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Review

In-port derivatization after sorptive extractions

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ABSTRACT

Derivatization is necessary when analysis of polar compounds containing hydroxyl, carboxylic acid, amine or thiol functional groups is accomplished by gas chromatography (GC), in order to improve peak symmetry, peak separation and detector response. Derivatization can be performed off-line in a reaction vessel that is separated from the GC analysis hardware. However, on-line derivatization can eliminate time-consuming sample-processing steps, decrease the amount of valuable and/or toxic reagents and solvents that are used off-line, as well as increase the speed and efficiency of the analysis performed. The present work revises on-line in-port derivatizations where the derivatization reaction is simultaneously carried out with the analysis step by injecting the sample/reagent mixture directly into the hot GC inlet after a sorptive extraction step. Sorptive extractions revised range from the more classical solid-phase extraction (SPE) to the microextraction approaches, including solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) applications, as well as liquid-phase microextraction (LPME) or microextraction by packed sorbent (MEPS).

Contents

1. Introduction

While until the mid-90s trace analysis of organic compounds was focused on the determination of mostly non-polar and hydrophobic compounds such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and chlorinated pesticides, nowadays polar organic compounds used as pharmaceutical and personal care products (PPCPs), disinfection agents or pesticides, among other applications, have gained attention. Although, in general, polar organic compounds are not bioaccumulative, their excellent water solubility, continuous discharge and resistance to degradation during conventional wastewater treatment, facilitates their ubiquity in the environment [1].

Polar organic compounds, which present functional groups such as hydroxyl (—OH), carboxylic acid (—COOH), amine ($—NH_2$) or

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thiol (—SH), cause a problem in gas chromatographic (GC) separation, and, thus, analysis by liquid chromatography (LC) is mostly recommended. For GC analysis, compounds containing functional groups with active hydrogens such as —SH, —OH, —NH_2, —NH—R and —COOH are of primary concern because of the tendency of these functional groups to form intermolecular hydrogen bonds [2]. These intermolecular hydrogen bonds affect the inherent volatility of the compounds, their tendency to interact with column packing materials and their thermal stability [3]. However, because of the inherent simplicity and effectiveness of GC analysis, analytical chemists have long attempted to apply GC even to polar compounds [4]. GC separation of polar compounds can be achieved if a derivatization step is introduced previous to the chromatographic separation. Since GC is used to separate volatile organic compounds, modification of the functional group of a molecule by derivatization enables the analysis of compounds that otherwise cannot be readily monitored by GC. Derivatization process either increases or decreases the volatility of the compound of interest. It also reduces analyte adsorption in the GC system and improves detector response, peak separations and peak symmetry. Besides, derivatization can also serve to enhance mass spectrometric properties of derivatives, by producing either more favourable diagnostic fragmentation patterns of use in structure investigations, or characteristic ions of use in trace analyses when other techniques are applied. Alkylation, acylation and silylation are common derivatization reactions for GC analysis [5].

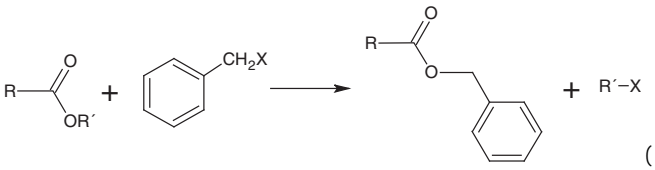
Most derivatization reactions are performed "off-line" in a reaction vessel that is separated from the GC analysis hardware [4]. *In situ* derivatization can be performed when working with water samples and water-compatible derivatization reagents. *In situ* derivatization does not only improve GC separation and detection, but also the extractability of the target compound into a non-polar sorbent and, therefore, the extraction efficiency is improved. However, *in situ* derivatization is limited to water compatible reagents and it does not include silylating reagents, which are mostly used. Off-line silylation reaction is performed after the extraction of the target analyte from the water sample and requires, thereby, an additional sample processing step and additional time for sample analysis. Off-line silylation procedures suffer from experimental errors such as loss of analyte through evaporation and re-suspension steps, contamination of samples during work-up, and the interference of water in the reaction system, since silylating reagents and the resulting derivatives are extremely sensitive to the presence of water [6]. When coupled to sorptive extraction techniques such as solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), off-line derivatization can also be performed on-polymer, after the sampling of the analyte or simultaneously to the sampling step. In the first case, the analyte is sampled to the polymer and then the analyte trapped in the polymer is exposed to the derivatization reagent. During simultaneous sampling and derivatization, the derivatization reagent is previously sorbed into the polymer and, then, the polymer containing the sorbed derivatization reagent is exposed to the target analyte [7].

As an alternative to off-line derivatization, "on-line" derivatizations are found. On-line derivatization can eliminate time-consuming sample-processing steps, decrease the amounts of valuable and/or toxic reagents and solvents that would otherwise be needed, and increase the speed and the efficiency of the analysis [4,6]. On-line derivatizations where the derivatization reaction is simultaneously carried out with the analysis step by injecting the sample/reagent mixture directly into the hot GC inlet are known as inlet-based derivatizations or "in-port" derivatizations and the derivatization occurs in the gas-phase.

The present work reviews in-port derivatization following sorptive extraction. Sorptive extractions revised range from the more classical solid-phase extraction (SPE), to the microextraction approaches, including SPME and SBSE applications, as well as liquid-phase microextraction (LPME) or microextraction by packed sorbent (MEPS).

2. Alkylation

Alkylation is mostly used as the first step for further derivatizations or as a method of protection of certain active hydrogens in a sample molecule. It represents the replacement of active hydrogen by an aliphatic or aliphatic-aromatic (*e.g.* benzyl) group in a process referred to esterification. Eq. (1) shows the general reaction representing the esterification process.



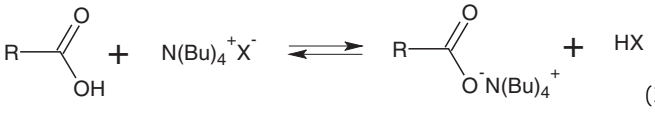
(1)

X = halogen or alkyl group, R′ = H or alkyl group.

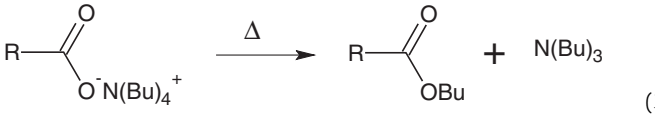
The principal chromatographic use of this reaction is the conversion of organic acids into esters, especially methyl esters that produce better chromatograms than the free acids. Alkylation reactions can also be used to prepare ethers, thioethers and thioesters, *N*-alkylamines, amides and sulphonamides [8,9].

Although common derivatization reagents for the alkylation type of reactions are dialkylacetals, diazoalkales, pentafluorobenzyl bromide (PFBBr), benzylbromide, boron trifluoride (BF_3) in methanol or butanol, those reagents have not been used for in-port derivatization. However, ion-pair reagents, namely tetraalkylammonium (TAA) salts, have been thoroughly used for in-port derivatization of acidic compounds.

