

Porous Graphitic Carbon for Solid Phase Extraction of Polar Micro Pollutants from Environmental Waters

C. Blythe, L. Pereira, R. Lewis, H. Ritchie

Thermo Electron Corporation, Runcorn, UK

Abstract

An SPE/LC/ESI/MS method has been developed for the analysis of polar degradation products of atrazine (ammelime, ammelide, cyanuric acid, atrazin-desethyl-desisopropyl, atrazin-desethyl and atrazin-desisopropyl), to allow their determination in environmental waters at the 0.1 µg/L level.

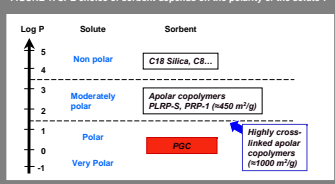
Introduction

Environmental protection is of increasing concern in many countries, with more legislation being introduced for maximum levels permitted for micro-pollutants in water, soil, etc. Very low detection limits are required for monitoring organic compounds in drinking water or studying the fate and transport of organics in the environment. The analysis of organic pollutants in environmental waters in trace amounts at the µg/L level and below requires sample preparation and pre-concentration because they are too complex and too dilute.

With regard to pesticides, the EU Directive 98/83/EC¹ for drinking water (which came into force in January 2004) established that the concentration of each pesticide and their relevant metabolites, degradation and reaction products, should be lower than 0.1 µg/L, and the total pesticide content should be below 0.5 µg/L.

The required sample pre-treatment consists of extracting traces of organic pollutants from the aqueous media, concentrating them and then removing other components of the matrix which may interfere with the chromatographic analysis by LC or GC. Solid phase extraction (SPE) is today widely accepted for trace enrichment of non volatile organic compounds in aqueous samples. In SPE the analytes are extracted provided these are retained by the sorbent and not eluted too rapidly by the water in the sample. Trace analysis of very polar pollutants is still a challenge, since traditional C18-silica and PS-DVB are not retentive enough to allow percolation of a high enough volume of sample before breakthrough occurs². Porous graphitic carbon (Hypercarb™) retains highly polar and water-soluble compounds and, therefore, this material can be used for the trace-level determination of polar and very polar water pollutants. Many pollutants when degraded produce very polar metabolites and these are often very well retained on Hypercarb, allowing the handling of large volumes of water prior to breakthrough. Hypercarb is commercially available in SPE format and also packed into LC columns (retention mechanism for polar molecules on Hypercarb explained below).

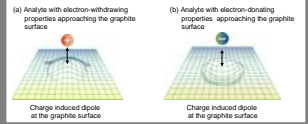
FIGURE 1. SPE choice of sorbent depends on the polarity of the solute³.



The water-octanol partition coefficient (P_{ow}) of a compound is a parameter which characterizes the hydrophobicity and reflects the polarity of the compound. Generally, compounds with $\log P_{ow}$ above 3 are apolar, between 1 and 3 moderately polar and below 1 polar. When $\log P$ is lower than zero, the compounds are more soluble in water than in the organic solution. $\log P$ allows to make predictions on the retention in reversed-phase LC and SPE.

Porous graphitic carbon retains very polar molecules

FIGURE 2. Schematic representation of a point charge approaching the graphite surface.



Hypercarb can retain very polar molecules which are not retained in RP-LC on other chromatographic supports. When a polar molecule, with a permanent dipole, approaches the surface of porous graphitic carbon, which is polarizable, it induces a dipole on this surface. This dipole-dipole interaction (represented in Figure 2) results in excellent retention for polar compounds with several hydroxyl, carboxyl and amino groups, which cannot be retained on silica-based alkyl phases such as C18.

Atrazine degradation products

The potential for water contamination by herbicides such as atrazine is high due to its high solubility. The very polar degradation products of atrazine are not often reported since these are not easily extracted due to their polarity or because they co-elute with early eluting interferences in the chromatographic method. The work presented on this poster investigates an extraction procedure and LC/ESI/MS method for the analysis of polar degradation products of atrazine, as specified on Table 1, to allow their determination in environmental waters at the 0.1 µg/L level.

TABLE 1. Structures and Log P values for the analytes studied.

Pollutant	Abbreviation	Structure	Log P
Ammelime	ANE		-1.3
Ammelide	ADE		-0.7
Cyanuric acid	Cya		-0.2
Atrazin-desethyl-desisopropyl	DEIA		0
Atrazin-desethyl	DEA		1.3
Atrazin-desisopropyl	DIA		1.6

Materials & Methods

• LC method:

Column: Hypercarb 5 µm, 100 x 2.1 mm (Thermo Electron Corporation, Bellefonte, PA)
 Mobile Phase: A = H₂O + 0.1% formic acid; B = ACN + 0.1% formic acid
 Gradient: 10 – 100% B in 10 mins
 Flow Rate: 200 µL/min Temperature: 68° C Injection Volume: 10 µL
 Detection: +ve/-ve ESI, SIM MS (M+H)⁺ for ANE, ADE, DEIA, DEA, DIA, [M-H]⁻ for cyanuric acid
 System: Finnigan™ Surveyor™ and Finnigan LCQ™ Deca (Thermo Electron Corporation, San Jose, CA)

