Target Based Screening of Environmental Water Samples using Deconvolution Reporting Software on an Agilent 5975 Series GC/MSD and the Creation of a New Screening Database

Application Note

Environmental

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Abstract

A target based, multiresidue, screening method using Deconvolution Reporting Software (DRS) has been developed on an Agilent 5975 Series GC/MSD in response to the introduction of the Water Framework Directive (WFD).

A new database has been created, containing over 1,000 target chemicals, including both volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs), which allows for the rapid identification and reporting of organic pollutants in an extracted sample.

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Introduction

In December 2000, the European Commission introduced a piece of legislation, WFD 2000/60/EC. The key objective is to provide for the planning and delivery of a better aquatic environment. It is aimed to help protect and further enhance the quality of the water environment across all member states of the European Union by 2015. At the heart of the WFD lies the requirement of countries to develop river basin management plans. In England and Wales, there are 11 river basins with management plans and these compliment a further 40 international river basin plans across the European Union.

The WFD applies to surface freshwater bodies (including lakes, streams, canals and rivers), groundwaters, transitional water bodies (estuaries), and coastal waters (out to 1 mile from low-tide). In contrast to the existing EU directives, the WFD applies to all water bodies. Under the WFD, surveillance, operational, and investigative chemical monitoring is required. The Environment Agency (EA) has to consider making this chemical monitoring cost effective. It is not possible to monitor for everything, everywhere. There is also the need to identify emerging pollutants that do not exist in routine suites and to inform of future monitoring priorities.

In order to address part of the chemical challenge of the WFD, the EA commissioned the development of the GC/MS based screening tool. The requirements of which are:

- A screening method capable of detecting a wide range of organic pollutants in a given water body under WFD
- The ability to identify both VOCs and SVOCs from a single sample
- Limit of Detection (LOD) of 0.1 µg/L
- Low cost solution for validating the pressures and risks to water bodies
- As monitoring requirements are constantly changing, the provision to add new substances

GC/MS was chosen as the analytical technique as it is widely applicable for the identification and measurement of a vast range of chemicals, and the use of a 5975 Series GC/MSD provided excellent sensitivity. Deconvolution Reporting Software (DRS) for GC/MS uses a retention time locked method. It combines results from the Agilent GC/MSD ChemStation, the Automated Mass Spectral Deconvolution and Identification Software (AMDIS) and the NIST Mass Spectral Search Program (NIST) into one report. It was chosen because it provides the necessary automation to reduce data interpretation time and increase the accuracy of chemical identification.

Using the Hazardous Industrial Chemicals (HIC) Database as a starting point, a new much larger target database was created with over 1,000 target compounds.

Experimental

Sample Preparation

An Internal Standard is added to 1 L of sample. The sample is extracted with 50 mL of Dichloromethane (DCM) solvent for 15 minutes and the solvent is removed. The remaining sample is then acidified and extracted with a further 50 mL of DCM for 15 minutes. This solvent is then removed. The extracts are combined and reduced to 1 mL, dried with anhydrous sodium sulphate and transferred to an autosampler vial ready for analysis by GC/MS.

Results and Discussion

Analytical Technique

GC/MS was chosen as the analytical technique because it is widely applicable for the identification and measurement of a vast range of chemicals. The DRS software was chosen because it provides the necessary automation, to reduce data interpretation time and increase the accuracy of chemical identification. The instrument operating conditions are shown in Table 1 and the software used in Table 2.