Actually, in-port alkylation has been applied to the analysis of carboxylic acids [10], linear perfluorooctane sulfonate (L-PFOS) [11] and acidic pharmaceutically active compounds [12]. In a first step, the ion-pair reagent is added to the aqueous solution in order to form the corresponding carboxylate ion-pair ($RCOO^-N(Bu)_4^+$) (see Eq. (2)). Then, upon introduction into a hot GC injection port, the carboxylate ion-pairs are derivatized to their corresponding volatile butyl esters (see Eq. (3)).



(2)



(3)

The carboxylate ion-pair is introduced in the hot injection port of the GC *via* thermal desorption after SPME or *via* injection of a small volume of the liquid extract after LPME or SPE. This injection-port derivatization is also known as pyrolytic alkylation [13]. Table 1 includes different applications of in-port derivatization with ion-pair reagents.

2.1. In-port alkylation with ion-pair SPE

In-port derivatization following ion-pair SPE has been applied to the determination of linear alkylsulfonates (LAS) [23], chlorophenoxy acid herbicides [18] and nonylphenolpolyethoxycarboxylates (NPEC) and its metabolites [24] in a variety of water samples, including waste, river, ground and surface waters. The methods involved extraction of samples using a graphitized carbon black

Table 1
In-port derivatization applications after ion-pair extraction.

Compound	Sample	Extraction technique	Derivatization reagent (volume)	Injection mode	Analysis	LODs (ng/L)	Ref.
NSs	Industrial effluents and river water	SPE (PS-DVB)	TBA-HSO_4 (100 μL chloroform with 20 mM)	LVI-PTV	GC–MS	20	[14]
Pharmaceutical residues	River and wastewater	SPE (OASIS HLB)	TBA-HSO_4 (100 μL chloroform with 10 mM)	LVI-PTV	GC–MS	1–8 (LOQ)	[15]
Linear and branched PFOS	Technical product and biota samples	SPE (OASIS WAX)	TBA-OH (100 μL diethyl ether solution)	LVI-PTV	GC–MS	50–1460 0.09–6.87 (ng/g, MDL)	[11]
Dicarboxylic acids (C2–C10)	Atmospheric aerosols	SPE (OASIS HLB)	TBA-OH (20 mM in 100 μL of MeOH)	LVI-PTV	GC–MS	25–250 (pg/m^3, LOQ)	[16]
Acidic herbicides	Drain water and pond water	HFLPME	TBA-Cl (2 μL)	Split/splitless	GC–EI-MS	510–13,700	[17]
Pharmaceuticals	Tap water and wastewater	HFLPME	TMAH (1 μL)	Split/splitless	GC–MS	10–50 ng/L	[12]
NSAIDs	River water	SA-LLE	TBA-HSO_4 (1 μL)	Split/splitless	GC–EI-MS	0.44–2.75	[13]
CPA herbicides	River, surface and groundwater	SPE (GCB)	TBA-HSO_4 (100 μL chloroform with 15 mM)	LVI-PTV	GC–MS	100–200 (LOQ)	[18]
LCFAs	Wastewater	DLLME	TBA-HSO_4 (2 μL)	Split/splitless	GC–EI-MS	9.3–15	[19]
PFCAs	River water, seawater and lake water	DLLME	TBA-HSO_4 (2 μL)	Split/splitless	GC–NCI-MS/MS	37–51	[20]
PFCAs	Wastewater and seawater	SPME (PDMS)	TBA-HSO_4 (100 μL, 0.5 M)	TD (splitless mode)	GC–NCI-MS	20–750	[21]
LAS	Wastewater and seawater	SPME (PDMS)	TBA-HSO_4 (100 μL, 0.5 M)	TD (splitless mode)	GC–EI-MS	450–5000	[22]
FAs	Vegetable oil samples	SPME	PTMAH (1 μL)	Split/Splitless	GC–MS	0.2–2 μM	[10]
LAS	Surface and groundwater	SPE (ENVI-Carb)	TBA-HSO_4 (100 μL methylene chloride:MeOH, 9:1, v/v with 2 mM)	LVI-PTV	GC–MS	1000	[23]
NPEC and metabolites	Wastewater	SPE (ENVI-Carb)	TBA-HSO_4 (100 μL chloroform with 10 mM)	LVI-PTV	GC–IT-MS	100 (LOQ)	[24]

CPA: chlorophenoxy acetic acids; DLLME: dispersive liquid–liquid microextraction; FAs: fatty acids; GC–EI-MS: gas chromatography–electron impact ionization-mass spectrometry; GC–IT-MS: gas chromatography–ion trap-mass spectrometry; GC–MS: gas chromatography–mass spectrometry; GC–NCI-MS: gas chromatography–negative chemical ionization-mass spectrometry; GC–NCI-MS/MS: gas chromatography–negative chemical ionization tandem mass spectrometry; HFLPME: hollow fibre liquid-phase microextraction; LAS: linear alkylbenzensulfonates; LCFAs: long chain fatty acids; LOD: limit of detection; LOQ: limit of quantitation; LVI-PTV: large volume injection-programmable temperature vapourizer; MDL: method detection limit; NPEC: nonylphenolpolyethoxycarboxylates; NSAIDs: nonsteroidal anti-inflammatory analgesics; NSs: naphthalenesulfonic acid isomers; PA: polyacrylate; PDMS: polydimethylsiloxane, PFCAs: perfluorinated carboxylic acids; PFOS: perfluorooctanesulfonic acid; PS-DVB: polystyrene divinylbenzene; SA-LLE: sonication assisted liquid–liquid extraction; SPE: solid phase extraction; SPME: solid phase microextraction; PTMAH: phenyltrimethylammonium hydroxide; TBA-Cl: tetrabutylammonium chloride; TBA-OH: tetrabutylammonium hydroxide; TBA-HSO_4: tetrabutylammonium hydrogenosulphate; TD: thermal desorption; TMAH: tetramethylammonium hydroxide; TMA-HSO_4: tetramethylammonium hydrogenosulphate.

(GCB) cartridge and direct derivatization in the GC injection port using a large-volume (LV) (10–20 μL) direct sample introduction (DSI) device with TAA. The analytes were then identified and quantified by gas chromatography–ion trap-mass spectrometry (GC–IT-MS) analysis. Owing to the availability of different TAA salts and the possible dependence of derivatization efficiency on the reagent selected, three TAA salts (tetrabutylammonium hydrogen sulphate, TBA-HSO_4, tetramethylammonium hydrogen sulphate, TMA-HSO_4 and tetramethylammonium hydroxide, TMA-OH) were evaluated. In the case of the analysis of LAS, chlorophenoxy acid herbicides and NPEC and its metabolites, TBA-HSO_4 reagent was chosen since characteristic ions of butylated species produced the highest average peak areas and quantitative results in all the cases. Retention effect of TAA salts in the injection port was not detected since the DSI device with disposable micro vials was used and no glass-wool was inserted into the inlet. Therefore, a routine check for sample carryover by subsequent injection of a different ion-pair reagent after a sample injection was not necessary in these methods. The sharp and symmetric chromatographic peaks were still observed after a series of 50 sample injections. The effect of the injection temperature was also evaluated and the average peak areas determined were not significantly different at 280, 300 and 320 °C. In this sense, 300 °C was used as injection/derivatization temperature in these works. The in-port derivatization in a LV-DSI provided sensitivity, fast and reproducible results for the quantification of the analytes at 100, 100–200 and 1000 ng/L in the case of NPEC, chlorophenoxy acid herbicides and LAS, respectively.

