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Review

Solid-phase extraction of quaternary ammonium herbicides

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Abstract

This paper highlights recent advances in the solid-phase extraction (SPE) of quaternary ammonium herbicides in water, soil, plant and biological samples. After a brief introduction summarizing the properties of quaternary ammonium herbicides and the difficulties involved in measuring them, attention is paid primarily to solid supports used for isolation and concentration, pre-treatments required for the different matrices, and eluents applied for quantitative desorption of these analytes. The determination techniques used after SPE and applications of the proposed SPE methodology are also briefly discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Extraction methods; Solid-phase extraction; Soil; Plant materials; Water analysis; Environmental analysis; Food analysis; Quaternary ammonium compounds; Pesticides

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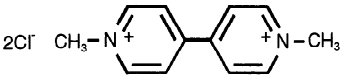
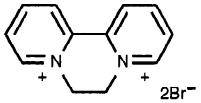
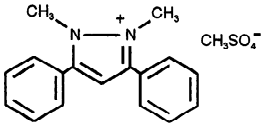
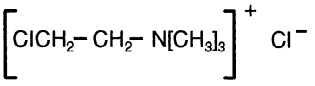
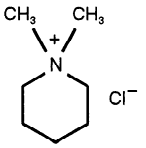
1. Introduction

Quaternary nitrogen herbicides were developed from the observation that quaternary ammonium germicides, like cetyl trimethylammonium bromide, desiccate young plants. Table 1 shows the chemical structure, chemical and common names [1,2].

Paraquat was first synthesized in 1882 and has been used as a redox indicator (under the name methyl viologen) in chemical laboratories since 1933. Its herbicidal properties were discovered in 1957, and since 1962 paraquat has been marketed in over 130 countries as a highly effective contact herbicide. The bipirydinium herbicides diquat and paraquat were introduced by Imperial Chemical Industries in 1958. They are very quick-acting herbicides that are absorbed by plants and translocated, thus causing desiccation of the foliage. These herbicides are strongly adsorbed by clay constituent soil, and are effectively deactivated as soon as they come into contact with soil [3].

The pyrazolium monocation difenzoquat is also used throughout the word as a selective herbicide for post emergence control of wild oats in barley and fall-seeded wheat. It is the active ingredient in Avenge and Finaven (registered trademarks of American Cyanamide Co.). From the chemical point of view, the plant growth regulators mepiquat chloride

Table 1
Chemical structure and identificative names of the ammonium quaternary herbicides

Chemical structure	Common name	Chemical name	Registered tradenames, Company
	Paraquat	1,1′-Dimethyl-4,4′-bipyridinium	Gramoxome, Zeneca
	Diquat	1,1′-Ethylene-2′2′-dipyridylium	Reglone, Zeneca
	Difenzoquat	1,2-Dimethyl-3,5-diphenyl-1H-pyrazolium	Finaven, Cyanamid Superaven, Cyanamid
	Chlormequat	2-Chloroethyltrimethylammonium	Cycocel, BASF and Cyanamid
	Mepiquat	1,1-Dimethylpiperidinium	Pix, BASF

and chlormequat, which are mainly used to prevent lodging in barley and rye and also to increase the yield in cotton could be added to the above mentioned list of cationic herbicides.

Diquat and paraquat are included in a priority list of herbicides of potential concern established for the Mediterranean countries by the European Union (EU), due to their widespread usage in this area [4]. Consequently, they may be present as residues in environmental, food and biological samples [5,6]. Although there are few studies on difenzoquat, chlormequat and mepiquat toxicity [7,8], diquat and paraquat are extremely toxic and are often encountered in cases of poisoning [9,10]. As a result there is a need for analytical procedures for their isolation and determination.

Table 2 outlines the physico–chemical properties of these ammonium quaternary herbicides because to develop an extraction method with the most appropriate sorbents and/or eluents, knowledge of the physical and chemical properties of the compounds is indispensable. All of them are very water soluble, non-volatile, thermally stable and stable in acid. Diquat is known to degrade slowly at pH levels over 9, and paraquat hydrolyzes at pH levels higher than 12 [11]. Difenzoquat, chlormequat and mepiquat are quite stable to hydrolytic or biological degradation. As can be observed, the common characteristics of these compounds are the positive charge and the high solubility in water. These characteristics make it difficult to applied any kind of isolation and concentration process prior to determination, since they are not extracted by organic solvents and remain in the aqueous extract after acidic and basic compounds have been removed.

In environmental analysis, solid-phase extraction (SPE) is a very useful preconcentration technique, which allows both, extraction of pesticide residues with high efficiency and their concentration as such levels that the limits set by safety regulations are really achieved [12,13]. The SPE methods that have been developed for these intractable environmental contaminants are based on two different modes: ion-exchange and ion-pair. These compounds adsorb strongly (bound residues) to organic and inorganic adsorbents (e.g. clay, plant tissues, adsorbents used for clean-up and chromatography, and glass) and therefore require strong eluents to solubilize the adsorbate. Considering the physico–chemical properties, SPE should be optimized by carefully testing the available parameters.

The main purpose of this review is to summarize and discuss the SPE methods developed to isolate and preconcentrate the ammonium quaternary herbicides prior to their separation and detection, and the principles and relative merits of each of these methods.

2. Solid-phase extraction supports

Selection of the ‘right’ phase for extraction is not a trivial task because there are difficulties involved in all the supports utilized that must be cleared up in order to obtain a suitable methodology. A wide range of solid-phases (SP) have been used to analyze these compounds, the most common of which are cation-exchange resins, silica and reversed-phase supports. The first two isolate the ammonium quaternary herbicides by ion-exchange mechanisms and the third by ion-pair chromatography.

