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TECHNICAL NOTES

Gas Chromatography/Electron Capture Negative-Ion Mass Spectrometry at the Zeptomole Level

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Twelve years ago, Hunt and Crow demonstrated that 20–50-amol amounts of strong electophores could be detected with a Finnigan quadrupole gas chromatograph/electron capture negative-ion mass spectrometer GC/ECNI-MS (*1*). These measurements were made after the conditions in the instrument were optimized for high sensitivity: electron energy 100 eV, methane reagent gas pressure 0.6–0.8 Torr, and source temperature 100–150 °C. Unremarkably, it would seem, nearly the same conditions are recommended by the manufacturer (Hewlett-Packard) for optimum sensitivity of the quadrupole ECNI-MS that we possess, based on testing the instrument with the prescribed electrophore, octafluoronaphthalene (OFN). However, it has been established that the response in ECNI is subject to many variables, including compound tested, type of instrument, and several parameters of the ion source (*2–6*). Thus we considered that it was worthwhile to tune our GC/ECNI-MS for the electrophoric analytes of interest in our laboratory. As reported here, such tuning leads to a set of conditions which significantly increase the S/N for these latter types of compounds, but only a little for OFN.

EXPERIMENTAL SECTION

Materials. 2,3-Bis(pentafluorobenzyl)pyrenedicarboxylate (**1**) was prepared by reacting 2,3-dicarboxypyrene (*7*) with pentafluorobenzyl bromide in the presence of potassium carbonate and acetonitrile. *N*1,*N*3-Bis(pentafluorobenzyl)-5-[(pentafluorobenzyloxy)methyl]uracil (**2**) (*8*), 1,3-bis(pentafluorobenzyl)-*N*7-[2-[(pentafluorobenzyl)oxy]ethyl]xanthine (*9*), and *N*-pentafluorobenzylidenyl-2-aminofluorene (**4**) (*10*) were prepared as described. Octafluoronaphthalene (OFN, 10 pg/μl methanol) was purchased from Hewlett-Packard (Palo Alto, CA). Standard solutions were prepared by weighing the compounds, dissolving in acetonitrile or toluene (glass-distilled, Burdick and Jackson, Muskegan, MI), and making dilutions in these solvents with borosilicate disposable micropipets (Drummond Scientific, Broomall, PA). Vials were cleaned and silanized as described (*11*).

Gas Chromatography/Mass Spectrometry. A Model 5988A mass spectrometer from Hewlett Packard (Palo Alto, CA) was used. It was fitted with a CI source volume containing a 1.2 mm i.d. sample inlet hole vs the other option (3.0-mm hole) available from the manufacturer. This choice was made because of our interest in establishing an elevated pressure in the ion source. The instrument was manually tuned each day for maximum sensitivity using perfluorotributylamine following directions from the manufacturer except that the source temperature was 250 °C instead of the recommended 100 °C. The gas chromatograph, a Hewlett-Packard 5890 Series II, was connected to the mass spectrometer with the capillary interface kept at 290 °C. The column terminated in the MS just prior to the ion source plunger. This was achieved as follows (procedure provided by J. Lau at Hewlett-Packard): (1) rotate the EI/CI source selector to midway between the EI and CI positions, (2) insert the column until it contacts the source, (3) tighten the column at this position, and (4) return the source selector to the CI position. The column was a fused-silica capillary, Ultra-1, 12 m, 0.2 mm i.d., 0.11 μm film thickness (Hewlett-Packard). The carrier gas (helium, 99.999%) for gas chromatography and the reagent gas (methane, 99.998%) for ionization by electron capture were from Med-Tech (Medford, MA). Both were adjusted using the pressure and vacuum gauges on the instrument without independent measurements of actual values, and each was filtered through an Oxisorb cartridge (MG Scientific Gases, Branchburg, NJ). Samples were on-column injected with a 10-μl syringe fitted with a fused-silica capillary needle. Blank solvent injections were made before all injections of samples containing a low concentration (<3 fmol/μL) of analyte to rule out carryover. The dwell time was 1 s, and the cycle time was 0.9 s.