Table 1. Instrumentation and Conditions of Analysis

Gas chromatograph	**Agilent 7890A Series GC**
Automatic sampler	Agilent 7683B Automatic Liquid Sampler
Inlet	Agilent PTV (Programmed Temperature Vaporization)
Column	0.25 mm × 30 m, 0.25 µm HP5-MS UI
Carrier gas	Helium, constant pressure mode
Retention time locking	Fluorene locked to 15.577 minutes
Oven temperature program	40 °C (0.2 minutes), 10 °C/min to 300 °C (8 minutes)
PTV inlet parameters	Cold splitless, 20 °C (0.2 minutes), 720 °C/min to 300 °C
Injection volume	1.5 µL
Run time	36 minutes
Mass selective detector	**Agilent 5975C Inert XL MSD with Triple-Axis Detector (TAD)**
Tune file	atune.u
Acquisition mode	EI full scan
Scan range	35 – 566 µm
Source, quad, transfer line temperatures	250 °C, 150 °C, 280 °C respectively
Solvent delay	1.82 minutes

Table 2. Software

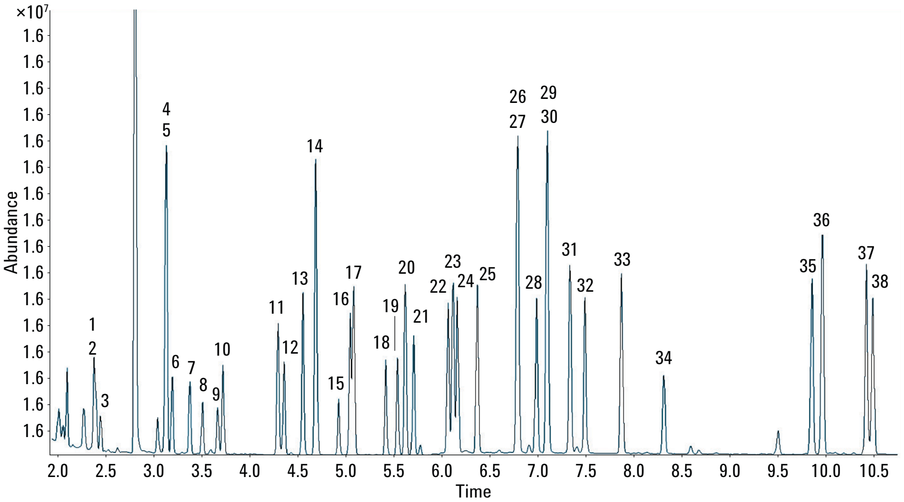
Software
GC/MSD ChemStation
Deconvolution Reporting Software (DRS)
Automated Mass Spectral Deconvolution and Identification Software (AMDIS)
NIST08 Mass Spectral Compound Library (NIST)
Hazardous Industrial Chemicals Database (HIC)

The inlet parameters were optimized to achieve good front end chromatography for the required volatile analysis. The 5975 Series GC/MSD provides sufficient sensitivity to allow for the use of a cold splitless injection, which is essential for the analysis of VOCs. Figure 1 shows the volatile section of a typical chromatogram.

Sample preparation

A liquid/liquid partition method using DCM under neutral and acid conditions was chosen to extract the widest variety of compounds. The extraction is performed on bottle rollers to maximize the solvent/matrix interaction and also to reduce the formation of emulsions. The extracts are concentrated using Zymark Turbo-Vap concentrators, which enables volatiles to be retained through the control of temperature and gas flow.

During sample collection and preparation, it is important to adopt good housekeeping procedures, and not introduce any contamination. In the laboratory, it is beneficial to have a dedicated screening room and use high quality DCM. Store dedicated glassware between 40–50 °C, as this has been found to reduce the impact from phthalate contamination and other airborne contaminants found in a typical laboratory. Perform a blank extraction with each batch of samples. Then, blank correct associated samples for any contamination introduced during the extraction process.