According to the previous experience of the research group [18,23,24], three new methods were also developed for the analysis of naphthalene monosulfonic acid (NS) isomers, selected pharmaceutical residues and low-molecular weight dicarboxylic acids in water samples and atmospheric aerosols, respectively [14–16]. The evaluation of the in-port derivatization process was previously optimized and TBA-HSO_4 turned out to be the best derivatization reagent [18,23,24].

Ion-pair reagents have also been used after SPE. For instance, Chu and Letcher [11] developed a novel method that incorporated clean-up by SPE WAX cartridges and in-port derivatization-GC–MS to identify and quantitatively determine L-PFOS and branched (monotrifluoromethyl and bistrifluoromethyl) isomers in PFOS technical product and in environmentally relevant biological samples. TBH dissolved in diethyl ether was used for derivatization *via* an *in situ* pyrolytic alkylation reaction that occurred in the GC injector and generated butyl PFOS isomer derivatives. The eluate from the SPE clean-up process was evaporated to dryness using a nitrogen evaporator, the residue was dissolved in water and TBH was added. After centrifugation and freezing, the diethyl ether phase was separated from the aqueous phase and transferred to another centrifuge tube. The sample solution was evaporated to dryness, reconstituted in 100 μL diethyl ether, and transferred to a vial with 100 μL insert for GC–MS determination. Ionization was performed the NCI mode using methane as reagent gas. In addition to L-PFOS, ten branched PFOS isomers were identified in the technical product.

2.2. In-port alkylation following ion-pair SPME

First introduced by Pan and Pawliszyn [25] for the determination of long chain fatty acids, several applications of in-port derivatization following ion-pair SPME can be found in the literature. In that first work, it was observed that TMA-HSO_4 provided better results than TMA-OH since TMA-HSO_4 contains a proton that can be released from HSO_4^- at high temperatures and can recombine with $RCOO^-$ to provide volatile RCOOH. This technique was valid for C_{14}–C_{22} fatty acids but not for C_{10}–C_{12} acids, probably due to their high polarity that prevented their extraction with polyacrylate (PA)-coated fibre along with the ion-pair reagent. In the case of desorption/derivatization temperature, higher temperatures (300 °C) provided the most efficient derivatization. Other applications include the analysis of LAS [22] and perfluorocarboxylic acids (PFCAs) [21] in wastewater and seawater and carboxylic acids in vegetable oils [10].

Desorption temperature and time are important parameters to be optimized for in-port derivatization of LAS. Alzaga et al. [22] studied the desorption temperature and time in the 260–320 °C and 2–5 min ranges, respectively. In the case of desorption temperature, 300 °C provided the best chromatographic signals and no improvement was observed at higher temperatures. The maximum recommended temperature for 100 μm polydimethylsiloxane (PDMS) is 280 °C but more than forty determinations could be performed at 300 °C without any fibre damage. For desorption time, 3 min desorption in the splitless mode provided quantitative desorption with no carryover at low concentrations. Carryover was observed using 100 μm PDMS at high concentrations (>2 mg/L as total LAS) but not when 7-μm PDMS fibres were used. In that sense, 100-μm fibre was selected for trace analysis of LAS (μg/L), while 7-μm for more contaminated samples (mg/L) [19].

For PFCAs preliminary studies [21] showed that the length of the TAA salts influenced extraction efficiency and the best results were obtained with the longest TAA salts tested, *i.e.* with TBA salts. Desorption from the 100-μm PDMS fibre at 300 °C in a splitless injector for 3 min guaranteed exhaustive desorption of the analytes and no carryover. During the analysis of butylated PFCAs (PFCAs-Bu) by gas chromatography–mass spectrometry (GC–MS), chemical ionization (CI) is preferred to electron impact (EI). The most abundant fragment ions produced by EI are m/z^+ 57 and 69, which are not specific and, therefore, not suitable for qualitative/quantitative analysis. In CI, negative chemical ionization (NCI) with ammonia provides at least 5-fold higher sensitivity than positive chemical ionization (PCI) with ammonia or methane and at least 3-fold higher than NCI with methane. All PFCA-Bu showed the same fragmentation pattern in NCI using ammonia and the main ions observed were $[M]^-$, $[M-HF]^-$, $[MC_4H_9OF]^-$, $[M-C_5H_9O_2F]^-$ and $[M-C_5H_9O_2F_3]^-$. Finally, 60 m × 0.25 mm i.d. cyanopropylphenyl-methylpolysiloxane, 1.4 μm film thickness ZB-624 chromatographic column provided good selectivity and efficiency for PFCAs-Bu in the $PFC_{4-12}As$–Bu range.

Liu et al. [10] used SPME for the determination of carboxylic acids in vegetable oil samples following in-port alkylation. Extraction and desorption time, desorption temperature and the concentration of esterifying reagent were thoroughly studied using three different polymers: 100 μm PDMS, 85 μm PA and 85 μm carboxene/PDMS. Different ratios of methylation reagent phenylmethylammonium hydroxide (PTMAH) and sample were studied in the 40:1–200:1 range and the highest yields were obtained for 125:1 ratio. The applied temperature is an important factor in both fibre desorption and methylation and the authors studied its influence between 200 and 280 °C. The optimum values were set at 280 °C, since the highest temperature provided the highest chromatographic response. Desorption time was also considered. Although desorption profiles seemed to increase with time up to 10 min, the change was not significant after 5 min. Under optimized conditions pyrolytic methylation of carboxylic acids was completed with no evidence of underivatized compounds and no apparent degradation.

2.3. In-port alkylation following ion-pair LPME

In-port derivatization following different ion-pair LPME formats, such as hollow fibre LPME (HFLPME) [12,17], dynamic LPME [19], sonication assisted liquid–liquid extraction [13], or dispersive liquid–liquid microextraction (DLLME) [20] can be found in the literature for the analysis of acidic herbicides [17], long-chain fatty

acids [19], nonsteroidal anti-inflammatory drugs (NSAIDs) [13], PFCAs [20] and acidic pharmaceutically active compounds [12].

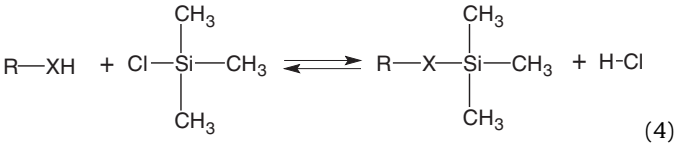
In the case of in-port derivatization following ion-pair LPME, two are the main studied variables: injection temperature and purge-off time. In most of the cases, the highest derivatization yields are obtained at high injection temperatures (~290–300 °C). However, in certain cases, such as for most of the acidic herbicides [17] or the NSAID gemfibrozil [13], an increase of the signal was observed up to 240–260 °C but, at higher injection port temperatures, a decrease of the analytical signal occurred, indicating that additional reactions instead of just butylation derivatization reaction occurred [13,17].

