Recent Advances in the Applications of Mass Spectrometry to Environmental Matrix Analysis

Peter J Baugh, Environmental and Food Analysis Special Interest Group, EFASIG, the British Mass Spectrometry Society (BMSS).

A review is presented of the advances in the applications of mass spectrometry to environmental matrix analysis with a focus on water, wastewater and soil with a mention of fly ash and flora and fauna. Coverage is given of sample types, sample preparation, cleanup techniques, mode of chromatography, ionisation process, mass analysis, scan function and data system involvement with illustrations of the applications. Contributions from ACDLabs, Agilent Technologies, Bruker Daltonics, Markes Int, Perkin Elmer, Thermo Scientific and Waters Ltd are highlighted to illustrate the types of MS and the environmental applications.

The ever increasing sophistication in instrumentation means yesterday's data can be somewhat obsolete and there is continuing need to refine acquired information. Particularly, important are the sensitivity, specificity and detection limits obtainable, which enable ever decreasing levels of pollutants to be detected, and quantified. Also the regulations on permissible levels are ever-changing in parallel.

It is important to emphasise where the problems lie in relation to ensuring that the data obtained reflect accurately the integrity of the sample and what it originally contained. To this end the introduction of the sample- front end, through separation & analysis to the back end- data acquisition, handling and processing must be considered in sequence. [1]

A Simple Flow Diagram of the Process for Environmental Matrix Analysis is as follows:

1. Sample matrix from the environment (preservation of sample integrity)
2. Sample preparation (liquid or solid dependent)
3. Sample clean-up (clean or contaminated sample dependent)
4. Sample introduction (physico-chemical property dependent- finite vapour pressure, involatile, labile, neutral or polar)
5. Sample analysis - selection of chromatography (physico-chemical property dependent as above)
6. Sample analysis – selection of MS ionisation & analyser with options
7. Analysis of data – identification- ms database search, mass accuracy (elemental composition & structure) & quantitation including calibration

Chemical pollutants include the priority pollutant list (Environmental Protection Agency- EPA) – Polycyclic Aromatic Hydrocarbons (PAHs), dioxins; pesticides, herbicides; insecticides; emerging contaminants including endocrine disruptors and the structures are illustrated in *Figure 1*.

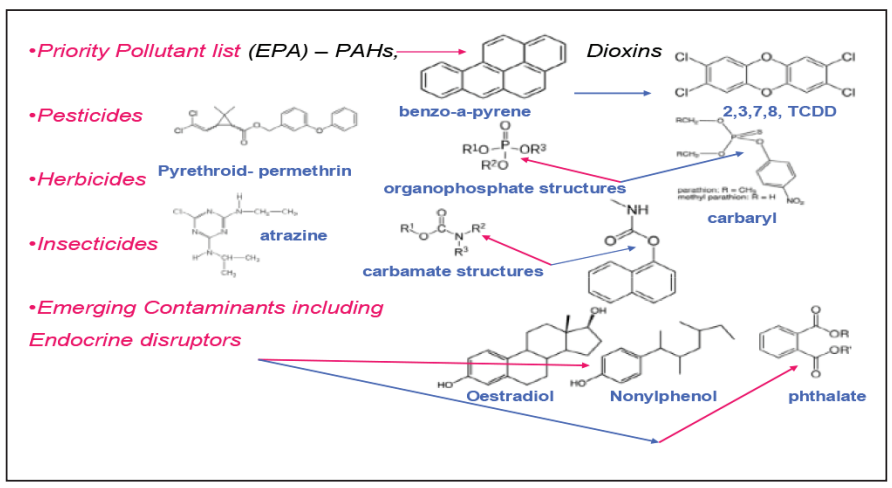

Figure 1. Chemical pollutants typically detected in environmental matrix analysis.

Sample Preparation

It may be that sample preparation still commands as important a place as ever in the analysis when taking a finite amount of the raw matrix through a number of stages to obtain the final residue in a specific volume, in which extraction, separation via reversed phase (RP) and normal phase (NP) column chromatography modes with the addition of standards (isotopically labelled) having the same chemistry as the target compounds are involved. A good example of the rigour required is in the sample preparation and work up for dioxin analysis. There has been a strenuous effort to render sample preparation largely unnecessary but a considerable element of clean up still remains. The availability of high resolution MS/MS which allows an increase in specificity can largely remove the need for matrix treatment thus avoiding the ever present problem of false positives. Limitations here can revolve around the number of MS/MS ion programs (precursor to product ion transitions) that can be accommodated when confronted with a multi-component mixture. Obviously MS/MS LRMS is of less value for unknown components.

