A Review on Microextraction Techniques for Selected Triazole Fungicides Determination in Water and Food Samples

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Abstract

Triazole fungicides are systemic fungicides applied to protect crops from fungus infections. Its use must be controlled due to the potential harmful effects in environment and human health. Before determination with a chromatographic system, analyte must be isolated and preconcentrated with an extraction step. This review presents the most used microextraction techniques for those compounds in water and several food matrices, mainly solid phase microextraction (SPME) and liquid phase microextraction (LPME) procedures. The most relevant analytical parameters of some studies from the recent literature are collected and also some advantages and disadvantages comparing SPME and LPME procedures are presented.

Keywords

Triazole fungicides, Microextraction, SPME, LPME, Water, Food samples

Abbreviations

Introduction

The big demand of food leads to use different kind of pesticides in order to protect the crops and increasing their production to satisfy the needs of the society. Some studies have shown that the use of pesticides can effectively reduce the plant diseases [1] as well as the delay the oxidative process suffered by the plants delaying senescence and increasing the crop yield [2]. The treated plants are taken by the consumers in different forms, such as the vegetables or processed products as wines or beverages. The Triazole compounds are one of the most common groups of pesticides to fight against fungus diseases widely applied to crops. The mode of action of theseazole fungicides is the inhibition of ergosterol, an essential component in fungal cell membranes, by influencing the cytochrome P450 enzyme activity [3]. Many studies have been carried out concerning these pesticides effects in animals. Tumorigenic effects [4], endocrine disrupting effects [5] and disturbances in reproductive systems have been shown in rats [6]. Those fungicides can also induce some tumors in mice livers [7]. Besides, the carryover of the analyte can also hazard the environment, such as increasing mortality rates of Daphnia's (tiny crustaceans), affecting also the plant growth and causing anticholigernic and oxidative stress in fishes [8]. Moreover, it has been shown that triazole fungicides can affect the beer making processes producing sluggish and even stuck of fermentation [9].

The following triazoles were selected according to literature: cyproconazole, difenoconazole, diniconazole, epoxiconazole, fluquinconazole, flusilazole, hexaconazole, metconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole and triticonazole. The molecular formula, CAS number and structure as well as the log value of K_{ow} of the triazoles are shown in the Table 1. Taking into account the pesticide manufacturers, these compounds can remain in the fruit and therefore the established pre-harvest security period can be up to a month for some cases. Furthermore, the superficial run-off is not able to take out easily the pesticides and some of them, can stay strongly adsorbed to the soils [10].

Table 1: CAS number, molecular formulas, structures and log value of K_{ow} (partition coefficient between n-octanol and water) of the triazole compounds considered in this review.

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS RN</th>
<th>Formula</th>
<th>Structure</th>
<th>log K_{ow}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyproconazole</td>
<td>[94361-06-5]</td>
<td>C_{15}H_{18}ClN_{3}O</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>2.91 (pH 7)</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>[119446-68-3]</td>
<td>C_{18}H_{17}Cl_{2}N_{3}O_{3}</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>4.20 (25 °C)</td>
</tr>
<tr>
<td>Diniconazole</td>
<td>[70217-36-6]</td>
<td>C_{15}H_{17}Cl_{2}N_{3}O</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>4.3 (25 °C)</td>
</tr>
<tr>
<td>Epoxiconazole</td>
<td>[106325-08-0]</td>
<td>C_{17}H_{13}ClFN_{3}O</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>3.44 (pH 7)</td>
</tr>
<tr>
<td>Fluquinconazole</td>
<td>[136426-54-5]</td>
<td>C_{16}H_{18}Cl_{2}FN_{5}O</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>3.24 (pH 5.6)</td>
</tr>
<tr>
<td>Flusilazole</td>
<td>[85509-19-9]</td>
<td>C_{16}H_{15}F_{2}N_{3}Si</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>3.74 (pH 7, 25 °C)</td>
</tr>
<tr>
<td>Hexaconazole</td>
<td>[79983-71-4]</td>
<td>C_{14}H_{17}Cl_{2}N_{3}O</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>3.9 (20 °C)</td>
</tr>
<tr>
<td>Metconazole</td>
<td>[125116-23-6]</td>
<td>C_{17}H_{22}ClN_{3}O</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>3.85 (25 °C)</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>[88671-89-0]</td>
<td>C_{15}H_{17}ClN_{4}</td>
<td><img src="image9.png" alt="Structure" /></td>
<td>2.94 (pH 7-8, 25 °C)</td>
</tr>
</tbody>
</table>

