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Dispersive Liquid-liquid Microextraction-injector Port Silylation: A Viable Option for the Analysis of Polar Analytes using Gas Chromatography-Mass Spectrometry

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Abstract

Analysis of analytes with polar functional groups using gas chromatography-mass spectrometry pose challenges due to adsorption of these analytes on the active sites of injector port and capillary column. These can be overcome by performing derivatization. An attempt has been made to review the literature to understand the injector port derivatization (particularly silylation) coupling with dispersive liquid-liquid microextraction for the analysis of polar analytes and its use in the analysis of chemical analytes containing polar functional groups.

Keywords: Injector port silylation; GC-MS; DLLME; Injector port derivatization; BSTFA

Introduction

Development of modern sample preparation techniques is aimed to focus on the use of zero or minimum amount of toxic solvents for extraction and to reduce the cost and time of analysis in the whole extraction procedure. In recent years, development of microextraction techniques such as solid-phase microextraction (SPME), single drop microextraction (SDME), and dispersive liquid-liquid microextraction (DLLME) etc has attracted a great promise for effective sample preparation techniques. Conventional gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) is not an ideal choice to study polar, hydrophilic and nonvolatile compounds as these compounds are well adsorbed on the active sites of injector port and column, additionally intra-molecular hydrogen bonding also interferes with the analysis of polar analytes by GC. This problem can be overcome by derivatizing polar analytes with a suitable derivatizing reagent. Derivatization increases the volatility, detectability and thermal stability of polar compounds. Out of the derivatization reagents reported, silylation is the most preferred derivatization and it has found wide applications for the analysis of polar analytes using GC or GC-MS analysis [1].

Injection Port Silylation (IPS)

Silylation is the most widely used derivatization method for the conversion of polar analytes into non-polar derivatives [1]. However, a conventional silylation which is performed outside the GC-MS injection port in a reaction vessel requires high temperature (~60–80°C), longer reaction time (~30–120 min) and large volume of toxic solvents/reagents. In order to overcome these limitations for rapid, sensitive and reproducible methods, Rasmussen has introduced a technique called injection port silylation (IPS) which is an online derivatization technique [2]. It is a gaseous phase reaction between a silylating reagent and polar analytes which occurs inside the hot GC or GC-MS injection port. Basically, IPS is a type of injection port derivatization (IPD), which also includes derivatization of polar analytes with ion-pair reagents such as tetra alkyl ammonium salts (TAA) such as tetrabutylammonium hydrogen sulphate (TBAHS), tetrabutylammonium chloride (TBAC) and tetrabutylammonium hydroxide (TBAH) [3-5]. In solution form, the TAA forms an ion-pair complex with analytes containing carboxylic or sulfonic acid groups which upon the introduction in hot GC-MS injection port forms an ester with polar analyte and tertiary amines as by-products. However, the major constraint of alkylation with TAA is that, only acidic functional groups can be derivatized.

In contrast to IPD with TAA, IPS overcomes the aforesaid limitations and can derivatize polar functional groups such as –OH, $-NH_2$, -COOH, -SH. Additionally, IPS also reduces the possibilities of degradation of derivatives as their exposure to moisture sensitive conditions is negligible. IPS has overcome the major problems associated with traditional *in-vial* silylation. Extra experimental apparatus such as the heater and reaction vials are not required for IPS derivatization as reagent and analytes are simultaneously or one by one injected inside the GC injection port. In addition, the amount of reagent required for derivatization and sample is greatly reduced from microliters to nanoliters. The reaction efficiency of on-line derivatization is also improved when compared to off-line derivatization which subsequently enhances the detector sensitivity

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Table 1: Coupling of IPD with various extraction methods in literature.