Peak	Compound name	Peak	Compound name	Peak	Compound name
1	Trichloroethylene	14	*m/p*-Xylene	27	1,2,4-Trimethylbenzene
2	Dibromomethane	15	Bromoform	28	1,3-Dichlorobenzene
3	Bromodichloromethane	16	Styrene	29	*sec*-Butylbenzene
4	*trans*-1,3-Dichloropropylene	17	o-Xylene	30	1,4-Dichlorobenzene
5	Toluene	18	1,1,2,2-Tetrachloroethane	31	*p*-Isopropyltoluene
6	1,1,2-Trichloroethane	19	1,2,3-Trichloropropane	32	1,2-Dichlorobenzene
7	1,3-Dichloropropane	20	Isopropylbenzene	33	*n*-Butylbenzene
8	Chlorodibromomethane	21	Bromobenzene	34	3-Chloro-1,2-dibromopropane
9	1,2-Dibromoethane	22	2-Chlorotoluene	35	1,2,4-Trichlorobenzene
10	Tetrachloroethylene	23	*n*-Propylbenzene	36	Naphthalene
11	Chlorobenzene	24	4-Chlorotoluene	37	1,2,3-Trichlorobenzene
12	1,1,1,2-Tetrachloroethane	25	1,3,5-Trimethylbenzene	38	Hexachlorobutadiene
13	Ethylbenzene	26	*tert*-Butylbenzene		

Figure 1. Volatile section of chromatogram for extracted Restek 502.2 VOC mix at 1 µg/L.

New Target Database

Using the HIC Database as a starting point, a new target database [1] has been created that is relevant to the water industry and WFD. It contains pesticides, fungicides, molluscicides, hydrocarbons and PAHs, emerging pollutants, industrial chemicals, metabolites, volatile solvents, as well as pharmaceuticals and personal care products. As a living database, this continues to grow. It presently contains approximately 1,000 compounds.

The addition of new target compounds is a straightforward process:

1. Obtain a reference standard and run against RTL analytical method.
2. Build profile in Chemstation (Edit compounds).
3. Add the deconvoluted spectra into the target library.
4. Create AMDIS library and Quantitation Database (QDB) from user library.

Interpretation of DRS Report

The results are semiquantitative and estimates of concentration are obtained by running a reference standard for each individual compound at a known concentration, typically 1 µg/L, to produce a response factor. Fully quantitative analysis is not practical due to the large number of compounds in the database and the requirement to use a set of standards.

The LOD is dependent on compound, sample matrix, and sample volume provided. One of the initial requirements was to achieve a typical LOD of 0.1 µg/L. This detection limit was taken from the EU Drinking Water Directive, which states that the levels of individual pesticides should not exceed 0.1 µg/L. Table 3 demonstrates that the required LOD was achievable for a river water fortified with compounds at 0.1 µg/L. This is a small selection from the many compounds that were originally tested. It is clear from this initial testing that much lower LODs were achievable. In fact, 75% of the targets tested were detected at < 0.02 µg/L, a few examples of which are shown in Table 4. These LODs are possible due to the excellent sensitivity of the 5975 Series GC/MSD.

Table 3. River Water Fortified with Targets at 0.1 µg/L

Target compound	Class of compound	River	Target compound	Class of compound	River
2-Chlorophenol	Biocide	✓	Dichlorvos	Insecticide	✓
Trichloroethylene	Volatile solvent	✓	Ethyl benzene	Volatile solvent	✓
2,4-D Methyl ester	Herbicide	✓	Fenchlorophos	Insecticide	✓
2,4,5-Trichlorobiphenyl	Industrial chemical	✓	Hexachlorobenzene	Fungicide	✓
p,p′-DDE	Pesticide	✓	Methoxychlor	Insecticide	✓
Pyrene	PAH	✓	Pentachlorobenzene	Industrial chemical	✓
Aldrin	Pesticide	✓	Pirimicarb	Insecticide	✓
Atrazine	Herbicide	✓	Simazine	Herbicide	✓
Benzo (a) pyrene	PAH	✓	Trietazine	Herbicide	✓
Caffeine	Psychoactive drug	✓	Vinclozolin	Fungicide	✓
Metaldehyde	Molluscicide	✓	Propachlor	Herbicide	✓
Carbetamide	Herbicide	✓	Tetrachloroethylene	Volatile solvent	✓
Chlordane	Insecticide	✓	Methoxychlor	Insecticide	✓

