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Determination of toxaphene in soil by electron-capture negative ion mass spectrometry after fractionation by high-performance gel permeation chromatography

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ABSTRACT

Toxaphene is extracted from soil by standard procedures using Soxhlet or sonication methods. The extract is fractionated by high-performance gel permeation chromatography (HPGPC), which separates toxaphene from the bulk of co-extractives including polychlorinated biphenyls. This HPGPC fractionation has broad application to many problems of environmental analysis. A solid-phase extraction cleanup with silica gel further removes any polar components present in the collected fraction. Determination of toxaphene is accomplished by electron-capture negative ion mass spectrometry (ECNI-MS) after introduction by capillary gas chromatography. Levels down to 100 μg/kg in soil are obtainable. Brief mention is made of high-resolution ECNI-MS carried out at a resolution of 10 000.

INTRODUCTION

Toxaphene is a multi-component mixture of chlorinated terpenes, primarily based on the C_{10} structure of bornane [1–5]. Hundreds of individual compounds make up the commercial pesticide. Although toxaphene is now limited in use, interest in its analysis continues because of its persistence and widespread transport in the environment [4,6–10].

Current US Environmental Protection Agency (EPA) methodology (SW-846) [11] consists of electron ionization mass spectrometry (MS) and gas chromatography (GC)–electron-capture detection methods (8270 and 8080) [11], both of which are greatly affected by interferences such as polychlorinated biphenyls (PCBs), chlordane and other pesticides. Swackhamer *et al.* [4] published an electron-capture negative ion (ECNI) method that was selective and lowered detection limits for toxaphene in fish. Their work was based, in part, on previous studies indicating the advantages of negative ion approaches [2,3,12]. Currently, no negative ion methods are recommended in SW-846, despite recent examinations that suggest the technique is useful [13–15] when due caution is exercised [16].

In our work, the negative ion approach is extended to soil matrices with the use of high performance gel permeation chromatography (HPGPC) [17,18] and solid-phase extraction cartridges [19,20]. Results obtained with high-resolution ECNI-MS [21,22] at a resolution of 10 000 are briefly discussed

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in the context of resolving potentially interfering ions that contain oxygen [4] from those of the same nominal mass that come from toxaphene.

EXPERIMENTAL

Chemicals

Toxaphene, chlordane, other pesticides, and PCB reference standards were obtained from the EPA Repository (Research Triangle Park, NC, USA). The following solutions from the Repository are defined: Pesticides II: aldrin, α-benzene hexachloride (BHC), β-BHC, γ-BHC, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, heptachlor and heptachlor epoxide; Acid Extractables II: benzoic acid, *p*-chloro-*m*-cresol, 2-chlorophenol, *o*-cresol, *p*-cresol, 2,4-dichlorophenol, 2,4-dimethylphenol, 4,6-dinitro-*o*-cresol, 3,4-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, 2,4,5-trichlorophenol and 2,4,6-trichlorophenol; Basic Extractables: 4-chloroaniline, 2-nitroaniline, 3-nitroaniline and 4-nitroaniline. Additional pesticide standards were purchased from Supelco (Bellefonte, PA, USA). Chem Service (West Chester, PA, USA) was the source of 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (OCB). [$^{13}C_1$]Chlordane (CHL) was obtained from Cambridge Isotope Laboratories (Woburn, MA, USA). Methylene chloride, methanol, diethyl ether and hexane were obtained from Burdick & Jackson (Muskegon, MI, USA).

Extraction/cleanup

Soils consisted of Nevada soil obtained locally; soil from Eagle Harbor, Puget Sound, Washington; organic potting soil purchased locally; and clay of unknown origin. The soils were weighed out and then spiked with the appropriate amount of toxaphene standard in hexane at a concentration of 50 ng/μl. The spiked soil was throroughly mixed after spiking.

Extraction was accomplished according to standard methods (3540 and 3550) [11]. Micro-Soxhlet extractions of 2-g samples were performed with a Wheaton micro-Soxhlet apparatus using methylene chloride. Sonication extraction of 20–30-g soil samples used a methylene chloride–acetone (1:1, v/v) solvent with a Heat Systems-Ultrasonic sonicator, Model XL2020 (Farmingdale, NY, USA).

