APPLICATION NOTE

EPA Method 8141B Organophosphorus Compounds by Gas Chromatography (GC-NPD)

AN181v1; July 2025, SCION Instruments

Introduction

US Environmental Protection Agency (EPA) Method 8141B details procedures to identify organophosphorus (OP) compounds by gas chromatography (GC) with either a flame photometric detector (FPD) or a nitrogen-phosphorus detector (NPD).[1]

Organophosphorus compounds are used as pesticides to prevent pests and treat disease on crops. As a result of pesticide use, this can lead to residual pesticides to be found in water.[2] The use of pesticides plays a crucial role in food production. The concern for human and animal health from the use of pesticides leads to the need of monitoring organophosphorus compounds.

In this application note water samples were analyzed for organophosphorus pesticides using a 8500 GC with NPD and 8400 PRO autosampler.

Experimental

An internal standard (IS) was employed during all stages of analysis for this application. The internal standard selected was 1-Bromo-2-nitrobenzene as suggested in EPA Method 8141B: Organophosphorus Compounds by Gas Chromatography.[1] The IS purchased had a concentration of 1000 µg/mL. For more information on The Importance of using an Internal Standard, see our technical note.

Two commercially available organophosphorus pesticide mixes were purchased, 8141 OP Pesticides Calibration Mix A and Mix B. Each compound in the mixes had a concentration of 200 µg/mL and are stated in Table 2. A linearity was conducted by preparing standards at 6 concentration levels: 50, 100, 200, 300, 400 and 500 ng/mL. Each linearity level standard contained 10000 ng/mL of internal standard. This is in accordance with EPA 8141B which states that calibration standards should be prepared at a minimum of 5 levels which encompass the concentration range expected from samples.

To prepare water samples and QC spiked water samples, 100 mL of tap water was added to a reagent bottle in duplicate. To each reagent bottle 19 mL hexane was added. To the water sample 1 mL linearity level 0 which just contained the IS was added. To the QC spiked water sample 1 mL of linearity level 3 was added which contained 200 ng/mL pesticide compounds and the IS.

Both samples were stirred for 15 min. 10 mL was extracted from the organic layer and samples were concentrated to 0.5 mL under nitrogen. Samples were vialled with inserts ready for analysis.

Instrument parameters can be found in Table 1. The instrument method shown in Table 1 gave good specificity and peak shape with most compounds resolving from one another. Figure 1 shows an example chromatogram from the analysis of the standard for mix A and mix B with IS.

Table 1 Instrument parameters for GC-NPD

Part	Settings
Injector	S/SL 250 °C Split: Initial 20:1 0.01 min OFF 0.30 min 20:1
Injection Volume	1.0 µL
Column	SCION-5 30 m x 0.32 mm x 0.25 µm
Carrier Gas	Helium 1.5 mL/min
Detector (NPD)	300 °C Make-up (Nitrogen) flow: 25 mL/min Combustion (H2) flow: 3.0 mL/min Combustion (Air) flow: 150 mL/min Bead Current optimized during Bead Optimization procedure
Oven Program	80 °C hold 2 min 5 °C/min to 150 °C 2.5 °C/min to 165 °C hold 2 min 2.5 °C/min to 200 °C 20 °C/min to 300 °C hold 7 min
Run Time	50.0 min
Software	CompassCDS

Results

Table 2 shows the correlation coefficient (r^2) and the RSD (%) for the system precision (n=10) conducted for the organophosphorus compounds found in 8141 OP Pesticides Calibration Mix A and Mix B. The r^2values are all >0.99 which demonstrates excellent linearity as stated in EPA method 8000, for a non-linear calibration to be acceptable r^2 ≥0.99 and RSD <20%.[3] For this application RSD values for system precision results was found to be ≤5%.

An example linearity plot which was produced using SCION's CompassCDS software and can be seen in Figure 2 for Methyl parathion which had a linearity of 0.9926. Table 3 shows the recovery and precision results for the QC spiked samples (n=6).

