# Multi residue analysis of EU black list priority pollutants in drinking and surface water using solid phase extraction and GC Tandem Quadrupole MSMS

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## Introduction

EU council directive 76/464/EC lists 132 compounds that have restricted levels in drinking and surface waters. Of these compounds, 109 are amenable to gas chromatographic analysis. Currently published methods[1] involve the use of two injections, one using selected ion recording as a screen, followed by a full scan injection for confirmation. The use of tandem quadrupole GC-MSMS allows the analyst to combine the screening and confirmatory injections into one run, whilst also reducing the chromatographic separation required for confirmation of the target compounds. The compound groups included in the range of GC-amenable compounds represent a wide range of polarities and compound types, and include benzidines, chloronitrotoluenes, organochloro pesticides, organophosphorus pesticides, chloroanilines, chlorophenols, chloronitrobenzenes, chlorotoluidines, phenylurea pesticides, PCBs, Semi volatile halogenated compounds, PAHs, triazines and volatile amines. Each of these groups of compounds would typically have its own dedicated method of analysis. By combining all of these groups into a single method would greatly increase laboratory throughput.

## Materials and Methods

All chemicals were obtained from Sigma-Aldrich, with all compounds having >99.5% purity. All analysis was performed using an Agilent 6890 GC oven fitted with a CTC Combi PAL autosampler. The GC was directly interfaced to a Waters Quattro Micro GC tandem quadrupole mass spectrometer that was operated in the EI+ ion mode.

The instrument ion source was operated at 70eV electron energy, with a source temperature of 180°C. Three GC columns were evaluated, J&W DB17-ms 30m 0.25mm ID, 0.25mm df, Restek RTX-5, 40m 0.18mmID, 0.2mm df and Varian factor four VF5-ms 30m 0.25mm ID, 0.25mm df. Injections were made using both splitless and cool on column injectors, with a 3m 0.53mm ID retention gap fitted.

All compounds were acquired in full scan and daughter scanning acquisition modes, with the results used to optimise at least two multiple reaction monitoring (MRM) transitions per compound. Internal and recovery standards had one MRM transition optimised.

MRM analysis was performed using a single transition per compound, where confirmation is based upon one MRM transition plus the retention time, and also using two MRM transitions per compound, where the strictest EU confirmatory criteria are satisfied. The difference in sensitivity between the two approaches was compared. The three GC columns were assessed for chromatographic resolution of critical pairs of co-eluting peaks, overall run time, and sensitivity of active components. All standards were prepared from >99.5% purity solids in Dichloromethane (DCM), with a mixed standard being prepared at a concentration of 5ng/ml in DCM, and also Acetone (for spiking purposes)

Extraction and clean-up was performed using Waters Oasis HLB 3cc, 60mg SPE cartridges. 200ml of filtered each filtered water sample was spiked with an internal standard mixture containing $d_5$-nitrophenol, 2-fluorobiphenyl, p-terphenyl-$d_{14}$ at a level of 500ng for each component. The SPE cartridges were conditioned by elution of 6ml DCM, 6ml Acetonitrile and 6ml HPLC grade water at a flow rate of 3ml/min. The water samples were then eluted through the cartridges at a flow rate of *ca* 6ml/min, followed by a wash with 1ml HPLC grade water. The cartridges were then dried with airflow of *ca* 1ml/min for 40mins, followed by final elution with 5ml DCM. After elution, the DCM fraction was adjusted to a volume of ca 1ml under a stream of dry nitrogen at ambient temperature, followed by the addition of 500ng of $d_{10}$-Anthracene as a recovery standard.

Drinking water and polluted canal/river water samples were spiked with the analytes at concentrations of 0.1mg/L and 5mg/L prior to extraction for recovery tests. The recovery tests were repeated with the waters sample pH's adjusted to pH7 and pH2.