Only one report involves the use of columns for

Table 2
Characteristic physico-chemical properties of ammonium quaternary herbicides

Compound	Molecular mass	Log P_{ow}	pH	Solubility				
				H_2O	Methanol	Chloroform	Ethyl acetate	Light petroleum
Paraquat	257	−4.7	3.35	700 g l^{-1} (20°C)	Slightly soluble	Insoluble	Insoluble	Insoluble
Diquat	344	−4.6	6–7.5	700 g l^{-1}	Slightly soluble	Insoluble	Insoluble	Insoluble
Difenzoquat	360	0.238 (pH 7)	3 –3.4	765 g l^{-1} (25°C) 780 g l^{-1} (37°C) 850 g l^{-1} (56°C)	620 g l^{-1}	500 g l^{-1}	Slighly soluble	Insoluble
Chlormequat	158	−1.58 (pH 7)	–	>1000 g kg^{-1}	320 g kg^{-1}	0.3 g kg^{-1}	<0.1 g kg^{-1}	<0.1 g kg^{-1}
Mepiquat	150	−2.82 (pH 7)	–	>1000 g kg^{-1}	162 g kg^{-1}	10.5 g kg^{-1}	Low soluble	Low soluble

gel filtration chromatography to determine diquat and paraquat in serum and urine [14]. Intensely hydrophilic components are not retained in the preparation column, but are eluted and eliminated. Paraquat and diquat are retained in the column. These findings confirm the satisfactory separation of paraquat and diquat from the protein of the serum sample. However, this methodology has not been improved since it was first applied.

2.1. Cation-exchange resins

Sulfonic acid or carboxylic acid functional groups bound to silica or polymers are used for strong and weak cationic extraction, respectively. Cation-exchange resins are usually supplied in the hydrogen ion form, but they can easily be converted to other cation forms by treating them with the appropriate salt. Resin applications to environmental and biological samples are outlined in Table 3.

The most widely used cationic-exchange resin is Dowex 50-X8 (Na^+ form), which is a strong acid exchanger with a poly(styrene-divinylbenzene) (PS-DVB) matrix [15–17]. The method is applied to determine paraquat and diquat in water, potatoes and soil samples. The results obtained (Table 3) were similar in the studies reported and showed major losses of paraquat, particularly in potato samples.

Paraquat can also be determined at the subnanogram per milliliter level by using a flow-through spectrometric sensor [17]. A flow manifold was designed integrating preconcentration, reaction, and detection in a sorbent material packed in a flow-cell for the pesticides determination. In preliminary assays, several sorbents were tested in order to find the most appropriate for the purpose. Cation-exchange resins provided the best results, especially Dowex 50W-X8-200. In addition, the visible spectrum of this support was clearly not the same in the presence as in the absence of the retained reaction product of paraquat and dithionite.

Kambhampati et al. [18] developed a method for the extraction of diquat and paraquat from environmental water samples by ion-exchange column chromatography. Experiments to extract these compounds were conducted with various ion-exchange resins, including a strong cation-exchange resin and a mixed ion bed ion-exchange resin. Recoveries from these resins were below 30% for both diquat and paraquat. However, extraction with Dowex ion retardation resin (containing paired anion [$-COO^-$] and cation [$(CH_3)_3N^+$] sites) resulted in acceptable recoveries (see Table 3).

Weak acid can be used for separating strongly basic substances such as diquat and paraquat that are often firmly retained on strong acid exchangers. A chromatographic procedure with an Amberlite CG-50 column was used to purify and concentrate the residues extracted from agricultural products. The cation-exchange resin removed most coextracted materials from agricultural products [19].

Matrix effects are expected to be more of a problem with ion-exchange because it is affected by the ionic strength and ionic composition of the sample, both of which can vary greatly with time and with the type of source. The influence of the matrix effects on the retention of paraquat in cationic exchanger resins has been studied utilizing synthetic water samples [17]. Two major variables were considered: pH and the ionic strength. The pH of the sample must be higher than 3.5; however, no influence on retention was observed at the normal values of this variable in real samples (from 5–6 to 8–9). On the other hand, the ionic strength played a major role because it affected the retention of paraquat on the exchanger adversely. However, a minimum ionic strength was clearly needed in order to prepare synthetic samples with a matrix similar to that of real water samples. This ionic strength decreased the retention efficiency by ca. 20% relative to samples with an ionic strength of the virtually zero. In spite of these matrix effects, this technique has been reported to be suitable for routine identification and quantitative determination of ammonium quaternary herbicides.

2.2. Silica sorbents

Silica is one of the most polar sorbents available for SPE. Under aqueous conditions, however, silica exhibits primarily cation-exchange selectivity. The cation-exchange capacity of silica depends on several factors, including pH. In general, this capacity increases with rise in pH. Under neutral or slightly basic conditions quaternary ammonium compounds

Table 3
SPE of quaternary ammonium herbicides using cation-exchange resins

Compound	Matrix	Extraction	Concentration range	Recovery (%)	Determination	Detection limits	Ref.
Paraquat	Water 250 ml	**SP**: Dowex 50-X8 (NH_4^+ form) **Eluent**: Saturated ammonium chloride	–	–	Spectrophotometric on the SP	0.11 ng ml^{-1}	[17]
Diquat	Water	**Pre-treatment**: Add EDTA **SP**: Dowex 50-X8 (Na^+ form)	0.01–1 μg ml^{-1}	–	Spectrophotometric	–	[20]
Diquat Paraquat	Drinking water 1–4 l	**Pre-treatment**: Adjust to pH 8.5 **SP**: 10 g of Dowex ion retardation resins **Eluent**: 0.2 *M* ammonium acetate and acetonitrile	5–10 μg l^{-1}	97	HPLC–MS	–	[18]
Paraquat	Water 250 ml Potatoes 250 g	**Pre-treatment**: Potato: Sulfuric acid reflux Water and potato: add EDTA and adjust to pH 9 **SP**: Dowex 50-X8 (Na^+ form) **Eluent**: Saturated ammonium chloride	0.2–1.0 mg l^{-1} 0.2–0.4 mg l^{-1}	64–70 54–57	Spectrophotometric	–	[15]
Paraquat	Soils 100 g	**Pre-treatment**: Sulfuric acid reflux **SP**: Dowex 50-X8 (Na^+ form) **Eluent**: Ammonium chloride solution	2.98–6.25 μg g^{-1}	95	Spectrophotometric	–	[16]
Diquat Paraquat	Rice, cabbage, wheat, potato, peach, corn 10 g	**Pre-treatment**: Hot dilute HCl **SP**: Amberlite CG 50 **Eluent**: Acid methanol	0.1–1 μg g^{-1}	79–98 80–103	HPLC–UV	0.02 μg g^{-1}	[19]

