REAL-TIME DETECTION OF ANILINE IN HEXANE BY FLOW INJECTION ION MOBILITY SPECTROMETRY

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ABSTRACT

Ion mobility spectrometry (IMS) with a conventional ^{63}Ni ion source exhibits chemical behavior that should be advantageous in detection of molecules with high proton affinity such as aromatic amines in common organic solvents. Since IMS instrumentation can be considered a continuous-sampling point sensor, IMS may be adapted for industrial process monitoring or area environmental monitoring. However, quantitative aspects of IMS are not well established and possible interferences may limit the usefulness of IMS. In order to characterize IMS behavior as an effluent sensor, a flow injection IMS device was evaluated in which an IMS was used as a detector for a heated injector port. An IMS drift tube was used with an acetone doped reaction region and a membrane inlet. Five microliter replicate samples were introduced and vaporized in the inlet at 15 - 90 second intervals and drawn into the IMS. Detection limits were ca. 0.5 mg L^{-1} for 5 ul aliquots (2 ng per sample). Sampling intervals could be reduced to 15 seconds for all concentrations below 40 mg L^{-1} above which however a working range could be considered to approximately 100 mg L^{-1}. Precision was 10 - 25% RSD and was largely concentration independent. Since the IMS alone in a vapor stream shows ca. 1 - 2% RSD, the bulk of variance was from the inlet and inlet-IMS interface. Four solvents (benzene, methylene chloride, ethyl acetate, and acetone) were evaluated as interferences. All solvents at some concentrations affected the peak area for aniline, although the causes arose through different mechanisms. The use of IMS as a flow sensor for aniline in organic solvents should presently be restricted to samples free of compounds with strong proton affinities and solvents which do not exhibit strong dipoles.

INTRODUCTION

Ion mobility spectrometry (IMS) with a conventional ^{63}Ni ion source exhibits chemical behavior that should be advantageous in detection of molecules with high proton affinity such as aromatic amines in common organic solvents. Since IMS instrumentation can be considered a continuous-sampling point sensor, IMS may be adapted for industrial process monitoring or area environmental monitoring. However, quantitative aspects of IMS are not well established and possible interferences may limit the usefulness of IMS. Among the attributes of an acceptable "field screening method for hazardous waste and toxic chemicals" are sensitivity, specificity, accuracy, precision, speed, and portability. Also, to be worthwhile, it should be applicable to the screening of analytes or classes of compounds which have a reasonably high toxicity. The optimum value of a real-time field technique would be in the screening of substances with acute toxicity, thereby assisting in the elimination of short term exposures. The purpose of this work is to investigate

such quantitative aspects of IMS as sensitivity, accuracy, and precision; interference is examined as a comparison of response to solvents of varying proton affinity; and speed of analysis is an additional experimental parameter.

In IMS, vapors are drawn into a reaction region where analyte is ionized through proton or electron transfers from a reservoir of charge, the reactant ions. The reactant ions originate in beta emission from a ^{63}Ni radioactive foil and the reactant ions exhibit near thermal energies. Consequently, product ions usually experience little fragmentation and exist principally as M^+, MH^+, or M_2H^+. Ionization in the reaction region is based on competitive charge exchange, and unequivocal response occurs when the target analyte has a proton affinity larger than that for any component in the sample matrix. When this is not assured, response can become confusing even for simple mixture (1). Thus, the primary basis for selectivity of IMS as a detector is based upon differences in proton affinities of constituents following vaporization into a flowing air stream. Product ions are injected into a drift region where ions acquire a constant velocity in a weak electric field. Differences in ion velocities are due to differences in cross-sectional areas, and this serves as a useful, second level of selectivity in IMS. However, response in IMS is fundamentally governed by the original step of product ion creation; thus, if a product ion is not formed in the ion source, regardless of cause, a peak corresponding to that substance will not be observed in the mobility spectrum.

Flow injection analysis (FIA) is a type of continuous analytical technique where discrete, reproducible aliquots of sample are introduced into a flux, allowed to interact with other components of that flux or with forces exerted on that flux, and are subsequently monitored by a detector having some inherent specificity for the resultant species. Reviews of flow injection analysis by Betteridge (2) and by Ranger (3) date the origins of this technique to the early to mid-1970's as an adaptation or subcategory of "continuous flow analysis" as described by Skeggs (4). This type of analysis has the advantages of being simple, accurate, reliable, reproducible, and can be accomplished with a small amount of simple equipment. All of these attributes are desirable in any real-time, field screening method. The disadvantages of FIA methods come from a dependence on detector selectivity in the absence of any separator techniques, as will be seen later.

