ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ

ΤΜΗΜΑ ΧΗΜΕΙΑΣ

ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΣΠΟΥΔΩΝ «ΧΗΜΕΙΑΣ»

ΕΙΔΙΚΕΥΣΗ «ΧΗΜΙΚΗ ΑΝΑΛΥΣΗ - ΕΛΕΓΧΟΣ ΠΟΙΟΤΗΤΑΣ»

ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Ανάπτυξη και επικύρωση μεθόδου για τον ταυτόχρονο προσδιορισμό οργανοφωσφορικών επιβραδυντών φλόγας σε περιβαλλοντικά δείγματα με τεχνικές φασματομετρίας μάζας

ΧΑΡΙΣ ΠΑΠΠΑ

ΧΗΜΙΚΟΣ

ΑΘΗΝΑ

ΑΠΡΙΛΙΟΣ 2013

NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

FACULTY OF SCIENCES

DEPARTMENT OF CHEMISTRY

GRADUATE PROGRAM "CHEMISTRY"

SPECIALIZATION "CHEMICAL ANALYSIS - QUALITY CONTROL"

RESEARCH WORK OF SPECIALISATION DIPLOMA

Development and validation of method for simultaneous determination of organophosphorus flame retardants in environmental samples with mass spectrometry techniques

CHARIS PAPPA

CHEMIST

ATHENS

APRIL 2013

ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

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ΜΙΧΑΗΛ ΚΟΥΠΠΑΡΗΣ
ΑΝΑΣΤΑΣΙΟΣ ΟΙΚΟΝΟΜΟΥ

ΗΜΕΡΟΜΗΝΙΑ ΕΞΕΤΑΣΗΣ: 18/04/2013

ΠΕΡΙΛΗΨΗ

Οι επιβραδυντές φλόγας είναι μια κατηγορία ενώσεων που προστίθενται εδώ και δεκαετίες σε διάφορα προϊόντα καθημερινής χρήσης, όπως πλαστικά, υφάσματα, προϊόντα ξύλου, βερνίκια για πατώματα κ.ά. για να επιβραδύνουν ή να σταματήσουν τη διαδικασία καύσης τους σε περίπτωση πυρκαγιάς. Ανάλογα με τη σύστασή τους διακρίνονται σε αζωτούχους, αλογονούχους, φωσφορούχους και είναι είτε οργανικές είτε ανόργανες ενώσεις.

Στόχος της παρούσας εργασίας είναι η ανάπτυξη και επικύρωση μεθόδου προσδιορισμού δεκατριών φωσφορούχων επιβραδυντών φλόγας (organophosphorus flame retardants) και ενός βρωμιούχου, του δεκα-βρωμο-διφαινυλ-αιθέρα (deca-BDE), ο οποίος χρησιμοποιούνταν κατά κόρον έως πρόσφατα. Το αναλυτικό ενδιαφέρον για τους φωσφορούχους επιβραδυντές έχει αυξηθεί τα τελευταία χρόνια, καθώς έχει απαγορευθεί η παρασκευή και χρήση των κυριότερων εκπροσώπων της βρωμιούχου ομάδας (penta-BDE, octa BDE και πιο πρόσφατα και του deca-BDE).

Η ανίχνευση και ο προσδιορισμός τους έγινε σε δείγματα νερού και λάσπης από απόβλητα αστικών περιοχών και σκόνης εσωτερικών χώρων. Αναπτύχθηκαν τρεις μέθοδοι προσδιορισμού, μία για κάθε μήτρα, οι οποίες διαφέρουν μεταξύ τους κυρίως στο στάδιο της προκατεργασίας του κάθε δείγματος. Ακολούθως έγινε επικύρωση με υπολογισμό της γραμμικότητας, επαναληψιμότητας, ανάκτησης και ορίων ανίχνευσης, για τις μεθόδους σε νερά και σκόνη, ενώ η μέθοδος σε ιζήματα δεν επικυρώθηκε, καθώς δεν επιτεύχθηκαν καλές ανακτήσεις για όλες τις ουσίες.

Στις τεχνικές προκατεργασίας περιλαμβάνονται η εκχύλιση στερεάς φάσης (solid phase extraction, SPE) και η επιταχυνόμενη εκχύλιση διαλύτη (accelerated solvent extraction, ASE), ενώ οι μετρήσεις έγιναν με χρωματογραφία υγρού και αερίου συζευγμένες με φασματομετρία μαζών (LC-MS, GC-MS).

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Περιβαλλοντική ανάλυση

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: επιβραδυντές φλόγας, οργανοφωασφορικοί εστέρες, απόβλητα νερά, σκόνη εσωτερικών χώρων, χρωματογραφία υγρού και αερίου συζευγμένη με φασματομετρία μαζών

ABSTRACT

Flame retardants (FRs) are used since decades as additives in a great variety of everyday life products to prevent the expansion of fires in indoor environments. After banning most of brominated flame retardants due to the increasing number of studies showing their adverse effects to the environment and human health, organophosphorus flame retardants (PFRs) started taking their place in the market.

In the current study, three slightly different methods for the simultaneous determination of fourteen of the most used FRs in waste water, sediment and indoor dust were developed and validated. The PFRs measured were the following: tri-iso-butyl phosphate (TiBP), Tri-n-butyl phosphate (TnBP), Tris-(2-chloroehtyl) phosphate (TCEP), Tris-(2-chloropropyl) phosphate (TCPP), Tris-(dichloro-propyl) phosphate (TDCPP), Tricresyl phosphate (TCP), Tri-phenyl phosphate (TPP), Tris-(butoxyethyl) phosphate (TBEP), Tris-(2ehtylhexyl) phosphate (TEHP), 2-ethylhexydiphenyl phosphate (EHDP), Resorcinol-bis(diphenyl phosphate) (RDP), Bisphenol A bis diphenyl phosphate (BDP), 9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide (DOPO) and the brominated flame retardant 2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene (BDE 209, commercial name deca-BDE).

The determination was accomplished in all cases by LC or GC-MS (time of flight and triple quadrupole were used as mass analyzers). Different pretreatment steps were followed for water, sediment and dust samples. Water samples were subjected to Solid Phase Extraction (SPE) clean up before the injection to the chromatograph, sediment samples were first subjected to Accelerated Solvent Extraction (ASE) and then to SPE and dust samples to ultrasound extraction.

The linearity, repeatability, recovery and limits of detection and quantification were calculated for water and dust methods with more details, but not for sediment method because the recoveries from the matrix were not so good for all the compounds.

SUBJECT AREA: Environmental analysis

KEY WORDS: flame retardants, organophosphate esters, waste water, sediment, indoor dust, LC-MS, GC-MS.

ACKNOWLEDMENTS

First of all, I would like to thank assistant professor Nikolaos Thomaidis, for the award of this very interesting thesis theme, the chance he gave me to participate to the student exchange program “Erasmus” and his precious advice.

Moreover, I would like to thank professor Jacob de Boer, PhD student Sicco Brandsma and all the members of the laboratory in the host university “Vrije Universiteit Amsterdam”, for their warm hospitality, advice and help during the conducting of the experiments for the current work.

Finally, I would like to thank the members of the examination committee, Nikolaos Thomaidis, Michail Koupparis and Anastasios OIkonomou for their attention and their useful coments.

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CHAPTER 1

FLAME RETARDANTS: ORIGIN, OCCURRENCE AND FATE IN THE ENVIRONMENT

1.1 What are flame retardants?

Fire is a very significant cause of injury, lifelong disabilities, death and property damage. According to the World Health Organization reports there are approximately 300,000 deaths per year, globally from fire-related burns, while every day in Europe there are about 12 fire victims and 120 people severely injured. Domestic fires are often caused by burning cigarettes, lit candles or children playing with matches.

Flame retardants (FRs) are substances that inhibit or slow down the growth of a fire and are incorporated to various materials (furniture, plastics, electronic equipment, textiles etc.) to increase their fire safety and meet the flammability standards [1].

1.2 How do they work?

To understand how flame retardants work it is important first to understand how a fire initiates, spreads and ends and then how materials burn:

a) The Fire Triangle:

A fire can be split in three stages, the initiating fire, the fully developed fire and the decreasing fire (figure 1). The fire starts with an ignition source (for example a match) setting combustible material (for example an upholstered armchair) on fire. The fire heats up the surroundings and spreads to them. Once the materials in the room have formed enough flammable gases and are sufficiently hot, flashover takes place and the whole room is engulfed in the fire.

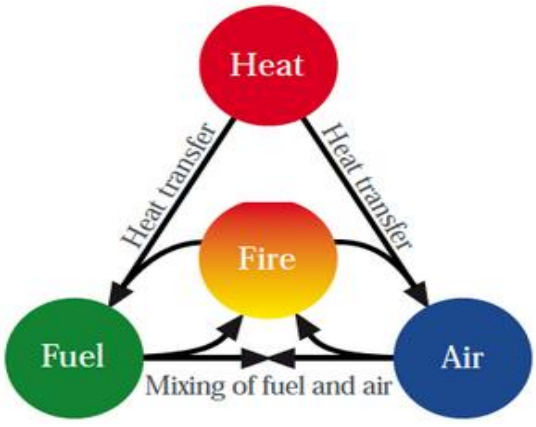


Figure 1: The fire triangle

This is a fully developed fire where temperatures up to 1200 °C can be reached. The fire will later decrease because of lack of the available combustible material (as it is consumed by the fire) or lack of oxygen, in case the fire occurs in a totally closed room.

b) How materials burn:

Solid materials do not burn directly: they must first be decomposed by heat (pyrolysis) to release short-chain molecules which transpire as flammable gases. Visible flames appear when these flammable gases burn with the air oxygen.

If solid materials do not break down into gases, then they will only smoulder slowly and often self extinguish, particularly if they “char” and form a stable carbonaceous barrier which prevents access of the flame to the underlying material.

The gas flame itself is maintained by the action of high energy radicals ($H^{\bullet}$ and $OH^{\bullet}$ in the gas phase) which decompose big molecules to smaller ones which can react with the air oxygen to burn to CO_2, generating heat energy.

By their chemical and/or physical action, FRs prevent or even suppress the process of combustion during a particular phase of the fire cycle. This can be either during heating, ignition, flame spread or decomposition (pyrolysis).

The FRs have either chemical (the most effective) or physical action.

When chemical action occurs they react either in the gas or in the condensed phase:

- In the gas phase the FR interrupts the radical gas phase combustion process resulting in a cooling of the system, a reduction and suppression of the supply of flammable gases.
- In the condensed phase, the FR builds up a char layer, smothering the material and inhibiting the oxygen supply, thereby providing a barrier against the heat source or already ignited flame from another source.

The less effective physical action of FRs can take place by:

- Cooling: the FR takes part in endothermic processes (e.g. release of water) and thus cools the underlying substance to a temperature that is unable to sustain the burning process
- Coating: the polymer is shielded with either a solid or gaseous layer, protecting it against the heat and oxygen required for combustion to take place

- Dilution: chemically inactive substances and additives turn into non-combustible gases which dilute the fuel in the solid and gaseous phases of the fire cycle [1].

1.3 Types of flame retardants

Since the term "flame retardant" describes a function and not a chemical class, a wide range of different chemicals can be used for this purpose. Often they are applied in combinations, so they can act synergistically. This variety of FRs is necessary, because the materials and products which are to be rendered fire safe are very different in nature and composition.

1.3.1 Brominated Flame Retardants (BFRs)

They are the main representative of the halogenated flame retardant group and they act with two mechanisms:

- these compounds (R-X) release active halogen (e.g. bromine or chlorine) atoms (called low-energy free radicals) into the gas phase before the material reaches its ignition temperature, or react with the organic substrate (P-H) under high temperatures and form hydro-halogens (H-X). These halogen atoms or hydro-halogen molecules effectively react with the $H^{\bullet}$ and $OH^{\bullet}$ radicals and thus remove them from the gas phase, resulting in a slowdown of the burning process and a reduction of the spreading of the fire. The effectiveness of the halogenated FRs depends on the number of halogen atoms present in the molecule and decreases in the order HI>HBr>HCl>HF.

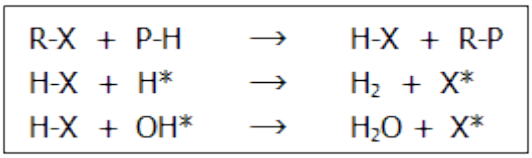


Figure 2: Reactions of halogenated FRs during a fire [56]

- in the solid phase they dehydrogenate polymers by abstracting hydrogen atoms needed to produce hydrogen bromide. This process enhances charring of the polymer on expense of volatile combustible products. Often and when permitted, the addition of metallic compounds such as zinc or antimony oxides will enhance the efficiency of BFRs, by allowing the formation of transition species, so-called metal oxohalides, which allow the deposit of a protective layer of metal oxides.

The area of electrical and electronic equipment accounts for more than 50% of their applications, for example in wire and cable compounds, printed circuits, outer housings of TV sets and computer monitors etc.

The most common are the tetrabromobisphenol-A (TBPA), polybrominated diphenyl ethers (PBDEs), hexa bromo cyclododecane (HBCD) and polybrominated biphenyls (PBBs). These BFRs (except TBBP-A) appear to be lipophilic and bioaccumulate in biota and humans [1, 8, 56].

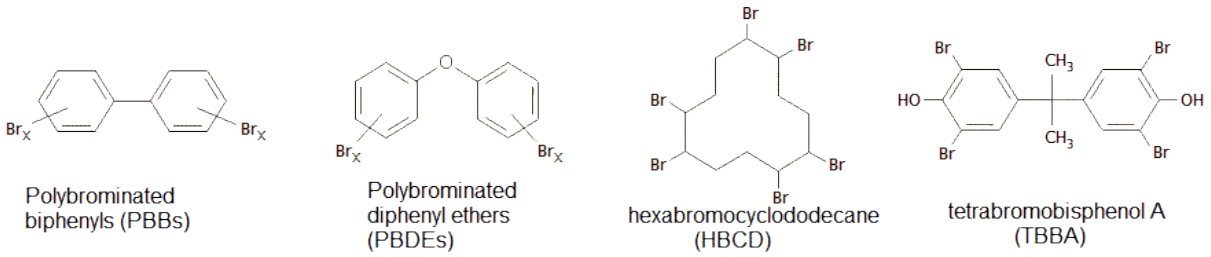


Figure 3: The most common types of halogenated flame retardants [56]

1.3.2 Nitrogen Flame Retardants

The mechanisms of action of nitrogen containing flame retardants are not fully understood, but they are believed to act by several mechanisms:

- they are relatively stable compounds at high temperatures, thus physically inhibiting the breakdown of materials to flammable gases, which are needed to feed the flames. A mechanism in the gas phase may aid the release of stable nitrogen-containing molecules, which dilute volatile polymer decomposition products.
- in the condensed phase, melamine is transformed into cross-linked molecular structures, which promote char formation.

Three main chemical groups can be distinguished: pure melamine, melamine derivatives (i.e. salts with organic or inorganic acids such as boric acid, cyanuric acid, phosphoric acid or pyro/poly-phosphoric acid) and melamine homologues.

Melamine-based products are the most widely used type of nitrogen flame retardant today, and are used for example in polyurethane foams for furniture, building foams, nylons etc [1].

1.3.3 Inorganic Flame Retardants

A wide range of different inorganic compounds are used as flame retardants, or as synergists of flame retardant systems in combination with brominated, phosphorous and/or nitrogen flame retardants, because their mechanisms are of a relatively low efficiency.

The inorganic compounds used include metal oxides, hydroxides, borates, stannates (aluminium and magnesium hydroxides, antimony oxides, boric acid, zinc borate and stannate), inorganic phosphorus compounds (red phosphorus and ammonium polyphosphate) and graphite.

Those used as flame retardants, mainly aluminium and magnesium hydroxides, interfere with the burning process through three main physical processes:

- release of inert gases such as water vapor, which dilute the fuel/oxygen mix thus preventing the exothermic radical reaction from taking place in the combustion zone.
- energy absorption through endothermic decomposition (reducing energy available for the fire spread) thereby contributing to cooling and retardation of the pyrolysis process (aluminium and magnesium hydroxides).
- production of a non-flammable and resistant layer on the surface of the material (protective char layer), reducing the release of flammable gases by the polymer and the energy transfer to the polymer, which sustains pyrolysis (boric acid, sodium borates, zinc borates, ammonium polyphosphate) [1, 56].

Antimony trioxide (Sb_2O_3) and Antimony salts (e.g. $NaSbO_3$):

They do not have flame retarding properties on their own, but are effective synergists for bromine and chlorine based (halogenated) flame retardants.

- They act as catalysts, facilitating the breakdown of these halogenated flame retardants to active free radicals.
- They also react with the halogens to produce volatile antimony halogen compounds, which are themselves directly effective in removing the high energy $H^{\bullet}$ and $OH^{\bullet}$ radicals that feed the gas phase of the fire, thus strengthening the flame suppressing effect of the flame retardants. When added to PVC, they act to suppress flames by activating the chlorine present in the plastic itself [1].

1.3.4 Intumescent coatings

Intumescent coatings are fire protection systems which are used to prevent from burning materials such as wood or plastic, but also to protect steel and other materials from the high temperatures of fires (thus preventing or retarding structural damage during fires). The coatings are made of a combination of products, applied to the surface like paint, which are designed to expand and form an insulating and fire-resistant covering when subject to heat.

The products involved contain a number of essential interdependent components:

- spumific compounds, which (when heated) release large quantities of non-flammable gas (such as nitrogen, ammonia, CO_2)
- a binder, which (when heated) melts to give a thick liquid, thus trapping the released gas in bubbles and producing a thick layer of froth
- an acid source and a carbon compound. On heating, the acid source releases phosphoric, boric, or sulphuric acid, which char the carbon compound causing the layer of bubbles to harden and producing a fire-resistant barrier. Often the binder can also serve as the carbon compound [1, 56].

1.4 Organophosphorus Flame Retardants (PFRs)

The class of phosphorus-containing flame retardants covers a wide range of inorganic and organic compounds and includes both reactive (chemically bound into the material) and additive (physically integrated into the material) compounds. The additive PFRs' concentration (and therefore their flame retardancy properties) may decrease during the lifetime of the product because they may leach out in the environment. PFRs have a broad application field and offer very good fire safety performance [1, 8].

The most important are phosphate esters, phosphonates, phosphinates, which may also contain halogen moieties (organic PFRs or organophosphorus flame retardants, PFRs) and red phosphorous and ammonium polyphosphate (inorganic PFRs). Halogenated phosphorus flame retardants combine the flame-retardant properties of both the halogen and the phosphorus groups. Halogen and phosphorus act indipendently and therefore additively. In addition, the halogens reduce the vapour pressure and water solubility of the

flame retardant, thereby contributing to the retention of the flame retardant in the polymer [1, 57].

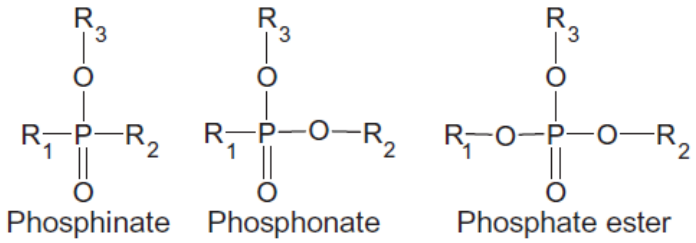


Figure 4: Types of organophosphorus flame retardants

When heated, the phosphorus reacts to give a polymeric form of phosphoric acid. This acid causes the material to char, forming a glassy layer, and so inhibiting the pyrolysis process, which is necessary to feed the flames. By this mode of action the amount of fuel produced is significantly diminished, because char rather than combustible gas is formed. The intumescent char acts as a two-way barrier, both hindering the passage of the combustible gases and molten polymer towards the flame and shielding the polymer from the heat of the flame [1]. A minimum amount of PFR is needed to form a char layer. Once the layer is formed there is no need for more PFR [8].

Some aromatic phosphates (e.g RDP and BDP) act in both gas and solid phases, though. In the gaseous phase they can generate $PO^{\bullet}$ radicals that react with high energy $H^{\bullet}$ and $OH^{\bullet}$ radicals and thereby cause flame inhibiton [17, 57].

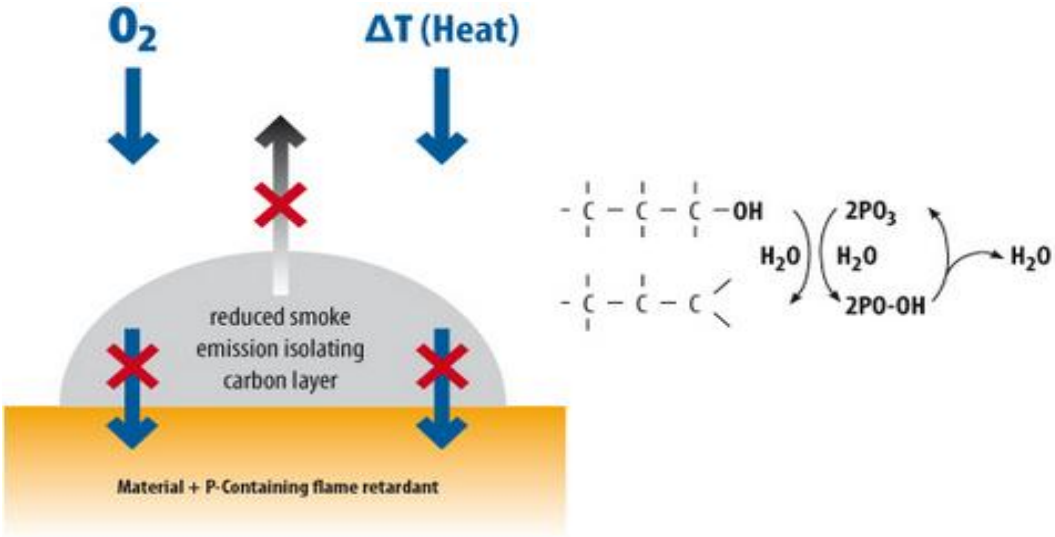


Figure 5: P-containing flame retardants' general mechanism

1.4.1 Organophosphate esters

Organosphosphate estres (OPs) have been used since decades as flame retardants, plasticizers, anti-foaming agents and lubricants due to their technical characteristics. Lately, the worldwide restrictions on the use of polybrominated diphenyl ethers (first of the commercial mixtures of penta- and octa- BDE and more recently of deca-BDE as well) in new products, have led in the increased use of this type of flame retardants. For example, the annual consumption of OPFRs in EU (alkyl phosphates as plasticizers not included) increased from 84,000 tons in 2004 to 91,000 tons in 2006, of which 56% were chlorinated phosphates. The organosphosphate estres are additive flame retardants [4, 57].

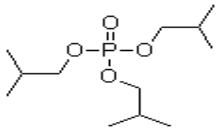
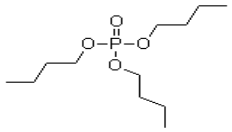
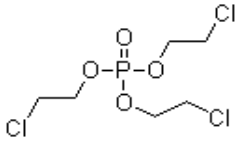
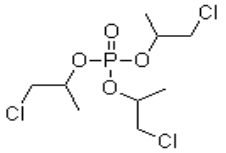
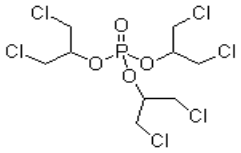
1.4.1.1 Physicochemical properties

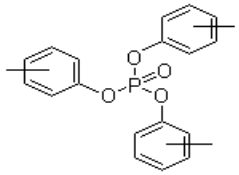
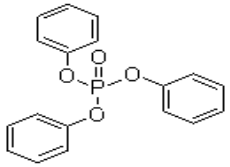
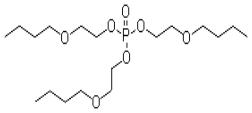
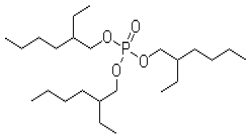
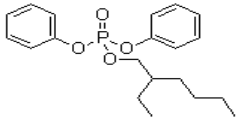
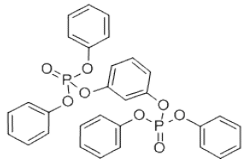
There is a great variation in physicochemical properties of PFRs, depending more on the alcohol moieties esterified to the phosphoric acid. For example, in case of methyl group (TMP) the triester is very polar and volatile ($\log K_{ow}$=-0.65, Vp=$8*10^{-1}$ mm Hg), whereas with the large ehthylexyl groups (TEHP) it is very hydrophobic and non-volatile ($\log K_{ow}$=9.5, Vp=$8*10^{-8}$ mm Hg). That shows that the solubility in water generally decreases by increasing molecular mass. Most PFRs have a positive $\log K_{ow}$ value, which means they are more lipophilic than hydrophilic.

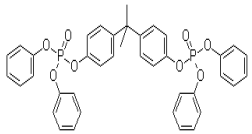
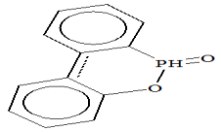
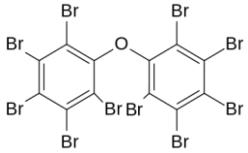
Vapor pressures and bioaccumulation factors (BCF) also vary among the PFRs. The BCF generally increases by increasing molecular mass, except for chlorine containing compounds. Therefore, the PFRs with higher molecular masses are more likely to be found in nature than those with lower molecular masses. For the chlorine containing PFRs no relation can be found between the BCF and the molecular mass or the amount of chlorine in the molecule [8, 18].

In the table below an overview of PFRs' physicochemical properties is shown:

Table 1: Physicochemical properties of organophosphates

Name	Acronym	CAS number	Molecular Formula	Molecular Weight (g/mol)	Chemical Structure	Boiling point (^oC)	Melting point (^oC)**	Flash point (^oC)*	Vapor pressure (mm Hg, 25 ^oC)	Log K_{ow}	d, soil absorption coefficient log K_{oc}*	BCF (L/kg wet weight)
Tri-iso-butyl phosphate	TiBP	126-71-6	$C_{12}H_{27}O_4P_1$	266.32		264	16	126	$1.3*10^{-2}$	3.60	3.05	211* 19,51**
Tri-n-butyl phosphate	TnBP	126-73-8	$C_{12}H_{27}O_4P_1$	266.32		289	-80	152	$1.1*10^{-3}$	4.00	3.24	479.69* 39.81**
Tris-(2-chloroehtyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P_1$	285.49		347* 330**	-35	232	$6.2*10^{-2}$	1.44	1.82	7.69* 0.61**
Tris-(2-chloropropyl) phosphate	TCPP	13674-84-5	$C_9H_{18}Cl_3O_4P_1$	327.57		358	-40	248	$\underline{5.64*10^{-5}}$	2.59	2.46	49.22* 3.55**
Tris-(dichloro-propyl) phosphate	TDCPP	13674-87-8	$C_9H_{15}Cl_6O_4P_1$	430.91		457* 237**	27	378	$\underline{2.86*10^{-7}}$	3.65	3.05	181.50* 17.8**

Tricresyl phosphate	TCP	1330-78-5	$C_{21}H_{21}O_4P$ [1]	368.37		439* 265** <u>476</u>	77	232	$6.00*10^{-7}$	5.11	4.15* 3.52**	4504.01* 163.6** 1280 xm
Tri-phenyl phosphate	TPP	115-86-6	$C_{18}H_{15}O_4P$ [1]	326.29		412* 245** <u>441</u>	50.5	201	$6.28*10^{-2}$	4.59	3.24	1813.01* 74.23**
Tris-(butoxyethyl) phosphate	TBEP	78-51-3	$C_{18}H_{39}O_7P$ [1]	398.48		414* 221** 434	-70	241 159viv	$2.1*10^{7}$	3.75	2.83	182.75* 20.72**
Tris-(2ehtylhexyl) phosphate	TEHP	78-42-2	$C_{24}H_{51}O_4P$ [1]	434.65		405* 215**	-74	206	$2.0*10^{6}$	9.48	6.39	$1.00*10^{6}$* 30.34
2-ethylhexydiphenyl phosphate	EHDP**	001241-94-7	$C_{20}H_{27}O_4P$ [1]	362.41		375	-54		$5.00*10^{-5}$	5.73	3.87	419.5**
Resorcinol-bis(diphenyl phsphate)	RDP	57583-54-7	$C_{30}H_{24}O_8P$ [2]	574.47		587		322	$2.1*10^{-4}$	7.01*	5.22* 4.80**	140889* 1256**

Bisphenol A bis diphenyl phsphate	BDP*	5945-33-5	$C_{36}H_{28}O_8P_2$	692.63		679*	<u>90</u>	377	<u>$2.06*10^{-8}$</u>	8.29	5.88	$1.00*10^{6}$*
9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide	DOPO**	35948-25-5	$C_{12}H_9O_2P_1$	216.18		399	<u>84</u>	206*	<u>$2.88*10^{-5}$</u>	<u>1.87</u>	<u>1.73</u>	7.93**
2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene	BDE 209	001163-19-5	$C_{12}Br_{10}O$	959.17		572* 530**	295	241*	<u>$4.67*10^{-12}$</u>	9.45*	6.51*	$1.00*10^{6}$* 41.71**

** : from ACD/labs (www.chemspider.com)*

*** : from EPISUITE*

<u>underline</u>: theoretical values (where experimental are not existing)

1.4.1.2 Applications

OPFRs are used in a range of polymers depending on the type of side chain of the phosphate ester. The halogenated ones are mostly used as flame retardants, while the non-haloganated mostly as plasticizers. For example, chlorinated alkyl phosphates (tris-2-chloroethyl phosphate (TCEP), tri (1-chloro-2-propyl) phosphate (TCPP), tris-1,3-dichloropropyl phosphate (TDCPP)) are often applied in flexible and rigid polyurethane foams as flame retardants. In some cases, they are also used to help controlling the pore sizes in foam. The non-haloganated alkyl phosphates (triethyl phosphate (TEP), tri-n-butyl phosphate (TnBP), tri-iso-butyl phosphate (TiBP)) are mostly used as lubricants or for their plasticizing properties in unsaturated polyester resins, cellulose acetate, poly-vinyl-chloride, acrylonitrile-butadiene-styrene and synthetic rubber. Triphenyl phosphate (TPP), tri cresyl phosphate (TCP) and ethyl-exyl-diphenyl phosphate (EHDP) are often used as flame retardant plasticizers (in PVC, cellulosic polymers, thermoplastics and synthetic rubber) and lubricants in hydraulic fluids. Tri (2-butoxyethyl) phosphate (TBEP) is often used in floor wax and rubber stoppers [4,9].

A number of other applications of PFRs include their use in textiles, antistatic agents, cellulose, cotton, cutting oils, electronic equipment, casting resins, epoxy and phenolic resins [10].

In the table below an overview of the applications of the PFRs studied is given:

Table 2: Organophosphates' applications

Name	Acronym	Applications	Citations
Tri-iso-butyl phosphate	TiBP	Lubricant, plasticizer, concrete (pore size regulation)	9
Tri-n-butyl phosphate	TnBP	Solvent for cellulose esters, lacquers & natural gums, plasticizer, antifoaming agent, hydraulic fluids, extractant for metal complexes	8, 9
Tris-(2-chloroehtyl) phosphate	TCEP	Flame retardant, pore size regulator, used in building materials, lacquers, paints, glues	5, 9, 11
Tris-(2-chloropropyl)	TCPP	Flame retardant (mostly in	9

phosphate		polyurethane foam)	
Tris-(dichloro-propyl) phosphate	TDCPP	Flame retardant (mostly in polyurethane foam), textiles, diverse, lacquers, paints, glues	9, 11
Tri-phenyl phosphate	TPP	Hydraulic fluids, flame retardant plasticizer in PVC, electronic equipment, casting resins, glues, engineering thermoplastics, phenylene-oxide-based resins, phenolics resins)	8, 9
Tris-(butoxyethyl) phosphate	TBEP	Plasticizer (rubber & plastics), antifoam agent, floor polish, lacquers, solvent, flame retardant	8, 9, 11
Tricresyl phosphate	TCP	Hydraulic fluids, solvent, flame retardant plasticizer in PVC, cellulose, cutting oils, polyurethane foam, rubber	5, 8, 9
Tris-(2ehtylhexyl) phosphate	TEHP	Solvent, plasticizer in PVC, cellulose, polyurethane foam, rubber, additive in paints &coatings, fungus resistance	8, 11
2-ethylhexydiphenyl phosphate	EHDP	Lubricant, plasticizer in PVC, food packaging	8
Resorcinol-bis(diphenylphosphate)	RDP	Plasticizer in engineering thermoplastics, polyurethane foam	8
Bisphenol A bis-diphenyl phosphate	BDP	Flame retardant plasticizer in engineered resin applications such as polyphenylene oxide alloys and PC/ABS (poly carbonate/acrylonitrile-butadiene-styrene), and thermoplastic resins	15
9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide	DOPO	Flame retardant in epoxy resin for PCB, Polyester Fiber Fabrics, Color protector for plastics, antioxidant type stabilizer	15
2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene	BDE 209	Plastic resins, textiles, flame retardant in HIPS resins which are used in electronic enclosures, in some upholstery textiles	14

1.4.1.3 Toxicity

The knowledge related to the toxicity of PFRs due to longtime exposure is still insufficient and few reports of adverse effects have been published. As the chemical structure of OPs used as flame retardants and plasticizers are similar to OPs used as insecticides, which are designed to affect the nervous system of the insects, a lot of them have neurotoxic properties in humans and other species, after chronic exposure. Most organophosphates have also strong hemolytic effects (decomposition of red blood cells) and the hemolytic effect decreases in the order: EHDP, TCP, TPP, TDCPP, TBP, TBEP, TCPP and TCEP. Further, carcinogenic effects have been observed for the chlorinated ones. There are also other adverse effects for each compound, most of them are described in more details in the table below [4, 5, 8, 9, 11].

