

## 1 "Green" nature of the process of derivatization in analytical sample preparation

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### 11 Abstract

12 Nowadays, Green Analytical Chemistry idea is of high importance what impact on the rapid  
13 growth in the sample preparation area with special emphasis on sample preparation  
14 simplification, miniaturization and automation. Because the derivatization process is often an  
15 essential element of the analytical procedure, it should be important to focus on this issue and  
16 conduct a series of experiments in order to develop the most favourable conditions. Application  
17 of microextraction techniques coupled with the derivatization perfectly meets the specified  
18 requirements. Other approaches to perform derivatization process in "green" way include  
19 application of eco-friendly solvents/reagents, enhanced parameters such as microwaves or  
20 ultrasound and application of in-port, on-column/in-capillary derivatization modes. This review  
21 describes factors that allow making derivatization process more green, different modes and  
22 ways of derivatization procedures involving less toxic, hazardous reagents/solvents and more  
23 efficient forms of energy. Moreover, microextraction techniques that are often coupled to  
24 derivatization are described with examples.

### 25 26 Keywords

27 Green analytical chemistry; derivatization; ionic liquids; supercritical fluids; ultrasounds;  
28 microwaves; microextraction techniques

### 29 1. Introduction

30 The low amounts of analytes present in different kinds of samples, the sample characterized by  
31 complex matrix composition, and the need for several isolation steps makes accurate  
32 quantification difficult. Thus, it is necessary to select an appropriate method of sample  
33 preparation for analysis including choice of extraction type, and a final determination technique.  
34 In addition, the fact that many compounds do not possess structural properties which enable  
35 determination by means of gas (GC) or liquid chromatography (LC). Therefore, derivatization  
36 process (chemical conversion of analytes) is often performed because it allows for a significant  
37 increase in the possibilities and scope of application of both techniques. For example,  
38 application of derivatization impact on decreasing of polarity and reactivity and increase  
39 volatility of the target compounds which is desirable in the case of GC analysis. Furthermore,

40 this contributes to an increase in the sensitivity and selectivity and, thus, a lowering of the  
41 detection limit [1].

42 The analytical derivatizations are so flexible that they can be switched between different  
43 matrices and applications. The same derivatization will work adequately for the aldehydes and  
44 ketones present in breath or atmospheric air. Similarly, the isolation of amino acids from  
45 different type of samples ranging from foods to complex biological matrices will be  
46 accomplished by the similar derivatization process [2, 3].

47 Derivatization is performed using pre-, on-, and post-column methods. Generally, pre- and on-  
48 column modes are used with GC systems. These modes improve thermal stability, volatility,  
49 and/or detection of target analytes. There are some examples where pre-column extraction is  
50 used with LC-MS/MS, the major objective is to improve the retention, and ionization efficiency  
51 of the analytes. Pre-column mode is performed before the injection of the sample into the  
52 instrument. For the GC, pre-column mode is suitable for thermally labile and polar or ionic  
53 analytes as it converts them into volatile and less-polar derivatives. Post-column derivatization  
54 is a common approach for liquid chromatography after separation of the analytes from the  
55 column. Basically, separated analytes are converted into forms which are detectable UV or  
56 fluorescence detector [3].

57 Despite the fact that derivatization process is an undesirable process by the analytical  
58 chemist, because it constitutes a further step of preparing the sample for analysis, which may  
59 affect the loss of the analytes and the introduction of additional impurities, and also extends the  
60 entire length of the proceedings, it is often a process necessary to carry out the analysis. Another  
61 challenge is the need for derivatization process in accordance with the green chemistry and  
62 green analytical chemistry (GAC) [4], which arise directly from the principles of sustainable  
63 development. In fact, the 6<sup>th</sup> principle of GAC says that derivatization should be avoided.  
64 Because the process of converting chemical analytes is often an essential element of the whole  
65 analytical procedure, it should be important to focus on this issue and conduct a series of  
66 experiments in order to develop the most favourable conditions for the chemical conversion  
67 process of analytes. In the literature, it can be found that miniaturization and automation are  
68 key elements that should be taken into account during optimization of "green" analytical  
69 procedure, in which there is a step derivatization of analytes [5]. The result of this approach is  
70 to reduce the waste of reagents and thus reduce the amount of waste generated. Application of  
71 microextraction techniques in conjunction with the derivatization perfectly meets the specified  
72 requirements. Other approaches to perform derivatization process in "green" way include  
73 application of environmentally friendly solvents and reagents; application of enhanced  
74 parameters such as microwaves, UV radiation or ultrasound; application of in-port (in GC), on-  
75 column/in-capillary (LC and CE, respectively) derivatization modes.

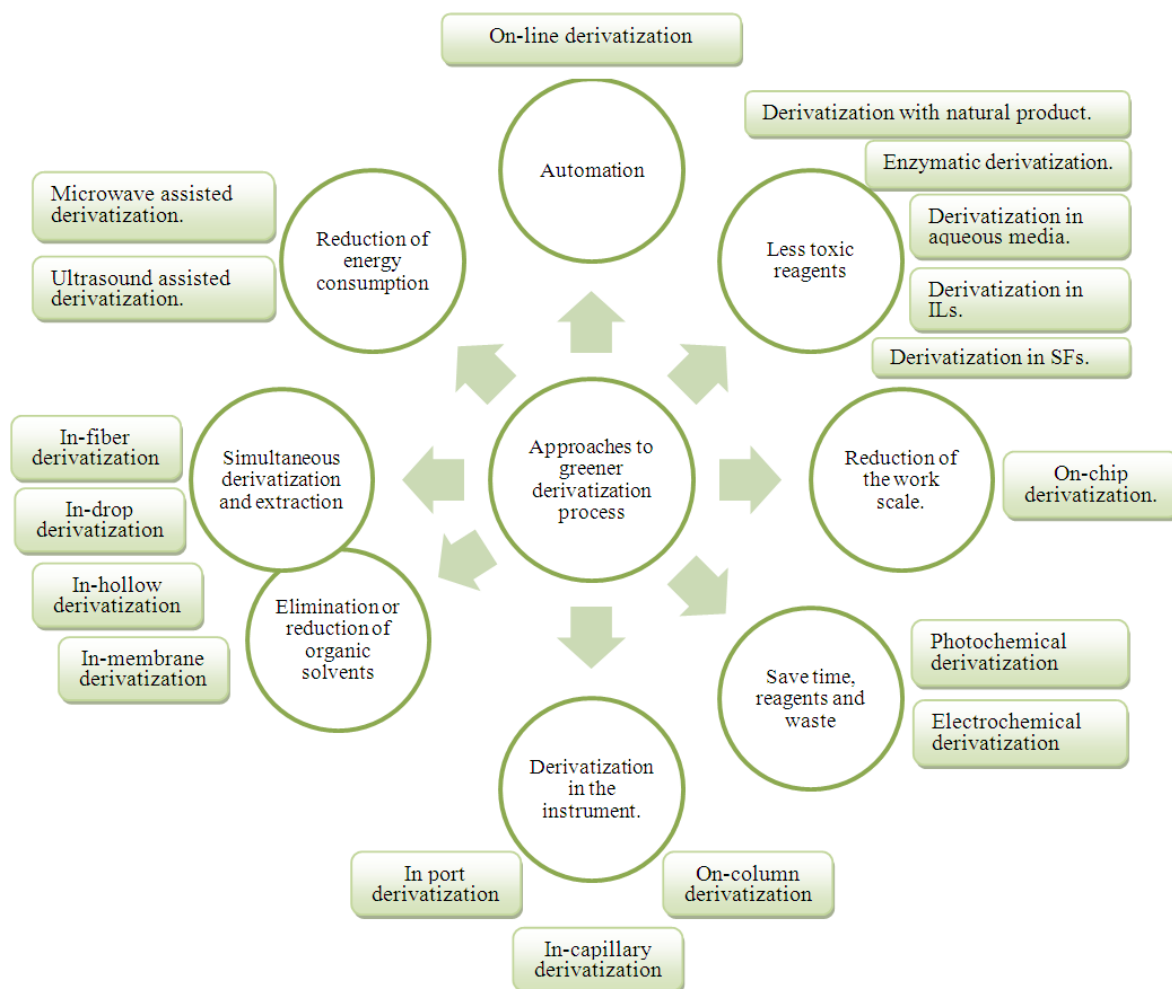
76 In this article, factors that allow to make derivatization process improved green,  
77 different modes and ways of derivatization procedures involving less toxic and hazardous  
78 reagents/solvents and enhanced efficient forms of energy are discussed. Moreover,  
79 microextraction techniques that are often coupled to derivatization process are described with  
80 examples. This review is based on literature data from the last two decades and refers to  
81 different type of samples characterized by complex matrix composition. Databases like Web of  
82 Science, Mendeley and Scopus were used to select literature commented in the body. Instead  
83 of covering all the articles, selective publications highlighting the major trends were included.

84 The keywords such as green analytical chemistry, green derivatization, enhanced parameters,  
85 microextraction techniques, green solvents, automation and connected to them were applied  
86 during literature searching.

87

## 88 **2. Ways to make derivatization process more green**

89 Several ways to make derivatization process more green exist (Figure 1). In past, the most often  
90 techniques used for introducing derivatives into the chromatographic system when the reaction  
91 takes place directly in the aqueous medium have been solid phase extraction (SPE) [6, 7] and  
92 liquid-liquid extraction (LLE) [8, 9]. However, these techniques present some drawbacks  
93 including high level of organic solvent consumption (especially LLE) and considerable  
94 manipulation of the sample (SPE, LLE). Moreover, the automation of either technique has been  
95 scarcely addressed. In the era when it is recommended to apply the principles of green chemistry  
96 in analytical laboratories, it is difficult to justify extraction methods, which use large quantities  
97 of toxic, organic solvents in the sample preparation stage [8]. Therefore, sample preparation  
98 techniques where solvent consumption is reduced are preferred, for example dispersive liquid-  
99 liquid microextraction (DLLME) or single drop microextraction (SDME). These techniques  
100 resolve many aspects of green chemistry while keeping advantages of using the well understood  
101 long used liquid-liquid extraction. Also, solventless sample preparation techniques based on the  
102 extraction of analytes in sorption processes have become effective and environmentally friendly  
103 alternatives compared with traditional solvent extraction techniques. These techniques include  
104 solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE). Both techniques  
105 are successfully applied to the in-situ derivatization of target compounds.