Chemically, the high proton affinities of aniline and other aromatic amines suggest that ion mobility spectrometry may be a technically acceptable technique for monitoring of these substances by flow injection technique. Development of a field screening method for these compounds would be worthwhile based on toxicity, the primary toxic effects of this class of compounds on man including methemoglobin formation and cancer of the urniary tract (5). Environmentally, "aromatic amines constitute a family of serious pollutants due in part to a high degree of toxicity toward aquatic life (6). Particular attention has been given to the effects of aniline, aniline derivatives, and aromatic amines on fish (7,8), Daphnia magna (9,10) and microbes in estuarine water (11)." (Eiceman et al) Commercially, they are important as intermediates in the manufacture of dyestuffs and pigments, but are also used in the chemical, textile, rubber, dying, paper industries and other (5).

EXPERIMENTAL

Instrumentation

The introduction of a flow injection stream to an IMS detector was accomplished using the instrumentation and procedures described below. A block diagram of the flow injection IMS apparatus is shown in Figure 1 and was comprised of a heated injector taken from a gas chromatograph, an Airborne Vapor Monitor (Grasby Analytical, Ltd., Watford, UK) as the IMS detector, a pressurized source of air and supporting electronics to control injector temperature. Air flow through the injector port was ca. 5 ml/min and the injector temperature was 100°C. Both the injector block assembly and the IMS instrument were placed inside a laboratory hood, and there was a distance of less than 1 cm between the injector exhaust and the IMS inlet. Digital signal averaging was used to acquire mobility spectra with an Advanced Signal

Processor (ASP) (Grasby Analytical, Ltd.) into an IBM XT microcomputer. Also, signal was routed from an output voltage on the ASP to a Hewlett-Packard 3380A recording integrator so peak areas for the aniline product ion could be recorded versus time and integrated. The window of observation for drift times for the aniline peak was ca. 0.1 - 0.2 ms wide and was centered on the drift time for aniline, 8.74 ms. Other parameters for signal collection through the ASP board were: number of waveforms, 32; points per spectrum, 512; and scale expansion, 0.25. The integrator parameters were: attenuation and threshold, each 9; chart speed, 1 cm/min; area rejection, 10000; and peak width 0.5.

Reagents and materials

The following solvents were obtained in high commercial purity and used without further treatment: aniline (Aldrich Chemical Co., Milwaukee, WI, 99.5%+), hexane (Chromopure, Burdick & Jackson Co., Muskegon, MI), acetone (Chromopure, Burdick & Jackson Co.), benzene (B&J Brand, Chromopure, Burdick & Jackson Co.), ethyl acetate (Fisher Scientific, Pittsburgh, PA), and methylene chloride (Fisher Scientific).

Procedures

In general, 5 ul aliquots of liquid sample were delivered with a 10 ul syringe (Hamilton Co., Reno, NV) to the heated injection port during continuous signal processing with the IMS. An interval of 15 to 90 seconds was permitted for the air to sweep vapors from the inlet before another injection was made. Several parameters were examined to determine optimum operating conditions and access the reliability of IMS as a flow injection detector. The particular details of each of these studies were:

Clearance study and response curve - Five microliters of aniline in hexane at concentrations from 0 to 100 ppm (volume/volume liquid) were delivered in five replicates at different intervals from 15 to 90 seconds. Peak areas were determined for the aniline product ion in the preparation of a quantitative response curve. The effect of injection interval also permitted the determination of memory effects in the IMS under a range of concentrations.

Chemical interferences - In the study of chemical interferences in aniline determinations, 5 ul of 5 ppm aniline in hexane were co-injected with 0 to 4 ul of pure interfering solvent. These interfering solvents were methylene chloride, benzene, acetone, and ethyl acetate. Five replicate determinations were made at 60 second intervals.