S.No.	Analyte(s)	Matrix	Derivatizing Reagent	Extraction Technique	Reference
1	Phenols and acidic herbicides	water	MTBSTFA	SBSE	[7]
2	Polyphenols	herbal infusions	BSTFA	DSDME	[8]
3	Melamine and cyanuric acid	powdered milk	BSTFA	LLE	[9]
4	Fluoxetine and norfluoxetine	human plasma	MBTFA	LPME	[10]
5	Fecal sterols	fecal matter	BSTFA	SPE	[11]
6	Quinine	urine	BSTFA+TMCS (99:1*v/v*)	DLLME	[15]
7	Endocrine disruptor chemicals	wastewater	BSTFA+TMCS (99:1*v/v*)	DLLME	[16]
8	3-phenoxybenzoic acid	liver and blood	BSTFA+TMCS (99:1*v/v*)	MISPE-DLLME	[17]
9	Alkylphenols	environmental water samples	BSTFA	MASE and SBSE	[18]
10	Alkylphenols and bisphenol A	seawater samples	BSTFA	SPME	[19]
11	Polycyclic aromatic hydrocarbons	sediment samples	MTBSTFA	SWE and DLLME	[20]
12	Endocrine disrupting chemicals	water	BSTFA+1%TMCS	MEPS	[21]
13	Mono and dicarboxylic acids	ozonolysis of cyclic alkenes	BSTFA	LLE	[22]
14	Chlorinated bisphenol A	human plasma	BSTFA	SPME	[23]
15	Benzophenone UV filters	water	BSTFA	vortex assisted DLLME	[24]
16	Triclosan	wastewater and surface water	TBDMS	SPE	[25]
17	Non-steroidal anti-inflammatory drugs	water samples	TBAHS	ion-pair liquid-liquid extraction	[3]
18	Acidic herbicides	aqueous samples	TBAC	ion-pair hollow fiber-protected LPME	[4]
19	Linear and branched perfluorooctane sulfonate isomers	biological samples	TBAH	SPE	[5]
20	Pharmaceutical residues	water	TBAHS	SPE	[26]
21	Phenolic acids	plasma	TBAH	ion-pair microextraction	[27]
22	Chlorophenoxyacetic acids	water	TBAC	USEME	[28]
23	Linear alkylbenzenesulfonates	aqueous samples	TBAHS	ion-pair-SPME	[29]
24	Low molecular weight dicarboxylic acids	atmospheric aerosols	TBAH	SPE	[30]
25	Long chain fatty acids	water	TBAHS	ion-pair dynamic LPME	[31]

SBSE: Stir Bar Sorptive Extraction; **DSDME**: Directly Suspended Droplet Microextraction; **LLE**: Liquid-Liquid Extraction; **MBTFA**: *n*-methyl-*bis*(trifluoroacetamide); **LPME**: Liquid-Phase Microextraction; **SPE**: Solid Phase Extraction; **MASE**: Membrane Assisted Solvent Extraction; **SWE**: Subcritical Water Extraction; **MEPS**: Microextraction by Packed Sorbents; **TBDMS**: *tert*-butyldimethylsilylated; **USEME**: Ultrasound Assisted Emulsification Microextraction.

and accuracy of quantification [6]. A summary of the research articles of coupling of IPS with various extraction methods for the determination of polar compounds is shown in Table 1.

Applications of IPS

The derivatization using IPS for GC analysis of 46 acidic and polar pollutants including phenols, acidic herbicides and several pharmaceuticals extracted from water samples [7]. Three derivatization strategies such as silylation, acetylation and alkylation tested for the analysis of all the targeted analytes. N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide, MTBSTFA (silylating reagent) was found to give best results for the simultaneous analysis of 46 acidic and polar pollutants using IPS. The high pH need for *in-situ* acetylation decreased the extraction efficiency of pharmaceutical herbicides, because phenols could not derivatize with alkylating reagent such as tetrabutylammonium salt [7].

Several factors such as mode of injection, injector port temperature and derivatization time influence the yield of IPS as studied by several authors [8-11]. In one such study, Vinas and the co-workers [8] have used BSTFA for IPS of polyphenols and compared the mode of injection either split and split-less and later was found superior over former. The temperature of GC-MS injector port between 160–280°C was also screened. The yield of derivatization of all polyphenols was found to increase upto 240°C and this temperature was found most suitable for the IPS derivatization of polyphenols [8]. The injector port temperature has played a critical role during IPS. Tzing and Ding [9] have shown that as the temperature raises from 75 to 90°C, the derivatization yield increased; which tends to decrease further after 90°C for the analytes melamine and cyanuric acid with BSTFA containing 1%TMCS. The residence time, i.e. time required for the analytes to react with derivatizing reagent inside the GC-MS injection port was also evaluated and found that 2 min giving the optimum derivatization efficiency. In another study conducted to evaluate the effect of solvents used for IPS for fluoxetine and norfluoxetine have shown that less volatile solvents were able to give satisfactory repeatability of the derivatization. Apart from these, initial column temperature and carrier gas flow rate has shown to effect the yield of derivatization using N-methyl-

bis(trifluoroacetamide), MBTFA as injector-port derivatizing agent [10]. In another study, Wu *et al* [11], has also investigated certain parameters like effect of solvents such as acetonitrile, acetone, dichloromethane, diethyl ether, ethyl acetate, hexane and *tert*-butyl methyl ether and shown that dichloromethane giving the best derivatization efficiency after solid-phase extraction of fecal sterols from environmental water samples by IPS/GC-MS analysis. Based on the literature and usefulness of the IPS as an easy to use derivatization method, it has expanded its scope for analysis of polar analytes using GC-MS.