Extracts were concentrated to about 0.5 ml for injection into a GPC system consisting of a guard column (50 × 7.8 mm) and two standard columns (250 × 22.5 mm) in tandem packed with Phenogel 10-μm particles of 100 Å pore size. The system was equipped with a Valco (Houston, TX, USA) injector, Isco (Lincoln, NE, USA) UA-5 detector, Isco 260D syringe pump, and Isco Foxy 200 fraction collector. The flow was 7 ml/min [17] and fractions 29–31 (14:00–15:30 min:s) were collected for toxaphene (12-mm diameter tubes, 30 s per tube) with a retention time of about 15 min using methylene chloride as solvent.

Solid-phase extraction cleanup used Supelco 3-g silica cartridges that were pre-rinsed with 6 ml of hexane, 6 ml of hexane–diethyl ether (50:50, v/v), and 6 ml of hexane. The sample was applied in 0.5 ml of hexane followed by elution with 0.4 ml of hexane, and 4 ml of diethyl ether–hexane (5:95). The eluent was concentrated to a 1.0–0.1-ml volume as appropriate and spiked with internal standards at 0.5 mg/kg level for unknowns or at a ratio of approximately 80 pg OCB and 1.2 ng CHL to 50 ng toxaphene standard. The CHL internal standard was primarily used for retention time reference.

GC–MS

A J&W DB-5 column (Folsom, CA, USA) 30 m × 0.25 mm I.D. (0.25-μm film thickness) was used with a flow-rate of 38 cm/s at 60°C. The initial temperature was 60°C for 3 min followed by a program rate of 20°C/min to 300°C. A Finnigan-MAT (San Jose, CA, USA) 4021 was operated in the negative ion mode at a source temperature setting of 170°C and 0.50 mA filament emission current with an electron energy of 70 eV; methane was used as the moderator gas for electron capture (0.40 Torr source pressure reading; 1 Torr = 133.322 Pa).

The following ions were monitored: *m/z* 309, 311, 326, 341, 342, 343, 345, 377, 379, 381, 410, 411, 413, 415, 430, 444, 447 and 449; dwell time was 0.05 s/ion with a total cycle time of 1.025 s. Only the areas of the ions at *m/z* 341, 343, 345, 377, 379, 381, 411, 413, 415, 447 and 449 with appropriate scan ranges were summed (with an area threshold) to determine response factors and to quantify toxaphene in extracts.

The standard glass GC injector insert of the Finnigan 9610 GC system was modified by a glass-

blower (Supelco) to have a restriction that passed a 26-gauge syringe needle for injection but not a 0.53 mm I.D. capillary inserted from the oven side as a retention gap. A standard 10-μl Hamilton syringe (Reno, NV, USA) fitted with 13-cm needle was then used for on-column injections with the modified insert acting as a needle guide.

High-resolution ECNI-MS

A DB-5 column 30 m × 0.25-mm I.D. (0.25-μm film thickness) was used with on-column injections using a retention gap on a Hewlett-Packard 5890A gas chromatograph. The temperature program was that of Swackhamer *et al.* [4]: 80°C for 1 min; 10°C/min to 200°C; 1.5°C/min to 230°C; 10°C/min to 250°C. The mass spectrometer was a Fisons/VG 70-250SE operated in the negative ion mode with filament current of 0.200 μA at an electron energy of 50 eV, source temperature of 110°C, −8 kV accelerating voltage, and methane as moderator gas at a source housing pressure of about $1 \cdot 10^{-4}$ mbar. Fomblin (Ultramark 1600; PCR, Gainesville, FL) was used as calibrant. Resolutions were determined using peak widths displayed by the selected ion recording software.

RESULTS AND DISCUSSION

Quantitation procedure

The ions monitored for toxaphene consist of the $(M-Cl^{\bullet})^-$ ions resulting from $C_{10}H_{12}Cl_6$ through $C_{10}H_8Cl_{10}$ elemental compositions (bornanes) and overlapping contributions from $(M-Cl^{\bullet})^-$ ions resulting from $C_{10}H_{10}Cl_6$ through $C_{10}H_6Cl_{10}$ (bornenes). We have found that ion pairs at *m/z* 309 and 311 and *m/z* 447 and 449 contribute little to the overall toxaphene response and were eliminated from consideration. In addition, the *m/z* 309–311 range was also subject to matrix contributions. The areas of the 11 ions given in the experimental section were integrated by automated procedures and summed to obtain a response for toxaphene. The response of the internal standard was obtained from the area of *m/z* 430 of OCB. An average response factor based on at least five runs of toxaphene standards spiked with OCB was used in quantitating toxaphene in soil extracts. As a control measure, the response factor was checked daily to confirm that it fell within ± 15% of the average response factor.