106

107 Figure 1. Schematic representation of approaches to greener derivatization process.

108 Another approach for greening the derivatization procedure include some instrumental  
 109 configurations. Here, on-column or in-capillary derivatization with LC and capillary  
 110 electrophoresis (CE), respectively [10] are of high importance. In these cases, derivatization  
 111 process takes place during the separation stage, thus, they are advantageous over the most  
 112 conventional pre-column, post-column or capillary modes of derivatization because  
 113 consumption of sample and derivatizing agents is low and full automation occurs without  
 114 additional equipment [10]. In addition, in-port derivatization (introduction of sample and  
 115 derivatization agent in the injection port) performed mainly in case of GC, allow for  
 116 simplification of sample preparation and reduction of solvent consumption as well as allow to  
 117 avoid the application of hazardous conditions and waste generation.

118 The application of such enhanced factors as microwaves, ultrasound (US) and UV radiation,  
 119 which provide to perform derivatization process at softer derivatization conditions as well as  
 120 often accelerate the chemical conversion of analytes is also in accordance with GAC principles.  
 121 Moreover, application of reagents and solvents that are less toxic in order to reduce their impact  
 122 on the environment and laboratory staff has received increasing attention [10].

123

### 124 3. Greener reagents and solvents in derivatization process

125 Due to the fact that reagents and solvents used in derivatization process are often toxic,  
126 corrosive and irritant, thus, their replacement by more suitable, eco-friendly agents could bring  
127 positive features of whole analytical method. Moreover, less toxic and corrosive chemicals used  
128 in derivatization, more eco-friendly wastes are produced.

129 One of the most comprehensive and easy ways to evaluation the greenness of analytical  
130 procedures used for environmental purposes is the National Environmental Methods Index  
131 (NEMI), which can be found at the website <http://www.nemi.gov>. Four criteria that refer to the  
132 properties of reagents as well as wastes applied in the procedure are taken into consideration to  
133 rate the existing methods [5, 11]. These are: i) persistent, bioaccumulative, toxic; ii) hazardous;  
134 iii) corrosive (pH <2 or >12); and, iv) volume (mass) of waste is >50 mL(g). Another example  
135 of an Internet database which is oriented towards chemicals and processes that can be changed  
136 to reduce the hazardous and volume of wastes produced is the Green Chemical Alternatives  
137 Wizard (<http://ehs.mit.edu/greenchem/>) developed by Massachusetts Institute of Technology.  
138 Persistent, bioaccumulative and toxic substances (PBT) as well as hazardous chemicals which  
139 are listed in the Toxic Release Inventory (published on The Environmental Protection Agency  
140 website [12]), are not only toxic, but also pose special risks because they remain in the  
141 environment for long periods of time and can be accumulated in biological tissues. Therefore,  
142 without a doubt it is important to look for alternatives which will be eco-friendlier.

143 Although, the application of solvent free derivatization process is the most welcome in the  
144 context of Green Analytical Chemistry, most derivatization processes use traditional organic  
145 solvents. Therefore, replacement of these solvents by other greener classical solvents is the  
146 simplest strategy, despite being rarely used [10]. However, this solution may be difficult to  
147 accomplish due to the fact that solvents have a substantial effect on reactions such as the reaction  
148 rate, the stereoselectivity and the outcome.

149 Very good example of application of green solvents in the determination of organic acids and  
150 phosphates as trimethylsilyl derivatives in plant extracts instead of the most popular chemicals  
151 (pyridine or dimethylformamide) was presented by Englmaier [13]. The research was focused  
152 on the preparation, kinetics of silylation and effect of a different solvent on the quantitative  
153 reaction and stability of the derivatives; and the acetone was proposed as more green option.  
154 However, nowadays, the better option seems to be the application of greener alternatives.

155 Considering these greener alternatives to popular traditional organic solvents applied in  
156 derivatization, should be mentioned: i) water; ii) bio-derived solvents; iii) natural deep eutectic  
157 solvents; iv) ionic liquid (ILs) and v) supercritical fluids (SFs). Among these chemicals, the  
158 most desirable solvent that could be used in the derivatization reaction is water, however, this  
159 possibility is limited by the low solubility of organic substances in aqueous media [10].

160 Due to such properties as non-flammability, low volatility and thermal stability, room  
161 temperature ionic liquids have attracted increased attention as an alternative to traditional  
162 volatile organic solvents. ILs are used in different stages of analytical chemistry, starting from  
163 the sample preparation (extraction procedures), by separating the analytes using various  
164 analytical techniques (GC, LC, CE) and ending at the final determination stage (matrix-assisted  
165 laser desorption/ionization mass spectrometry). Also, derivatization process coupled with  
166 microextraction in ILs is often performed. In this combination, mainly dispersive liquid liquid

167 microextraction (DLLME) [2, 14] is applied. From the other side, several disadvantages of ILs  
168 from the GAC point of view are known, including:

- 169 i) Due to their slow degradation, they have high persistence in the environment;
- 170 ii) Due to their significant solubility in water, they may be released into the aquatic  
171 environment;
- 172 iii) Some of the ILs are toxic, however, their toxicity varies for different organisms as  
173 well as depends on composition of ILs.

174 Other green solvents, which are sometimes, applied in derivatization process are SFs, in  
175 particular carbon dioxide [15]. This is mainly due to their advantages such as chemical  
176 inertness, low toxicity and easy disposal. From the other side, high cost of production (high  
177 consumption of energy) and low polarity make them relatively rarely used.

178 Among the application of SFs in derivatization process, Supercritical Fluid Extraction with in  
179 situ derivatization is the most popular technique used. This brings such positive features as  
180 simplification of sample handling and sample preparation step, minimizing number of stages  
181 of whole analytical procedure and shortening of analysis time. In addition, it is not necessary  
182 to remove the excess of solvent from the extracts obtained, thus, they can be directly transferred  
183 to the chromatograph for on-line analysis [10]. In the case when derivatizing agents will be  
184 used as modifiers of supercritical fluid, it is required to select its carefully to ensure their  
185 compatibility with extraction conditions (pressure and temperature).

186 Not only solvents, but also other chemicals should be investigated when derivatization process  
187 is considered from the sustainable environment point of view. However, despite the fact that  
188 the search for less toxic as well as natural compounds for derivatization should be a goal in new  
189 analytical developments, achievements in this area are very scarce. However, some examples  
190 of such solution can be found in the literature. For instance, with the objective of utilizing the  
191 nontoxic chemicals, novel oxidative coupling reactions resulted using Cisapride (CPE) as green  
192 analytical spectrophotometric reagent were performed [16]. Furthermore, this reagent was  
193 investigated in the research focused on the determination of bromate in drinking water, bread  
194 and flour additives. The proposed methods have distinct advantages of sensitivity and  
195 selectivity. Besides, the methods do not require heating or distillation and they exhibit reliability  
196 due to their reproducibility. Such proceeding is worth to be followed. In another work, a  
197 sequential injection method was developed for the spectrophotometric determination of  
198 chlorine in tap-water and surface water samples based on the reaction between  
199 tetramethylbenzidine (TMB) and free chlorine [17]. The application of the TMB/chlorine  
200 reaction in a sequential injection system was successful and adds other advantages as it  
201 enhances the degree of automation, minimisation of reagent consumption and low effluent  
202 production. Moreover, TMB proved to be a highly selective reagent, yielding a very sensitive  
203 methodology resulting in fairly low quantification limit.

204 Different examples of the use of unrefined natural reagents derived from plant and animal  
205 tissues or microbial cell for derivatization have been published [18]. Crude plant extracts may  
206 contain chemical compounds that enable their use as chromogenic or fluorogenic agents. The  
207 application of natural reagents in conjunction with a flow injection system can confer a number  
208 of advantages. First of all, the enhanced kinetic control that flow analysis offers may assist in  
209 avoiding undesirable side reactions that would otherwise occur using impurified reagents [18].



210 The natural reagents lifetime may be prolonged when applied in a flow analysis system because  
211 their exposure to light or air can be monitored.

212

#### 213 **4. Microextraction coupled with derivatization**

214 Over the last few decades, numerous microextraction techniques have been developed and  
215 broadly used to different type of samples. They are featured by minimum or no use of organic  
216 solvents, shorter extraction times, opportunities for automation, and miniaturized dimensions.  
217 Among number of miniaturized extraction techniques, solid phase microextraction (SPME) and  
218 liquid phase microextraction (LPME) are pertinent to mention here.

219 SPME is a green alternative to conventional solid phase extraction (SPE). It requires very little  
220 amount of the sorbent coated on a silica fiber or metallic support. Moreover, it allows thermal  
221 desorption of analytes when coupled with gas chromatography and very little volumes of  
222 solvents are required when coupled with liquid chromatography. As analytes are extracted into  
223 very small amount of sorbent, SPME provides very high enrichment factors and thus the  
224 sensitivity. The selectivity can be fine-tuned by selecting suitable sorbent phase.

225 LPME is a miniaturized format of liquid phase extraction (LLE). LLE is famous for its  
226 simplicity, effective cleanup ability, and high selectivity through the selection of suitable  
227 solvents. Despite all the advantages, it does not represent a green extraction due to excessive  
228 use of toxic solvents (hundreds of mL). Furthermore, it is time consuming and cannot be  
229 automated due to emulsion formations. However, compared to LLE, LPME utilizes very little  
230 volume of the organic solvent (in  $\mu\text{L}$ ) which renders it as a green approach. Some famous  
231 versions of LPME technique include single drop microextraction (SDME), hollow-fiber liquid  
232 phase microextraction (HF-LPME), and dispersive liquid-liquid microextraction (DLLME)  
233 [19].