Table 3: Organophosphates' toxicity

Name	Acronym	Symptoms	Citations
Tri-iso-butyl phosphate	TiBP		
Tri-n-butyl phosphate	TnBP	Neurotoxic, irritates eyes, skin, respiratory system, causes headache, nausea	9,16
Tris-(2-chloroehtyl) phosphate	TCEP	Carcinogen, neurotoxic, hemolytic & reproductive effects (i.e. reduced fertility, reduced sperm motility & density)	5, 9
Tris-(2-chloropropyl) phosphate	TCPP	Possible carcinogen (observed tumors in kidneys)	9
Tris-(dichloro-propyl) phosphate	TDCPP (more neurotoxic than TCEP, TCPP, (9))	Suspected carcinogen (observed tumors in brain, liver, kidneys), reduces thyroid hormone levels, irritates the skin, concentration-depended neurotoxicity, DNA synthesis inhibition, decreases cell number, alters neurodifferentiation	4, 5, 9
Tricresyl phosphate	TCP	Causes delayed neuropathy which can lead to irreversible paralysis, irritates the skin	
Tri-phenyl phosphate	TPP	Contact dermatitis, minor changes in	4, 5, 8, 16

		blood enzymes, linked to decline in sperm concentration, possible neurotoxic; in animals: muscle weakness, paralysis	
Tris-(butoxyethyl) phosphate	TBEP	Irritates the skin	4
Tris-(2ehtylhexyl) phosphate	TEHP	Irritates skin & eyes, may injure liver, kidney	16
2-ethylhexydiphenyl phosphate	EHDP		
Resorcinol-bis (diphenyl phosphate)	RDP	Generally not problematic (not genotoxic, mutagen, teratogen); in test animals: moderate accumulation in lungs & bones (when combinated with TPP), amplification of lungs, liver, eye irritation	9
Bisphenol A bis diphenyl phosphate	BDP	Low toxicity (neither mutagenic nor clastogenic, teratogenic in bacteria, hamsters, rats, fish, LD50> 100mg/kg), minimal skin and eye irritation	8
9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide	DOPO		
2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy) benzene	BDE 209	Irreversible damage risk, protect skin/eyes/lungs	16

1.4.1.4 Occurrence and fate in the environment

Since OPs are not chemically bonded to the polymeric materials, they can reach the environment via abrasion, leaching and volatilization. They are used for decades, so their occurrence in the environment is not a new issue: there are sparse reports on their detection in surface waters, in ground waters influenced by wastewater and even in drinking water since 1980s. Furthermore, the resistance of the chlorinated FRs to biodegradation and to removal from potable water and wastewater by several treatment methods apart from activated carbon filtration is well documented [18, 24].

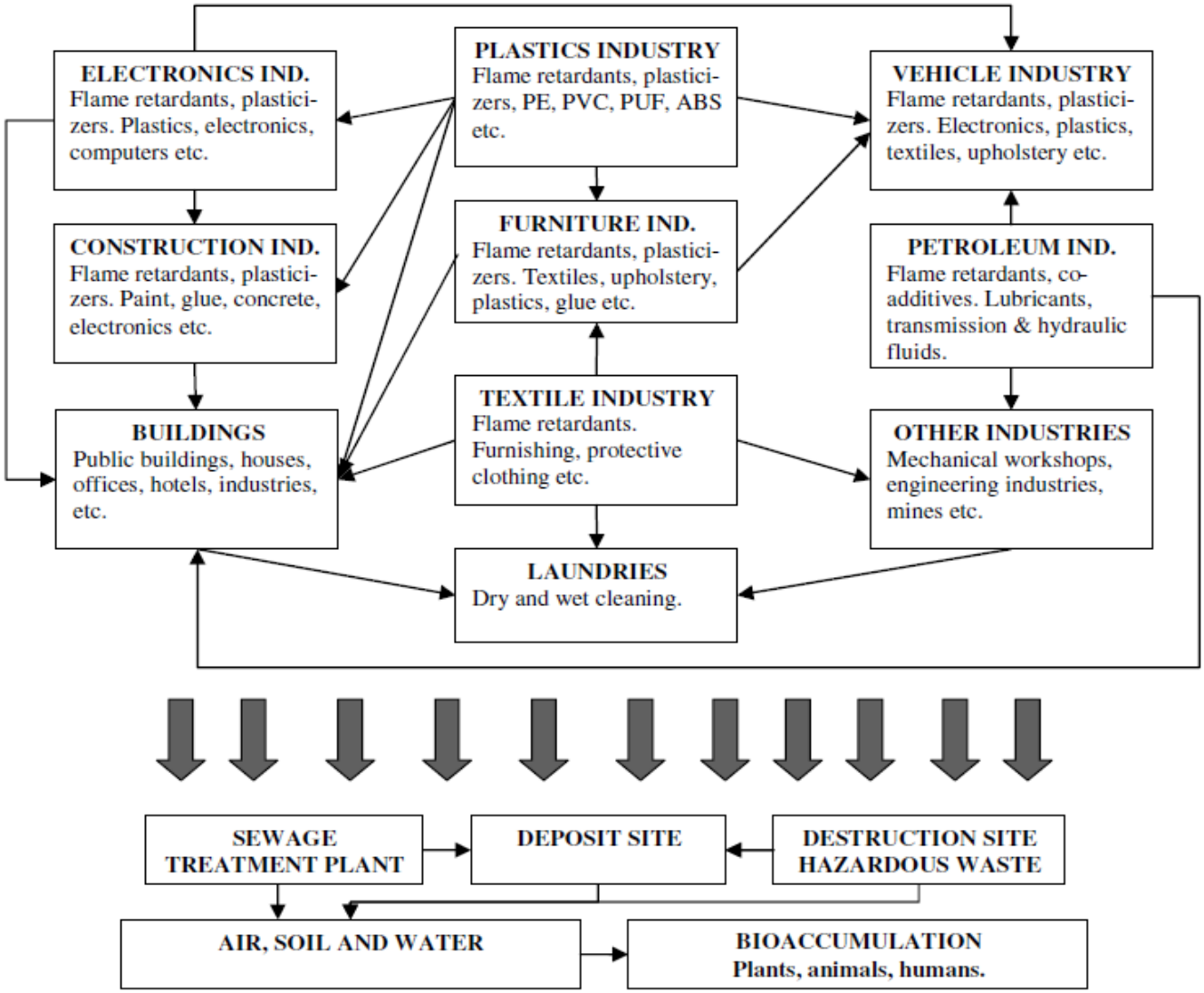


Figure 6: Flowchart of organophosphorus compounds in the environment [11]

Wastewater

In general the chlorinated aliphatic esters (TCEP, TCPP and TDCP) show no significant removal after WWTPs and thus they are these esters with the highest effluent concentrations. It was found that the sorption to activated sludge is the major removal process of TCPP in a WWTP. Among the other OPs, the most are sufficiently removed from the dissolved phase.

Surface water

WWTPs are considered as the major source of OPs in surface waters. In addition to that, emission into surface water may occur via leaching from landfills and release into the marine environment from dump sites. OPFRs have also been detected in storm-water runfoff and snow [4, 18, 23].

Sub-surface water

Leaching from landfills and releasing from dump sites are sources of OPs in sub-surface waters as well. Besides, infiltration of surface water into the ground may be a significant factor that provides groundwater with OPs. Polar and especially poorly degradable compounds can pass a sub-surface barrier and occur in the groundwater, because neither sorption nor biodegradation is effective for such compounds.

Drinking water

OPFRs are present to surface, sub-surface and ground waters so there is a general risk that some of these compounds occur in drinking water as well. Indeed, a study conducted in USA in 2001 reported four OPs in raw waters and in finished drinking water, suggested that the specific drinking water treatments were insufficient for removal of OPFRs. The most appropriate method for OPFRs' removal from drinking water has been shown to be activated carbon filtration. Alternatively, membrane processes (e.g. nanofiltration or reversed osmosis) can be used. Sand filtration was also turned to be a sufficient removal method for the biodegradable OPs.

Sediments

OPs that are released to the environment by the ways described above (leaching from landfills, releasing from dump sites and WWTPs, volatilization, abrasion etc.) can easily be distributed and concentrated in sediments, especially due to their physicochemical properties (persistence of some species (mainly the chlorinated ones), low solubility in water and high adsorption to particulate matter).

Indoor environment

OPs are additives to building materials, electric appliances, upholstery and floor polishes. As a consequence they are present at significantly higher concentrations in indoor environment than in outdoor areas. Especially the more volatile species (TBP, TCEP and TCPP) have clearly higher concentrations in indoor air, while the others are more likely to be found in dust. When released in indoor air by volatilization they distribute between the gas phase and various organic films on surfaces and airborne particles. When the emitting source is removed from the room, the organic film layers may act as secondary emitting sources for these chemicals.

Because of the lack of biotic and abiotic degradation indoors the OPs become more persistent. The human exposure to OPs in the indoor environment occurs via inhalation of air or dermal contact or incidental ingestion of dust. An adult with a daily respiratory volume of 19,2 m3 spending 21 hours per day indoors will inhale 0,24 m3 /kg/day of indoor air and ingest 1 mg /kg/day of house dust. The corresponding values for children (1-3 years) are 0.53 m^3 /kg/day and 10.3 m^3 /kg/day. According to Sundkvist et al [4], under these conditions and assuming that the OPs are bioavailable and fully taken up, and adult weighing 70 kg would be exposed to 0.03-5.8 μg /kg/day of six OPFRs (TBP, TBEP, TCEP, TCPP, TPP and TEHP). The correspondence value for children was 0.25-57 μg /kg/day [4, 5, 13, 18].

With the exception of TPP, OPFRs are rapidly resorbed and distributed to the whole body. The phosphate ester moiety is very common in living organisms (e.g. in ATP and in enzymes for the hydrolysis of phosphate esters). So, the organophosphate triesters are hydrolyzed in blood and urine spontaneously or enzymatically by α-esterases and phosphorylo-phosphatases. The main metabolites of the trialkyl and triaryl phosphates in animal experiments and in in vitro studies are the corresponding dialkyl and diaryl phosphates and they seemed to have considerable toxicological effects [19]. Alcohols released during the hydrolysis of OPs are also readily degradable (e.g. methyl alcohol from TMP, ethanol from TEP, phenol from TPP, butoxyethanol from TBEP), but they may also have adverse effects in health. For example, butoxy-ehtanol is a proven mutagen and is suspected to be endocrine disruptor [18, 19].

OPFRs have been detected in human milk [4, 23], urine [19], adipose tissue [11], seminal fluid [11], as well as in biota and aqueous organisms [4, 23]. Since 1990s the TCEP is mainly substituted by TCPP in Europe, due to its toxic effects to aquatic organisms and its classification in the European Union as “potential human carcinogen”. Nevertheless, it is still present in the aquatic environment. The TCPP is expected as non-toxic to aquatic organisms even though its data base is not so extensive as the TCEP’s.

In general, several studies showed that the concentrations of OPFRs are much higher in public buildings (e.g. hospitals, offices, prisons, hotels etc) and in cars than in houses, due to more strict regulations for such places [14, 21, 11].

In the picture below metabolites of some OPFRs are shown:

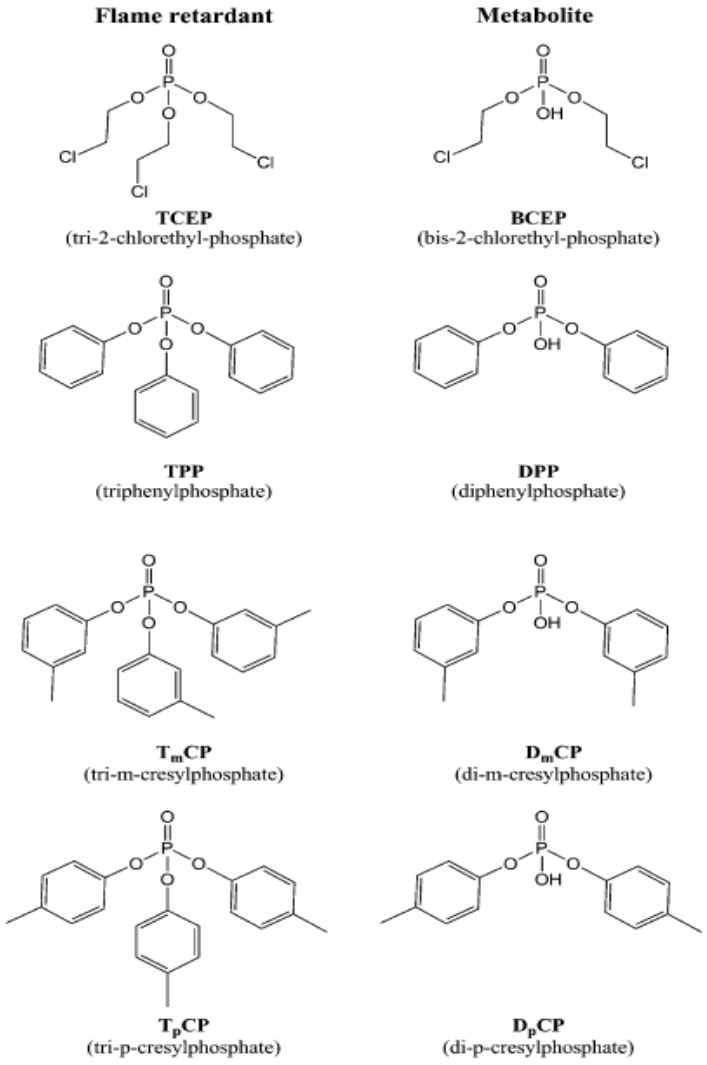


Figure 7: Some flame retardants and the corresponding metabolites [19]

Degradation in outdoor environment

The degradation of OPs in the environment is primarily microbial, as both photolysis and hydrolysis are more unlikely to happen in natural conditions. Phosphoric acid triesters are considered as poor absorbers of light with wavelengths longer than 290 nm (natural light range). Besides, most triesters are stable against hydrolysis at neutral pH. Hydrolysis half lives at pH 7 for TMP, TEP and TPP are in the range 1,2-1,5 years. On the other hand, it can be significantly accelerated at basic pH or by enzymes (α-esterases and phosphorylophosphatases, as mentioned above) [4, 18, 24].

The importance of microbial activity has been shown for example, by Saeger et al., since they detected no loss of OPs in heat sterilized samples of Mississippi river water, but they observed complete primary degradation of TBP, TCP, and TPP within seven days and of EHDP within 21 days, in untreated samples. Other studies showed that phosphoric acid mono- and diesters are the microbically degradation products of the triester compounds (e.g. Schindler et al. [19], Quintana et al.). Generally, primary degradation of the aromatic esters appears to be faster than of the aliphatic ones, while the haloganated ones are not biodegradable [18, 24]. The further removal of the secondary products was found not to be the same fast for all the diesters, in laboratory experiments in bacteria. Some of them disappear in 25 days (e.g. diphenyl phosphate (DPP) and dibutoxyehtyl phosphate (DBEP)), while others remain for much longer (e.g. di-iso-butyl phosphate (DiBP) was only 50% degradated during this period) [24].

The biodegradability of OPs may be affected by several factors, such as the steric effects of some alcohol groups (e.g. iso-butyl group in TiBP and ethylhexyl group in TEHP) that may hinder the attack of hydrolases. Indeed, the degradation of these two compounds in the environment measured to be slower. Similar steric effects may occur in the case of the chlorinated phosphates, which also appear poor biodegradability. Besides, low water solubility and strong sorption tendency can reduce the bioavailability and so the biodegradation of OPs [24].

Some other studies propose that indirect photolysis may happens to OPs in the environment, via reactive oxygen species (e.g. OH radicals, H_2O_2) that can be composed by dissolved organic matter absorbing solar irradiation [24], in a way that the total degradation is a combination of microbial degradation and indirect photolysis.

The concentrations found in several matrixes in the environment are shown at the end of the next chapter in order to be together with the methods of analysis used in each paper.

CHAPTER 2

REVIEW OF METHODS OF ANALYSIS OF FRs IN THE ENVIRONMENT

In contrast to PBDEs, there is not such extensive literature for the analysis of Organophosphate esters. The techniques used for the determination of OPs are either Liquid or Gas Chromatography. Whichever the matrix (water, sediment, dust), the analysis protocols include two parts: first the pretreatment of the sample in order to extract the compounds from the matrix and end up in an extract ready for the injection, and second the instrumental analysis.

2.1 Sample preparation

The sample has to be subjected to a clean-up method, which aims to acquire a homogenized sample that is appropriate for injection to the chromatographic column. There are several reasons that necessitate the sample pretreatment:

- The matrix components may interfere to the analysis and suppress or amplify the analyte's signal
- The sample has to be harmless for the chromatographic column
- The sample has to be compatible with the chromatographic system (e.g. the sample's solvent should be soluble to the LC mobile phase without affecting the retention or the resolution of analytes)
- Sometimes derivatization or preconcentration of the analytes is needed

Many times the pretreatment stage may need more time for its development and implementation than the chromatographic technique. The accuracy, recovery and repeatability of the method might depend on this stage. The best pretreatment method is the one with the minimum steps and which gives the possibility of automatization [29].

The pretreatment protocols found in literature included first of all the homogenization of the sample, then the extraction of the analytes from the matrix, filtration or centrifugation of the extract if needed and finally evaporation to a maximum volume of 1 ml.

2.1.1 Drying techniques

Where wet samples (sediments, biota etc) are under consideration a step of drying and homogenizing of the sample is needed. Freeze drying technique is usually used when this step takes place before the extraction of the analytes, while sometimes passing the extract over anhydrous Na2SO4 is preferred.

2.1.1.1 Freeze drying technique

Freeze drying is a dehydration process that is used mostly in case of sensitive, perishable material. During the freeze drying technique the sample first gets frozen below the water's triple point and then the content water is removed by sublimation. The freeze drying device consists of two chambers connected to each other: the first one contains the sample and the second one, which has much lower temperature, collects the water. For example, if the sample is cooled at -14^{o}C (the vapor pressure at this temperature is 1.36 mm Hg) and at the second chamber there is a surface with temperature -40^{o}C (the vapor pressure at this temperature is 0.097 mm Hg), then the water from the sample will spontaneously go to the second chamber because of the difference in pressures.

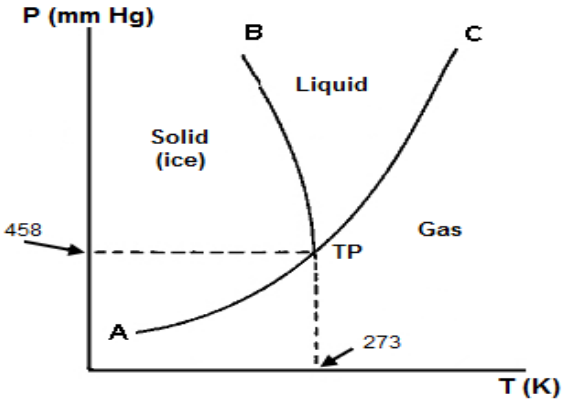


Figure 8: Water's phase diagram (TP is the triple point) [30]

If the freezing process is quick the water crystals formed are small, while in the opposite case the crystals formed are big. The size of the crystals can affect the structure of the freeze dried product. For example, in the case of food, or objects with formerly-living cells, large ice crystals will break the cell walls, resulting in the destruction of more cells, which can result in increasingly poor texture and nutritive content. In this case, the freezing is done rapidly. In

general, when the ice crystals are small, the freeze dried material has small pores, but when the ice crystals are big the final product appears big pores.

In many materials the water exists in two forms: the “free” water and the “crystalline” water. The drying takes place in two phases: in the primary drying the “free” water is removed by the process described in the previous paragraph, while a secondary drying follows, which aims to remove the “crystalline”, unfrozen water. In this phase, the temperature is raised higher and can even be above 0 °C, to break any physico-chemical interactions that have been formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption (typically in the range of microbars, or fractions of a pascal) [30].

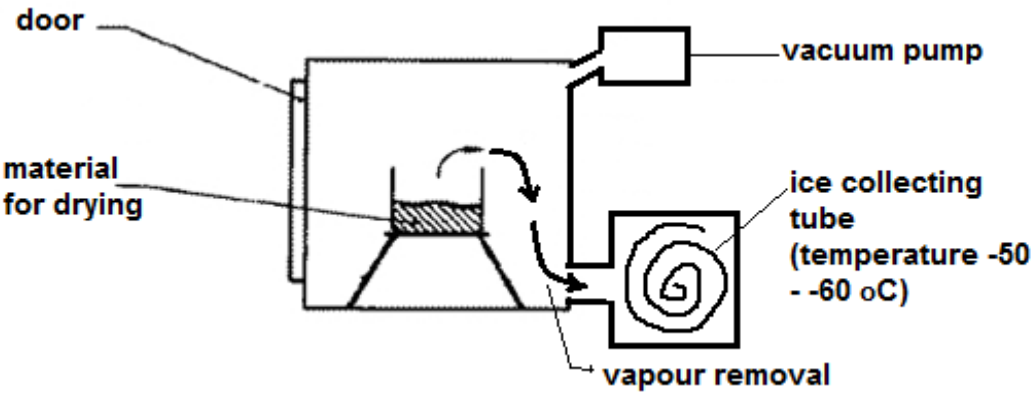


Figure 9: Basic freeze dryer arrangement

2.1.1.2 Drying using anhydrous Na_2SO_4 column

Another way of removing humidity from the sample is to pass its extract over a column made from anhydrous Na_2SO_4. This technique was used by Chen et al [6], while analyzing herring gull eggs for chlorinated and brominated organophosphorous flame retardants.

Na_2SO_4 forms hydrates with 7 and 10 water molecules. It has big water capacity but small drying ability in comparison to other drying media (e.g. $MgSO_4$). It is suitable for primary drying, though. It is non reactive, so suitable for all organic compounds [31].

2.1.2 Extraction techniques

In case of solid material (sediment, dust, biota) the compounds have to be transferred to a solvent so the further analysis is attainable. Although in the water samples usually this step is skipped, there are some references for performing liquid-liquid extraction (LLE) before the clean up step as well. Several extraction techniques were found in literature such as Ultrasound Assisted Solvent Extraction, Microwave Assisted Extraction (MAE), Solid-Liquid Extraction, Liquid-Liquid Extraction (LLE), Accelerated Solvent Extraction (ASE) and Soxhlet Extraction.

2.1.2.1 Ultrasound Assisted Solvent Extraction

Finely divided sample is put in a vessel in ultrasonic bath together with an appropriate solvent. The ultrasound waves have a minimum frequency of 20 kHz and they travel through solid, liquid or gas matter, causing expansion and compression cycles among the molecules. Expansion pulls molecules apart and compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse [32]. That way transfer, diffusion and dissolution phenomena are accomplished.

Advantages of ultrasound assisted solvent extraction are that it is less time and solvent consuming than the classic extraction techniques and that it allows the simultaneous extraction of many samples. Besides, it can be applied to samples that contain thermally unstable compounds.

2.1.2.2 Accelerated Solvent Extraction (ASE)

Accelerated Solvent Extraction (ASE) is a technique for the extraction of solid and semisolid matrices. The sample is enclosed in a cell and during the extraction is filled with a solvent that is heated over its boiling point (temperatures 50−200 °C), causing pressure increase (500−3000 psi), for short time periods (5−10 min). Compressed gas is used to purge the sample extract from the cell into a collection vessel.

The elevated temperature enhances the extraction in two ways: first it increases the solubility and the diffusion rates of the analytes (solubility and mass transfer effects) and second it

disrupts the strong solute-matrix interactions and decreases the solvent viscosity, thus better penetration of the matrix particles can happen (disruption of surface equilibira effects). The elevated pressure on the other hand, facilitates the extraction from samples that the analytes are trapped on matrix pores [34].

2.1.3 Techniques for (further) clean up or preconcentration

With the exception of LLE the previous extraction techniques are used to take the analytes out of solid matrix. If liquid matrix is under consideration different clean up ways are used. Moreover, sometimes the extract is still dirty as along with the alanytes matrix components are also extracted, so further clean up is needed.

2.1.3.1 Solid Phase Extraction (SPE)

Solid Phase Extraction (SPE) is a new technique for sample pretreatment and is based on adsorption of analytes on a solid sorbent. The sorbent, which is placed in a cartridge, is usually silicon dioxide with chemically bonded groups, so as it acquires different adsorption properties. The technique is useful for extraction of analyte(s), separation of them with gradient elution, preconcentration and salts' removal from mixtures of macromolecules.

There are two types of SPE mechanisms, the "retention SPE", where the analyte is retained in the sorbent and elute later and the "non retention SPE", where the matrix is retained in the sorbent and the analyte passes through the column. The first one is the most common and consists of four steps:

1) Washing and conditioning: small portions of a solvent are used to remove any contamination and to activate the bonded groups.
2) Sample addition: the sample is diluted in a poor solvent and is loaded to the cartridge.
3) Washing: a moderately strong solvent is used to remove any matrix compounds that might have been kept on the sorbent.
4) Elution of analyte: the aim is the quantitative recovery of the analyte(s) with a strong solvent.

At the non retention SPE after the sample addition step the analyte elutes and is collected in a tube. The passing of the sample and the solvents through the cartridge is done spontaneously by gravity or mechanically by syringes or vacuum devices [29, 35].

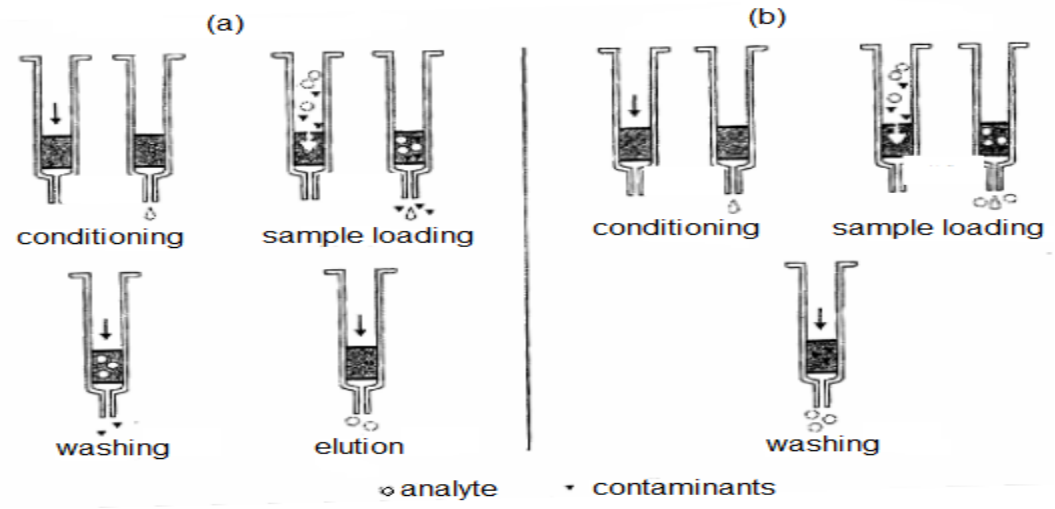


Figure 10: Mechanisms of (a) retention SPE and (b) non-retention SPE [29]

2.1.3.2 Gel Permeation Chromatography (GPC)

Gel Permeation Chromatography (GPC), also known as Size Exclusion Chromatography (SEC) or Gel Filtration Chromatography (GFC), is a chromatographic technique that separates dissolved molecules on the basis of their size by pumping them through specialized columns containing a microporous packing material. The pore sizes of these particles are controlled and available in a range of sizes. The smaller the molecule the more porous it passes through and so the longer retention time it has, such as it separates from other molecules.

As the sample is separated and eluted from the column, it can be characterized by a single concentration detector (Conventional Calibration) or series of detectors (Universal Calibration and Triple Detection). GPC is the most convenient method for determining important parameters (number average molecular weight, weight average molecular weight, molecular weight distribution) of natural and synthetic polymers, biopolymers, proteins or nanoparticles. These values are important, since they affect many of the characteristic physical properties of a polymer.

Like all chromatographic systems, a GPC instrument consists of a pump, that pumps the mobile phase containing the polymer, an injector, that injects the sample, a separation column, which efficiently separates the sample components from one another, a detector, that corresponds to a certain property of the eluate and data processing equipment, which automatically calculates, records, and report numerical values for Mz, Mw, Mv, Mn, and MWD.

However, sometimes GCP technique can be used to get rid of small or big matrix particles (e.g. sulfur compounds) that can interfere to the following analysis, especially when dirty samples are under consideration (waste water, sludge etc). In that case after fractioneering of the sample the time fractions that contain the matrix compounds are rejected [36, 37].

2.1.4 Solid matter removal

Usually, after the extraction of a sample the extract still contains solid particles that have to be removed before the injection to the chromatographic column to avoid clogging it. The filtration can be conducted by passing the sample over a filter, a Na_2SO_4 column, SPE or by centrifugating it. The first methods were described in previous sections (2.1.1.2 and 2.1.3.1).

2.1.4.1 Centrifugation

A good way to get rid of matrix particles when the analyte is diluted or has significant differences in mass or density with them, is to centrifugate the sample and keep the supernatant for further analysis. The solution of the sample is placed in a tube and subjected to centrifugation under high rotation rate, which is measured in rounds per minute (rpm). Many particles, given time, will eventually settle at the bottom of the tube in response to gravity. The centrifugal field is measured as xg, gravity (Relative Centrifugal Field, RCF). The higher the mass and density of the particle the higher the speed obtained inside the centrifugal field and the faster the bottom of the tube is reached.

2.2 Chromatography

The basic technique for OPs analysis is Chromatography. The term Chromatography includes a variety of techniques that are used to separate substances with similar chemical properties out of a mixture. Many of these separations are not able to happen with other techniques (e.g. sedimentation, distillation, extraction). In all chromatographic separations the sample travels along with a mobile phase, which can be gas, liquid or supercritical fluid. The mobile phase has to pass through a structure holding another material called the stationary phase and can be either liquid or solid. The various constituents of the mixture are differentially distributed between the two phases (different partition coefficients), resulting in different retention on the stationary phase and thus different traveling speeds through the mobile phase and separation. The constituents that are hold stronger by the stationary phase are travelling slower through the mobile phase.

Chromatography may be classified according to its aim, state of stationary and mobile phase, separation mechanisms and other parameters. The aim of chromatographic procedure may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use and is thus a form of purification (e.g. for use in pharmaceutical preparation). The aim of analytical chromatography is to detect presence and amount of certain component in the mixture. The two are not mutually exclusive.

Stationary phase may be a sheet of paper (paper chromatography) or a thin layer of porous material on a surface of metallic or glass plate (thin layer chromatography), a granular filling of a tube (column chromatography) or as a layer in a capillary tube (capillary chromatography). Mobile phase can be liquid (liquid chromatography), gaseous (gas chromatography) or supercritical fluid (supercritical fluid chromatography).

The mechanism used for the separations is usually partitioning of the analytes between the two phases (liquid stationary phase) and adsorption to the stationary phase (solid stationary phase), but also other mechanisms have been developed: ion exchange, affinity with the modified stationary phase, size exclusion, hydrophilic interactions etc [29, 38, 41].

Column chromatography

As mentioned, in column chromatography the stationary phase is held in a tube through which the mobile phase and the sample pass. Special eluent pump or gravity forces the

eluent to travel through the column. During the separation significant dilution of the analytes takes place, so the detectors have to be sensitive enough.

Some important parameters for chromatographic separations are the following:

- Dead time (t_M): the time needed for the unretained species to reach the detector
- Retention time (t_R): the time needed for a retained substance to reach the detector
- Corrected retention time (t'_R): $t'_R = t_R - t_M$
- Peak Width: the width on the peak basis
- Capacity factor: $k' = (t_R - t_M) / t_M$ (best values 1 - 5)
- Linear mobile phase velocity: $u = L / t_M$
- Partitioning constant: $K = C_S / C_M$ or $K = k'V_M / V_S$ (M stands for mobile phase and S for stationary phase)
- Selectivity factor: $\alpha = ((t_R)_A - t_M) / ((t_R)_B - t_M)$
- Column Resolution, R_S: measures the column ability to separate two analytes $R_S = 2[(t_R)_B - (t_R)_A] / (W_A + W_B)$ or $R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_B}{1+k'_B}\right)$
- Column length, L
- Plate number, N: $N = 16\,(\frac{t_R}{W})^2 = 5{,}54\,(\frac{t_R}{W_{1/2}})^2$ or $N = 16\,R_S^2(\frac{1}{\alpha-1})^2(\frac{1+k'_B}{k'_B})^2$
- Plate height, H: $H = L / N$
- Asymmetry factor, A_f: $A_f = \frac{a}{b}$, where a and b are the peak widths at the two sides of the central vertical axis, usually on 10% of peak height (best values: 0,8 - 1,2)

There are many mathematical equations that relate the plate height with the column parameters and some of them indicate ways of improving column's performance as well. A very successful one is van Deemter equation: $H = A + B/u + Cu = A + B/u + (C_S + C_M)\cdot u$,

where factors *A, B and C* describe the multiplicity of pathways that a molecule or ion can follow through the particles of stationary phase, the longitudinal diffusion and mass transfer phenomena respectively ($C_S \cdot u$ refers to mass transfer in stationary phase, while $C_M \cdot u$ in mobile phase).

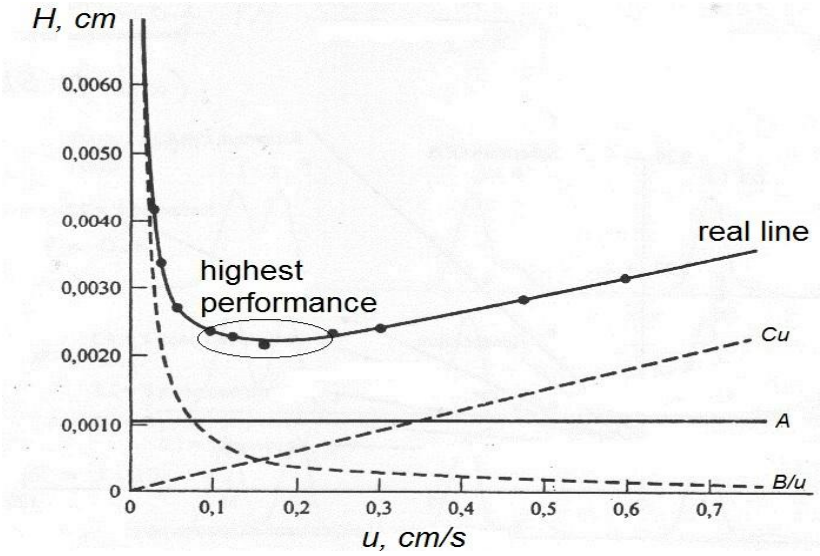


Figure 11: van Deemter diagram [38]

In general, column performance is affected by the changes in N, H, k' and α. One way of improving it is by increasing the number of plates, N, either by increasing the column's length, L, (resulting to longer retention times though) or by decreasing the plates' height, H. Decrease in H can also be done by decreasing the packing particles' size. Where liquid mobile phases are under consideration decrease in H can also happen with a decrease of solvent viscosity and thus increase of diffusion factor in mobile phase.

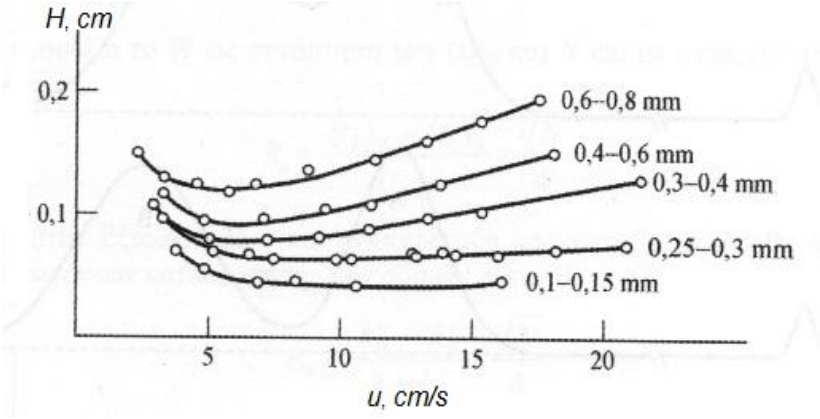


Figure 12: Effect of stationary phase particles size to the plate height, H [38]

Higher k' generally means better resolution (R_S) but longer retention time. The best k' values are in the range of 1 and 5. The easiest way of improving R_S is by increasing the temperature in Gas Chromatography and by changing the mobile phase in Liquid Chromatography.