Previous work [4] included correction factors due to the presence of coextractives in quantitating toxaphene. In our work, no corrections were necessary for the presence of chlordane. Nevertheless, its presence (along with heptachlor and nonachlor) was monitored by the responses at *m/z* 342, 410 and 444 in order to assess potential contributions to ions indicative of toxaphene.

Responses of PCBs, which are also potential interferences, were monitored at *m/z* 326 (pentachlorobiphenyls) and 430 (octachlorobiphenyls). PCBs constitute an interference due to the oxygen reaction producing $(M - Cl + O)^-$ ions that are of the same nominal mass as ions monitored for toxaphene. We suspect that it is common for operators not to be able to completely eliminate the oxygen reaction in certain instruments. Our HPGPC cleanup, however, eliminates PCBs prior to determination of toxaphene by ECNI-MS and no correction for their presence is needed.

Results of the determination of toxaphene in soil

Table I provides results of analyses of unspiked (blank) soils (samples 4, 6, 11, 13, 15, 17 and 19) and spiked soils (samples 1–3, 5, 7–10, 12, 14, 16, 18 and 20) for toxaphene ranging from spiking levels of 100 μg/kg (samples 3 and 16) to 10.0 mg/kg (sample 10). Samples spiked with chlordane and other pesticides, PCBs, anilines and phenols were analyzed as well as soils already contaminated with polynuclear aromatics (PNAs) (samples 11 and 12). Fig. 1A and B gives example chromatograms of *m/z* 377 from a standard and from a cleaned-up extract (sample 18) at a spiked level of 0.5 μg/kg, respectively. Fig. 1C shows an example of a toxaphene standard taken through the cleanup. Chromatographic patterns and relative ion abundances remain largely consistent between standards and extracts. There are, however, some changes in the pattern caused by the cleanup.

For spiked samples, one could assess comparisons of ion chromatographic patterns using a standard taken through the cleanup. For real samples, however, weathering [23] could alter relative contributions of specific components of toxaphene that could vary on a case-by-case basis. This possibility and the results discussed later led us to rely solely on quantitations obtained using the toxaphene standard directly. We found, for example, that for a

TABLE I

DETERMINATION OF TOXAPHENE IN SOILS BY ECNI-MS

Soil origin	Sample No.	Spike level toxaphene (mg/kg)	Spike level chlordane (mg/kg)	Spike level PCBs (mg/kg)[a]	Spike level phenols/anilines (mg/kg)[b]	Toxaphene level (mg/kg), ECNI-MS
Nevada	1	1.0	–	–	–	0.823
						0.862
Nevada	2	0.5	–	–	–	0.394
						0.396
Nevada	3	0.1	–	–	–	0.111
						0.137
						0.103
Nevada	4	Blank	–	–	–	0.046
Nevada	5	0.5	1.0	–	–	0.467
Nevada	6	Blank	–	1.0	–	0.017[c]
Nevada	7	0.5	–	1.0	–	0.423
Nevada	8	0.5	–	1.0	–	0.360
Nevada	9	0.5	–	–	1.0	0.474
						0.549
						0.421
Nevada	10	10.0	–	–	–	1.83[d]
Eagle Harbor	11	Blank	–	–	–	0.032[c]
Eagle Harbor	12	0.125	–	–	–	0.083
Clay	13	Blank	–	–	–	0.069
Clay	14	0.5	–	–	–	0.703
Organic	15	Blank	–	–	–	0.025[c]
Organic	16	0.1	–	–	–	0.082
CH_2Cl_2	17	Blank	–	–	–	0.021[c]
CH_2Cl_2	18	0.5	–	–	–	0.459
Nevada	19	Blank	–	–	–	0.016[c]
Nevada	20	0.5	1.0	1.0	1.0[e]	0.450

[a] PCB 1242, 1248 and 1254 each 1 mg/kg.
[b] 1 ppm of Basic Extractables and Acid Extractables II.
[c] Not confirmable as toxaphene.
[d] Weathered 6 months.
[e] 1 ppm of Pesticides II.

soil spiked at 10 mg/kg (sample 10) and weathered for 6 months, only slight changes in ion chromatographic patterns were evident compared with standards.