For the GC separation, the following listings define, in this sequence, the compound, starting oven T in °C, T program rate in °C min^{-1} (that was started immediately except for OFN as noted), final T, and hold time at the final T in min: **1**, 160, 70, 290, 8; **2**, 110, 70, 290, 6; **3**, 120, 70, 290, 6; **4**, 120, 50, 280, 5; OFN, 35 (hold 1 min), 30, 200, 5.

RESULTS AND DISCUSSION

Aside from octafluoronaphthalene (OFN), the structures of the compounds tested in this work are shown in Figure 1. They are of interest to us as analyte products derived from DNA adducts (*9, 10, 12*). Each of these compounds gives a strong response (see below) by gas chromatography with electron capture negative-ion mass spectrometry with selected-ion monitoring, in part because the ECNI spectrum of each is dominated by a single ion. For compounds **1–3** this ion is $(M - 181)^-$ arising from the loss of a pentafluorobenzyl radical from the molecular ion. The dominant ion for compound **4**, and for OFN, is $M^{\bullet-}$.

We arbitrarily selected compound **1** for optimizing several conditions of the GC/ECNI-MS system for high sensitivity. The results of these experiments are shown in Table I. As seen, higher than conventional settings for each of the conditions studied increased the signal/noise (S/N) for this compound at least 2-fold. Although the data are not shown, progressive increases in response were observed in all cases as each parameter was incrementally raised to the higher settings. In two cases, column head pressure and ion source pressure, compounds **2** and **3**, respectively, were tested as well and gave similar results. Increasing the settings beyond the higher values adopted had the following effects on the response for compound **1**: increased then decreased (head pressure), no change (ion source pressure), decreased (ion source temperature), increased slightly (electron energy), and decreased (emission current).

It is well established that both absolute and relative abundances of ions in ECNI-MS can depend on several parameters reflecting the conditions and configuration of the GC/ECNI-MS (*2–6*). Some ions may be affected by these parameters more than others (*4*). Relying largely on this prior literature, we offer some comments to rationalize the increased S/N values seen in Table I.

The positive effect of an increased head pressure (5 up to 20 psi) applied to the GC column on the S/N for peak height

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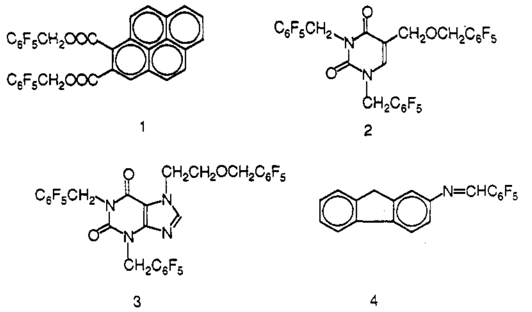

Figure 1. Structures of compounds 1–4.

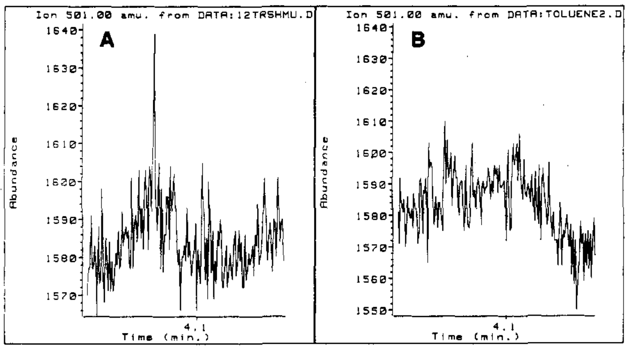

Figure 2. Determination of compound 2 by GC/ECNI-MS with selected-ion monitoring. (A) Injection of 150 ag (200 zmol) in 1 μL of toluene using the higher settings given in Table I. (B) Blank: 1 μL of toluene injected just before chromatogram A. An equivalent blank was observed when this was repeated after A.

