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DEVELOPMENT OF PREPARATION PROCEDURE BASED ON IN-CELL PLE FOLLOWED BY GPC FOR THE ANALYSIS OF OPES BY GC-EI/APCI-MS/MS

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1. Introduction

Organophosphate esters (OPEs) represent a group of chemicals that have been extensively used for several decades for 2 main purposes, depending greatly on the type of side chain of the phosphate ester: (i) the halogenated compounds are applied as flame retardants (FR), and (ii) the non-halogenated compounds are mostly used as plasticisers although they are also used as FRs [1]. With the gradual discontinuation of the use of some brominated flame retardants (BFRs) due to their proved persistence, bioaccumulation and/or toxicity in the environment, but also to animals and humans, organophosphorus flame retardants (OPFRs) generally and OPEs particularly were then proposed as suitable alternatives and their use is in continuous increase [2, 3]. Although there is insufficient knowledge about the toxicity of OPEs, some studies have reported adverse effects in animals as a result of long term exposure to these contaminants [3]. The objective of the present study was (i) to investigate the fragmentation profiles in the mass spectra of 18 OPEs using different ionisation techniques by GC-MS/MS, namely EI, NCI, PCI and APCI and then to select the most relevant techniques, (ii) to develop the instrumental methods (GC-EI-MS/MS and GC-APCI-MS/MS) by optimising both spectrometric and chromatographic separation, (iii) to evaluate the instrumental detection levels (IDLs), (iv) to investigate an appropriate sample preparation procedure enabling their extraction and further purification in Fish and finally (vi) to apply the analytical strategy for the identification and quantification of these substances at trace levels in Fish and other food samples, in order to contribute to the evaluation of Human dietary exposure.

2. Materials and methods

The 18 studied compounds included 6 alkyl (TEP, TPrP, TnBP, TiBP, TBEP, TEHP), 7 aryl (TPP, EHDPP, DBPhP, DPhBP, o-, m-, p-TCP), 3 chlorine-containing (TCEP, TDCIPP, TCPP) and 2 bromine-containing (TDBPP, TTBNPP) phosphates. Seven compounds were used as internal standards (d_{15}-TEP, d_{21}-TPrP, d_{21}-TnBP, $^{13}C_6$-TBEP, $^{13}C_{18}$-TPP, d_{12}-TCEP, d_{15}-TDCPP). Several authors reported blank contamination as an important issue concerning OPEs analysis [4]. Glassware (tubes, vials and pipettes) was backed at 400 °C for 4 h and covered with aluminum foil whenever possible and plastic materials were avoided. A gas chromatography (GC) fitted with Electron Impact (EI) or Chemical Ionisation with CH_4 as reagent gas (NCI, PCI) (Scion, Bruker) and a GC (7880 series, Agilent Technologies) fitted with Atmospheric Pressure Chemical Ionisation (APCI), in protic condition (H2O/MeOH 1:1, v/v) (Xevo, Waters) were used. Triple quadruple mass spectrometer (QqQ) was the mass analyser in the two instruments. In both cases, one µL was injected at 295 °C in splitless mode. DB-5MS (30 m x 0.25 mm i.d, 0.25 µm) was used for the separation of 16 OPEs (non-brominated). The optimized temperature program was as follows: initial temperature at 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 finally held for 5 min. The total run time was 27.58 min and the carrier gas flow rate was constant at 1.5 mL.min^{-1}. For the two brominated OPEs, a shorter capillary column ZB-5HT (15 m x 0.25 mm i.d, 0.10 µm) was used in order to resolve the efficiency problem for these two heavy compounds through minimizing their longitudinal diffusion. On GC-APCI-MS/MS, the optimized temperature program was as follows: the initial oven temperature was set as 85 °C for 1 min, ramped to 350 °C at 35 °C.min^{-1} and held for 5 min. The total run time was 16.63 min and the carrier gas flow rate was constant at 3 mL.min^{-1}. The chromatographic conditions used were the same on GC-EI-MS/MS, with some exceptions, so that the final temperature of the oven program was set at 310 °C (limited by the maximum allowed transfer line temperature of 310 °C). The

total run time was 12.43 min. The carrier gas flow rate was constant at 1.5 mL.min^{-1}. Figure 1 presents the chromatogram of the optimal separation of the 18 OPEs on the two used columns.