Table 4
SPE of quaternary ammonium herbicides using silica as sorbent

Compound	Matrix	Extraction	Concentration range	Recovery (%)	Determination	Detection limits	Ref.
Paraquat Diquat	Well-water 250 ml	**SP:** Silica Sep-Pak (500 mg) **Eluent:** 0.5% TMAN and 3% of ammonium sulphate in 0.1 N sulfuric acid	0.5–10 μg l^{-1}	86–90 88–90	HPLC–UV	0.5 μg l^{-1}	[21]
Paraquat Diquat	Well water 100 ml	**SP:** Silica column (100 mg) **Eluent:** 0.5% TMAN and 3% of ammonium sulphate in 0.1 N sulfuric acid	0.1–10 μg l^{-1}	118 117	HPLC–UV	0.1 μg kg^{-1}	[23]
Diquat Paraquat Difenzoquat	Well water 1 l	**SP:** Silica cartridge (100 mg) **Eluent:** 0.17 *M* TMAOH and ammonium sulfate in sulfuric acid (pH 2.2)	100–500 μg l^{-1}	65–85	HPLC-UV	<0.1 μg l^{-1}	[22]
Diquat Paraquat Difenzoquat	Natural and drinking water 0.250–1 l	**SP:** Silica Sep-Pak **Eluent:** 0.2% of TMAOH and 3% ammonium sulfate in sulfuric acid (pH 3) and 10% methanol	0.1 25 μg l^{-1}	86–91 88–95 89–95	HPLC–UV	0.05 μg l^{-1}	[24] [25] [27]
Diquat Paraquat Difenzoquat	Drinking and surface water 50 ml	**SP**: Silica cartridge (80 g) **Eluent:** On-line with HPLC system using mobile phase 0.2% of TMAOH and 3% ammonium sulfate in sulfuric acid (pH 3) and methanol	0.1–20 μg l^{-1}	84–101	HPLC–UV	0.05 μg l^{-1}	[26]
Diquat Paraquat Difenzoquat Mepiquat Chlormequat	Tap water 250 ml	**Pre-treatment:** Adjusting pH 9 **SP:** Silica Sep-Pak (500 mg) **Eluent:** 8% methanolic HCl	0.01–5 μg l^{-1}	85.2–98.4	HPLC–API–MS	0.1 μg l^{-1} 1.8 μg l^{-1} 0.05 μg l^{-1} 0.1 μg l^{-1} 0.1 μg l^{-1}	[28]
Paraquat Diquat	Potatoes 5 g	**Pre-treatment:** With acid using a micro-reflux, adjusting pH 9–10 **SP:** Silica Sep-Pack (500 mg) **Eluent:** 8% methanolic HCl	0.05–5.0 mg kg^{-1}	79.5–97.6	HPLC–UV Radioassay	0.05 mg kg^{-1}	[30]
Paraquat Diquat	Potato Corn Turnip Asparagus 10 g	**Pre-treatment:** With 6 *M* HCl, and adjust to pH 9 **SP:** Adsorbex silica cartridges (400 mg) **Eluent:** 0.1 *M* HCl in methanol	0.01–0.50 μg g^{-1}	79.3–104.8	HPLC-DAD[a]	0.01 μg g^{-1}	[11]

Paraquat	Water 500 ml	**Pre-treatment:** Maceration with 250 ml of water	40–100 μg l^{-1}	95.8–96.8	Spectrophotometric	1.2 μg g^{-1}	[31]
	Wheat, Rice Potatoes, Grass 20 g	**SP:** Silica gel (5 g) **Eluent:** Saturated ammonium chloride	0.8–3 μg g^{-1}	74–89			
Paraquat Diquat	Mill barley Dray navy beans Wheat flour 10 g	**Pre-treatment:** With 6 *M* HCl, and adjust to pH 9 **SP:** Silica column (4 g) **Eluent:** 6.5 *M* HCl in methanol	0.01–0.3 μg g^{-1}	47–95	HPLC–DAD[a]	0.01 μg g^{-1}	[32]
Paraquat Diquat	Potatoes 1 g	**Pre-treatment:** With 6 *M* HCl and adjust to pH 9 **SP**: Silica Sep-Pak (500 mg) **Eluent:** 5 *M* HCl with 8% methanol	0.05–1 μg g^{-1}	70–81	CE–UV	0.01 μg g^{-1}	[35]
Difenzoquat	Rice, wheat, barley, buckwheat, corn, rye	**Pre-treatment:** With acetone **SP**: Extrelut 20 (22.5 g) **Eluent:** Ethyl acetate containing 4% formic acid	0.2 μg g^{-1}	82.3–94.1	GC–MS	1 μg kg^{-1}	[36]
Paraquat	Blood, urine, mother's milk 2 ml Food, soil 25 g Water 100 ml	**Pre-treatment:** Human samples: 1 ml 5% EDTA and 1 ml 1% TCA Food sample: with 1 ml 5% EDTA and 25 ml sulfuric acid Water sample: 1 ml 5% EDTA **SP:** Silica gel column (6 g) **Eluent:** Saturated ammonium chloride	0.05–0.5 μg ml^{-1}	97–99	Spectrophotometric	0.03 μg ml^{-1}	[33]
Paraquat	Plants, fruits, grains 25 mg Water 100 ml Blood, urine 2 ml	**Pre-treatment**: Human samples: 1 ml 5% EDTA and 1 ml 1% TCA Food sample: extraction with aqueous medium (1 ml EDTA 5% and 150 ml water) Extraction with 9 *M* acid (1 ml 5% EDTA and 25 ml sulfuric acid). Water sample: 1 ml 5% EDTA **SP:** Silica gel column (6 g) **Eluent:** Saturated ammonium chloride	1–12 μg	95–99	Spectrophotometric	0.03 μg ml^{-1}	[34]

[a] DAD=Diode array detection.

are largely retained on silica. For this reason, many of the reported methods use a pH controlled silica SPE for clean-up and isolation of quats from sample matrices, as is summarized in Table 4.