Because of the potential health risks to consumers, European Union (EU) has published several regulations to establish maximum residue limits (MRLs) for a wide range of pesticides in different food types. First regulation about MRLs was published in 2005 [11], and from that year new regulations have been done and the MRLs have been updated. These limits for triazoles are ranging from 0.01 to 3.00 mg kg\(^{-1}\) depending on the fruit type and the analyte [12].

Thus, it is necessary to use reliable methods to determine the pesticide residues in different matrices. Traditionally, those analysis have been done using liquid-liquid extraction (LLE) or solid-phase extraction (SPE) [13-15]. LLE is based on the equilibrium distribution/partition coefficient between two immiscible liquids. On the other hand, SPE is based on the selective distribution of analytes between the solid packing material and liquid mobile phase [15, 16]. The main drawbacks of these two techniques are the important amounts of solvents and the laborious procedures involved. The large amounts...
of solvents are against of the green chemistry principle of reducing waste. Moreover, many solvents using in LLE and SPE are difficult to manage after disposal because its toxicity. Regarding procedures, these techniques are performed in many steps that make difficult the automatization and increase error sources due to the loss of analytes.

In 90’s began the solid phase microextraction (SPME). This technique avoids the use or uses a limited amount of organic solvents and can be easily automated. Few years later, liquid phase microextraction (LPME) techniques came up to the laboratories giving more versatility to the extraction procedures.

The objective of this work is to provide a summary of the recent developed methods based on several microextraction techniques for the determination of triazole fungicides in water and food samples. Different extractant materials and techniques have been used in those matrices and the obtained precision, accuracy, limits of detection and enrichment factors show wide ranges. Some advantages and drawbacks of SPME and LPME are indicated, as well as the future perspectives.

**Analytical microextraction procedures for triazole determination**

In the past years considerable efforts have been made in order to develop new sample preparation techniques that save time and require tiny amounts of hazardous organic solvents. Those microextraction techniques, mainly SPME and LPME, have in common the microliters scale volume of the extractant phase [17].

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Formula</th>
<th>EF</th>
<th>Drug Name</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penconazole</td>
<td>C_{13}H_{11}Cl_{2}N_{3}</td>
<td>3.72</td>
<td>(pH 5.7, 25 °C)</td>
<td></td>
</tr>
<tr>
<td>Propiconazole</td>
<td>C_{16}H_{17}Cl_{2}N_{3}O_{2}</td>
<td>3.72</td>
<td>(pH 6.6, 25 °C)</td>
<td></td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>C_{16}H_{17}Cl_{2}N_{3}O_{2}</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetcenazole</td>
<td>C_{13}H_{15}Cl_{2}N_{3}</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triticonazole</td>
<td>C_{14}H_{14}Cl_{2}N_{3}O</td>
<td>3.29</td>
<td>(20 °C)</td>
<td></td>
</tr>
</tbody>
</table>

Solid phase microextraction (SPME) is based on the partition of analytes between the sample phase and a solid extracting phase. SPME consist on a coated polymeric fiber which is exposed to a sample in order to extract the analytes [18]. The fibers can be exposed in three different operation modes: immersion (DI), headspace (HS) or membrane-protected approach [19]. Different variables affect the SPME extraction process, among them: type of coating, extraction time, extraction temperature, salt addition, pH, agitation, sample volume and headspace volume [20-25]. There are some commercially available fibers with different coating and thickness. Polyacrylate (PA), Polydimethylsiloxane (PDMS), and Divinylbenzene (DVB) sorbent materials and its combinations are the most used materials.