However, when α≈1, improvement of k' and N is not enough for sufficient separation. So increase in α has to be done together with keeping k' in its best range. Increases in α can be

conducted by changing the mobile phase (or the pH of mobile phase), temperature, stationary phase or using specific chemical effect [38, 29, 41].

2.2.1 Gas Chromatography (GC) [29]

In gas chromatography the sample is vaporized and injected on top of the chromatographic column. The elution is carried out with inert gas, which is the mobile phase. In contrast to other types of chromatography, here the mobile phase does not interact with the analyte molecules and its only purpose is to carry the sample through the column.

There are two types of gas chromatography: gas-solid chromatography, where the retention of the analytes is result of their adsorption to the solid stationary phase. This type is not commonly used though, because in most times the retention of the analytes is permanent causing the damage of the column. The other type is the gas-liquid chromatography where the stationary phase is liquid and the separation mechanism is the partitioning between the two phases. The analyte's speed through the column depends on its affinity to the stationary phase.

A significant factor in GC is the carrier gas flow, as it affects the column's sensitivity. There is an optimum flow. If the flow is higher than this, the peaks may come too close to each other and thus not good separation will happen, while if the flow is less than the optimum the peaks are too wide resulting low sensitivity. The flow also affects the retention time. 1% change in carrier gas flow causes 1% change in retention time, so the flow must be stable during the separation. This can be done by controlling the inlet gas pressure and the flow speed. Usually the gas pressure is high in the entrance and low in the exit of the column.

2.2.1.1 Instrumentation

Gas traps

Gas traps are usually placed before the gas inlet in the column, to remove humidity or oils that may have been entered in the gas cylinders during their filling. These contaminants may interact with the stationary phase and give more peaks or cause increased noise at the detector.

Columns

A chromatographic column consists of the tubing material which can be stainless steel, glass or fused silica and the stationary phase, which can be solid substrate, liquid, porous polymer or sorbent material.

The columns may be either packed or capillary (or open tubular columns). The packed columns are wider and shorter (inert diameter 2-4 mm, length 1-4 m). They are packed with solid substrate which is covered with the liquid layer of the stationary phase. The capillary columns on the other hand are thinner and longer (inert diameter 0.10-0.53 mm, legth 10-100 m). The stationary phase layer is 0.2-1 μm and is kept on the walls. Generally the capillary columns give better separations and more acute peaks [29].

Sample inlet system

The sample has to have the right quantity and be injected as a low width zone, otherwise the peaks will be too wide to separate. The common injection tools are microsyringe and bulb. The injection area has to be kept in high temperature, so the sample is vaporized immediately.

The injection can be done on column (the syringe is placed on top of the column) or off column. In capillary columns, where small amount of sample is needed, split injector is usually used. The column is placed in a glass tube where the carrier gas and the sample enter, but only a portion of them ends up inside the column.

Detectors

The eluate of the column is led to a detector for determination of the content. The ideal detector should have good sensitivity (in mass or concentration), specificity, stability and reproductivity, wide linear area, low noise, wide enough area of temperatures. Commonly used GC detectors are Thermal Conductivity Detector (TCD), Flame Ionization Detector (FID), Electron Capture Detector (ECD), Photoionization Detector (PID), Flame Photometric Detector (FPD), Nitrogen-Phosphorus Detector (NPD) and Mass Spectrometer (MS).

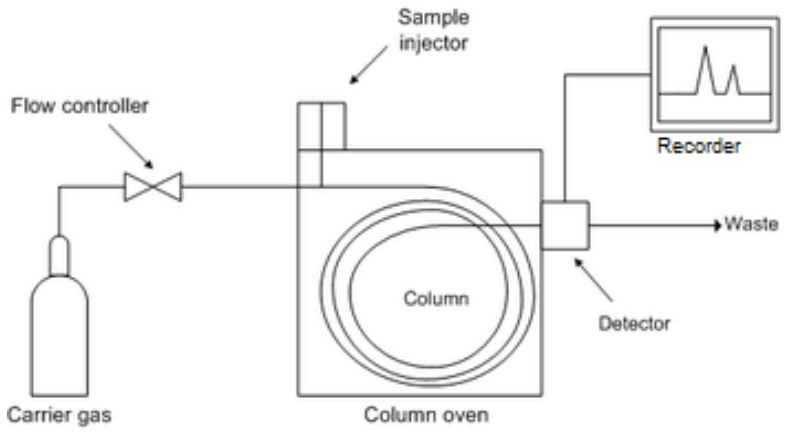


Figure 13: Basic GC arrangement

2.2.1.2 Coupling GC with MS

In general the GC coupling with MS is easy. The flow rates of the capillary columns are low enough and compatible with the MS ion source, so the eluate can be directly injected to it. Nevertheless, for eluates out of packed or capillary megabore columns a jet separator is needed for the removal of the biggest portion of the carrier gas away from the analyte [38].

2.2.2 Liquid Chromatography (LC) [38, 39]

The term Liquid chromatography includes all the types of chromatography in which the mobile phase is liquid. During the development of LC scientists cleared out that the column performance could be significantly increased with a decrease in the size of the column packing material. The term High Performance Liquid Chromatography (HPLC) is used to separate the newer techniques from the old ones, where the columns were out of glass, their size bigger (diameter 1-5 cm, length 50-500 cm) and the flow rate slow, caused by gravity. Common LC mechanisms are partitioning, ion-exchange, adsorption, size exclusion, affinity, hydrophilic interactions.

2.2.2.1 Instrumentation

Mobile phase bottles and systems of solvent processing

The mobile phase solvents are placed in bottles that are equipped with systems for removing the diluted gases. The gases can produce bubbles that may cause peak broadening or may harm the detector. The degassing may be done by vacuum, helium, ultrasounds, heating or reflux.

Pumps

The solvent pumps provide stable flow speed, without pulses, and control the flow (flow repeatability ≤ 0,5%). The pressure developed can be until 600 psi. For gradient elution more than one pump are needed.

Columns

The analytical columns are of smaller size (inert diameter 2-6 mm, length ≤ 30 cm) in contrast to preparative columns (inert diameter > 6 mm and length 25-100 cm). Both are usually made of stainless steel.

The packing materials consist of either irregular or spherical particles. The more spherical and smaller the particles and the smaller the size distribution, the higher the column performance is (more acute peaks). There are several kinds of particles:

- totally porous microspheres: the most common ones, they consist of smaller connected particles
- micropellicular particles: they have a solid, rigid, non-porous core and a very thin stationary phase skin around it. They appear very high performance with macromolecules, because they facilitate mass transfer. However, they have low sample capacity because of small specific surface area. Columns with this kind of particles give very acute peaks
- perfusion particles: they have very big pores and a net of smaller pores connected to each other, among the bigger pores. The transfer of the analytes through the pores is governed by flow and diffusion, a fact that minimize the peak broadening. This way big particles act like small ones

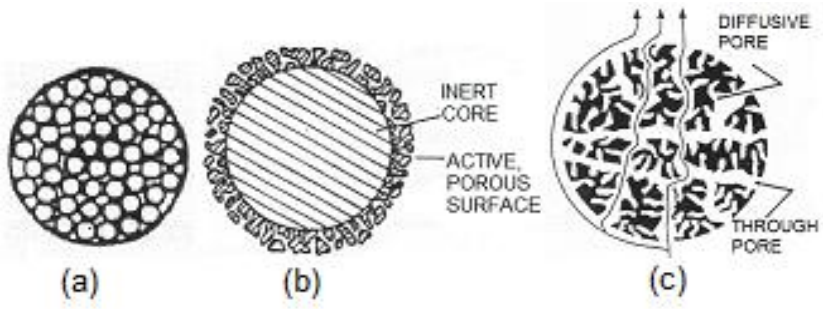


Figure 14: (a) totally porous microsphere (b) porous layer bead (c) perfusion particle [39]

Packing materials can be either inorganic, such as silicon dioxide ($(SiO_2)_x$, silica), aluminum oxide ($(Al_2O_3)_x$, alumina), zirconium oxide ($(ZrO_2)_x$, zirconia), controlled-pore glass and graphitized carbon, or organic, such as styrene-divinylbenzene resin, methacrylates and agarose.

Development in packing materials has led to monolithic columns, which consist of a single porous, very high purity piece of SiO_2. This piece has macro (~2μm) and mid-pores (~13 nm). The macropores reduce the column pressure and allow higher flow rates and thus shorter analysis time. The mid-pores provide big surface area so better separations.

Moreover, since the effect of the stationary phase particles size to the plate height is known, and thus to column performance, there is an increase trend of using smaller particles. For example, rearchers from the Schering-Plough institute developed a quick HPLC technique for the analysis of pharmaceutical compounds. In this technique the stationary phase particles have 2,7 μm diameter and they consist of compact SiO_2 beads, coated with porous SiO_2 being 0,5 μm thick. The method is comperable to that of UHPLC (which is described in later section) but it uses simpler and cheaper instrumentation because the pressure at the column edges is lower.

Usually a pre-column (or guard column) is placed before the analytical column. The pre-column removes the solvent's impurities and sample's constituents that permanently bond to the stationary phase. In liquid-liquid chromatography it also saturates the mobile phase with the stationary phase, so the solvent losses from the analytical column are minimized. The pre-column's stationary phase is similar to the one of analytical column, but the particles size is bigger, so they are cheaper and minimize the pressure decrease.

Detectors

The ideal detector for LC must have the same properties as the one for GC (section 2.2.1), with the exception of working in wide temperature area. Common LC detectors are Absorption detector (with filters at UV-Vis or IR), Fluorimetric detector, Refractive Index detector, Conductivity detector, Electro-chemical detector, Light Scattering detector, Mass Spectrometer.

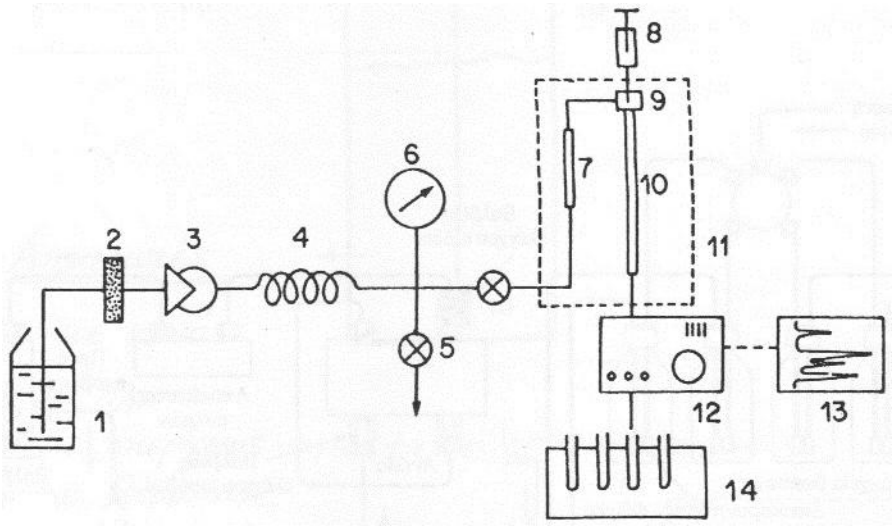


Figure 15: Schematic diagram of an HPLC unit [29]

1= solvent reservoir; 2= sintered metal frit; 3= high pressure pump (may incorporate flow meter); 4= pulse damper; 5= drain valve; 6= manometer; 7= pre=column (optional); 8= injection syringe; 9= injection valve; 10= column; 11= thermostat oven (optional); 12= detector; 13= data acquisition; 14 fraction collector (optional)

2.2.2.2 Ultra High Performance Liquid Chromatography (UHPLC or UPLC)

Ultra HPLC uses the same principle for separations and instrumentation as the conventional HPLC, but the stationary phase particles size is smaller than 2 μm. The result is a great increase in the column performance and thus in sensitivity, resolution and speed of analysis. However, the small particles cause very high pressure at the column edges, so high pressure pumps are needed. Ideally the pumps should provide stable and non pulsed mobile phase flux.

Columns

UHPLC columns may be capillary or steel. Capillary columns need lower mobile phase flux and smaller sample amount. Thus they are more easily connected to mass spectrometry detectors. Moreover, this kind of columns diffuse better the heat developing due to high values of mobile phase flux and pressure at the edges. As a result, steel columns tend to have less applications, they have the advantage of coping with bigger amount of sample though [29].

2.2.2.3 Reversed Phase Partitioning Liquid Chromatography [38, 39]

Partitioning between two solvents is the most commonly used chromatographic mechanism. The solvents must be immiscible to each other, so there must be a difference in polarity. Two types of chromatography can then be distinguished: when the stationary phase is polar the mobile phase has to be non polar (normal phase chromatography) and vice versa, when the stationary phase is non polar the mobile phase has to be polar (reversed phase chromatography).

During the first period of HPLC use, analysts were using SiO_2 as stationary phase and a non polar solvent as mobile phase (e.g. hexane with dichloromethane). In such type of columns knowing the chemistry of SiO_2 surface was necessary for good separations. Furthermore, a strict control in water's portion in mobile phase is needed, and in vulnerable cases even a change in laboratory's humidity may change the whole chromatography. Another problem is related to the separation of aqueous solutions, such as environmental or biological samples. Most of them are not sufficiently diluted in non polar solvents used as mobile phase in normal phase chromatography.

In reversed phase chromatography the stationary phase is turned non polar and polar solvents are used as mobile phase. In such stationary phases the SiO_2 substrate is covered with a non polar solvent and the elution is related to the increase of hydrophobicity of analytes.

Stationary phases

The stationary phases in reversed phase chromatography usually consist of SiO_2 particles on which a non polar solvent is hold. At the beginning these solvents were physically held on the

SiO_2 substrate, but afterwards they were chemically bonded to it (bonded phases). The silanol surface groups (-Si-OH) can react with a silanized reagent. The reagent's functional group is usually Cl or an ether. The molecular part responsible for the anlytes' retention (-R) is usually an alkane moiety and found at the non reactive side of the reagent. The most common alkane groups added to SiO_2 surface are the $C_{18}H_{37}$, but nowadays a variety of hydrocarbon chains that provide different column polarities is available in the market. Because of steric hinders not all the -Si-OH groups react and these free silanol groups (that still can react with small molecules found in the sample) can cause increased polarity and changes in peaks. Thus, reaction with a smaller silanized reagent (e.g. $SiClCH_3$) takes place to cover the free silanol groups (endcapping).

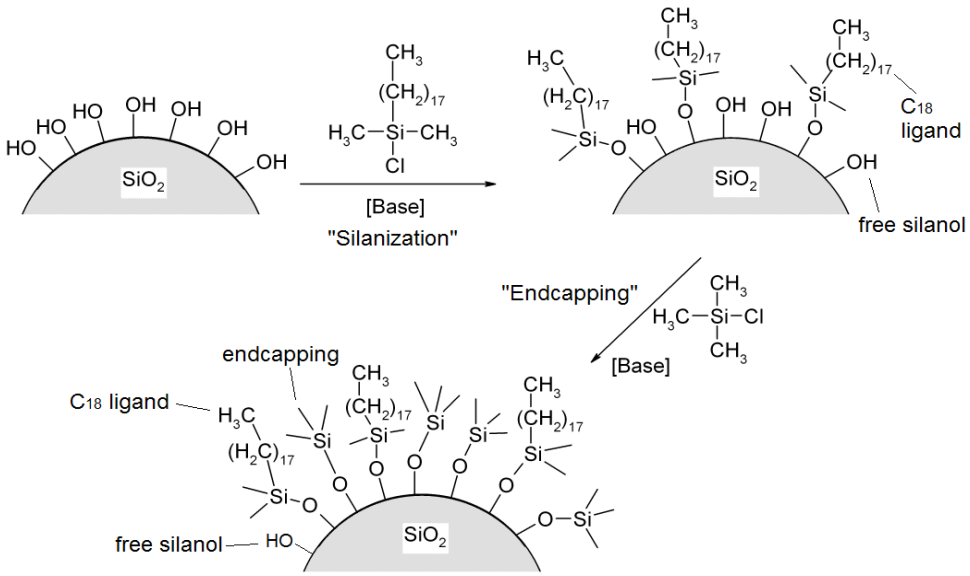


Figure 16: Bonded phase preparation

However, stationary phases based on SiO_2 are not stable at extreme pH values. At pH<2 the Si-O-Si bond becomes unstable, while at pH>8 silica is diluted, so the proposed pH range is between 2 and 8.

Mobile phases

In reversed phase chromatography the mobile phase consists of polar solvents. It usually is a mixture of water with an organic solvent, with the most common being methanol and acetonitrile, followed by tetrahydrofuran, dimethylformamide and dioxane. Many times buffers are added to this mixture to avoid ionization of some analytes. The mobile phase's composition can stay the same during the separation (isocratic elution) or change (gradient

elution). Gradient elution is usually used in complex mixtures in which not all the analytes separate sufficiently with the same mobile phase.

2.2.2.4 Coupling LC with MS

The incompatibility of solvents or buffers and of the relatively high flow rates of LC mobile phase with those that can be used in the ionization source of MS constitute two of the early problems in coupling LC with MS. New ionization sources (ESI, MALDI, APCI) can handle higher flow rates though.

Because of high vacuum demands MS ionization sources receive low volumes of liquid. For example, while standard ESI sources can generally handle flow rates up to 1 mL/min, lower flow rates in the range 0.05 to 0.2 mL/min result in improved sensitivity. To achieve low flow rates columns of smaller diameter (1.0 or 2.1 mm) are employed, resulting in less mobile phase consumption and higher sensitivity and resolution as well (capillary columns). Another way to connect LC with MS is to interpolate an injection system between LC column and MS ionization source. This can either split the LC eluate and inject a small portion of it in the MS source, or insert the LC eluate in a vaporization chamber prior to MS source, or utilize the thermospary technique. This conjunction allows the direct injection of the LC eluate in the ionization source. The liquid vaporizes during its passing through a stainless steel heated capillary tube, forming a fine particles spray of solvent and analyte, which finally ionizes in the source.

Typical solvents used in conventional LC separations are usually compatible with MS when attention is paid to the grade of solvent's purity. For example a grade might be suitable for LC-UV detection because the solvent is transparent to the UV range, but incompatible with LC-MS detection because it may contain impurities that affect the MS quality. Buffers containing inorganic ions such us phosphate, sodium or potassium acetate can create products that easily contaminate the ion source, so they should be replaced with other, more compatible with MS system, such as those based on ammonium acetate, ammonium formate or ammonium bicarbonate [38, 55].

2.3 Detection techniques

As mentioned several types of detectors can be connected at the end of GC or LC columns. The technique that is increasingly used in latest years is mass spectrometry. Indeed, in almost all bibliographic references the detection is conducted by mass spectrometers.

2.3.1 Mass Spectrometry - Theory

Mass Spectrometry is probably the technique with the widest variety of applications because it provides information on the:

- elemental composition of the sample
- structures of inorganic, organic, organometallic and biological molecules
- qualitative and quantitative composition of samples
- structure and composition of surfaces
- isotope ratio of elements in samples

It is based on the ionization of atoms or molecules or on the production of ionic molecular fragments and the recording of the relative ionic current intensity corresponding to a certain m/z ratio.

The molecular mass spectrum is a bar diagram that relates the peaks' intensities to the m/z ratio. The peak with the highest intensity is called the base peak and is given the value 100. The other peaks' heights are given as a percentage of the height of the base peak [38, 39].

2.3.2 Mass Spectrometer

A mass spectrometer consists of:

1) Inlet system: sample inlet in the ionization source
2) Ionization source: produces ions in gaseous phase
3) Focus lens: the ions are aligned and injected to the mass analyzer
4) Mass analyzer: separates the ions according to the m/z ratio
5) Detector: transfers the ionic current into electrical signal
6) Recorder

7) Vacuum pumps: the whole system (parts 2-5) has to be in vacuum because ions can interact with air / carrier gas and destroy themselves or produce new ions and increase the noise [39]

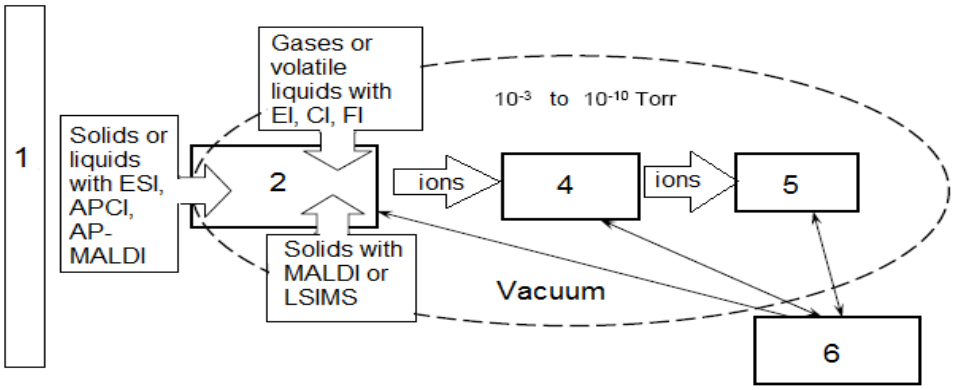


Figure 17: Schematic presentation of MS instrument [39]

2.3.3 Ionization sources

Ionization sources can be classified in two ways: first in gas phase or desorption sources and secondly in harsh or soft sources.

Gas phase sources (the sample first comes to the gaseous phase and then is ionized):

- Electron Impact (EI)
- Chemical Ionization (CI)
- Field ionization (FI)

Desorption Sources (the sample found in solid or liquid phase is directly transformed to gaseous ions):

- Field Desorption (FD)
- Electrospray Ionization (ESI)
- Matrix-Assisted Desorption/Ionization (MALDI)
- Plasma Desorption (PD)
- Fast Atom Bombardment (FAB)
- Secondary Ion Mass Spectroscopy (SIMS)
- Thermospray Ionization (TI)
- Plasma

The desorption sources' advantage is that they can be applied in non volatile or thermally unstable samples.

Harsh sources transfer high amount of energy to analyte's molecules and thus many fragments with m/z lower than the molecular ion's are produced as a result of extensive bonds' break. Hard sources are mainly used to identify a molecule's structure. In contrast, soft sources provide lower amount of energy and cause limited fragmentation. They mostly give information about the molecular ion mass [38, 39].

2.3.3.1 Electron Impact (EI)

Electrons are produced by a heated thread made of tungsten or rhenium and they are accelerated by 70 V voltage. Electrons and molecules routes are perpendicular to each other and they meet at the center of the source where impact and ionization take place. Molecular ion is produced when high energy electrons come very close to it and cause electron detachment due to electrostatic repulsion. EI is a hard source and not many molecular ions finally survive, it is very useful though to produce libraries with spectrums of thousands of compounds [38, 39].

Ionization: $M + e \rightarrow M^{\bullet +} + 2e^-$

Fragmentation: $M^{\bullet +} \rightarrow EE^+ + R^\bullet$ or $M^{\bullet +} \rightarrow OE^+ + N$

where: $M^{\bullet +}$: molecular ion

EE^+: even ion - ion with even number of electrons

OE^+: odd ion - ion with odd number of electrons

N: molecule

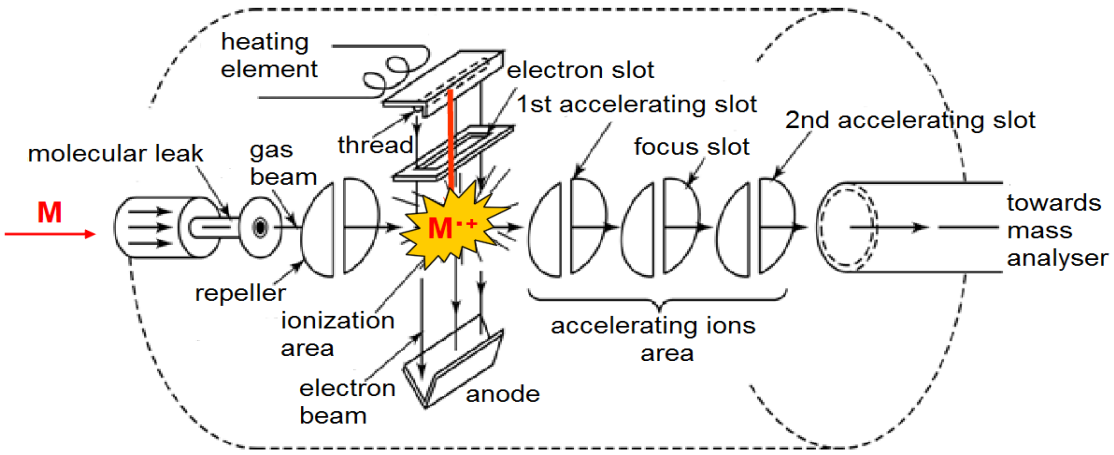


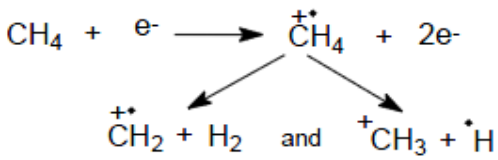
Figure 18: EI source arrangement [38]

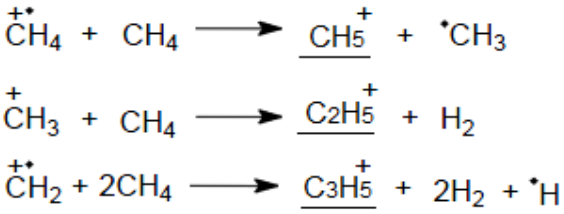
2.3.3.2 Chemical Ionization (CI)

In chemical ionization a gaseous reagent (usually CH_4, NH_3, N_2) is first ionized by high energy electrons. These ions crash to the sample's gaseous molecules and ionize them via proton, hydride or charge transfer reactions, addition reactions or even nucleophilic substitution reactions. The arrangement of a CI source is similar to the one of EI, with the difference of adding a vacuum pump in the ionization area and reducing the slot width that leads to the mass analyser. This way the analyte's pressure is below 10^{-5} torr, while the reagent's pressure is around 1 torr, and thus the reagent to sample concentration ratio is kept 10^3 to 10^4 [38, 39].

Example: Ionization of a molecule, MH, using CH_4

1st step: ionization of CH_4:





2nd step: molecule ionization:

charge transfer: $CH_4^{\bullet+} + MH \rightarrow MH^{\bullet} + CH_4$ $[M^{\bullet+}]$

proton transfer: $CH_5^+ + MH \rightarrow MH_2^+ + CH_4$ $[M + 1]$

$C_2H_5^+ + MH \rightarrow MH_2^+ + C_2H_4$

hydride transfer: $C_2H_5^+ + MH \rightarrow M^+ + C_2H_6$

ionic complexes production: $C_2H_5^+ + MH \rightarrow [C_2H_5{:}MH]^+$ $[M + 29]^+$

$C_3H_5^+ + MH \rightarrow [C_3H_5{:}MH]^+$ $[M + 41]^+$

2.3.3.3 Electrospray Ionization (ESI) [38, 39, 42 - 46]

Electrospray (ESI) mass spectrometry was described for first time in 1984 and nowadays is one of the most employed techniques for the analysis of biomolecules, such as polypeptides, proteins and oligonucleotides with molecular weights higher than 100.000 Da, and of inorganic compounds and synthetic polymers as well. Its extensive use is a result of its easy coupling with liquid chromatography and electrophoretic devices and also of its ability to detect and give valid results for both small and big molecules.

Electrospray ionization is conducted at atmospheric pressure and temperature in a device shown in the picture below.

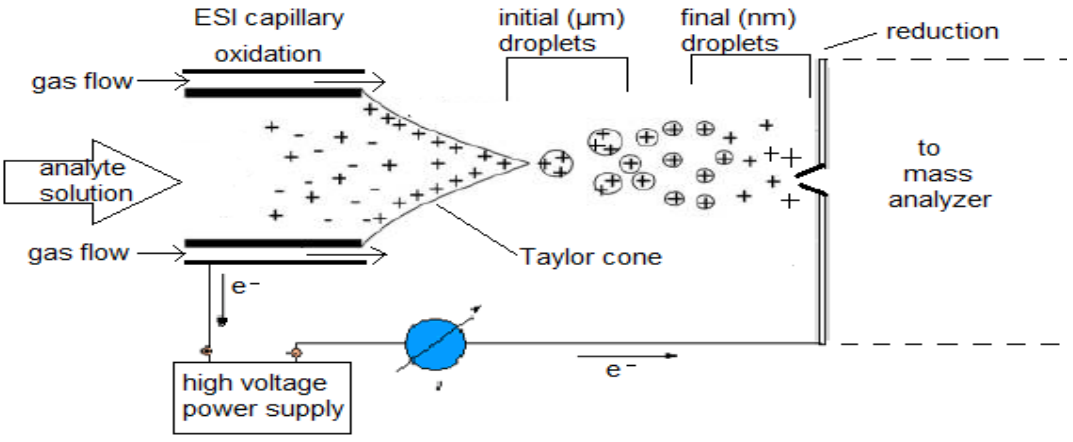


Figure 19: Electrospray ionization process [42, 46]

The solution is injected through a stainless steel capillary needle with speed of some μl/min. Between the needle and a counter electrode, placed in a distance in the range of 3-10 mm, a voltage of several KV is applied. The solution at the needle tip forms a Taylor cone that emits a mist of droplets. The spraying process is assisted by a coaxial gas flow. The initial ESI droplets have radii in the micrometer range. Considering positive ion mode, the voltage at the needle is positive, so each droplet is positively charged due to the presence of excess ions that can include H^+, NH_4^+, Na^+ and K^+. Protons are often the main contributor to the droplet charge, partly because many analyte solutions are acidic. More importantly, protons are generated at the metal/solution interface inside the capillary (e.g., $2\ H_2O \rightarrow 4\ H+ + 4\ e- + O_2$). These ions together with the solvent oriented dipoles (e.g. water's dipoles) gather at the surface of the droplet. The analytes ions mainly prefer the droplet interior as there they are well solvated, and only a small amount of them are found at the droplet periphery.

Three mechanisms are used to explain many ions' behaviors inside the device:

- <u>The ion evaporation mechanism (IEM)</u>:

The IEM model assumes that the formed charged droplets shrink by evaporation until the field strength at their surface is sufficiently large to cause the ejection of small solvated ions from the droplet surface. It is believed that this mechanism is mainly followed by species with low molecular masses.

- <u>The charge residue mechanism (CRM)</u>:

In this mechanism the charged droplets are supposed to contain only one analyte molecule that it is well solvated by solvent's molecules. The droplets shrink by evaporation to dryness and as the last solvent shell disappears, the charge of the vanishing droplet is transferred to the analyte. This way, multiply charged ions are produced and thus more complicated spectra are taken. This model is mainly followed by large globular molecules, such as natively folded proteins.

- <u>The chain ejection mechanism (CEM)</u>:

Most proteins are spontaneously folded in a compact structure, where the charged and polar moieties are coming at the surface, facilitating water interactions. Although natively folded proteins are following the CRM model, unfolded ones are released in the gaseous phase via another mechanism, described as the CEM model. Unfolded proteins can occur in the

solution as a result of an acidic mobile phase for example. These proteins are highly disordered and non polar residues that were previously gathered in their core are now solvent accessible. The previously hydrophilic compact protein surface is now turned to extended and hydrophobic. This largely hydrophobic character makes it unfavorable for unfolded proteins to reside in the droplet interior. Instead, when placed in a Rayleigh-charged nanodroplet, unfolded chains immediately migrate to the droplet surface. One chain terminus then gets expelled into the vapor phase. This is followed by stepwise sequential ejection of the remaining protein and separation from the droplet.

A problem in electrospray ionization is the so-called “matrix effect”, which means the suppression or the enhancement of the analyte’s signal. One of the main proposed explanations of this phenomenon is the competition for the ESI droplet surface between the analyte molecules and matrix compounds. There are two types of ions that cause matrix effect: the first type of ions includes ions that are ubiquitous in all spectra, even if no analyte or sample is injected. These ions originate from the impurities of solvents, buffers, plastic and glassware used. The second type of ions consists of ions present in sample’s matrix.

Matrix effect can be partially treated more effective sample preparation that leads in cleaner samples, or by improving the LC resolution of the analyte and matrix compounds. Furthermore, matrix effects can be taken into account in calculations, by using several quantification techniques, such as matrix matched calibration, standard addition, the internal standard method, post-column standard infusion, extrapolative dilution and accounting via uncertainty.

2.3.4 Mass analyzers [38, 39]

A mass analyzer separates ions according to the mass to charge ratio (m/z). Every moment, the ionic current recorded is a result of only one m/z. There are two types of mass analyzers:

- Continuous mass analyzers (they send ions to the detector continuously, from the biggest m/z to the smallest - space separation):
    - Quadrupole (Q)
    - Magnetic sector analyzer
- Pulsed mass analyzers (they send ions to the detector in pulses - time separation):

- Ion trap (IT)
- Time of flight (TOF)
- Fourier-transform ion cyclotron resonance (FTICR)

The perfect mass analyzer should separate masses with very little different and allow the transit of enough ions so the ionic current is straight measurable.

Resolving Power, Resolution and Mass Accuracy

Resolving Power is the capacity of the instrument to distinguish two just separable peaks, m and m+Δm: $R = m / \Delta m$

Δm can be the Full Width at Half Maximum (FWHM) for low resolving power instruments.

Resolution is the difference between two nearby m/z values: $(m_2 - m_1) / m_1 = \Delta m / m_1$ and is expressed in ppm

Mass accuracy is the difference between the mass calculated theoretically and the mass measured in MS.

Quadrupoles and ion traps are low resolution instruments, while time-of-flight, magnetic sector analyzers and FTICR are high resolution instruments [39].

2.3.4.1 Quadrupole [39, 47-49]

A typical quadrupole consists of four parallel, metallic rods arranged in a form of square. Adjacent rods have opposite voltage polarity applied to them. The voltage applied to each rod is the summation of a constant DC voltage (U) and a varying radio frequency (RF) ($V_{rf}\cos(\omega t)$), where ω is angular frequency of the radio frequency field. The ions are shot axially to the rod system and accelerated by a relatively small potential ranging from 10 to 20 volts. The applied quadrupole field deflects the ions in the X and Y directions, causing them to oscillate and describe helical trajectories through the mass filter. For given DC and AC voltages, only ions of a certain mass to charge ratio pass through the quadrupole filter. For all other ions the oscillations become infinite and they collide to the rods.

