# Detection and Quantitation of Brominated and Chlorinated Hydrocarbons by DART with Linear Ion Trap and Triple Quadrupole Technology

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## Overview

**Purpose:** Halogenated compounds such as brominated flame retardants (BFRs) and chlorinated pesticides (OCs) have been in use for many years. Both BFRs and OCs are persistent in the environment[1] and pose potential health risks. Therefore, detection and monitoring of these compounds is critical. This experiment is developed to quantitate BFRs and OCs using liquid chromatography-mass spectrometry (LC-MS).

**Methods:** The DART-SVP source (IonSense Corp.) was used to reduce sample preparation and provide ionization. Both ion trap and triple stage quadrupole (TSQ) technology were used for this study.

**Results:** Ionization modes and fragmentation determined on the linear ion trap were confirmed on the TSQ. Further optimization and breakdown curves for the TSQ method were achieved using DART-infusion of the BFRs chosen for further study.

## Introduction

Brominated hydrocarbons also known as BFRs have been used in various industries for decades. Recently, several classes of BFRs have been detected in the biosphere. OCs have also been used for many years primarily as pesticides, the most infamous of these being DDT. While most OCs have been banned in the United States, their use still occurs in developing countries. The continued use of BFRs and OCs, as well as their persistence in the environment and potential deleterious activity therein, makes the detection and monitoring of these compounds an important topic. We propose DART as a simple, rapid, easy-to-use technique; eliminating the need for chromatographic method development, and reducing or eliminating sample preparation, for detection and quantitation of both BFRs and OCs.

## Methods

### Sample Preparation

Compounds listed in Table 1 were dissolved in acetone at 1 mg/mL to make stock solutions. Stock solutions were diluted serially to give the following standards: 100 ppm, 50 ppm, 5 ppm, 1 ppm, 500 ppb, 100 ppb, 50 ppb, 10 ppb. Kepone was spiked in at a constant level of 100 ppb as a reference point. Spiked and un-spiked water samples were analyzed directly with no additional preparation.

### DART Methodology

Preliminary data was acquired on the Thermo Scientific LTQ linear ion trap mass spectrometer using the DART-SVP source in 1D transmission mode, with a grid voltage of 300V and temperature of 200 ºC. Full scan and MS/MS data were acquired for all compounds. To confirm the linear ion trap data, further optimize ionization, and obtain collision energies (CE) breakdown curves, the DART-SVP source was run in direct infusion mode on the Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer. Subsequent quantitation data on the TSQ Quantum Access MAX™ MS was obtained with the DART-SVP source in 1D transmission mode, with a grid voltage of 300V and temperature of 400 ºC.

### Mass Spectrometry

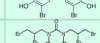
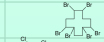
Negative ion full scan and MS/MS mass spectral data was acquired on the LTQ™ linear ion trap MS with the following conditions: capillary temperature 270 ºC, tube lens -100V. Negative mode selective ion monitoring (SIM) and selected reaction monitoring (SRM) were acquired on the TSQ Quantum Access MAX MS with the following conditions: capillary temperature 200 ºC, skimmer offset 0V. SRM data was acquired with a Q1 and Q3 resolution of 0.7 FWHM, collision gas pressure of 1.5, with compound dependent CE and tube lens voltages.

## Results

### Compound optimization

Initial studies were performed on the linear ion trap MS due to the full scan sensitivity and high scan rate which is necessary when optimizing on spots with an average signal duration of 5 to 10 seconds that results when using the DART-SVP in 1D transmission mode. All but three of the selected compounds were detected and precursor masses were determined (see Table 1). Additionally, MS/MS spectra were acquired to determine potential fragments for quantitation (see Figure 2). Confirmation of the precursor masses was achieved on the TSQ MS using the DART-SVP in direct infusion mode.

**TABLE 1. Compounds analyzed with structures, formulas, proposed ionization mechanisms, observed precursors, and monitored SRM transitions. All precursor masses detected by the linear ion trap were confirmed on the triple stage quadrupole with DART-SVP infusion. Compounds marked with an asterisk were not detected initially but were seen with DART-SVP infusion.**