For purge-off time, a response increase is usually observed from 0 min to 1.5 min purge-off time [17,19] and no further improvement is observed for higher purge-off time values. However, in certain cases, a chromatographic signal drop, as well as peak tailing, are observed when high purge-off time values are used [20].

In the case of PFCAs, Liu et al. [20] also studied the influence of two ion-pair reagents, TBA-HSO_4 and TBA iodide (TBA-I), and similar results were obtained in both cases.

3. In-port silylation

Silylation is the most versatile derivatization technique and one of the most common GC derivatization procedures. Silylation is a nucleophilic substitution reaction whereby an electrophilic alkylsilyl group, often a trimethylsilyl (TMS) group, is attached on the nucleophilic site by displacement of active hydrogen. The mechanism involves the replacement of the active hydrogens (in —OH, —COOH, —NH—R, —NH_2, and –SH groups) with a TMS group. The general reaction for the formation of trialkylsilyl derivatives is shown by Eq. (4). The leaving group in the case of trimethylchlorosilane (TMCS) is the Cl atom [26].



(4)

X = O, COO, N, NH or S.

One of the advantages of using TMS derivatives is their thermal stability. They are routinely used at column and injector temperatures of 300 °C, but temperatures of 350 °C and above have also been successfully used. The TMS reagents themselves are also quite thermally stable; however, the more reactive silyl donors such as *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) will decompose at elevated temperatures, especially in the presence of metals. When GC is used to determine reagent purity, it is necessary to use injector temperatures of 125–150 °C and glass-lined injection ports. However, silylation reagents can decompose at temperatures above 75 °C and, therefore, care must be taken when derivatization is conducted at high temperatures. On the other hand, silylation presents some disadvantages since the derivatization cannot be carried out in the presence of water because the TMS derivatives are hydrolyzed in aqueous solution.

In the silylation procedure, solvents are usually added to increase reaction efficiency and prevent the hydrolysis of the products. The solvents used in silylation should show high solubility of derivatives, as well as being aprotic, without containing active hydrogen atoms, such as pyridine [27].

Frequently, erratic and irreproducible results begin to occur when stainless steel injection ports are used for TMS reagents and derivatives. Thus, the use of glass-lined injection ports or direct on-column injection when working with silylating reagents could avoid these problems [28]. Table 2 summarizes some works found in the literature where in-port silylation was used.

3.1. In-port silylation following SPE

Split/splitless in-port derivatization combined with SPE was developed by Wu et al. [27] and applied for the first time to determine five types of faecal sterols (coprostanol, cholestanol, epicholestanol, epicoprostanol and cholesterol) with GC–MS. In this method, silylation of faecal sterols was performed with BSTFA at GC injection-port. Variables such as injection-port temperature, purge-off time, derivatization reagent volume, and the type of organic solvent were investigated. It was observed that GC–MS responses of the five silylated derivatives increased with increasing injection-port temperature (in the 200–300 °C range), indicating that the high temperature could accelerate silylation efficiency. Different purge-off time values (0.2–2.0 min in splitless mode) were also evaluated. Similarly to what has been previously commented for in-port ion-pair derivatization, responses increased sharply when purge-off time was increased from 0.2 to 1.0 min, followed by a generally flat profile when the purge-off time was increased from 1.0 to 2.0 min. Different volumes of the derivatization reagent, BSTFA (1% TMCS), ranging from 10 μL to 200 μL were also studied. It was found that the relative abundance (GC–MS peak area ratio of faecal sterol derivative to internal standard) increased with the increase of reagent volume from 10 μL to 50 μL and, then, remained unchanged with further increase of reagent volume from 50 μL to 200 μL. Acetonitrile, acetone, methylene chloride, diethyl ether, ethyl acetate, *n*-hexane and *tert*-butyl methyl ether were investigated as injection solvents as well. Methylene chloride provided the highest derivatization efficiency, followed by ethyl acetate, *tert*-butyl methyl ether and *n*-hexane, whereas acetonitrile produced the poorest derivatization efficiency.

The rapid determination of three benzophenone-type UV filters in aqueous samples was described by Ho and Ding [43]. The method involved the extraction of an aqueous sample using an Oasis HLB SPE cartridge, followed by on-line derivatization gas chromatography–tandem mass spectrometry (GC–MS/MS) with a TMS reagent. The effects of three derivatization parameters (injection-port temperature, residence time and the volume of silylating agent co-injected with the sample) were evaluated. The abundances of the TMS-derivatives increased when the injection-port initial temperature was increased from 60 to 70 °C, but decreased thereafter. The highest yield was achieved at a residence time of 2.5 min. For residence times longer than 2.5 min, the abundances of the TMS-derivatives decreased significantly. This phenomenon can be attributed to the derivatives escaping from the injection-port at the highest temperatures and the longest residence times once the analytes have been completely converted into the corresponding TMS-derivatives. The effect of the volume of the derivatization reagent co-injected with the sample was also evaluated and they found that the abundances were similar when the volume was within the range from 1 to 4 μL. Sharp and symmetrical peaks remained visible after more than 50 sample injections.

3.2. In-port silylation following SPME

Several works can be found in the literature for in-port silylation following SPME, such as the determination of phenols and nitrophenols in rainwater [44,45], chlorinated bisphenol A compounds (BPAs) in human plasma [31] and phenols in environmental water samples [32].

A method based on headspace-SPME (HS-SPME) was selected by Helaleh et al. [32] to determine phenolic compounds in environmental water samples. Two types of SPME fibres were compared to this purpose: PDMS (7 μm) and the more polar PA (40 μm), which

Table 2
In-port silylation applications following sorptive extractions.