RESULTS AND DISCUSSION

General

The reactant ion peak (RIP) with acetone reagent ion chemistry and the mobility spectrum for aniline in the hand-held IMS are shown in Figure 2. The mobility spectrum for aniline contained a single symmetrical peak at 8.74 ms drift time, consistent with previous findings for aniline with water-based chemistry in the ion source (12). Residual amounts of reactant ion at 6.97 ms in aniline mobility spectrum demonstrated that the ion source was not saturated and that comparable behavior may be anticipated at vapor levels lower than this. This mobility spectrum was generated using 5 ul of a 5 ppm solution (25 ng absolute mass) and the peak height relative to the RIP was reasonable considering the high proton affinities of aniline. Previously, aniline was shown with IMS/MS to yield a protonated molecule, MH^+ product ion (12) although the ambient temperature drift tube and alternate ion chemistry used here may favor the existence of a MH^+_xS ion where S is an acetone solvent molecule, but this has not been unequivocally established.

Clearance Behavior, Standard Deviation, and Response Curve

The hand-held IMS used in this work would be suited for field use due to its size (40cm x 15cm x 8cm), weight(2.6 kg), and ability to operate continuously in hostile environments unattended. The IMS itself is battery powered and could be interfaced with a battery powered lap top computer for data acquisition, providing a portable system. However, this IMS could be expected to exhibit memory effects from the ambient temperature drift cell and membrane-equipped inlet. At high concentrations of aniline, slow clearance from repetitive determinations might occur. In Table 1, peak areas and percent relative standard deviations (%RSD) from repetitive determinations are given for solutions between 0 and 100 ppm at injection intervals from 15 to 90 seconds. The %RSD ranged from 13 to 125, but showed a median of 21% Previous

experience with this IMS as a detector in FIA methods had yielded reproducibility of peak heights of 8 to 10 %RSD and this large variance was suspected to be due to the placement of the FI-IMS in the fume hood. Turbulence in a fume hood has been associated with position and movement of the user as well as amount and location of equipment in the hood (13). This turbulence likely affected yields in the interface between the inlet and IMS and this large RSD was suggestive that mechanical improvements in interface between the IMS and injection port are needed. A straightforward leak-tight connection was not employed in these studies due to the flow characteristics for this IMS and the eminent rupture of the membrane inlet if pressure differences developed between the inlet and ion source regions.
The anticipated memory effect from slow clearance of the aniline from the IMS was evident in the peak areas given in Table 1. In general, peak areas with 90 second injection intervals were the lowest for a given concentration level. Injection intervals less than 90 seconds caused an accumulation of aniline in the IMS and peak areas increased for example as much as 100% at 30 second intervals with the 100 ppm concentration. This was manifested in the signal for continuous monitoring as a rising baseline and in the mobility spectrum as a persistent product ion. Memory effects here were dependent upon concentrations, as expected, and at concentration below 20 ppm, injection intervals of 15 seconds could be employed with reasonable differences in absolute areas.

A plot of peak area versus concentration of aniline in hexane for 5 ul injections at 90 second intervals is shown in Figure 3 and resembled previous response or calibration curves in IMS (14). Such curves are comprised of narrow linear ranges (in this instance between 5 to 20 ppm), a shallow but mostly linear response at concentrations above the main linear region and a nearly linear plot with shallow slope below the linear region. This behavior is due to the nature of the kinetics of reactant ion formation from the beta emitting ion source and, thus, to the limited reservoir of charge available to analyte vapors.

Chemical Interferences

The existence of solvents with a range of proton affinities in industrial waste streams constitutes a potential compromise on the integrity of IMS response in flow injection determinations through two mechanisms. Conceivably, large levels of such solvents might compete for charge resulting in reduced peak areas for aniline at given vapor levels. Alternately, solvents may cause, at ambient cell temperatures, ion-solvent clusters which lead to shifts in drift times for product ions. This will cause a decline in certainty regarding peak identity or may cause the peak to fall outside a window of observation in the signal processing software.

Four solvents with low and medium proton affinities were selected for interference studies and mobility spectra for individual solvents are shown in Figure 4. Methylene chloride gave little response in positive polarity IMS as expected due to a low proton affinity. For the same reason, benzene showed a weak response with an acetone reactant ion chemistry and the product ion had a drift time shorter than that for the RIP. Acetone formed cluster ion, with drift times longer than that for the RIP, through ion-molecule interactions in the IMS drift region as described by Preston and Rajadhax (15). Only ethyl acetate (EtOAc) showed significant competition with the reactant ion, due to large proton affinities of EtOAc relative to acetone, with the obvious result of a product ion. Of these solvents, only benzene has been mass identified as M^+ (16) though acetates are known to form MH^+ and M_2H^+ product ions (17).