Table I. Increase in S/N When the Values of Selected Conditions in the GC/ECNI-MS Are Increased One at a Time (Compound 1 Tested except Where Indicated)

conditions			
type	conventional setting	higher setting	increase in S/N^e
head pressure (top of column), psi	5^a	20^b	6.5 ± 0.50 4.8 ± 0.40 (2)
ion source pressure (methane), Torr	1.0^c	2.0	2.8 ± 0.15 4.5 ± 0.32 (3)
ion source T, °C	150^d	250	2.1 ± 0.19
electron energy, eV (ion source)	150	240	2.1 ± 0.15
emission current, μA	300	450	2.0 ± 0.13

[a] Conventional range is 5–8 psi for a 12 m × 0.2 mm (length × i.d.) column. Linear velocity for the column was 25 cm s^{-1} at 5.0 psi (air injection). [b] Linear velocity was 35 cm s^{-1}. [c] Conventional range is 0.5–1.0 Torr. [d] Conventional range is 120–180 °C for ECNI. [e] Selected-ion monitoring measurements were made. The ions monitored are cited in Table II (footnote a). For each measurement, the other settings were conventional, as defined in the table. Attomol amounts injected into 1 μL of toluene as separate solutions were: 781 (1), 1123 (2), and 135 (3). The S/N was calculated by the computer for peak height. For each pair of settings (conventional and higher), the measurements were made on the same day. The precision shown is the range for triplicate measurements.

Table II. Detection Performance for Electrophoric Compounds with Combined Use of Higher Settings in the GC/ECNI-MS

compd (see Figure 1)	increase in S/N with higher vs conventional settings[a]	example of detn with higher settings[b]	
		amol injected	S/N
1	13.4 ± 1.1	13.8	19 ± 1.6
2	18.1 ± 1.3	0.2	3 ± 0.3
3	10.4 ± 0.9	1.3	10 ± 0.8
4	8.4 ± 0.8	1.3	13 ± 1.2
OFN[c]	1.5 ± 0.3	368	177 ± 20

[a] The values for the conventional conditions were as cited in Table I. The higher values were as follows: column head P, 25 psi; ion source P, 2.5 Torr; ion source T, 250 °C; electron energy, 240 eV; emission current, 300 μA except 450 μA for compound 2. Attomol amounts injected in 1 μL of toluene as separate solutions were 634 (1), 22 (2), 136 (3), 265 (4), and 368 (OFN). Detection was by selected-ion monitoring and the ions monitored (m/z) were 469 (1), 501 (2), 555 (3), 359 (4), and 272 (OFN). Peak heights were measured for S/N. The precision shown is the range for triplicate measurements. The retention times (min) of the compounds for the conventional and higher settings, respectively, were 5.4/4.5 (1), 4.3/3.4 (2), 4.7/3.5 (3), 3.3/2.5 (4), and 2.4/1.9 (OFN). [b] The values are cited as examples of detection of the compounds: while representative, these values can change with time depending on the conditions of the GC column, ion source, and electron multipler. Direct comparisons of these values are limited by the fact that the measurements were made over a 2-month period. The S/N values were calculated by the computer. GC conditions were not optimized for the detection of OFN in terms of S/N for peak height. The precision is as for footnote a. [c] OFN = octafluoronaphthalene.

is of course due, at least in part, to the resulting faster elution of the compound on the column, which increases peak height by narrowing peak width. As will be shown later, the faster elution also appears to increase the recovery of the compounds from the GC into the ion source. The higher column head pressure increases the pressure in the ion source by an unknown amount: although the reading on the pressure gauge that monitors the ion source increases by less than 5%, this gauge is at the end of a tube connected to the source. Nevertheless, the amount of this gas (including any impurities) increases 1.4-fold in the ion source: on the basis of air injection, the linear velocity in the column is observed to increase from 25 to 35 cm s^{-1} when the column head pressure is increased from 5 to 20 psi.