Compound	Sample	Extraction technique	Derivatization reagent (volume)	Injection mode	Analysis	LODs (ng/L)	Ref.
OH-PAHs	Seawater	Sorption (TENAX-TA)	BSTFA (1 μL)	TD	GC–EI-MS	0.04–12	[29]
Melamine and cyanuric acid	Powdered milk	Sonication (10 mL ACN+5% DMSO)	BSTFA (3 μL)	PTV	GC–EI-MS	0.2–0.5 ng/g	[30]
Faecal sterols	Surface water	SPE (C18)	BSTFA (50 μL)	Split/splitless	GC–MS	1.3–15	[27]
Chlorinated BPAs	Human Plasma	SPME (PA)	BSTFA:DCM (2 μL) (50:50)	TD (splitless mode)	GC–EI-MS	500–3000	[31]
EDCs	Water samples	SPME (PA)	BSTFA (1 μL)	Split/splitless	GC–MS	10–100	[32]
APs and BPA	Sea water	SPME (PA) PC-HFME	BSTFA (2 μL)	Split/splitless	GC–MS	2000–14,000 (SPME) 70–2340 (PC-HFME)	[33]
BPs	Canned beverages and vegetables	SBSE	BSTFA (3 μL)	TD (splitless mode)	GC–MS	0.9–2.5	[34]
EDCs	Estuarine and wastewater samples	SBSE	BSTFA (2 μL)	TD-LVI-PTV	GC–MS	0.8–84 (MDL)	[35]
APs	River water	SBSE and LD	BSTFA (0.5 μL) + Ac_2O (20 μL)	TD-LVI-PTV	GC–MS	0.2–10	[36]
E2	River water	SBSE and LD	BSTFA (1 μL) + Ac_2O (100 μL)	TD-LVI-PTV	GC–MS	0.5–5	[37]
EDCs and pharmaceuticals	Water	m-SBSE	BSTFA (1 μL)	TD (splitless mode)	GC–MS	–	[38]
BPA, E2 and EE2	Real world process and potable water	MISPE-SBSE	BSTFA (1 μL)	TD (splitless mode)	GC–MS	0.010–2	[39]
APs, BPA, natural and synthetic hormones	River and wastewater	MEPS (C18 and 17β-E2MIP)	BSTFA (10 μL) + 1% TMCS	LVI-PTV	GC–MS	0.02–87 (MEPS-C18)	[40]
APs, CPs and BPA	Tap water, seawater	HFLPME	BSTFA (2 μL)	Split/splitless	GC–MS	6.0–15	[41]
Polyphenols	Herbal infusions, fruits and functional foods	DSDME	BSTFA (2 μL)	Split/splitless	GC–EI-MS	10–100	[42]
BP-UV filters	River, groundwater and effluent samples	SPE (OASIS HLB)	MSTFA (1 μL)	LVI-PTV	GC–MS/MS	0.3–1.0	[43]
Phenols and nitrophenols	Rain water	SPME (PA)	MBDSTFA (2 μL)	TD (splitless mode)	GC–EI-MS	208–99,300	[44]
Phenols and nitrophenols	Rain water	SPME (PA)	MBDSTFA (2 μL)	TD (splitless mode)	GC–EI-MS	200–99,000	[45]
GHB, *t*-HCA	Urine	LLE (ethylacetate 900 μL)	MTBSTFA + 1%TBCS (50 μL)	PSS	GC–EI-MS	49	[46]
Acidic and polar organic pollutants	Ground, river and wastewater	SBSE and LD	MTBSTFA (20 μL)	LVI-PTV	GC–MS	1–800	[47]
PhACs	Drain water samples	SBME	MTBSTFA (10 μL)	Split/Splitless	GC–MS	6.0–22	[48]

ACN: acetonitrile; Ac_2O: acetic acid anhydride; APs: alkyphenols; BPA: bisphenol A; BPs: bisphenols; BP-UV filters: benzophenone-UV filters; BSTFA: *N*,*O*-bis(trimethylsilyl)trifluoro acetamide; CPs: chlorophenols; DCM: dichloromethane; DMSO: dimethyl sulfoxide; DSDME: suspended droplet microextraction; E2: 17β-Estradiol; EDCs: endocrine disruptor compounds; EE2: 2α-ethynyl estradiol; GHB: γ-hydroxybutyrate; GC–EI-MS: gas chromatography–electron impact ionization-mass spectrometry; GC–MS/MS: gas chromatography tandem mass spectrometry; GC–MS: gas chromatography–mass spectrometry; HFLPME: hollow fibre liquid-phase microextraction; LD: liquid desorption; LLE: liquid–liquid extraction; LOD: limit of detection; LVI-PTV: large volume injection-programmable temperature vapourizer; MBDSTFA: *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide; MDL: method detection limit; MEPS: microextraction by packed sorbent; MISPE: molecularly imprinted solid phase extraction; m-SBSE: multiple stir bar sorptive extraction; MSTFA: *N*-methy-*N*-(trimethylsilyl) trifluoroacetamide; MTBSTFA: *N*-methyl-*N*-[*tert*-butyldimethyl-silyl]trifluoroacetamide; OH-PAHs: hydroxyl polycyclic aromatic hydrocarbons; PA: poly(acrylate); PC-HFME: polymer coated hollow fiber microextraction; PhACs: pharmaceutically active compounds; PSS: programmable split/splitless injector; PTV: programmable temperature vapourizer; SBME: solvent bar microextraction; SBSE: stir bar sorptive extraction; SPE: solid phase extraction; SPME: solid phase microextraction; TBCS: *tert*-butyldimethylchlorosilane; TD: thermal desorption; *t*-HCA: *trans*-hydroxycrotonic acid; TMCS: trimethylchlorosilane.

provided higher extraction recoveries. The fibre was exposed to the water samples in the immersion mode and in the headspace (HS) mode for 60 min in the presence of salts and in acidic conditions and higher extraction yields were observed by performing the extraction in the HS. The derivatization of the analytes was performed at once with fibre desorption in the injection port at 300 °C using 1 μL of BSTFA injected just after the desorption of the analytes. Desorption time was studied between 3 and 12 min and comparable results were obtained after 5 min desorption. It was observed that not only the silylated phenol derivatives showed better peak shape, but better separation was also obtained.

In the case of phenols and nitrophenols [44] *N*-(*t*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MDBSTFA) was used for derivatization. During the silylation reaction performed using MDBSTFA, all the hydroxyl groups of phenols are converted to their corresponding *t*-butyldimethylsilyl (TBDMS) ethers, *via* a bimolecular nucleophilic substitution (SN_2) reaction, yielding a stable single derivative for each phenolic compound. The mass spectra of all phenolic derivatives are dominated by $[M-57]^+$ characteristic ions of the cleavage of the *t*-butyl moiety. Authors tried both on-fibre and in-port derivatization of phenolic compounds with MDBSTFA. Although Lord and Pawliszyn [49] recommended that the best approach consisted on the simultaneous HS extraction and derivatization, Jaber et al. [44] observed a rapid degradation of the fibre due to the reactive vapour of MDBSTFA. Therefore, in-port derivatization was adopted as an alternative to the simultaneous HS extraction and derivatization of phenols and nitrophenols. Hence, a PA-coated SPME fibre was exposed to 2 mL of rain water sample for 40 min in the immersion mode at room temperature. Then, the analytes adsorbed in the fibre were derivatized using 2 μL of MDBSTFA that was injected directly in the injection port previous to the introduction of the SPME fibre. Thus, desorption and derivatization steps of the analytes were performed at once in the injection port, which was maintained at 280 °C for 5 min in the splitless mode. Based on this previous method proposed by Jaber el al. [44], Schummer et al. [45] monitored 6 phenols and 14 nitrophenols in rainwater in order to study the spatial and seasonal variations of the target compounds in east France.

del Olmo et al. [31] applied SPME coupled to the derivatization of four chlorinated BPAs (Cl-BPA, Cl_2-BPA, Cl_3-BPA and Cl_4-BPA) on the GC injection port for the determination of the target analytes in human plasma using BSTFA, as optimized by Zafra et al. [50] for the determination of trace amounts of BPA and its chloroderivates in wastewater by liquid–liquid extraction and GC–MS analysis. Briefly, the authors investigated the effectiveness of different silylation reagents to improve the selectivity, sensitivity and performance of the chromatographic properties of the target compounds. BSA, *N*-trimethylsilylimidazole (TMSI), BSTFA, TMCS and the mixture formed by TMCS, hexamethyldisilazane (HMDS) and pyridine as polar solvent in a ratio 1:3:9 were tested and the best results were obtained when 10 μL of BSTFA was used. Time and temperature are the primary factors affecting SPME-GC desorption and del Olmo et al. monitored desorption temperatures ranging from 270 to 300 °C and desorption times between 5 and 8 min, respectively. High desorption temperatures, *i.e.* 300 °C, provided the best responses for all studied chloroderivatives with no carry-over effects (<1%). For desorption time, the maximum signals were obtained for 7 min desorption in the splitless mode.