After the extraction, GC and HPLC equipment’s are mainly used for desorption [26, 27]. In GC, desorption occurs in the injector equipped with a suitable liner where the analytes are thermally desorbed. The maximum allowed temperatures of coatings have to be considered in order to avoid fiber damage. In HPLC, desorption is made in a desorption chamber linked to an injector and the desorption can be performed in two different modes: the static and the dynamic modes [23, 28].

Liquid phase microextraction (LPME) introduces the idea of miniaturizing the classical LLE in a water-immiscible organic droplet [29]. Basically, the droplet is placed directly in the sample or in its headspace and later the droplet is collected with a variety of techniques. Liquid phase microextraction is gaining acceptance in pesticide residue analysis because its different protocols can be adjusted to different aims [30]. LPME is also based on equilibrium between the donor and acceptor phase. The extracted amount in the organic droplet is proportional to the initial concentration. Although the extraction depends on the volume of sample and the organic drop, the partition coefficients remain constant during all the process.

Enrichment factor (EF) can be defined as the ratio of the equilibrium concentration of analyte in the organic phase (C<sub>eq</sub> ) to the original concentration (C<sub>0</sub>) of the analyte in the sample [31, 32]. A high enrichment factor indicates good extraction efficiency that helps to improve method validation parameters, such as limit of detection.

\[
EF = \frac{C_{eq}}{C_0}
\]

As in the SPME technique there are several variables that need to be taken into account for a successful extraction using LPME techniques [33, 34]. The principal variable to decide is the extractant material [34, 35], it must have high affinity with the target analytes, be immiscible with water, be stable enough over the extraction time and it also need to have good chromatographic behavior. The used volume of the droplet also needs to be optimized, because EFs are favored with little volumes [36], but they might be difficult to handle if the droplet is not big enough. As in SPME case, extraction time, temperature and ionic strength also need to be considered, because different extraction efficiency is obtained changing those variables [34, 36]. The mass transfer can be favored with
agitation or ultrasonication, both methods help in the sample homogenization increasing the contact surface.

Several papers concerning triazole determination with microextraction techniques have been published. In these studies, water samples, fruit and juice samples, wine, milk, honey and oils have been analyzed. Some of the most used triazole analytes were selected to build the Table 2: cyproconazole (CP), difenconazole (DF), diniconazole (D), epoxiconazole (EPX), fluquinconazole (FQ), flusilazole (FS), hexaconazole (HX), metconazole (MTT), myclobutanil (M), penconazole (PN), propiconazole (PR), tebuconazole (TB), tetraconazole (TT) and triticonazole (TRT). The linear range, limits of detection, relative standard deviations (RSD, %), enrichment factors (EF) and recovery values obtained with different methods are included in Table 2 [37-65].

As it can be seen in the table, five studies considered SPME methods [37-41] where the extractant material was directly immersed in the liquid sample. Headspace mode has not been used due to the low volatility of the triazole compounds. Fibers in immersion mode are more subjected to damage, since solution salt content and other sample compounds can modify the structure of the extractant leading to the breaking of the coating when its not carefully managed [24, 28, 66, 67]. This could be the reason of high reproducibility values for some cases (expressed in Relative Standard Deviation %) as for example 22.1 % for TB when MPL is used [38].

Highest LOD was obtained for TB when PDMS/DVB is used [41]. Although extraction step is crucial for LOD determination, chromatographic detectors are also important. The reason could be that ECD has low sensitivity for TB. ECD is a selective detector and thus, not all the compounds can give a good signal.

Farajzadeh et al. proposed a combination of a stir bar sorptive extraction (SBSE) method with a dispersive liquid microextraction to extract analytes from the stir bar coated with octadecylsilane (ODS) [42]. The main advantage of this technique is that allows different coatings that can be design for specific purposes. Hence, it avoids some SPME problems such as fiber fragility or limitation with solvents, and allows better RSD results than SPME (2.9-11.6%) as with wider linear ranges (10-50000 µg L⁻¹).