The higher ion source pressure (adjusted from 1.0 up to 2.0 Torr with methane) probably increases the S/N in at least three ways: (1) higher population of thermal electrons; (2) more collisional stabilization of the molecular and/or dominant fragment ion; (3) reduced diffusion of the ion of interest to the wall where losses can take place. Possibly, an increased ion source pressure can also change the residence time of the compound in the source. In regard to mechanism 2, any stabilization that increases the S/N is likely to be against electron loss (reneutralization) vs undesired fragmentation, since the ECNI mass spectrum for each of these compounds essentially consists of a single-ion peak. An exception (somewhat) to this is OFN, which gives a second peak at m/z 238 (34%) in addition to m/z 272 ($M^{\bullet-}$, 100%). Also, compound 1 gives peaks at m/z 196 (12%) and 272 (14%) in addition to its base peak at m/z 469 (100%). (These data were obtained using the higher settings presented in Table II).

Since compound 1 undergoes dissociative electron capture, it is normally expected that its response would increase (at least up to some point) with increasing ion source temperature (*3*). This is what is observed: as seen in Table I, raising this temperature from 150 to 250 °C increases the response of 1 by 2.1-fold. More data and discussion on the effect of temperature on the response are presented later.

Probably, the increases in S/N with a higher setting for the electron energy (240 eV instead of 150), and similarly for the emission current (450 μA instead of 300), are each largely due to the resulting increased population of thermal electrons in the source.

We next tested the response of the compounds with all of the settings raised to higher values. Aside from OFN, this boosted the S/N values by 8.4–18.1-fold, as shown in Table II, starting from the values obtained under conventional

Table III. Response for Electrophoric Compounds with Partial Use of Higher Settings

	settings[b]							
	head	ion source		electron	emission	response (peak area)[c]		
test[a]	P, psi	P, Torr	T, °C	energy, eV	current, μA	1	4	OFN
A	H	H	H	H	H	1.00	1.00	1.00
B	C	H	H	H	H	0.31	0.49	0.38
C	H	H	C	H	H		0.40	0.56
D	H	H	C	C	C	0.23		
E	H	C	H	C	C	0.11		
F	H	C	C	H	C	0.27		
G	H	C	C	C	C		0.31	
H	C	H	C	H	H		0.21	

[a] Each test is defined by the settings shown here. [b] The values for the conventional and higher settings are given in Table I. C = conventional setting, H = higher setting. [c] All of the data in this table were collected on the same day. Amounts injected were 1.5 (**1**), 123 (**4**), and 37 fmol (OFN).

conditions. Thus, in balance, the higher settings act together favorably. Given the data available and the tentative mechanisms cited, it is difficult to anticipate either an additive or multiplicative effect on the *S/N* by combining the higher settings. For example, there may be an upper limit to the electron population achieved with the higher settings. Further, the ionization yield of a compound may already be near its maximum under the conventional conditions used in Table I.

Encouraged by the increased *S/N* values in Table I and striving for high sensitivity in our work, we used the new conditions to test the detection limit and linearity for one of the compounds. As shown in Figure 2, 200 zmol (150 ag) of compound **2** could be detected. The response was linear (r = 0.998), at least up to 22 amol (data not shown).

We wondered why the response of OFN changed so little (1.5-fold increase in *S/N*; Table II) in going to the new conditions compared to the behavior of the other compounds (8.4–18.1-fold increases). Was this tied to OFN possibly being a weaker electrophore than the other compounds? A additional experiment ruled this out: the relative molar responses of the compounds, as peak areas, were 0.77 (**1**), 5.10 (**2**), 1.99, (**3**), 0.66 (**4**), and 1.00 (OFN). (For this experiment the amounts injected ranged from 8 to 113 fmol; higher values, as cited in Table I, were used for all of the settings, and all of the injections were done on a single day.)