The detection limit for toxaphene is actually lower than 100 μg/kg as evidenced, for example, by results for sample 16 (80 μg/kg). Quantitative results for blanks reflect reagent and matrix contributions integrated by the automated procedure and do not indicate the presence of toxaphene-like compounds. A reagent blank (sample 17) taken through the cleanup gave a result of 0.021 mg/kg (not confirmable as toxaphene), so that laboratory contamination involving toxaphene is not at issue. The higher-level blanks (samples 4 and 13) were a result of carry-over from calibration of the HPGPC with toxaphene standards. Once proper precautions were taken in rinsing the injector, a background level of about 0.02 mg/kg resulted. This response was not confirmable as toxaphene. Hence, a 0.100 mg/

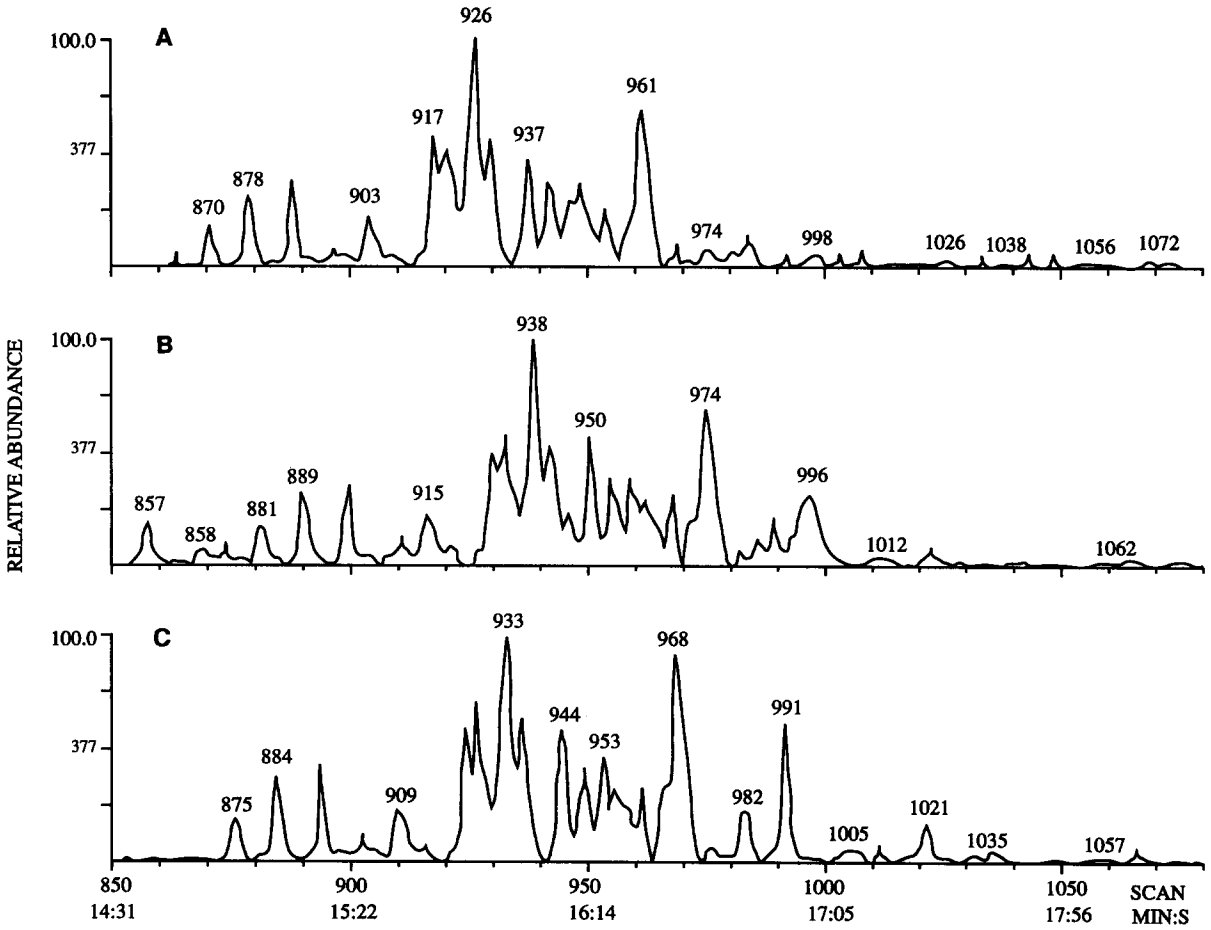

Fig. 1. Ion chromatograms of m/z 377 of (A) a standard of toxaphene; (B) sample 18, 0.5 mg/kg toxaphene taken through the cleanup; and (C) a standard of toxaphene taken through the cleanup.

kg limit seems reasonable in view of apparent unidentified background contributions at this level of cleanup.

The effects of PCBs, chlordane and other pesticides are considered in the spiking studies reported in Table I (samples 5–9 and 20). In general, quantitation of 0.5 mg/kg levels are unaffected by these compounds. This substantiates the improvement of this approach over existing methodology [11]. As an indication of the presence of PCBs, ions monitored at m/z 326 and 430 correspond to molecular anions of pentachlorobiphenyls and octachlorobiphenyls and serve as a check on the efficiency of the HPGPC cleanup. Without the HPGPC cleanup, a 1.0 mg/kg spike of PCBs would result in a "toxaphene-like" response quantitated as 3–6 mg/kg toxaphene in our instrument.

