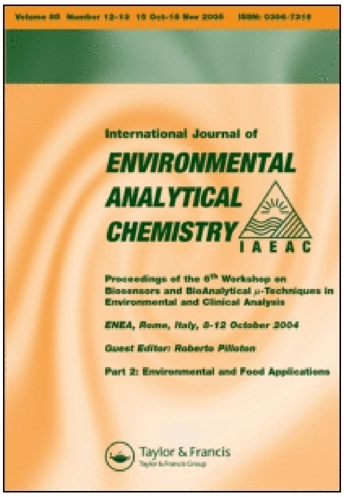

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Comparison of external and internal standard methods in pesticide residue determinations

Christos Anagnostopoulos[a]; George E. Miliadis[b]; Pipina Aplada-Sarlis[b]; Basilios N. Ziogas[a]
[a] Laboratory of Pesticide Science, Department of Plant Science and Production, Agricultural University of Athens, Athens, Greece [b] Pesticide Residues Laboratory, Benaki Phytopathological Institute, Kifissia, Greece

To cite this Article Anagnostopoulos, Christos , Miliadis, George E. , Aplada-Sarlis, Pipina and Ziogas, Basilios N.(2006) 'Comparison of external and internal standard methods in pesticide residue determinations', International Journal of Environmental Analytical Chemistry, 86: 1, 77 — 82

To link to this Article: DOI: 10.1080/03067310500249799

URL: http://dx.doi.org/10.1080/03067310500249799

Intern. J. Environ. Anal. Chem.
Vol. 86, Nos. 1–2, 15 January–15 February 2006, 77–82

Comparison of external and internal standard methods in pesticide residue determinations

CHRISTOS ANAGNOSTOPOULOS*†, GEORGE E. MILIADIS‡,
PIPINA APLADA-SARLIS‡ and BASILIOS N. ZIOGAS†

†Laboratory of Pesticide Science, Department of Plant Science and
Production, Agricultural University of Athens, Iera Odos 75,
Votanikos, 118 55, Athens, Greece
‡Pesticide Residues Laboratory, Benaki Phytopathological Institute,
145 61, Kifissia, Greece

(*Received 2 December 2004; in final form 8 April 2005*)

A qualitative and quantitative gas-chromatographic pesticide-residue analysis method for 21 pesticides sensitive to the nitrogen–phosphorus detector was evaluated. The two methods of quantitative determination that were compared were the external standard (ES) method and the internal standard (IS) method. The methods were evaluated for the precision and linearity of the detector response. Precision was assessed by examining repeatability and reproducibility of the detector response. The RSD values found were lower than 20%. A better repeatability and reproducibility were observed with the IS method. The linearity of the detector was found to be satisfactory; by comparing the correlation coefficients found by the two methods, no significant difference was observed. The results were evaluated for the effect of the following factors: solvent of the final extract and time of determination. From a statistical comparison of the results of the IS and the ES methods, it was found that with the ES method, both factors were significant for the determination, and there was correlation between the two factors. With the IS method, only the solvent of the final extract had a significant effect on the results and no correlation was observed in most cases between the two factors.

Keywords: External standard; Internal standard; Pesticide residues

1. Introduction

Quantitation in chromatographic analysis is mainly performed by two methods, the ES and the IS method [1]. By the ES method, the area of the peak in the sample extract is compared with the area of the peak of one or more standard solutions. In the IS method, a compound named the internal standard, which cannot be detected in the samples, is added to both the samples and the standard solutions, preferably at the same concentration, and the ratios of standard/IS areas are compared.

In the present study, a quantitative gas-chromatographic pesticide residue analysis method for the determination of 21 pesticides, sensitive to the nitrogen–phosphorus

---

*Corresponding author. Fax: +30-210-8077584. Email: res1@bpi.gr

International Journal of Environmental Analytical Chemistry
ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310500249799

detector (NPD), in tomato matrix was evaluated. The ES and IS methods for quantitative determination were compared for:

- pesticide calibration curves; and
- repeatability and reproducibility of the detector response.