Continuing to focus on the unusual behavior of OFN, we considered whether the increased responses for compounds **1**–**4** in Table II were largely due simply to an increase in their recovery from the GC, since the increased head pressure reduces their residence time in the GC. Perhaps OFN, which lacks heteroatoms unlike compounds **1**–**4**, already was highly recovered from the GC under the conventional conditions used in Table I. Note, for example (Table I data), that increasing the column head pressure alone increases the *S/N* (peak height) for compound **1** significantly (6.5-fold). Indeed, this enhancement, coupled multiplicatively with any one of the remaining, increased *S/N* values for this compound in Table I (2.0–2.8-fold), could easily account for the increased *S/N* of this compound (13.4 fold) in Table II. This concern about analyte recovery, and some related questions about the results so far, led us to obtain the data shown in Table III.

The values shown in Table III are peak areas, which improves our ability to define analyte recovery. In test A, we employed the higher (H) settings used to obtain the detection data presented in Table II, and assigned the resulting peak area a value of 1.00 for each of the three compounds studied (**1**, **4**, and OFN). Test B, in which all settings are high except for the use of a conventional head pressure (increasing the residence time for the compounds in the GC), shows a similar reduced response for all three compounds. Thus, not only does a variation in compound recovery appear to contribute to the effects that we observe, as we anticipated, but OFN surprisingly undergoes essentially the same drop in recovery as **1** and **4**. Our hypothesis that OFN is highly recovered from the GC under conventional conditions, in contrast to the behavior of the other compounds, is therefore disproved.

Given the linearity in response that is routinely seen for these compounds in our laboratory (data not shown), the anticipated (but not observed) inertness of OFN, and the similar reduction in response here with a lower head pressure for all three compounds, we speculate that some very active sites are present prior to the ion source (including the outlet edge of the capillary) which resist saturation with the sample and thereby destroy a constant fraction (or all) of the analyte that makes contact. Related to this, others have observed increased exchange of active deuteriums (CD_2 α to a carbonyl) in a GC/MS whenever the residence time of the compound is increased in the column, even when a new bonded-phase fused-silica capillary column is installed (*13*). Thus, there is room for improvement in the inertness of current GC systems.

This still leaves unexplained the observation that OFN, alone, undergoes a small increase in *S/N* when all of the settings are changed from conventional to high values (data in Table II). Test C vs A (Table III) reveals that the answer is not related to ion source temperature either. As seen, the responses of both **4** and OFN fall to about the same degree when this parameter alone is lowered. Scanning the ECNI mass spectrum for OFN down to *m/z* 10 under the new conditions shows no ion for F^-, ruling out that this pathway might compete with the ion intensity at *m/z* 272 that is being monitored.

Consideration of certain other factors did not help either. While OFN is the most volatile of the compounds tested, there is no baseline disturbance from the injection solvent even when a small quantity of OFN is determined. Since two of the compounds (data cited above) give molar responses as peak areas which are greater than that for OFN, the response for OFN is not 100% efficient under any of the conditions used. In other words, there is an opportunity for its response to improve with a change in conditions.

