matrix concentration in (B) was 1.7 mg/ml.

tor response are improved using the hot on-column injection technique. Fig. 2 illustrates the increase in detector response observed for the injection of the same amount of organophosphorus compounds dissolved in acetone and spiked into a residue-free milk matrix extract using hot on-column injection and the same chromatographic parameters. The increase in response observed for the matrix standard solution is discernable. Table I illustrates the observed recoveries determined with reference to a matrix-free standard solution for a mixture of organophosphorus pesticides spiked into milk and butterfat extracts using splitless and hot on-column injection techniques. Comparing the two injection techniques, the level of response enhancement (observed recovery) in excess of 100% is smaller in the case of hot on-column injection. Comparing the two extracts, recoveries for the butterfat are relatively high compared to the milk extract for splitless injection but differences using hot on-column injection are not significant. The influence of peak area response for a standard mixture of organophosphorus pesticides as a function of the injection temperature using hot on-column injection is shown in Fig. 3. There is a gradual increase in the observed response for all compounds as the temperature is increased in the range of 150 to 230°C. Above 230°C a plateau region is reached (chlorpyrifos), a further shallow increase in response is observed (methamidophos, omethoate, dimethoate), or a decrease in response is observed (acephate, diazinon). A compromise temperature of 230°C and hot on-column injection were selected as the most favorable conditions for further comparative experiments reported subsequently.

The above initial experiments provide an adequate framework for a qualitative discussion of the influence of the chromatographic conditions and sample matrix on the recovery of organophosphorus pesticides. The sample matrix has a protective influence on the sample components increasing their transfer to the column by either reducing the thermal stress imposed on the analytes or by blocking active sites within the

TABLE I

OBSERVED CHROMATOGRAPHIC RESPONSE ENHANCEMENT FOR MILK AND BUTTERFAT EXTRACTS WITH ORGANOPHOSPHORUS PESTICIDES USING SPLITLESS AND HOT ON-COLUMN INJECTION

Extraction fortifications were between 0.06 and 0.12 ppm.

Compound	Milk extract		Butterfat extract	
	Splitless	On-column	Splitless	On-column
Acephate	1.14 ± 4.3%	1.01 ± 4.7%	1.36 ± 4.7%	1.00 ± 1.1%
Omethoate	1.07 ± 6.7%	1.04 ± 5.3%	1.33 ± 5.3%	0.97 ± 4.2%
Diazinon	1.00 ± 4.2%	0.97 ± 5.1%	1.25 ± 2.1%	0.84 ± 5.8%
Dimethoate	1.06 ± 4.2%	1.03 ± 7.3%	1.31 ± 4.0%	0.92 ± 1.1%
Chlorpyrifos	1.04 ± 2.7%	0.95 ± 5.2%	1.07 ± 5.3%	1.04 ± 0.6%

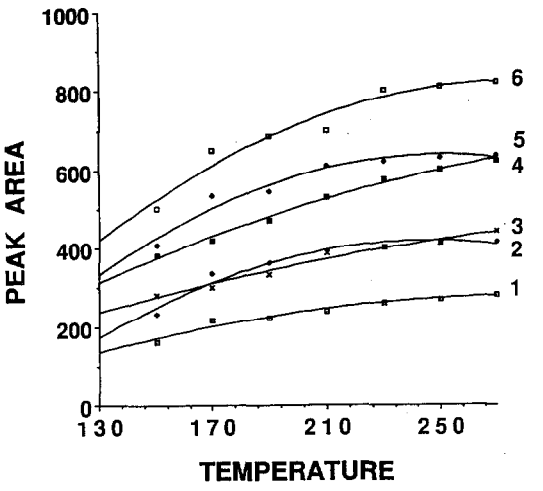

Fig. 3. Plot of peak area response (arbitrary units) as a function of injector temperature (°C) for hot on-column injection of organophosphorus pesticides. Identification: 1 = methamidophos; 2 = acephate; 3 = omethoate; 4 = dimethoate; 5 = diazinon; 6 = chlorpyrifos.

injector that would tend to delay transfer of the analytes to the column. Given that the organophosphorus compounds typically used as pesticide encompass a wide range of physical and chemical properties both factors are likely to be important. Increasing area response with increasing injector temperature, at least up to 230°C, would indicate that adsorption to active sites within the injector is important. Higher temperatures tend to diminish adsorptive forces. Above 230°C reduced area response was observed for some compounds indicating either thermal decomposition and/or enhanced catalytic decomposition on active sites has to be taken into consideration. The results for splitless injection compared to on-column injection show a higher relative observed chromatographic response enhancement since the relatively long residence time of the sample in the vaporization chamber and contact of the sample with a larger (and probably more active surface) result in those conditions most likely to reduce the transfer of the analytes to the column. Releasing the analytes on column results in their vaporization into the column or retention gap where surface activity is likely to be lower and the residence time in the vaporizing chamber much less.

