Identification strategies for flame retardants employing time-of-flight mass spectrometric detectors along with spectral and spectra-less databases

Supporting Information 1

Identification strategies for flame retardants employing time-of-flight mass spectrometric detectors along with spectral and spectra-less databases

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SUPPORTING INFORMATION

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Reagents and materials

All solvents used were of analytical or pesticide grade. n-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, toluene, dichloromethane (DCM) and iso-octane were purchased from Merck (Darmstadt, Germany). Modified nylon centrifugal filters with 0.2 μm and 0.45 μm pore size were bought from VWR (Leuven, Belgium).

Samples

Dust samples from previous studies, such as house dust from California (n=5) (Dodson et al., 2012) and dust from e-waste storage areas in Thailand (n=6)(Muenhor et al., 2010), along with samples of car interiors (foam and textile materials, n=8) and consumer products (electrical power boards, an LCD television, a plastic children's toy and sample of e-waste) from The Netherlands (n=5) were investigated for other chemicals than those previously reported(Ballesteros-Gómez et al., 2013).

Agilent-specific parameters for molecular feature extraction:

a) *Extraction algorithm*: small molecules (chromatographic)
b) *Retention time* and *m/z restrictions*: the molecular mass of FRs typically varies between 200 and 1400 Da and the retention time varies according to the LC column and parameters used. For non-targeted screening, this filter should not be used. For FR screening, these parameters should be restricted as much as possible to facilitate data analysis.
c) *Isotope model*: the unbiased model proved to work the best for FRs. Among other models, the one for peptides and glycans are unsuited for this purpose and the one for common organic molecules favours the basic organic molecules containing C, H, O, N and S. The same applies when generating formulas.
d) *Quality score*: this parameter is an estimate generated by the algorithm about how likely the extracted compound is to be an actual compound. It takes into consideration factors like signal-to-noise ratio, peak shape, peak width, consistency of retention time, mass difference between ion species, whether it is a single-ion compound and isotope pattern. If the detector is properly calibrated and tuned, this parameter can be set > 80.

Bruker-specific parameters:

a) *Maximum number of overlapping compounds*: to ensure the best results, this value should be set at values as high as possible, especially for complex matrices. For non-targeted screening for FRs from dust samples, this parameter was set at 20. Even for injections of pure analytical standards, this value should be > 3.
b) *Cut-off intensity*: this parameter dictates the relative intensity of mass signals included in the spectrum. To diminish some of the spectral noise, a value of > 0.1 is recommended (the maximum is 10).

c) *Chromatographic resolving power*: this, along with the "maximum number of overlapping compounds" controls if almost co-eluting signals are separated into distinct compounds or combined into one compound. Higher values generate more peaks, if there is any difference in retention time. However, the "dissect peaks" algorithm determines by itself what the most appropriate setting for this parameter is, by factoring in the approximate width of the chromatographic peaks. As the software can select these parameters automatically with good results, we recommend not changing this parameter, unless the output of the "dissect peaks" tool is not the desired one.
d) Proteomics, CHNO: this parameter is similar to the "common organic molecules" isotope model in the Agilent software. It favours the formula generation for typical organic molecules containing mostly C, H, N and O. For (halogenated) FRs, this option needs to be unchecked.

Table SI-1: The ratios of the intensities between the two most abundant isotope peaks from the cluster.

Number of halogens / cluster	**Intensity Ratios**	
	Chlorine	**Bromine**
3	0.96	0.97
4	0.78	0.69
5	0.64	0.97
6	0.8	0.77
7	0.96	0.97
8	0.89	0.82
9	0.78	0.97
10	0.85	0.86
11	0.96	0.97
12	0.94	0.88
13	0.85	0.97
14	0.88	0.9
Min	0.64	0.69
Max	0.96	0.97

Table SI-2: Possible deviations from the theoretical halogen clusters due to the presence of multiple O or S atoms in the analyte molecule.