3. Results and discussion

3.1. Selection of the ionisation mode

Clearly, under EI conditions, the highly diagnostic molecular ion was often absent due to the extensive fragmentation resulting in the formation of less specific fragment ions. PCI and NCI showed considerably fewer fragmentations but with reduced sensitivity in comparison with EI (in terms of peak response area), except for TDCIPP, TDBPP and TTBNPP where the presence of halogen atoms favoured the detection of high abundant $[X]^{-\bullet}$ via NCI mode. In the contrary, with positive APCI under protic conditions, the protonated molecular ions were largely preserved, increasing the specificity of the transitions in the SRM method. The two techniques (i.e. GC-EI-MS/MS and GC-APCI-MS/MS) were then selected for further development and optimisation of spectrometric and chromatographic conditions.

3.2. Optimisation of GC-EI-MS/MS and GC-APCI-MS/MS methods

Various cone voltages were tested in order to select the one yielding the optimal ionisation/fragmentation for each compound. After that and to characterise and optimise the fragmentation pathways of the selected precursor ions in the collision cell, different collision energies were tested. The SRM methods with optimised cone voltages and collision energies are shown in Table 1.

3.3. Evaluation of the instrumental detection limits (IDLs)

Instrumental detection limits (IDLs) were defined as the lowest detection limit where S/N > 3. For almost all the studied compounds, IDLs achieved were 2.5 to 25 times lower in the APCI mode than those in the EI mode, 50 times for TTBNPP and 100 times for TBEP and TDBPP. This conclusion is going to be verified in terms of LOD and/or LOQ values after applying the two methods for the analysis of real fish samples.

3.4. Development of extraction – purification strategy

The investigated preparation procedure was based on pressurised liquid extraction (PLE) with the in-cell use of 15 g Florisil as a first cleanup step. Gel permeation chromatography (GPC) was then used as a second cleanup step to maximise the elimination of lipid. EtAc/cyclohexane (50:50) solvent mixture was used at both the extraction and purification steps. To minimise as much as possible the risk of contamination, an additional rinsing cycle prior to the extraction step.

3.5. In-house quality control (QC)

To assure the reliability of the results, some quality control (QC) practices should be in place, including for example in our study, the use of 'in-house' QC samples which were spiked with 50 ng of native OPEs and introduced in each series of samples in order to evaluate the repeatability an reliability of the results output. Figure 2 shows an example of control chart for TPrP analysed on GC-EI-MS/MS.

3.6. Application to various food matrices

The whole developed strategy was then applied for the analysis of a series of different samples, including silurus river fish and marine fish samples as well as other seafood samples. The experiments were done while introducing procedural blank samples (n=20) in order to be able to study the repeatability of the procedural contamination which is mainly attributed to TCEP, TCPP, TDCIPP and EHDP and to define the limit of reporting (LoR) of these ubiquitous contaminants.

4. Conclusion

The following study presents the ability of GC-MS/MS to analyse a wide range of OPEs including alkyl, aryl and halogenated compounds. An instrumental method was developed on EI and APCI ionisation modes by optimising the chromatographic and the spectrometric conditions. Then, an extraction-purification procedure enabling the extraction of OPEs from complex biological matrices (e.g. fish) was developed. To evaluate its robustness, quality control charts were established on 'in-house' quality control sample and the method was applied to a wide range of samples in order to analyse these contaminants at trace levels.