There are several classifications for liquid microextraction techniques [35, 68-75] and many of those procedures are included in Table 2 [43-65]. Liquid microextraction techniques started with the Single drop microextraction (SDME). This method uses a little amount of the organic solvent (1-3 µL) and it is exposed to the sample (DI or HS) at the tip of a micro syringe. After the exposure time, the droplet is collected to further determination. A variation of the simplest method has been developed by Farajzadeh et al. [43], called dynamic single drop microextraction (DSDME). In this case, the droplet is directly placed in the liquid in a narrow bore tube. The tube is reversed upside down several times to extract the analytes and then the droplet is collected by a capillary tube. For all the analytes excellent reproducibility values (2.9-4.5%) and a wide linear range (10-100000 µg L⁻¹) has been obtained. Nevertheless high LODs were obtained for both difenconazole isomers (102 and 112 µg L⁻¹ respectively).

Low volatility compounds, such as triazoles, remain mainly in the aqueous phase and not in the headspace. Their extractions are favored with a wide contact between the aqueous phase and the extractant. In dispersive liquid-liquid microextraction (DLLME), the organic droplet is dispersed in the aqueous phase forming a cloudy solution. As a result, an increase of the contact surface area occurs making the extraction more efficient. This dispersion can be made with the help of other solvent (called the dispersive solvent) and with agitation. Afterwards a centrifugation is needed in order to recollect the organic phase into a one droplet again.

Nine studies for triazoles included in Table 2 chose DLLME as the extraction procedure [44-52]. Among different available extractants, the selected for these studies are: hexanol/ hexane mixtures, a chloroform/monochlorobenzene mixture, 1,2-dibromoethane, chloroform, trimethylchlorosilane, acetonitrile, 1- dodecanol and the ionic liquid [C₆MIM] [PF₆]. Ionic liquids, also known as molten salts, are gaining acceptance in different microextraction procedures. Extraction in those cases is almost immediate and the sample is lead to centrifugation to recollect the droplet. Depending on the used extractant and its density, some of the extractant were collected from the bottom of the used vial directly with a syringe or using the procedure of solidification of floating organic drop (SFO) [45, 48, 50-52].

Good analytical parameters are obtained in all the studies when DLLME is used. Obtained RSD values are between 0.9-7.25% except in one of the methods [44], where the maximum RSD value is 11%. Recovery values are good for almost all the cases, but in complex matrices, such as cow milk [50] low recovery values were obtained. The reason may rely on the difficulty to recover the whole liquid extractant in a droplet again. A loss of extractant and hence the analyte can occur in those cases, leading to worse recovery values. The dilution of the samples can partially avoid this problem; but then the analyte amount is also diluted, and as a consequence, analytes may not be detected.

High enrichment factors were obtained in one of the works [49], ranging from 482 to 2738. In this procedure after analytes were attached to a silylated vessel, the extraction was performed with a methanol and chloroform mixture. Thus, it can be considered as a double extraction procedure and that could be the reason of the high enrichment factors.