3.3. In-port silylation following SBSE

In-port silylation has also been coupled to SBSE in the literature. In this case, in most of the approaches, a few microlitres (1–3 μL) of the derivatization reagent added to glass wool or inserted in a small capillary tube are included together with the stir-bar or twister in the thermal desorption (TD) tube [34–38]. In such cases the term in-tube silylation is applied.

Kawaguchi et al. [36] developed a method for the determination of alkylphenols (APs) in river water samples based on SBSE and TD with in-tube silylation and GC–MS. When TD with in-tube silylation was studied, different BSTFA addition methods were studied in order to perform the derivatization. The direct addition of BSTFA into PDMS phase was dismissed because dull chromatographic results were obtained. A glass capillary tube filled with BSTFA inside a glass TD tube and located in the front, middle or back portion of the glass tube was studied instead (Fig. 1). It should be underlined that the TD unit (TDU) was horizontal in the present work. As BSTFA is quite volatile, the best results were obtained when the capillary tube was located in the back part of the glass TD tube. Volume of BSTFA was also analyzed and 0.5–2.0 μL of BSTFA were tested. The highest response was obtained when 0.5 μL of BSTFA were added. Finally, in the same work SBSE-TD–GC–MS without derivatization, SBSE-TD with *in situ* acylation followed by TD, and SBSE-TD with in-tube silylation were compared. The results showed that the derivatization is recommended in all the cases. APs with small log K_{ow} values (*tert*-butylphenol, *n*-butylphenol and *tert*-propylphenol) showed better results with *in situ* acylation, while in-tube silylation provided better results for compounds with more hydrophobic properties.

Iparraguirre et al. [35] optimized a method based on SBSE and in-tube derivatization-TD-GC–MS for the determination of several endocrine disrupting compounds (EDCs), including APs, BPA, estrogens and sterols, in different environmental water samples. During in-tube derivatization, the volume of the derivatization agent (BSTFA + % 1 TMCS) and cryo-focusing temperature were studied. Desorption time and temperature are usually studied but, in the present work, they were fixed at maximum values (300 °C and 10 min) in order to guarantee an efficient desorption of the analytes. The derivatization reagent volume was studied in the 0.5–20 μL range. Although higher chromatographic responses were obtained using higher reagent volumes, the volume was fixed at 2 μL due to instrumental problems at higher volumes. Cryo-focusing temperatures lower than −50 °C caused the blockage of the CIS-4 unit and −50 °C temperature was finally chosen. The vertical configuration of the TDU used in this work facilitated the loss of the derivatization reagent, which implied a lack of precision after long injection sequences.

Cacho et al. [34] optimized and compared the analysis of bisphenols from canned beverages and vegetables using in-tube silylation

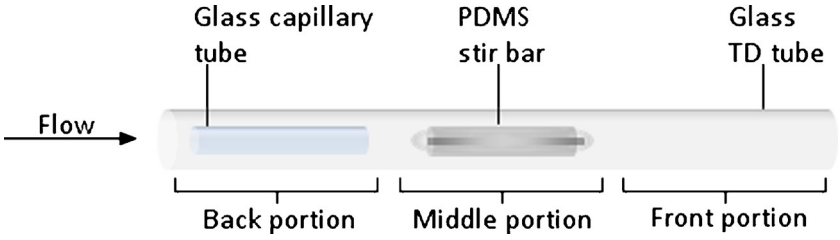


Fig. 1. In-tube silylation following SBSE. The silylation reagent is loaded in a capillary tube that can be place in the back, middle or front position of the TD tube.

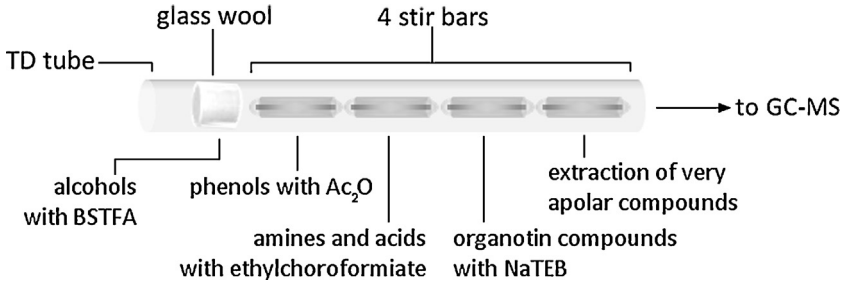


Fig. 2. In-tube silylation with the derivatization reagent added in glass wool. The four stir-bars had been exposed to four aliquots of the same sample fitted under different chemical conditions in order to extract target analytes with different physic-chemical conditions.

and *in situ* acetylation. *In situ* acetylation provided the best sensitivity.

In several works of the literature, in tube silylation has been applied in combination with *in situ* acylation in the determination of 17β-estradiol (E2). E2 has an aromatic and an aliphatic hydroxyl group. During acylation the aromatic hydroxyl group is derivatized, while in the second derivatization, the silylation of the remaining aliphatic hydroxyl group is carried out in tube. First applied by Kawaguchi et al. [37] it was named dual derivatization. Similarly, Van Hoeck et al. [38] applied dual derivatization of E2 during the determination of this compound together with other EDCs and pharmaceuticals. Due to the different physic-chemical properties of the analytes, four different aliquots of the sample were extracted with 10 mm and 25 μL PDMS stir-bars under different chemical conditions. The four stir-bars, together with 1 μL of BSTFA added in glass wool, were introduced in the desorption tube (see Fig. 2) and simultaneously desorbed in a TDU. Finally, Canale et al. [39] combined molecularly imprinted solid phase extraction (MISPE) and dual derivatization for the determination of BPA, E2 and 17α-ethinyl estradiol (EE2) in real-world process and potable water samples. After the MISPE, target analytes were eluted with 2 mL of methanol. The methanol eluate was diluted 1:10 with Milli-Q water and acylation and in-tube silylation were applied similar to Van Hoeck et al. [38].

Although in-port silylation following SBSE is mostly applied following TD, Quintana et al. [47] described the optimization of liquid desorption (LD) of the stir-bars previously to the in-port silylation during the determination of phenols (octyl- and nonylphenols, nitrophenols, BPA), pharmaceuticals (clofibric acid, ibuprofen and phenazone among others) and herbicides (dicamba, mecoprop, *etc.*), including acidic and polar organic contaminants. All the target compounds, except for phenazone and propyphenazone, were in-port silylated using *N*-(*t*-butyltrimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA). Although, *in situ* acylation and ion-pair derivatization were also studied as alternatives or in combination with silylation, MTBSTFA was selected as the sole derivatization agent due to its ability to derivatize the studied compounds and the proved thermal and hydrolytic stability of the *tert*-butyldimethylsilyl (TBDMS) derivates.