Sample Preparation Methods

Sample preparation can be conducted off-line or on-line depending on the nature of the matrix and the extent to which clean-up and concentration are required. Essentially, the extent to which sample preparation is necessary from the raw matrix depends on the physical nature, liquid or solid (or gaseous atmosphere). Water as a matrix (river system/ effluent) can be solvent (LLE) or solid phase extracted (SPE) and the resulting sample pre-concentrated prior to analysis. Large volume Injection (& headspace) is also possible for a water based matrix (direct or on-line preconcentration injection). Solid Phase Micro Extraction (SPME) can be conducted off-line or on-line for Volatile Organic Compounds, VOCs, & Semi-Volatile Organic Compounds, SVOCs [2].

Purge & trap for Highly Volatile Organic Compounds (HVOCs) can be conducted on-line (lost in Liquid Liquid Extraction, LLE). Soil or fly ash as a matrix may be solvent extracted (direct, soxhlet or microwave digestion) but the resultant liquid will require extensive clean-up involving a number of steps (classic case- dioxins). Pressurised fluid extraction (PFE) can replace soxhlet extraction. (Note: fauna & flora also will require a similar sample treatment).

Sample Clean-up Exemplified

Clean waters relatively free of particulates can be filtered through SPE discs that, for example, quantitatively retain dioxins & furans. The EPA Methods 8260 and 8270 are the basis of the sample preparation for polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polycyclic chlorinated biphenyls (PCBs) in surface and ground water.

Typical sample clean-up for dioxins & furans analysis post-soxhlet extraction involves: (1) removal of lipids by sulphuric acid treatment; (2) removal of interfering components by acidic/basic silica gel; (3) separation of PCBs and similar interferences by activated alumina; (4) separation of non-planar molecules by activated charcoal.

PFE recently introduced requires less solvent use and reduced extraction time (EPA method 3545).

Flow Diagrams for the determination of pesticide residue extracted from water (100 mL;50 mL, GLC & HPLC, respectively) differ for the analysis by GC & LC-QqQ/MS but include the use of solid phase micro extraction (SPME) cartridges and a number stages-filtration/centrifugation, conditioning, washing, vacuum drying, elution, evaporation, & reconstitution.

Chromatography: GLC or HPLC & UPLC?

HPLC in gradient elution mode is increasingly used for Environmental Analysis with increase in elution strength of the solvent during separations to allow analysis of the more retained molecules. LC/MS can be used for Environmental Analysis in many instances but GC/MS is still preferred for dioxin analysis.

Whereas coupling of capillary GC to MS is relatively simple LC is more challenging for several reasons:

Gas phase ions are required to be generated from either solvated molecules or ionised in liquid mobile phase. Nebulisation, desolvation, ionisation processes occur in the MS source at atmospheric pressure – API. Ions formed in the source should be transferred efficiently into the optics of the MS while eliminating the huge volume of gas produced by evaporation of the MP. Neutral compounds as contaminants should be eliminated as far as possible.

Ionisation/Scan Mode of choice

API covers a number of ionisation processes, which are soft ionisation modes unlike EI for GC/MS (Note- though enhanced M+ is now possible applying lower ionisation energy for EI for GC x GC TOF without reduction in sensitivity), e.g., Atmospheric Pressure Ionisation, API, APCI, APPI Electro spray ESI & DESI. APCI has been developed for GC/MS applications.

Some general aspects are as follows:

- EI full scan is required for ms data base search; SIM for selected ion monitoring & higher levels of detection (GC-MS)
- EI/CI with MS/MS for M+ and precursor to product ion transition monitoring (MRM)
- API/APCI with LC-MS/MS for M+ or M.-
- ESI for M+ or M.- & metal adduct formation.

Mass Analysers

Illustrated in *Figure 2* are the QTOF with ion mobility separator or collision cell; Orbitrap; single quadrupole mass filter and ion trap (note also the linear ion trap is employed, but not illustrated); tandem mass analyser, APGC QqQ;). A summary *(Table 1)* of the sensitivity and scan parameters is also presented below.

