ANALYSIS OF ORGANOPHOSPHORUS FLAME RETARDANTS USING GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Introduction

Flame retardants are human-made chemicals added to consumer and industrial products for the purpose of reducing flammability[1]. Among FRs, there are the halogenated FRs that may be divided into brominated and chlorinated flame retardants (BFRs and CFRs), and phosphorus-containing flame retardants (PFRs)[2]. The market for flame retardant chemicals is being driven by globally tightening fire safety regulations. In this context, some halogenated FRs, such as BFRs, have proven to be persistent, bioaccumulative and toxic to environment, animals and humans. This led governments to adopt restrictions which phase out the production and use of some BFRs[3]. On the other hand, novel brominated flame retardants (NBFRs) and organophosphorus flame retardants (OPFRs) are proposed as alternatives for BFRs and their use shows a continuous increase[4,5].

OPFRs act mainly in the solid phase of burning materials by promoting the formation of an insulating char layer. However, most OPFRs are introduced as additives and not chemically bound to the polymer; hence they are slowly released in the environment by abrasion and volatilization. Some of these chemicals are suspected to be neurotoxic, particularly triphenyl phosphate (TPP), tri-*n*-butyl phosphate (TnBP) and tri-cresyl phosphate (TCP)[6]. Furthermore, halogenated alkyl phosphates have low degradation potential and thus may be persistent[7]. As a result, OPFRs are considered as re-emerging pollutants because of their increased use after BFR bans and their ubiquitous occurrence in both indoor and outdoor environments [3].

The focus of this work was to develop an analytical method enabling the analysis of OPFRs by using gas chromatography coupled to tandem mass spectrometer (GC-MS/MS), as a first step to the development of an analytical strategy dedicated to the identification and quantification of these substances at trace levels in complex biological matrices such as fish, in order to contribute to the evaluation of Human dietary exposure. To this purpose, chromatographic parameters, ion source mode and triple quadrupole parameters were investigated.

Materials and methods

Triethyl (TEP), tri-*n*-propyl (TPrP), tri-*n*-butyl (TnBP), tris(2-butoxyethyl) (TBEP), tri(2-ethylhexyl) (TEHP), triphenyl (TPP), tris(2-chloroethyl) (TCEP), tris(1,3-dichloro-2-propyl) (TDCIPP), tri(chloropropyl) (TCPP) and 2-ethylhexyl diphenyl (EHDPP) phosphates were obtained from Wellington Laboratories (Canada), as well as d_{15}-triethyl (dTEP), d_{21}-tri-*n*-propyl (dTPrP), d_{21}-tri-*n*-butyl (dTnBP), tris(2-butoxy-[$^{13}C_2$]-ethyl) (M6TBEP), $^{13}C_{18}$-triphenyl (MTPP), d_{12}-tris(2-chloroethyl) (dTCEP) and d_{15}-tris(1,3-dichloro-2-propyl) (dTDCPP) phosphates used as internal standards. Toluene picograde was obtained from LGC Promochem®, (Germany). OPFR compounds were analyzed on gas chromatograph 436-GC Bruker series coupled to Scion™ mass spectrometer operating in the positive EI and positive and negative CI modes. The GC was equipped with DB-5MS (30 m x 0.25 mm, 0.25 µm) on which 1 µL was splitless injected with carrier gas flow of 1 ml.min^{-1}. The temperature of the source was 250 °C and that of transfer line was 300 °C.

Several authors reported blank contamination as an important issue concerning OPFRs analysis at trace levels since these compounds are ubiquitous contaminants in indoor environment and may be present in dust[5]. Glassware (tubes, vials and pipettes) was backed at 400 °C for 4 h and covered with aluminum foil whenever possible. Plastic materials were avoided.

Results and discussion

Selection of ionization mode

To make a clear comparison between the three main ionization modes of mass spectrometer (EI, PCI and NCI), 10 ng of each individual OPFR compound was injected into GC-MS/MS using EI, PCI and NCI modes, and analyzed on full scan ranging from m/z 72 to 500. The EI spectra were dominated by ions such as $[H_4PO_4]^+$, $[M\text{-}Cl]^+$, $[M\text{-}CH_2Cl]^+$, $[M\text{-}R]^+$ and $[M]^+$, depending on the structure of each compound. The NCI spectra were

dominated by $[M-R]^-$ and the PCI spectra were mainly dominated by $[M+H]^+$. On one hand, Table 1 gives the sensitivities of the three ionization modes for studied OPFR compounds. Under the same conditions, EI provides the best sensitivity in terms of peak response area. The exception was TDCIPP, which responded as well using ECNI as using EI. However, these results are not reliable unless the comparison is done on matrix and in terms of S/N ratio and specificity of selected ions, which will be done later in the project. The results were consistent with results from Ma *et al.* (2013)[8], who presented a systematic overview of EI, NCI and PCI mass spectra of 13 phosphate esters. On the other hand, Figure 1 shows an example of mass spectra observed for chlorinated phosphorus compound (TDCIPP) and an aryl phosphate compound (EHDP).