Table 2 Correlation coefficient and system precision results for all organophosphorus pesticides in 8141B mix A and B

Compound	Correlation coefficient (r^2)	RSD (%)
Dichlorvos	0.9965	1.50
Mevinphos	0.9945	2.03
Demeton O&S	0.9954	2.66
Ethoprophos	0.9969	1.44
Naled	0.9967	1.52
Sulfotepp & Monocrotophos & Phorate	0.9987	2.33
Dimethoate	0.9973	2.77
Diazinon	0.9985	3.72
Disulfoton	0.9991	2.80
Methyl parathion	0.9926	3.32
Fenchlorphos	0.9975	3.21
Fenthion	0.9986	2.80
Malathion	0.9986	3.12
Chlorpyrifos	0.9985	3.82
Trichloronate & Ethyl parathion	0.9981	4.41
Merphos	0.9940	2.20
Tetrachlorvinphos	0.9984	2.50
Prothiofos	0.9985	2.37
Fensulfothion	1.0000	4.53
Sulprofos	0.9996	5.07
Azinphos methyl	0.9998	2.30
Coumaphos	0.9990	2.17
EPN	0.9996	3.60

RSD results for the recovery were all <8% but the vast majority of compounds had an RSD <2%.

The water sample was shown to have no organophosphorus pesticides present. An example of this can be seen in Figure 3 which shows two chromatograms overlaid the blank water sample (red) and the QC spiked water sample (black). By spiking your sample by a known concentration of target analytes allows the analytical method to be evaluated to determine if it is affecting the sample. Assessing the performance method shows if the results are accurate and valid due to the recovery of the analytes added to the sample. It is crucial to spike your sample at a concentration within your linearity range and sample volume is not increased. This allows calculations to be consistent and avoids introducing unknown effects. See our technical note on Recovery Spiked Sample.

The success of recovery of the organophosphorus compounds present in 8141B mix A and B was varied. As stated in method 8141B not one solvent will be universally appropriate for the use of extracting all analytes so QC spiked samples must be carried out and % recovery calculated. By calculating the % recovery the preparation, clean up and analysis of your sample can be evaluated. EPA method 8000D states that when a method does not state an acceptance criteria for recovery then the laboratory should use 70-130 %. EPA method 8141B indicates that method 8000 should be used as guidance for spiked samples.

The majority of organophosphorus compounds spiked into the water sample were recovered within the 70-130 % range which shows for these pesticides the sample preparation and analytical method described in this application note was suitable for the analysis. Due to coelution some pesticides were reported as a sum which can be seen in Table 3.

From the 26 compounds present in the pesticide standard as seen in Table 3, dichlorvos, mevinphos, diazinon and azinphos methyl showed low calculated % recovery below 70% and dimethoate was not recovered. It can be concluded that for these specific compounds the method described in this application note was not suitable for determining if they were present in the water sample.

Method 8141B depicts other solvents than hexane which may be more suitable for extracting certain pesticides such as isooctane, acetone and for triazine standards specifically Tetrahydrofuran (THF) and Methyl tert-butyl-ether (MTBE).