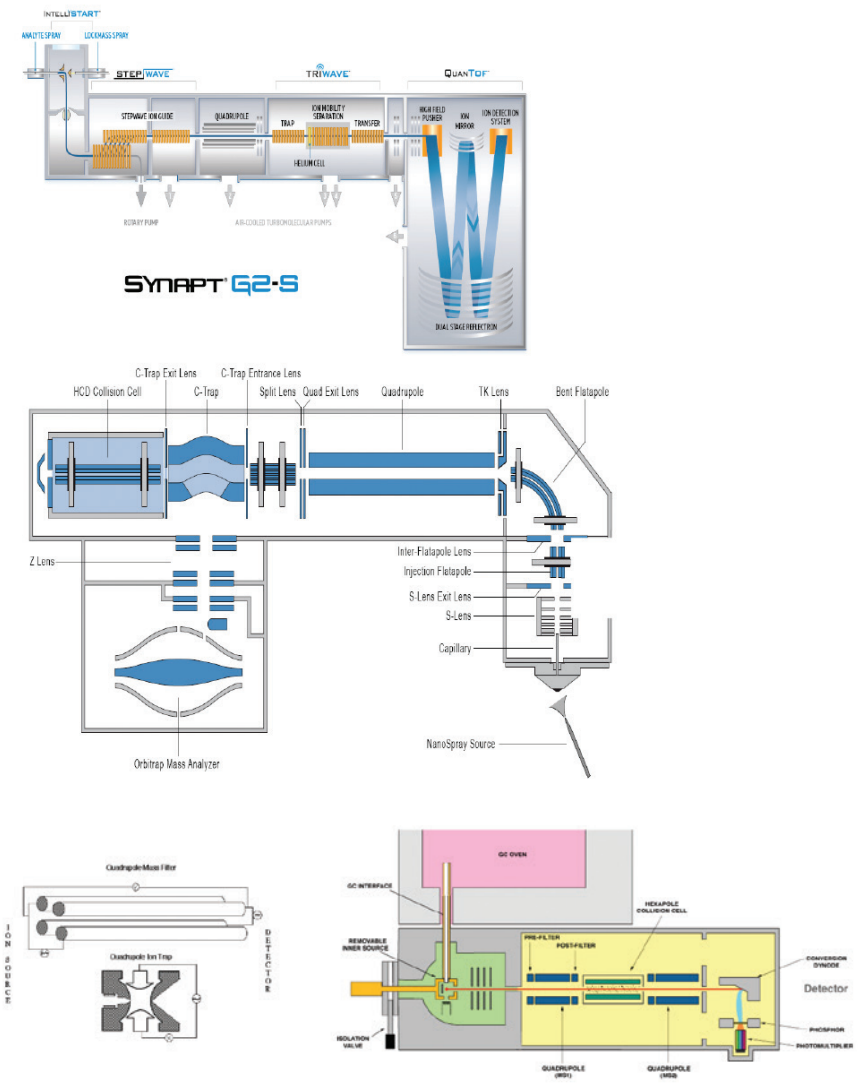

Figure 2. Types of mass analyser employed in environmental analysis.

Table 1. Sensitivity and scan parameters for mass analysers

Analyser	Mass Range [*10-3]	Resolution [Δm/z]	Resolving Power FWHM [*10-3]	Mass Accuracy [ppm]	MS/MS	Scan Rate [Scans/s]
Q	3	0.1 - 0.7	-	Low	-	0.5 - 4
QqQ	3	0.1 - 0.7	-	Low	MS/MS	0.5 - 4
IT	4 - 20	0.1 - 0.7	-	Low	MSN	5 - 10
LIT	4 -20	0.1 - 0.7	-	Low	MSN	1 - 10
Orbitrap	6	-	50 - 100	≤5	MSN	0.5 - 2
oaTOF	20 - 40	-	15 - 20	≤5		20 - 30
QqTOF	10 - 40	-	15 - 25	≤5	MS/MS	20 - 30
Sector, EB						
hybrid	5 - 15	-	30 - 80	≤5	MS/MS	0.1 – 0.5

Low Resolution Instruments (IT, Q & QqQ/TSQ)

Single quadrupole or ion trap instruments are employed for environmental analysis where low cost is required. However, tandem quadrupole systems are preferred and for general quantitative analysis MS/MS. Multiple Reaction Monitoring (MRM) is used providing selectivity, sensitivity and linearity over a wide range. Screening methods allow a large number of target compounds to be analysed and target pollutants to be confirmed and quantified. MRM is limited to detecting specified compounds selected. Features/ capabilities of TSQs are:

Acquisition speed: capability 10 ms per acquisition; multi-residue methods require fast analysis with UPLC and 2MRMs (transitions) per compound and n x 2MRM, where n = 50 – 200, the number of target compounds; time programmed methods are used with time windows to select a number of MRMs for compounds expected (retention time related) as for single quadrupoles in SRM mode; Linearity: TSQ 5 – 6 orders of magnitude. Only the most recent/sensitive LC/TSQ can achieve required detection limits (DLs) in drinking water by direct sample injection. Targeted methods are also employed to detect and quantify a known list of compounds in an environmental matrix, such as, surface water.

High Resolution Instruments (sector, QTOF, Orbitrap)

Comparison of resolution capability demonstrates an increase from LRMS to HRMS to illustrate the elemental composition for one compound only at high resolution.

Double Focusing HR Mass Spectrometers for ultra-trace analysis: GC/HRMS operated in SIM mode are almost universally employed for ultra-trace analysis to selectively distinguish pollutant masses from the background. The advent of high sensitivity electron multipliers allows low femtogram detection of ions separated by HRMS and to distinguish between isotopically-labelled analogues and the native compounds. There are a number of criteria for positive ID: (1) peak must be Gaussian;Intensity > 3:1 (S/N); (2) elution correct time window; (3) for CDDs, the 2,3,7,8 congeners must elute within ± 2 s of labelled analogues (LIS); (4) isotope ratios must be within 15% of theoretical values. QC requirements are rigorous to avoid false positives and negatives.

Summary of the QTOF as the preferred LC/MS instrument for Environmental Analysis: Fast screening is possible with UPLC resolution and speed because of the fast acquisition rate of TOFs providing compound ID based on accurate mass, isotope patterns and on MS/MS data and RT with a selective XIC set for a narrow mass window. Other attributes relate to the investigative capability of TOF. The fast acquisition rate makes TOF analysers ideal for fast GC/MS applications. Deconvolution algorithms can be used to effect ID of overlapping peaks resulting from the decrease in GC separation.

The Orbitrap is a comparative alternative to the QTOF for environmental analysis and food screening (see for example, *Figure 2*. for Exactive™ plus Orbitrap mass spectrometer, courtesy of Thermo Scientific). Ion mobility MS *(see Figure 2, SYNAPT G2.S)* also has a future in specialised environmental analysis.

Applications Illustrated

1. Tandem Quadrupole: Sensitivity progression for TSQ-MS Instrumentation is illustrated below in *Figure 3* (courtesy of Agilent Technologies).

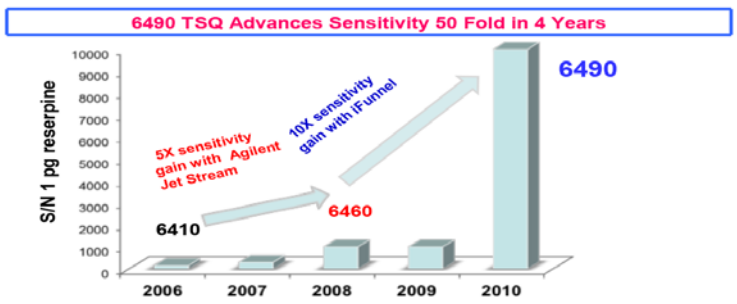

Figure 3. Increase in the sensitivity of tandem mass spectrometers (TSQ) over a 4 year period.

2. Direct Sampling Analysis: Field Free APCI design application to Direct Sample Analysis of pesticides in orange juice shows an illustration of speed of analysis compared to SPE with a reduction of 25 min to 25 sec – *Figure 4* (courtesy of Perkin Elmer).