3.4. In-port silylation following LPME

Basheer et al. [33] developed polymer coated hollow fibre microextraction (PC-HFME) extraction method for the determination of APs and BPA in seawater and compared it with an SPME approach. In the case of SPME, the 85 μm PA-fibre was placed in the headspace of a 3 mL vial containing 30 μL of BSTFA in 1 mL of acetone at 60 °C for 20 min, and then, the desorption of the fibre was accomplished for 3 min at 250 °C. However, for PC-HFME assays, the authors considered both on-fibre and in-port derivatization modes and the latter approach provided better results in terms of sensitivity. In this sense, the extract was put in contact with the silylation reagent BSTFA in the injection port of GC at 280 °C for 2 min before being focused into the chromatographic column. Different volumes of BSTFA and sample extract were evaluated, *i.e.* extract:BSTFA (v:v), 1:1, 2:1, 1:2, and the best results in terms of repeatability were obtained using 4 μL of 1:1 extract:BSTFA ratio.

Basheer and Lee [41] employed in-port derivatization for the derivatization of phenols (APs, chlorophenols and BPA) in water samples after HFLPME. They studied the effect of the BSTFA volume (1–3 μL) in the GC–MS analysis, concluding that 2 μL of BSTFA provided better derivatization efficiencies since higher derivatization reagent volume caused poor resolution of the analytes as well as low precision. In 15 s the target analytes were completely derivatized in the injection port and no carryover was observed when blank samples were analyzed.

Viñas et al. [42] applied the in-port derivatization with BSTFA to convert the polar non-volatile polyphenols into non-polar, volatile and thermally stable derivatives. Polyphenols were extracted by directly suspended droplet microextraction (DSDME) from herbal infusions, fruits and functional foods, and were further analyzed by GC–MS. The derivatization was carried out in the splitless mode at 240 °C during 30 s. Several variables were studied to determine their influence in the derivatization process, *i.e.* the injection mode, the order of injection between the sample and BSTFA, the temperature of the injection port, the derivatization time and the BSTFA/sample volume ratio. Finally, the best recoveries for most of the polyphenols were obtained in the splitless mode. Better sensitivity was achieved when BSTFA was injected before the sample. Higher peak areas were obtained when injection port temperature was 240 °C. Optimal efficiencies were observed when the derivatization lasted 30 s and 2 μL of BSTFA/3 μL of sample were injected. No cross-memory effects were observed.

3.5. In-port silylation following other sorptive extraction techniques

Tzing and Ding [30] used in-tube silylation in a PTV for the determination of melamine and cyanuric acid in powdered milk. After ultrasound assisted solid–liquid extraction and clean-up of the extract, the sample was evaporated and redissolved in 100 μL of acetonitrile containing the internal standard (2,6-diamino-4-chloropyrimidine) and 0.5% pyridine. Then, 20 μL of the sample solution were mixed with BSTFA and injected in a programmable temperature vapourizer (PTV). Three variables were optimized: initial injection-port temperature, residence time and volume of BSTFA. Variables were optimized one at a time and the highest chromatographic response was obtained using initial injection-port temperatures up to 90 °C and residence time up to 2 min. Finally, since the BSTFA volume was not significant in the optimized range (2–5 μL), 3 μL were finally chosen. Besides, in the present work both EI and CI were studied for the analysis of the TMS-derivatives

of the target analytes and CI using furan as CI reagent provided the best results.

Elie and Brikett [46] studied in-port silylation for the determination of γ-hydroxybutyrate (GHB) and *trans*-hydroxycrotonic acid (*t*-HCA) in urine samples in order to avoid potential hydrolysis of the sylilated derivatives due to water condensation in the vessels used for reaction. MTBSTFA was used as silylating agent since di-*tert*-butylsimethylsilyl derivatives (*t*-BDMS) have been reported far less susceptible to hydrolysis than the classic TMS-derivatives [51]. In this case, the target analytes present in the urine samples were liquid–liquid extracted into ethyl acetate, the extract was evaporated and finally reconstituted in 100 μL of acetonitrile:MTBSTFA. 1-μL extract was injected in a programmable split/splitless injector (PSS) with a programmable pneumatic control (PCC) and different variables were studied in order to improve the in-port derivatization process. A preliminary injector temperature optimization was performed in the 200–300 °C range and it was observed that the analytical signal decreased when the injector temperature increased for both silylated target analytes. This behaviour could not be attributed to possible conversion of GHB into γ-butyrolactone (GBL) since *t*-HCA cannot dehydrate into its corresponding lactone and the same behaviour was observed for *t*-HCA(*t*-BDMS)$_2$. 240 °C was finally chosen due to the best precision obtained at this temperature. Other variables related to the temperature at which the derivatization took place were optimized and it was observed that a lower initial temperature (60 °C) and a low temperature ramp up to 240 °C provided the best results. Finally, in the case of the purge off time, contrary to what has been observed in the literature [27], the highest signals for the silylated GBH(*t*-BDMS)$_2$ and *t*-HCA(*t*-BDMS)$_2$ were obtained for short purge-off periods and, consequently, working in the split (10:1) mode was chosen.

Prieto et al. [40] developed a fully automated protocol consisting of MEPS coupled with LVI-derivatization-GC–MS in order to determine several EDCs such as APs, BPA and natural and synthetic hormones in river and wastewater samples. 50 μL of MEPS extract in ethyl acetate:dichloromethane (70:30, v/v) was injected into the LVI system and subsequently 10 μL of BSTFA was added. E2-molecularly imprinted polymer (MIP) and C18 sorbents were examined for the enrichment of the target analytes. Recovery values for most of the analytes ranged from 75 to 109% for the C18 sorbent and from 81 to 103% for the MIP material except for equilin.

Itoh et al. [29] developed a method for the determination of hydroxyl PAHs in seawater based on TENAX TA (beads of the polymer 2,6-diphenyl-*p*-phenylene) adsorption and in-tube silylation in a TDU coupled to a GC–MS. With this purpose, 100 mL of the sample adjusted at pH 2 and containing phenanthrene-d$_{10}$ as internal standard were loaded into a TD tube filled with 180 mg of TENAX TA. After the extraction, the tube was rinsed with 3 mL of Milli-Q water and the excess of water was removed by N_2 gas. The introduction of BSTFA was carried out by putting a capillary filled with BSTFA near the other end of the TD tube, as shown in Fig. 3. The tube was then immediately placed in the TDU. Firstly, the introduction of BSTFA for the in-tube silylation was studied and it was observed that larger peaks and better precision were obtained when a 32 mm-long capillary tube was used compared to the introduction with a syringe (see Fig. 3). The larger peak area and the better precision indicate that adequate amounts of BSTFA vapour were constantly supplied during the reaction time. This is the result of the BSTFA in the capillary vapourizing through the cut planes only, whose area does not change, contrary to the changing area of the BSTFA liquid introduced with the syringe. The volume of BSTFA was evaluated and volumes higher than 1 μL (2 μL or 3 μL) caused peak fronting, not only for TMS derivatives of OH-PAHs (TMS-O-PAHs) but also for the internal standard used (phenanthrene-d$_{10}$) that did not take part in the derivatization reaction. This was probably caused by overloading of the GC column at high volumes of BSTFA due to the cryofocusing of the derivatization reagent at −80 °C.