Water samples of approximately 250 ml adjusted or not to basic pH are directly passed through the silica cartridge [21–28]. In all the studies, silica SPE efficiency in isolating traces of the five ammonium quaternary herbicides from water is demonstrated. Recoveries are quite acceptable in the pH 6.5– 9.5 range for diquat, paraquat and difenzoquat.

However, the interactions between these compounds and other components present in water may affect the SPE efficiency. The effect of different concentrations of organic matter, surfactants, salts and other contaminants on the extraction and isolation of diquat, paraquat and difenzoquat using silica has been evaluated [24,25]. The results show the negative effect of organic matter, surfactants and salts on the recoveries of the three cationic herbicides. One exception is cationic surfactants that do not reduce the extraction efficiency. Another special case is the presence of other contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenols and carbamates), which cause an important decrease in the recoveries only for difenzoquat, whereas diquat and paraquat are not affected.

Some authors argue that in the case of humic acids and surfactants the interference may be caused, by saturation of the sorptive sites of the solid-phase or by binding to the pesticides. The chemical species formed could either remain retained by solid-phase and not be desorbed during the elution or pass unretained through it [24,29]. The bulk of the available evidence indicates the interaction of quaternary ammonium groups of the herbicide, which bear positive charges, with the negative sites of humic or surfactants substances, such as the oxygen atoms of the carboxyl and hydroxyl groups.

The effect of the salts present in water on the silica SPE of diquat, paraquat and difenzoquat was studied [25]. Recoveries register substantial decrease when the salt concentration is increased. This phenomenon was expected and confirmed the ion-exchange process between the cationic herbicides and the silanol groups of the solid-phase.

Paraquat and diquat have also been isolated from the high-moisture crop digests using pH-controlled silica SPE [11,30–35]. The most critical aspects of silica SPE procedure were the pH adjustment of the crop digests and the apparent pH of the silica sorbent before sample application. Below a pH of 9, recoveries for these analytes began to decrease, particularly for diquat. In the same study [11], however, the proposed method applied to standards in absence of crop matrix gave recoveries from 91 to 101% for both diquat and paraquat over the entire range of pH tested. The presence of crops coextractants made it necessary to create conditions that favored increased capacity of the silica sorbent.

These methods for isolation of paraquat and diquat from high-moisture food crops have not been successfully applied to low-moisture commodities. The reason seems to be that the capacity of the SPE cartridges is exceeded because of the excessive sample matrix, and the recoveries of paraquat and diquat are therefore low and irreproducible. Some authors postulate that increasing the pH would theoretically increase capacity of the silica, but at pH>9, degradation of diquat and 'irreversible' retention of paraquat occur. Another way to increase the capacity is to use a greater mass of silica gel. In this way, a column of 4 g silica was found to provide sufficient capacity to extract these compounds [32].

The silica SPE method has been applied for paraquat determination in blood, urine and mother's milk. Prior to this determination ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were added, both to remove interference by various metal ions and for deproteinization, respectively. The authors do not discuss the possible matrix effects but they employ silica gel columns containing 6 g of silica gel [33].

Tsukioka et al. [36] used an Extrelut 20 column instead of conventional silica gel to determine difenzoquat in cereals. Difenzoquat was extracted with acetone, concentrated in a rotary evaporator, and applied to the Extrelut column. Moreover a clean-up with an ODS minicolumn was performed.

2.3. Apolar phases

Two major mechanisms of analyte retention on apolar solid supports are adsorption and partitioning.

Common adsorbents are charcoal and porous polymers, such as Amberlites, whereas partitioning is mainly performed on bonded-silicas (C_{18} and C_8) [13].

SPE of ammonium herbicides with an apolar sorbent is complicated by their ionic nature. It is a fact that the use of C_{18} Sep-Pak cartridges to purify paraquat concentrates extracted from sunflower, marijuana and oil have been described [37–39]. The cartridge retains apolar interfering materials and paraquat passes through the cartridge without interacting.

In spite of the lack of affinity between these solid supports and these herbicides, the wide application of the SPE with reversed supports has led the researchers to develop different approaches that allow ammonium quaternary compounds extraction on these supports. As a general rule, the mechanisms involved in the SPE require the formation of an ion pair. However, every rule has its exception.

Experiments performed by Tsunoda et al. [40] showed that paraquat and diquat present in water can be retained on C_{18}-silica cartridges by adjusting the sample to pH 13, which probably represents interaction with the silica matrix of the material. Sep-Pak cartridges have been used to selectively extract paraquat from water, human urine, blood, tissue, cow's milk, and beverage samples without other treatment than a pH adjustment to around 13 [40–43]. One possible explanation for this behavior could be that the retention of diquat and paraquat were due to the interactions with residual silanol groups present the C_{18} solid-phase, i.e. the C_{18} is utilized as a weak cation-exchanger.

One report [44] proposed the use of a cyanopropyl cartridge to extract paraquat from serum or plasma. The authors found that allowing the columns to run dry after either sample application or after rinsing had no effect on paraquat recoveries. This is an important advantage compared to other SPE methods. This method was optimized for the quantitation of paraquat in serum or plasma, but a brief study was undertaken to determine the possibility of extracting paraquat from urine. Results obtained were similar that those reported with plasma.

Selective on-line SPE and HPLC of diquat, paraquat and difenzoquat from environmental water samples has been accomplished with Graphitized Carbon Black (GCB) as both extraction and analytical columns [45]. Some years ago GCB was studied as a selective adsorbent to extract organic compounds of varied chemical nature from water samples [46–48] and retention mechanisms on GCB columns have been fully discussed. Although the conclusions are contradictory, it has been established that the retention order of the solutes depends on the basicity of the electron pair [49]. GCB has been shown to be more effective for trapping polar compounds than adsorbents such as C_{18} and PS-DVB.