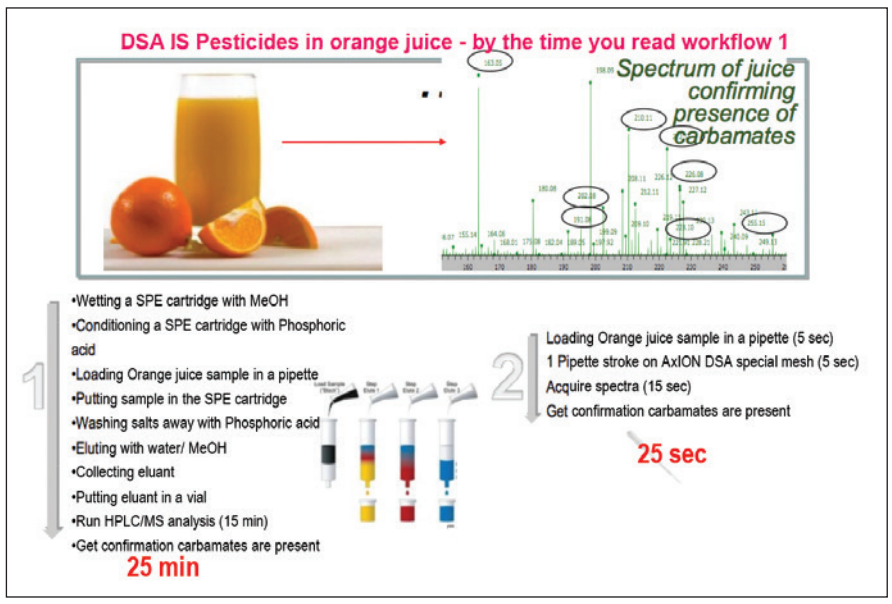

Figure 4. The rapid increase in analysis of carbamates using direct sample analysis and specialised field free APCI.

3. Synapt-Ion Mobility QTOF-MS: The application highlights an illustration in *Figure 5* of mobility separated co-eluting isobaric masses for two components of ciprofloxacin (courtesy of Waters, see *Figure 2* for schematic diagram).

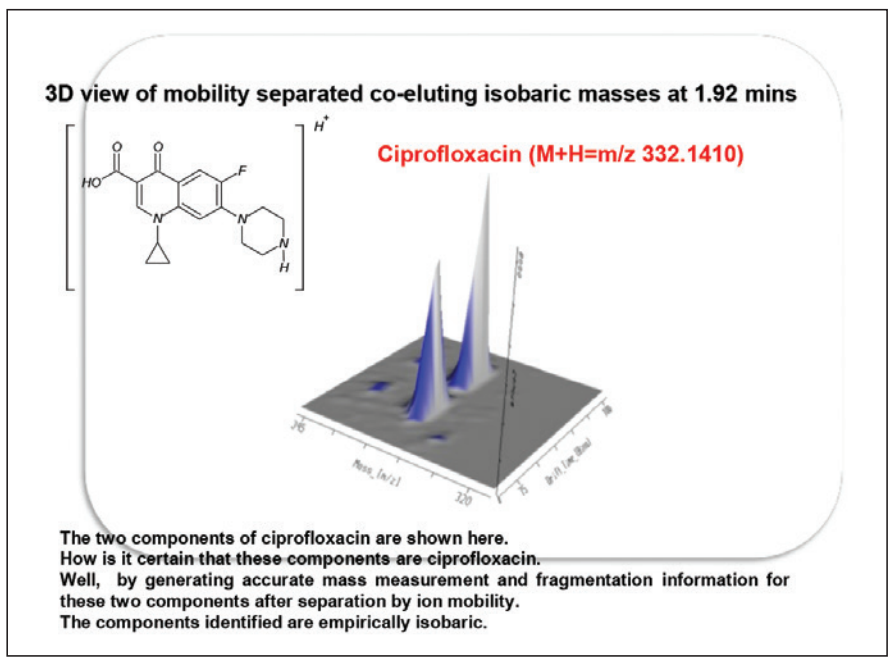

Figure 5. Ion mobility QTOF mass spectrometric application to the isobaric separation of co-eluting components of cyprofloxacin.

4. Variable EI for GC & GC x GC TOFMS: Analysis of emerging contaminants is demonstrated in *Figure 6* (courtesy of Markes International).