234 There are different aspects through which environmental impact of derivatization can be  
235 minimized. Apart from several universal approaches that include use of greener derivatizing  
236 reagents, decrease in their volumes, less-generation of waste, etc miniaturization and  
237 automation of the analytical extraction process may help to implement green practices. Several  
238 emerging trends indicate faster and simpler derivatization procedures based on their coupling  
239 with microextraction techniques. The derivatization process can get greenness from the nature  
240 of the microextraction itself. If derivatization is combined with SPE or LLE, it will require large  
241 volumes of derivatizing reagents relating to the sample volume requirement for these  
242 extractions. However, the similar derivatization will require much-reduced amounts of  
243 derivatizing reagents in case of SPME, LPME or other microextraction techniques.

##### 244 **4.1. Combination of derivatization and microextractions: A way forward to green** 245 **process**

246 Apart from the green nature of the process, the need for the combination of derivatization with  
247 microextraction arises from the facts that derivatization alone, in some cases, may introduce  
248 impurities, excess reagents, side-reaction products, and incomplete reactions that may interfere  
249 with target analytes.

250 In general, pre-column derivatization can be combined with microextraction in the following  
251 ways:



- 252 i) Pre-extraction derivatization: it is performed in sample solution or donor-phase  
253 before microextraction. This approach may provide higher partition coefficients and  
254 improve separation of the analytes.
- 255 ii) Post-extraction derivatization: This approach is adopted when pre-extraction is not  
256 required or derivatization can make extraction process impossible or can induce  
257 adverse effects on the extraction itself by complicating the matrix. In this way, the  
258 purpose of post extraction is to convert the analytes into the form, which is  
259 analyzable by the instrument.
- 260 iii) In-situ derivatization: it involves one step extraction and derivatization. It seems to  
261 be more focused recently. It has advantages of being simple, rapid, relatively  
262 greener.
- 263 iv) Injection port derivatizations: Most of the derivatization procedures include off-line  
264 coupling with microextractions. Such protocols are performed prior to the analysis.  
265 However, in some cases, offline derivatizations lead to experimental errors due to  
266 loss of analyte through evaporation, transfer, and re-suspension steps, contamination  
267 of samples during work-up. Moreover, interference of moisture in the reaction  
268 system can cause some major issues as some of the derivatizing reagents and the  
269 resulting derivatives are very sensitive to water. On-line derivatization techniques  
270 are emerging to solve such issues.
- 271 Online derivatizations are time-effective, require less amount of reagents, and result  
272 in better efficiency of the analysis. Inlet-based or in-port derivatization allow direct  
273 injection of the sample and derivatization reagent into the hot GC inlet, where the  
274 derivatization reaction takes place in the gaseous phase. The sample and the  
275 derivatization reagent can be injected separately or by a single injection. In later  
276 case, the syringe is filled with both the sample and the derivatization reagent, but  
277 with an air gap between them.

278 Derivatization can be combined both with solid and liquid phase microextractions. In the  
279 coming sections, we will discuss the green aspects of coupling microextractions and  
280 derivatization. The description of derivatization reactions and their types have already been  
281 emphasized in many reviews and is beyond the scope of this article.

## 282 4.2. Sorbent microextraction and derivatization

### 283 4.2.1. Solid phase microextraction

284 Solid phase microextraction (SPME) was introduced almost 27 years ago and it laid the  
285 foundations of research in area of microextractions [20]. SPME has different formats and  
286 configurations. In fiber SPME, analytes are directly extracted into a solid extracting phase and  
287 then desorbed into the instruments. SPME fulfils the requirements of green analytical chemistry  
288 (GAC) being solventless (or minimal use of solvent), less waste production etc. This technique  
289 has been widely used in environmental, food, and biological analysis and it has many  
290 advantages over conventional extraction techniques that have been described in detail in  
291 scientific literature.

292 Although SPME is in good agreement with the principles of GAC but still the search for green  
293 materials as well as derivatizing reagents continues. Thus, it has been used for extraction and



294 derivatization of analytes from the samples of varying composition and matrix complexity. Due  
295 to solventless nature of the technique, there are various options for its combination with  
296 derivatization.

297 The coupling of SPME with derivatization was first reported by Pan and Pawliszyn in 1997.  
298 This coupling was performed in three ways; in the sample matrix, in the fiber coating, and in  
299 the GC injection port [21]. Again, in the SPME, derivatization is performed with the objectives  
300 of conversion of polar analytes into less polar equivalents, therefore enhancing their  
301 coating/water or coating/gas partition coefficients and improving SPME efficiency and method  
302 sensitivity. Derivatization using SPME has several advantages over conventional methods such  
303 as low solvent use, relatively low cost, easy to perform, and all desired features of GAC.

304 In the following section, some latest examples of each category of derivatization are described.

305

306

#### *4.2.1.1. In-situ SPME-derivatization*

307 It involves conversion of the analytes into their derivatives within the sample (in situ) plus  
308 extraction employing SPME technique. Recently, silk fiber was used as an adsorbent in in-tube  
309 SPME for extraction of in-situ chemically derivatized aldehydes. Silk fibers are green,  
310 biocompatible, porous, and cost-efficient materials, which are enriched with hydroxyl, amino,  
311 carboxyl, and other hydrophilic groups, making them good adsorbents [22].

312

#### *4.2.1.2. Pre- or post-SPME-derivatization*

313 As an example of pre-SPME-derivatization, multiclass organic UV filters were first derivatized  
314 within the sample vial and then extracted by direct immersion SPME. Low cost derivatization  
315 reagents, short extraction times, and low sensitivities were major advantages of this work [23].  
316 The second way is to perform derivatization on the fiber. This is possible either before or after  
317 extraction. A derivatizing agent can be adsorbed onto the fiber before extraction and then  
318 analytes can be extracted using DI or HS mode. The analytes can be extracted first onto the  
319 fiber and then can be derivatized chemically by immersion, vapor exposure, or spraying the  
320 fiber with derivatizing agent.

321 Recently, a rapid and environment friendly SPME method involving on-fiber derivatization  
322 coupled with GC-MS was developed for quantitation of four non-volatile biogenic amines  
323 (putrescine, cadaverine, histamine, and tyramine) in fish samples. SPME fiber was first dipped  
324 into a solution containing isobutyl chloroformate as derivatization reagent and isooctane as  
325 extraction solvent. This resulted in formation of a thin organic liquid membrane coating. The  
326 modified fiber was then directly immersed into sample solution for extraction of biogenic  
327 amines. The analytes were then thermally desorbed into the GC-injection port. Only few  
328 microliters of the reagents were employed that were not harmful to the environment and the  
329 analyst [24].

330 A fully automated post-HS-SPME-derivatization method was developed by modifying the  
331 programming of SPME autosampler and coupled online with GC-MS for determination of  
332 clenbuterol in meat. This type of automation provides better reproducibility as well as reduced  
333 exposure of workers to the toxic analytes [25].

334

#### *4.2.1.3. SPME-Injection port-derivatization*

335 In this type of derivatization, SPME is performed first for extraction and concentration of  
336 certain analytes in different matrices. SPME fiber is then injected to GC injection port and  
337 analytes are thermally desorbed for a fixed period with split closed. After that derivatization  
338 reagent is injected with the same closed split and a reaction is allowed to occur. This mode of  
339 derivatization was used for determination of chlorinated bisphenol-A in human plasma samples  
340 [26]. The amount of derivatizing reagent is much reduced compared to in matrix derivatization.  
341 Additionally, all the reaction takes place within a controlled environment with decreased  
342 chances of analyte loss and contamination.

#### 343 *4.2.2. Stir-bar sorptive extraction*

344 Stir bar sorptive extraction (SBSE) was developed in 1999 to overcome some limitations of  
345 existing techniques including SPME. In SBSE, a sorbent generally PDMs, is coated on a stir  
346 bar and it is used for extraction of hydrophobic/non-polar molecules from different media. The  
347 PDMS extracts based on van der Waals forces as well as the hydrogen bonds which form with  
348 its oxygen atoms depending on the molecular structure of the analytes.

349 In case of thermal desorption, apolar polymer coating in SBSE may be useful only for semi-  
350 volatile and thermally stable compounds. However, its coupling with derivatization can extend  
351 its application to polar and thermally labile compounds. SBSE can be coupled with  
352 derivatization process in the following ways: pre-SBSE derivatization, in-situ SBSE-  
353 derivatization, and on-stir bar microextraction.

##### 354 *4.2.2.1. Pre-SBSE-derivatization*

355 In this mode of derivatization, analytes are first derivatized in the sample solution using suitable  
356 derivatizing reagent under optimum conditions. In the second step, they are extracted using  
357 SBSE and then desorbed thermally or in suitable solvent.

358 Carbonyls were determined in the rain water using this approach. For the 100 mL of the  
359 rainwater sample, 1 mL of the derivatizing reagent (PFBHA 1 mg/mL) was employed. After  
360 adjusting the pH, the mixture was left overnight to complete the derivatization reaction. SBSE  
361 was performed under optimum conditions and finally the analytes were desorbed into 2 mL of  
362 acetonitrile with the aid of ultrasonication. This led to high enrichment factors due to  
363 concentration of derivatized analytes from large volume sample to very small volume of  
364 desorption solvent. The extract was injected to GC-MS for analysis and very low LODs (10 –  
365 30 ng/L) were obtained [27].

##### 366 *4.2.2.2. In-situ SBSE-derivatization*

367 This is the simplest way to change the analytes into the derivatives in the respective sample  
368 media before or simultaneously with SBSE. The SBSE can be performed both in DI or HS  
369 modes. After extraction of derivatized analytes, stir bar is placed with a desorption chamber  
370 coupled to GC, or analytes can be desorbed with suitable solvent for LC.

371 Chlorophenols were determined in water and body fluids by SBSE with in situ derivatization.  
372 To the sample solution, derivatizing reagent and stir bar were subsequently added, all affecting  
373 parameters were suitably optimized. After extraction, stir bar was transferred to thermal  
374 desorption tube which was further connected to GC-MS. Up to 100  $\mu$ L of acetic anhydride was

375 used as derivatizing reagent [28]. This type of derivatization procedure has potential of being  
376 green and amenable to automation.