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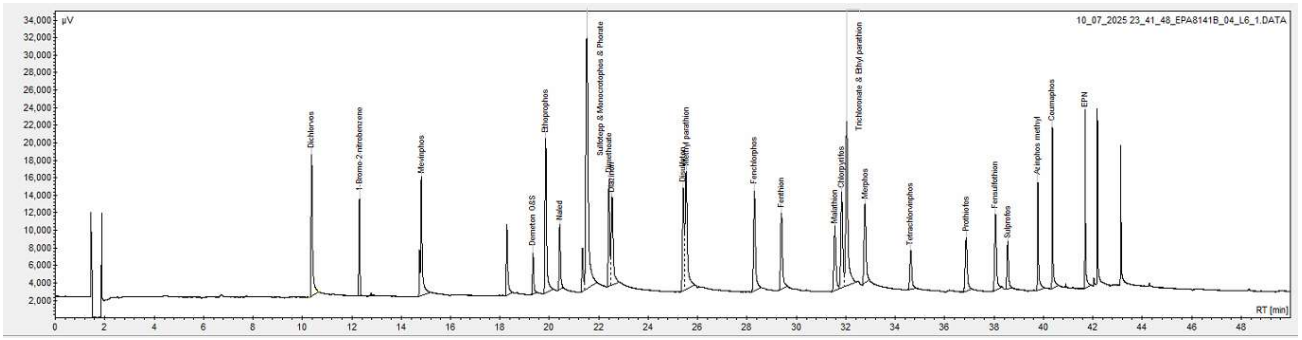

Figure 1 8141B OP pesticide mixes A and B with IS

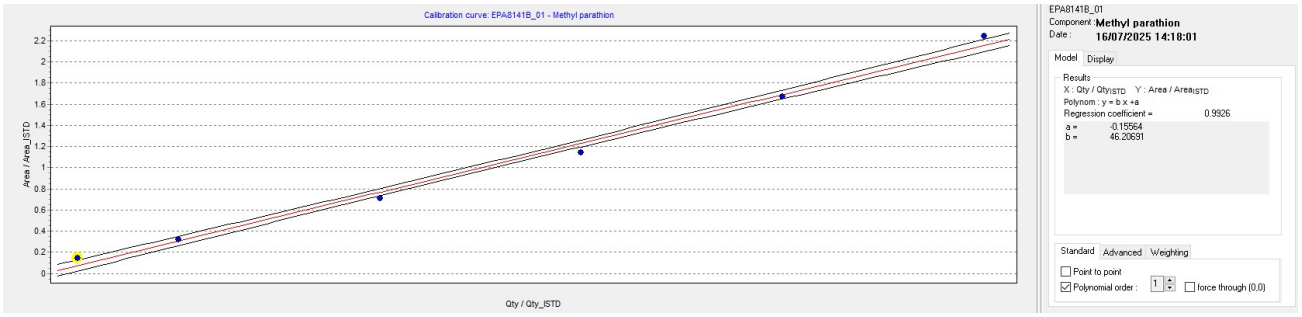

Figure 2 Linearity results for Methyl parathion

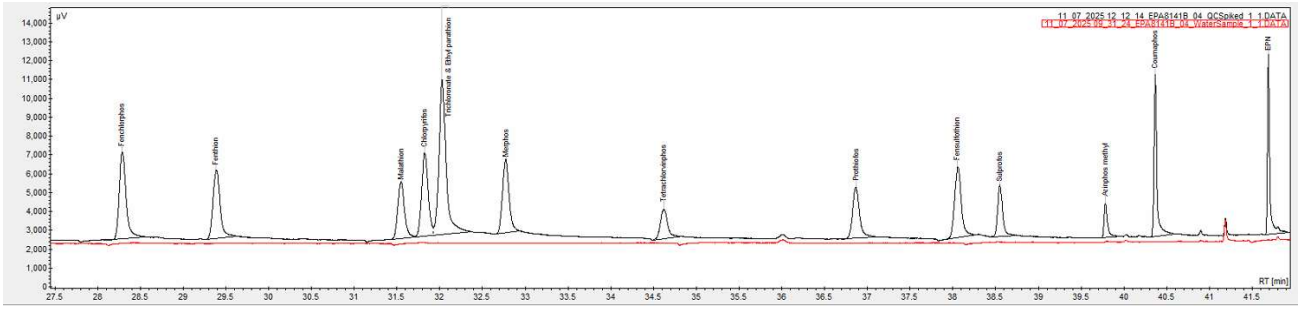

Figure 3 Overlay of water sample and QC water sample spiked with 8141B OP pesticide mixes A and B, both with IS