We next focused on the unknown peak (already cited above) at *m/z* 238 for OFN, which is 38% of the intensity of *m/z* 272 (M^-). The former peak, which is larger when there is an air leak in the system (J. Lau, personal communication), corresponds to $M^- + O_2 - F_2 - CO$. While this relative intensity (38%) is not high enough to directly explain our results with OFN, we had made this measurement by injecting 37 fmol of OFN into the instrument. However, 100-fold less was injected into study the boost in response. We next made injections of 0.37 and 37 fmol of OFN and monitored the peak areas for both *m/z* 238 and 272, under both conventional and new conditions. This work was done several months after the data shown in Tables I–III was collected. For the 0.37-fmol injection, the response for *m/z* 272 increased 1.2-fold in going from the conventional to new conditions, in good agreement with the earlier value of 1.5. But the response increased 2.8-fold for the 37-fmol injection. All chromatograms were unremarkable except the series of *m/z* 238 chromatograms that we obtained for 0.37 fmol under the new conditions. Here a later eluting peak was observed at about twice the retention time of OFN. Sometimes the peak was split, and its intensity varied as well, reaching a value which was as much as 3-fold greater than that of the usual, earlier peak at *m/z* 238 for OFN. If OFN was injected not as usual (1-μL sample volume sandwiched in the syringe between two 2-μL volumes of air), but without any air, the later peak decreased about 5-fold and the m/z 272 peak area for OFN increased about 4-fold. Under conventional conditions, injecting without air only increased the response for OFN 1.4-fold at the 0.37-fmol level. Finally, injecting 0.37-fmol of OFN without air and monitoring m/z 272 gave up to an 8-fold increase in response in going from the conventional to the new conditions.

Thus, at least part of the reason for the failure of OFN to behave like the other compounds in our experiments is due to its tendency to react with oxygen. This reaction takes place both in the column (a late-eluting peak can be observed when trace OFN is coinjected with air and m/z 238 is monitored), and the ion source (the usual OFN peak, which is detected by monitoring m/z 272, is also recorded by m/z 238). The reaction on the column is enhanced when the new vs conventional conditions are employed, limiting the degree to which the response of trace OFN increases in going to the new conditions.

Another parameter that may contribute to the aberrant behavior of OFN, suggested by a referee, is that some positive-ion reaction channels available to OFN may be influenced by the operating parameters. For example, the presence of an electron capture agent in an ion source can influence the reaction of a positive ion and a molecule (*14*).

Since both 4 and OFN undergo nondissociative electron capture, one might ordinarily anticipate their responses to decrease with source temperature (*3*), although not always (*15*). As seen in Table III, the responses of both increase with ion source temperature. Interestingly, we previously observed the ECNI response (measured as intensity of the base peak relative to total ion current) of 4 to increase instead with source temperature on a different instrument (*10*). Perhaps one or more general artifacts, like a reaction of these compounds with oxygen or with the surfaces in the source, are playing a role.

Tests D–F (Table III) were done to see whether increasing the selected groups of settings could enhance the response as much as increasing all of them together (test A). While we did not study this exhaustively, we see that at least several sets of conditions, comprising partial use of the higher settings, fail to provide a response as high as that seen when all settings are increased together. One might expect that ion source pressure, electron energy, and emission current might act synergistically. Indeed, when the values of these three parameters are lowered together (test E), the greatest drop (9-fold) in response is seen for compound **1**.

For application to real samples, a drawback of the new conditions is that the use of a higher column head pressure, by shortening the GC retention time, increases the likelihood in general of interferences. (Footnote *a* in Table II provides the retention times of the compounds for both settings of the column head pressure.) Test B in Table III (already discussed in a different context) reveals that using a conventional head pressure (potentially to increase the chromatographic resolution), with the other conditions set to high values, reduces the response 2–3-fold. Thus, there can be a trade-off between sensitivity and resolution.

CONCLUSIONS

We have developed a new set of conditions involving five parameters in a GC/ECNI-MS that significantly boost the response for four electrophoric compounds related to DNA adducts. One parameter is an increased column head pressure and the other four are higher settings for the ion source. To the extent investigated, incomplete combinations of the new conditions did not provide the full boost in response.

The area response of every compound falls with a lower head pressure on the GC column. Since this corresponds to a longer residence time for the compound in the GC/MS system prior to the ion source, it appears that the front end of the system contains some powerful active sites. This is in spite of the use of on-column injection onto a bonded-phase, fused-silica capillary column. Advances therefore are still needed in the inertness of GC systems.

It was previously known that octafluoronaphthalene (OFN) can apparently react with oxygen in an ECNI ion source. Here we learn that what may be essentially the same reaction (but with OFN as a neutral) can take place as well in the GC column at the trace level.

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