Optimisation of chromatographic separation

The initial oven temperature was set as 85 °C for 5 min, ramped to 240 °C with rate 15 °C.min^{-1}, to 255 °C at 3 °C.min^{-1}, then to 300 °C at 20 °C.min^{-1} and held for 5 min. The total run time was 27.58 min. The initial temperature and hold time were chosen to allow for the detection of the more volatile compounds (TEP and TPrP). The temperature program was set after several attempts to obtain a satisfying separation between TPP, TEHP, EHDP and TBEP in an acceptable time. All analytes eluted before 21 min. Figure 2 presents the chromatogram of the optimized separation for all studied compounds, noting that the peak numbers corresponding to each compound, are defined in Table 2.

Optimization of spectrometric conditions (MS/MS)

For the purpose of optimizing the tandem mass spectrometric conditions on the triple quadrupole filter (QqQ), the pure individual standard solutions of OPFR compounds were first analyzed in the full scan mode in order to select the precursor ions. Product ion scan mode was then employed in order to characterize the fragmentation pathways of these precursor ions, at collision energies varying from 5 to 45 V. Finally, a multiple reaction monitoring (MRM) acquisition method was set with defined diagnostic signals (transitions), allowing for the isolation of precursor ions in the first quadrupole, followed by the isolation of specific fragment ions in the third quadrupole. Three transitions were first optimized for each compound among which only two, with highest intensities, were selected. The optimized collision energies are given in Table 2.

Table 1- Analytical sensitivities in terms of peak area (x 10^6 AU) of each compound injected at 10 ng using EI, PCI and NCI modes, as well as the base peak ions (m/z)

	EI (+)		NCI (-)		PCI (+)	
Compound	**Area**	**m/z**	**Area**	**m/z**	**Area**	**m/z**
TBP	134700	99	748	127	5162	267
TCEP	85140	249	3950	221	9116	285
TCPP	116900	125	1947	249	7970	327
TDCIPP	96590	191	176700	319	25180	431
TPP	134900	326	12600	249	97000	327
TBEP	35950	199; 85	897	127	10560	393
EHDP	80450	251	1260	285	17120	251
TEHP	54220	99	682	127	20190	111
dTBP	124400	102.9	835	127	3223	294
dTCEP	77310	261	2530	229	5148	297
dTDCIPP	100300	197	164700	329	23890	446
MTPP	130600	343	4048	261	53420	345
M6TBEP	36500	201; 85	917	127	11330	405