A method for the analysis of diquat and paraquat in water was developed using ENVI-8 DSK SPE disks for sample concentration followed by liquid chromatography on a C_1 reversed-phase column and electrospray ionization process [50]. Diquat and paraquat were isolated, without ion-pair agents, on ENVI-8 DSK SPE disk and eluted with trifluoroacetic acid (TFA). No breakthrough was observed for 500 ml samples. The sample preparation procedure is compatible with the chromatographic system, which, in turn, is compatible with the electrospray ionization process.

Table 5 collects the special uses of apolar solid support without ion pair reagents described in the literature.

Ion pair formation constitutes the analytical tool of choice for isolating ammonium quaternary herbicides using apolar SPE to extract and concentrate them from water, biological samples or vegetable extracts. The most common solid supports used are silica bonded to C_{18} or C_8 chains using hexanesulfonate, heptanesulfonate, dihydrogenorthophosphate, etc as counter ions. Table 6 lists the common ion-pair SPE methods used to extract quaternary ammonium herbicides and also gives the results reported on the method performance.

The counter ion could be added directly to the samples or could be present in the solid support. In the latter case, it is supposed that the counter ions, which must be hydrophobic anions, adsorb on the surface of the reversed-phase support to give a material, which acts as a cation-exchanger. The sample is passed through the solid support and the quaternary herbicides are retained as ion-pairs, while the majority of the endogenous material is eluted.

Ahmad [51,52] described a procedure for the

Table 5
Uses of the reversed solid-phase supports without ion-pair reagents

Compound	Matrix	Extraction	Concentration range	Recovery (%)	Determination	Detection limits	Ref.
Paraquat	Sunflower seeds 1 g	Digest with 6 *M* HCl Adjust pH 7 KH_2PO_4/H_3PO_4 and passed through C_{18} Sep-Pak	0.1– 20 μg g^{-1}	89–101	HPLC–UV	–	[37]
Paraquat	Marijuana1 g	Extract HCl with sonication Phosphate buffer-C_{18} Sep-PakEluent: Water	5–100 μg g^{-1}	90–97	HPLC–UV	2 ng g^{-1}	[38]
DiquatParaquat	Olive oi11 g	Dissolve in phosphate buffer pH 7 and passed through C_{18} Sep-Pak	5–50 μg g^{-1}	91 90	HPLC–UV	0.2 μg g^{-1}0.4 μg g^{-1}	[39]
Paraquat	Water, urine, blood, milk and beverages 1–5 ml	**Pre-treatment**: Blood samples: Deproteinized with perchloric acid All the samples: Adjust to pH 13 **SP:** C_{18} Sep-Pak **Eluent:** 0.1<HCl	10–50 μg ml^{-1}	96–98 83–94 87–90	Spectrophotometric	2 μg	[40]
Paraquat Diquat	Urine and Blood 2–50 ml	**Pre-treatment:** Adjust to pH 12 **SP:** C_{18} cartridge **Eluent:** 0.1 *M* HCl	–	–	Spectrophotometric On a solid support of silica	0.02 mg l^{-1} 0.1 mg l^{-1}	[43]
Diquat	Plasma, serum 0.5 ml	**Pre-treatment:** Adjust to alkaline pH **SP:** C_{18} Sep-Pak **Eluent:** 0.1 *M* HCl	1.2–10 μg ml^{-1}	85–100	Spectrophotometric	–	[42]
Paraquat Diquat	Human tissue 0.1–1 g	**Pre-treatment:** With perchloric acid and adjust to pH 11 **SP:** C_{18} Sep-Pak **Eluent:** 0.1 *M* HCl	0.1–10 μg g^{-1}	89–95 77–80	HPLC–UV	0.05 μg g^{-1}	[41]
Paraquat	Plasma 5 ml	**SP:** Cyanopropyl columns **Eluent:** 0.1 *M* HCl	0.4–4 μM	76	Spectrophotometric	0.23 μM	[44]
Paraquat Diquat Difenzoquat	Water 50 ml	**SP:** GBC **Eluent:** On-line with the HPLC system Dilution of TMAOH and ammonium sulphate in water, adjusted to pH 3 and methanol.	0.1–20 μg l^{-1}	95–99 94–98 97–99	HPLC–UV	50 ng l^{-1}	[45]
Paraquat Diquat	Water 500 ml	**SP:** ENVI-8 DSK DISK **Eluent:** 5 *M* trifluoroacetic acid	3.74 μg l^{-1}	96–98 99–110	HPLC–ES–MS	0.1 μg l^{-1} 0.2 μg l^{-1}	[50]

quantitative determination of difenzoquat in water samples. Potassium dihydrogenorthophosphate was used as an ion-pair reagent, and was added to each water sample. No difenzoquat was adsorbed on the cartridge when spiked water samples without the addition of ion pair reagent were passed through the C_{18} cartridge. The same author also described a method for determining paraquat by adding borax to the water sample before the SPE [53]. Paraquat recovery decreased to<60% when potassium dihydrogen phosphate or sodium dicarbonate were used as ion pair reagents. The same result was obtained when concentration of paraquat was attempted without the addition of ion pair reagent.

However, pre-treatment of the solid-support with the counter ion to retain quaternary ammonium herbicides from the water sample seems to be the preferred technique, as it is more widely employed [54–59] and has been proposed by the United States Environmental Protection Agency (EPA) as the reference method for diquat and paraquat determination [56–58]. The EPA methods use a C_8 solid sorbent, which has been especially prepared for the reversed-phase, ion-pair mode [56,57]. Several versions use C_8 cartridges or disks to remove diquat from the water samples. In the revised version, EPA Method 549.2, the sample pH is not adjusted to 10.5 before extraction because the EPA has determined that this adjustment does not improve the extraction of diquat. Also, at pH 10.5, a precipitate is formed in several hard water samples. The precipitate causes very long delays in passing the sample through the solid-phase sorbent, which results in unacceptably low recoveries of diquat. No precipitation occurred in the pH range of 7 to 9 in artificial matrices simulating extremely hard water. Data included in the EPA method 549.2 show that recovery of diquat is more variable and lower that those obtained for other organic contaminants using reversed-phases without ionic pair-formation.