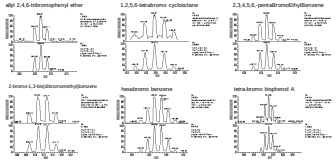
| Compound | Molecular Structure | Formula | Theoretical monoisotopic (most intense isotope) m/z | Observed precursor MS/MS and proposed ionization mechanism | Fragments (monitored SRM transitions) |
|---|---|---|---|---|---|
| allyl 2,4,6-tribromophenyl ether* |  | $C_9H_7Br_3O$ | 367.8 (369.8) | 306.9 $[M-OH-HBr]^-$ $C_9H_5Br_2O_2^-$ | 265.8 |
| 1,2,5,6-tetrabromo cyclooctane* |  | $C_8H_{12}Br_4$ | 423.8 (427.8) | 459.6 $[M+Cl_2]^-$ fragmentation $C_8H_{12}Br_4Cl_2^-$ | Weak |
| 2,3,4,5,6-pentabromoethylbenzene |  | $C_8H_5Br_5$ | 495.6 (499.63) | 436.7 $[M+OH-Br]^-$ $C_8H_5Br_4O^-$ | 81.0, 274.7, 356.6 |
| 2-bromo-1,3-bis(dibromomethyl)benzene |  | $C_9H_6Br_6$ | 495.6 (499.6) | 370.8 $[M+O+OH-2HBr]^-$ $C_9H_6Br_2O_2^-$ | 79.0, 81.0, 326.7 |
| hexabromobenzene |  | $C_6Br_6$ | 545.51 (551.5) | 486.5 $[M+OH-HBr]^-$ $C_6Br_5O^-$ | 378.0, 380.0 |
| tetrabromobisphenol A |  | $C_{15}H_{12}Br_4O_2$ | 539.8 (543.8) | 542.8 $[M-H]^-$ $C_{15}H_{11}Br_4O_2^-$ | 260.8, 417.8, 419.8 |
| tris(2,3-dibromopropyl)isocyanurate |  | $C_{12}H_{15}Br_6N_3O_3$ | 722.6 (728.6) | 727.5 $[M-H]^-$ $C_{12}H_{14}Br_6N_3O_3^-$ | 79.0, 81.0 |
| tetrabromophthalic anhydride* |  | $C_8Br_4O_3$ | 459.7 (463.7) | 398.7 $[M+OH-HBr]^-$ $C_8Br_3O_4^-$ | 326.8, 354.8 |
| 1,2,5,6,9,10-hexabromocyclododecane |  | $C_{12}H_{18}Br_6$ | 635.7 (641.6) | 640.62 $[M-H]^-$ $C_{12}H_{17}Br_6^-$ | 79.0, 81.0 |
| kepone |  | $C_{10}Cl_{10}O$ | 485.7 (489.7) | 506.8 $[M+OH]^-$ $C_{10}Cl_{11}O_2H^-$ | 424.8, 426.8 |

Direct infusion was achieved by connecting an electrospray needle via peek tubing to a syringe pump. The needle was held by forceps in a multi-positional clamp. The needle was then positioned directly between the DART-SVP source and the ceramic capillary interfaced with the mass spectrometer. Compounds were infused at rates ranging from 1 to 5 µL/min and a concentration of 100 ppm. The infusion studies showed that the compounds required higher DART-SVP source temperatures for optimum ionization than were initially utilized. The optimum temperature was determined to be 400 ºC. The results of the infusion studies shown in Figure 1 confirm the linear ion trap MS data. It also shows it was possible to ionize the three compounds that were not initially observed on the linear ion trap MS due to the DART-SVP source temperature being too low.

It is interesting to note that the results shown in Figure 1 demonstrate a pattern in the ionization pathway of the molecules. Compounds containing a hydrogen bonded to a non-aromatic carbon, such as tetrabromobisphenol A, tended to lose a proton to form the $[M-H]^-$ species. Alternatively, compounds containing no hydrogen atoms or hydrogen bonded to an aromatic carbon tended to add $OH^-$ and lose HBr.

In addition to optimizing precursor detection the DART-SVP infusion method was used to determine: tube lens values, fragment ions and CE breakdown curves for the quantitative experiments on the TSQ MS. In the process of acquiring the CE breakdown curves it was noted that the fragments differed from those observed in the linear ion trap, as shown in Figure 2. This is not surprising as the fragmentation in the TSQ MS is more energetic than that in the linear ion trap MS.

**FIGURE 1. TSQ full scan infusion data. Acquired spectra versus theoretical spectra for observed precursors demonstrating proposed ionization mechanisms. Top spectrum in each pair is the acquired data; lower spectrum is theoretically generated spectrum based on proposed formulas.**




Panel B of Figure 2 depicts a spectrum automatically generated on the TSQ MS from the auto-tune procedure in which the CE is automatically stepped from low to high and the most intense fragments are automatically selected as transition ions (Table 1).

### Quantitative experiments

After the infusion experiments, the 10-spot linear rail for 1D transmission experiments was installed. Kepone was selected as a reference compound, due to its highly efficient ionization, and spiked into all samples at a level of 100 ppb. Data was acquired in the free run mode with a constant rail speed of 0.7 mm/sec. This mode was chosen to generate the best approximation of Gaussian shaped peaks (Figure 3) and avoid spiking that can occur when the rail moves discretely to each spot.

The results of calibrators and samples are shown in Figure 3, each peak represents the signal from a single spot. Each chromatogram should contain a total of ten peaks from one pass through the 10-spot rail. 5 µL of sample was applied to each spot in a horizontal line through the center of the spot. This process was repeated twice for a total application of 10 µL. Several of the compounds were detected as low as 50 ppb, specifically tetrabromobisphenol A, 1,2,5,6,9,10-hexabromocyclododecane, and tris(2,3-dibromopropyl)isocyanurate. Unfortunately, the reproducibility at this level was poor. It was determined that each compound responded differently. Thus, it was not possible to normalize responses with kepone, our reference compound. Poor reproducibility was most likely a function of the spotting technique and could easily have been compensated for by the use of labeled internal standards. However, even given the variation in response from spot to spot it was possible to obtain some quantitative information. Peak areas for each chromatogram were exported to Excel.

