Rapid Large Volume Injection/GC–MS Analysis of Pesticides in Food Prepared by the QuEChERS Method

Karin Friedrichs,[1] Heinz-Dieter Winkeler[1] and Hans-Ulrich Baier,[2]
[1]Chemical and Veterinary Investigation Office, Detmold, Germany,
[2]Shimadzu Europa GmbH, Duisburg, Germany.

In many analytical areas there is a tendency to save time in sample preparation. Regarding pesticide screening in food the well-known QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe)[1,2] method has been applied in many laboratories. This drastically reduces sample preparation effort when compared with the formerly used method with a final gel permeation chromatography (GPC) clean-up step. In contrast, when injecting the extracts prepared by QuEChERS many matrix signals can be observed in the GC–MS chromatogram. Full scan modes are, therefore, necessary to prevent false positive or false negative determination of target pesticides that could easily occur when running the gas chromatography–mass spectrometry (GC–MS) system in the more sensitive selected ion monitoring (SIM). To reach a high sensitivity for routine work in full scan mode, firstly, the GC–MS system should be a high-sensitivity instrument and secondly, a large volume injection further improves the limit of quantification (LOQ).

In this article a method called rapid large volume injection was used with a PTV injection port (Optic 3, ATAS GL International). Volumes up to 50 µL were injected with subsequent full scan GC–MS runs and the quantitative precision was checked by analysing round robin test samples. For the programmable temperature vaporization (PTV) insert special sintered glass liners were used. They do not have any filling material, preventing decomposition of fragile pesticides. Capacity for the large volume injection is achieved by the rough inner surface of the liners. This surface was SILTEK deactivated. To automatate the whole process after a liner was dirty (checked by a special degradation mixture) the LINEX automatic liner exchanger was installed and after about 80 injections the system performed the liner exchange automatically. The compound tables comprise of over 500 pesticides. Identification of the target compound was performed by checking full scan spectra and by using linear retention indices automatically checked as an additional filter.

Sample Preparation

The procedure involved the extraction of 10 g sample with 10 mL acetonitrile, followed by a liquid-liquid-partitioning step performed by adding 4 g anhydrous $MgSO_4$ plus 1 g NaCl, 1 g Na_3citrat and 0.5 g Na_2Hcitrat. The sample clean-up was performed using a rapid procedure called dispersive solid-phase extraction (SPE), in which 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent are mixed with 1 mL acetonitrile extract.

Contact author: Hans-Ulrich Baier
E-mail: shimadzu@shimadzu.eu

After a second mixing and centrifugation step, the extract was transferred to autosampler vials for concurrent analysis by large volume GC–MS.

Experimental Conditions

The instrumentation was a GCMS-QP2010 Plus (Shimadzu Europa GmbH) with Optic 3 injector (ATAS GL International), AOC-5000 autoinjector (Shimadzu Europa GmbH) with an automatic glass liner exchanger option (LINEX, ATAS GL International). The chromatographic conditions were: VF-5-MS EZ guard column 30 m × 0.25 mm, 0.25 µm with an integrated retention gap of 10 m. The column temperature was set to 50 °C for 1 min (hold) then with 40 °C/min to 150 °C followed by 4.6 °C/min to 280 °C for 28.24 min, with a mean linear velocity of 30 cm/s (He). For the Optic 3 injector the temperature was set to 55 °C during the period of the solvent venting time and then ramped with 15 °C/s to 280 °C for the rest of the analytical run (59.75 min). The solvent venting at low temperature (55 °C) was optimized and finally set to 38 s at a split ratio of 50:1. The split was further programmed to transfer the analytes to the column.

This was achieved by closing the split after the venting time for 2 minutes (analyte transfer). The split was then reopened to 10:1 to purge residual solvent out of the liner. The injection volume was finally set to 30 µL. The Optic 3 is heated by direct ohmical heating. This leads to liner inner diameters of this PTV of about 3.4 mm, corresponding to typical hot split/splitless liner dimensions.