The influences of these solvents on IMS response to a 5 ul injection of 5 ppm aniline in hexane are shown in Figure 5 as a plot of peak height for aniline in various ratios of four solvents in a binary mixture with hexane. All solvents affected the peak area for aniline although the causes arose through different mechanisms. In Figure 6, mobility spectra are shown from equal mixtures of hexane and solvent for 5 ppm aniline and these can be compared directly to spectra for individual solvents (Figure 4) and for aniline (Figure 2). For EtOAc, the product ion dominated the ion chemistry when aniline

was present even though proton affinities favored aniline. Ethyl acetate at high concentrations relative to aniline appropriated virtually all the charge except that remaining with the RIP. The ion-molecule chemistry for acetone as an interference also followed this pattern and aniline was not detected with high levels of acetone. Thus, the rise in peak areas in Figure 5 represented a false positive by acetone for aniline since acetone product ion intensity intruded upon the drift time window used to monitor aniline. In such a situation, only inspection of the mobility spectrum could avert an error in monitoring on analyses. A product ion for aniline was evident with methylene chloride due to the low proton affinities of methylene chloride. However, the increase in response for aniline in positive polarities from addition of methylene chloride to hexane (Figure 5) was unprecedented in IMS and conclusions cannot be made pending IMS/MS studies. Benzene, with proton affinities between methylene chloride and acetone or EtOAc, exhibited a type of intermediate behavior. A product ion for aniline was observed in the presence of benzene, but the benzene was at a level sufficient to effectively compete for protons from the RIP and a benzene product ion was also observer (Figure 6). These spectra and trends suggest that an IMS will be sensitive to common solvents at low levels even with an alternate reactant ion chemistry, a membrane inlet, and low (<1%) levels of solvents other than hexane. However, if the solvent composition is known and reasonably constant, calibrations presumably could be prepared in that matrix. These findings for simple compositions argue for standard addition techniques with flow injection IMS determinations.

CONCLUSIONS

Ion mobility spectrometry has never been widely regarded as a quantitative instrument, but as a detector for flow injection determination, IMS exhibited suitable response curves, standard deviations, and response times. This was accomplished under the demanding situation of a fast transient vapor level in FIA methods. The linear range is a weak aspect to quantitative IMS and alternative configurations to conventional ^{63}Ni sources should be sought. Reactant ion chemistry based on acetone was not wholly successful in discriminating chemically against common organic solvents. Consequently, until improved source chemistry is found, standard addition should be considered the method of choice for quantitative FIA with IMS for aromatic amines.

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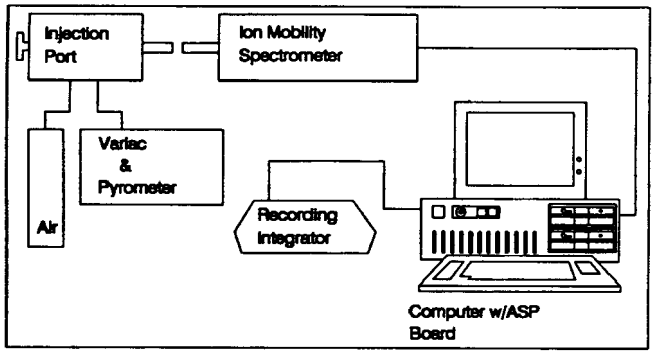
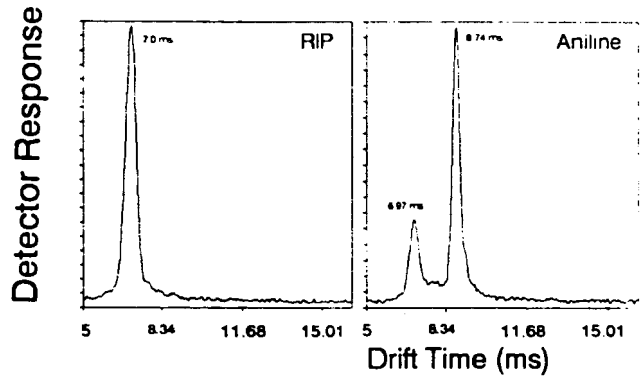
Table 1. Peak Areas from Plots of Aniline Product Ion Intensity versus Time in Flow Injection Ion Mobility Spectrometry