The stability of the oscillating ions is determined by the magnitude of two parameters (a) and (q) which are defined by the following equations:

- $a = 8eU/(mr_0^2\omega^2)$ (represents the constant voltage) and
- $q = 4eV_{RF}/(mr_0^2\omega^2)$ (represents the radio frequency)

  r_0 is the field radius

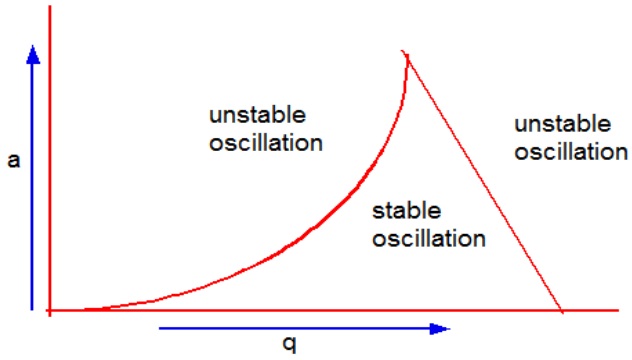


Figure 20: Stability diagram of a certain m/z [49]

A mass spectrum is obtained by monitoring the ions passing through the quadrupole filter as the voltages on the rods are varied. There are two methods: varying ω and holding U and V constant, or varying U and V (U/V) fixed for a constant ω. Dividing the two previous equations by one another yield: $a/q = 2U/V_0$, which is the slope of the so-called load line of the quadrupole:

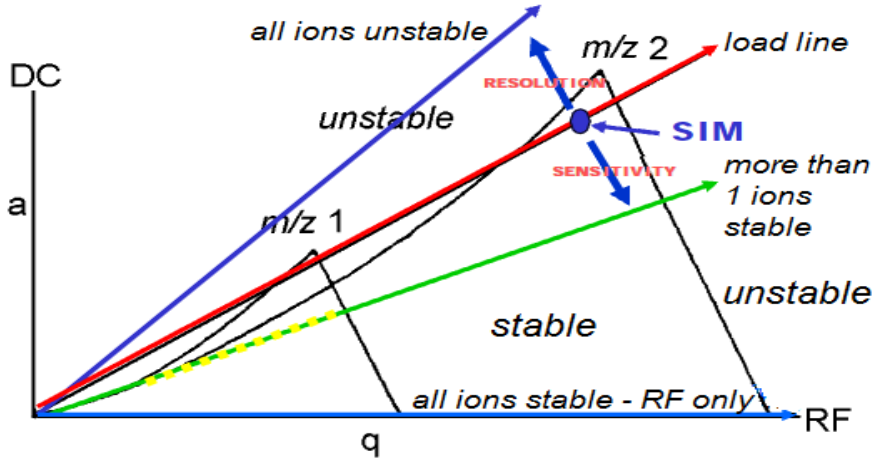


Figure 21: Ion stability diagram for two ions [39]

By changing the DC/RF ratio the load line is moving upper, so the resolution is increasing, or below, so the sensitivity is increasing.

The quadrupole can operate in two different modes:

- The singular ion monitoring (SIM) mode, where only one ion is monitored

- The full scanning mode or RF only mode, where no DC voltage is applied and all ions can pass through the mass filter.

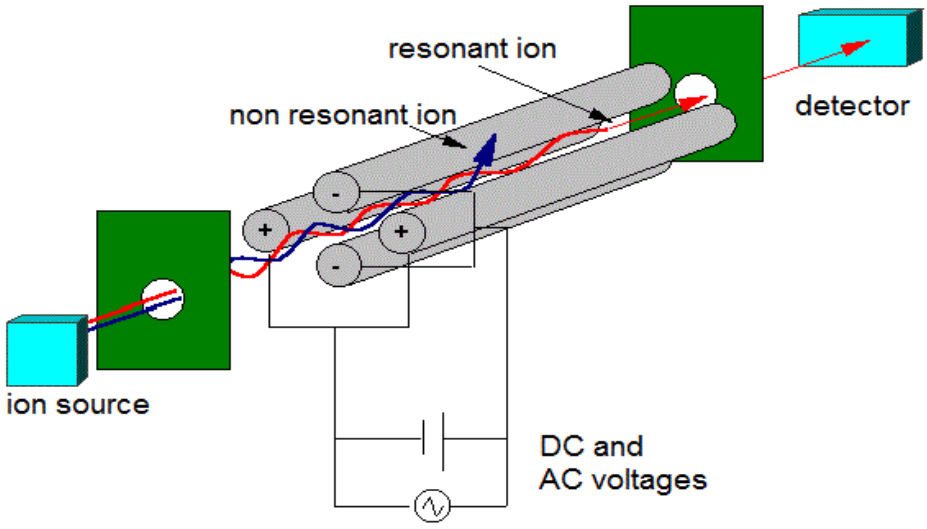


Figure 22: Quadrupole basic arrangement [48]

2.3.4.2 Time of flight (TOF) [38, 39, 50-52]

A typical TOF arrangement is shown in the figure below.

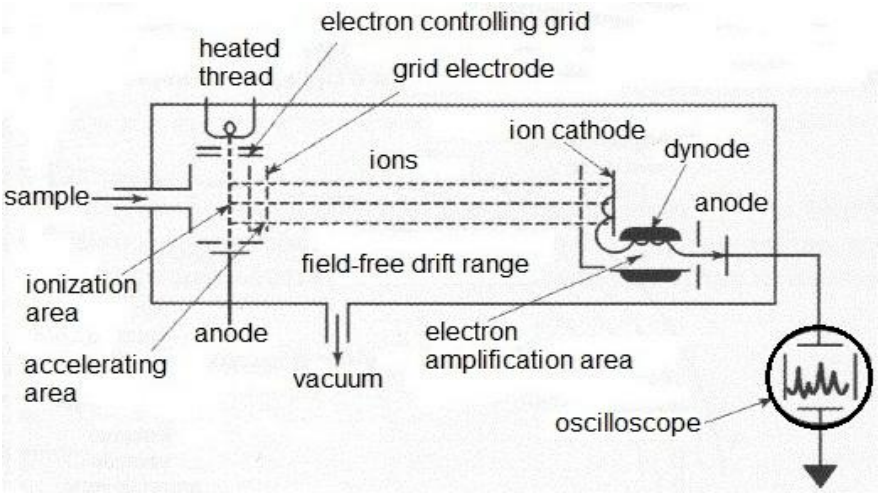


Figure 23: Basic TOF set up [38]

The ions are introduced either directly from the source of the instrument or from a previous analyser (in the case of Q-TOF). An extraction pulse gives all the ions the same initial kinetic energy and they accelerate in a constant homogenous electrostatic field. After passing the accelerating area the ions enter (through a grid electrode) into a field-free drift range where

they are not accelerated further and thus travel with a speed they have reached at the moment when passing the electrode. This speed, in turn, depends on the mass of the ions with heavier molecules having a higher moment of inertia and thence a lower velocity. For the resolution of the mass spectral analyses the length, L, of the field-free drift range is essential and in modern machines it measures about one meter. Times of flight are usually between 1 and 30 µs.

In practice the ions of a particular m/z might not reach the detector at the exact same time due to the effects of uncertainty in the time of ion formation, location in the extraction field and initial kinetic energy, resulting in reduced resolution.

- Temporal distribution: Two ions of the same mass that are formed at different times with the same kinetic energy will traverse the field-free region maintaining a constant difference in time and space.
- Spatial distribution: When ions of the same mass are formed at the same time with the same initial kinetic energy, but are formed at different locations in the extraction field, the ions near the back of the source will experience a larger potential gradient and be accelerated to higher kinetic energy, than those formed close to the extraction grid. The ions formed at the back of the source will enter the field-free region later, but will eventually pass the ions formed closer to the extraction grid due to having larger velocities. By adjusting the extraction field it is possible to achieve a space focus plane, where ions of any given mass arrive at the space focus plane at the same time. The location of the space focus plane is independent of mass, but ions of different masses will arrive at the space focus plane at different times.
- Kinetic energy distribution: Ions formed with different initial kinetic energies will have different final velocities after acceleration and arrive at the detector at different times. The initial kinetic energy distribution also includes ions with the same kinetic energy, but velocities in different directions. Ions of the same kinetic energy but with velocities in different directions will arrive at the detector at different times corresponding to their turn around time in the source. This effect can be minimised by utilising longer field-free regions [52].

This problem is easily corrected by applying a set-up called a ‘reflectron’ at the end of the drift range. This consists of one (single-stage reflectrons) or a series of electric fields (double-

stage reflectrons or quadratic-field reflectrons) which decelerates and reflects the ions back along the flight tube - usually at a slightly displaced angle (see figure). For ions of the same m/z entering such a field, those with higher kinetic energy (and velocity) will penetrate the decelerating field further than ions with lower kinetic energy. Therefore the faster ions will spend more time within the reflecting field, and ‘catch up’ with lower energy ions further down the flight path and thus they reach the detector together. By adjusting the reflectron voltages it is possible to achieve a time-focusing plane. In this ideal case, the resolution of the peaks in the mass spectrum will only be dependent on the time-width of ion formation. Ion detection is accomplished with a conversion dynode with secondary electron amplification. The spectrum acquired is a time-resolved plot for each single laser pulse.

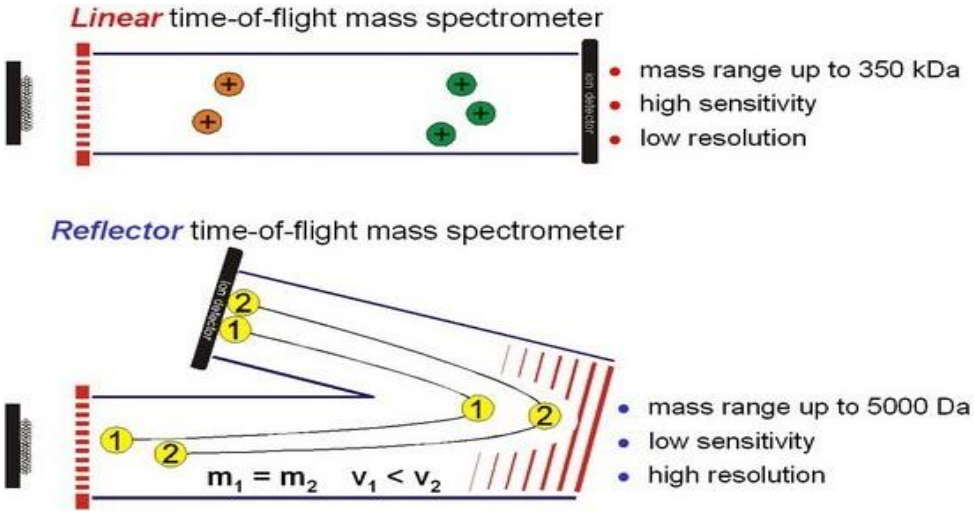


Figure 24: Linear and single-stage reflector TOF MS [51]

Double stage reflectrons consist of two separate homogenous electric fields of different potential gradient, as shown in the picture below. The result is a large enhancement in resolution, in particular for ion beams with broad kinetic energy distributions.

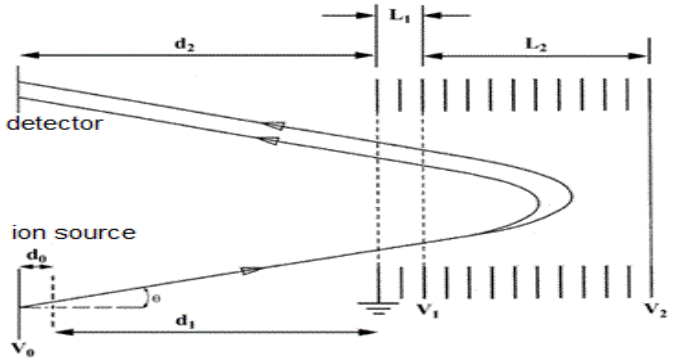


Figure 25: Double-stage reflector TOF MS arrangement [52]

Even more decrease in any kind of time aberration is accomplished by the use of quadratic field reflectrons. They basically utilize electric fields in which the potential is either axially-symmetrical hyperbolic, or axially symmetrical hyper-logarithmic, or planar hyperbolic.

2.3.4.3 Tandem mass spectrometry [39, 50, 54]

Unlike the classical ionization methods EI and CI that produce a good amount of fragments, more modern ionization methods like ESI or MALDI produce mainly the molecular ion and a few fragment ions that may not be enough to elucidate the molecular structure. Besides, these fragments might be obscured by the presence of matrix components or by ions produced by the matrix components. For these reasons sometimes a secondary induced fragmentation step is necessary, followed by fragment analysis.

There are many instruments and instrument set-ups that are employed to analyze the secondary fragment ions. The first type includes instrument that are assembled to tandem. For example two mass analyzing quadrupoles, two magnetic sector analyzers or hybrids like one magnetic sector and one quadrupole, one quadrupole and one TOF etc. A collision cell for fragmentation is usually placed between the two analyzers.

The second category comprises instruments that have an ion storage capability, like ion cyclotron resonance or ion traps. These devices allow the selection of particular ions and the ejection of all others. The selected ions can be excited and subjected to fragmentation during a selected time, and the fragment ions can be observed in a mass spectrum. This process may be repeated to monitor fragments of fragments over several generations. The first category uses a sequence of mass spectrometers in space, while the second category one spectrometer with ion storage capability to exploit a sequence of events in time.

The most commonly used tandem mass spectrometer is the triple quadrupole (QQQ) instrument, which consists of three quadrupoles assembled in series. Only the first and the last quadrupoles act as mass analyzers, being operated in DC and RF voltages. The second one acts like a collision cell with ion focusing properties and is operated in RF only, so it is permeable by all ions. The collision is often conducted by excess of inert gas (argon, helium or xenon) and results in fragment ion formation. This quadrupole usually consists of six rods (hexapole) in contrast to outboard quadrupoles that have the typical structure with four rods.

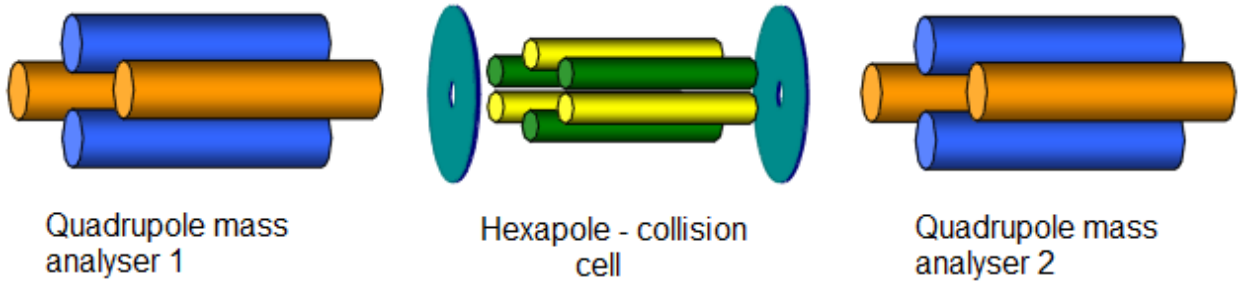


Figure 26: Triple quadrupole arrangement [39]

There are several modes that a tandem mass spectrometer can operate:

- Full Scan Mass Spectrometry: The first quadrupole scans a selected m/z range, while the two next quadrupoles are in RF only mode, so all ions pass through them.
- Single Ion Monitoring (SIM): A certain m/z is selected to pass through the first quadrupole, which pass also through the two next quadrupoles (2^{nd} and 3^{rd} quadrupoles in RF only mode).
- Product Ion Scanning: The selected ion(s) (which are usually the molecular ions) pass through the first quadrupole, then they are induced to the collision cell where they fragment by an inert gas and finally the product ions are analysed by the third quadrupole, which is set to scan a selected m/z range. The product ion spectrum allows one to record fragments arising from the molecular ion of a compound present in the sample, and thus generate information about the structure of this compound. This method most commonly employed with ESI ionisation and/or LC-MS.
- Selected Reaction Monitoring (SRM): If a reactive instead of inert gas is introduced to the collision cell of a time or space based tandem mass spectrometer ion-molecule reactions can be observed. The three quadrupoles operate as previous: the first one monitors the selected ion(s), the second act as a collision cell and the third monitors the selected ion products. The result is a spectrum of product ions that arise from a certain ion-molecule reaction. These reactions are unique for a particular compound, so this operation mode is very selective and sensitive. Thus, it is appropriate for quantitative determination of various compounds (large or small).
- Precursor Ion Scanning: In this case the third quadrupole is monitoring a selected m/z, which corresponds to a particular fragment coming from the collision cell. When this m/z appears to the Q3 the first quadrupole scans over a chosen m/z range and the

resulting spectrum gives all precursor compounds that result to this particular fragment. For example quadrupole 3 is set to measure m/z 77, which corresponds to the phenyl group ($[C_6H_5]^+$). Precursor ion scanning will give a record of compounds containing this group. This is especially useful when used with EI or CI ionisation and/or GC-MS and allowed by tandem in space mass spectrometers.

- Neutral Loss Scan: Both Q1 and Q3 analysers are scanned together but monitor m/z having a constant difference between each other. This scan allows to recognize all ions that by fragmentation lose a neutral molecule. Again this mode is especially useful for EI and CI ionisation and allowed by tandem in space mass spectrometers.

Table 4: MS/MS modes

MS/MS mode	Q1	Q2	Q3	Advantages	Disadvantages
Full Scan	Scanning	RF only	RF only	Information about the Mr	-slow scanning -no quantification
SIM	Selected m/z	RF only	RF only	-certain ion monitoring -quick scanning -quantification	-interferences
Product Ion Scanning	Selected m/z	Collision	Scanning	-information about the precursor ion structure	-slow scanning -no quantification
SRM	Selected m/z	Collision	Selected m/z	-certain analyte monitoring -quick scanning - simultaneous monitoring of several reactions	-limited structure information
Precursor Ion Scanning	Scanning	Collision	Selected m/z	-identification of compounds that give a certain product ion	-slow scanning
Neutral Loss Scan	Scanning	Collision	Scanning	-identification of congeners that have a certain molecule	-slow scanning

2.4 Literature review for the determination of organophosphates in environmental samples

The tables below show the pretreatment and analysis methods utilized by previous analysts for PFRs determination in several environmental matrices.

Table 5: Determination of OPFRs: Summary of GC methods and levels in environmental matrices

Citation	Compounds	Matrix	Sample preparation	Mobile phase	Column	Technique	MQL (ng/g)* or (ng/l)**	Levels (ng/g)* or (ng/l)**
3	TCEP, TCPP, TPP, TDCP, TiBP, TnBP, TPrP	Sediment (river & marine)	ASE with Water:ACN 3:1, water addition to the extract until 200 ml total volume, SPE cleanup-elution with MeOH and water:ACN 98:2	Helium (1.2 ml/min)	HP-5 MS type capillary column	GC-MS	0.6 (TnBP) - 5 (TCPP) *	65 (TCPP) – 6200 (TCEP)*
4	Lipids, TCEP, TCPP, TPP, TDCP, TiBP, TnBP, TBEP, TEHP, TCP, EHDP	Biota Human Milk	• Biota freeze drying & homogenization of each sample, ASE with ethyl acetate:cyclohexane 5:2, filtration over a Na_2SO_4 column, cleanup with GPC, evaporation of the middle fractions (containing the analytes) to 0.8 ml toluene • Milk Lipids removal with extraction in hexane & ACN, extraction of analytes from the ACN phase with MTBE, filtration, evaporation & solvent change to cyclohexane:ethyl acetate 3:1, GPC cleanup as above	Helium (1.3 ml/min)	DB-5 fused silica capillary column	GC-HRMS		Marine: * Herring:61-120 Perch:330-490 Mussels:190-1600 Eelpout:15000 Salmon:34 <u>Freshwater</u>:* perch:350-1000 Human Milk: 46-180*

5	TCEP, TCPP, TPP, TDCPP, TiBP, TnBP, TBEP, TEHP, BDE-209	Indoor dust	2 ultrasound assisted solvent extractions (5min, 2 ml DCM/extraction), centrifugation, evaporation of supernatants until dryness & redissolving in 0.5 ml hexane, filtration over florisil-elution with EtAc	Helium (1.0 ml/min)	HT-8	GC-EI-MS	20 (TCPP) - 500 (TiBP) *	OPFRs: 130 (TnBP) -2030 (TBEP) * BDE-209: <1 - 5300 *
7	TCEP, TCPP, TPP, TDCPP, TBP, TBEP, TEHP	Dust (SRM 2585)	ultrasound assisted solvent extraction (2 times with acetone), centrifugation, SPE to the supernatant-elution with hexane:aceton 1:1, evaporation to 5 ml		J&W DB5-MS & J&W DB5-MS-UI capillary column	GC-MS/MS (PICI: a-with isobutene b-with ammonia)	3.6 pg (TiBP) – 46 pg (TDCPP)	<LOQ (TiBP) - 82000 (TBEP)**
9	TiBP, TnBP, TCEP, TCPP, TPP, TDCPP, TBEP	River Water	LLE with toluene, evaporation of the organic phase to 1 ml & SPE for TCEP-elution with methyl tert-butyl ether (MTBE) & toluene, freezing to remove water, evaporation to 1 ml	Helium (1.5 ml/min)	J&W DB-5MS	GC-MS	4.9 (TCPP) – 14 (TDCP) **	<LOQ – 870 (TBEP)**
11	TBP, TCEP, TCPP, TPP, TDCPP, TBEP, THEP	Dust	ultrasound assisted solvent extraction (twice with 25 ml DCM), filtration with a vacuum filtration device	Helium (1.5 ml/min)	J&W DB-5 fused silica capillary column	GC-NPD	LOD: 7-60*	Σ OPFRs: 28-5900 (for computer cover) ng/m^2
20	TCPP, TiBP, TBEP, TDCPP, TPP	Coastal & marine surface	SPE – elution with 50 ml DCM, evaporation until 5-20 ml, water removal by freezing & filtration over Na_2SO_4,	Helium (1.3 ml/min)	J&W HP-5MS	GC-MS	0.2-11.7**	<LOD (TPP) – 570 (TCPP)**

		Waters	evaporation until 150µl in hexane					
21	TBEP, TBP, TCEP, TCPP, TCP, TDCPP, TEHP, TPP, BDE 209	Dust	Soxhlet extraction with acetone & toluene BDEs: Clean up with silica column, elution with HEX:DCM 3:1 OPs: Clean up with silica column, elution with hexane& acetone (2 fractions)	Helium	DB-5MS	GC-MS		<5 (TPP) – 230,000 (TBEP)*
22	BDE209	Dust	Soxhlet extraction with hexane:acetone 3:1 for 2h, clean up over silica column-elution with hexane & DCM, evaporation until 250 µl	Helium (1.0 ml/min)	DB-5	GC-MS		Homes: <LOQ-2,200,000 Offices: 620-280,000 Cars: 12000-2,600,000(*)
24	TCEP, TCPP, TBEP, TnBP, TiBP	Surface Waters	pressure filtration if needed, SPE-elution with 1 ml MeOH:ACN 1:1			GC-MS	3 (TCEP) – 30 (TBEP)**	3 (TCEP) – 652 (TBEP) **

25	TiBP, TnBP, TPP, TBEP, TCPP, TCEP, TDCP,TEHP	Sediment	- Microwave extractions with acetone & acetonitrile, cooling, centrifugation of the supernatants, cleanup over silica cartridges-elution with EtAc, evaporation to 200 µl - Soxhlet extraction with acetone and cleanup like the microwave method	Helium (1.5 ml/min)	HP-5 capillary column	GC-ICP-MS	10000** (TCPP) & 5000** for the others	TBP: 2,8-8* & TCPP: 4-10*
26	TiBP, TnBP, TBEP, TCPP, TCEP, TDCP	Precipita-tion & Water (rain, melted snow)	pressure filtration, SPE-elution with 1 ml MeOH:ACN 1:1			GC-MS	Thermo-Scientific GC-MS System: 3 (TCEP) – 4 (TBEP)** Fisons GC-MS System: 2 (TDCP) – 8 (TBEP)**	5 (TDCP) – 880 (TCPP)**

Table 6: Determination of OPFRs: Summary of LC methods and levels in environmental matrices

Citation	Compounds	Matrix	Sample preparation	Mobile phase	Column	Technique	MQL (ng/g)* or (ng/l)**	Levels (ng/g)* or (ng/l)**
2	TCEP, TCPP, TPP, TDCPP, TiBP, TnBP, TBEP, TEHP	Water (waste & river water) Sediment (river)	• Water LLE: 1 extraction with 25 ml dichloromethane + 2 with 5 ml dichloromethane, drying over anhydrous Na_2SO_4 and concentration to 1 ml ACN • Sediment ultrasound assisted solvent extraction with 30 ml ethyl acetate : ACN (30:70) for 30 min, centrifugation (20 min), evaporation to 4 ml, centrifugation, evaporation to 0.5 ml	Gradient: (A) Water (B) Methanol	Luna C_8	HPLC-MS/MS	Waste water: 4.1 (TBEP) - 13 (TCP) ** River water: 2.6 (TBEP) - 7.9 (TCP) ** River Sediment: 0.48 (TBEP) -11 (TBP)*	Waste water: 14 (TPP) - 5400 (TBEP) ** River water: <LOQ (TEHP) – 170 (TCPP)** <u>River Sediment</u>: 6 (TPP) – 1300 (TCPP) **
6	TCEP, TCPP, TPP, TDCP, TiBP, TnBP, TBEP, TEHP, TCP, EHDP	Herring gull eggs	ASE with DCM:HEX 50:50, drying over Na_2SO_4, SPE cleanup, collection of the 2^{nd} fraction (DCM:HEX 20:80), drying, re-dissolved to 200 µl MeOH	Gradient: (A) Water-0.1% formic acid (B) Methanol-0.1% formic acid	Waters X-Terra Phenyl column	LC-MS/MS	0.06 (TDCPP) - 0.20 (TCPP) * (ng/g wet weihtgt)	TCEP:<MQL -0.55 TCPP<MQL - 4.1 TPP: n.d.-0.13 TDCP: n.d.-0.17 TBEP:0.16-

				(0.2 ml/min)				2.2
10	TnBP, TPP, TBEP, EHDP, TEHP, TCP	Fish	freeze drying & homogenization (with anhydrous Na_2SO_4) of fish tissue, extraction with hexane:acetone 1:1, gravimetric determination of lipids to a portion of the extract, further clean up with silica column to the other portion of the extract (elution with 100 ml DCM), evaporation to dryness, reconstruction to 1 ml MeOH	Gradient: (A) 0.1% formic acid in milli Q Water – (B) 10 mM ammonium acetate in Methanol	Asentis express C_{18}	UHPLC-MS	LOD: 0.002 (TnBP) – 0.014 (TPP)*	Σ OPFRs: 110-1900*
12	TiBP, TnBP, TCEP, TCPP, TPP, TDCPP, TBEP, TEHP, RDP, BDP	Waste water	SPE (elution with MeOH), evaporation to 1 ml ultrapure water	Gradient: (A) methanol:water 20:80 (B) pure methanol	Luna 3µm C_{18}	LC-MS	19 (TCPP) - 81(BDP)**	<u>Raw wastewater</u>: <LOD-3100** Primary effluent: <LOD-2400** Tertiary effluent: <LOD-2600**

Conclusions

- The most common determination method is GC-MS. In all cases the GC carrier gas is helium and preferred GC columns are J&W DB5MS and HP-5MS capillary columns. The detection is carried out by a mass analyser, usually equipped with Electron Impact (EI) ionization sources and triple quadrupole. Only in one study a Nitrogen-Phosphorus Detector (NPD) is used.
- Fewer studies use LC-MS techniques. The gradient usually consists of water and methanol in different ratios. Both Luna C_8 and C_{18} were mainly used as LC columns. The detection was again carried out by MS systems, equips with Electrospray Ionization (ESI) sources and triple quadrupoles.
- The extraction techniques depended on the sample's matrix. So, for water samples solid phase extraction (SPE) was the most common technique, but also liquid-liquid extraction (LLE) was used. The elution from SPE cartridges was conducted mainly with methanol or dichloromethane. Dichloromethane and toluene were used in LLE.
- Sediment samples were firstly subjected to a solid-liquid extraction techniques and afterwards to SPE for further clean-up. The initial extraction was carried out with Accelerated Solvent Extraction (ASE), Microwave Assisted Extraction (MAE) and Ultrasound Assisted Solvent Extraction. Some solvents used at this first step were acetone, acetonitrile, ethyl acetate and a mixture of water with acetonitrile. At the SPE step common solvents were methanol, acetonitrile and ethyl acetate.
- The extraction from dust samples is easier and is usually carries out by Ultrasound Assisted Solvent Extraction, as it is a quick, simple and non solvent consuming technique. Common solvents used were acetone and dichloromethane. However, in two studies Soxhlet extraction was carried out by acetone, toluene and hexane.
- The current study followed more or less these patterns. So, extraction from water samples was carried out with SPE, from sediment samples with ASE and from dust samples with ultrasounds. Several solvents were tested in each case. The determination techniques included LC-TOF-MS, LC-MS/MS and GC-MS/MS.

CHAPTER 3

PURPOSE OF THE STUDY

Organophosphate esters (OPs) are in the market for many decades, as flame retardants, plasticizers, lubricants and anti-foaming agents in a great variety of products like electric and electronic equipment, floor polishes, lacquers, hydraulic fluids, polyurethane foam, paints etc. Last years there is a dramatic increase in their consumption mostly due to worldwide restrictions on the use in new products of another wide category of such compounds, the brominated flame retardants (BFRs). Despite their extensive use, there is not sufficient knowledge on OPs' toxicological effects yet.

This study aims to develop a method for the simultaneous determination of thirteen OPs and one BFR in various matrices. The OPs measured were Tri-iso-butyl phosphate (TiBP), Tri-n-butyl phosphate (TnBP), Tris-(2-chloroehtyl) phosphate (TCEP), Tris-(2-chloropropyl) phosphate (TCPP), Tris-(dichloro-propyl) phosphate (TDCPP), Tricresyl phosphate (TCP), Tri-phenyl phosphate (TPP), Tris-(butoxyethyl) phosphate (TBEP), Tris-(2ehtylhexyl) phosphate (TEHP), 2-ethylhexydiphenyl phosphate (EHDP), Resorcinol-bis(diphenyl phosphate) (RDP), Bisphenol A bis diphenyl phosphate (BDP), 9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide (DOPO) and the brominated flame retardant 2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene (BDE 209, commercial name deca-BDE).

The matrices tested were waste water, sediment and indoor dust, so a comparison between the outdoor and indoor concentrations was made. The indoor dust samples, apart from house dust, included a sample of car dust, as inside the cars great amounts of plastics and polyurethane foam (seats) are found. The dust samples came from both the Netherlands and Greece and the purpose was to compare the contamination profiles of the two countries.

Several pretreatment techniques for the isolation of the analytes out of each matrix were tested. Eventually, Solid Phase Extraction (SPE) was used for water samples, as it was found to give good recoveries and pretreatment times. Optimization in SPE eluents and solid phases was carried out. For dust samples extraction using ultrasounds was sufficient. Sediment samples were subjected first to ASE and then to SPE, but this protocol was not further optimized.

The techniques used for the samples' analysis were liquid and gas chromatography (LC and GC) coupled with mass spectrometry (MS). This was tried because most compounds (with the exception of RDP and BDP which are too large to evaporate in the GC) can be measured by both LC and GC. The MS detectors used were time-of-flight (TOF), quadrupole (Q) and triple quadrupole (QQQ). The results were compared.

The methods were first optimized with spiked samples. The methods for water and dust were validated separately and the quantification technique turned to be the internal standard technique, after comparing the calibration curves with and without internal standard.

Finally, the methods were applied in real samples. The water samples came from Waste Water Treatment Pants (WWTP) of Eindhoven, Amsterdam and Rotterdam. The dust samples came from houses of Amsterdam and Athens.

CHAPTER 4

LABORATORY EQUIPMENT, INSTRUMENTS AND REAGENTS

4.1 Pretreatment instrumentation

- Dionex ASE 350
- GPC: GX-271liquid handler, (Gilson International B.V. Den Haag)

  112 UV detector 280nm, (Gilson International B.V. Den Haag)

  307 pump, (Gilson International B.V. Den Haag)

  402 syringe pump 1ml, (Gilson International B.V. Den Haag)

  PL gel GPC kolom 7,5 x 600 mm 10 µm 50 A, (Polymer Laboratories, Heerlen)

  PL gel voorkolom 7,5 x 50 mm 10 µm 50 A, (Polymer Laboratories, Heerlen)

4.2 Chromatographic systems

For the current study the following four chromatographic systems were used:

4.2.1 Liquid chromatograph - time of flight mass spectrometer

- Liquid chromatograph: Agilent Technologies, 1290 infinity HPLC
- Column: Luna C-18, 150x3 mm, 3 µm, Phenomenex, Eschborn, Germany
- Time of flight spectrometer: Bruker, micro TOF II
- LC software: Hystar software (version 3.2; Bruker Daltonics)
- Detector: microTOF II mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electro-spray ionization source (ESI)

4.2.2 Liquid chromatograph - triple quadrupole mass spectrometer

- Liquid chromatograph: Agilent Technologies, 1200 series
- Column: Luna C-18, 150x3 mm, 3 µm, Phenomenex, Eschborn, Germany
- LC software: Hystar software (version 3.2; Bruker Daltonics)
- Triple quadrupole spectrometer: Agilent Technologies, 6410 Triple Quad LC/MS

4.2.3 Gas chromatograph - quadrupole mass spectrometer

- Gas chromatograph: Agilent Technologies, 6890
- Autosampler: Agilent Technologies, 7683 series
- Column: Forte GC capillary column, 25 m, I.D. 0.22 mm, film 0.25 μm (for PFRs)
- Column: Durabond DB-5, 15 m, I.D. 0.250 mm, film 0.25 μm (for BDE 209)
- Quadrupole spectrometer: 5975C inert XL EI/CI MSD with Triple - Axis Detector
- GC software: Agilent Technologies, MSD Chemstation

4.3 Laboratory equipment

Laboratory equipment consisted of: ultrasound device (Branson 5510), vortex (IKA MS 3 basic), calibrated analytical balance (Satorius ME235), ultrapure water production apparatus (Millipore Q at 18,5 MΩ/cm), SPE device (J. T. Baker spe-12q), evaporation device (homemade) and centrifuge (Firlabo).

Among the glassware used were the following: glass flasks of 100, 250, 300 and 500 ml, glass cylinders of 50, 100, 250, 500 and 1000 ml, glass columns for filtration/SPE and glass pipettes (Pasteur). Plastic pipettes (Pasteur of 3 ml and Eppendorf with tips of 100, 200 and 500 μl) were used as well.

For SPE clean up prepared cartridges and handmade columns were tried: (a) prepared cartridges: Supelco - Discovery DSC-NH_2 - 6ml - 500 mg, Waters - Oasis HLB - 3cc - 60 mg, Waters - Oasis HLB - 6cc - 150 mg, Waters - Oasis MCX - 6cc - 150 mg, Waters - Oasis WAX - 6cc - 150 mg and (b) handmade columns: Supelco - Discovery DSC-NH_2 powder and Sigma-Aldrich - Florisil.

4.4 Solvents

The solvents and chemicals used were all pro-analysis quality or HLPC grade, unless otherwise stated. Hexane, acetone, methanol and acetonitrile used for the extraction and cleanup were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and

dichloromethane from Promochem, Wesel, (Germany). Formic acid (98%) and isooctane were obtained from Merck, Darmstadt, (Germany).