Both ion-pair SPE methods have recently been coupled with MS detection [59,60]. An inherent advantage of liquid chromatography–mass spectrometry (LC–MS) determination is the analytical specificity. It should be noted that the EPA recommends methods in which identity is confirmed by mass spectrometry (MS).

Although matrix effects have not been reported when ion-pair SPE is used, they can be expected due to its similarity with cation-exchange mechanisms.

A methodology for the isolation of paraquat from serum and tissues has been established using column chromatography on Amberlite XAD-2 resin pre-treated with sodium dodecyl sulfate (SDS). Paraquat is probably extracted from the water eluent into an adsorbent layer rich in SDS by forming dodecylsulfate-paraquat ion pairs, which are then eluted by organic solvent [61,62]. Another alternative method is to use XAD-2 resin without SDS pre-treatment and mixed the serum samples with SDS before passing them through the XAD-2 material. This method is convenient for clinical chemists because purified resin is already widely used in laboratories to extract drugs or poisons from biological materials [63]. A procedure for extracting the herbicides from urine and serum has also been developed using disposable cartridges of C_{18}-silica, which were pre-treated with a solution of sodium heptanesulfonate [64,65].

Chlormequat and mepiquat have been determined in grain by a method based on ion-pair chromatography clean-up using SPE-C_{18} cartridges and ammonium acetate. The use of volatile acetate as the counter ion is compatible with electrospray MS detection and quantification, which greatly reduces the risk of false positives and eliminates the need for excessive cleanup [66,67].

3. Sample pre-treatment

3.1. Water samples

A clean-up procedure may not be necessary for relatively clean samples. The clean up procedures recommended have been used to analyze various water types. If the sample contains particulates or the complexity is unknown, the entire sample should be passed through a membrane filter. Water samples should be stored at 4°C unless extraction is performed immediately. It is important that the samples be collected in plastic bottles to avoid the adsorption in the glass [23].

Although the undesirable effect originated by the water composition are well known [24,25], only an analytical study focus on avoiding them [25]. It is

Table 6
SPE of quaternary ammonium herbicides using reversed-phase ion-pair chromatography

Compound	Matrix	Extraction	Concentration range	Recovery (%)	Determination	Detection limits	Ref.
Paraquat	Serum 2 μl	**Pre-treatment:** Diluted with water **SP:** Syringe packed with SDS-pre-treated XAD-2 **Eluent:** Methyl isobutyl ketone-isobutanol containing SDS	0.5–107.3 nmol l^{-1}	83.6–88.1	Spectrophotometric	0.19 nmol l^{-1}	[62]
Paraquat	Psoas muscle, liver, lung and kidneys. 2 g	**Pre-treatment:** With sulfuric acid and adjust to pH 12.5 **SP:** Syringe packed with SDS-pre-treated XAD-2 **Eluent:** Methyl isobutyl ketone-isobutanol containing SDS	0.01–75 mg kg^{-1}	73.4–75.7	Spectrophotometric	0.1 mg kg^{-1}	[61]
Paraquat	Serum 2 ml	**Pre-treatment:** With TCA and SDS **SP:** Syringe packed with XAD-2 **Eluent:** Methyl isobutyl ketone-isobutanol containing SDS	0.02–1.00 mg l^{-1}	86	Spectrophotometric	0.005 mg l^{-1}	[63]
Paraquat Diquat	Urine 1 ml	**Pre-treatment:** Adjust to alkaline pH **SP:** C_{18} Sep-Pak pre-treated with heptanesulfonate **Eluent:** Acidic methanol	1–250 μg ml^{-1}	92–98 90–97	HPLC–UV	1 μg ml^{-1}	[64]
Paraquat Diquat	Serum 1 ml	**Pre-treatment:** With TCA and adjust to alkaline pH **SP:** C_{18} Sep-Pak pretreated with heptanesulfonate **Eluent:** Acidic methanol	0.5–2 μg ml^{-1}	97	CE–UV	0.05 μg ml^{-1}	[65]
Paraquat	Rat brain	**Pre-treatment:** With perchloric acid and adjust to alkaline pH **SP:** C_{18} Sep-Pak pretreated with heptanesulfonate **Eluent:** Acidic methanol	15–360 ng g^{-1}	88–99	HPLC–UV	15 ng g^{-1}	[68] [69]
Paraquat	Plasma Urine 1 ml	**Pre-treatment:** With 200 μl of concentrated ammonia **SP:** C_{18} Sep-Pak pretreated with heptanesulfonate **Eluent:** Acidic methanol	5 μg	88.2–97.3	TLC–FID[a]	50 ng	[70] [71]
Difenzoquat	Tap water 100 ml	**Pre-treatment:** With dihydrogenorthophosphate **SP:** C_{18} Sep-Pak **Eluent:** 1.4% of KH_2PO_4 in water–acetonitrile and adjust pH to 2.8 with H_3PO_4	2–50 μg l^{-1}	92	HPLC–UV	2 μg l^{-1}	[51]
Difenzoquat	Water 250 ml	**Pre-treatment:** With dihydrogenorthophosphate as counter ion **SP:** Co:Pell ODS material **Eluent:** On-line with HPLC system using 1.4% of KH_2PO_4 in water–acetonitrile and adjust pH to 2.8 with H_3PO_4	5–20 μg l^{-1}	97	HPLC–UV	0.25 μg l^{-1}	[52]