Another way to form the cloudy solutions is using ultrasonication [76]. In case of ultrasound-assisted emulsification microextraction (USAEME) no dispersive solvent is needed. The use of US in liquid-liquid microextraction helps in the mass transfer between phases, and also in the formation of the emulsion with tiny droplets of organic phase that increase the contact area. The dispersed phase can be easily collected in one droplet using centrifugation, this procedure can be performed in a few minutes accelerating the separation of the organic and aqueous phase [56, 77-84]. Table 2 shows some of the US extraction procedures for triazole fungicides [53-57]. Ultrasound can be efficient as any dispersive solvent and generally similar results to DLLME are obtained.
Table 2: Analytical characteristics of the triazole determination using microextraction methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Extractant material</th>
<th>Matrix</th>
<th>Analytes</th>
<th>Linear range (µg L⁻¹)</th>
<th>LOD (µg L⁻¹)</th>
<th>RSD (%)</th>
<th>EP</th>
<th>Recovery (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPME-GC/MS</td>
<td>Trimethylsilyl-modified Thai/SBA-15 nanocomposite</td>
<td>Water</td>
<td>PN-HX-D-TB-TRT-DF</td>
<td>0.1-2000</td>
<td>0.01-0.09²</td>
<td>4.3-14.1</td>
<td>-</td>
<td>-</td>
<td>[37]</td>
</tr>
<tr>
<td>SPME-GC/MS</td>
<td>MIP</td>
<td>Grape juice</td>
<td>TB-MT</td>
<td>100-2000</td>
<td>30¹</td>
<td>2.9-22.1</td>
<td>-</td>
<td>-</td>
<td>[38]</td>
</tr>
<tr>
<td>SPME-HPLC/DAD</td>
<td>Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)</td>
<td>Water, fruit samples</td>
<td>D-M-FQ-FS-TB-TT</td>
<td>5.5-50.4</td>
<td>1.5-5.9⁰</td>
<td>2.8-13.1</td>
<td>-</td>
<td>94.5-123.4</td>
<td>[39]</td>
</tr>
<tr>
<td>SPME-GC/TS/F/MS</td>
<td>PDMS/DVB</td>
<td>Grape Strawberries</td>
<td>D-CP-M-FS-HX-PN-PR-TB</td>
<td>0.25-1000</td>
<td>0.25-5¹</td>
<td>1.7-14.6</td>
<td>-</td>
<td>84-117</td>
<td>[40]</td>
</tr>
<tr>
<td>SPME-GC/ECD</td>
<td>PDMS/DVB</td>
<td>Grape and apple juices</td>
<td>D-M-TT-TB</td>
<td>0.4-2560</td>
<td>0.2-162²</td>
<td>3.3-18.0</td>
<td>-</td>
<td>93.6-112.1</td>
<td>[41]</td>
</tr>
<tr>
<td>SBSE-DLLME-GC/FID</td>
<td>Octadecylsilane (ODS) /1,1,2,2-tetrachloroethane</td>
<td>Water, juices</td>
<td>D-DF-HX-PN-TB-TRT</td>
<td>10-50000</td>
<td>0.5-24.0³</td>
<td>2.9-11.6</td>
<td>282-1792</td>
<td>71-116</td>
<td>[42]</td>
</tr>
<tr>
<td>DLLME-LC/MS/MS</td>
<td>Chloroform/monochlorobenzene</td>
<td>Mineral water</td>
<td>CP-TB-EPX</td>
<td>0.005-0.3</td>
<td>0.005-0.01³</td>
<td>1-11</td>
<td>-</td>