Because PCBs create an interference when oxygen is present in the ion source, the question arises as to the contribution of the internal standard OCB in producing a toxaphene-like response due to the oxygen reaction. No corrections were found necessary at the levels studied and the retention time of this compound.

A blank soil spiked at 1.0 mg/kg PCBs (sample 6) establishes the efficiency of the HPGPC cleanup in removing PCBs (compare 0.017 mg/kg to other blank levels in samples 4, 11, 15 and 19). The 1.0

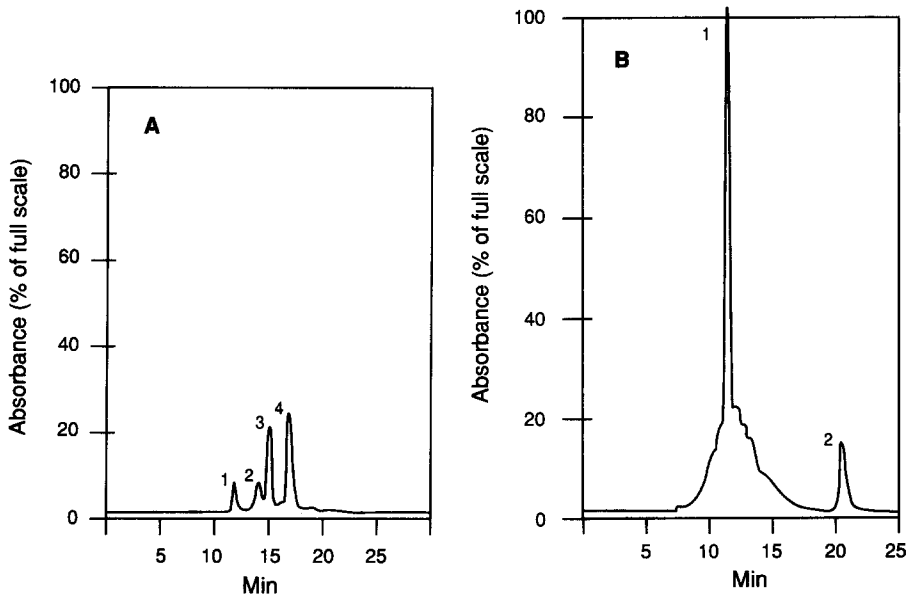

Fig. 2. HPGPC separations. (A) Standards of (1) bis(2-ethylhexyl)phthalate, retention time 12 min 20 s; (2) toxaphene, retention time 14 min 50 s; (3) Arochlor, retention time 15 min 40 s; and (4) perylene, retention time 17 min 30 s. (B) Soil extract with (1) main matrix components centered at retention time 11 min 30 s, and (2) sulfur at retention time 21.0 min.

mg/kg spikes of PCBs and chlordane and other pesticides (*e.g.*, sample 20) do not generate interferences either because they are not collected in the fraction with toxaphene or because they do not afford ions that can interfere. Chlordane is collected in the fraction with toxaphene but does not interfere at levels comparable to those of toxaphene, as evidenced by the results in Table I (samples 5 and 20). By monitoring chlordane response at m/z 342, one can assess whether the chlordane level is of such magnitude as to require any of the corrections suggested by Swackhamer *et al.* [4].