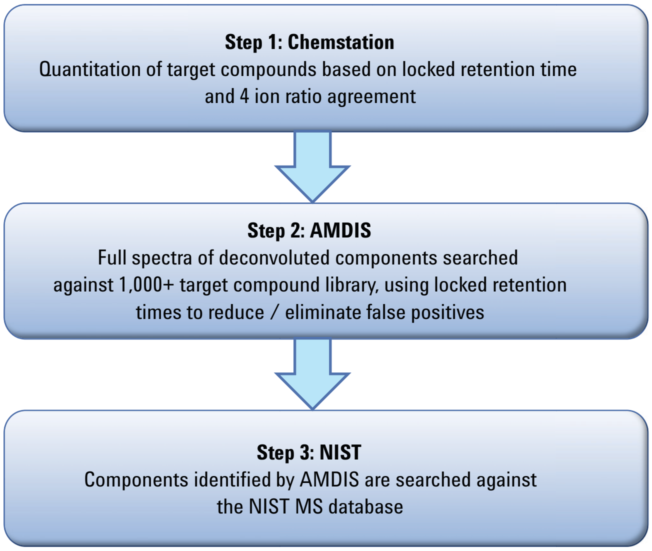
Table 4. DRS Report for Five Compounds Demonstrating that Lower LODs are Achievable

			Amount spiked (µg/L)	**AMDIS**		**NIST**	
R.T.	**Cas no.**	**Compound name**	**ChemStation**	**Match**	**R.T. diff sec.**	**Reverse match**	**Hit no.**
2.4158	79016	Trichloroethylene (volatile solvent)	0.01	48	4.8	90	1
7.7070	108623	Metaldehyde (molluscicide)	0.02	45	-0.8	82	1
17.8784	1912261	Trietazine (herbicide)	0.01	40	-3.2	60	1
21.4323	129000	Pyrene (PAH)	0.01	93	-4.4	93	2
23.715	298464	Carbamazepine (pharmaceutical)	0.01	66	-0.9	73	1

The DRS software application for target compound analysis works by combining the results of ChemStation, AMDIS, and NIST into a final report as shown in Figure 2.

The final and most important step in the confirmation process is the interpretation of the DRS report by the analyst. For instance, low match factors do not necessarily mean a negative confirmation, they can also mean that the target compound is indeed present but just at very low levels. This can be confirmed by analyzing the compound of interest by a dedicated technique or alternatively, an informed decision can be made based on the information available.

It is also important to be aware of compounds that do not have a mass spectral entry in the NIST library, so that these compounds do not get overlooked. Fifty three of the target database compounds do not have an entry in NIST08. Table 5 shows the DRS report for a sample, where out of the nine positive identifications, four of these compounds (Pyrimethanil, Flufenacet, Boscalid, and Bixafen) did not have an entry in NIST08. In the DRS report, the NIST match columns remain blank and it would be easy for these compounds to be overlooked. However, being aware that they are not in NIST is useful as a decision can be made using the information that is available. Figure 3 shows that the retention time, extracted ion chromatogram, spectra and ion ratios for Boscalid and confirms that it is indeed present in the sample. Using the most up to date version of the NIST library (NIST11) is beneficial as there are fewer omissions, although Bixafen is still missing.



Step 1: Chemstation
Quantitation of target compounds based on locked retention time and 4 ion ratio agreement