Table 4 Recoveries and precision results for all organophosphorus pesticides in 8141B mix A and B

Compound	Recovery (%)	RSD %
Dichlorvos	42.56	1.49
Mevinphos	23.08	1.13
Demeton O&S	114.66	0.70
Ethoprophos	106.00	0.61
Naled	99.83	0.85
Sulfotepp & Monocrotophos & Phorate	105.75	0.78
Dimethoate	NR	NR
Diazinon	51.45	0.93
Disulfoton	110.36	2.23
Methyl parathion	110.11	7.67
Fenchlorphos	110.43	0.78
Fenthion	113.81	0.81
Malathion	112.06	1.31
Chlorpyrifos	112.35	1.14
Trichloronate & Ethyl parathion	110.42	0.82
Merphos	113.52	1.19
Tetrachlorvinphos	100.64	3.55
Prothiofos	116.13	1.07
Fensulfothion	116.57	3.05
Sulprofos	124.97	1.02
Azinphos methyl	51.92	0.90
Coumaphos	115.62	1.06
EPN	120.79	1.20

NR= Not recovered

In method 8141B it highlights that the type of extraction method and solvent used can affect the extraction for these OP compounds from the sample and so results should be supported by performance data.[1] This is highlighted in the reported results in method 8141B where it can be observed that depending on extraction technique used and concentration of the spike would determine the success of the recovery.

Conclusion

A method was developed and validated for the analysis of organophosphorus compounds in a water by GC-NPD using EPA Method 8141B. Water samples and QC spiked water samples were prepared using liquid-liquid extraction for more information see our technical notes on Sample Preparation-Liquid-Liquid Extraction and How to Improve Our Liquid-Liquid Extraction Processes.

Results shown implied that none of the pesticides present in the 8141B mix A and B were present in the water sample, which was expected, but to validate this QC spiked samples were analysed. EPA Method 8000D describes the importance of using QC spiked samples and describes acceptable criteria which should be followed. The QC spiked samples concluded that for the majority of compounds the preparation, clean up and analytical method described in this application note was suitable for analysis but for dichlorvos, mevinphos, diazinon and azinphos methyl it cannot be concluded with certainty that they are not present within the water sample due to low calculated % recovery and therefore analysis should be repeated using a different solvent for extraction or possibly a different extraction method until an acceptable recovery can be shown.

The linearity conducted showed that the instrument and method were in good working order and system precision results indicate excellent repeatability of the system.

SCION instruments recommends checking with local regulatory authorities to ensure all testing and reporting requirements are met, or contact the SCION applications team for assistance.

Ordering Information

Part	Part Number
8400 PRO Autosampler for 8300 and 8500 GC	840000001
Suggested Consumables	
Part	**Part Number**
SCION-5 30m x 0.32mm x 0.25µm	SC30233
Liner TAPER FOCUS ULTRA PK/5	41312115
15% Graphite/85% Vespel Ferrule 1/16" with 0.4 mm hole pk/10	41312148
BTO Septa 9 mm, pk/50	CR298713
10 µL fixed needle syringe, 5 cm, 0.47 mm OD, 26 g conical needle	41312133
Vial, 9-425 Screw Thread, 2 mL Clear Glass 12 x 32 mm Flat Base with Label, pk/100	41311000
Cap, Screw, Blue 9-425 Open Top Ribbed with 9mm Red PTFE/Sil Septa 1mm Thick. 100pcs/pk	41311002

For ordering info on the SCION 8300/8500 GC, please contact your local SCION sales representative.

References

1. EPA, https://www.epa.gov/sites/default/files/2015-12/documents/8141b.pdf, (accessed 16 Jul 25)
2. dwi, https://www.dwi.gov.uk/consumers/learn-more-about-your-water/pesticides/(accessed 29 Jul 25)
3. EPA, https://www.epa.gov/sites/default/files/2015-12/documents/8000d.pdf, (accessed 17 Jul 25)

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