FR name	FR abbreviation	Formula	Halogen	Number	Intensity ratio	Deviation from theoretical halogen cluster (%)
Dodecachlorododecahydrodimethanodibenzocyclooctane	DDC-CO aka DP	C18H12Cl12	Cl	12	0.93	1.1
5-(Tetrabromophenyl)-1,2,3,4,7,7-hexachloro-2-norbornene	HCTBPH aka Dec604	C13H4Br4Cl6	Cl/Br	6/4	0.92*	0.4*
2,2-Bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate]	BCMP-BBCP aka V6	C13H24Cl6O8P2	Cl	6	0.82	-2.5
Tris(2,4,6-tribromophenoxy)-s-triazine	TBP-TAZ	C21H6Br9N3O3	Br	9	0.98	-1.0
Tetrabromobisphenol S bis(2,3-dibromopropyl ether)	TBBPS-BDBPE	C18H14Br8O4S	Br	8	0.8	2.4
N,N'-Ethylenebis(tetrabromophthalimide)	EBTBPE	C18H4Br8N2O4	Br	8	0.82	0.9
Tetrabromobisphenol S	TBBPS	C12H6Br4O4S	Br	4	0.68	1.4

*To save time in calculating the ratios of all of the possible Cl/Br combinations, we recommend extracting the isotope cluster analysis chromatogram with an intensity of 0.81 and a tolerance of 21%, in order to detect all of the halogenated compounds with more than 3 halogen atoms.

Fig SI-1: Final confirmation of the identification of V6

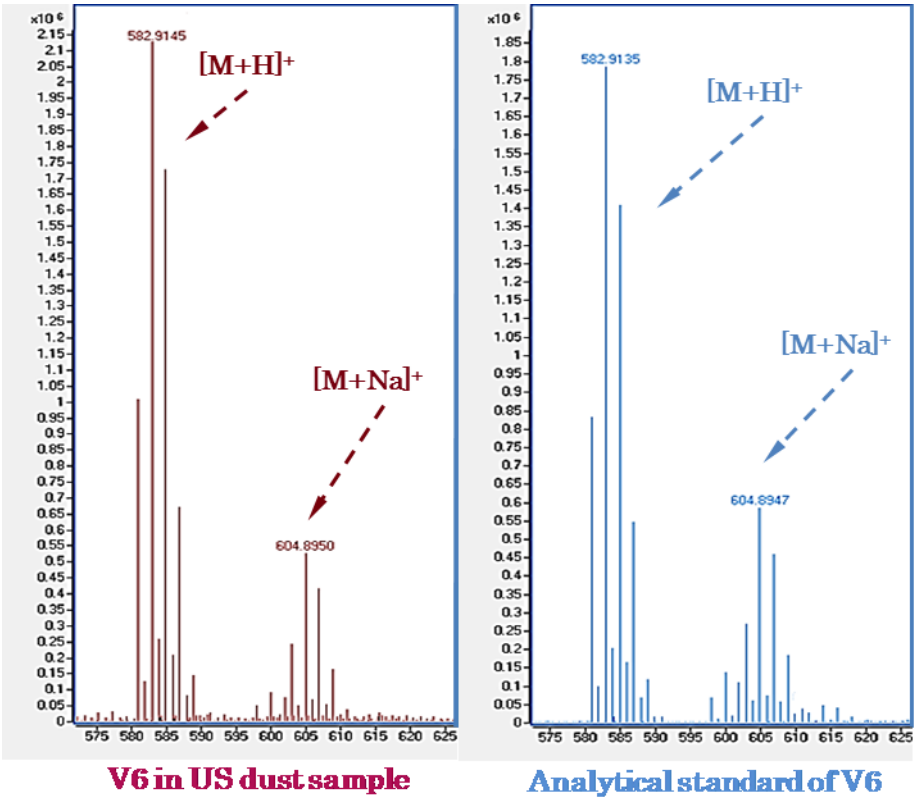

Table SI-3: Formulas generated and compounds tentatively identified in the technical mixture of the FR Antiblaze V6.