Paraquat	Water	**Pre-treatment** With borax	50–1000 μg l^{-1}	93–108	HPLC–UV	50 μg l^{-1}	[53]
	100 ml	**SP:** C_{18} Sep-Pak					
		Eluent: $(NH_4)_2HPO_4/H_3PO_4$					
Diquat	Water	**SP:** C_8 cartridge (500 mg) pretreated with hexanesulfonic acid	10–200 μg l^{-1}	97	HPLC–UV	<10 μg l^{-1}	[54]
	250 ml	**Eluent:** Ortophosphoric acid and diethylamine in water					
Diquat	Water	**SP:** C_8 cartridge (500 mg) pretreated with hexane sulfonic acid	10–200 μg l^{-1}	98	HPLC–UV	<10 μg l^{-1}	[55]
	250 ml	**Eluent:** Orthophosphoric acid and diethylamine in water					
Paraquat	Water	**Pre-treatment:** Adjust to pH 10.5	2–100 μg l^{-1}	85–90	HPLC–DAD	0.44 μg l^{-1}	[56]
Diquat	250 ml	**SP:** C_8 cartridge (500 mg) pretreated with hexanesulfonic acid				0.80 μg l^{-1}	
		Eluent: Orthophosphoric acid and diethylamine in water					
Paraquat	Water	**Pre-treatment:** Adjust to pH 10.5	2–100 μg l^{-1}	94–98	HPLC–DAD	0.44 μg l^{-1}	[57]
Diquat	250 ml	**SP:** C_8 Empore disk pretreated with hexanesulfonic acid				0.80 μg l^{-1}	
		Eluent: Orthophosphoric acid and diethylamine in water					
Paraquat	Water	**SP:** C_8 cartridge (500 mg) or disk pretreated with hexanesulfonic acid	2–100 μg l^{-1}	94–98	HPLC–DAD	0.44 μg l^{-1}	[58]
Diquat	250 ml	**Eluent:** Orthophosphoric acid and diethylamine in water				0.80 μg l^{-1}	
Diquat	Water	**SP**: C_{18} disk pretreated with hexanesulfonic acid	–	–	MS	0.44 μg l^{-1}	[59]
Paraquat		**Elution:** MALDI[b]				0.80 μg l^{-1}	
Diquat	Water	**Pre-treatment:** with heptafluorobutyric acid	–	–	LC–APCI–MS	<50 ng l^{-1}	[60]
Paraquat		**SP**: C_{18} disk					
Difenzoquat		**Eluent**: On-line with the HPLC system using a gradient with an					
Chlormequat		aqueous solution of HFBA and acetonitrile					
Mepiquat	Oats	**Pre-treatment:** With methanol–water–acetic acid	0.2 mg kg^{-1}	91	HPLC–MS–MS	6 μg kg^{-1}	[66]
Chlormequat	Wheat	**SP**: C_{18} Sep-Pak					
	10 g	**Eluent**: Methanol–water–acetic acid with ammonium acetate					
Chlormequat	Grain	**Pre-treatment:** With methanol–water–acetic acid	0.2 mg kg^{-1}	91	HPLC–MS–MS	6 μg kg^{-1}	[67]
Mepiquat	10 g	**SP:** C_{18} Sep-Pak		90		2 μg kg^{-1}	
		Eluent: Methanol–water–acetic acid with ammonium acetate					

[a] FID=Flame ionization detection.
[b] MALDI=Matrix-assisted laser desorption/ionization.

mainly devoted to evaluating whether cationic surfactants have the ability to eliminate interactions between the bipyridinium herbicides and other contaminants present in water samples and also to provide acceptable recoveries in their presence. The results show that the addition of cation surfactants to the water samples in the determination of traces of paraquat, diquat and difenzoquat avoids the negative influence on SPE of humic acids and anionic surfactants. It can be considered the first step for a simple removal of interferences that prevents the loss in the extraction procedure.

3.2. Soil and plant materials

Ammonium quaternary herbicides have a strong affinity for components in plant and soil, and even through they are water soluble, they can not be easily extracted once they have been sprayed onto and incorporated into the plant or retained in soil [38]. Procedures involving refluxing the sample in sulfuric [15,16] or hydrochloric [11,30,32] acids are the ones most reported, but these extraction conditions are drastic and time-consuming. Alternative methods have been proposed to simplify the sample preparation. Nagayama et al. described the almost complete extraction of diquat and paraquat by homogenizing the sample with the mixer and then heating the homogenized sample in acidic solution on a steam bath [19]. Wigfield et al. used a milder method in which a large surface area of the sample is exposed and cells walls may be destroyed by grinding and sonication [35]. They found that the recoveries were in the 60–73% range and suggested that this method afforded procedural advantages over the conventional method of boiling the crop with acid.

Curiously, isolation of paraquat from different samples of grain such as wheat and rice, potato and grass only by maceration with water has also been presented [31,34]. The recoveries ranged from 74 to 90% and were in agreement with the values reported after acid treatment [34].

Chlormequat and mepiquat required less drastic extraction conditions. Grain samples were extracted with a methanol–water–acetic acid mixture using a disperser [66,67]. The effectiveness of the method was demonstrated by analyzing grain material from an inter-comparison study.

3.3. Biological samples

Samples obtained from living organisms generally include a wide range of substances from higher (e.g., proteins) to lower molecular weight (e.g., amino acids). Direct SPE of a biological sample hence results in a clogging condensation on the column, leading to an increase in intramolecular pressure, fluctuations in the recoveries and higher variation coefficients.

Ammonium quaternary herbicides were recovered from blood, plasma, urine and milk samples mainly after deproteinization with different acids, such as sulphosalicylic acid [43], perchloric acid [40], and trichloroacetic acid [33,34,65]. The addition of EDTA to remove interferences by various metal ions was also reported [33,34]. Direct isolation from plasma and serum without any deproteinization procedures was also applied [42,44,62,64].

The methods most frequently used to determine ammonium quaternary herbicides in tissues were acid digestion or deproteinization. The most commonly used acids for these purposes included sulfuric [61] and perchloric acids [41,68]. Although tissue homogenate could be deproteinized with TCA, it must be eliminated from the extract before performing the reaction for determinate ‘quats’ by a spectrophotometric method (after reduction of paraquat in alkaline sodium dithionite solution) due to the interference of TCA [61].