<td>92-120</td>
<td>[44]</td>
</tr>
<tr>
<td>DLLME-GC/NPD</td>
<td>1,2-dibromoethane (1,2-DBE)</td>
<td>Honey</td>
<td>D-DF-HX-PN-TB</td>
<td>0.45-30</td>
<td>0.15-0.70⁰</td>
<td>3-6</td>
<td>1943-1994</td>
<td>97-100</td>
<td>[45]</td>
</tr>
<tr>
<td>DLLME-HPLC/DAD</td>
<td>Chloroform</td>
<td>Water</td>
<td>HX-PN-TB</td>
<td>30-1500</td>
<td>8.6-29.0³</td>
<td>0.9-2.3</td>
<td>-</td>
<td>88-104</td>
<td>[46]</td>
</tr>
<tr>
<td>DLLME-GC/FID/MS</td>
<td>n-hexanol:n-hexane (75:25, v/v)</td>
<td>Water</td>
<td>D-HX-PN-TB-TRT</td>
<td>2-5000</td>
<td>0.3-5.0³</td>
<td>2-12</td>
<td>263-380</td>
<td>74-99</td>
<td>[47]</td>
</tr>
<tr>
<td>DLLME-HPLC/UV</td>
<td>Chloroform</td>
<td>Water</td>
<td>TB</td>
<td>2.0-100</td>
<td>1.2¹</td>
<td>2.8-5.3</td>
<td>83</td>
<td>90.6-105.3</td>
<td>[48]</td>
</tr>
<tr>
<td>SEV-DLLME-GC/FID</td>
<td>Trimethylchlorosilane (TMCS)</td>
<td>Water, juices</td>
<td>D-DF-HX-PN-TB-TRT</td>
<td>0.5-20000</td>
<td>0.09-1.04⁴</td>
<td>2.51-7.25</td>
<td>482-2738</td>
<td>86-103</td>
<td>[49]</td>
</tr>
<tr>
<td>DLLME-GC/FID/MS</td>
<td>Acetonitrile</td>
<td>Cow milk</td>
<td>DF-HX-PN-TB-TRT-EPX</td>
<td>20-80000</td>
<td>2-58¹</td>
<td>3.6-6.0</td>
<td>156-435</td>
<td>45-96</td>
<td>[50]</td>
</tr>
<tr>
<td>DLLME-SFO-HPLC/DAD</td>
<td>1-dodecanol</td>
<td>Water</td>
<td>M-HX-TB</td>
<td>0.5-200</td>
<td>0.06-0.1¹</td>
<td>4.3-5.7</td>
<td>190-450</td>
<td>86-110</td>
<td>[51]</td>
</tr>
<tr>
<td>IL-DLLME-HPLC/DAD</td>
<td>1-hexyl-3-methylimidazolium hexathiorophosphate ([C,MIM][PF₆])</td>
<td>Banana extract</td>
<td>TB</td>
<td>122-6830</td>
<td>3.9¹</td>
<td>2.5-5.0</td>
<td>-</td>
<td>93-98</td>
<td>[52]</td>
</tr>
<tr>
<td>UA-SHIL-DLLME-HPLC/UVD</td>
<td>Tributylchlopyrophosphonium/tributylchlopyrophosphonium ([P₄,5,6,7][PF₆][PF₆])</td>
<td>Water, juices</td>
<td>EPX-TB-HX</td>
<td>5-500</td>
<td>1.10-1.38¹</td>
<td>3.4-5.9</td>
<td>94-101</td>
<td>75.4-108.6</td>
<td>[53]</td>
</tr>
<tr>
<td>UASO-HLLME-GC/MS</td>
<td>Acetonitrile, salt</td>
<td>Water</td>
<td>EPX-DF-M-TB</td>
<td>10-200</td>
<td>0.8-14.4¹</td>
<td>4.0-6.1</td>
<td>120-190</td>
<td>97-115</td>
<td>[54]</td>
</tr>
<tr>
<td>USAEME-SFO-HPLC/DAD</td>
<td>Acetonitrile</td>
<td>Water, grape and apple samples</td>
<td>D-M-FQ-FS-TB-TT</td>
<td>20-890</td>
<td>10.9-17.2²</td>
<td>1.9-10.6</td>
<td>226-255</td>
<td>82-112</td>
<td>[55]</td>
</tr>
</tbody>
</table>
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Regarding reproducibilities, recoveries and most of the LODs. As in DLLME, in some works an ionic liquid as the extractant solvent has been used [53, 57].