4.5 Standard compounds and solutions

Tri-n-butyl phosphate (TnBP), Tricresyl phosphate (TCP), Tris-(2-chloroehtyl) phosphate (TCEP), Tris-(dichloro-propyl) phosphate (TDCPP), Tri-phenyl phosphate (TPP), Tris-(butoxyethyl) phosphate (TBEP), Tris-(2ehtylhexyl) phosphate (TEHP), 2-ethylhexydiphenyl phosphate (EHDP) were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). Tri-iso-butyl phosphate (TiBP) was supplied by Merck, Darmstadt, (Germany), Tris-(2-chloropropyl) phosphate (TCPP) by Ehrenstorfer, (Augsburg, Germany), Resorcinol-bis(diphenyl phosphate) (RDP) and Bisphenol A bis diphenyl phosphate (BDP) by Chiron, (Trondheim, Norway) and 9,10-diydro-9-oxa-10-phosphatphenanthrene-10-oxide (DOPO) by KCCS (Austria). The brominated flame retardant 2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy) benzene (BDE 209, commercial name deca-BDE) was purchased as a mixture of PBDE congeners from Wellington Laboratories (Guelph, Ontario, Canada).

The internal standards TPP-d15 and TBP-d15 were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and Cambridge Isotope Laboratories, Inc (*CIL*), (Andover, MA, USA) respectively, while the ($^{13}C_{12}$) 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (^{13}C-deca-BDE) from Wellington Laboratories (Guelph, Ontario, Canada), like the other PBDEs.

CHAPTER 5

DEVELOPMENT AND VALIDATION OF GC AND LC - MS METHODS FOR DETERMINATION OF PFRs IN WATERS, SEDIMENTS AND DUST

5.1 Optimization of the pretreatment procedure of the matrix samples

In most cases in chromatography, sample's clean-up is a crucial step prior to the injection to the instrument, especially where dirty samples are under consideration, because the matrix components may interfere (which may lead to suppression or enhancement of the signal), or harm or be non-compatible with the chromatographic system. However, during this step a significant part of the compounds can also be lost and not recovered from the sample. So it is important that an appropriate pretreatment stage is chosen.

5.1.1 Water samples

The most common pretreatment method for water samples found in literature was Solid Phase Extraction (SPE), which accomplishes both clean up and concentration of the compounds. Different SPE columns and different elution solvents were tested.

The performance of each SPE column was evaluated using water spiked solutions and calculating the recovery of each compound at the end of the SPE process. In each experiment 50 μl of stock solutions of the compounds and 200 μl of internal standard were spiked in 100 ml of ultra pure water (milli-q water). The elution was made with different solvents each time and the final SPE extract was concentrated to 1ml of methanol and injected to the LC-TOF system. The recoveries were calculated by the following equation:

$$\%R = \frac{Peak\ Area\ of\ the\ compound\ in\ the\ sample}{Peak\ Area\ of\ the\ compound\ in\ the\ reference\ sample} * 100$$

The reference sample was a solution of the same amount of compounds in 1 ml of methanol. It was injected directly to the instrument without being subjected to SPE process.

The best combination would be the one that would give the highest recoveries for most compounds and eventually turned to be the Oasis MCX column (6 ml, 150 mg) eluting with 6 ml of methanol.

5.1.1.1 Optimization of SPE columns

SPE mainly aims to separate the analytes from the matrix and/or to concentrate the analytes enough for quantitative analysis. “Retention SPE” mechanism was used here, according to which the analytes are retained to the column and a solvent is used to recover them from the sorbent. Not all the SPE columns are suitable for all the compounds. If inappropriate solid phase structure is used, two problems may appear: a) the compounds may be retained too strongly to the column to elute and b) the compounds may not be retained at all and pass through the column along with the matrix. In both cases the resulting recoveries are low.

During the experiments the following SPE cartridges were tried:

Supelco - Discovery DSC-NH2, 6 ml, 500 mg

Waters - Oasis HLB, 3 ml, 60 mg

Waters - Oasis HLB, 6 ml, 150 mg

Waters - Oasis WAX, 6 ml, 150 mg

Waters - Oasis MCX, 6 ml, 150 mg

The two columns giving the best recoveries found to be Oasis WAX and Oasis MCX. The Oasis MCX was preferred though, because all the compounds elute in the same fraction (1st fraction), in contrast to the Oasis WAX that they distribute in the first two fractions. The following table shows this comparison. The samples’ pH was lowered around 2.

Table 7: Comparing Oasis MCX and Oasis WAX

	% Recovery							
	Oasis MCX				**Oasis WAX**			
	BDP	**RDP**	**TPP**	**DOPO**	**BDP**	**RDP**	**TPP**	**DOPO**
Fr 1	58	106	120	65	59	101	127	-
Fr 2	3	2	4	7	2	1	2	94
flask	9	18	1	-	9	22	-	-
total	70	124	125	72	70	124	128	94

** fr 1 = 6ml MeOH; fr 2 = 6ml 5% NH_4OH in MeOH*

5.1.1.2 Optimization of elutants and number of fractions

In general, methanol is a good solvent for PFRs and here is preferred because PFRs are already diluted in polar solvent (water samples). Acetone and dichloromethane were also tested and gave similar recoveries.

A different number of fractions with different volume of elutant solvent were also tested. A 6ml volume of methanol seemed sufficient and in most cases the highest amount of the compounds was eluted at the first fraction. Generally, the recovery of the compounds depended mainly on the type of sorbent used rather than on the number of fractions.

Some tests with different SPE columns and different number of elution fraction are showed in the following tables. None of the columns gave sufficient recoveries for all the compounds. It was observed that the highest percentage of the compounds is eluted in the first two fractions and a significant amount of them (10 - 20%) remains in the flask in which the sample is placed. For that reason eventually only two elution fractions were collected and the conical flask (after having loaded all the sample over the SPE column) was rinsed with water and that rinse was also loaded over the SPE column.

Table 8: SPE with Oasis HLB 3 ml, 60 mg column

	% Recovery											
	RDP			**BDP**			**TPP**			**DOPO**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Fr 1	44	44	46	23	17	15	94	75	3	27	18	27
Fr 2	-	-	-	2	2	2	2	12	13	0.2	0.2	0.2
Fr 3	-	-	-	0.3	0.2	0.1	2	8	10	-	-	-
Fr 4	-	-	-	-	-	-	-	-	-	-	-	-
flask	12	7	11	16	12	15	2	8	10	0.5	-	-
total	**56**	**51**	**57**	**41**	**31**	**30**	**100**	**103**	**36**	**27**	**18**	**27**

**fr 1 = 6ml MeOH; fr 2 = 6ml MeOH; fr 3 = 6ml MeOH; fr 4 = 6ml MeOH;*

Table 9: SPE with Oasis HLB 6ml, 150 mg column

	% Recovery							
	RDP		BDP		TPP		DOPO	
	1	2	1	2	1	2	1	2
Fr 1	70	65	10	12	75	30	57	54
Fr 2	11	7	5	3	12	13	0.2	0.2
Fr 3	4	4	2	2	8	10	8	-
flask	18	29	18	17	8	10	0.2	0.3
total	**103**	**105**	**35**	**34**	**93**	**63**	**65**	**54**

***** *fr 1 = 6ml MeOH; fr 2 = 6ml MeOH; fr 3 = 8ml Acetone*

5.1.1.3 Optimization of the pH of the sample

Even with the found to be most appropriate sorbent (Oasis MCX) the recoveries were insufficient in samples' natural pH (neutral pH). After literature research a lower pH was tried and seemed to give the desirable results. At the beginning, pH was lowered to 2, but afterwards just an addition of 3 ml of formic acid turned to be good enough.

The table below shows an experiment made with Oasis MCX (6ml, 150 mg) column firstly with neutral pH and then with lowered pH.

Table 10: Recoveries with Oasis MCX column and different PH values.

	% Recovery							
	pH ~ 7				pH ~ 2			
	RDP	BDP	TPP	DOPO	RDP	BDP	TPP	DOPO
Fr 1	71	13	80	-	84	86	83	83
Fr 2	-	-	-	-	1	8	3	4
Fr 3	-	-	-	-	-	4	3	-
flask	10	-	4	-	14	26	3	-

total	**81**	**13**	**84**	**-**	**99**	**122**	**92**	**87**

**the recoveries of the second experiment (pH ~2) are the average of three repeats*

*** fr 1 = 6ml MeOH; fr 2 = 6ml 5% NH_4OH in MeOH; fr 3 = 6ml MeOH:DCM 1:1*

5.1.2 Sediment samples

Sediment samples in general have more complicated and difficult matrices. Besides, the PFRs are diluted in organic and solid material here, so an extraction from this material is first needed. The most common extraction methods found in literature are the Accelerated Solvent Extraction (ASE) and the Ultrasonic Extraction. Soxhlet extraction is not preferred because it is more time and solvent consuming. Here, only ASE was tried because it is considered as a stronger extraction procedure and thus more suitable for complicated matrices.

5.1.2.1 Optimization of extraction solvents

During the tests, a UNEP (United Nations Environment Programme) freeze dried sediment was used as a clearly homogenized material. This sediment was spiked each time with certain amount of PFRs and internal standards which were diluted in hexane or iso-octane, in order to simulate the later on solvent (the ASE solvent). The solvent(s) considered as the most appropriate were those that gave the highest recoveries of PFRs, according to the signal of the reference sample. The reference sample was again a solution of PFRs and internal standards straight in hexane or iso-octane (without being subjected to ASE extraction) but spiked with the same levels of the compounds as the sediment samples. The following solvent mixtures were tested:

hexane:acetone 3:1

methanol with 0,2% formic acid

toluene:acetone 1:1

dichloromethane:acetone 1:1

dichloromethane:hexane 1:3

dichloromethane:methanol

acetonitrile:water 1:4

5.1.2.2 Optimization of SPE columns

ASE was followed by clean-up steps, because matrix components are also extracted along with the compounds, with the usual solvents. The first column tested was the DSC-NH_2 column, both bought and handmade (in glass tubes), to check if any contamination due to the commercial plastic SPE tube takes place.

In general none of the previous mixtures of solvents gave sufficient recoveries for all the compounds when this type of column was used. That is why other sorbents were also tested. A successful one was florisil, put in a handmade glass column. The ASE extraction was carried out with hexane-acetone. The recoveries obtained were in the range of 35% (EHDP, BDP) until 115% (TDCPP) and they were repeatable enough. Only two compounds were out of these limits, TCEP (146%) and the RDP dimer (14%). Moreover, with this method, DOPO was not detectable at all.

Another clean-up method tested was the one developed for water analysis. The SPE clean-up was applied to the ASE extract (after extraction with hexane-acetone) testing both Oasis MCX and Oasis WAX columns. The Oasis MCX was preferred also here not only because the compounds elute in the same fraction but also because with the Oasis WAX column much higher signal amplification for DOPO occurred.

In the table below three experiments are presented. In all experiments spiked UNEP sediment was used. In “test 1” the ASE was made with methanol containing 0.2% formic acid and SPE was conducted according to the protocol used for waters analysis (Oasis MCX columns and elution with methanol). In “test 2” the ASE was conducted with hexane:acetone 3:1 and for SPE handmade columns of florisil were used. The elution was made by ethyl acetate. In “test 3” the ASE was conducted again with hexane:acetone 3:1, but for SPE handmade columns of DSC-NH_2 were used. The analytes were eluted by 20% dichloromethane in hexane. The best of the three turned to be the second one, where the ASE was made using hexane:acetone 3:1 and the SPE with forisil column and elution with

ethyl acetate, but still the recoveries of several compounds (TBEP, EHDP,TCP and BDP) were not sufficient. DOPO was not detected in any of them.

Table 11: % recoveries resulting by different tests

Compound	Recovery %					
	Test 1		Test 2		Test 3	
	Repeat 1	Repeat 2	Repeat 1	Repeat 2	Repeat 1	Repeat 2
TBP - TiBP	167	146	77	91	98	120
TEHP	97	109	73	92	60	72
TCEP	60	47	170	156	90	85
TCPP	234	211	102	126	85	94
TDCPP	25	23	108	123	82	77
TBEP	193	201	60	71	183	134
TPP	49	47	85	88	27	27
EHDP	13	12	39	42	40	35
TCP mix	107	96	42	47	74	74
RDP	41	62	75	56		
RDP dimer	3	10	18	8		
BDP	2	7	46	45		
TPP-d15	36	29	106	100	123	129
TBP-d27	69	65	86	85	133	135

Similar experiments were conducted using kiezelguhr instead of UNEP sediment and they showed acceptable recoveries (67 - 81 %) for all the compounds (with the exception of DOPO with a recovery of 150%). In the case of kiezelguhr the most appropriate solvent for ASE extraction turned to be the mixture of toluene:acetone 1:1. Kiezelguhr is not a real matrix for any sample and is supposed not to affect the analysis, so these experiments showed that even after the ASE, the sediment matrix still interferes to the PFRs analysis. This conclusion

was also reached when the PFRs were injected straight on an ASE filter and analyzed as previously (recoveries between 90 and 120%, extraction with hexane-acetone). In this experiment though, the SPE step was skipped because no matrix was present.

In summary the sorbents tried for SPE were the following:

Supelco - Discovery DSC-NH_2, 6 ml, 500 mg (cartridges)

Supelco - DSC-NH_2 (powder)

Waters - Oasis MCX, 6 ml, 150 mg (cartridges)

Sigma-Aldrich - Florisil (powder)

In the tables below ASE tests using spiked kiezelguhr samples and spiking directly on the ASE filter are shown.

Table 12: Tests using kiezelguhr instead of real sediment as matrix

Compound	**Recovery %**			
ASE solvent	**Toluene-Acetone**	**DCM-Acetone**	**Hexane-Acetone**	**Hexane-DCM**
DOPO1	26	0	0	0
DOPO2	121	0	0	0
TCEP	67	55	62	47
TCPP	62	61	66	60
TDCPP	73	58	68	37
TPP	79	20	54	14
TIBP-TBP	70	64	74	73
RDP	79	0	9	0
TBEP	68	80	77	59
TCP	81	44	68	33
RDP dimer	77	0	0	0
EHDP	71	65	68	59
BDP	76	9	28	6

TEHP	72	73	65	70
TBP d27	74	65	74	73
TPP d15	72	29	67	15

Table 13: Tests of ASE solvents without sediment matrix

Compound	**Recovery %**					
ASE solvent	**DCM-Acetone**		**Hexane-Acetone**		**Toluene-Acetone**	
	Repeat 1	**Repeat 2**	**Repeat 1**	**Repeat 2**	**Repeat 1**	**Repeat 2**
TCEP	89	55	83	80	78	92
TCPP	90	84	99	85	99	99
TDCPP	88	84	103	98	89	92
TPP	96	99	101	99	100	104
TiBP-TBP	92	88	92	88	109	101
TBEP	110	106	86	158	107	127
RDP	103	106	108	92	105	108
TCP	94	119	103	93	126	100
RDP dimer	110		49	95	281	145
EHDP	93	116	99	85	120	98
BDP	85	114	97	93	148	101
TEHP			108	111	102	113
TPP d15	100	110	100	100	100	100
TBP d27	91	100	101	91	97	107

5.1.2.3 Gel Permeation Chromatography (GPC)

Gel Permeation Chromatography (GPC) is another clean-up technique, which is used to separate the matrix from the analytes. In the current experiment fractions were collected

every minute in different tubes and afterwards analyzed by LC-TOF-MS. The purpose was to determine the fractions that contain the analytes and check if the matrix elutes in the same fractions as well. The recovery of each fraction was calculated using a reference sample, as previously. The solvent used for elution was dichloromethane.

A quick test to check where the matrix components elute is to observe the colour of the different fractions. Most analytes come out between 18-20 minutes and some of them between 17-20 minutes, while the matrix, elutes a bit earlier, mainly between 16-19 minutes, but there is still an overlapping in the fractions. The recoveries of most compounds were in the range of 50 – 120%. In summary, the GPC efficiency as a clean-up technique is similar to the one of the optimized SPE.

Table 14: GPC results. Fr 1 corresponds to 13 min and fr 14 to 27 min

	Fr1	Fr2	Fr3	Fr4	Fr5	Fr6	Fr7	Fr8	Fr9	Fr10	Fr11	Fr12	Fr13	Fr14	total
colour	-	-	+	+++	+++	+++	+++	++	-	-	-	-	-	-	
TDCPP						81	32								113
TCPP						67	37								104
TCEP						64	65								129
DOPO1							12	13	1						26
DOPO2							6	42	1						49
TiBP-TBP						39	34	1							74
TBEP		4	3	5	40	58	3	3	2	3		2	2	4	131
EHDP						34	19								53
TCP						29	24	1							54
TEHP					10	87	10								107
RDP dimer				4	28	4									36
TPP						13	67	4							84
RDP					29	47	2		4						82
BDP				2	43	17									62

TPP-d15						20	74	4							98
TBP-d27						46	36	1							83

5.2 Experimental Procedure

After the optimization in extraction and cleanup methods, the sample extract is ready to be injected to the chromatographic system. In this study three instruments were used: LC-MS/MS, LC-TOF-MS and GC-MS. The parameters for liquid and gas chromatography, as well as for mass spectrometry were adapted from literature, after checking them with a standard solution.

Liquid chromatography was performed on an Agilent Technologies 1260 Infinity high performance liquid chromatograph (HPLC) with a Phenomenex Luna C18 column (see 4.2.1 & 4.2.2) at a flow rate of 0.25 ml/min and column temperature 45 ^{o}C. Eluents were MeOH/water (20/80) (eluent A) and pure methanol (eluent B), both containing 0,2% formic acid. The gradient was as follows: 0 min, 55% B; 0,5 min, 70% B; 11 min, 100% B; 16 min, 100% B; 17 min, 55% B; 27 min, 55% B.

The HPLC was coupled either to a triple quadrupole mass spectrometer (LC-MS/MS) or to a time-of-flight mass spectrometer (TOF-MS). The triple quadrupole was an Agilent Technologies 6410 Triple quad MS with electrospray ionization (ESI) interface measuring in the positive mode. Capillary voltage was set at 3500 Volt, source temperature at 350 ^{o}C, nebulizer gas pressure at 45 psi and flow at 8 L/min. The injection volume was 10 μL. The MS was run at the MS-MS mode using multiple reaction monitoring (MRM) of the precursor and product ions.

The time-of-flight instrument was a microTOF II mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electro-spray ionization source (ESI). It was controlled by the Compass 1.3 for microTOF software package (Bruker Daltonics) and operated in positive ion mode. The capillary voltage was maintained at 4500V with the end plate offset at −500V. The pressure for the nebulizing gas (N_2) was set at 4 bar, and the drying gas (N_2) flow rate was 8.0 l/min with a temperature of 200 °C. The full scan mass ranged from m/z 100 to 1000. At the beginning of the LC–MS experiment an internal calibration was performed, using the sodium formate cluster injected into a six-port valve, to provide high-accuracy mass

measurement. For this sodium formate cluster a mixture of 12.5 ml Milli-Q, 12.5 ml Propanol, 250 μl Sodiumhydroxide (1M) and 50 μl Formic acid was used.

Gas chromatography was carried out at an Agilent Technologies 6890 gas chromatograph, with a Forte GC capillary column for PFRs' determination and a Durabond DB-5 for BDE 209 determination (more details about the columns are given in section 4.2.3). For PFRs analysis the injector was set at pulsed splitless mode, at 250 ^{o}C and purge flow 50,0 ml/min. The final column temperature was 325 ^{o}C (initial temperature 110 ^{o}C, equilibrium time 0.50 min) and Helium was used as carrier gas at a flow rate of 1.3 ml/min. The mass spectrometer was operated at selected ion monitoring (SIM) mode and chemical ionization (CI) took place. The MS source temperature was set at 230 ^{o}C (maximum 300 ^{o}C), the electron energy at 70 eV and the MS quadrupole temperature at 150 ^{o}C.

For BDE 209 analysis, the injector was set again at pulsed splitless mode, at 275 ^{o}C with purge flow 50,0 ml/min. The final column temperature was 325 ^{o}C (initial temperature 90 ^{o}C, equilibrium time 0.50 min) and Helium was used as carrier gas at a constant flow rate of 1.0 ml/min. The mass spectrometer was operated at selected ion monitoring (SIM) mode and the compounds were ionized by electron impact (EI) ionization. The MS source temperature was set at 200 ^{o}C (maximum 300 ^{o}C) and the quadrupole temperature at 106 ^{o}C (maximum 200 ^{o}C). Methane was used as CI reagent gas.

In the table below the whole set of MS parameters for all compounds measured is given.

Table 15: MS/MS parameters of the LC-MS/MS method

Compound Acronym	Precursor ion	Product ion	Dwell	Fragmentor	Collision Energy
TPP-d15*	342.2	160.2	20	125	15
		82.1	20		
TBP-d27*	294.4	166.1	20	100	15
		102.1	20		
TiBP	267.2	155.1	20	75	8
		99.1	20		
TBP	267.2	155.1	20	100	10
		99.1	20		
TEHP	435.4	113.1	20	125	10

		99.1	20		
TCEP	287	99.1	20	100	19
	285	63.1	20	110	18
TCPP	329	99	20	100	15
TDCPP	433	99.4	20	125	30
TBEP	399.4	299.2	20	125	15
		199.1	20		
TPP	327.2	152.1	20	175	35
		77.1	20		
EHDP	251.1	152.2	20	150	28
		77.2	20		
TCP mix	369.2	165.2	20	175	35
		91.1	20		
RDP	575.1	481.1	20	225	45
		419	20		
BDP	693.2	367.1	20	250	38
		327	20		

** internal standards*

In the following sections the final protocols for measuring PFRs in waters and indoor dust, as came out of the optimization procedure, are presented schematically. The LC-TOF, GC-MS and LC-MS/MS measurements were conducted as previous.

5.2.1 Water samples

During the water analysis for PFRs, 100 ml of sample were spiked with internal standard and 3 ml of formic acid were added. After the homogenization of the solution, SPE was conducted with Oasis MCX columns. The eluate (in methanol) was evaporated until 1ml, transferred in appropriate vials and injected to the instrument.

Below, the flow chart of the method is presented:

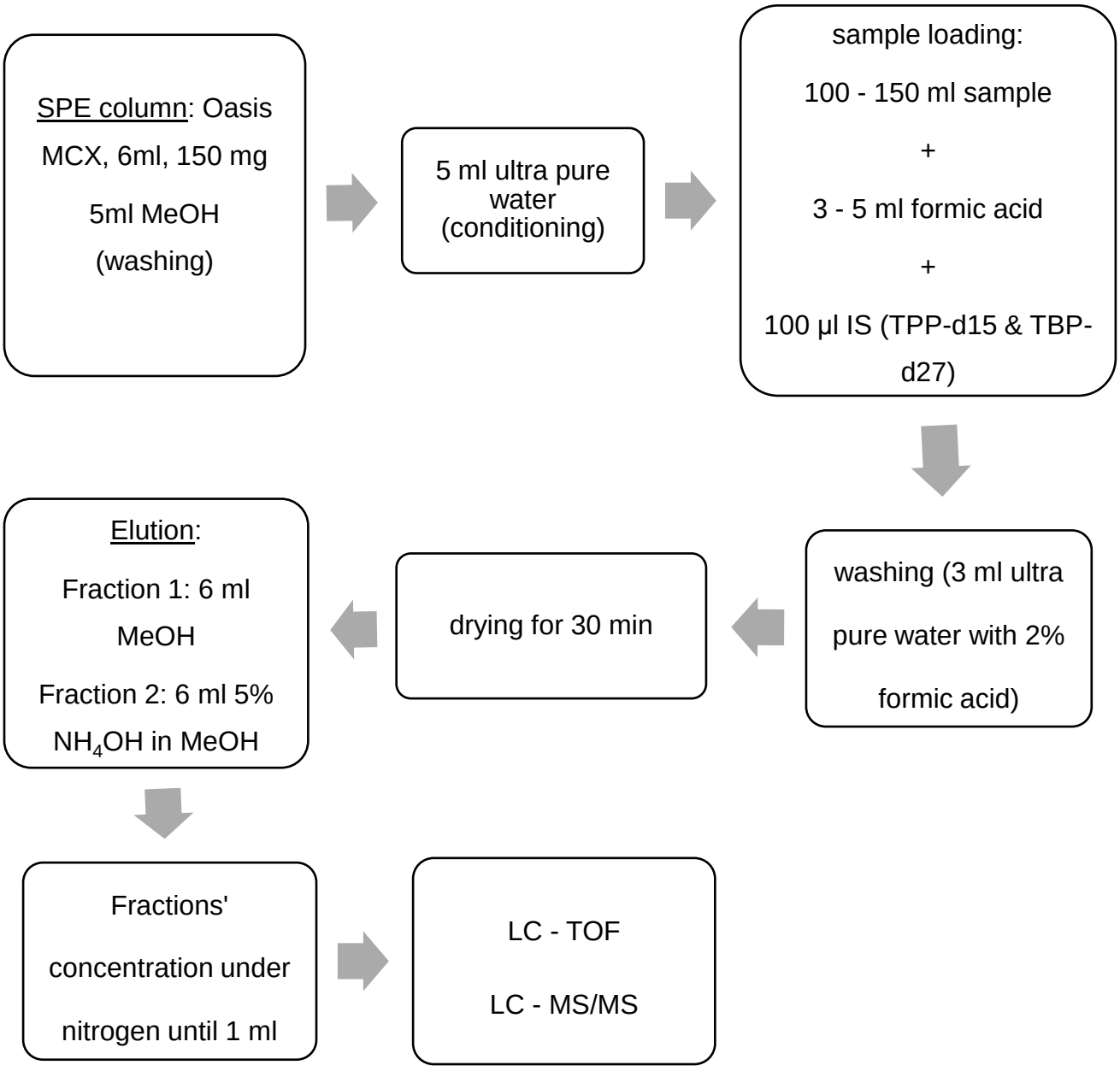


Figure 27: Analytical protocol for the determination of PFRs in water samples

5.2.2 Indoor dust samples

The dust samples were collected mainly from houses (one car sample was also included), using a DOWNTSREAM^{TM} collector (Figure below). The device was adapted to the vacuum cleaner and the dust was collected to the internal tube. Because PFRs are used mainly in electronic equipment two separate types of samples were collected from each house: one from electronic equipment's surfaces and another one from surfaces around the electronic equipment (e.g. tables etc). Floors were not included in those surfaces, because they would give very dirty samples. Samples were collected from both the Netherlands and Greece and a comparison of the contamination profiles of the two countries was made.

Figure 28: Picture of the DOWNTSREAM^{TM} collector used for collecting the dust samples.

The extraction of PFRs from the dust was conducted via sonic bath using acetone and toluene (toluene is preferred to acetone for the extraction of BDE-209). The extracts were filtrated over Na_2SO_4 column, evaporated until 1 ml of methanol and analyzed at the LC - TOF and LC - MS/MS, or at 1 ml of hexane and analyzed at GC - MS/MS. The results were compared. The analytical protocol followed is shown at the next figure:

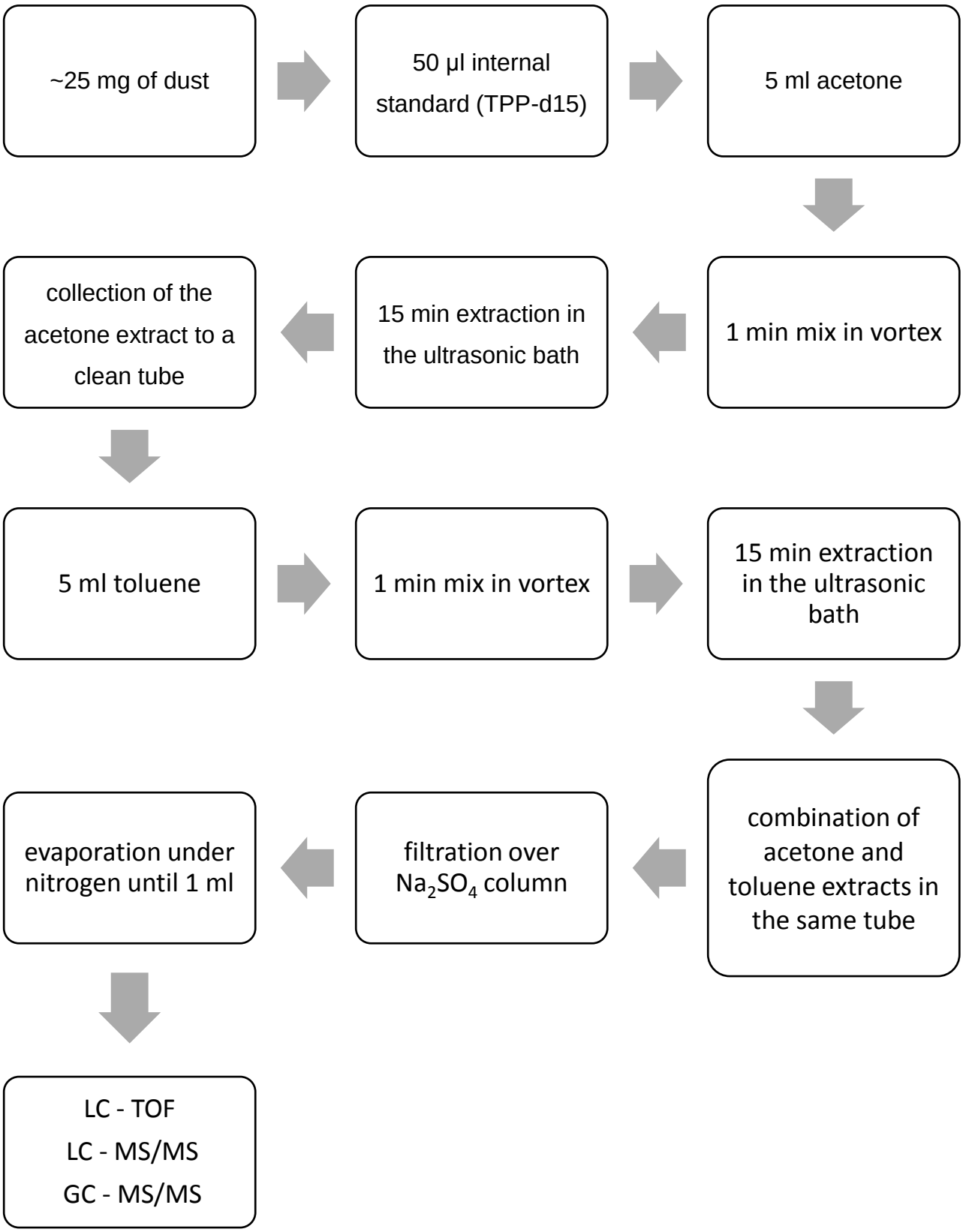


Figure 29: Analytical protocol for the determination of PFRs in dust samples

5.2.3 Sediment samples

The experiments with sediments didn't lead to any extraction method that gives sufficient recoveries for all the compounds. So the protocol was not further developed.

5.3 Method validation

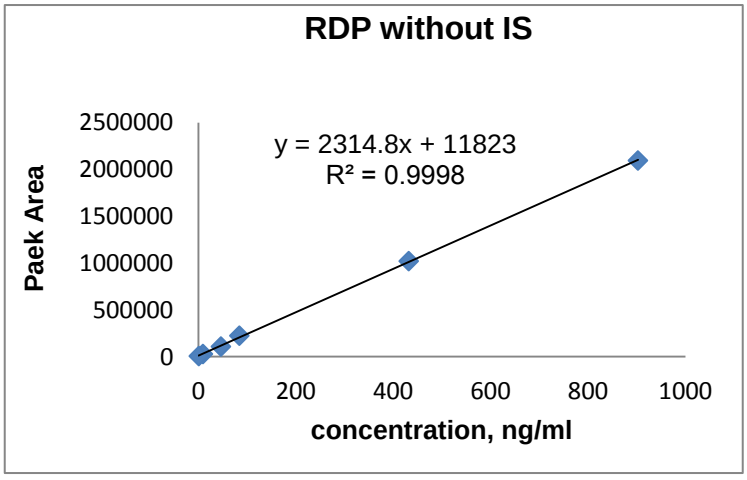
Validation of the developed methods has to be carried out in order to ensure that the procedures followed consistently lead to the expected results and fulfill the particular demands for the specific use. The validation procedure includes the estimation of the method linearity, recovery, limits of detection, repeatability and reproducibility.

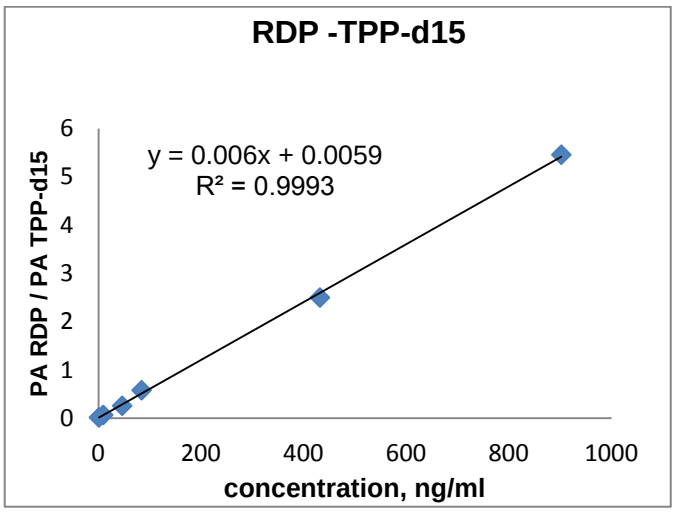
5.3.1 Water samples

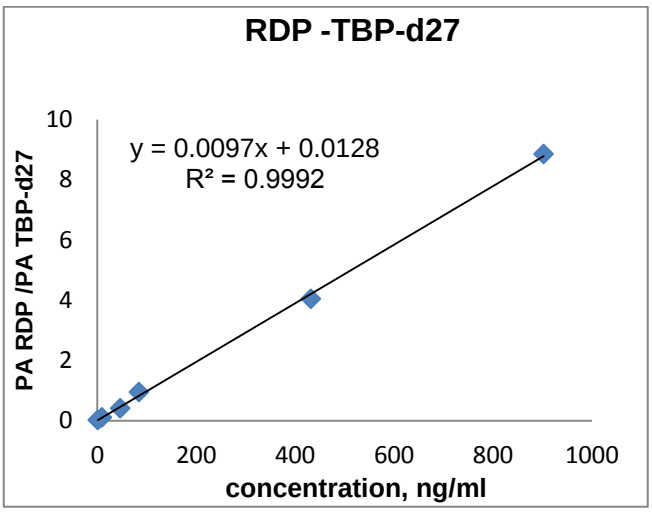
The previous demands were applied to spiked water samples and lead to good results.