**FIGURE 2. MS/MS Spectra for tetrabromobisphenol A. Panel A depicts linear ion trap data, Panel B depicts triple quad data. Linear ion trap data was acquired with a normalized collision energy of 35V, triple quadrupole data was generated with stepped collision energy in the auto-tune process.**

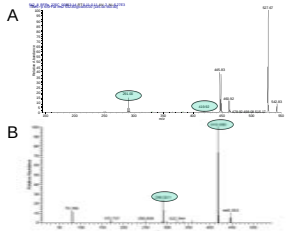
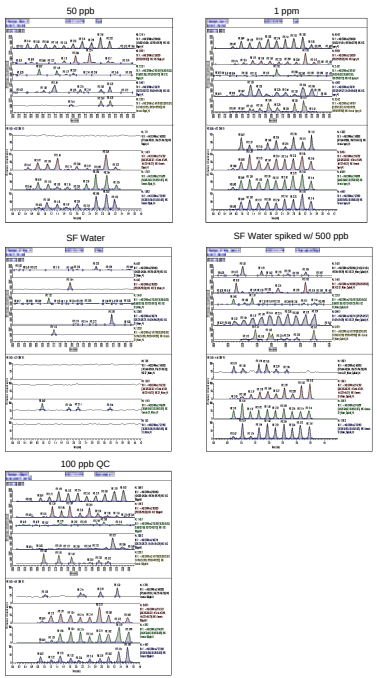



**FIGURE 3. TSQ MS data for calibrators and unknowns. Each panel depicts the compounds in the following order from top to bottom:**

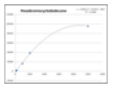
**1) kepone**
**2) allyl 2,4,6-tribromophenyl ether**
**3) 2-bromo-1,3-bis(dibromomethyl)benzene**
**4) tetrabromophthalic anhydride**
**5) 2,3,4,5,6-pentabromoethylbenzene**
**6) hexabromobenzene**
**7) tetrabromobisphenol A**
**8) 1,2,5,6,9,10-hexabromocyclododecane**
**9) tris(2,3-dibromopropyl)isocyanurate**



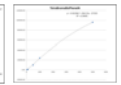

All compound peaks corresponding to each kepone peak were averaged to generate a data point at each level. A minimum of nine peaks were required for the level to be included in a curve. Chromatograms and results for some of the compounds are shown in Figure 4.

**FIGURE 4. Calibration curves and results for; tris(2,3-dibromopropyl)isocyanurate, 1,2,5,6,9,10-hexabromocyclododecane, tetrabromobisphenol A**

| Samples | Area | Calc Amount |
|---|---|---|
| 100ppbQC | 879 | 117 |
| Spiked Water | 2489 | 332 |



| Samples | Area | Calc Amount |
|---|---|---|
| 100ppbQC | 1326 | 92.7 |
| Spiked Water | 11558 | 331.0 |



| Samples | Area | Calc Amount |
|---|---|---|
| 100ppbQC | 16018 | 128 |
| Spiked Water | 49620 | 258 |

A San Francisco (SF) water sample was analyzed by spotting 10µL, as previously described, and drying at 60 ºC for ten minutes. No BFRs or OCs were detected (Figure 3). It is interesting to note that when the 500 ppb standard was spiked into the SF water sample the compound response varied greatly, most noticeably with an enhancement of tetrabromophthalic anhydride and a lower-than-expected response for tetrabromobisphenol A, 1,2,5,6,9,10-hexabromocyclododecane, and tris(2,3-dibromopropyl)isocyanurate (Figure 3). This variation indicates the importance of applying the standards in the same matrix as the sample that is being analyzed. Thus, while sample variation was observed, the method shows promise as a quick, simple method of detecting and quantitating BFRs and OCs, with additional work to address the effect of labeled standards and matrixes.

## Conclusions

- The linear ion trap MS with the DART-SVP in 1D transmission mode provided an excellent method of detecting BFRs and OCs, providing precursor and fragment ion information.
- The Quantum Access MAX MS with the DART-SVP in direct infusion mode generated full scan spectra for BFRs and OCs that 1) generated a high quality match to theoretical spectra confirming the precursor information provided by the linear ion trap and 2) facilitated the automated optimization of tube lens voltages, transition fragments, and collision energies.
- BFR and OC quantitative experiments were performed and LODs were found to be as low as 50 ppb for several compounds.
- Further work to minimize sample response variation and investigate the effect of matrix on sample response will be performed.
- DART-SVP provides a quick simple method of analyzing BFRs and OCs without the need for sample preparation or chromatographic method development.

## References

1. Emerging Brominated Flame Retardants in the Environment, Cynthia A. de Wit, Amelie Kierkegaard, Niklas Ricklund, and Ulla Sellstro¨m, E. Eljarrat and D. Barcelo´ (eds.), Brominated Flame Retardants, Springer-Verlag Berlin Heidelberg 2010, Published online: 9 December 2010

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