5. Acknowledgements

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6. References

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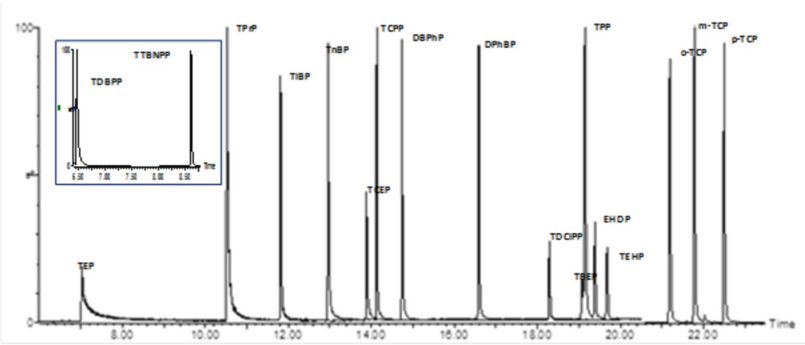

Figure 1. Overlaid ion chromatograms obtained for the optimised SRM transitions of the 18 OPEs by GC-APCI-MS/MS.

Table 1. Optimised transition parameters for the 18 OPEs and obtained instrumental detection limits (IDL) (CE: collision energy; CV: cone voltage).

	GC-APCI-MS/MS						GC-EI-MS/MS				
	Transitions						Transitions				
Compound	T1	CE 1 (eV)	T2	CE 2 (eV)	CV (V)	IDL (µg.L^{-1})	T1	CE 1 (eV)	T2	CE 2 (eV)	IDL (µg.L^{-1})
TEP	183>99	15	183>155	5	20	1	155>99	10	127>99	10	0.4
TPrP	225>99	10	225>183	5	20	0.4	141>99	10	183>99	15	0.4
TnBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	20	0.4
TiBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	10	0.4
TEHP	435>99	15	435>323	5	30	0.4	113>57	10	113>95	10	10
TBEP	399>199	15	399>99	25	30	0.4	125>99	10	199>99	10	40
TPP	327>77	25	327>125	25	30	0.4	326>215	20	326>169	20	1
EHDP	251>95	20	363>251	5	40	0.4	251>77	20	251>152	20	1
DBPhP	287>175	15	287>231	5	20	0.4	175>77	15	175>51	10	1
DPhBP	307>251	10	251>153	15	30	0.4	251>152	15	306>251	10	2
o-TCP	369>91	25	369>166	25	40	0.4	368>181	10	165>139	25	2
m-TCP	369>166	25	369>91	25	40	0.4	368>165	25	368>261	10	1
p-TCP	369>166	25	369>91	25	40	0.4	368>108	15	368>198	15	1
TCEP	285>223	10	287>99	15	30	0.4	249>125	10	249>99	10	1
TCPP	329>99	15	327>251	5	20	0.4	125>99	10	201>125	10	1
TDCIPP	431>321	5	321>209	5	30	1	191>75	10	381>159	10	2
TDBPP	698.5>99	25	698.5>299	15	30	1	337>137	5	217>137	5	100
TTBNPP	1018>147	30	1018>307	20	30	10	713>309	15	713>145	15	500

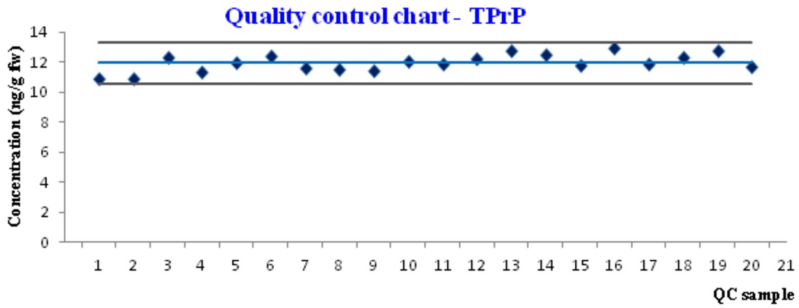

Figure 2. Example of a quality control chart for TPrP, showing the repeatability of results within the upper and lower warning limits (analysis on GC-EI-MS/MS).