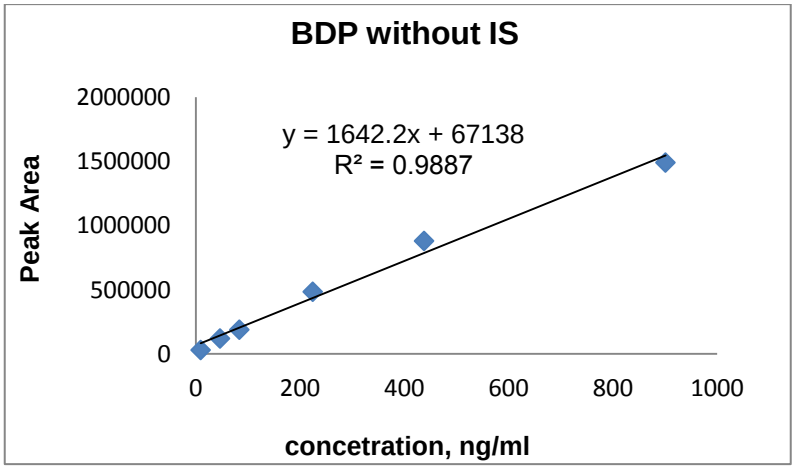
5.3.1.1 Linearity

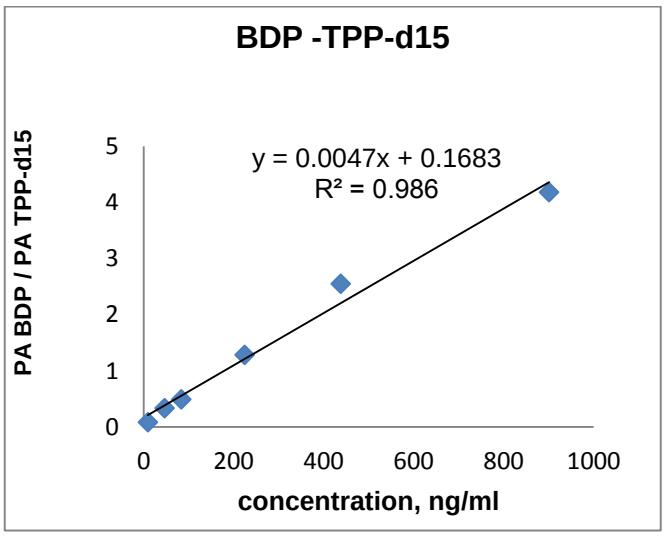
In this study the method linearity was estimated preparing eight solutions in methanol spiked with different amount of PFRs, in a range of concentrations in general between 10 and 100 ng/ml for BDP and RDP and between 450 and 1800 ng/ml for DOPO, because it has higher detection limits. Below, the calibration curves are presented:

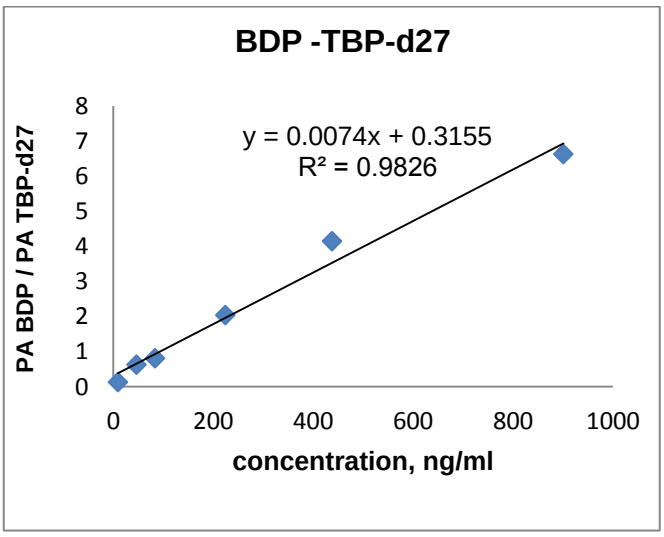


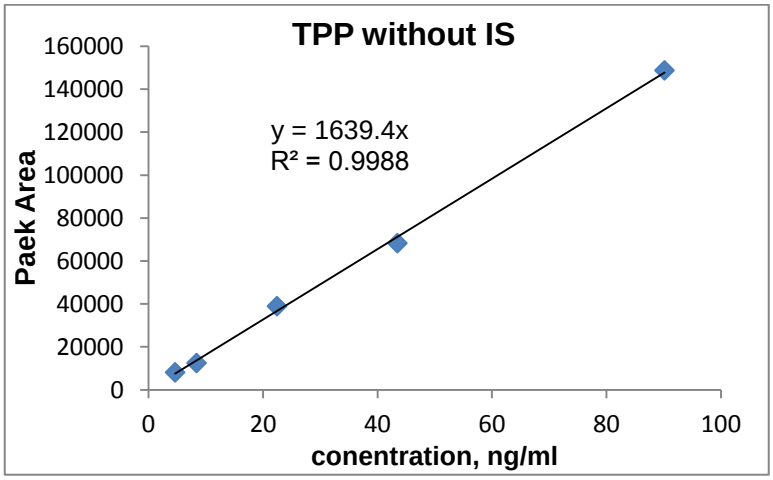

RDP -TPP-d15
y = 0.006x + 0.0059
R² = 0.9993
PA RDP / PA TPP-d15
concentration, ng/ml

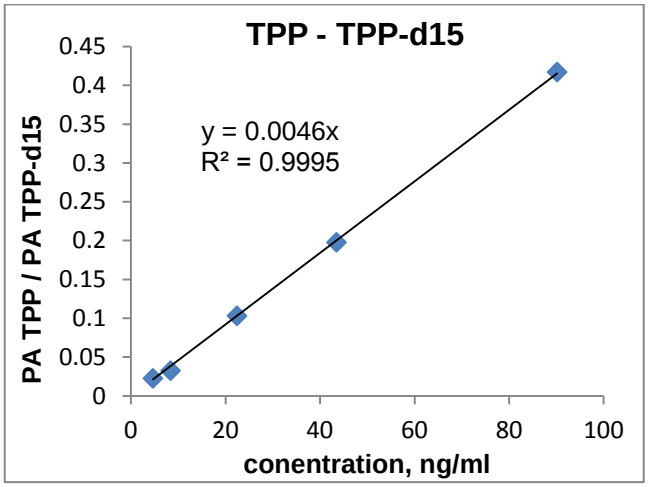

RDP -TBP-d27
y = 0.0097x + 0.0128
R² = 0.9992
PA RDP /PA TBP-d27
concentration, ng/ml

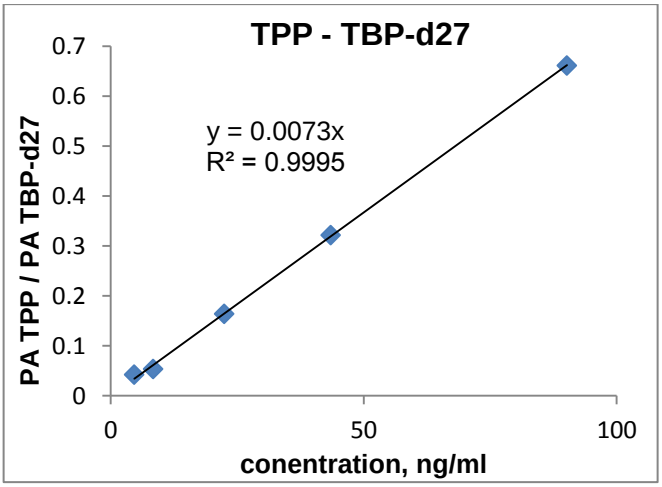

BDP without IS
y = 1642.2x + 67138
R² = 0.9887
Peak Area
concetration, ng/ml

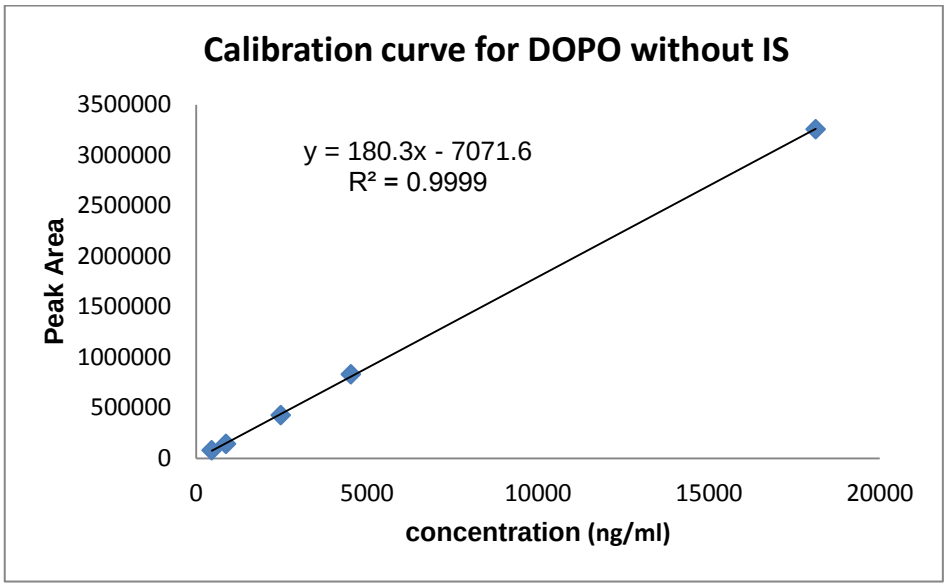

BDP -TPP-d15
y = 0.0047x + 0.1683
R² = 0.986
PA BDP / PA TPP-d15
concentration, ng/ml

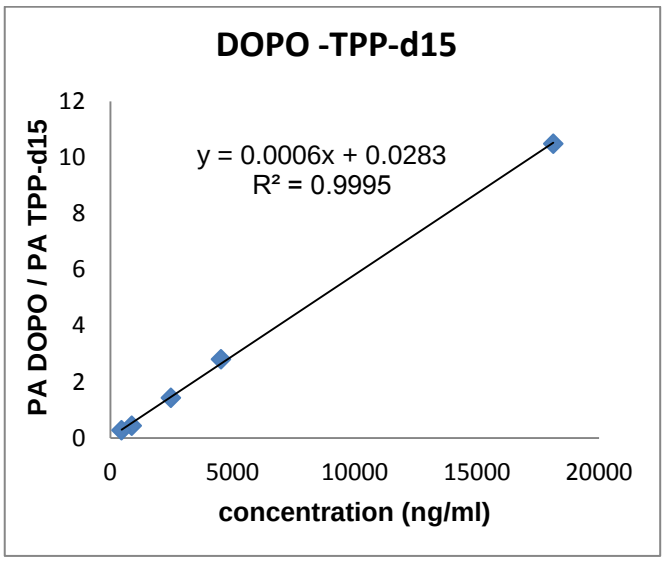

BDP -TBP-d27
y = 0.0074x + 0.3155
R² = 0.9826
PA BDP / PA TBP-d27
concentration, ng/ml

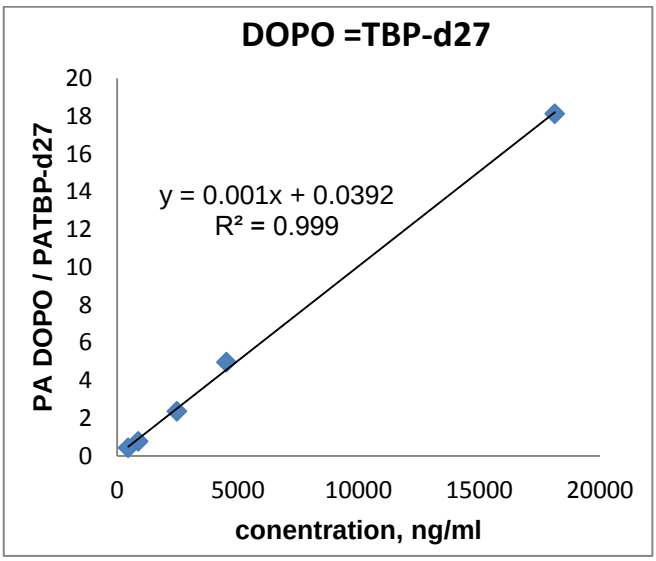

TPP without IS
y = 1639.4x
R² = 0.9988
Paek Area
conentration, ng/ml
160000
140000
120000
100000
80000
60000
40000
20000
0
0
20
40
60
80
100


TPP - TPP-d15
y = 0.0046x
R² = 0.9995
PA TPP / PA TPP-d15
conentration, ng/ml
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
80
100


TPP - TBP-d27
y = 0.0073x
R² = 0.9995
PA TPP / PA TBP-d27
conentration, ng/ml
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100


Calibration curve for DOPO without IS
y = 180.3x - 7071.6
R² = 0.9999
Peak Area
concentration (ng/ml)
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
5000
10000
15000
20000





5.3.1.2 Limits of Detection (LOD)

The limit of detection, LOD, expressed as the concentration, c_L, or the quantity, q_L, is derived from the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation

$$x_L = \overline{x}_{bi} + k\, s_{bi}$$

where $\overline{x}_{bi}$ is the mean of the blank measures, s_{bi} is the standard deviation of the blank measures, and k is a numerical factor chosen according to the confidence level desired.

There are two types of detection limits: instrumental LOD and method LOD. The instrument LOD is calculated using reagents' solutions, while the method LOD is adjusted to the sample's matrix.

There are several ways to calculate the LOD in both cases:

1. According to the signal to noise ratio, S/N: comparing the signals originating from a sample containing low concentration of analyte with the signal originating from a blank sample. Acceptable ratio is S/N = 3,3. This method is used in analytical methods that show baseline noise.
2. According to the standard deviation (*SD*) of the response and to the slope (*b*) of the calibration curve. The following type is used:

$$LOD = \frac{3{,}3 * SD}{b}$$

The SD can be:

i. the SD of the response of the blank sample
ii. the residual standard deviation, $S_{y/x}$, of the calibration curve
iii. the standard deviation of the y-intercept, S_a, of the calibration curve

(The calibration curve in the last two cases is made of samples with analyte's concentrations near to the expected LOD.)

Here, as SD was chosen residual standard deviation, $S_{y/x}$, of the calibration curve. The LODs shown in the table below are the instrumental LODs:

Table 16: Detection limits of RDP, BDP and TPP

Compound	Instrumental LOD (ng/ml)		
	Without IS	**With TPP-d15**	**With TBP-d27**
RDP	18	35	37
BDP	162	148	166
TPP	9.8	2.4	14.1
DOPO	288	605	866

5.3.1.3 Analysis of real water samples

Effluent samples from Eindhoven, Amsterdam west and Rotterdam (Kralingseveer) Sewage Treatment Plants (STP) were collected and analyzed for RDP, BDP and TPP using the previous method. The liquid part of each sample bottle was separated from the solid part with centrifugation and afterwards it was analyzed using the method developed for water samples. Some basic information on the STP systems is given in the table below.

Table 17: The three STP used for the screening of RDP, BDP and TPP

Location	System	Biological capacity
Eindhoven	Aeration tank	1014000
Amsterdam West	Active sludge	750000
Rotterdam, (Kralingseveer)	Low active sludge	400400

Results

The table below shows the concentrations of the RDP, BDP and TPP found in each of the three STPs. The samples were analyzed at LC-MS/MS because their matrix caused problems to time-of-flight, despite the fact that DOPO can't be determined with this technique. All compounds, except for BDP in Rottedam's sample, had concentrations lower than those of the lowest standard.

Table 18: BDP, RDP and TPP concentrations in ng/L from the three STP in the Netherlands

Compound	Concentration, ng/l		
	Eindhoven	Amsterdam	Rotterdam
BDP	<1	<5	10
RDP	<1	<1	<1
TPP	<1	<4	<3

5.3.2 Dust samples

The validation procedure was followed for the dust samples as well, giving good results.

5.3.2.1 Linearity

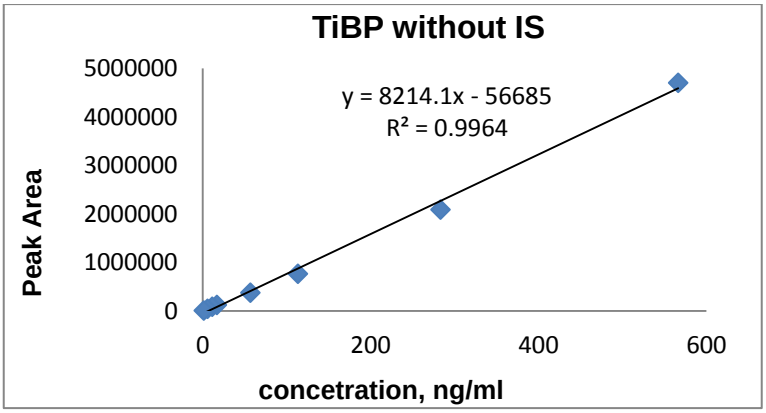
For the calibration curves standard solutions were prepared in iso-octane. In each solution 50μl of internal standard solution were added (TPP-d15, TBR-d27, 13C BDE 209 - 1700, 1700, 500 ng/ml). The total volume of each standard solution was 600μl. Below the concentrations of each compound in each standard solution are shown:

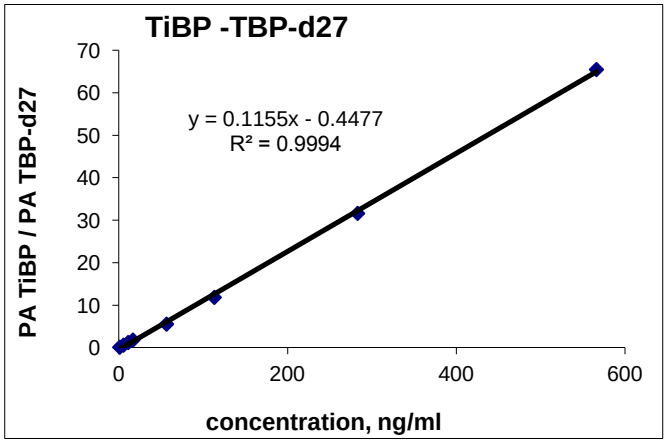
Table 19: Concentrations of PFRs and BDE 209 in each standard solution made for the calibration curves

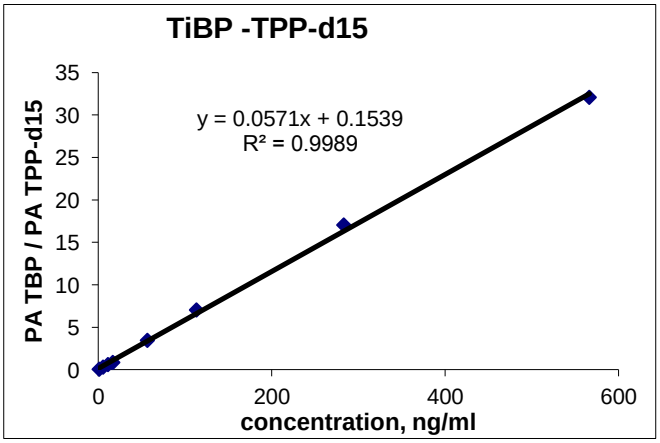
Compound	Concentration (ng/ml)							
	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
TiBP	566	283	113	56.6	16.8	11.2	5.58	1.12

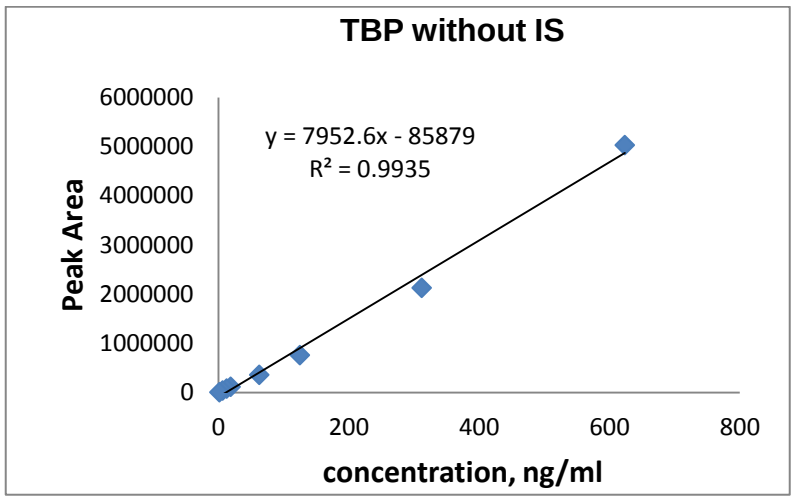
TBP	623	312	125	62.3	18.4	12.3	6.15	1.23
TEHP	675	337	135	67.5	20.0	13.3	6.65	1.33
TCEP	568	284	114	56.8	16.8	11.2	5.60	1.12
TCPP	628	314	126	62.8	18.6	12.4	6.19	1.24
TDCPP	605	302	121	60.5	17.9	11.9	5.96	1.19
TBEP	616	308	123	61.6	18.2	12.2	6.08	1.22
TPP	673	336	135	67.3	19.9	13.3	6.63	1.33
EHDP	695	347	139	69.5	20.6	13.7	6.85	1.37
TCP mix	758	379	152	76	22.7	15	7.6	1.53
RDP	552	261	104	52	15	10	4.9	1.0
BDP	559	279	112	56	16	10.6	5.3	1.1
BDE 209	992	496	198	99	30	20	10	2

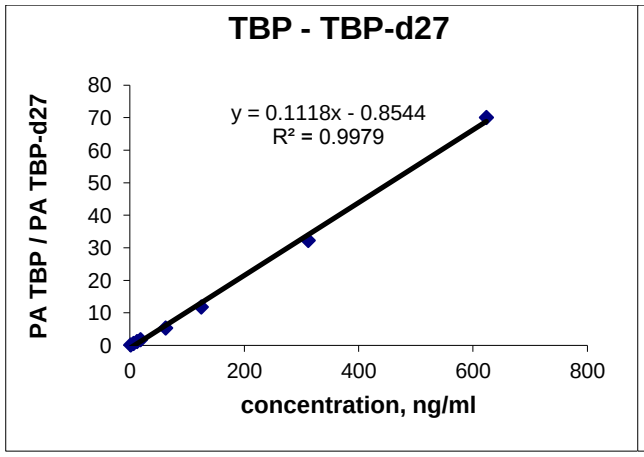
The calibration curves acquired from the analysis of the standard solutions are the following:

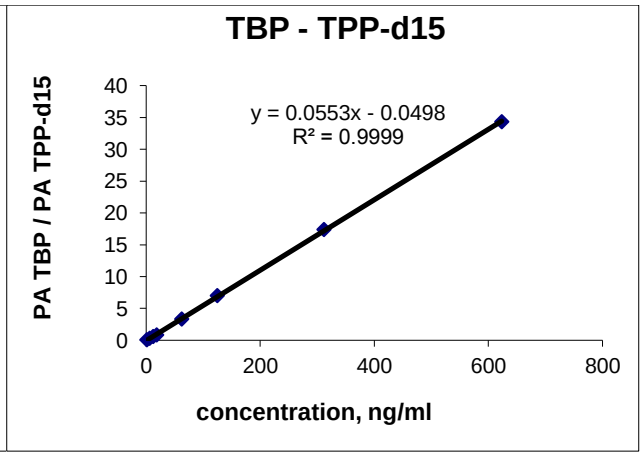


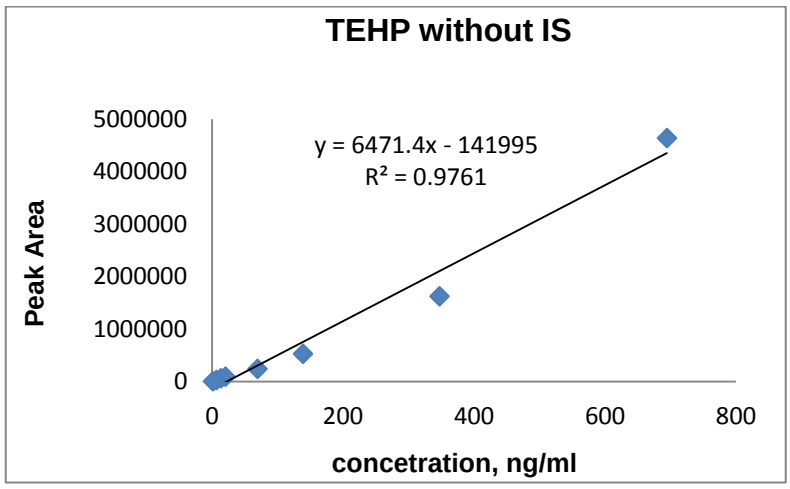


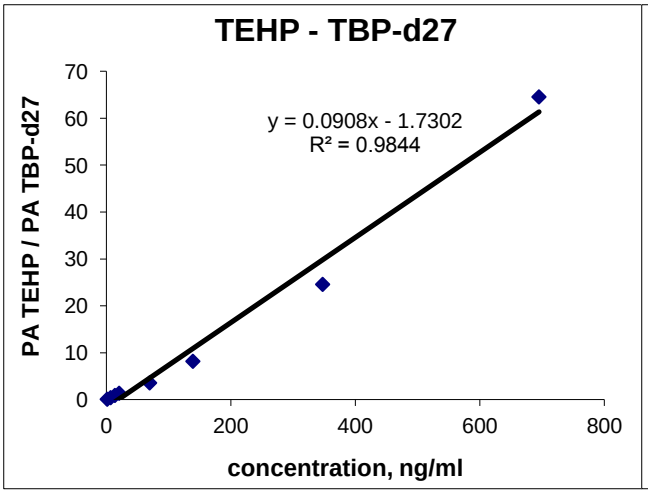


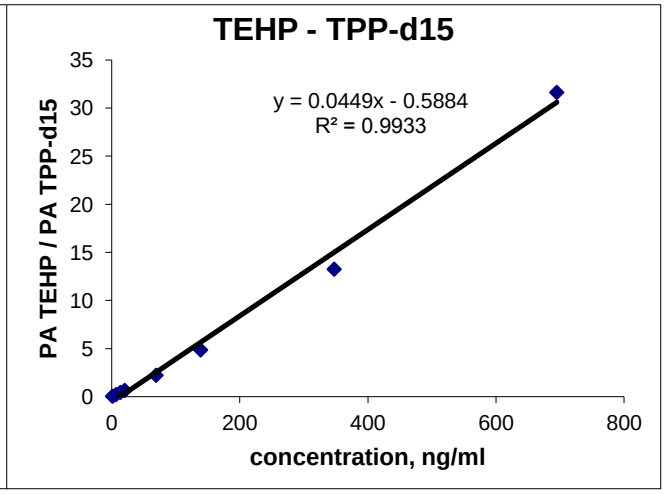

TBP without IS
y = 7952.6x - 85879
R² = 0.9935
Peak Area
6000000
5000000
4000000
3000000
2000000
1000000
0
0
200
400
600
800
concentration, ng/ml

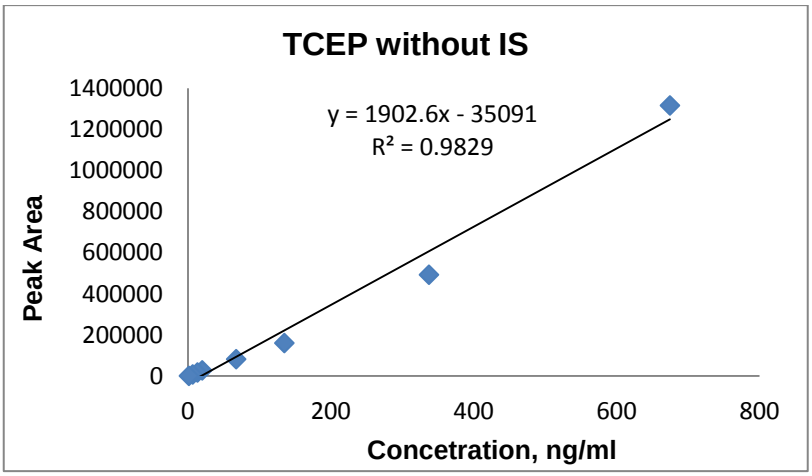

TBP - TBP-d27
y = 0.1118x - 0.8544
R² = 0.9979
PA TBP / PA TBP-d27
80
70
60
50
40
30
20
10
0
0
200
400
600
800
concentration, ng/ml

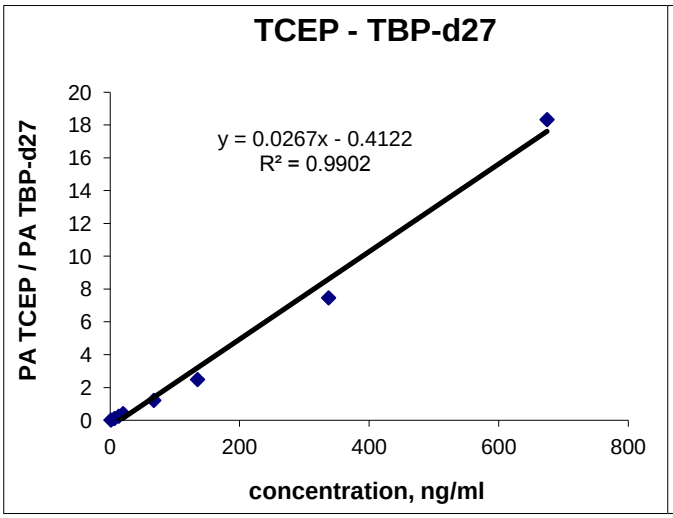

TBP - TPP-d15
y = 0.0553x - 0.0498
R² = 0.9999
PA TBP / PA TPP-d15
40
35
30
25
20
15
10
5
0
0
200
400
600
800
concentration, ng/ml

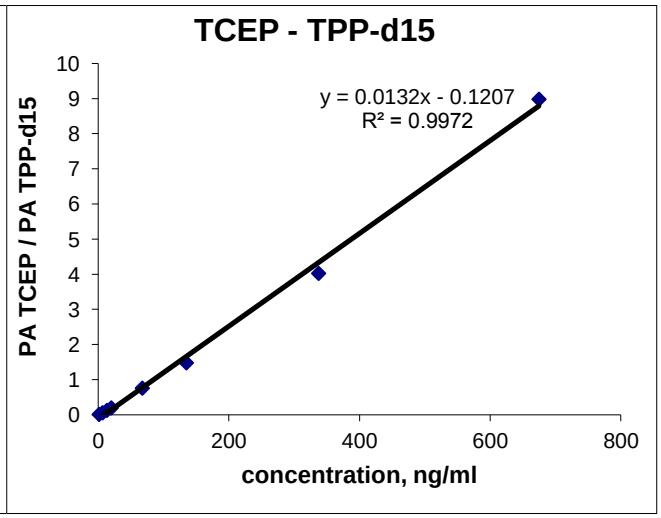

TEHP without IS
y = 6471.4x - 141995
R² = 0.9761
Peak Area
5000000
4000000
3000000
2000000
1000000
0
0
200
400
600
800
concetration, ng/ml

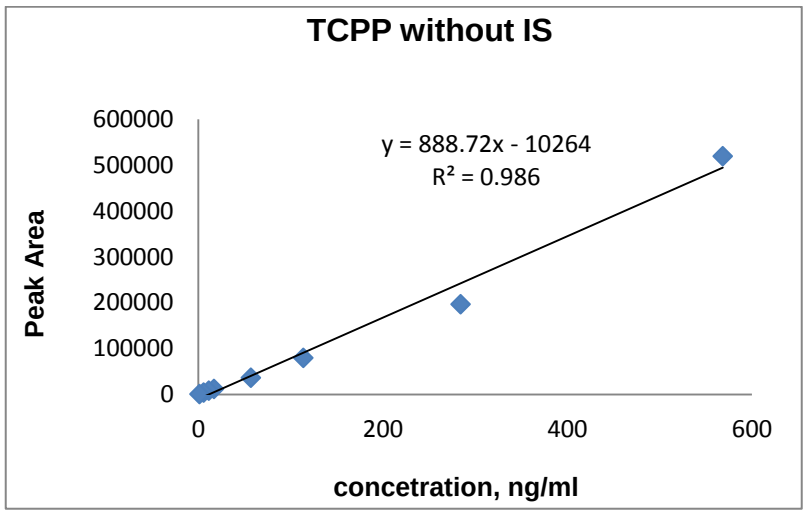

TEHP - TBP-d27
y = 0.0908x - 1.7302
R² = 0.9844
PA TEHP / PA TBP-d27
concentration, ng/ml

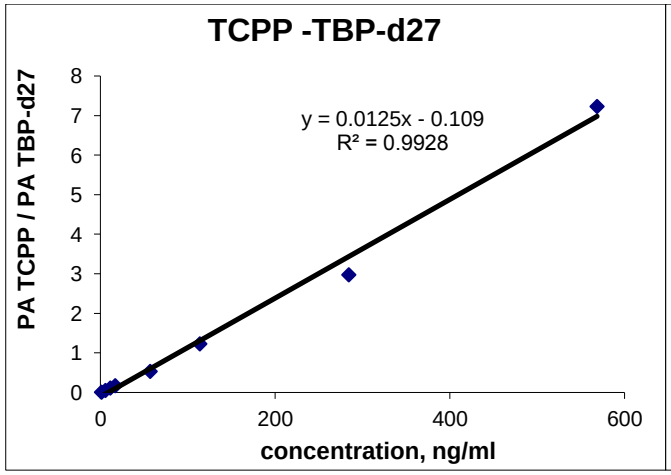

TEHP - TPP-d15
y = 0.0449x - 0.5884
R² = 0.9933
PA TEHP / PA TPP-d15
concentration, ng/ml

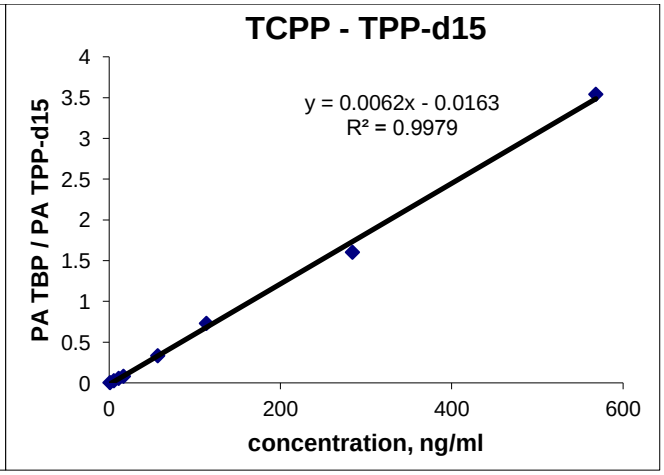

TCEP without IS
y = 1902.6x - 35091
R² = 0.9829
Peak Area
Concetration, ng/ml

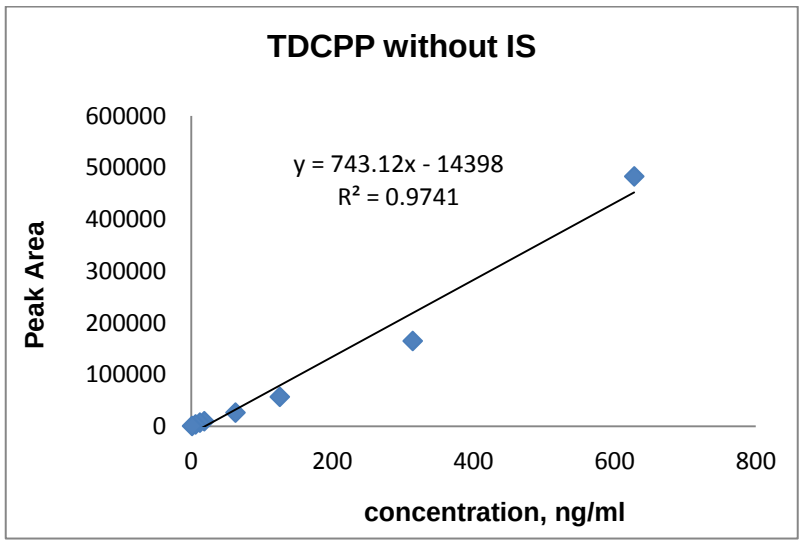

TCEP - TBP-d27
y = 0.0267x - 0.4122
R² = 0.9902
PA TCEP / PA TBP-d27
concentration, ng/ml