#### 377 *4.2.2.3. On stir bar derivatization*

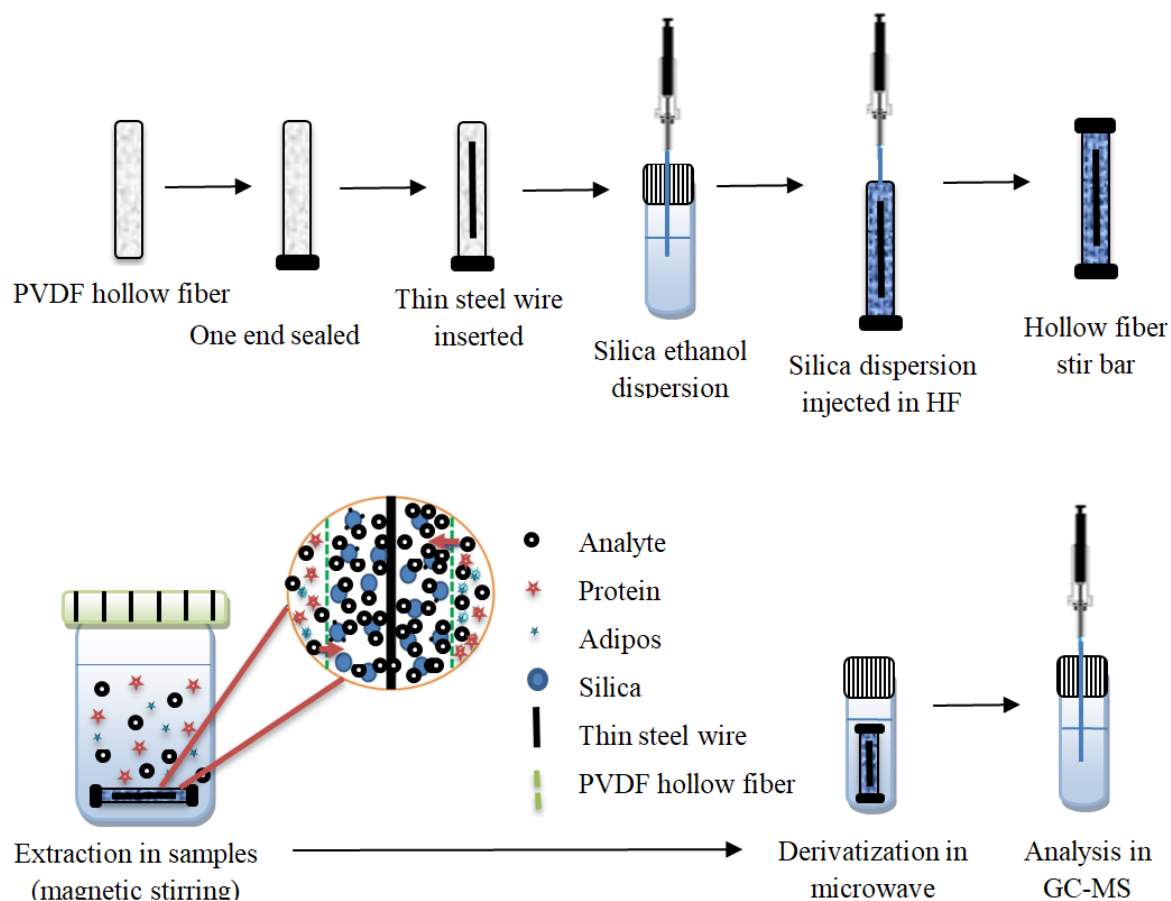
378 This mode works in two ways: first includes preloading or adsorption of derivatizing reagent  
379 on polymer coating. This mode performs simultaneous derivatization and extraction. Secondly,  
380 extraction is performed on SBSE device, followed by the derivatization on the stir bar (through  
381 vapour spraying). Another approach is used with thermal desorption. In this approach, a small  
382 capillary containing derivatizing reagent is placed with stir bar in desorption chamber. This is  
383 a good approach for silylating agents. In case of liquid desorption, derivatizing reagent is added  
384 to desorption solvent after stir bar desorption.

#### 385 *4.2.2.4. Hollow fiber stir bar sorptive extraction*

386 In order to fulfil ever-growing demands for ultra-trace analysis, recently, some efforts have  
387 been made to combine extraction procedures together. These combinations can synergistically  
388 derive benefits from individual microextractions. Such fusion may help to get better matrix  
389 clean-up, higher sensitivity and selectivity. Here, we describe some representative examples of  
390 combined microextraction techniques combined with derivatization. With this aim, hollow fiber  
391 based SPME was combined with SBSE to design a new technique HF-SBSE. Porous HF acts  
392 as filter and carrier for stir-bar, and dispersed sorbent. HF-SBSE device can be used for direct  
393 extraction in complex biological matrices as HF protects its contents against the interferences.  
394 This technique can also be coupled with microwave or ultrasonic assisted derivatization [29].

395 HF-SBSE was coupled with microwave assisted derivatization for determination of amino acids  
396 in biological matrices. After the extraction of analytes onto the HF-SBSE device, the  
397 derivatization and desorption was performed simultaneously using derivatizing reagent and  
398 solvent. Trimethylsilylation was the reaction of choice (Figure 2). This method has several  
399 benefits in terms of the green process:

- 400 i) desorption solvent also served as reaction media for derivatization,
- 401 ii) microwave assisted derivatization was time and energy efficient; derivatization  
402 requires only 2 min microwave assistance while 30 – 60 min are needed in case of  
403 conventional heating.



404

405 Figure 2. Schematic figure of preparation, extraction and derivatization of the hollow fiber stir  
 406 bar for amino acids [29]

407 *4.2.3. Microextraction by packed sorbent*

408 Microextraction by packed sorbent (MEPS) is based on the same principles as SPE. MEPS is  
 409 more than just a miniaturized version of SPE as it allows packing of the sorbent (1 – 4 mg)  
 410 inside the cartridge or special container within the injection syringe. This sorbent containing  
 411 cartridge is positioned between barrel and needle (Barrel insert and needle BIN). The sorbent  
 412 can be reused for several times. MEPS integrates sample preparation with analytical  
 413 instrumentation. It can be coupled with GC, LC, and CE.

414 There are some examples where MEPS was used in combination with derivatization. The most  
 415 famous mode, however, is the pre-extraction derivatization, where derivatization is completed  
 416 first and then MEPS is performed. Some authors termed it “in-situ derivatization” because  
 417 derivatization is followed by MEPS using a sequence of steps controlled by an automated  
 418 system.

419 Haloacetic acids were in situ derivatized in aqueous samples, extracted by MEPS, and  
 420 determined by GC-MS. The whole process from addition of derivatizing reagents into the  
 421 sample to extraction to analysis was automated. Derivatization was performed in a sample vial  
 422 of the auto sampler. The derivatization process was completed within 10 min at room  
 423 temperature in the aqueous media. From the perspectives of automation, time and energy-  
 424 efficiency, and medium for reaction; the derivatization process was greener [30].

425 The beauty of the MEPS lies in its automated procedure and online coupling with analytical  
426 instruments. The derivatization is also amenable to be the part of the same automated procedure  
427 which gives it an additional green perspective. In addition, derivatizations which require little  
428 volume of derivatizing reagent, work in green medium such as water, and complete in shorter  
429 times add more value on green aspects. One example of this kind is determination of polyamines  
430 and related compounds in urine using in situ aqueous derivatization followed by automated  
431 MEPS. The derivatization was performed using ethyl chloroformate in aqueous medium and  
432 reaction completed within 1 min [31].  
433 MEPS has also been combined with large volume injection in-port-derivatization GC-MS for  
434 selective determination estrogenic compounds in water [32].

#### 435 4.2.4. *Dispersive/magnetic solid phase extraction*

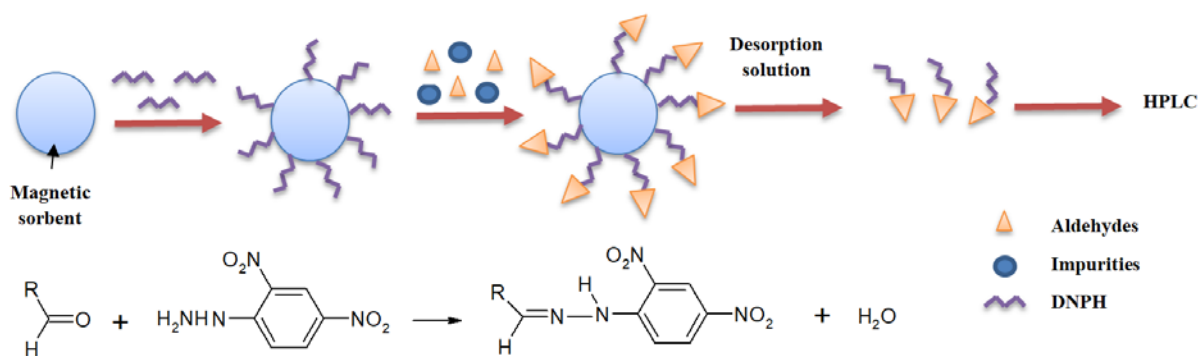
436 Dispersive solid phase extraction (DSPE) is based on the dispersion/addition of the few  
437 milligrams of the sorbent into the sample solution. The sorbent is dispersed within the solution  
438 by shaking or assistance of the vortex. After the extraction, sorbent is separated from the sample  
439 solution by centrifugation. Analytes are then back extracted from sorbent into suitable solvent.  
440 DSPE offers fast extraction due to increased interfacial area for the interaction of the sorbent  
441 and the analytes. The other potential problems associated with SPE such as packing inside the  
442 cartridge, blockage of the column, and requirement of large amounts of sorbents, sample  
443 volumes, and organic solvents can be avoided. Magnetic SPE is another form of DSPE in which  
444 magnetic sorbent is employed. It provides some additional advantages of fast phase separation  
445 using an external magnet.