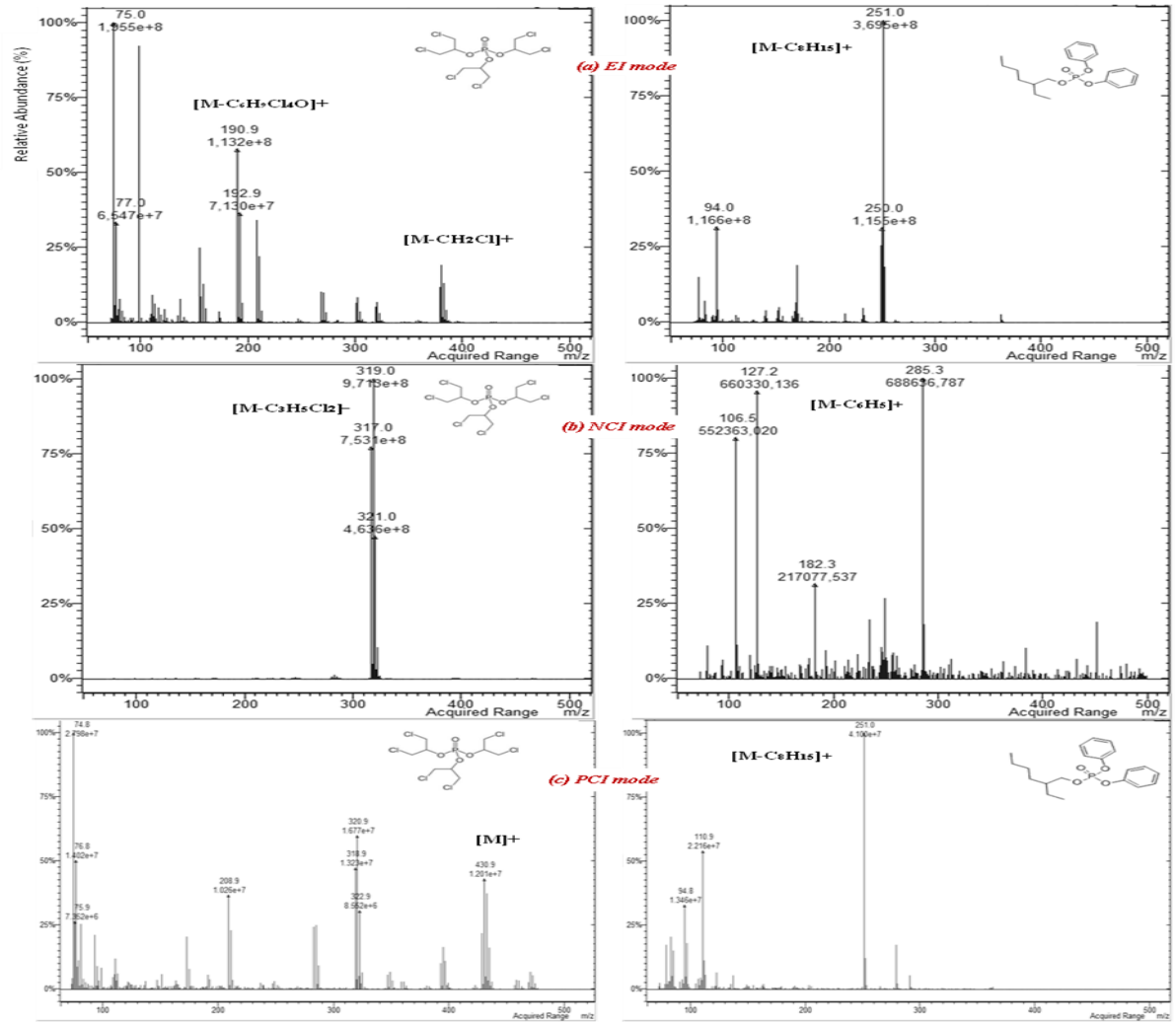

Figure 1- Example of the mass spectra of Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) (left) and 2-Ethylhexyl diphenyl phosphate (EHDP) (right) resulted from the full scan analysis from m/z 72-500 in (a) EI, (b) NCI and (c) PCI modes

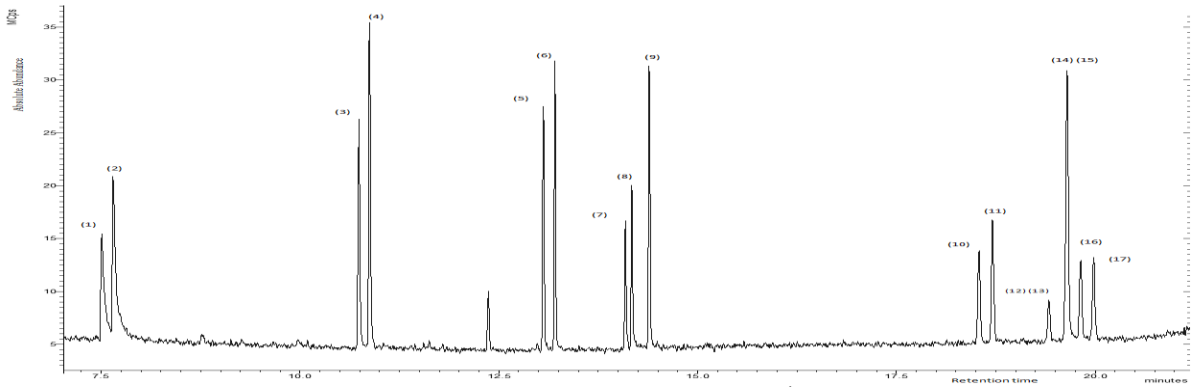

Figure 1- Total ion chromatogram of standard solution mixture at 1 ng.µL^{-1} analyzed by GC-EI-MS/MS (full scan; m/z 72-500)

Table 2- Optimized transitions and collision energies (V) for the studied OPFRs, along with their corresponding isotopic-labeled compounds

	Compound	Retention time (min)	Transition 1	C.E 1 (V)	Transition 2	C.E 2 (V)
1	*dTEP*	7.53	167>103.1	10	167>83.1	25
2	*TEP*	7.65	155>99.1	10	126.9>99.1	10
3	*dTPrP*	10.74	151>103.2	10	199.1>103.2	10
4	*TPrP*	10.87	141>99.1	10	183>99.1	15
5	*dTBP*	13.06	102.9>83.1	15	167.0>103.1	10
6	*TBP*	13.20	154.9>99.1	10	211>99.1	10
7	*dTCEP*	14.09	260.9>131.1	10	260.9>67.2	10
8	*TCEP*	14.17	249>125.1	10	249>99	15
9	*TCPP*	14.39	124.9>99.1	10	200.9>125.1	10
10	*dTDCIPP*	18.54	197>79.2	10	217>103.1	10
11	*TDCIPP*	18.71	190.9>75.1	10	380.9>159.1	10
12	*M6TBEP*	19.42	126.9>99.1	10	201>99.1	10
13	*TBEP*	19.42	124.9>99.1	10	199>99.1	10
14	*MTPP*	19.64	343.1>181.3	15	343.1>152.2	30
15	*TPP*	19.64	326.1>169.1	20	326.1>94.2	15
16	*EHDP*	19.82	251>152.1	20	251>77.1	20
17	*TEHP*	19.98	113>57.3	10	113>95.1	10

Conclusion

An instrumental method for the analysis of organophosporus flame retardants, was developed on GC-MS/MS by optimizing the chromatographic and the spectrometric conditions. As a future perspective, the work will extend to optimize an extraction-purification procedure enabling the extraction of these compounds from complex biological matrices. To evaluate its robustness, the method will be applied on fish samples in order to analyze these contaminants at trace levels.

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