The GC temperature ramps employed were: -

30m DB17-ms: - 35ºC/1min, 3ºC/min to 160ºC, 7ºC/min to 240ºC, 15ºC/min to 305ºC, hold 15mins. 1ml/min He flow.

40m RTX5: - 35°C/1min, 3ºC/min to 160ºC, 7ºC/min to 240ºC,15ºC/min to 310ºC, hold 15mins. 0.7ml/min He flow.

30m VF5-ms: - 35ºC/1min, 8ºC/min to 240ºC,10ºC/min to 300ºC, hold 15mins. 1ml/min He flow.

All injections in splitless mode were made with an injection temperature of 250°C, using a single gooseneck 4mm ID liner with a purge tim of 1min and a purge flow of 70ml/min. Cool on column injections were made in track oven mode.

**Results and Discussion**

The first assessment that was made was of the chromatographic performance offered by each of the GC columns used, primarily focussing on the chromatographic separation of key partial or total co-elutions. The 4 pairs of close/totally co-eluting compounds. There were two issues involved with the co-elutions; one where the compounds are isomers so mass spectrometry alone cannot resolve the components of interest. The compounds fitting this scenario are 3 & 4 chlorophenol, E & Z mevinphos and o,p'-DDT & p,p'-DDD; in each of these cases, the pairs of compounds all exhibit the same MRM transitions, for example o,p'-DDT and p,p'-DDD both have the transitions 235>165 and 237>165, and also partially co-elute using some commonly used column phases (i.e. DB5-ms). The second issue is where closely eluting peaks have fragments that may give a response for the analyte peak in question, in this case the elution of alpha-endosulfan and alpha chlordane, where if the peaks are co-eluting, some contribution from chlordane can be seen in the endosulfan channel. Figure 1 shows the MRM channels for alpha endosulfan that elutes at 49.66 minutes, with the contributions from the chlordane peaks clearly visible at 49.17 and 49.54 minutes. These peaks were chromatographically separated using the DB17-ms column, but far closer eluting/co-eluting using the other phases.

The cool on column injector showed the expected increase in sensitivity for more active compounds such as the underivatised chloro phenols and organophosphorus pesticides.

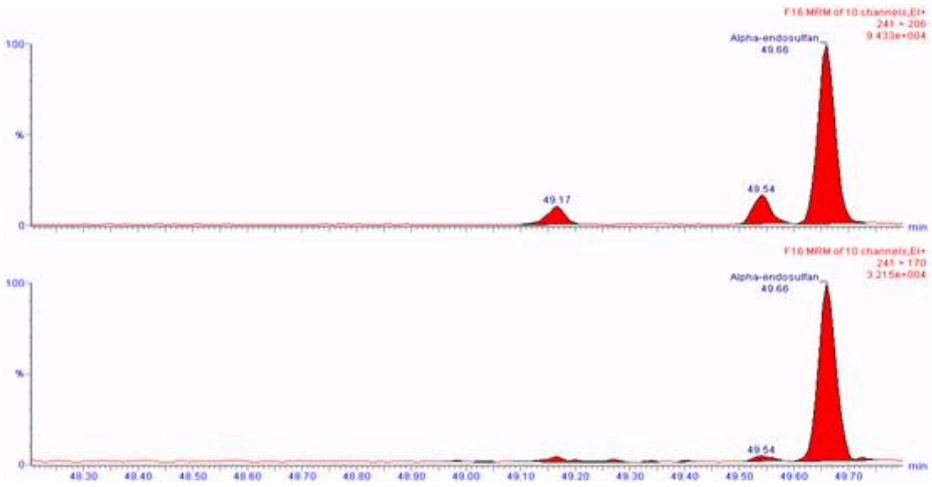


Figure 1 alpha-endosulfan MRM chromatograms, showing the contribution from chlordane peaks at 49.17 and 49.54 mins

Figure 2 shows the separation obtained for p,p'-DDD and o,p'-DDT using cool on column injection and the DB17-ms

column, allowing unequivocal quantification of both compounds.