446 There are several opportunities by which derivatization can be combined with DSPE/MSPE:

- 447 i) it can be performed in the sample solution prior to extraction;
- 448 ii) the sorbent can be coated/loaded with derivatizing reagent (in situ); and
- 449 iii) it can be performed after extraction.

450 The example of derivatization in the sample solution prior to addition of magnetic sorbent  
451 include the extraction of methylmercury in seawater. NaBPh<sub>4</sub> was used derivatizing reagent in  
452 this work, the derivatized analytes were extracted using Fe<sub>3</sub>O<sub>4</sub>/PANI composite based MSPE  
453 [33]. Here derivatization step was performed just before extraction steps and it was named as  
454 in situ derivatization.

455 In another example, MSPE-in situ derivatization, was used for extraction and derivatization of  
456 aldehydes in human urine samples. Instead of adding derivatizing reagent 2,4-  
457 Dinitrophenylhydrazine (DNPH) into sample solution, it was first adsorbed/loaded on the  
458 magnetic sorbent [Fe<sub>3</sub>O<sub>4</sub>/SiO<sub>2</sub>/P(MAA-co-EGDMA)] which was then used for extraction of  
459 hexanal and heptanal in human urine samples. Different steps involved in this procedure are  
460 shown in Figure 3. The whole process was completed within 9 min [34]. Table 1 lists sorbent  
461 phase microextraction methods combined with derivatization.  
462



**Figure 3.** The scheme of magnetic solid phase extraction-in situ derivatization procedure [34]

#### 4.3.Liquid phase microextraction and derivatization

LMPE is a miniaturized version of LLE. It eliminates some major drawbacks of LLE such as huge consumption of toxic solvents, long extraction times, and tedious process. LPME performs extraction with very small volumes of extractant typically in several microliters. Moreover, it performs extraction and preconcentration in a single step leading to very high enrichment factors. LPME is performed in different formats such as SDME, HF-LPME, DLLME etc. Due to low volumes of extraction solvents, these techniques require extremely low volumes of derivatizing reagents.

##### 4.3.1. Hollow fiber liquid phase microextraction (HF-LPME)

HF-LPME implies porous hollow fibers for extraction. These fibers are impregnated with an organic solvent prior to use. Extraction solvent is filled inside the lumen of the fiber. The fiber is then placed in sample solution for a defined period, after which extraction solvent is taken out of the fiber and injected into the instrument. For every extraction, a fresh piece of hollow fiber is used, which removes the chances of contamination or carryovers. The beauty of HF-LPME lies on the use of extremely low volumes of extraction solvents, thus contributing toward greenness of the extraction process. HF-LPME has also been combined with derivatization where direct analysis of target compounds is not possible using analytical instrument.

##### 4.3.1.1.HF-LPME coupled with injection port derivatization

Basheer and Lee reported coupling of HF-LPME with injection port derivatization for extraction, derivatization, and determination of endocrine disrupting alkylphenols, chlorophenols, and bisphenol-A in aqueous samples [35]. These analytes are polar and semi-volatile in nature, and therefore require derivatization before analysis by GC. The analytes were extracted from sample solution using direct immersion HF-LPME containing 5  $\mu$ l of water immiscible organic solvent inside the hollow fiber. The sample was stirred during 30 min extraction time. After extraction, an aliquot (2  $\mu$ l) of the extract and 2  $\mu$ l of BSTFA were consecutively injected into the GC injection port. This method provided very high enrichment factors and better results than HS-SPME and LLE.

Since in HF-LPME very little volume (5  $\mu$ l) of extract is accessible for derivatization. Instead of further dilution that can decrease sensitivity of the analysis, injection port derivatization is



494 preferred. Besides, it reduces volume of the derivatization reagent, derivatization time, and  
495 degradation of analytes due to moisture exposure [35].

#### 496 *4.3.1.2.Pre-HF-LPME derivatization*

497 Derivatization can be performed before HF-LPME to convert the analytes into extractable and  
498 analyzable product. For example, formaldehyde was derivatized using acetyl acetone in  
499 presence of ammonium acetate buffer to convert into 5-diacetyl 1,4-dihydrolutidine which was  
500 extracted using HF-LPME and analyzed by spectrophotometer. The derivatization reaction was  
501 supported by ultrasonic energy for 30 min at 70°C. This work utilized only 25 µL of octanol as  
502 extraction solvent [36]. The same mode of derivatization was used to determine cocaine and its  
503 derivatives in hair samples which were subjected to methanolic extraction followed by  
504 derivatization. The derivatization process was completed in 6 minutes with the aid of ultrasonic  
505 bath. LPME was used for cleanup [37].

506 Electromembrane extraction (EME) is modified form of HF-LPME where extraction of  
507 ionizable analytes is performed by the provision of electric field. Pulsed EME was used for  
508 extraction of derivatized amino acids prior to their analysis by HPLC-UV. The purpose of  
509 derivatization was to enhance the UV absorbance and hydrophobicity of selected amino acids  
510 [38].

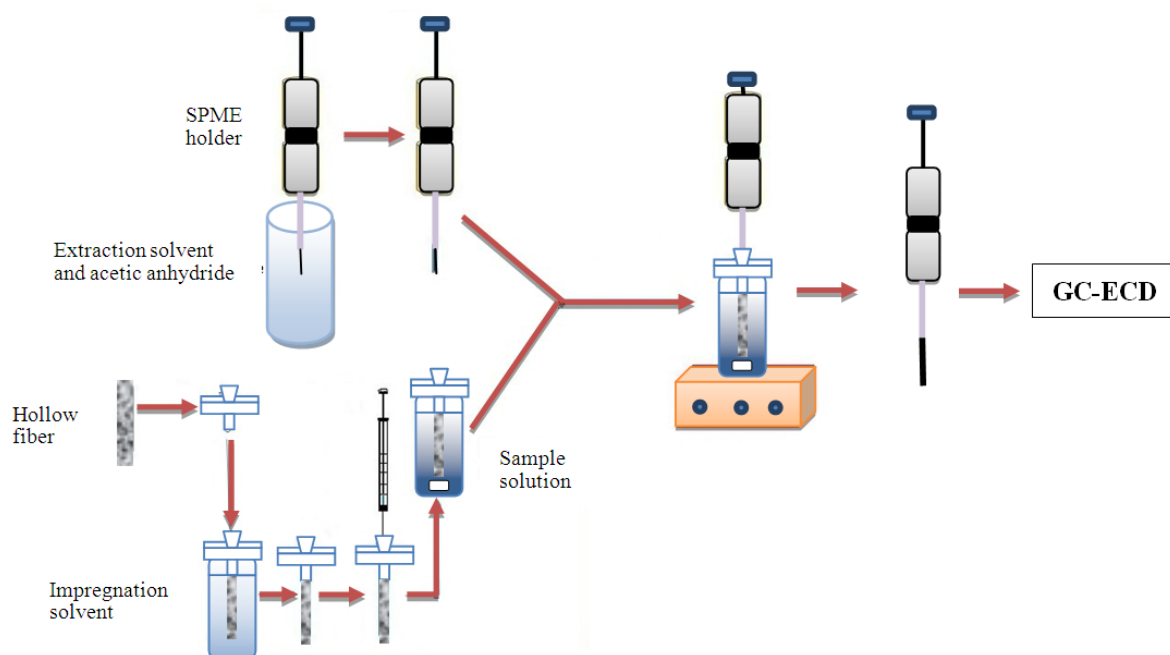
#### 511 *4.3.1.3.Post-HF-LPME derivatization*

512 Sample preparation such as conjugate metabolite hydrolysis is required for determination of  
513 BPA and phthalate metabolites in urine samples. This is performed by using specific enzymes  
514 that work under defined temperature and pH environment. Then analytes can be extracted using  
515 some extraction technique. Such processes can be simplified using derivatization. BPA and  
516 other phthalates were determined in urine using HF-LPME followed by derivatization [39]. The  
517 derivatization completes within few minutes under ambient conditions. In this way, this is  
518 another time and energy efficient approach.

#### 519 *4.3.1.4.HF-LPME coupled with SPME*

520 HF-LPME is performed either in two-phase or three-phase modes. Two-phase mode (HF-  
521 LPME) is suitable for extraction of hydrophobic analytes into an organic solvent from sample  
522 solution. Three-phase mode (HF-LLLPME) is used for extraction of ionizable compounds  
523 (basic or acidic) from aqueous samples into an organic solvent that is impregnated in the pores  
524 of HF, and finally into an aqueous acceptor phase filled inside the lumen of the HF. pH gradient  
525 drives this extraction. The final extract is aqueous and cannot be injected directly into GC.  
526 Secondly, the polar or ionic analytes should be derivatized to convert them more volatile and  
527 less-polar analytes before injection to GC. To make the aqueous extract analyzable by GC, HF-  
528 LLLPME was combined with SPME with simultaneous on fiber derivatization for analysis of  
529 chlorophenols [40]. This combination provided very low LODs 0.0004 – 1.2 µg/L and  
530 enrichment factors in the range of 432 – 785. The schematic of this combination involves some  
531 steps indicated in below Figure 4.

532



533  
534 **Figure 4.** The schematic diagram of the extraction device and extraction procedure [40]

535 *4.3.2. Dispersive liquid liquid microextraction*

536 Dispersive liquid–liquid microextraction (DLLME) was introduced about a decade ago by  
 537 Assadi and coworkers[41]. DLLME relies on ternary component solvent system, the proper  
 538 mixture of extraction and disperser solvent is injected into the aqueous sample, leading to a  
 539 cloudy solution. After centrifugation, the organic layer is separated for analysis. This technique  
 540 provides high enrichment factors. In addition, it is fast and consumes low volumes of organic  
 541 solvents due to which it is relatively a green approach. Additionally, due to the use of organic  
 542 solvents, it compatible with direct injection to GC. However, in case of polar or non-volatile  
 543 compounds, DLLME can be combined with derivatization to convert the analytes in less polar  
 544 and volatile derivatives suitable for GC analysis. DLLME has been widely coupled with  
 545 derivatization. There are opportunities to make this coupling greener through:

- 546 i) reduction of the sample size,
- 547 ii) using or developing less toxic derivatizing agents,
- 548 iii) reduction in the volumes of extraction and dispersive solvents,
- 549 iv) in many cases, the solvent used for derivatizing agent, also acts as dispersive solvent  
 550 for DLLME and it attributes a green aspect in terms of solvent consumption,
- 551 v) dispersive solvent may be avoided by performing dispersion using air or some other  
 552 sources,
- 553 vi) green dispersive solids can be used instead of dispersive solvents,
- 554 vii) automation and online coupling of DLLME with analytical instrument.