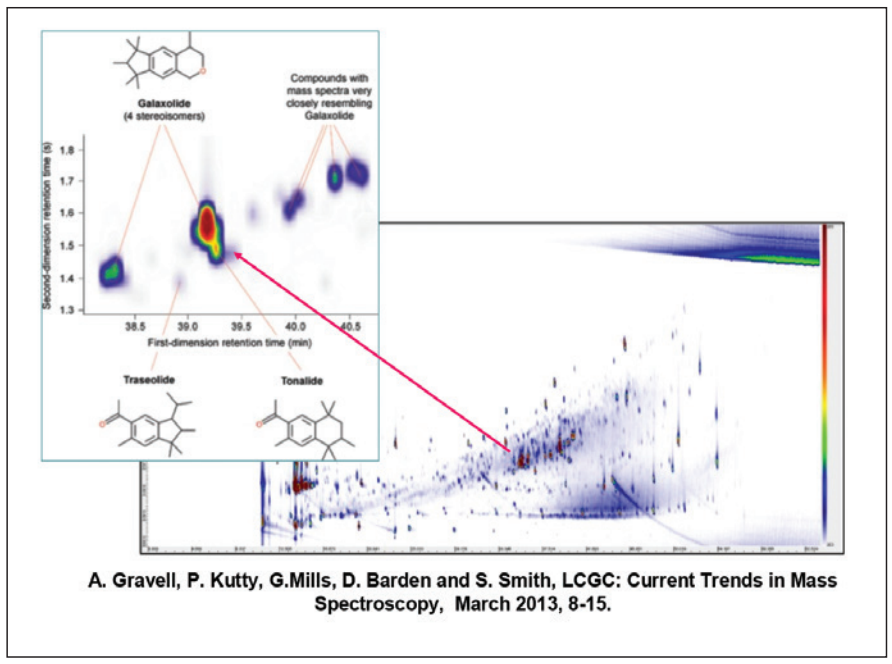

Figure 6. GC and GCxGC TOFMS analysis of emerging contaminants (with variable EI capability).

5. Pesticide Screener: Complete LC-QTOF solution is based on high resolution, accurate mass data for multi-target screening in food and feed samples in *Figure 7* (courtesy of Bruker Daltonics).

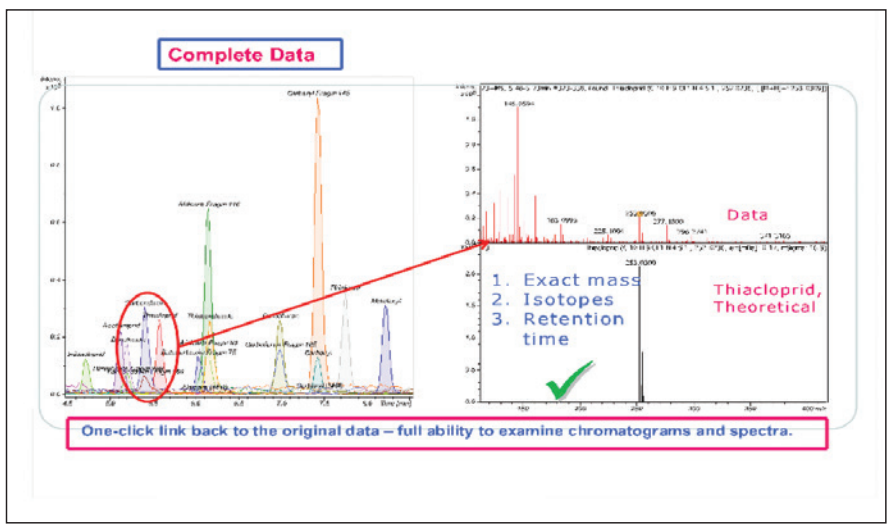

Figure 7. Complete data analysis in pesticide screening for the characterisation of a thiochloprid target (link back to the original data).

Data System Software Capability

The acquisition of data has reached enormous proportions and one file can have a capacity as great as 1 GB, for example, for a QTOF in which all ions are detected over the time period of the analysis. Data system software provides the ability to link interpreted analytical data with its chemical and geographical context and store in a database: featuring automated full scan GC-MS screening; automated LC-MS screening both with de-convolution and formula searching against a library; toxicity predictions for identified chemical structures; automated archiving of raw data files retrievable via a database search; chromatography method development (courtesy of ACDLabs).

Acknowledgements

1. The British Mass Spectrometry Society.
2. ILM Publications for adapted text & information from articles in reference 1.
3. The applications from mass spectrometer instrument companies as indicated in the text.

References

1. Comprehensive Environmental Mass Spectrometry, Advances Topics in Environmental Science, Albert T. Lebedev, ed. (2012), ILM Publications (St Albans, England; Glendale, AZ, USA),
2. SPME Oct 1, 2012, John Hinshaw, LCGC (EUROPE), Volume 25, Issue 10, pp. 570-575.