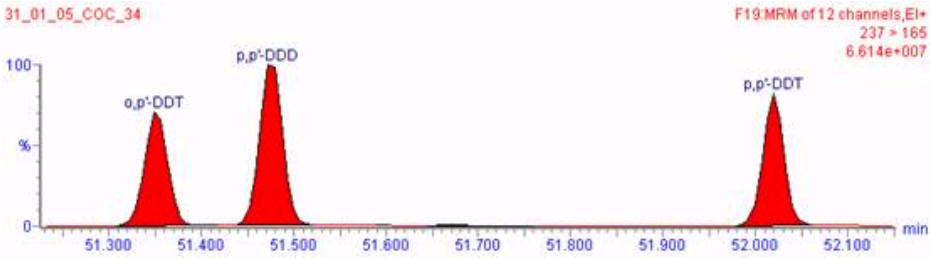


Figure 2 separation of o,p'-DDT and p,p'-DDD using the DB17-ms column

The cool on column injector required more on-going maintenance, with the chromatography being affected by a build up of contamination on the guard column every few days.Figure 3 shows the overlayed chromatograms for all MRM transitions acquired, using a cool on column injection on the DB17-ms column.

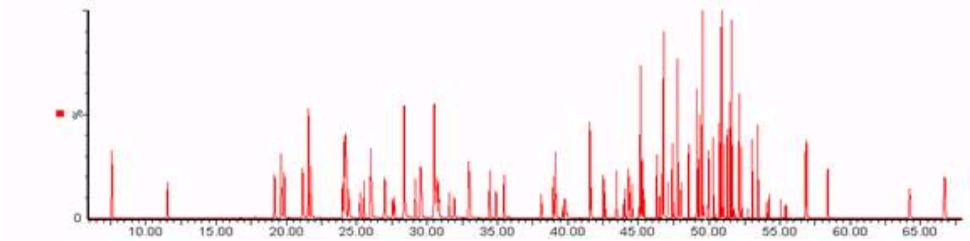


Figure 3 overlayed chromatograms of all analytes acquired in MRM mode using the DB17-ms column.

Table 1 gives a summary of the instrumental LODs obtained for the analytes, with all LOD calculated based upon the confirmatory MRM transition. All but 4 of the compounds showed LODs below the required 20pg/mL required to meet the EU limit of 0.1mg/L for these compounds. Using the single MRM transition approach allowed all compounds except 2-amino-4-chlorophenol to achieve the required <20pgmL LOD. Using a single MRM transition maintains a higher degree of selectivity when compared with low resolution selected ion recording using 2 masses, whilst increasing the sensitivity when compared with monitoring two MRM transitions.

| | LOD <1pg/μL | LOD 1-20pg/μL | LOD>20pg/μL |
|---|---|---|---|
| No compounds | 15 | 88 | 4 |

Table 1 summary of instrumental LODs

In general, the extraction recoveries and limits of detection satisfied the requirements for surveillance purposes, with compounds such as 2-amino-4-chlorophenol requiring a dedicated analytical method, as this compound requires derivatisation prior to GC analysis.

**Conclusions**

GC-MSMS provides the analyst with the ability to simultaneously screen and confirm the presence of organic environmental pollutants, removing the need for separate screening and confirmatory analysis. Having the ability to perform multi residue analysis on a wide range of compounds greatly increases laboratory throughput.

The use of MRM allows the analyst to reduce chromatographic separation in some cases, again increasing throughput. Solid phase extraction is a less labour intensive extraction/clean-up technique when compared with traditional liquid liquid extraction, whilst also using much lower volumes of extraction solvents, to help reduce laboratory costs.

1. .Anal. Chem.2000, 72,1430-1440; Broad Spectrum Analysis of 109 Priority Compounds Listed in the

76/464/CEE Council Directive Using Solid-Phase Extraction and GC/EI/MS; SílviaLacorte, Ingrid Guiffard,DanielFraisse,Damià Barceló