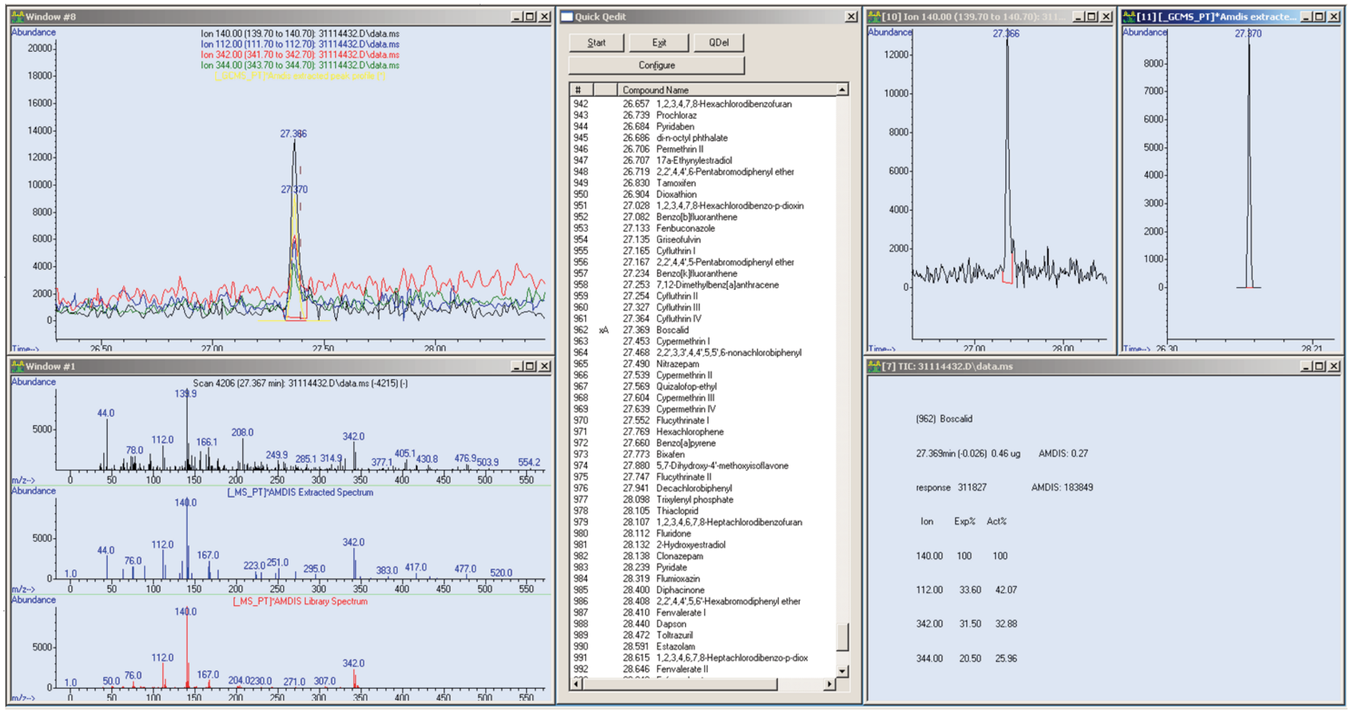
Step 2: AMDIS
Full spectra of deconvoluted components searched against 1,000+ target compound library, using locked retention times to reduce / eliminate false positives

Step 3: NIST
Components identified by AMDIS are searched against the NIST MS database

Figure 2. Summary of the DRS Process.

Table 5. DRS Report for a Sample, Showing that Pyrimethanil, Flufenacet, Boscalid, and Bixafen do not have an Entry in NIST08

			Amount (µg/L)		**AMDIS**		**NIST**	
R.T.	**Cas no.**	**Compound name**	**ChemStation**	**AMDIS**	**Match**	**R.T. diff sec.**	**Reverse match**	**hit no.**
16.1975	10543574	N,N,N′,N′-Tetraacetylethylenediamine	0.06		42	-5.1	74	1
18.0320	53112280	Pyrimethanil	0.15		94	-2.1		
18.5786	58082	Caffeine	0.1		87	-2.7	86	1
20.1009	67306030	Fenpropimorph	0.01		42	-7.2	71	1
20.2315	142459583	Flufenacet	0.01		45	-1.1		
20.782	40487421	Pendimethalin	0.04		52	-6.5	68	1
24.7148	18181801	Bromopropylate	0.01		41	-0.3	79	1
27.367	188425856	Boscalid	0.46		58	-1.7		
27.7441	581809463	Bixafen	0.64		77	-1.7		
17.889		Phenanthrene-d10	1					