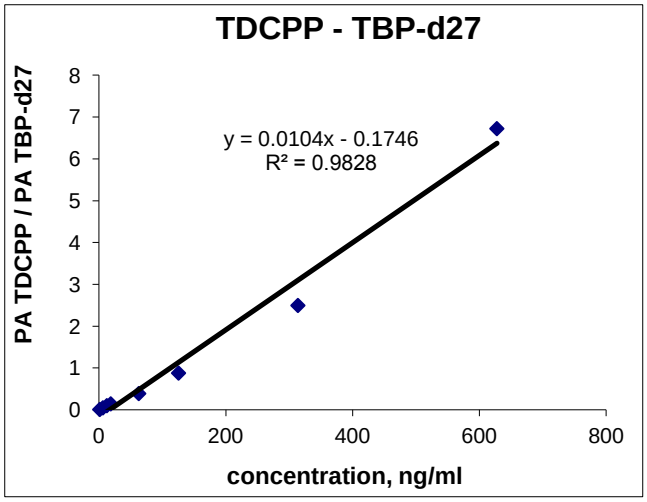

TCEP - TPP-d15
y = 0.0132x - 0.1207
R² = 0.9972
PA TCEP / PA TPP-d15
concentration, ng/ml

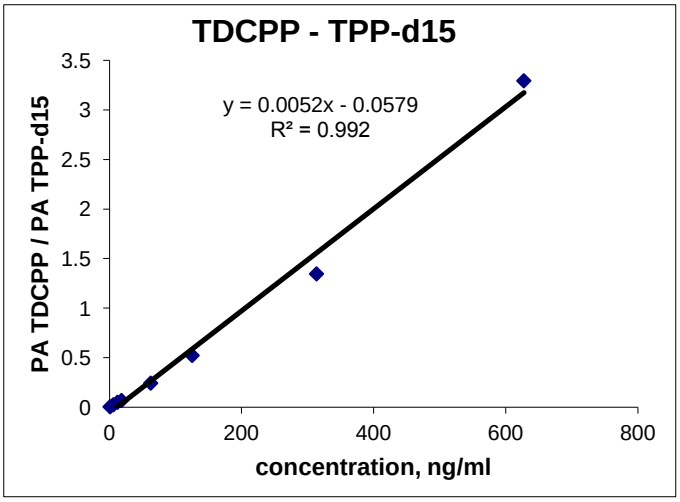

TCPP without IS
y = 888.72x - 10264
R² = 0.986
Peak Area
600000
500000
400000
300000
200000
100000
0
0
200
400
600
concetration, ng/ml

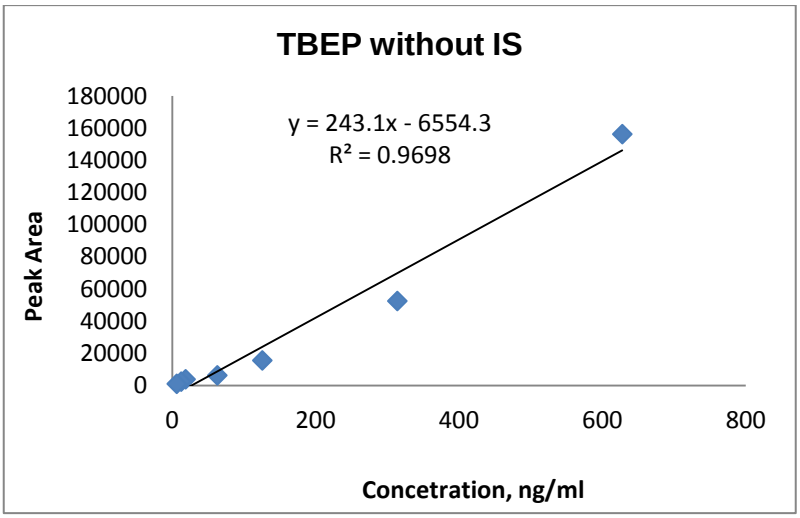

TCPP -TBP-d27
y = 0.0125x - 0.109
R² = 0.9928
PA TCPP / PA TBP-d27
8
7
6
5
4
3
2
1
0
0
200
400
600
concentration, ng/ml

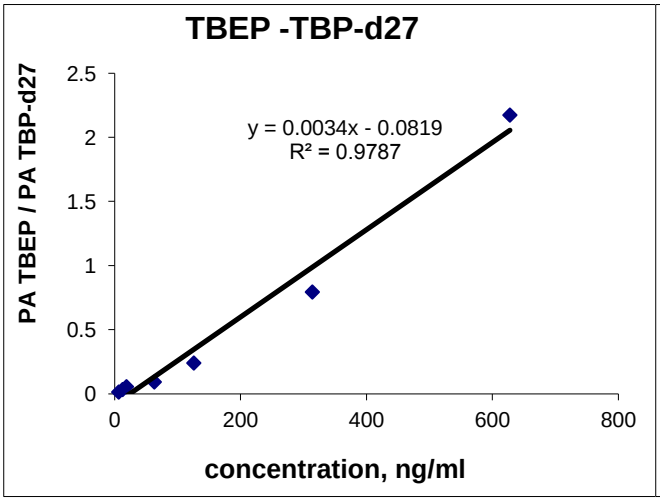

TCPP - TPP-d15
y = 0.0062x - 0.0163
R² = 0.9979
PA TBP / PA TPP-d15
4
3.5
3
2.5
2
1.5
1
0.5
0
0
200
400
600
concentration, ng/ml

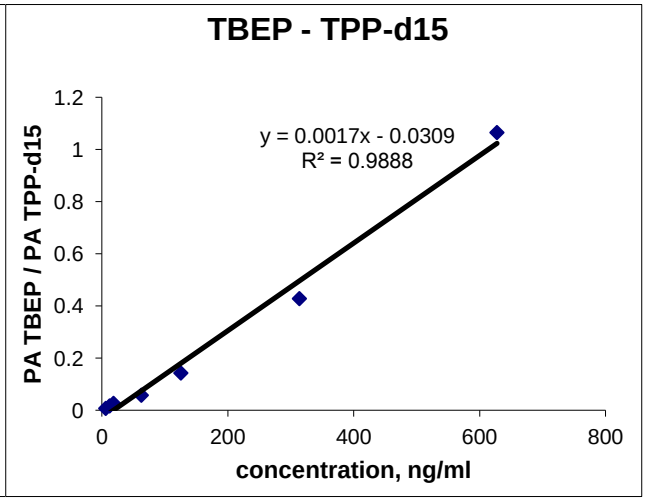

TDCPP without IS
y = 743.12x - 14398
R² = 0.9741
Peak Area
600000
500000
400000
300000
200000
100000
0
0
200
400
600
800
concentration, ng/ml

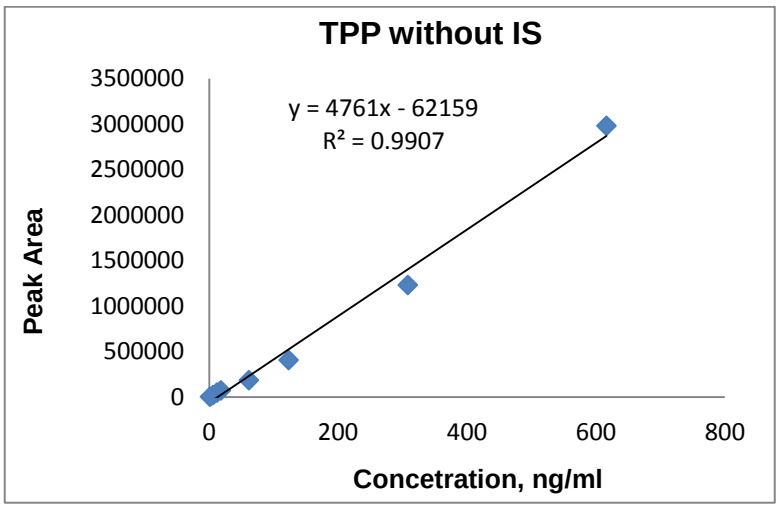

TDCPP - TBP-d27
y = 0.0104x - 0.1746
R² = 0.9828
PA TDCPP / PA TBP-d27
concentration, ng/ml

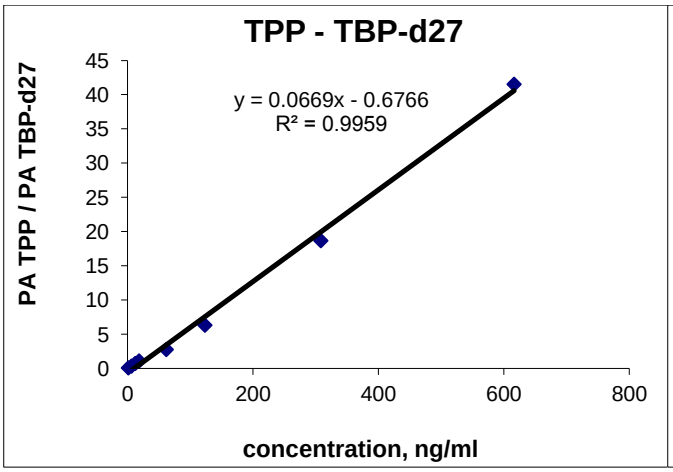

TDCPP - TPP-d15
y = 0.0052x - 0.0579
R² = 0.992
PA TDCPP / PA TPP-d15
concentration, ng/ml

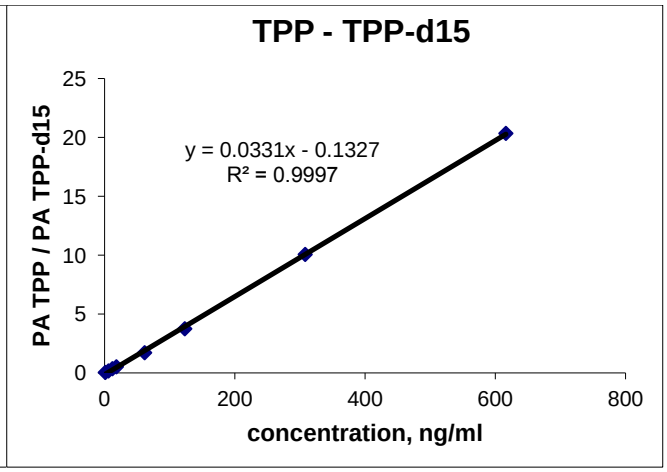

TBEP without IS
y = 243.1x - 6554.3
R² = 0.9698
Peak Area
Concetration, ng/ml

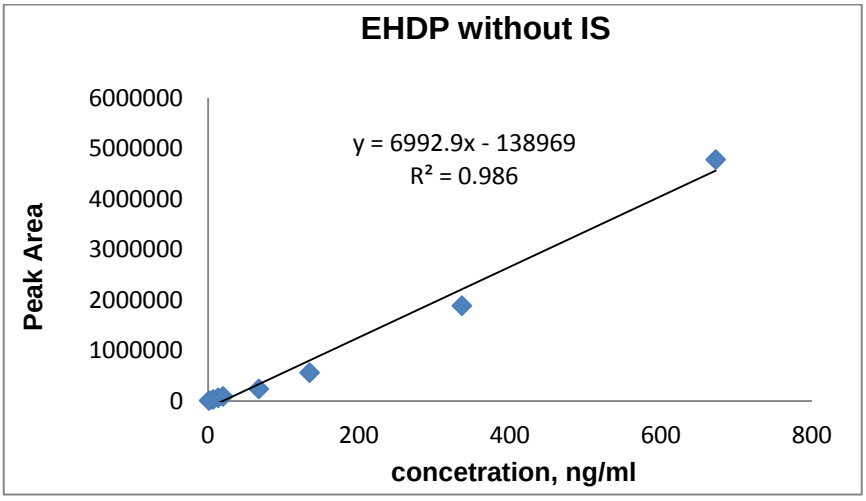

TBEP -TBP-d27
y = 0.0034x - 0.0819
R² = 0.9787
PA TBEP / PA TBP-d27
concentration, ng/ml

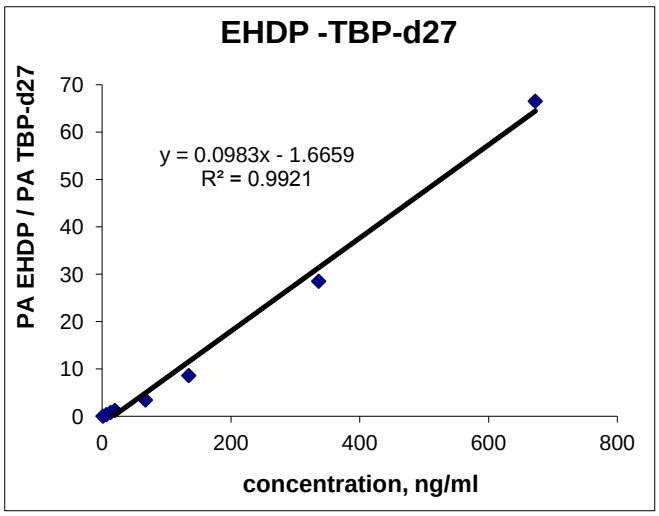

TBEP - TPP-d15
y = 0.0017x - 0.0309
R² = 0.9888
PA TBEP / PA TPP-d15
concentration, ng/ml

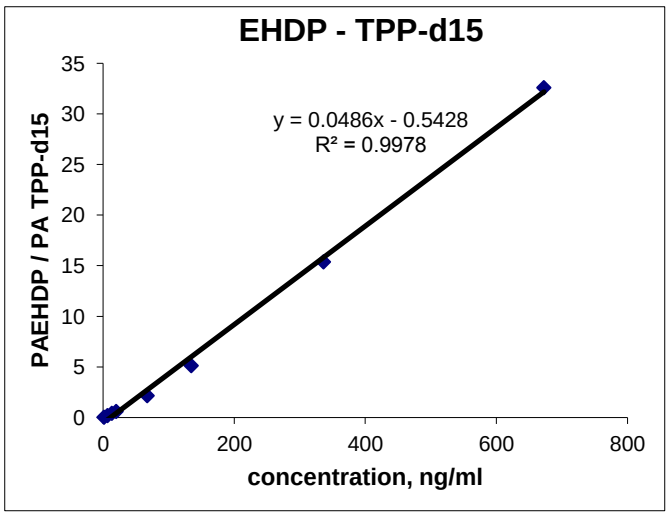

TPP without IS
y = 4761x - 62159
R² = 0.9907
Peak Area
Concetration, ng/ml
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
200
400
600
800

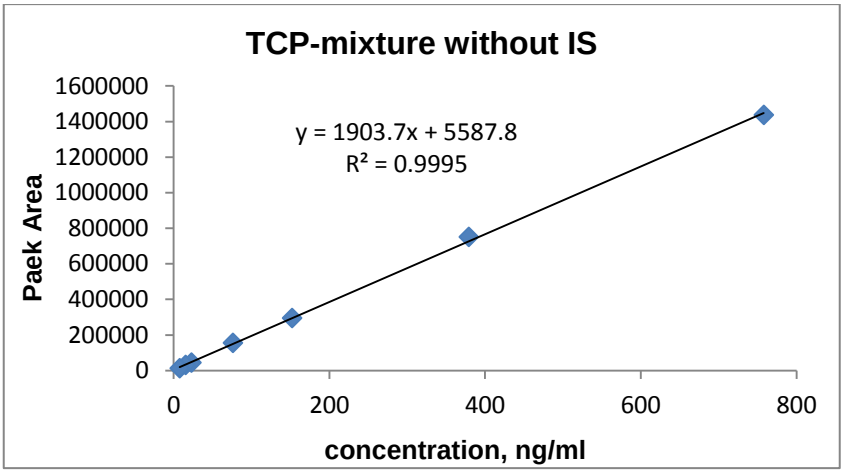

TPP - TBP-d27
y = 0.0669x - 0.6766
R² = 0.9959
PA TPP / PA TBP-d27
concentration, ng/ml
45
40
35
30
25
20
15
10
5
0
0
200
400
600
800

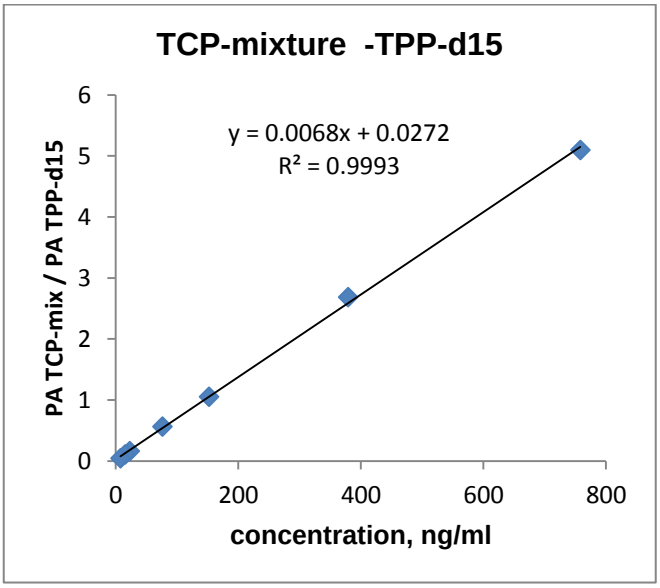

TPP - TPP-d15
y = 0.0331x - 0.1327
R² = 0.9997
PA TPP / PA TPP-d15
concentration, ng/ml
25
20
15
10
5
0
0
200
400
600
800

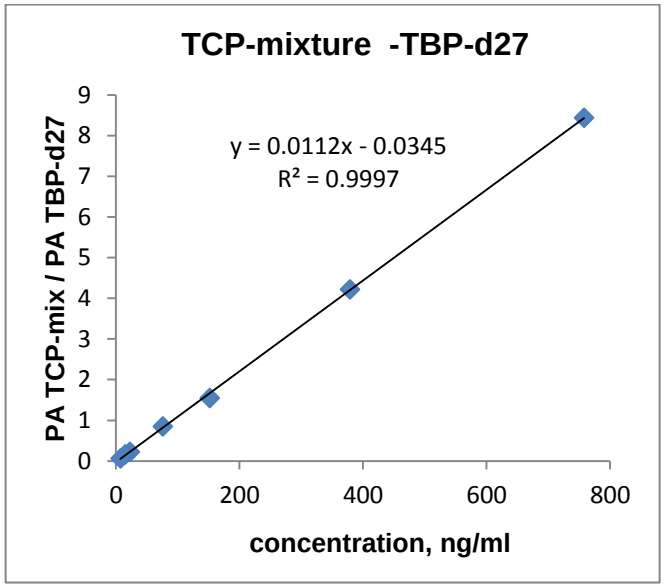

EHDP without IS
y = 6992.9x - 138969
R² = 0.986
Peak Area
concetration, ng/ml
6000000
5000000
4000000
3000000
2000000
1000000
0
0
200
400
600
800

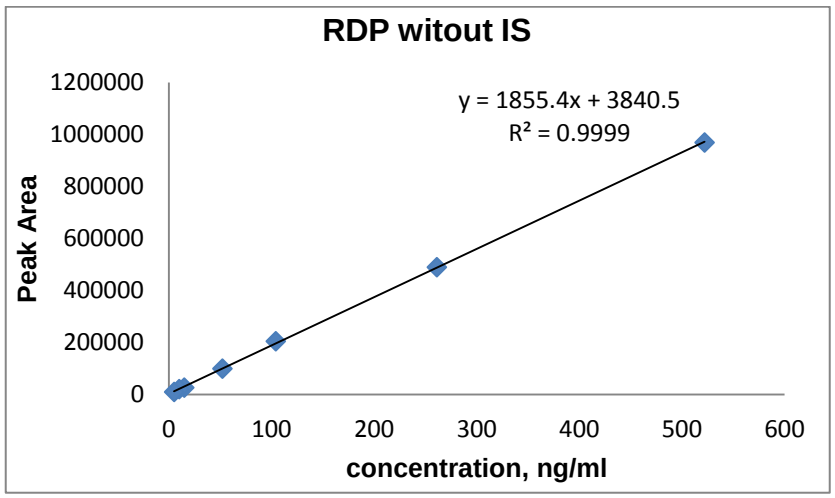

EHDP -TBP-d27
PA EHDP / PA TBP-d27
y = 0.0983x - 1.6659
R² = 0.9921
concentration, ng/ml

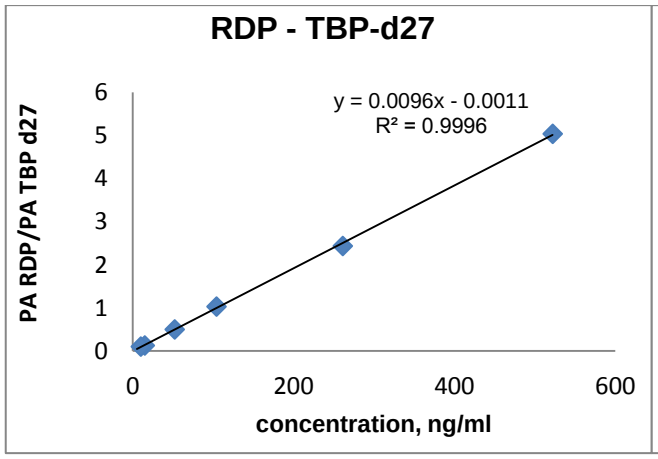

EHDP - TPP-d15
PAEHDP / PA TPP-d15
y = 0.0486x - 0.5428
R² = 0.9978
concentration, ng/ml

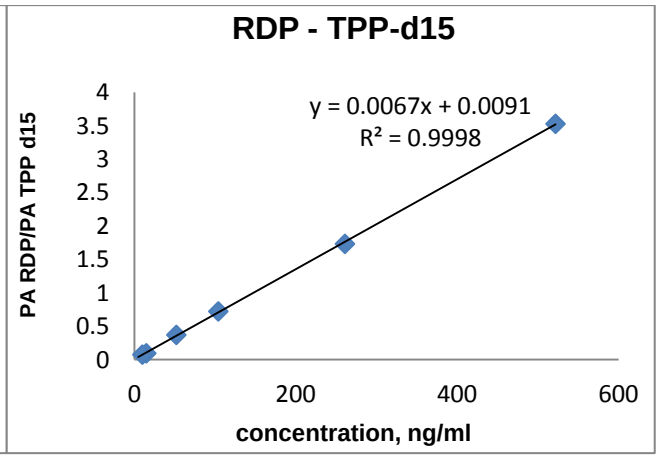

TCP-mixture without IS
Paek Area
y = 1903.7x + 5587.8
R² = 0.9995
concentration, ng/ml

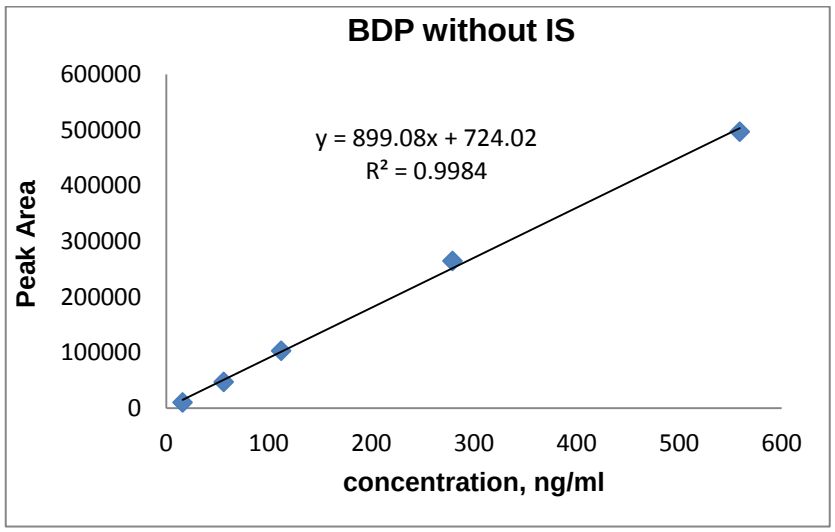

TCP-mixture -TPP-d15
PA TCP-mix / PA TPP-d15
y = 0.0068x + 0.0272
R² = 0.9993
concentration, ng/ml

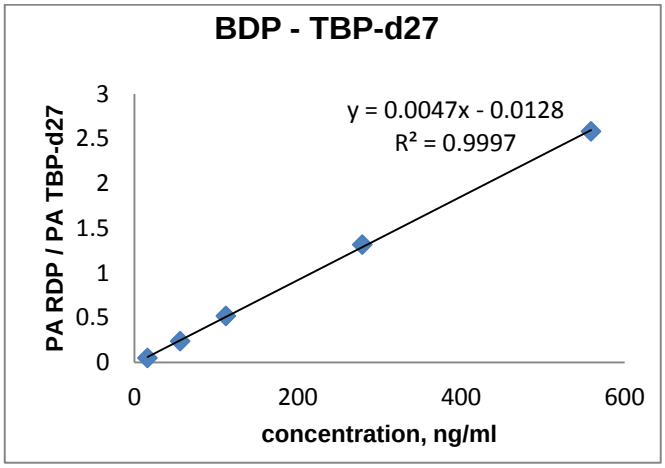

TCP-mixture -TBP-d27
PA TCP-mix / PA TBP-d27
y = 0.0112x - 0.0345
R² = 0.9997
concentration, ng/ml

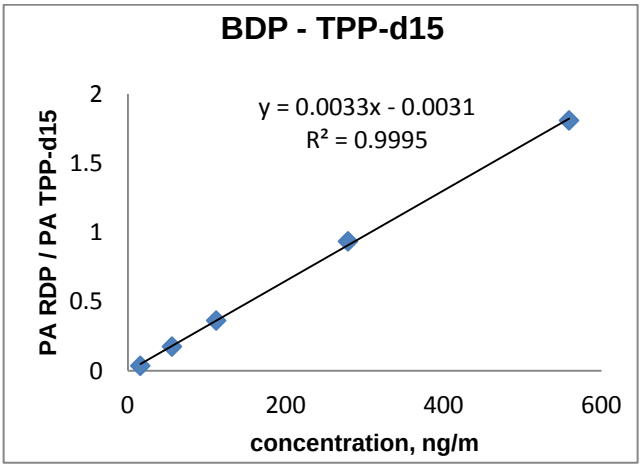

RDP witout IS
y = 1855.4x + 3840.5
R² = 0.9999
Peak Area
1200000
1000000
800000
600000
400000
200000
0
0
100
200
300
400
500
600
concentration, ng/ml

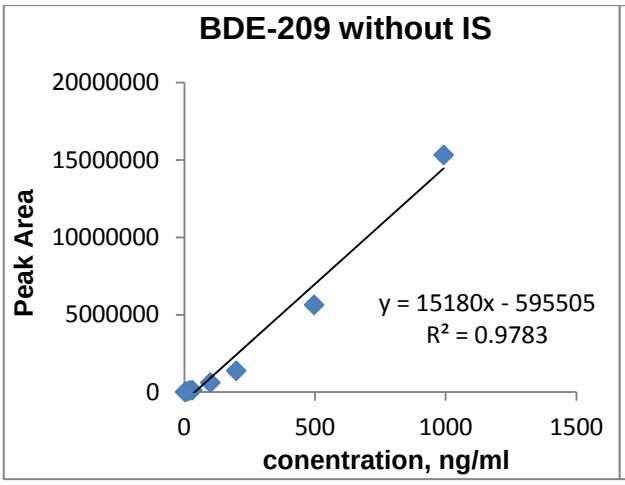

RDP - TBP-d27
y = 0.0096x - 0.0011
R² = 0.9996
PA RDP/PA TBP d27
6
5
4
3
2
1
0
0
200
400
600
concentration, ng/ml

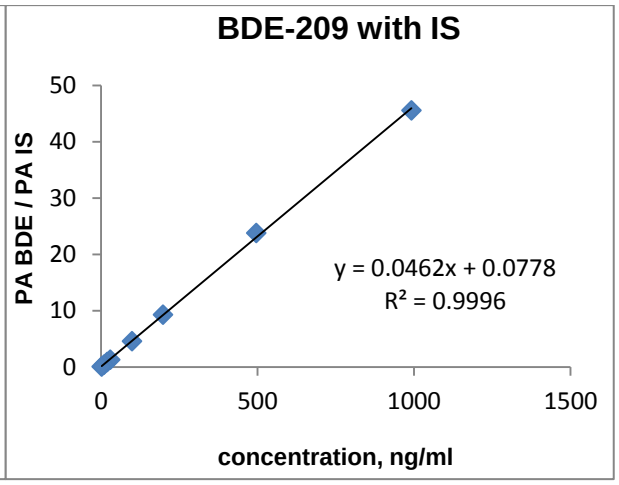

RDP - TPP-d15
y = 0.0067x + 0.0091
R² = 0.9998
PA RDP/PA TPP d15
4
3.5
3
2.5
2
1.5
1
0.5
0
0
200
400
600
concentration, ng/ml


BDP without IS
y = 899.08x + 724.02
R² = 0.9984
Peak Area
600000
500000
400000
300000
200000
100000
0
0
100
200
300
400
500
600
concentration, ng/ml









5.3.2.2 Recovery

The dust sample SRM 2585 was used. It is only certified for BDE 209 and not for the PFRs though, so spike experiments were conducted at two levels of PFRs' concentration. In each tube around 75 mg of the dust sample SRM 2585 were weighed and 50µl of internal standard (TPP-d15, TBP-d27, 13C 209 - 1700, 1700 and 500 ng/ml) were added. Three of the tubes were spiked with 50 µl of higher concentration dilution of PFRs and three with lower concentration dilution of PFRs. The next three tubes were spiked only with internal standard and the last three were the blank samples, therefore they contained only 50 µl internal standard without dust. After spiking, all samples were left to stabilize for 2 hours. The extraction was carried out following the analysis method described above (section 5.2.2).

For the calculations of RDP, BDP and DOPO the internal standard TBP-d27 was used, whereas for the rest PFRs the internal standard TPP-d15 was used.

The measurements at the unspiked tubes showed that the SRM 2585 contains some of the PFR analytes. These levels constitute the background concentrations and were taken under consideration in the calculations followed. The table below shows the amount of PFRs found in SRM 2585 and compares it with previous articles.

Table 20: PFRs concentrations (ng/g) in SRM 2585 in current and previous studies

Compound	LC-MS/MS (TPP-d15)	LC-MS/MS (TBP-d27)	GC-MS (TPP-d15)	Berg et al.	Van den Eede et al.
TiBP	546	528	394	<290	
TBP	739	810	752	190	180
TCEP	1036	1018	1404	840	700
TCPP	1108	1006	1216	880	820
TDCPP	362	387	2778	2300	2000
TBEP	66021	46314	92527	82000	49000
TPP	1997	1705	1654	1100	990
EHDP	287	339	1695		
TEHP	652	727	1656	370	
TCP total	781	854	1877	740	1070
RDP	147	147			
BDP	156	156			

The same dust samples were analyzed for BDE-209 and the recovery was calculated comparing the given by the SRM value (true value, 2510 ± 190 ng/g) with the results.

The tables below show the recoveries of all compounds:

- OPFRs:

Table 21: Method recovery at high level

Compound	Concentration spiked, ng/g	% Recovery				Standard deviation	% Relative standard deviation
		Sample 1	Sample 2	Sample 3	Average		
TiBP	178	84	87	84	85	0.020	1.7
TBP	191	87	89	91	89	0.022	1.9
TCEP	205	97	101	106	101	0.045	3.9
TCPP1	174	92	98	94	95	0.033	2.9
TCPP2	174	107	119	111	112	0.058	5.1
TDCPP	192	114	104	119	112	0.079	7.0
TBEP	1840	69	67	91	76	0.133	11.7
TPP	184	88	87%	91	89	0.021	1.8
EHDP	204	94	95	106	98	0.066	5.8
TEHP	214	121	115	134	123	0.097	8.6
MMM-TCP	236	166	157	172	165	0.075	6.6
MMP-TCP	236	141	138	153	144	0.079	6.9
MPP-TCP	236	175	173	189	179	0.088	7.8
PPP-TCP	236	197	180	202	193	0.116	10.2
TCP total	236	154	149	165	156	0.078	6.9
RDP	493	81	83	97	87	0.085	1.8
BDP	524	66	68	76	70	0.056	5.8

Table 22: Method recovery at low level

Compound	Concentration spiked, ng/g	% Recovery				Standard deviation	% Relative standard deviation
		Sample 1	Sample 2	Sample 3	Average		
TiBP	53	80	81	83	82	0.014	1.3
TBP	57	92	81	87	87	0.054	5.0
TCEP	61	78	88	85	84	0.055	5.1
TCPP1	52	91	89	82	87	0.050	4.6
TCPP2	52	105	121	95	107	0.130	12.0
TDCPP	57	94	104	122	107	0.140	12.9
TBEP	548	29	61	107	65	0.394	36.3
TPP	55	74	91	94	86	0.107	9.9
EHDP	61	88	94	103	95	0.078	7.2
TEHP	64	134	133	151	140	0.101	9.3
MMM-TCP	70	157	148	179	162	0.162	14.9
MMP-TCP	70	127	130	158	138	0.172	15.8
MPP-TCP	70	158	159	191	169	0.185	17.1
PPP-TCP	70	162	86	288	179	1.023	94.2
TCP total	70	142	139	172	151	0.182	16.8
RDP	147	93	97	96	95	0.024	9.9
BDP	156	60	75	59	65	0.090	7.2

- BDE-209:

Table 23: Method recovery for BDE 209

Sample	1	2	3	4	5	6	7	8	9
Concentration calculated, ng/g	2482	2632	2781	2488	3685	2519	2412	2876	2280
% Recovery	99	105	11	99	147	100	96	115	91
% Average recovery	107								
Standard deviation	0.17								
% Relative standard deviation	15.6								

5.3.2.3 Repeatability

The same experiment was used to calculate the repeatability. Again here, TBP-d27 was used to calculate the repeatability of RDP, BDP and DOPO, while TPP-d15 was used for the repeatability of the rest PFRs.