Another way to produce a disperse solution is the air-assisted liquid-liquid microextraction (AALLME). In this case the dispersion is made by introducing air repeatedly sucking the sample-extractant mixture with a syringe until the solution becomes turbid. Very good RSD values were obtained in the works that used this technique (1-7%) as well as good recovery values (81-113%) [58-61].

Apart from the mentioned procedures, there are other studies for triazole fungicides determination that uses vortex-assisted matrix-solid liquid dispersive microextraction (VA-MSLDME) [62], hollow-fiber liquid phase microextraction (HF-LPME) [63], graphene-based magnetic nanoparticles extraction (GBMNE) [64] or cloud-point extraction (CPE) [65]. In the first one a vortex was used to form the dispered phase but also was included a primary secondary amine (PSA) as a cleanup adsorbent to eliminate sample interferences. Although good analytical parameters were obtained in general, poor enrichment factors were achieved (22-47) for the selected triazoles.

In HF-LPME, a porous hollow fiber is used containing the organic phase. Then, the hollow-fiber is introduced to the sample for the extraction and retracted after the exposure time. Similar analytical characteristics were obtained comparing with DLLME, with LODs between 0.3 to 0.8 µg L−1.

GBMNE is the method in Table 2 with the lowest LODs in the table (0.005-0.01 µg L−1). Bolzan et al. [44] also obtained same order LODs, but in that case the used detector is a tandem mass spectrometer, which is much more sensitive than the UV used in GBMNE procedure. The method is based in magnetic nanoparticles added to the sample where the analytes are extracted and finally desorbed with acetone.

The last method, CPE, consists on forming a cloudy solution with a polyethylene glycol surfactant. The function of the surfactant is to preconcentrate the analytes and then, the

<table>
<thead>
<tr>
<th>Method Abbreviation</th>
<th>Extractant</th>
<th>Sample Type</th>
<th>LODs</th>
<th>RSD%</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>USAEMME-GC/FID</td>
<td>1-octanol</td>
<td>Pear, apple and grape juices</td>
<td>5-500</td>
<td>1.77-2.26</td>
<td>3.5-7.7</td>
</tr>
<tr>
<td>USA-IL-DLLME-HPLC/DAD</td>
<td>1-hexyl-3-methylimidazolium hexafluorophosphate (HIMIM)[PF6]</td>
<td>Wine</td>
<td>D-EPX</td>
<td>50-2000</td>
<td>9.7-10.4</td>
</tr>
<tr>
<td>AALLME-GC/FID</td>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>Edible Oils</td>
<td>D-HX-PN-TB-TRT</td>
<td>10-50000</td>
<td>2.2-6.1</td>
</tr>
<tr>
<td>AALLME-GC/FID</td>
<td>1,2-DBE</td>
<td>D-DF-HX-PN-TB-TRT</td>
<td>5-10000</td>
<td>0.4-5</td>
<td>2-7</td>
</tr>
<tr>
<td>AALLME-GC/FID</td>
<td>Toluene</td>
<td>D-HX-PN-TB-TRT</td>
<td>2-750</td>
<td>0.53-1.13</td>
<td>2-7</td>
</tr>
<tr>
<td>AALLME-GC/FID</td>
<td>1,2-DBE</td>
<td>D-HX-PN-TB-TRT</td>
<td>1-50000</td>
<td>0.2-5.9</td>
<td>1-4</td>
</tr>
<tr>
<td>VA-MSLDME-GC/ECD</td>
<td>Toluene</td>
<td>Honeysuckle Cotton seed</td>
<td>M-D-EPX-TRT-DF</td>
<td>2-1000</td>
<td>0.1-20</td>
</tr>
<tr>
<td>HF-LPME-GC/MS</td>
<td>Toluene</td>
<td>Water, cucumber, tomato, grape juice</td>
<td>D-HX-PN-PN-TB-TRT</td>
<td>1-5000</td>
<td>0.3-0.8</td>
</tr>
<tr>
<td>GBMNE-HPLC/UV</td>
<td>Graphene-ferriferrous oxide (G–Fe3O4)</td>
<td>Water</td>
<td>HX-M-TB</td>
<td>0.05-50</td>
<td>0.005-0.01</td>
</tr>
<tr>
<td>CPE-HPLC/UV</td>
<td>Polyethylene glycol 600 monoooleate (PEG600MO)</td>
<td>Water</td>
<td>D-TB</td>
<td>0.05-20</td>
<td>0.02-0.03</td>
</tr>
</tbody>
</table>

*Method abbreviations are explained in list of abbreviation section.
*Analytes: CP: Cyproconazole; DF: Difenconazole; D: Diniconazole; EPX: Epoxiconazole; FS: Flusilazole; FQ: Fluquinconazole; HX: Hexaconazole; MT: Metconazole; M: Myclobutanil; PN: Penconazole; PR: Propiconazole; TB: Tebuconazole; TT: Tetraconazole; TRT: Triticonazole.
*LOD: Limit of detection: 1Based on S/N= 3; 2Based on calibration; 3LOQ: Limit of quantification.
*RSD: Relative standard deviation.
*EF: Enrichment factor.
analytes with the surfactant are separated from the aqueous phase with centrifugation. The analytes are injected to the HPLC system together with the surfactant. Low LODs were obtained (0.02-0.03 µg L⁻¹) and good reproducibility values (4.2-5.3%), but the studied linear range was quite limited (0.05-20 µg L⁻¹).