Triphenyl phosphate was used as an internal standard; the presence of a phosphorus atom in its molecule makes it sensitive to the NPD detector.

The preparation of standard solutions in solvent or in tomato matrix was also studied, for evaluating the matrix effect on quantitation, by both ES and IS methods. In the literature, both ES and IS methods are used to determine residues of pesticides in agricultural products [2, 3]. However, to the best of the authors' knowledge, no such data on comparisons between the ES and IS methods have been reported.

2. Experimental

The materials used were acetone, toluene, and 2,2,4-trimethyl pentane of pesticide residues grade, and triphenyl phosphate (as internal standard); and the pesticide analytical standards acephate, diazinon, pirimiphos methyl, chlorpyriphos ethyl, procymidone, imazalil, iprodione, phosalone, diclorvos, dimethoate, chlorpyriphos methyl, malathion, methidathion, ethion, azinphos methyl, azinphos ethyl, ethoprophos, phosphamidone, parathion ethyl, pendimethalin, and triazophos.

A GC Fisons gas chromatograph, HRGC Series, Mega 2 with a splitless injector, capillary column SE 54 (30 m, i.d. 0.32 mm, and film thickness 0.25 μm), NPD-detector, and Chrom-Card intergration software were used. An established method of extraction [4], using a mixture of acetone dichloromethane and petroleum ether was used. The internal standard was added in the final extract. The temperature programme of the GC was from 50°C (1 min) to 180°C at $30°\text{C min}^{-1}$, at $1.8°\text{C min}^{-1}$, to 210°C, and at $30°\text{C min}^{-1}$, to 260°C, where it remained for 30 min.

In the case of the IS method, the parameter recorded was the ratio of the detector signal for each compound to the detector signal for the internal standard. In the case of the ES method, the parameter recorded was the signal of the detector for each compound.

3. Results and discussion

The linearity of the detector response, for each pesticide, was studied at concentrations between the reporting limits (C) of the laboratory (table 1) and $50C$. The linearity was evaluated in both IS and ES methods by the correlation coefficient (r) values, after injecting solutions of the compounds in 2,2,4-trimethylpentane–toluene (table 2) or in tomato matrix (table 3). The linearity was satisfactory (with r higher than 0.95) in both cases at the selected concentration range of pesticides.

Figures 1 and 2 are chromatograms of six of the studied pesticides in 2,2,4-trimethylpentane–toluene and tomato matrix, respectively. The retention times of the pesticides are shown in the figures; no interferences are evident in the tomato matrix chromatogram. A quantitative determination was performed by bracketing the

Table 1. Reporting limits (*C*) of the laboratory for the 21 pesticides.

Pesticide	*C* ($\mu g\,mL^{-1}$)	Pesticide	*C* ($\mu g\,mL^{-1}$)	Pesticide	*C* ($\mu g\,mL^{-1}$)
Acephate	0.5	Ethion	0.05	Pirimiphos methyl	0.5
Chlorpyriphos ethyl	0.2	Ethoprophos	0.02	Phosalone	0.5
Chlorpyriphos methyl	0.2	Imazalil	0.5	Phosphamidone	0.02
Azinphos methyl	0.2	Iprodione	1	Procymidone	1
Azinphos ethyl	0.02	Malathion	1	Triazophos	0.02
Diazinon	0.2	Methidathion	0.02		
Dichlorvos	0.05	Parathion ethyl	0.05		
Dimethoate	0.5	Pendimethalin	0.05		

Table 2. Correlation coefficient values by the use of internal and external standard methods after injection of pesticide solutions in organic solvent.

Pesticide	Internal standard	External standard
Acephate	0.994	1.000
Diazinon	0.995	0.999
Pirimiphos methyl	0.996	1.000
Chlorpyriphos ethyl	0.999	0.999
Procymidone	0.998	0.999
Imazalil	0.998	0.999
Iprodione	0.999	0.998
Phosalone	0.999	0.999
Dichlorvos	0.999	0.999
Dimethoate	0.997	0.999
Chlorpyriphos methyl	0.996	0.995
Malathion	0.991	0.997
Methidathion	1.000	1.000
Ethion	0.999	0.999
Azinphos methyl	0.998	0.998
Azinphos ethyl	0.998	0.998
Ethoprophos	0.999	0.998
Phosphamidone 1	0.999	0.999
Phosphamidone 2	0.999	0.999
Parathion ethyl	0.998	0.998
Pendimethalin	0.999	0.999
Triazophos	0.998	0.997

sample's response between the responses of two standard solutions (which did not differ from each other in terms of concentration by more than 20%).