Three potential solutions to the problem suggest themselves. In the absence of matrix the protection effect is not operative and it is true that if samples containing very little matrix are spiked with the organophosphorus compounds there is little if any increase in the detector response. This is perhaps an obvious statement, but is generally not a practical solution. In residue analysis, given the nature of the samples analyzed, matrix contamination cannot be avoided. The extracts obtained by solid-phase extraction are comparatively clean compared to those obtained by other procedures and therefore, increasing the number of steps to minimize the matrix burden is not desirable and would only become feasible if a highly chemically selective isolation procedure for the organophosphorus compounds could be devised. Such a procedure does not exist at present.

A second solution would be to deactivate the chromatographic system to such an extent that the adsorption of the organophosphorus compounds became negligible. Having failed to achieve this chemically by silanization the possibility of achieving permanent or temporary deactivation by the sample matrix itself was evaluated. Injecting milk extract without cleanup by solid-phase extraction and milk extract to which small quantities of corn oil and/or soybean oil had been deliberately added was used to contaminate the chromatographic system in the hope of saturating active sites responsible for sorption of the analytes. As seen from Table II, this was not successful, and the observed recovery increased, in general, with the increasing matrix burden. Also, the changes observed were temporary and required that each sample be contaminated with the crude matrix, which over time resulted in a build up of involatile material increasing the frequency with which retention gaps had to be changed.

The most practical solution is the use of a residue free matrix standard solution prepared to closely resemble the sample extracts in concentration and character. Data presented in Table III for the recovery of the organophosphorus pesticides in butterfat and milk extracts by on-column injection as a function of temperature indicate that if reproducible results are to be obtained both the matrix and injection tempera-

TABLE II

OBSERVED CHROMATOGRAPHIC RESPONSE ENHANCEMENT FOR ORGANOPHOSPHORUS PESTICIDES IN MILK EXTRACTS WITH AN IMPOSED MATRIX BURDEN

Sample preparation[a]	Chromatographic response enhancement					
	Methamidophos	Acephate	Omethoate	Diazinon	Dimethoate	Chlorpyrifos
A	1.24	1.29	1.46	1.34	1.43	1.54
B	1.25	1.55	1.68	1.68	1.54	1.59
C	1.31	1.72	1.92	1.63	1.72	1.81

[a] A = Milk matrix method excluding cleanup step by solid-phase extraction; B = extract A to which one drop of corn oil was added; C = extract B to which one drop of soybean oil was added.

TABLE III

CHROMATOGRAPHIC RESPONSE ENHANCEMENT (MATRIX STANDARD/MATRIX FREE SOLVENT STANDARD) USING HOT ON-COLUMN INJECTION

Injection temperature (°C)	Matrix extract	Organophosphorus compounds					
		Methamidophos	Acephate	Omethoate	Diazinon	Dimethoate	Chlorpyrifos
150	Butterfat	0.92	1.50	1.52	1.01	1.24	0.97
	Milk	1.72	1.61	1.30	1.31	1.23	1.11
170	Butterfat	1.58	1.50	1.18	1.14	1.14	1.15
	Milk	1.92	1.67	1.79	1.26	1.32	1.33
190	Butterfat	1.20	1.41	1.37	0.97	1.15	1.04
	Milk	1.62	2.01	2.12	1.35	1.56	1.41
210	Butterfat	1.19	1.42	1.52	1.00	1.31	1.03
	Milk	1.77	1.61	1.79	1.30	1.46	1.33
230	Butterfat	1.11	1.28	1.30	1.00	1.10	0.94
	Milk	1.82	1.59	1.58	1.20	1.24	1.23
250	Butterfat	0.93	1.20	1.23	1.14	1.16	1.16
	Milk	1.65	1.54	1.52	1.35	1.29	1.31
270	Butterfat	1.09	1.29	1.25	1.05	1.12	1.12

ture need to be specified. It is not adequate to use a butterfat matrix to correct for matrix enhancement of milk extracts (at least not without correction for the gravimetric differences in the matrix concentrations). The response enhancement seems to be less in all cases around 230°C and this remains the best compromise temperature for sample vaporization.

CONCLUSIONS

The chromatographic response of organophosphorus pesticides using hot vaporizing injectors is matrix dependent. The matrix protects the analytes from adsorption or alteration during transfer from the injector to the column resulting in a higher observed detector response for the same amount of substance injected in a matrix-modified standard solution compared to a matrix-free standard solution. The protective influence of the matrix is not permanent and probably depends on the nature and concentration of the matrix. To obtain acceptable results either the matrix must be present in low concentration when calibration using matrix-free standards is used or matrix-modified standards

prepared from a residue-free matrix of the same kind and similar concentration to the samples should be used for calibration. In practice, the second alternative will generally be selected when analyzing complex samples such as fatty foods.

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