DLLME-IPS

In recent years, microextraction techniques coupled with different derivatization make the analysis more efficient, sensitive, selective, economical and eco-friendly. Dispersive liquid-liquid microextraction (DLLME), a new microextraction technique introduced by Assadi and co workers [12] has gained a promising place among the researchers to develop rapid and cost-effective sample preparation methods for the analytes of their interest and improve this technique thereupon. This method mainly based on ternary component solvent system in which an appropriate mixture of dispersant, extraction solvent (both miscible in each other) rapidly injected into an aqueous solution which enable the formation of a cloudy solution (water/dispersant/extraction solvent). This cloudy solution has tiny droplets of extraction solvent dispersed throughout the aqueous solution. The hydrophobic analytes are then enriched in the extraction solvent is centrifuged, due to which, high density extraction solvent accumulates at the bottom of the tube known as sedimented phase which can directly injected into GC for analysis. Compared with SPME and SDME, the extraction time in DLLME is very less. DLLME has been widely applied for the analysis of organic analytes and metals from various complex matrices [13, 14].

An attempt has made by our group to couple DLLME with injector port silylation (IPS) which can enhance the scope of DLLME for the analysis of polar analytes at cheaper cost. This coupling enhances the use of DLLME and overcome several limitations of *in-vial* silylation. This coupling lessens the (a) time for silylation (less than a minute), (b) need of external anhydrous conditions, (c) use of toxic silylating reagent and the solvents used for extraction. The coupling of DLLME with IPS has successfully applied for extraction of quinine from urine samples and the sediment phase then injected manually into GC-MS along with BSTFA containing 1% TMCS. Thus, quinine was derivatized inside the hot GC-MS injector port instantaneously thus eliminating the lengthy reaction time needed in conventional *in-vial* silylation [15]. The DLLME-IPS also used for the analysis of multi-class analytes like phenolic endocrine disruptors (PEDCs) in environmental water samples. This method added the advantage of automatic injection of both sample and derivatizing agent using an auto sampler which eliminates the need of injecting them manually into the GC [16]. In another study the DLLME-IPS has hyphenated with molecularly imprinted polymers (MIP) (has ability for selective picking of the analytes from the sample) for the quantitative determination of 3-phenoxybenzoic acid (3-PBA) from complex biological samples such as blood and liver. This has improved not only sensitivity but also enhanced the selectivity of the analysis. The analyte, 3-PBA has been extracted from biological samples using molecularly imprinted polymer (MIP) solid-phase extraction (MISPE) [17]. The DLLME-IPS-GC-MS approach has been shown in Figure 1.

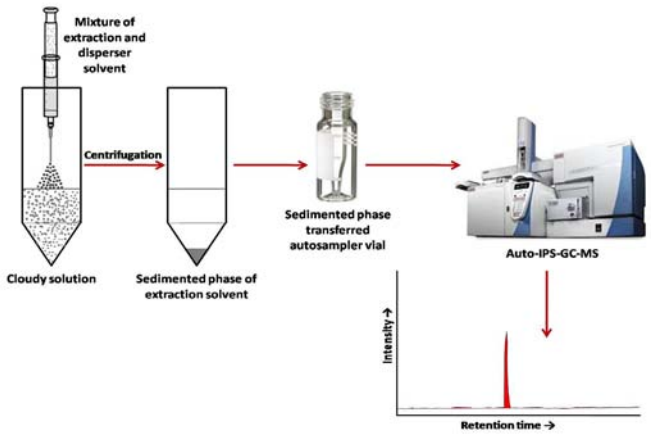

Figure 1: Diagrammatic representation of DLLME-IPS-GC-MS approach.

Conclusion and Future Directions

Coupling of DLLME with IPS results in a rapid, economical, eco-friendly and sensitive analytical method. This coupling has enabled to analyze polar analytes by GC-MS. It is a first step in coupling the microextractions with injector port derivatization but need more such. DLLME-IPS has the potential to analyze multiple polar analytes in single run due to the potential of DLLME as extraction/preconcentration tool and ability of silylation as an effective derivatization agent for most of the polar analytes which definitely expand the use of GC-MS for toxicological and/or clinical analysis. In future, DLLME-IPS/GC-MS could be an alternative to study the polar analytes in simple or complex matrices for several studies including untargeted metabolomics. The researchers should concentrate in this area of research so that the methods developed will be helpful for the routine analysis and generate more authentic data for regulatory purposes. These methods can also cut the burden on the analyst who is performing day-to-day analysis in the laboratory. Further, this approach reduces the use of toxic organic solvents for extraction and thus develops eco-friendly method, a step towards green chemistry.

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