An evaluation of repeatability and reproducibility of the detector response for the selected pesticides was performed at the level of 10*C*. The concentration of the internal standard used was $2\,\mu g\,mL^{-1}$. The $SD_R\%$ values of the 21 pesticides under repeatability (within day) and reproducibility (between days) conditions by the ES and the IS methods after injection of pesticide solutions in 2,2,4-trimethylpentane–toluene and in tomato matrix are presented in tables 4 and 5, respectively. The SD_R values for both methods were lower than 20%, thus acceptable for pesticide-residue analysis [5]. By comparing the SD_R values of the two methods, under either repeatability or reproducibility conditions, it is observed that in most cases, the SD_R values for the IS method were lower than those for the ES method. This is more evident when the pesticides were injected as solutions in tomato matrix.

Statistical analysis of the results [6] for the evaluation of the matrix effect and the time of pesticide residue determinations showed that in the case of the IS method

Table 3. Correlation coefficient values by the use of internal and external standard methods after injection of pesticide solutions in tomato matrix.

Pesticide	Internal standard	External standard
Acephate	0.992	0.992
Diazinon	0.992	0.992
Pirimiphos methyl	0.999	0.999
Chlorpyriphos ethyl	1.000	1.000
Procymidone	0.998	0.998
Imazalil	0.975	0.975
Iprodione	0.998	0.998
Phosalone	1.000	1.000
Dichlorvos	0.999	0.998
Dimethoate	0.997	0.996
Chlorpyriphos methyl	0.995	0.991
Malathion	0.993	0.990
Methidathion	0.999	0.999
Ethion	0.999	0.998
Azinphos methyl	0.999	0.998
Azinphos ethyl	0.999	0.998
Ethoprophos	1.000	0.999
Phosphamidone 1	1.000	1.000
Phosphamidone 2	0.999	0.999
Parathion ethyl	0.999	0.999
Pendimethalin	0.999	0.999
Triazophos	0.999	0.998

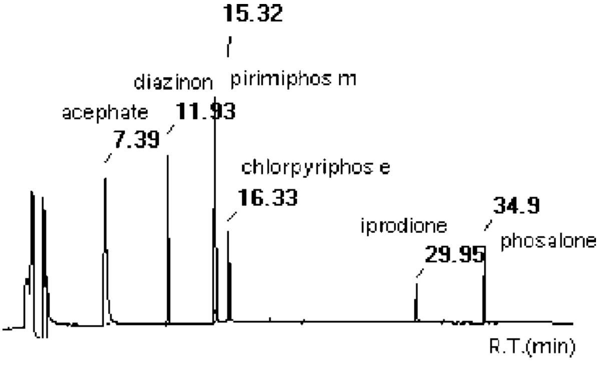

Figure 1. Chromatogram of six of the studied pesticides in 2,2,4-trimethylpentane–toluene.

at $p = 0.05$, the time of pesticide determination was significant for the pesticides: acephate, chlorpyriphos methyl, dichlorvos, and methidathion. The effect of the tomato matrix was significant for the pesticides: acephate, diazinon, pirimiphos methyl, chlorpyriphos ethyl, phosalone, dichlorvos, methidathion, azinphos methyl, ethoprophos, phosphamidone, parathion ethyl, and triazophos. A correlation between the two factors was found in the determination of dichlorvos, methidathion, and phosphamidone.