4. In-port acylation

Derivatization by acylation is a type of reaction in which an acyl group is introduced to an organic compound. In the case of a carboxylic acid, the reaction involves the introduction of the acyl group and the loss of the hydroxyl group. Compounds that contain active hydrogens (*e.g.* —OH, —SH and —NH—R) can be converted into esters, thioesters and amides, respectively, through acylation. Acylation is also a popular reaction for the production of volatile derivatives of highly polar and non-volatile organic compounds [2]. Acylation also improves the stability of those compounds that are thermally labile by inserting protective groups into the molecule. Acylation can render amenable separation by GC to extremely polar materials such as sugars and, consequently, it is a useful alternative or complimentary to the silylation.

Common reagents for the alkylation and acylation processes are fluorinated anhydrides such as heptafluorobutyric anhydride (HFBA), fluoracylimidazoles, *N*-methyl-bis(trifluoroacetamide) (MBTFA), pentafluorobenzoyl chloride (PFBCI) and pentafluoropropanol (PFPOH). Acylating reagents readily derivatize highly polar, multi-functional compounds, such as carbohydrates and amino acids. In addition, acylating reagents offer the distinct advantage of introducing electron-capturing groups [2] and, therefore, enhancing detectability during analysis. These reagents are available as acid anhydrides, acyl derivatives or acyl halides. Table 3 summarizes some works found in the literature where in-port acylation was used.

As an example of in-port acylation, Nagasawa and co-workers determined amphetamine and metamphetamine in blood by HS-SPME and simultaneous desorption and derivatization [53]. With

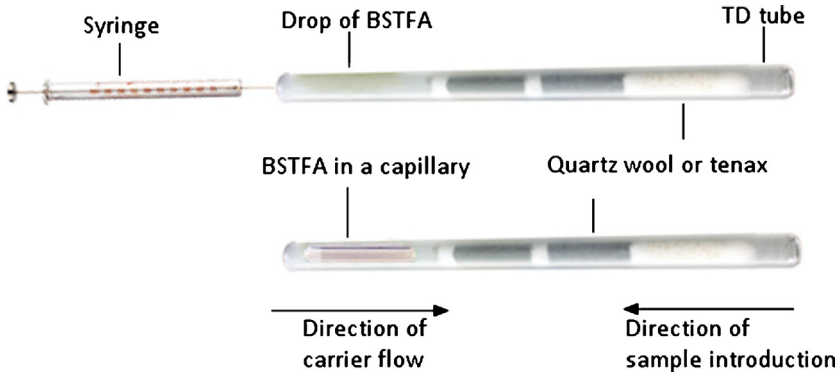


Fig. 3. Tenax TD tube where BSTFA is added with a syringe into glass wool (up) or introduced in a capillary tube (down).

Table 3
In-port acylation applications following sorptive extraction.

Compound	Sample	Extraction technique	Derivatization reagent (volume)	Injection mode	Analysis	LODs	Ref.
Amphetamine and metamphetamine	Blood	HS-SPME	HFBA (1 μL)	TD (splitless mode)	GC–EI-MS	10–20 ng/g	[53]
Fenfluramine, amphetamine and metamphetamine	Blood	HS-SPME	HFBA (1 μL)	TD (splitless mode)	GC–EI-MS	5–10 ng/g	[54]
FLU and NOR	Human plasma	LPME	MBTFA (1 μL)	Split/splitless	GC–EI-MS	3.0–5 ng/mL	[52]

DMA: dimethylamine; FLU: fluoxetine; GC–EI-MS: gas chromatography–electron impact ionization-mass spectrometry; HFBA: heptafluorobutyric acid; HS-SPME: headspace solid phase microextraction; IT-SPME: in tube solid phase microextraction; LC–MS: liquid chromatrography–mass spectrometry; LOD: limit of detection; LPME: liquid phase microextraction; MBTFA: *N*-methyl-bis(trifluoroacetamide); NOR: norfluoxetine; TD: thermal desorption.

this purpose, an extraction fibre coated with 100-μm PDMS was used to extract the drugs from 0.5 g blood sample heated at 80 °C for 20 min in the HS mode. Similarly, Namera et al. [54] used the same procedure for the simultaneous analysis of these target analytes and fenfluramine but the extraction temperature and time were set at 70 °C and 15 min, respectively. The addition of salts was studied to improve the extraction efficiency of target drugs from biological materials. Since the recoveries in the presence of sodium hydroxide were higher than those obtained with potassium carbonate, sodium chloride or ammonium sulphate, the analysis was performed in presence of 0.5 mL of 1 M sodium hydroxide. Derivatization of the analytes and desorption from the fibre was performed at once in the injection port at 250 °C using 1 μL of HFBA in both works. With this aim, HFBA solution was injected into the injection port of the GC–MS to make heptafluorobutyramide (HFB) derivatives of amphetamines. The compounds absorbed on the fibre were desorbed by exposing the fibre in the injection port for 3 min in the splitless mode.

Oliveira et al. [52] applied for the first time the in-port derivatization for the determination of the fluoxetine and norfluoxetine drugs in human plasma. Although normally analyzed by LC–MS/MS, in-port derivatization with *n*-methyl-bis(trifluoroacetamide) (MBTFA) following LPME was studied in order to determined fluoxetine and norfluoxetine by GC–MS. The injector was set to solvent flash injection mode, which means that 1 μL of MBTFA was drawn followed by a gap of air and, then, 2 μL of sample were drawn. Oliveira and co-workers tested several injection solvents, such as, methanol, toluene and ethyl acetate. However, only the less volatile solvents, such as *n*-hexyl ether and *n*-octanol, provided satisfactory repeatability. An experimental design was carried out to assess several variables, *i.e.* the injector temperature (250–300 °C), the initial temperature of the oven (70–140 °C) and the carrier gas flow (0.9–1.7 mL/min). All the three variables studied showed a positive influence in the in-port derivatization and they were set at the highest values studied, *i.e.* 300 °C, 140 °C, and 1.7 mL/min, respectively.

5. Conclusions

A thorough revision of in-port derivatization following sorptive extraction is included in the present manuscript. In-port derivatization following ion-pair extraction and in-port silylation are mostly use, while acylation applications are developed in a lower extent. In-port derivatization following ion-pair extraction has been used both with traditional SPE but also SPME and LPME applications are referred in the literature. In the case of in-port silylation SBSE and MEPS applications are also available in the literature. In the case of SBSE coupled to TD the term in-tube silylation is usually applied. Applications of dual derivatizations (*in situ* plus in-tube) have also been described. It could be concluded that in-port derivatization is a good alternative to off-line derivatization in terms of shorter analysis time and amount of reagents to be applied.

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