• SPE method

Cartridge: HyperSep™ Hypercarb, 500 mg / 6 mL (Thermo Electron Corporation, Bellefonte, PA)
 Column Conditioning: 10 mL MeOH, 10 mL H₂O, vacuum - 3 mm Hg
 Sample Application: 500 mL, vacuum - 10 mm Hg
 Elution: 6 mL (MeOH/THF, 1:1) + 0.1% TFA (stand for a 1 min, vacuum - 3 mm Hg)
 6 mL (MeOH/THF, 1:1) + 0.1% TFA (vacuum - 3 mm Hg)

Sample dried under nitrogen
 Sample re-dissolved in 1 mL of H₂O.



Results

The LC/MS method for the separation of the six analytes described in Table 1 was developed on a Hypercarb column. The ability of this stationary phase to retain very polar molecules allows the use of a generic gradient of water and acetonitrile with 0.1% formic acid. The elution order observed correlates well with the $\log P$ values, i.e., the more polar analyte (ANE) is the first to elute, the least polar (DIA) is the more retained. A typical trace for the separation on LC/ESI/MS is shown on Figure 3. The analytes containing amino groups (ANE, ADE, DEIA, DEA, DIA) were detected in positive ESI and cyanuric acid was detected in negative ESI, using polarity switching. The method shows good linearity for concentrations in the range of 11 µg/L to over 300 µg/L (Figure 4 for calibration data).

FIGURE 3. LC/ESI/MS trace for standard solution containing the six pollutants.

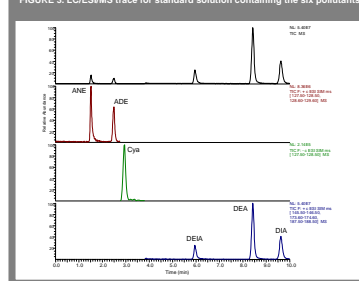


FIGURE 4. Calibration plots for each degradation product: +ESI for ANE, ADE, DEIA, DEA and DIA, -ESI for Cya.

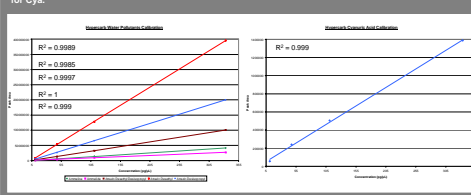


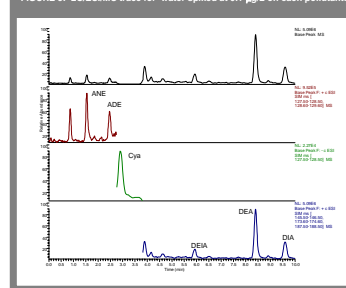
TABLE 2. SPE recoveries for 0.1 µg/L solution.

Pollutant	Abbreviation	% Recovery
Ammelime	ANE	83.4
Ammelide	ADE	87.1
Cyanuric acid	Cya	>100
Atrazin-desethyl-desisopropyl	DEIA	82.0
Atrazin-desethyl	DEA	80.4
Atrazin-desisopropyl	DIA	75.6

Recoveries were measured using a 500 mg / 6 mL cartridge and by pre-concentrating 500 mL of water spiked with 0.1 µg/L each of ANE, ADE, Cya, DEIA, DEA and DIA. Several organic solvents and electronic modifiers were investigated to perform the elution, and MeOH / THF (1:1) + 0.1% TFA was found to give the best recoveries for all analytes. Average recoveries (shown on Table 2) range between 75 and over 100% and were determined by comparison with standard solutions of equivalent concentration.

The LC/ESI/MS trace for one of the samples spiked at the 0.1 µg/L level is shown in Figure 5. Limits of detection for ANE, Cya, DEA and DIA are several fold lower than 0.1 µg/L, with the current method. In order to improve the limits of detection, further work in the optimization of this methodology is necessary.

FIGURE 5. LC/ESI/MS trace for water spiked at 0.1 µg/L on each pollutant.



Conclusions

- Porous graphitic carbon can be used as a stationary phase for the LC separation of very polar transformation products of polar pollutants; its ability to retain very polar molecules allows the use of weakly buffered mobile phases ideal for sensitive MS detection.
- Porous graphitic carbon in SPE offers the capability of extracting very polar pollutants from water matrices, allowing the loading of high volumes of water when target analyte pre-concentration is required to detect trace pollutants.
- The methodology presented here extends the number of polar pollutants that can be analyzed at trace levels in water to meet legislation requirements for drinking water.

References

- (1) Council Directive 98/83/EC, November 1998, Official Journal of the European Communities
- (2) Pichon V.; Chen L.; Guano S.; Henrion M.-C.; J. Chromatogr. A, 711 (1995) 257-267
- (3) V. Pichon (ESPCI, Paris), SPE Workshop ISC 2004, Paris

Additional Information

For additional information, please browse our website: www.thermo.com/columns.

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