In the case of the ES method at $p = 0.05$, the effect of the time of determination was significant for the pesticides: acephate, diazinon, pirimiphos methyl, chlorpyriphos ethyl, phosalone, dichlorvos, chlorpyriphos methyl, methidathion, ethion, azinphos methyl, parathion ethyl, and pendimethalin. The tomato matrix was also significant

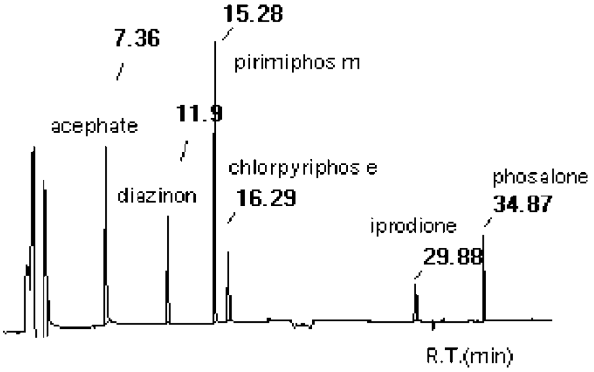

Figure 2. Chromatogram of six of the studied pesticides in tomato matrix.

Table 4. SD_R% values under repeatability (within day) and reproducibility (overall, between days) conditions by the external standard (ES) and the internal standard (IS) methods after injection of pesticide solutions in 2,2,4-trimethylpentane–toluene ($N = 6$).

	Day 1		Day 2		Day 3		Overall	
Pesticides	ES	IS	ES	IS	ES	IS	ES	IS
Acephate	3.98	4.81	3.83	3.67	8.86	12.43	7.51	8.32
Diazinon	4.99	2.06	1.08	1.57	6.02	5.34	6.06	3.62
Pirimiphos methyl	4.70	2.15	1.46	1.86	5.66	4.89	5.87	3.64
Chlorpyriphos ethyl	4.75	2.18	1.86	1.66	5.98	4.96	6.17	3.52
Procymidone	4.25	3.74	9.65	10.32	7.44	6.92	8.29	10.12
Imazalil	5.36	4.78	5.94	6.12	9.05	9.09	8.79	11.23
Iprodione	4.71	4.64	5.63	4.73	4.35	9.76	6.66	8.37
Phosalone	4.17	1.76	2.49	1.04	7.94	3.84	6.67	2.68
Dichlorvos	5.27	4.49	8.49	3.84	3.15	5.09	6.58	5.00
Dimethoate	5.72	3.77	8.56	3.69	5.20	4.75	7.01	4.51
Chlorpyriphos methyl	4.09	3.02	8.05	2.74	0.97	3.23	6.60	3.74
Malathion	4.63	3.51	7.74	2.56	1.15	3.48	6.47	3.76
Methidathion	8.16	7.76	6.30	7.75	2.96	3.38	9.01	8.72
Ethion	3.44	1.65	8.17	1.10	4.36	6.22	6.67	4.10
Azinphos methyl	6.16	3.01	8.95	6.41	6.16	5.05	7.43	5.38
Azinphos ethyl	5.73	4.90	4.62	5.93	2.95	3.24	5.53	5.78
Ethoprophos	5.59	2.23	3.48	2.89	7.21	3.63	6.50	3.10
Phosphamidone 1	10.62	7.88	4.47	5.10	11.41	10.83	10.97	10.78
Phosphamidone 2	8.06	7.53	5.83	6.00	8.31	8.54	10.51	9.48
Parathion ethyl	5.07	5.39	14.86	15.13	10.46	11.74	11.75	13.22
Pendimethalin	9.91	6.25	5.90	5.85	4.75	2.45	9.68	6.55
Triazophos	5.16	3.93	3.72	3.68	5.98	7.41	7.41	6.00

for the determination of the pesticides: acephate, phosalone, dichlorvos, chlorpyriphos methyl, methidathion, ethion, azinphos methyl, ethoprophos, phosphamidone, parathion ethyl, and triazophos. A correlation between the two factors was observed in the determination of the pesticides: acephate, diazinon, pirimiphos methyl, chlorpyriphos ethyl, phosalone, dichlorvos, chlorpyriphos methyl, methidathion, ethion, azinphos methyl, ethoprophos, phosphamidone, parathion ethyl, triazophos, and pendimethalin.