It is possible to ramp the Optic 3 up to a maximum of 30 °C/s even using these liners, which is in contrast to conventional PTVs that have indirect resistive heaters and correspondingly have typical inner diameters of about 1–2 mm. This has a strong influence on the method development in large volume injections. For the Optic 3 the injection speed up to about 100 µL is not as critical and, therefore, the liquid can be injected rather quickly [rapid large volume injection (RLVI)] while in the latter case a speed control of injection is important.

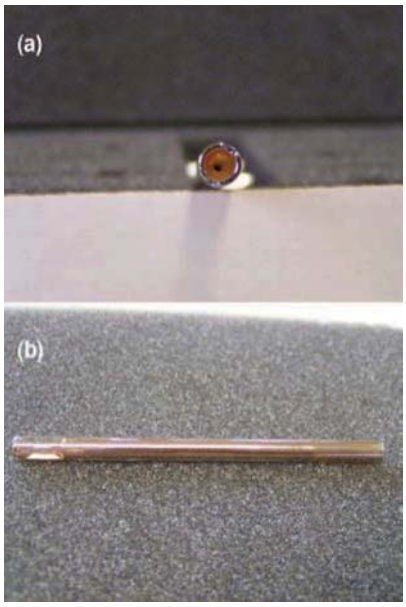
The mass spectrometer was operated in full scan mode to minimize false positive or false negative identification. The scan range was set to 50–550 *m/z*. The ion source temperature and the interface was set to 200 °C and 320 °C, respectively.

Results

The correct liner choice is critical to the success of any pesticide analysis using PTV injection. The liner must be thoroughly deactivated or many labile pesticides may decompose or adsorb in the inlet. For large volume injections the capacity of the glass insert is crucial. Any filling material such as glass wool or TENAX used in classical large volume injections that increases the injection volume capacity has to be avoided even if deactivated. For this reason, a glass insert with a rough surface (sintered glass liner, ATAS GL International) was chosen. With these glass liners the inner surface is covered by many small quartz beads to have a larger surface. When using a syringe with a side hole needle the liquid injected will be sprayed onto the wall surface of the liner. External experiments showed that even a 50 µL acetonitrile injection does not result in dropping any liquid out of the liner. These liners were deactivated by a double SILTEK (Restek) deactivation process.

In Figure 1 such a liner is shown. The inertness of the glass insert after subsequent injections of pesticide matrix

Figure 1: Sintered glass insert with glass beads on inner surface for Optic 3 with SILTEK deactivation.



was checked by a degradation of dichloro-diphenyl-trichloroethane (DDT) (also used in EPA 8270). The degradation of DDT must be below 20%. This was checked automatically in batch runs.

After optimization of the Optic 3 injector parameters the analytical performance of the repeatability was studied. Pesticide free extracts were spiked with a pesticides mixture (10–200 µg/L) and 10 subsequent 30 µL injections were made; quantification was fully automated. The result of the relative standard deviations (RSDs) was calculated from the concentration and was about 4.3% or better.

In the following steps the linearity of response was studied with standard solutions prepared in matrix extracts. The calibration curves generated from the matrix matched

Figure 2: Multiple pesticide-residue calibration run of a mixture of more than 50 pesticides on a VF-5-MS 30 m × 0.25 × 0.25 µm with an integrated retention gap of 10 m.

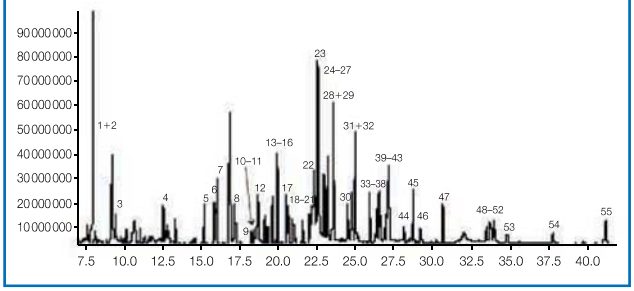


Figure 3: Strawberry sample (Germany) from the field. The pesticides cyprodinil 0.016 mg/kg, fludioxonil 0.022 mg/kg, trifloxystrobin 0.005 mg/kg, fenhexamid 1.078 mg/kg, boscalid 0.032 mg/kg and azoxystrobin 0.189 mg/kg.