Aniline Concentration (ppm)	PEAK AREA ($X10^6$) (% RSD) Interval for injection (seconds) 15	30	60	90
0	0.83 (13)	1.6 (104)	0.84 (13)	1.1 (34)
0.4	2.8 (23)	5.4 (49)	2.5 (20)	2.0 (29)
1	6.8 (19)	5.2 (23)	4.6 (27)	1.6 (8.0)
5	19.6 (21)	14.9 (16)	16.0 (19)	10.9 (22)
10	32.6 (29)	32.9 (21)	30.4 (20)	26.1 (13)
20	•	35 (21)	41.9 (16)	46.6 (13)
40	•	49 (33)	40.4 (15)	37.6 (21)
100	•	95 (45)	62.7 (31)	42.5 (125)

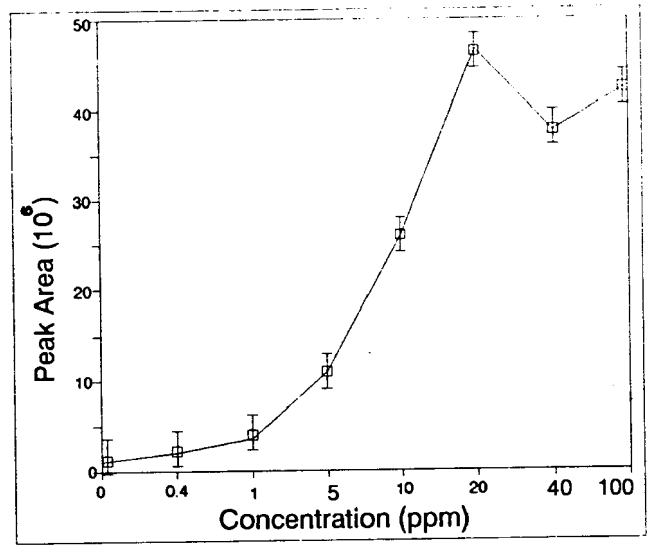
•Baseline drift due to residual aniline was too severe for integration or recognition of a peak in flow injection IMS.



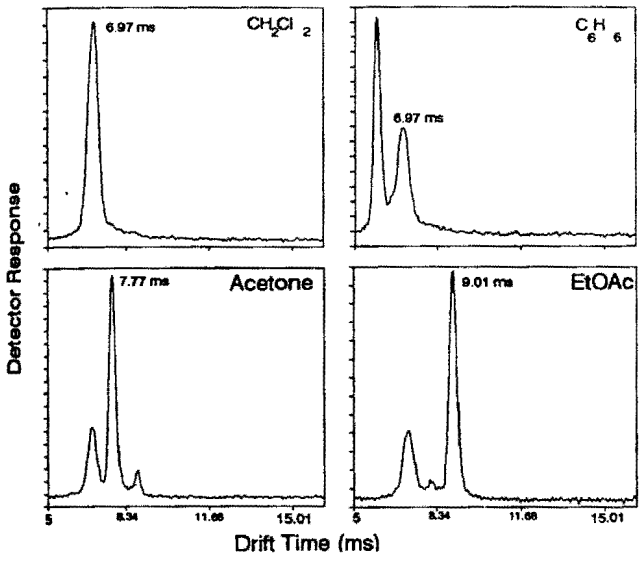
1. Block diagram of apparatus for quantitative flow injection ion mobility spectrometer (IMS) including continuously flowing air stream. Sample is deposited in the heated injection port, vaporized, and swept into the IMS detector. The mobility spectrometer was operated with digital signal averaging causing ca. 1 second intervals between display of successive IMS analyses.



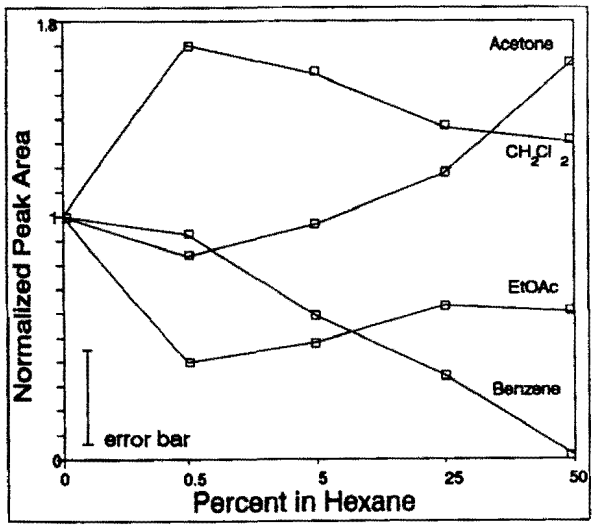
2. Ion mobility spectra for acetone reactant ion peak (RIP) alone and for aniline in hexane using a hand-held IMS. Spectra were obtained in positive polarity, and care was taken to keep the source from a saturated condition.