555  
556 *4.3.2.1. In-situ derivatization-DLLME*

557 It is most widely used mode of DLLME coupled derivatizations. In situ derivatization-DLLME  
558 performs simultaneous derivatization and extraction. It reduces the number of extraction steps,  
559 sample size, and consumption of the solvents. In this way, in situ approach is greener than  
560 performing derivatization and DLLME separately.

561 Melamine is used as food adulterant to enhance the apparent protein content in the milk. Dabsyl  
562 chloride was used for chemical derivatization of melamine in the milk and powdered infant  
563 formula prior to its extraction by DLLME and subsequent determination by HPLC. In this work,  
564 100  $\mu\text{L}$  of 4  $\text{mg mL}^{-1}$  of dabsyl chloride was used. Dabsylation is fast, and resulting derivatives  
565 are very stable. They absorb in the range of 436–460 nm and the interferences from UV-  
566 absorbing biological species in the food matrix can be prevented [42].

567 Simultaneous monitoring of several neurotransmitters has special importance in Parkinson's  
568 disease pathology, pharmacology and drug screening. A simple method that combines in situ  
569 derivatization and UADLLME (in situ DUADLLME) was used for determination of  
570 catecholamines and their biosynthesis precursors and metabolites in rat brain microdialysates.  
571 Other than simplicity and speediness, this method utilized relatively green extraction solvent  
572 (bromobenzene, 50  $\mu\text{L}$ ) and required very small volume of sample (30  $\mu\text{L}$ ) [43].

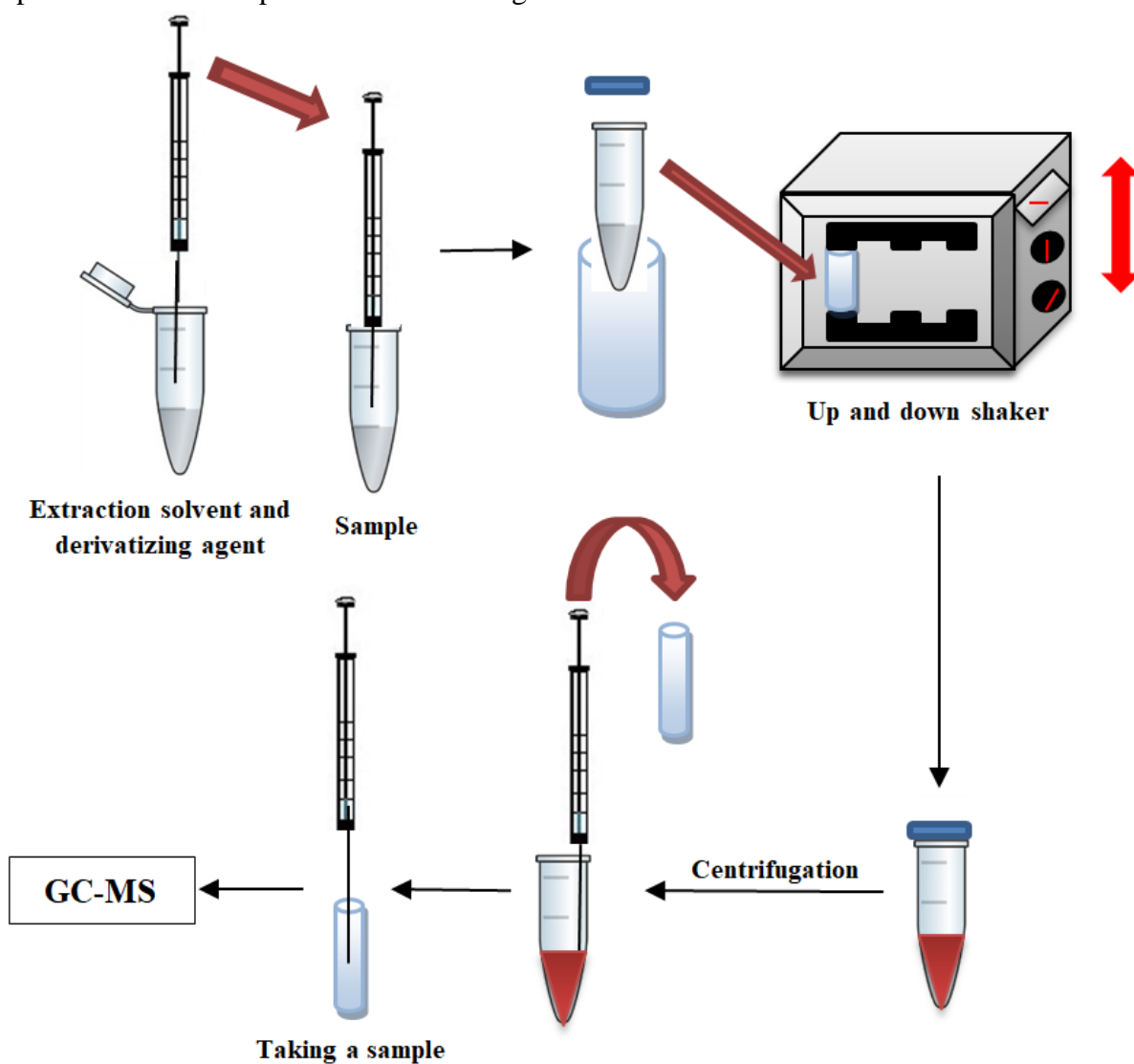
573 4'-carbonyl chloride rosamine (CCR) was used as derivatizing agent for in situ ultrasound-  
574 assisted derivatization DLLME of amino acid and monoamine neurotransmitters and their  
575 metabolites in rat urine of Alzheimer's disease. CCR works at mild derivatization conditions,  
576 easy to handle and provides better sensitivity [44]. Being mass-spectrometry sensitive, CCR  
577 was also used a derivatizing agent for simultaneous determination of biogenic amines and  
578 amino acids in food samples using in situ DUADLLME coupled with UHPLC-MS/MS [45].  
579 DLLME was also used for simultaneous extraction and derivatization of 13 biogenic amines in  
580 homemade wine samples. This method required very little quantities of extraction solvents and  
581 derivatization reagents. Secondly, it was rapid and did not require aid of external energy for  
582 derivatization [2].

583 Numerous modified DLLME methods have been described in the literature for in-situ  
584 derivatization and extraction where dispersive solvent was replaced by some other procedures  
585 such as assistance of air, vortex, up and down shaking, temperature, etc. These methods  
586 introduce greenness in the process by avoiding the use of dispersive solvent.

587 A modified version of DLLME known as fast syringe-assisted liquid-liquid microextraction  
588 was used for simultaneous extraction and derivatization of parabens in aqueous and cosmetic  
589 samples using GC-FID. In this method, derivatizing agent, catalyst, and disperser solvent were  
590 rapidly injected to salt-added and pH adjusted sample solution. Then, extraction solvent was  
591 added to the solution which was withdrawn to a glass syringe for several times and evacuated  
592 in a conical tube. This resulted in a turbid solution and analytes extracted into extraction solvent.  
593 The solution was again withdrawn into syringe, and needle was replaced with a filter through  
594 which organic and aqueous phase were separated. This whole process was completed in 1.5  
595 min and derivatization was not supported by any external energy source and was accomplished  
596 in aqueous media. Moreover, very minute volumes of solvents were used [46].

597 In another modified version of DLLME known as air-assisted LLME, where dispersive solvent  
598 was replaced by performing the dispersion step with a syringe through aspirating and dispensing  
599 the solution (sample, extraction solvent, and derivatizing reagent) for several times in a glass  
600 test tube. After completion, extraction solvent was separated through centrifugation. This

601 method was employed for determination of non-steroidal anti-inflammatory drugs in biological  
602 fluids using GC-FID [47].  
603 Similarly, in another method, emulsification was attained using up-and-down shaker-assisted  
604 dispersive liquid-liquid microextraction (UDSA-DLLME). This method was used for  
605 simultaneous extraction and derivatization of chlorophenols in water samples. The whole  
606 process was completed within a minute under normal temperature conditions. The relatively  
607 less toxic solvent 1-heptanol (12  $\mu\text{L}$ ) was used as extraction solvent [48]. The schematic  
608 representation of this procesis shown in Figure 5.



609  
610  
611 **Figure 5.** Diagrammatic sketch of the up-and-down shaker-assisted dispersive liquid-liquid  
612 microextraction. The conical glass tubes were secured by in-house designed plastic holders and  
613 then equipped to the up-and-down shaker [48].

614 Temperature-assisted DLLME was used for simultaneous extraction and derivatization of three  
615 anti-depressants in the urine which was further determined by GC-FID. In this method, a  
616 mixture of extraction solvent, dispersive solvent, and derivatization reagent was rapidly injected  
617 into a heated sample which was then cooled to room temperature to obtain a cloudy solution.  
618 The analytes were simultaneously derivatized and extracted into extraction solvent. The

619 temperature of the sample was initially kept high as it accelerates solubility of extraction  
620 solvent, rate of derivatization, and mass transfer of the analytes. The decrease in temperature  
621 will reduce the solubility of extraction solvent in aqueous media leading to enhanced turbidity  
622 due to formation of larger droplets that can be centrifuged [49].