In conclusion, the IS method was found to be more precise than the ES method, in that it has better SD_R values and is more robust. For the ES method, the time of

Table 5. SD$_R$% values under repeatability (within day) and reproducibility (overall, between days) conditions of the 21 pesticides by the ES and the IS methods after injection of pesticide solutions in tomato matrix ($N=6$).

	Day 1		Day 2		Day 3		Overall	
Pesticides	ES	IS	ES	IS	ES	IS	ES	IS
Acephate	3.66	4.15	6.48	1.54	3.89	12.43	13.67	5.30
Diazinon	1.42	4.88	7.74	2.72	2.19	5.34	10.45	3.64
Pirimiphos methyl	1.42	4.19	8.72	4.62	1.97	4.89	11.33	3.85
Chlorpyriphos ethyl	1.94	4.49	7.04	1.59	2.24	4.96	10.55	3.16
Procymidone	5.06	9.23	7.35	6.73	2.06	6.92	7.43	13.58
Imazalil	12.60	16.68	8.90	9.10	5.14	9.09	15.32	15.61
Iprodione	6.15	4.39	11.95	14.00	7.09	9.76	10.40	11.56
Phosalone	5.30	4.94	5.68	0.68	3.85	3.84	11.88	3.51
Dichlorvos	0.89	1.06	5.72	4.71	7.55	5.09	24.33	7.54
Dimethoate	1.90	0.98	4.18	4.31	6.71	4.75	13.64	4.94
Chlorpyriphos methyl	1.02	1.23	4.31	3.74	6.87	3.23	17.92	4.13
Malathion	0.61	1.25	2.01	3.18	8.11	3.48	12.40	6.60
Methidathion	4.44	4.51	3.74	4.94	10.72	3.38	24.11	9.37
Ethion	1.65	0.96	4.36	1.08	9.96	6.22	14.05	8.63
Azinphos methyl	3.02	1.66	6.03	2.50	6.04	5.05	19.44	3.28
Azinphos ethyl	1.86	1.20	6.90	6.66	8.60	3.24	27.70	27.03
Ethoprophos	5.61	5.25	3.93	4.72	2.70	3.63	6.55	4.52
Phosphamidone 1	3.42	4.85	5.57	4.97	1.77	10.83	7.07	4.74
Phosphamidone 2	4.77	4.91	4.76	4.46	0.94	8.54	6.95	4.31
Parathion ethyl	5.07	5.60	6.65	6.48	4.01	11.74	7.07	6.36
Pendimethalin	3.17	6.22	5.14	6.95	4.28	2.45	6.73	6.39
Triazophos	7.04	5.54	2.42	2.60	5.23	7.41	7.59	4.22

determination and the effect of the tomato matrix were significant in the determination in 57 and 52% of tested pesticides, respectively, and a correlation was observed between the two factors in 71% of the tested pesticides. In contrast, with the IS method these two factors were significant in the determination in 19 and 57% of the tested pesticides, respectively, and a correlation between the two factors was observed in only 14% of the tested pesticides.

References

[1] R.L. Grob. *Modern Practice of Gas Chromatography*, 2nd Edn, Wiley, New York (1985).

[2] G.E. Miliadis, P.T. Malatou. *Int. J. Environ. Anal. Chem.*, **70**, 29 (1998).

[3] Y. Tamura, T. Nagayama, M. Kobayashi, T. Hashimoto, N. Haneishi, M. Ito, T. Tomomatsu. *J. Food Hyg. Soc Japan*, **39**, 225 (1998).

[4] P. Van Zoonen (Ed.), *Analytical Methods for Pesticide Residues in Foodstuffs*, 6th Edn, Ministry of Public Health, Welfare and Sport, Bilthoven, The Netherlands (1996).

[5] P.A. Greve. In *Pesticide Residue Analysis*, A. Ambrus and R. Greenhalgh (Eds), pp. 281–291, WHO–FAO, Rome (1984).

[6] P.I. Kaltsikis. In *Factorial Experiments*, A. Stamoulis (Ed.), Piraeus, Athens (1989).