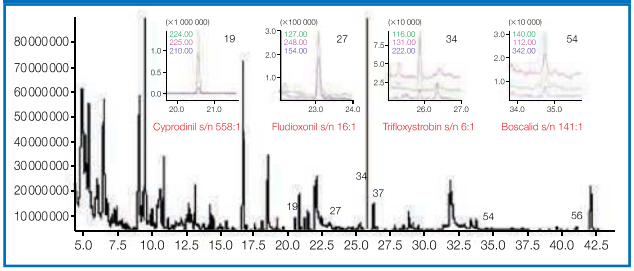


Table 1: Analytical data of a multi-residue pesticide mixture.

No.	RT	Substance	*m/z*	RI	Conc. mg/kg	Straw-berry sample mg/kg
1	7.885	Dichloranilin-3.5	161	1340	0.1648	
2	8.059	Dichlobenil	171	1354	0.01715	
3	9.585	Nitrapyrin	194	1464	0.048	
4	12.707	Ethoprophos	97	1649	0.0408	
5	15.357	Terbuphos	57	1789	0.035	
7	16.028	Disulphoton	88	1823	0.0342	
6	16.176	Ethrimphos	153	1831	0.05958	
8	16.524	Flufenoxuron	126	1848	0.19	
9	17.241	Desmetryn	213	1885	0.0495	
10	17.605	Spiroxamin 1	100	1903	0.013	
11	18.522	Fenpropidin	98	1950	0.0103	
12	18.621	Spiroxamin 2	100	1955	0	
13	18.701	Fenitrothion	125	1959	0.0654	
14	19.217	Metolachlor	162	1985	0.025	
15	19.447	Fenpropimorph	128	1996	0.0105	
16	19.68	Tetraconazol	336	2008	0.053	
17	20.047	Pirimiphos-Ethyl	333	2027	0.0615	
18	20.616	Pendimethalin	252	2056	0.05	
19	20.685	Cyprodinil	224	2060	0.0245	0.016
20	21.014	Pyrifenox-z	92	2077	0.0624	
21	22.06	Pyriphenox-e	92	2131	0	
22	22.147	Chinomethionat	206	2136	0.0548	
23	22.554	Mepanipyrim	222	2158	0.051	
24	22.575	Endosulphan-a	159	2158	0.23	
25	22.964	Chlorfenson	111	2179	0.0505	
26	23.035	Fludioxonil	127	2184	0.1261	0.022
27	23.267	Oxadiazon	175	2195	0.0505	
28	23.524	Myclobutanil	179	2209	0.0565	
29	23.596	Buprofezin	105	2212	0.05265	
30	24.522	Endrin	81	2262	0.04905	
31	24.775	Fensulfothion	97	2276	0.05535	
32	24.96	Endosulphan-b	159	2286	0.0655	
33	26.021	Trifloxystrobin	116	2345	0.1875	0.005
34	26.332	Quinoxyfen	237	2362	0.02625	
35	26.481	Endosulphansulphate	272	2371	0.04545	
36	26.578	Fenhexamid	97	2376	0.097	1.078
37	26.825	Hexazinon	171	2390	0.0555	
38	27.06	Propagite 1	135	2403	0.0217	
39	27.106	Tebuconazol	125	2406	0.05275	
40	27.119	Propagite 2	135	2407	0	
41	27.238	Haloxyfop-ethoxyethylster	302	2414	0.067	
42	27.251	Triphenylphoshat (TPP - INSTD)	77	2414	0.05	
43	27.291	Piperonylbutoxid	176	2417	0.015	
44	28.129	Iprodion	314	2473	0.196	
45	28.769	Fenpropathrin	97	2502	0.0495	
46	29.209	Fenazaquin	145	2529	0.01035	
47	30.728	Acrinathrin	93	2620	0.0624	
48	33.547	Cyfluthrin 1	163	2787	0.0975	
49	33.624	Fenbuconazol	129	2792	0.029	
50	33.702	Cyfluthrin 2	163	2802	0	
51	33.868	Cyfluthrin 3	163	2810	0	
52	34.081	Cyfluthrin 4	163	2815	0	
53	34.764	Boscalid (Nicobifen)	140	2849	0.0594	0.032
54	37.773	Pyraclostrobin	132	2980	0.1275	
55	41.268	Azoxystrobin	344	3109	0.1584	0.189