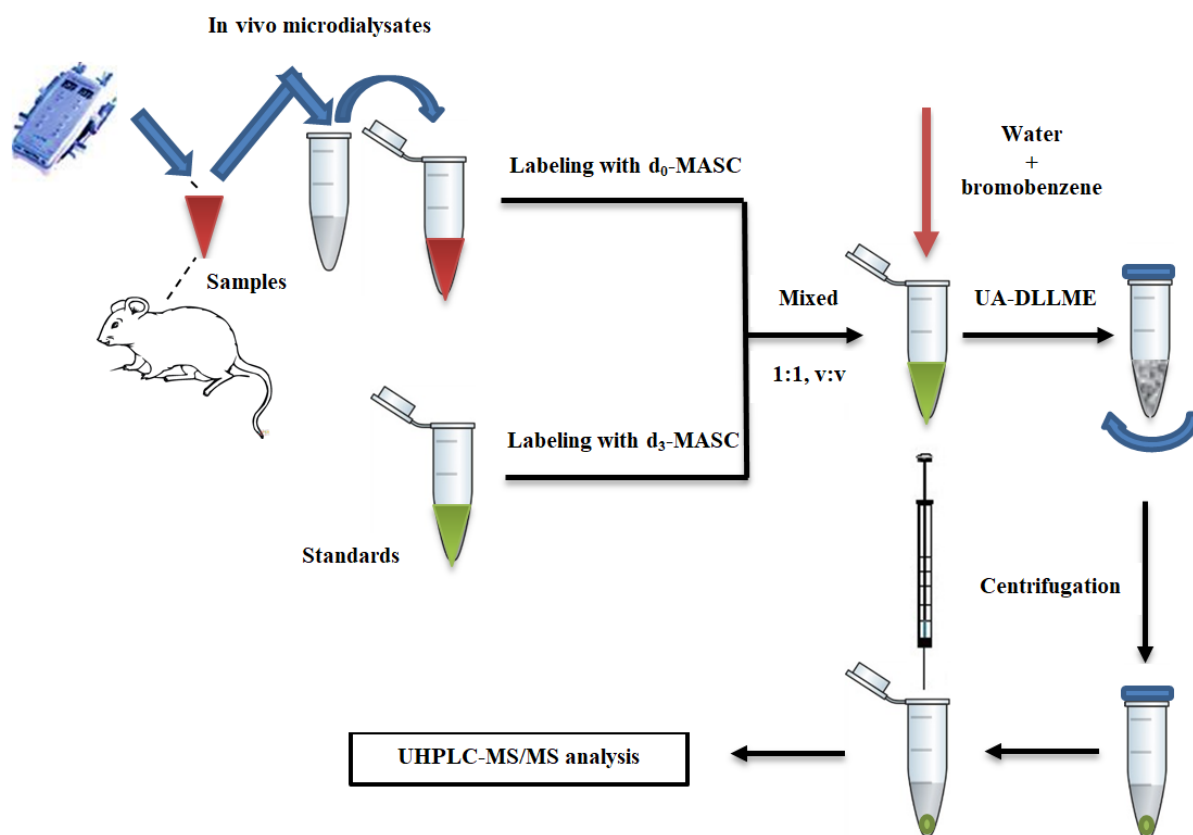
623 Another green approach that can be realized from the existing literature suggests the use of  
624 solid disperser instead of dispersive solvent. In this regard, sugar cube loaded with extraction  
625 solvent and derivatizing reagent was dissolved in sample solution containing the analytes and  
626 a catalyst. Dissolution of the sugar cube slowly release extraction solvent and derivatizing  
627 reagent into sample solution changing it cloudy and simultaneous extraction and derivatization  
628 takes place. After centrifugation, sedimented phase is separated and injected to the analytical  
629 instrument. This method was used for determination of some pharmaceutical drugs in urine  
630 and plasma samples by GC-FID [50].

631 The automation and coupling of in-situ derivatization-DLLME with analytical instrumentation  
632 can introduce an additional advantage of eliminating personnel effort and thus reducing the  
633 chances of error. Moreover, better control on overall process can be achieved with lesser  
634 exposure of chemicals to the workers. A fully automated in syringe magnetic stirring assisted  
635 DLLME (with extraction and derivatization simultaneously) was coupled online with GC-MS  
636 for determination of UV filters in the environmental water samples [51]. DLLME can be  
637 automated in a lab-in-syringe system. As in-syringe-DLLME can precisely handle small  
638 volumes, its reproducibility and precision will be better than the manual mode. In addition, it  
639 can be supported with automated magnetic stirring system for proper mixing and dispersion.  
640 The automation will simplify the whole process as well as the intervention of the analyst.

#### 641 *4.3.2.2.Pre-DLLME derivatization*

642 In some cases, derivatization is performed prior to DLLME. For example, isotope-labelling  
643 derivatization was combined with ultrasound-assisted dispersive liquid–liquid microextraction  
644 (UA-DLLME) to deal with low concentrations of NTs in brain microdialysates and matrix  
645 effect [52]. Stepwise procedure is shown in Figure 6.

646 Similarly, 2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate (BCEC-Cl) labelled  
647 steroidal and phenolic EDCs were extracted by DLLME. Here derivatization was used to  
648 enhance the detection sensitivity toward fluorescence detector, selectivity and hydrophobicity  
649 of the analytes. However, due to complex nature of the real food samples and ultra-trace levels  
650 of EDCs, derivatization was followed by DLLME [53].



651

652 **Figure 6.** Isotope labelling derivatization followed by ultrasound-assisted dispersive liquid-  
 653 liquid microextraction for the determination of neurotransmitters in rat brain microdialysates  
 654 by UHPLC-MS/MS [52].

655 *4.3.2.3. Post-DLLME derivatization or in between two microextractions*

656 Addition of derivatizing reagents may further complicate the matrix for extraction, particularly  
 657 when they are reactive toward matrix components other than compounds of interest. In such  
 658 cases, extraction can be performed first.

659 An automated in syringe magnetic stirring assisted DLLME used for simultaneous extraction  
 660 and preconcentration of estrogens in wastewater. DLLME was performed inside a syringe using  
 661 an automated system. Mixing and dispersion was achieved through magnetic stirring. After  
 662 DLLME, a certain amount of extract was taken in an amber vial where derivatizing reagents,  
 663 internal standards, and other chemicals were added manually [54]. Recently, a dual ultrasound-  
 664 assisted DLLME (dual-UADLLME) procedure, coupled with microwave-assisted  
 665 derivatization (MAD) between two DLLMEs, has been reported for extraction of phytosterols  
 666 in functional foods and medicinal herbs [55].

667 *4.3.2.4. DLLME-Injection port derivatization*

668 The recent example of this mode is determination of lipophilic compounds in fruit juices.  
 669 DLLME was performed offline and then extract from DLLME and derivatizing reagent were  
 670 injected into GC-injection port. For derivatization, the temperature of injection port, purge off  
 671 time, sample: derivatization reagent ratio (v/v) were optimized. The green aspect of this



672 derivatization is demonstrated by the requirement of low volumes of extract and derivatizing  
673 reagent which was 1  $\mu\text{L}$  each in this case [56].

#### 674 *4.3.2.5. DLLME-Post-column derivatization*

675 In this approach, a post-column reactor is used after the chromatographic column to convert the  
676 analytes into some detectable derivatives. DLLME extracted aflatoxins in yogurt were  
677 determined by post-column derivatization HPLC Photo-Induced Fluorescence Detection [57].  
678 Although DLLME is not directly coupled with derivatization in such instances, but it affects  
679 overall green nature of the process. It makes complex matrix suitable for injection to analytical  
680 instrument. The analytes after extraction are confined to a small volume of the solvent, which  
681 will ultimately require reduced amounts of derivatizing reagents. Table 2 lists DLLME based  
682 derivatization methods along with their analytical figures of merit.

#### 683 *4.3.3. Single drop microextraction*

684 As apparent from the name, single drop microextraction (SDME) utilizes very little volume of  
685 the solvents compared to traditional liquid-liquid extraction. In this procedure, a syringe is  
686 employed to append a microliter drop of an extracting solvent in either direct immersion or  
687 headspace mode. After the extraction, microliter drop is directly injected into the analytical  
688 instrument. The technique represents “a highly green process” which utilizes about a single  
689 drop of the solvent with the advantages of completing extraction as well as pre-concentration  
690 in a single step. Moreover, it has other advantages such as low-cost, simple to operate, selective,  
691 and very sensitive. Some disadvantages of SPME concerning to carry over and fiber  
692 degradation can be avoided. It is suitable for the cleanup of the samples that represent matrix  
693 complexity. The most prominent advantage is its provision of automation with analytical  
694 instruments [1].

695 SDME is combined with derivatization in different ways

- 696 i) SDME followed by in-syringe derivatization
- 697 ii) Derivatization of the analytes in the sample solution followed by SDME.
- 698 iii) Simultaneous derivatization and extraction of the analytes within the suspended  
699 drop that is a mixture of extraction solvent and derivatizing reagent.

700 SDME was employed for the extraction of phenols from water samples using SDME. The  
701 analytes were extracted within a drop (2.5  $\mu\text{L}$  hexylacetate) that was suspended from the syringe  
702 tip 1 cm below the surface of the sample solution (3.0 mL). After the extraction, the drop was  
703 retracted back into the syringe. Then 0.5  $\mu\text{L}$  of derivatizing reagent (BSA) was withdrawn into  
704 the syringe and mixed with extract by success movement of the plunger through the syringe  
705 barrel. Then, the microsyringe was sealed by putting a GC septum over the syringe needle tip  
706 and heated at 50°C for 5 min in an oven. Since, in-syringe derivatization utilizes very small  
707 volume of the derivatizing reagent, the issues related to excess derivatizing reagent, interfering  
708 by products, and additional cleanups are not encountered. Secondly, the possibility of analyte  
709 loss due to transferring of extract is also eliminated because derivatization reaction takes place  
710 within the syringe [59].

711 Prior to HS-SDME, short chain fatty acids were derivatized within the sample solution. This  
712 work utilized very small volumes of derivatizing reagent (60  $\mu\text{L}$ ) and extraction solvent (1 $\mu\text{L}$ ).