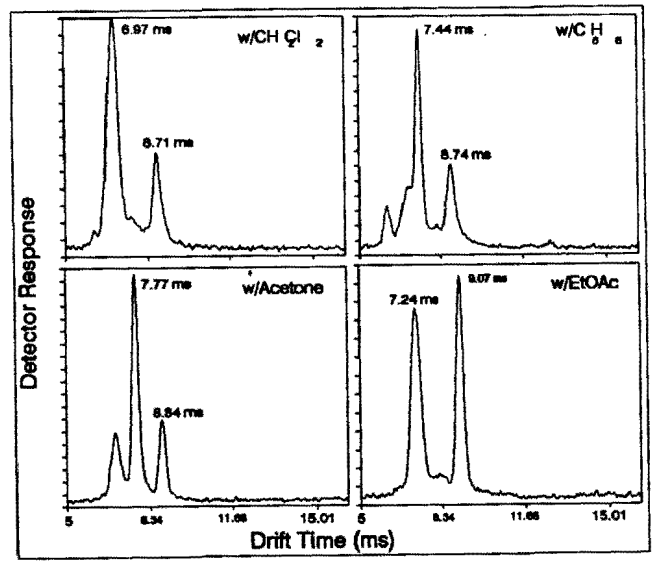
3. Response curve for quantitative determination of aniline in hexane using gaseous phase flow injection IMS. Error bars added at each point were twice (2X) the standard deviation taken as an average for all measurements.



4. Ion mobility spectra for solvents expected to be encountered in analysis of non-aqueous streams for aniline. Mobility spectra were obtained in positive polarity with acetone reagent ion chemistry. Spectra were obtained with solvent vapors permitted to deplete reactant ion intensity ca. 50% from background levels.



5. Effect on peak height for aniline at 5 ppm in binary solvent mixtures of hexane with other common solvents with vol/vol percentage from 0 - 50%. Curves were normalized to the peak height of aniline in hexane solution.



6. Mobility spectra for mixtures of 5 ppm aniline in 50 : 50 mixtures of individual solvents with hexane. Aniline in hexane exhibited a single product ion with drift time of 8.74 ms as shown in Figure 2.

DISCUSSION

STEVEN HARDEN: The question I have is with respect to orthonitrophenol and the sensitivity of the IMS system to that particular kind of material. Did you ever do a calibration run to determine what that sensitivity might be under various conditions?

PETER SNYDER: The answer to that question is no, we have not on pure orthonitrophenol. However, the signals — the amount of signal that we see from the other point of view, looking at it from the organism's point of view, and knowing how much organism we have. It seems like there is still plenty of analyte, given the relatively short time of detection, and knowing that the signal is still a bit spread out. The signal is not in one, or say two, or maybe three at the most, peaks. We see it at about seven, eight, nine 10 peaks, until it finally clears down.

So I'm not trying to skirt the question. It's just that no, we haven't done it to see how sensitive the CAM itself is, or the ion mobile spectrometer 20MP. However, I suspect that it has to be very sensitive, since 200, even 50 cells is a good response, and the response is spread out, so if we can find ways of compacting it, it'd be that much better.

MAHADEVA SINHA: What are the vapor pressures for the orthonitrophenol when it gets combined with the glucose. Do you get any response?

PETER SNYDER: Yes, we've done many, many blanks. We always do a blank before and after.

First of all, the vapor pressure of orthonitrophenol is 5.54 torr at ambient temperature. That doesn't should like much, but relatively speaking, that's a lot for the CAM. And the controls — we have done ONP by itself, with buffer, without buffer, and then just organisms themselves. Organisms do produce some peaks, but that's just right after the reactant ion peak. But it just happens to tail off, and there is no signal in the area that the ONP shows. So we have been pretty lucky in that respect.

The ONP has very negligible vapor pressure by itself. Even if you get a bottle of the dry powder, and just stick the CAM in the bottle, you see no response at all. That should be the most amount, the dry powder, and if anything's going on it would show. But even in the solution, there's no problem.

Orthonitrophenylacetate is a different story. There is hydrolysis going on and over a couple of hours, you can see orthonitrophenol being produced.

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