standards were used for quantification, so that possible errors as a result of enhancement/suppression caused by the matrix effects could be minimized. All results were calculated using triphenylphosphate (TPP) as internal standard.

For the analysis of carrot extracts, for example, the correlation coefficients obtained for the calibration plots of all analytes were in the range 0.989–0.999 in the concentration range of 0.002–1.3 mg/L.

Applying this method, the lowest detection limit (LOD) for more than 500 analytes were in the range of 0.002–0.020 mg/kg depending on the substance.

Figure 2 shows the calibration run of a multiple pesticide-residue standard spiked with a blank strawberry sample. In Table 1 the retention times, linear retention indices (LRI) and concentrations are given.

Figure 3 shows the TIC chromatogram and the ion sets of cyprodinil, fludioxonil, trifloxystrobin and boscalid of a strawberry sample from the field (Germany). The target ions shown in this figure correspond to a range of the LOQ of 0.002–0.02 mg/kg. The LOD for this strawberry example is in the range of 0.0005–0.001 mg/kg.

Another critical point is the number of possible injections before the SILTEK deactivation is no longer stable enough to give reliable quantitative data. For this purpose, a degradation check standard was analysed to check the condition of the glass liner. A mixture of 5 ng pp'-DDT and endrin was periodically injected and analysed. The breakdown of pp'-DDT and endrin must be below 20%. pp'-DDD, pp'-DDE, endrin aldehyde and endrin-ketone are the metabolites from endrin and pp'-DDT. The check formula used was to calculate the total target areas of pp'-DDT + pp'-DDE + pp'-DDD/total target areas of pp'-DDE + pp'-DDD × 100%. In a batch series every fifth injection was done with the degradation check standard diluted in matrix extracts (for example apple matrix) and degradation check values were calculated.

Summary

The quantitative determination of multiresidue pesticides in food matrix according to the QuEChERS method can be successfully combined with a rapid large volume injection (RLVI) and a full scan GC–MS method.

The lowest determination limits (LOQ) were ≤0.002 mg/kg. The observed RSDs of 4.3% and below indicate a high precision in routine work. Up to 80 injections of RLVI of 30 µL into a SILTEK deactivated sintered glass liner were possible.

References

1. Anastassiades et al., *Journal of AOAC International*, **86**(2), (2003).
2. T. Cajka et al., *J. Sep. Sci.*, **28**, 1048–1060 (2005).

Karin Friedrichs holds a chemical engineering degree from Münster Technical University. Between 1981 and 1985, she worked at Essen University's organic chemistry institute on structural determination of organic compounds using spectrometrical methods. Since 1985, she has been working at the Chemical and Veterinary Investigation Office in Detmold, Germany. She is responsible for the GC–MS analysis and sample preparation of contaminants in food.

Heinz-Dieter Winkeler obtained his doctor's degree at Paderborn University in 1983. Since 1990, he has been head of the laboratory of residue analysis at the Chemical and Veterinary Investigation Office in Detmold, Germany. His research interests include the development of analytical methods in the field of organic trace analysis using GC–MS and LC–MS–MS.

Hans-Ulrich Baier studied physics and achieved his PhD degree in 1991. He has been working for Shimadzu Europa GmbH since 1992. He started as a product specialist for surface analysis and mass spectrometry. Since 1996, he has been working as a product specialist for GC/GC–MS.