- OPFRs:

Table 24: Repeatability at high level

Compound	Concentration, ng/g				Standard deviation	% Relative standard deviation
	Sample 1	Sample 2	Sample 3	Average		
TiBP	149	155	150	151	3.5	2.3
TBP	166	169	174	170	4.2	2.5
TCEP	198	206	216	207	9.1	4.4
TCPP1	160	171	163	165	5.6	3.4

TCPP2	187	206	193	195	10.1	5.2
TDCPP	218	199	229	215	15.2	7.0
TBEP	1268	1232	1672	1390	244.3	17.6
TPP	162	160	167	163	3.8	2.3
EHDP	191	194	216	201	13.4	6.7
TEHP	258	246	287	264	20.8	7.9
MMM-TCP	391	371	406	389	17.8	4.6
MMP-TCP	331	325	360	338	18.6	5.5
MPP-TCP	412	407	445	421	20.8	4.9
PPP-TCP	463	424	477	455	27.2	6.0
TCP total	365	352	388	368	18.4	5.0
RDP	401	408	477	429	42.1	2.3
BDP	345	355	401	367	29.4	6.7

Table 25: Repeatability at low level

Compound	**Concentration, ng/g**				**Standard deviation**	**% Relative standard deviation**
	Sample 1	**Sample 2**	**Sample 3**	**Average**		
TiBP	43	43	44	43	0.8	1.8
TBP	52	46	50	49	3.1	6.2
TCEP	47	54	52	51	3.4	6.6
TCPP1	47	46	42	45	2.6	5.7
TCPP2	54	63	49	55	6.7	12.1
TDCPP	54	60	69	61	8.0	13.1
TBEP	157	331	586	358	215.9	60.3

TPP	41	49	52	47	5.9	12.4
EHDP	53	57	63	58	4.7	8.2
TEHP	85	85	96	89	6.5	7.3
MMM-TCP	110	104	126	113	11.3	10.0
MMP-TCP	89	91	111	97	12.1	12.4
MPP-TCP	111	112	134	119	13.0	10.9
PPP-TCP	114	60	202	125	71.7	57.3
TCP total	100	97	120	106	12.7	12.1
RDP	136	142	141	140	3.5	12.4
BDP	93	117	92	101	14.1	8.2

- BDE-209:

Table 26: Repeatability of BDE - 209

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Concentration calculated, ng/g	2482	2632	2781	2488	3685	2519	2412	2876	2280
Average	2684								
Standard deviation	417.8								
%Relative standard deviation	16%								

5.3.2.4 Limits of detection

The instrumental limits of detection were calculated as previously (section 5.3.1.2):

Table 27: Limits of detection (LOD)

Compound	Instrumental LOD (ng/ml)		
	Without IS	**With TPP-d15**	**With TBP-d27**
TiBP	43	24	17
TBP	63	8	36
TCEP	112	45	84
TCPP	85	33	61
TDCPP	129	70	105
TBEP	147	88	123
TPP	75	14	50
EHDP	101	39	79
TEHP	136	45	84
TCP-mix	22	16	29
RDP	8	9	14
BDP	33	19	14
BDE-209	**Without IS**	**With ^{13}C BDE-209**	
	248	26	

5.3.2.5 Analysis of real dust samples

The dust samples were collected mainly from homes (one car sample is also included) from both the Netherlands and Greece and the differences in patterns were assessed. The samples were analyzed with the method described above. In the tables below a detailed description of the samples and the PFRs' levels measured are given.

Table 28: Description of samples, samples' locations and weights

Country	Sample	Location	Weight (mg)
Holland	1	House 1 – around e.e.	12.66
	2	House 2 – around e.e.	14.09
	3	House 3 – around e.e.	49.12
	4	House 4 – around e.e.	50.14
	5	House 5 – around e.e.	23.39
	6	House 1 – on e.e.	10.52
	7	House 2 – on e.e.	10.28
	8	House 3 – on e.e.	21.49
	9	House 4 – on e.e.	17.33
	10	House 5 – on e.e.	20.11
	14	House 6 – on e.e.	34.38
	15	House 6 – around e.e.	28.14
	16	House 7 – on e.e.	7.19
	17	House 7 – around e.e.	20.56
Greece	19	House 8 – on e.e.	3.10
	20	House 8 – around e.e.	21.10
	21	House 9 – on e.e.	21.80
	22	House 9 – around e.e.	30.20
	23	House 10 – on e.e.	8.10
	24	House 11 – pc monitor	10.50
	25	House 11 – pc	25.00
	26	House 12 – tv	1.90
	27	House 12 – fridge	9.00
Holland	28	Car	20.8
	29	House 13 – 2-3 m from tv	26.00
	30	House 13 – around tv	29.50
	31	House 13 – on tv	2.30

** e.e.: electronic equipment*

Results:

All samples were analyzed at GC-MS for TiBP, TBP, TCEP, TCPP, TBEP, TPP, EHDP, TEHP, TCP and BDE 209. Analysis for RDP and BDP was carried out only at LC-MS/MS because these compounds are not volatile. Moreover, samples 1-17 were additionally analyzed at LC-MS/MS. The results are given in the tables below.

Table 29: Levels of FRs in ng/g in the analyzed with GC-MS/MS dust samples

Sample	1	2	3	4	5	6	7	8	9
TiBP	0.1	0.04	0.05	0.05	0.04	0.1	0.10	0.11	0.09
TBP	0.1	0.02	0.20	0.02	0.02	0.1	0.10	0.21	0.03
TCEP	1.45	0.59	6.94	1.11	1.77	1.97	0.52	4.47	1.06
TCPP1	0.48	2.33	0.60	3.84	4.62	0.58	2.40	1.31	3.08
TCPP2	0.64	4.17	1.05	6.89	4.66	0.87	3.69	2.42	5.71
TDCPP	0.84	3.23	0.24	0.40	0.26	1.18	7.41	1.81	0.48
TBEP	25.06	31.0	11.5	11.3	26.6	27.1	34.6	36.5	20.4
TPP	0.75	0.74	0.76	2.95	10.9	1.63	7.31	3.58	5.72
EHDP	0.55	0.34	0.39	1.97	0.35	0.57	3.68	0.35	1.73
TEHP	0.14	0.36	0.02	1.39	0.36	0.28	0.78	0.42	0.92
total-TCP	0.14	0.18	0.07	0.18	0.18	0.15	0.42	0.29	2.27
BDE209	0.3	0.03	0.1	3.8	0.09	0.2	0.1	0.1	0.8

Sample	10	14	15	16	17	19	20	21	22
TiBP	0.01	0.07	0.03	0.03	0.60	0.45	0.24	0.44	0.46
TBP	0.01	0.01	0.01	0.02	0.07	0.52	0.08	0.07	0.05
TCEP	2.19	0.55	4.56	0.49	0.22	0.58	0.41	1.77	8.42
TCPP1	4.46	0.93	0.97	1.30	1.67	0.52	0.68	1.68	1.40
TCPP2	4.73	1.04	1.36	2.10	2.28	0.00	0.96	2.35	1.94
TDCPP	0.24	0.13	0.29	0.14	0.07	0.55	0.08	19.06	65.34

TBEP	26.4	52.4	159	17.2	18.7	0.52	0.94	7.1	12.7
TPP	20.8	2.26	0.68	11.1	0.88	8.06	4.24	11.78	0.79
EHDP	1.42	0.17	0.30	0.39	0.34	3.47	6.89	1.06	0.43
TEHP	0.70	0.43	0.81	0.88	1.28	0.58	0.09	0.00	0.00
total-TCP	0.30	0.11	0.07	0.36	0.05	0.00	1.05	2.11	1.51
BDE209	0.1	0.7	1.6	0.3	0.03	<0,2	0.4	0.3	0.5

Sample	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
TiBP	0.56	0.1	0.39	0.74	0.16	0.07	0.05	0.23	0.61
TBP	0.20	0.2	0.58	0.84	0.18	0.00	0.06	0.05	0.70
TCEP	4.26	1.54	65.01	0.95	0.20	0.09	0.07	0.19	0.78
TCPP1	20.25	2.89	3.05	0.84	0.50	1.99	0.46	0.49	0.70
TCPP2	27.91	3.60	4.19	0.00	0.19	2.12	0.52	0.05	0.70
TDCPP	9.18	2.97	11.62	0.89	0.19	23.89	0.11	0.06	0.74
TBEP	2.1	2.2	2.2	0.8	1.7	9.2	6.5	5	11.2
TPP	5.5	2.04	0.69	0.84	0.18	9.1	0.40	1.05	10.8
EHDP	0.22	1.15	0.62	0.95	0.20	18.16	0.20	0.28	0.78
TEHP	0.22	0.17	0.16	0.00	0.00	9.19	0.35	0.00	0.78
total-TCP	4.46	0.00	0.67	0.00	0.00	1.37	0.08	0.07	0.00
BDE209	0.5	4.2	3.8	<0,3	0.4	2.2	0.3	1.1	<0,2

Table 30: Levels of FRs in ng/g in the analyzed with LC-MS/MS dust samples

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
TiBP	0,2	0,2	0,1	0,1	0,1	0,3	0,3	0,1	0,2
TBP	0,2	0,2	0,2	0,05	0,10	0,22	0,23	0,1	0,1
TCEP	1,6	1,3	4,6	0,8	1,6	2,4	1,5	3,4	1,2
TCPP	1,4	3,1	0,7	3,2	3,9	1,6	3,2	1,6	3,1
TDCPP	0,6	1,1	0,1	0,2	0,2	0,8	1,5	0,3	0,9
TBEP	19	24	5,9	5,0	16	18	23	24	10

TPP	1,6	1,4	0,8	2,2	9,4	2,5	7,9	3,7	5,5
EHDP	0,5	0,3	0,2	1,3	0,3	0,6	5,8	0,4	1,6
TEHP	0,08	0,07	0,02	0,4	0,1	0,1	0,4	0,01	0,1
total-TCP	0,2	0,2	0,05	0,1	0,1	0,2	0,4	0,3	1,2
RDP	0,2	0,002	0,3	2,1	2,0	0,5	1,2	52	11
BDP	1,2	0,2	0,5	102	0,3	40	274	4,8	276

Sample	**10**	**14**	**15**	**16**	**17**	**19**	**20**	**21**	**22**
TiBP	0,2	0,1	0,1	0,4	0,6				
TBP	0,1	0,1	0,1	0,3	0,01				
TCEP	1,8	0,7	3,2	2,0	0,7				
TCPP	3,8	1,0	1,1	2,6	1,8				
TDCPP	0,3	0,2	0,2	0,8	0,3				
TBEP	14	31	78	14	13				
TPP	17	2,1	1,0	11	1,2				
EHDP	1,6	0,1	0,2	0,2	0,3				
TEHP	0,2	0,2	0,5	0,3	0,9				
total-TCP	0,1	0,1	0,1	0,6	0,03				
RDP	0,8	0,3	0,1	4,4	0,05	0,9	4,4	0,3	<0,03
BDP	1,1	21	1,4	719	8,7	7,4	50	735	12

Sample	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
RDP	0.5	<0,1	<0,04	<0,5	<0,1	<0,05	<0,04	<0,03	2,7
BDP	681	1,5	<0,1	15	0,7	0,3	0,5	2,8	1275

**Lower than the lowest standard*
**Higher than the highest standard*

In the diagrams below the previous results are visualized and categorized:

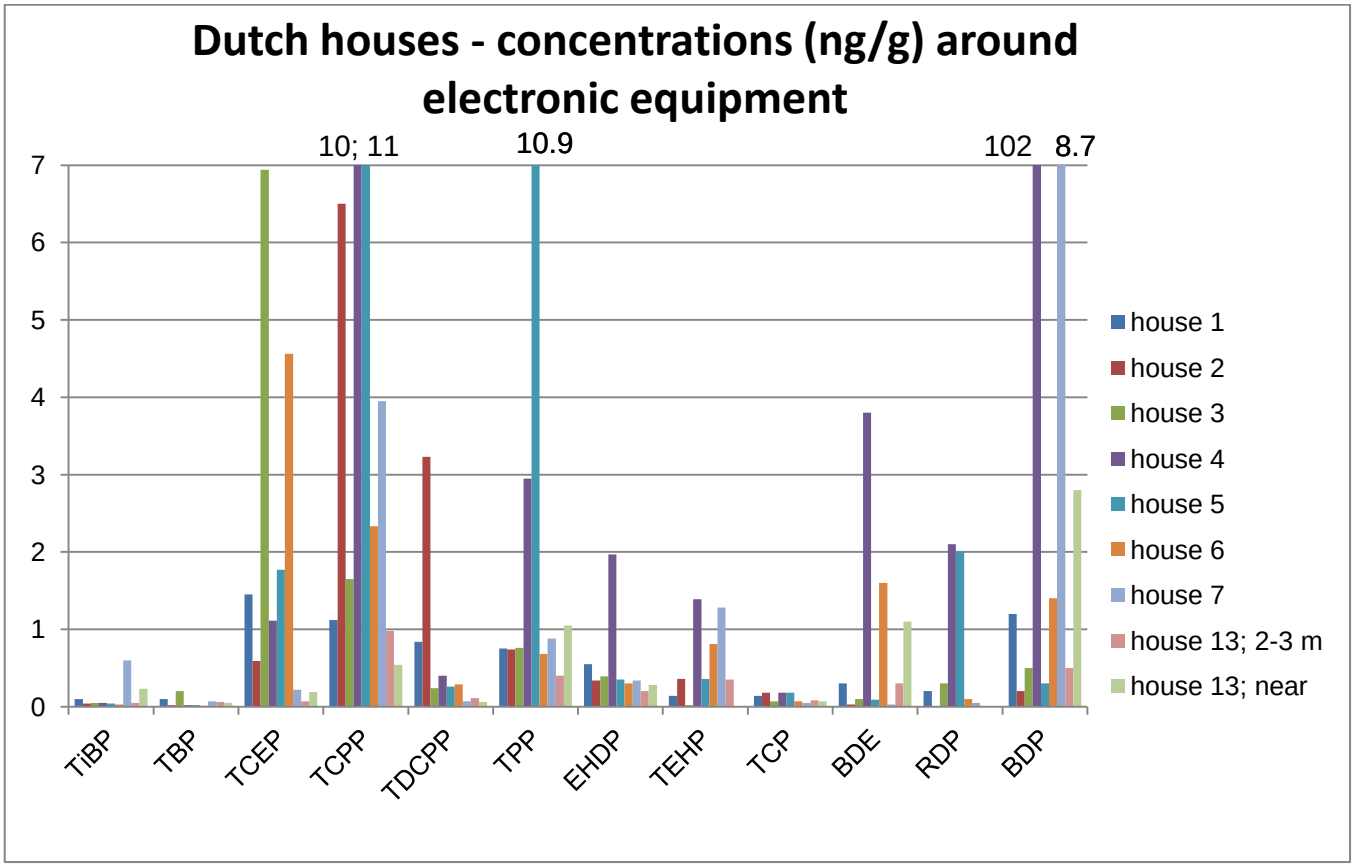


Figure 30: Concentrations in ng/g around electronic equipment in Dutch houses

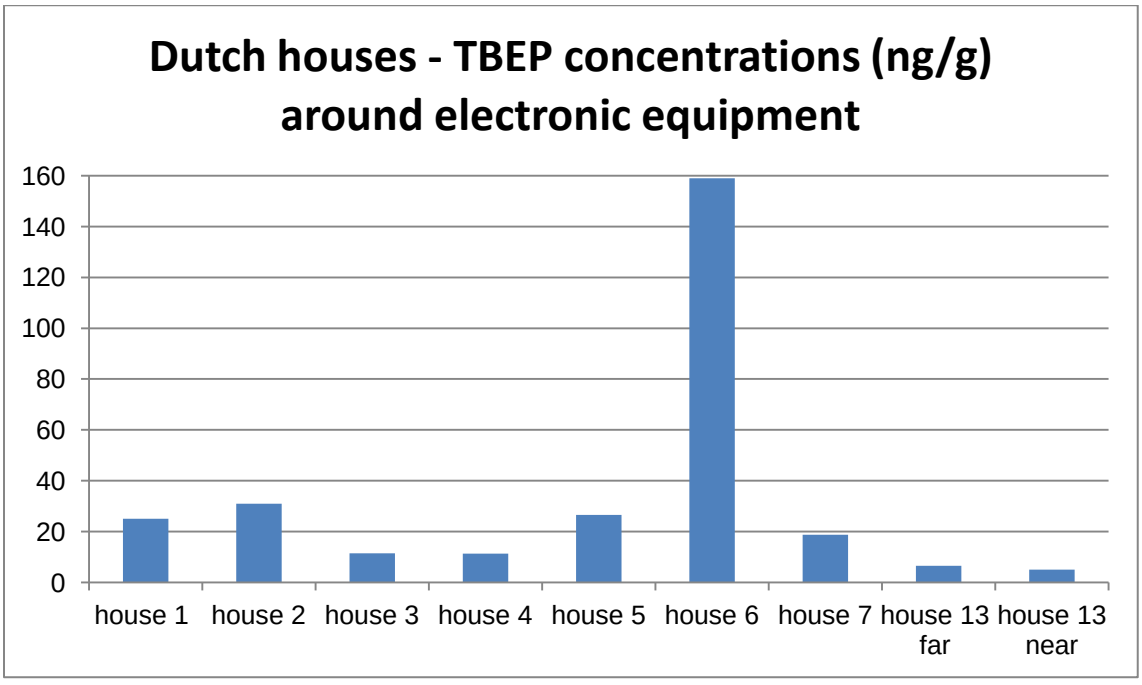


Figure 31: TBEP concentrations in ng/g around electronic equipment in Dutch houses

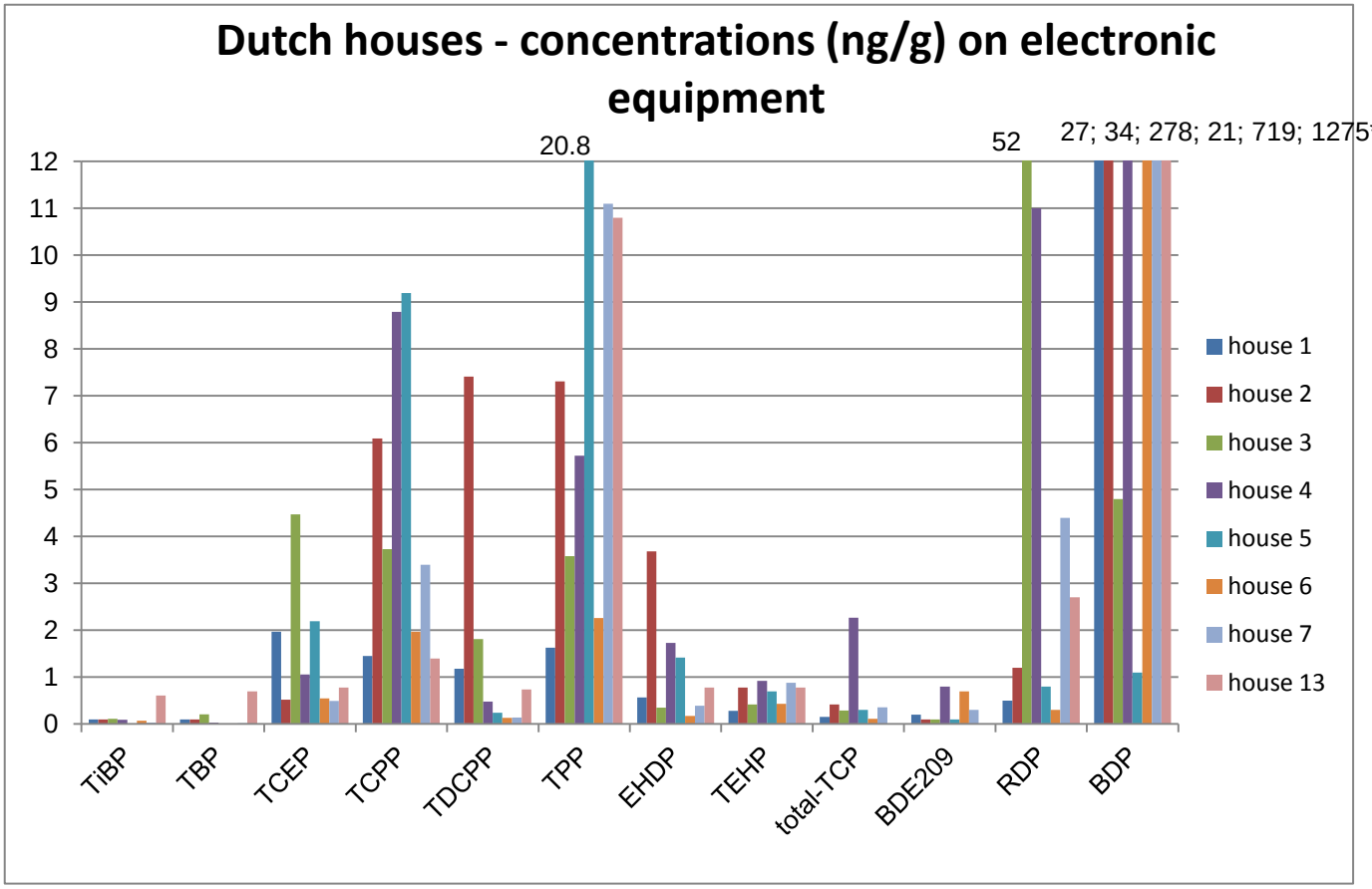


Figure 32: Concentrations in ng/g on electronic equipment in Dutch houses

**Because of large distribution in concentrations some columns are not complete. Their concentrations are described above them with numbers.*

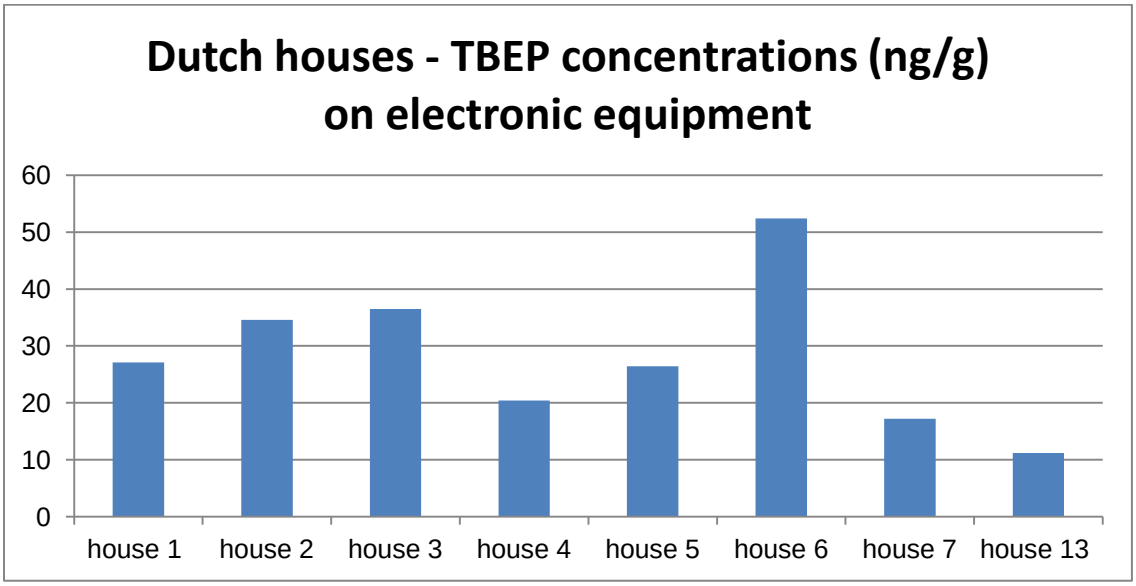


Figure 33: TBEP concentrations in ng/g around electronic equipment in Dutch houses

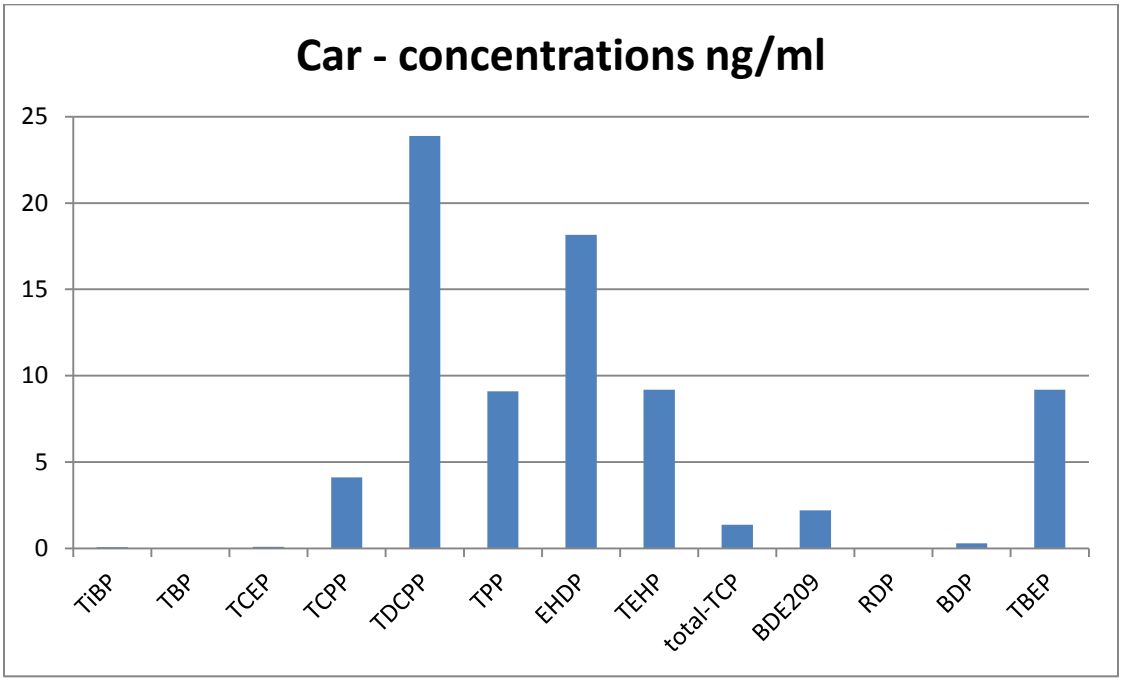


Figure 34: Concentrations, in ng/g, of the compounds found in a Dutch car

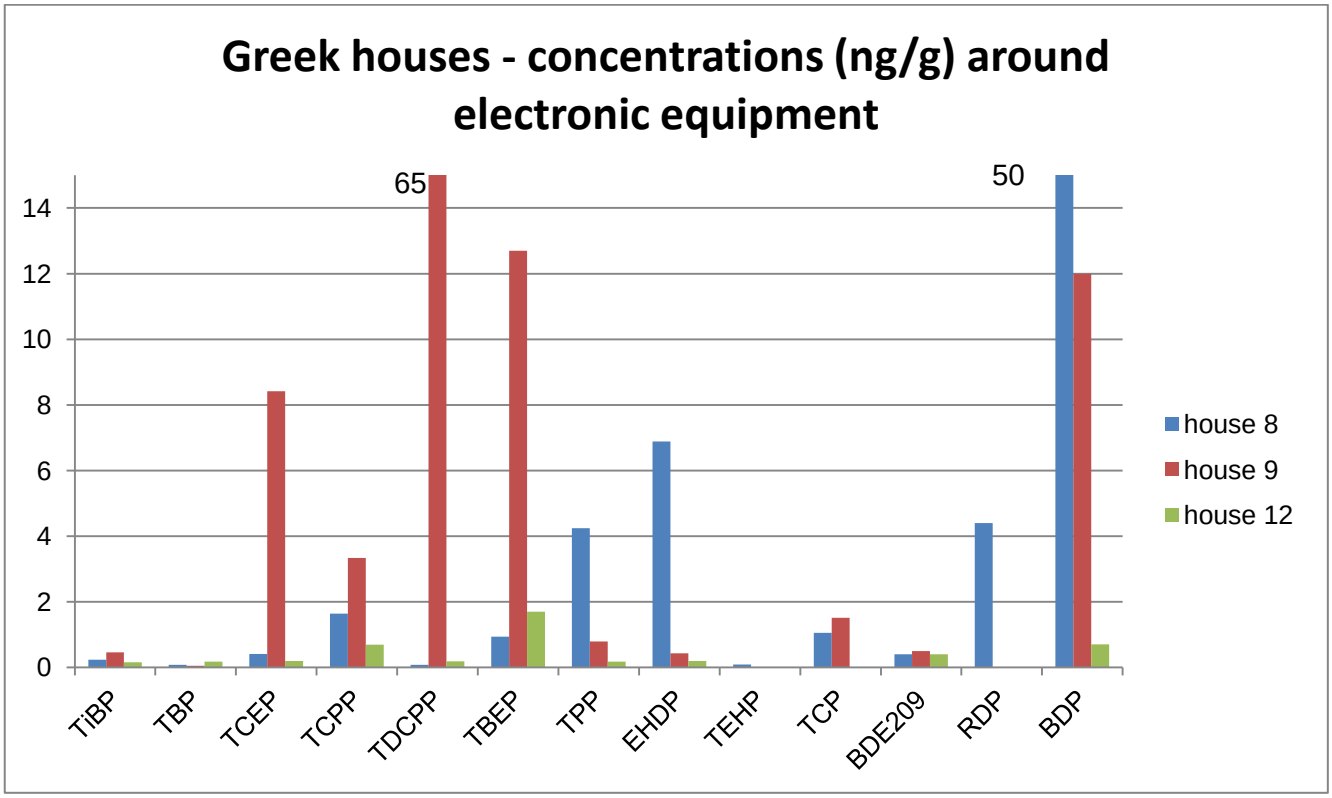


Figure 35: Concentrations in ng/g around electronic equipment in Greek houses

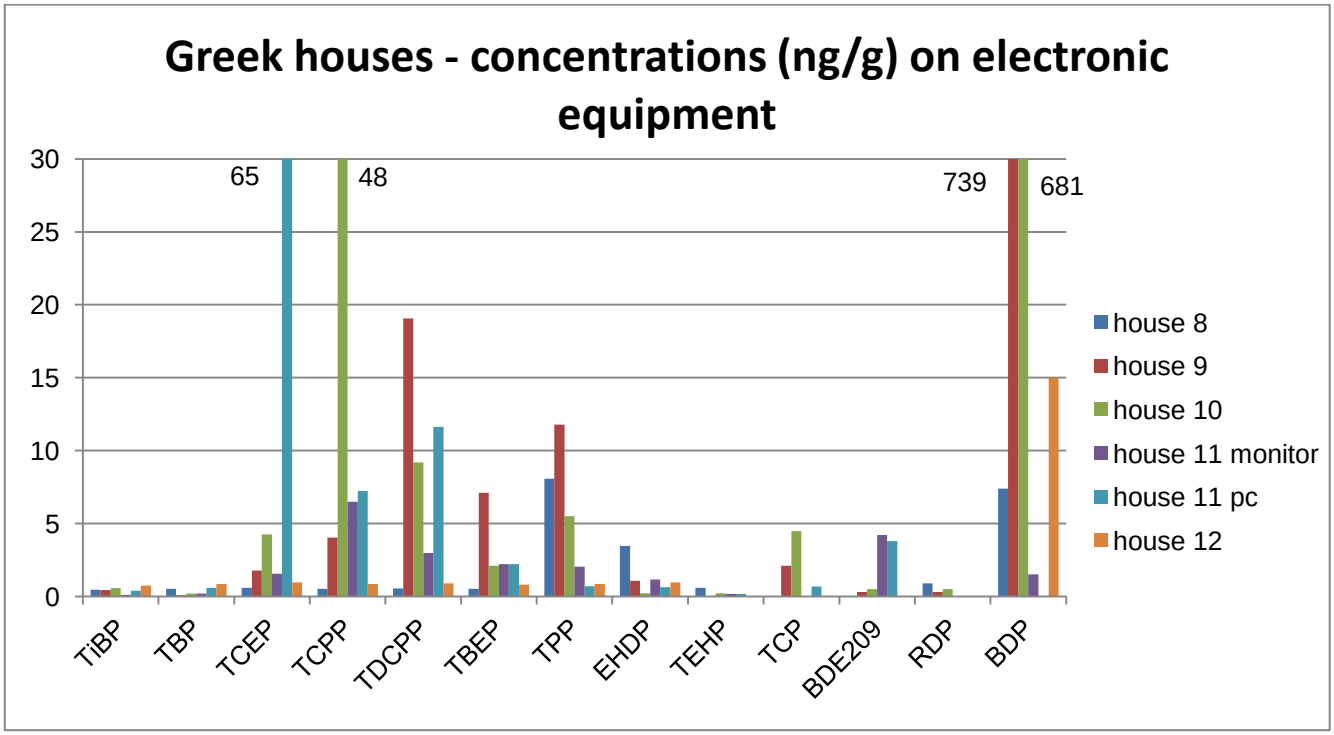


Figure 36: Concentrations in ng/g on electronic equipment in Greek houses

Conclusions

Patterns in houses from the Netherlands and Greece were compared. The most abundant compound on electronic equipment of Dutch houses was BDP, followed by TBEP, TPP, TCPP and TDCPP. This pattern changes at the space around electronic equipment, with TBEP being predominant and BDP being second. TCPP is again found in higher concentrations than the other compounds.

In Greek houses again BDP is the predominant compound on electronic equipment, followed by TDCPP, TCEP, TCPP and TPP. Concentrations of TBEP are much lower than in Dutch houses. In general Dutch houses are better classified than the Greek ones, maybe due to the fact that they were more and the trends could be more obvious.

Moreover, a dust sample from a Dutch car was collected, which appear a totally different pattern from homes. Here, the most abundant compound was TDCPP, followed by EHDP, TEHP, TBEP and TPP. One can notice that there are not only differences in concentrations of the same compounds, but also compounds that are not considered as pollutants in houses are found here (EHDP and TEHP).

CHAPTER 6

CONCLUSIONS

The following conclusions can be reached according to the current study:

- Method development and validation was conducted for simultaneous determination of organophosphorus flame retardants in waters and dust.
- The matrix effect was too strong to sediment samples, so the method couldn't be validated.
- The matrix of real water samples also caused problems to TOF-MS, so they couldn't be measured with this instrument. Instead, LC-MS/MS was preferred, but without being able of determining DOPO.
- Some PFRs are subjected to signal suppression during the LC, so GC was preferred. However, RDP, BDP and DOPO are too large to evaporate in GC, so their levels, as presented here, came from either LC-TOF-MS or LC-MS/MS.
- The water method was validated for four PFRs (RDP, BDP, TPP and DOPO). Real water samples came from Sewage Treatment Plants (STP) of Amsterdam, Eindhoven and Rotterdam. The levels of these compounds were low.
- The dust method was validated for thirteen PFRs. Real dust samples were collected from houses from the Netherlands and Greece, and one car. The car pattern was different from the house patterns of both countries, as compounds that are very low in the houses are abundant here. BDP is the predominant compound found in house dust on and around electronic equipment. Apart from this, TBEP has higher concentrations in Dutch houses, while TDCPP is common in Greek ones. TPP and TCPP had similar levels in both countries.

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