Name	Formula	Total Volume*	Retention Time (min)	m/z	Mass	Mass (MFG)	Mass Difference (MFG, ppm)	Mass Difference (DB, ppm)	Score (DB)	Score (MFG)	Overall match score***
BCEP (Bis(2-chloroethyl) hydrogen phosphate) 2	C4H9Cl2O4P	191,680	7.8	222.9693	221.9621	221.9616	-2.7	-	-	93.9	93.9
-	C26H40Cl8O16P3	266,356	9.2	981.9103	980.9016	980.9037	2.2	-	-	91.6	91.6
BCEP (Bis(2-chloroethyl) hydrogen phosphate) 1	C4H9Cl2O4P	276,887	8.3	222.9687	221.9615	221.9616	0.3	-	-	99.4	99.4
-	C27H32Cl7O12P	289,724	9.0	824.952	823.9456	823.9451	-0.6	-	-	89.0	89.0
-	C12H22Cl5O6P	389,734	9.3	468.9675	467.9607	467.9597	-2.2	-	-	94.9	94.9
-	C16H29Cl6O12P3	451,010	7.2	733.9344	715.9000	715.9003	0.4	-	-	96.4	96.4
-	C21H31Cl6O5P3	498,228	9.7	666.9574	665.9502	665.9515	2.0	-	-	92.2	92.2
-	C16H30Cl6O10P2	705,432	8.7	654.9536	653.9455	653.9445	-1.4	-	-	97.3	97.3
-	C11H22Cl4O9P2	835,416	5.0	500.9568	499.9494	499.9493	-0.2	-	-	99.7	99.7
-	C18H32Cl8O9P2	1,050,434	10.2	734.9115	733.9039	733.903	-1.3	-	-	97.4	97.4
-	C10H17Cl4O5P	1,091,022	8.2	388.9646	387.9576	387.9568	-2.0	-	-	95.7	95.7
-	C28H32Cl7O12P	1,308,134	9.2	836.9525	835.9459	835.9451	-0.9	-	-	96.4	96.4
-	C19H34Cl8O10P2	1,370,558	10.3	781.9477	763.9135	763.9135	0.0	-	-	99.6	99.6
-	C33H42Cl9O14P	1,715,929	10.1	1008.9583	1007.9513	1007.9509	-0.4	-	-	98.2	98.2
-	C18H34Cl7O13P3	1,888,587	7.6	796.911	795.9042	795.9032	-1.2	-	-	98.8	98.8
-	C11H21Cl5O8P2	2,291,247	6.3	518.9231	517.9159	517.9154	-0.9	-	-	99.4	99.4
-	C34H44Cl10O16P2	2,651,267	9.8	1120.9073	1119.9002	1119.899	-1.1	-	-	96.0	96.0
-	C21H37Cl8O12P	2,738,839	10.3	792.9623	791.9557	791.9531	-3.3	-	-	84.7	84.7
1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate	C7H12Cl3O4P	6,765,566	6.7	296.9616	295.9543	295.9539	-1.4	-	-	98.7	98.7
TCEP 2	C6H12Cl3O4P	13,779,816	6.5	284.9621	283.9547	283.9539	-2.9	-2.9	97.3	97.3	97.3
TCEP 1	C6H12Cl3O4P	28,377,846	6.7	284.9619	283.9541	283.9539	-0.8	-0.8	61.9	93.1	77.5
Bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate	C9H17Cl4O5P	28,613,420	7.8	376.9659	375.9579	375.9568	-2.9			98.7	98.7
V6	C13H24Cl6O8P2	384,746,848	9.1	580.9171	579.9099	579.9078	-3.6	-3.6	94.3	94.3	94.3

In blue: compounds tentatively identified

*This parameter is directly proportional with the area of the compound peak and is calculated by the Agilent Qualitative Analysis software as being the total volume (m/z × RT × abundance) of the ions associated with the compound

**The Overall match score is a weighted average of scores obtained by different ID techniques that have contributed to the identification of the hit. In this case the database ("DB") score and the molecular formula generation ("MFG") scores contributed to this number. The MFG score, which is the score for the generated formula designated as the best hit, is calculated as the weighted average of three scores, which the analyst can adjust as contribution to the final MFG score. Here, a mass score of 90 (out of 100), an isotope abundance score of 100 and an isotope spacing score of 80 were used.

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