713 In addition to the green nature of overall process that arises from less use of chemicals, this  
714 method provided LODs much lower than reported by previous methods [60].  
715 Recently, fully automated SDME that performs extraction and derivatization in a single step  
716 was used for extraction of hydroxylated PAHs from seawater. A mixture of the extracting  
717 solvent and derivatizing agent with total volume of 1.0  $\mu\text{L}$  was suspended as single droplet at  
718 the tip of the syringe that was connected to an autosampler [61]. Greenness comes both from  
719 automation and reduced use of chemicals. Automation saves human efforts while reduced use  
720 of chemicals make overall process green. Table 3 provides list of some solvent based  
721 microextraction combined with derivatization.

## 722 5. Energy saving in derivatization processes

723 Without a doubt, the amount of energy consumed in chemical reactions is always necessary  
724 from the standpoint of Green Chemistry due to the fact that energy generation as well as  
725 consumption are considered crucial for the environment. Taking into account the fourth and  
726 ninth principles of Green Analytical Chemistry, operations that saves energy should be  
727 performed to reduce the energy consumption. Thus, it is recommended to conduct synthesis at  
728 ambient temperature and pressure. The application of high temperatures in the sample-  
729 preparation step is key in the energy consumption of any laboratory [10]. Such proceedings  
730 pushes up the analysis costs and the environmental impact. Moreover, the sample preparation  
731 and measurements steps should be carried with saving energy, thus, microextraction techniques  
732 or microextraction techniques coupled with derivatization are recommended because it not only  
733 allow to minimize the energy consumption but also shortens of the whole analysis time what  
734 also impact on energy saves.

735 Despite the fact that the aim is to conduct the derivatization process at room temperature, most  
736 of these processes need energy inputs (e.g. heating the reaction mixture for long time).  
737 Therefore, it is recommended to use the alternative energy sources in order to minimize energy  
738 consumption in derivatization processes. Several forms of energy could be proposed, such as  
739 microwaves, vortex, ultrasound, photochemical or electrochemical. These are considered not  
740 only more sustainable but also more effective than conventional heating sources in the  
741 laboratory [10, 64].

742 Application of microwaves are an alternative form of energy reduces significantly the reaction  
743 time, even though that depends strongly on the substances involved [10]. For example, in the  
744 case of application of such energy type in derivatization process occurred in reaction mixture  
745 of polar nature, the heating time is reduced from several hours to less than 5 min [65]. In  
746 addition, strict control over the temperature and the time of irradiation allows focus on a small  
747 volume of sample, resulting in increased precision [10].

748 Moreover, microwaves heating can be applied in digestion as well as extraction what also  
749 enhanced these processes. It is common to perform the microwave derivatization coupled to  
750 extraction what allow to carry out these processes simultaneously. Such proceeding was applied  
751 in a procedure for the determination of chlorophenolic compounds in ash samples obtained  
752 from the incineration of waste materials [66]. Analytes were simultaneously derivatized with  
753 acetic anhydride in presence of triethylamine and extracted from the sample in a mixture of *n*-

754 hexane acetone using a microwave system equipped with closed extraction vessels. The  
755 recoveries as well as quantification limits of the proposed procedure were very satisfied.  
756 Nowadays, also two enhanced factors are applied in the derivatization and extraction processes.  
757 For example, multi polar groups containing biothiols were derivatized using microwave energy  
758 [63]. Due to difference of polarity, all the groups are difficult to derivatize in aqueous medium.  
759 Thus, researchers used a tandem derivatization approach which includes first derivatization in  
760 aqueous medium and then salt-assisted extraction of intermediate derivatives and excess  
761 derivatizing reagent into small volume of organic phase where remaining groups were  
762 derivatized through the aid of microwave energy. This approach is relatively greener as it allows  
763 the recovery and reuse of excess derivatizing reagents. The extraction solvent also works as  
764 aprotic derivatization medium in the second step [63]. The other example is extraction of  
765 biogenic amines in fruit juices and alcoholic beverages after derivatization with 1-  
766 naphthylisothiocyanate. This reagent overcomes disadvantages associated with other  
767 derivatizing reagents such as temperature dependent stability [67].  
768 Nowadays, simultaneous microwave derivatization is often performed with microextraction  
769 techniques such as SPME, DLLME [68, 69]. In addition, microwave-assisted derivatization can  
770 be performed on-line what brings additional advantages (provides high sensitivity, reduces the  
771 amounts of reagents and the analysis time). Such derivatization mode can be carried out for  
772 certain reactions (for instance an on-line microwave system [70] or by application of flow  
773 injection system [71]). However, a large number of derivatization reactions do not easily adapt  
774 to flow injection systems due to the requirement for long heating times. Unfortunately, the  
775 application of microwaves for derivatization purposes is still rare in analytical laboratories.  
776 Therefore, researches are still performed in order to know better such processes.  
777 Another factor enhanced the derivatization and extraction process of analytes reducing  
778 consumption of energy are vortex and stirring. The main advantage of mixing is the possibility  
779 to obtain high analyte enrichment without the need to use factors such as pressure, temperature,  
780 or ultrasound radiation, which may cause a degradation of analytes. In addition, a great  
781 advantage of this solution is also a much lower cost of analysis compared to solutions where  
782 ultrasonic bath or ultrasonic probes are used. This has led to the introduction to the laboratory  
783 practice of many new methodological solutions, such as vortex-assisted liquid-liquid  
784 microextraction (VALLME), vortex-assisted surfactant-enhanced emulsification  
785 microextraction (VASEME), stirring solidified floating microextraction (SC- SF-SLDME).  
786 Malondialdehyde (MDA) in human plasma was derivatized to highly fluorescent compound  
787 MDA-TBA using thiobarbituric acid (TBA) prior to its extraction VALLME. This method  
788 employed only 90  $\mu\text{L}$  of n-heptanol as an extraction solvent and extraction time was about 1  
789 min. The whole process of derivatization, extraction, and analysis was completed in 10 min.  
790 [62].  
791 Ultrasonic assistance has become a popular enhancing factor in many chemistry fields including  
792 derivatization process because of the presence of cavitation phenomena. Generally, ultrasounds  
793 (US) causes important acceleration of reactions using softer conditions. Moreover, cavitation  
794 in a solution involves high temperatures and pressures that promote the formation of reactive  
795 radicals [10]. Mainly conventional baths are applied, however more powerful systems including  
796 probe or cup horn can also be used to increase cavitation and, in turn, sono-chemical effects on

797 reactions [10]. Application of US in derivatization process impact on a significant reduction in  
798 reaction time.

799 As was previously mentioned, simultaneous US-assisted extraction and derivatization has also  
800 been reported and both solid-liquid and liquid-liquid extraction approaches have been  
801 exploited. For example, ultrasonic assisted extraction-derivatization-DLLME was used for  
802 extraction of acrylamide from potato chips. UAE was used for extraction of acrylamide into  
803 water. This extraction was followed by derivatization using xanthydrol. The green aspect of  
804 this derivatization include use of very small volume of derivatizing agent and performing  
805 derivatization under ambient conditions within 40 min. Derivatization was followed by DLLME  
806 [58]. Such solution can bring many advantages including: shortening of  
807 extraction/derivatization time, reduction of reagent consumption, and reduction of  
808 temperature required during process. In addition, solvents used in the microextraction can be  
809 replaced by ILs enhancing the green character of the procedure. Continuous systems are  
810 appreciated in the application of US-assisted derivatization because this approach allows  
811 automation and coupling with other steps of the analytical. From the other side, ultrasounds  
812 can cause degradation and compositional changes of the analyte.

813

## 814 **6. Conclusions and future trends**

815 Although, the main objective of derivatization is to enhance the sensitivity, selectivity, and  
816 detectability of the analysis but greener derivatizations are getting substantial consideration in  
817 Analytical Chemistry [72]. The reason behind that is the corrosive, persistent and toxic nature  
818 of most commonly used derivatizing reagents. Moreover, the conventional derivatizations  
819 utilize extremely large volumes of derivatizing reagents, solvents, and are time-consuming.  
820 Nevertheless, the selected references indicate that many objections to the incorporation of these  
821 reactions are being circumvented. In a broader sense, there are two strategies which are of prime  
822 importance in accomplishing the goals of greener derivatizations: search and use of  
823 environment-friendly derivatizing reagents, solvents, reaction conditions, and energy sources;  
824 and miniaturization and automation of the analytical procedure [73]. Development of automated  
825 and/or miniaturized techniques demonstrated that the concerns regarding extra steps and time  
826 requirements are not necessarily at issue. Moreover, exploitation of these techniques allows to  
827 reduce the amounts of derivatizing reagents as well as generate less amount of waste. It also  
828 need to be noted that and novel separation techniques have reduced the potential of interferences  
829 arising from excess reagents. It is evident that appropriate application of analytical  
830 derivatizations brings benefits in getting higher sensitivity and more informative mass spectral  
831 data. However, some drawbacks and limitations of analytical procedures connected with  
832 derivatization still exist. And due to the fact that green analytical chemistry is an important idea  
833 nowadays, it challenges analytical chemists to devise techniques and instrumentation –  
834 particularly those that are highly automated – that take advantage of the increases in sensitivity  
835 and specificity but also reduce or eliminate the disadvantages of derivatizations.  
836 Nowadays, to help analytical chemists to follow principles of GAC, it is common to develop a  
837 solvent selection guides, enabling the selection of greener alternatives to harmful solvents  
838 typically used in scientific and technological processes. In 2017, a guide which provides an

839 assessment, in terms of greenness, of almost three hundred of LC, GC and chiral derivatisation  
840 reagents typically used in analytical chemistry and related fields was published [74]. The  
841 preference rankings were performed for each group of derivatisation agents by means of  
842 multicriteria decision analysis (MCDA) which consists of a set of tools for solving complex  
843 decision problems. In the future, such tools as MCDA will be applied before the starting of  
844 analytical procedure development. Such proceeding will allow to choose the best option for an  
845 analytical problem, for example, the best analytical methodologies reagents, chemicals,  
846 derivatizing agents, etc. used for specific purposes.

847

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