Selective fractionation by HPGPC has broad implications for cleanup of environmental samples. Fig. 2 illustrates the chromatography and separations obtained with HPGPC for standrds (Fig. 2A) and for a typical soil extract (Fig. 2B). HPGPC, unlike most adsorption chromatography, separates toxaphene, a multi-component mixture, as one peak rather than separated into multicomponents over a broad retention range. Since HPGPC is operated in an automatic fractionation mode, other analytes such as PCBs are also collected in individual fractions as a first cleanup step. This approach seems to be an obvious advantage in PCB analysis since the usual subsequent cleanup steps are spent isolating PCBs from interferences. Other target analytes found in the window from bis(2-ethylhexyl)phthalate to beyond perylene but before sulfur can be collected separately or taken as a whole by combining fractions if desired [17,18].

Finally, the solid-phase extraction cleanup step with silica removes polar components (sample 9) that might be present. The main benefit is an extract free from relatively non-volatile components that could cause problems in the injection port or retention gap.

The precision of determination for a given sample extract is about 10% as seen, for example, at the 0.5 mg/kg level with sample 9. Precision of recovery (reproducibility) at the same spiking level is also about 10% (samples 2, 5 and 7–9). The relative response factor for toxaphene *versus* the internal standard (OCB) exhibited a 13% R.S.D. over a 3-month period. Linearity of the relative response of toxaphene to the internal standard was demonstrated for levels of toxaphene from 0.1 to 1.0 mg/kg with OCB levels at 0.5 mg/kg. The recovery of toxaphene from spiked samples is summarized as follows from the data in Table I. At the 0.5 mg/kg level

for spiked soils for which extensive data are available, the average recovery was 85% ±10% (7 determinations). At the 1.0 and 0.1 mg/kg levels the recoveries were similar but are based on fewer determinations.

High-resolution ECNI-MS

Although a variety of soils were analyzed in developing the ECNI-MS methodology, potential exists in environmental analysis for unexpected difficulties or interferences. A high-resolution ECNI-MS determination could present an alternative or tiered approach that would give the analyst a more selective determinative technique without having to resort immediately to further sample cleanup.

In the early stages of this work, high-resolution ECNI-MS was examined to explore its ability to eliminate interferences caused by the reaction of oxygen with PCBs and the potential interference posed by polychorinated diphenyl ethers (PCDPEs) [4]. High-resolution ECNI-MS at 10 000 resolution can eliminate responses from oxygen-containing ions that are isobaric in nominal mass with toxaphene ions regardless of whether they arise from PCDPEs or from oxygen reactions with PCBs (*e.g.* *m/z* 342.8646 for $C_{12}H_4OCl_3{}^{37}Cl_2$ and 342.8962 for $C_{10}H_{11}Cl_5{}^{37}Cl$).

Potential also exists for the presence of *m/z* 342.8776 from $C_{10}H_9Cl_4{}^{37}Cl$ in toxaphene, which would require a resolution of about 26 000 to resolve from the interfering ion. Studies of ratios of toxaphene standards to PCBs of 1:0, 1:10, 1:100 and 1:1000 at resolutions of 1000, 5000, 10 000 and 15 000 were made. Results indicated that a resolution of 10 000 was sufficient to give reliable quantitations in the presence of PCBs.

Despite these apparent advantages, high-resolution ECNI-MS has not been widely adopted, even though reports of its applications [21,22] and calibration procedures [22] have appeared. Some additional effort by instrument manufacturers may be necessary to deal with arcing and beam instability problems.

NOTICE

Although the research described in this article has been funded wholly or in part by the US Environmental Protection Agency through contract No. 68-C0-0049, it has not been subjected to Agency review. Therefore, it does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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