#		Compound Name
942		26.657 1,2,3,4,7,8-Hexachlorodibenzofuran
943		26.739 Prochloraz
944		26.684 Pyridaben
945		26.686 di-n-octyl phthalate
946		26.706 Permethrin II
947		26.707 17a-Ethynylestradiol
948		26.719 2,2',4,4',6-Pentabromodiphenyl ether
949		26.830 Tamoxifen
950		26.904 Dioxathion
951		27.028 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
952		27.082 Benzo[b]fluoranthene
953		27.133 Fenbuconazole
954		27.135 Griseofulvin
955		27.165 Cyfluthrin I
956		27.167 2,2',4,4',5-Pentabromodiphenyl ether
957		27.234 Benzo[k]fluoranthene
958		27.253 7,12-Dimethylbenz[a]anthracene
959		27.254 Cyfluthrin II
960		27.327 Cyfluthrin III
961		27.364 Cyfluthrin IV
962	xA	27.369 Boscalid
963		27.453 Cypermethrin I
964		27.468 2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
965		27.490 Nitrazepam
966		27.539 Cypermethrin II
967		27.569 Quizalofop-ethyl
968		27.604 Cypermethrin III
969		27.639 Cypermethrin IV
970		27.552 Flucythrinate I
971		27.769 Hexachlorophene
972		27.660 Benzo[a]pyrene
973		27.773 Bixafen
974		27.880 5,7-Dihydroxy-4'-methoxyisoflavone
975		27.747 Flucythrinate II
976		27.941 Decachlorobiphenyl
977		28.098 Trixylenyl phosphate
978		28.105 Thiacloprid
979		28.107 1,2,3,4,6,7,8-Heptachlorodibenzofuran
980		28.112 Fluridone
981		28.132 2-Hydroxyestradiol
982		28.138 Clonazepam
983		28.239 Pyridate
984		28.319 Flumioxazin
985		28.400 Diphacinone
986		28.408 2,2',4,4',5,6'-Hexabromodiphenyl ether
987		28.410 Fenvalerate I
988		28.440 Dapson
989		28.472 Toltrazuril
990		28.591 Estazolam
991		28.615 1,2,3,4,6,7,8-Heptachlorodibenzo-p-diox
992		28.646 Fenvalerate II

(962) Boscalid

27.369min (-0.026) 0.46 ug AMDIS: 0.27

response 311827 AMDIS: 183849

Ion	Exp%	Act%
140.00	100	100
112.00	33.60	42.07
342.00	31.50	32.88
344.00	20.50	25.96

Figure 3. Boscalid (fungicide) positive confirmation.

Analysis Time

One major advantage of DRS is that it dramatically reduces data processing time and, as a result, more samples can be processed. With a GC/MS cycle time of approximately 40 minutes, 24 samples can be run on an overnight sequence before being processed and reported the following day. This in turn delivers real cost saving to any business. The speed of analysis is very important, particularly during pollution incidents or in the event of an environmental threat when information is required quickly.

Applications

Other applications for this screening technique include the analysis of sewage and trade effluent discharge, saline water, potable raw water, landfill leachates, as well as sediments and solids.

Conclusions

A target based, multiresidue, screening method using DRS has been developed on an Agilent 5975 Series GC/MSD in response to the introduction of the WFD, which is capable of detecting virtually all GC amenable pesticides. With the excellent sensitivity of the instrument, this screening method is capable of detecting compounds at levels as low as 0.01 µg/L. A new database has been created which contains over 1,000 target chemicals, including both VOCs and SVOCs. It also includes some compounds not available in NIST libraries. The database is fully customizable, and the use of DRS means data processing time is substantially reduced, which in turn provides a real cost saving.

Reference

1. Full listing of new target database can be viewed at: www.natlabs.co.uk

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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Printed in the USA
November 13, 2012
5991-1431EN

Agilent Technologies