Advantages and disadvantages of SPME and LPME

Comparing LPME and SPME and its advantages and disadvantages, it can be said that LPME can offer more versatility than SPME. Different LPME approaches give the advantage of adjusting each situation depending on the matrix type, analytes and its properties. For the specific case of triazole fungicides, where the analytes are not volatile, increasing the surface contact area helps the mass transfer, facilitating the extraction and thus, reducing time. That is why the chosen method in most cases is a technique where the extractant is dispersed (DLLME, US-LPME or AALME). However, it is harder to automatize the whole system when LPME procedures are used because the procedure consist on various steps.

The main advantage of using a liquid extractant is that for every analysis a new droplet is used. This fact is reflected in reproducibility of the assays. When SPME is used, especially in DI mode, the extraction is affected by the extraction numbers performed with a single fiber and this leads to worse reproducibility values. In addition, the fiber can be easily broken if the analyst is not careful enough in the work. In those cases, the fiber needs to be replaced and this can introduce batch-to-batch differences in reproducibility. Cleaning steps must also be considered when SPME is used, especially when salt is added to help in the extraction process that can result in crystallization. Also, SPME procedure needs conditioning to the chromatographic system before the first use. Those facts are not necessary when LPME is used. Moreover, to couple SPME to chromatographic system an interface is needed, whereas with LPME usually the extractant is directly injected with a common syringe [33, 35, 71, 73, 85].

Another main drawback especially for SPME is the procedure cost. Each fiber allows around 50-60 extractions in DI mode and taking into account the elevated cost of one fiber, each analysis can be expensive. In LPME, there is a big variety of liquid extractants that present a wide range of prices. Since, the used liquid volume is minimum, a big number of extractions can be made, and therefore the cost per extraction is considerably reduced. Although it can be complicate to use with complex matrices, microextraction liquid techniques are faster, cheaper and simpler than other sorptive extraction techniques.

Conclusions and Perspectives

This review presents the recent developments in microextraction techniques for the triazole fungicide determination. Solid phase microextraction (SPME) and liquid phase microextraction (LPME) are the most used procedures in microextraction. Both microextraction techniques show a big potential to isolate and preconcentrate triazole compounds not only in water and liquid fruit samples, but also in other complex matrices. After the extraction, the sample can be injected in GC or HPLC instruments equipped with FID, GC, NPD, DAD or MS detectors.

One remarkable characteristic of these microextraction procedures is the tiny volume amount of the used solvents. The great reduction of solvent volumes, mainly the toxic ones, comparing with the volume used in classic extraction procedures make these techniques safer from the environmental point of view.

Although SPME gives good analytical results LPME shows in general better quality data. Moreover, in LPME a wider range of solvents is available allowing more versatility. The dispersive liquid-liquid microextraction allows to increase the contact area and therefore to reduce the extraction time.

The main SPME drawbacks are the fragility of the fiber and the reproducibility. Analysts have to be meticulous in the work trying to avoid the mentioned drawbacks. Respect to the coatings options, the principal supplier can offer about ten different coatings that partially reduce the application spectrum. At the present, one of the most interesting research area is relating to development of new extractant material like molecularly imprinted polymers.

The major development for the future for these microextraction procedures will be focused in the automation. Automation reduces error sources and therefore better results can be obtained. Automatic SPME samplers are actually in the market, but to automatize LPME is more difficult. Some procedures or part of the procedures can be automated, but can be difficult to automatize every method in a whole.

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