4. Eluents

When the extraction is finished, a small volume of a liquid is allowed to pass through the solid-phase (SP). Desorption is usually accomplished using solvents or acid saline solutions, for which the partition coefficient in a given solid-phase/solvent or solution system favors the elution.

4.1. Cation-exchange resins

Diquat and paraquat are always eluted from the strong cationic resins with a saturated ammonium solution [15–18,20]. As a result of the effective

retention of ammonium quaternary herbicides on the cationic exchange resins the elution process had to be carefully designed. According to Sensi [72,73], several cations remove paraquat from clay but with a differences in efficiency (e.g. $NH_4^+ > K^+ > Ca^{2+} > Mg^{2+} > Na^+$). Therefore, the ammonium ion is almost always chosen as eluent.

The elution profiles obtained from the weakly acidic resin Amberlite CG-50 showed high recoveries using acidic methanol [19].

4.2. Silica sorbents

The cation-exchange capacity of silica increases with increasing pH. Under acidic conditions quats are not retained on silica. Prior to the elution of the trapped herbicides with a suitable solution, other solutions can be used for the elution of impurities. Chichila et al. [11] recommend wash the cartridge with 0.1 *M* HCl in methanol to remove most of the sample interferences from the sorbent before analyte elution. Methanol is employed to remove water from the sorbent before the application of the washing solutions because final recoveries are found to be low (<50%) and irreproducible if residual water is not removed before the application of this acidic wash. Apparently, even a slight increase in water content in the methanolic acid wash was sufficient to partially elute the analytes. With the removal of residual water, the acidic wash could be applied with no detectable losses of paraquat and diquat.

Elution of the analytes with chlorhydric [11,30,32,35] in methanol is generally used. Methanol was included in the final elute to facilitate evaporation. Vacuum, high temperatures and nitrogen stream are required to evaporate efficiently the eluate.

Elution of the SPE columns in the reversed direction was proposed in order to achieve high sensitivity using low eluent volumes [23]. Sometimes the adsorbed compounds on the silica gel can be eluted by saturated ammonium chloride [31,33] or by ammonium sulfate alone, in sulfuric acid [22,27] or increasing the straight ionic force by the tetramethylammonium nitrate (TMAN) or tetramethylammonium hydroxide (TMAOH) ion in sulfuric acid [21,23–27].

4.3. Apolar supports

Concentration procedures on non-polar SPE disks or cartridges by direct adsorption are generally followed by elution with a HCL solution [40–44]. As an alternative, a volatile acid of comparable pK_a was sought [50]. TFA was found to be a practical substitute for HCl. In this sense the non-polar phase has behavior of cation-exchange support.

However, it is more usual to use ion-pairs formation during SPE enrichment. Various solvents and mixtures has been tested for desorption from the solid-phase, e.g., methyl isobutyl ketone (MIBK) and isobutanol added of the counter ion used to the extraction [53,61,62], acidic methanol [64,65,68,69], monobasic phosphates and phosphoric acid in water acetonitrile [51–53], orthophosphoric acid and diethylamine in water [54–58], ammonium acetate in acidic methanol [66,67] or heptafluorbutyric acid in aqueous methanol [60]. High recoveries have been obtained with all of them.

5. Determination systems coupled to solid-phase extraction

Published reports on the determination of ammonium quaternary herbicides after SPE include methods based on liquid-chromatography, flow-injection analysis and capillary electrophoresis (CE). However, while the sophisticated instruments required for these methods are sensitive, they are costly and cumbersome for routine field measurements. Spectrophotometric methods are simpler and less expensive but are neither sufficiently sensitive nor specific.

5.1. Spectrophotometry

The available spectrophotometric methods are based on measurement of the reduced ion obtained by the reduction of paraquat with alkaline sodium dithionite solution [40,43,61–63]. These methods show low sensitivity (range 4–10 ppm), and the color of the solution is only stable for a short period of time (5–10 min). The method was suitably modified

using flow injection analysis, which increased the sensitivity by several fold [15,17].

Other methods for determining paraquat and diquat using different reducing reagents, such as ascorbic acid [31], glucose [34], sodium hydrosulfite [42] and sodium borohydride [33] in an alkaline medium have been reported. Sodium borohydride was found to be the best reagent for analyzing paraquat because the reduction was complete [33] and it offers the advantage of greater stability of the blue radical ion.

All viologens (among them paraquat and diquat) must be fairly good electron acceptors because of the stability of the free-radical ions, and they have a strong tendency to form charge-transfer complexes with donor anions. Hence, both compounds form charge-transfer complexes with a wide range of anions. Such complex formation often gives rise to new absorption bands or to broadening of the absorbance bands towards the red end of the spectrum. The reaction involving the formation of a charge-transfer complex between Diquat and Sistine has been proposed [20]. Specificity and precision is improved because only those species react.

5.2. High-performance liquid chromatography

HPLC procedures for analyzing ammonium quaternary herbicides in various samples after SPE have been described. The most common separations are carried out on C_{18} reversed-phase columns, using ion-pairing reagents in the mobile phase, such as heptanesulfonate [30,64,68,69], octanesulfonate [22,37,38], orthophosphate [39,51–53] or bromide [41].

The analytical C_{18} columns based on silica particles has been substituted for polymeric packings (especially recommended by the EPA) such as PS-DVB to avoid the free silanol group effect [55–58].

Chichila et al. [11,32] demonstrated that quaternary ammonium compounds could be chromatograph on silica using inorganic halide salts, as ion pairing reagents in a non aqueous acetonitrile mobile phase. Chromatography is also carried out on silica using aqueous acidic solutions containing tetramethylammonium and ammonium ions as a mobile phase [21,23,24,26,27].

An analytical column of GCB has also been tested for chromatographic determination of diquat, paraquat and difenzoquat [45]. The elution was performed with a gradient of pH 3 aqueous solution of TMAOH and ammonium sulfate and methanol. The Hypercarb column was found to give a low probability of false positive for bipyridilium herbicides because it is very selective for polar compounds.

Conventional detection of ammonium quaternary compounds in HPLC is usually done with UV and diode array detectors. The UV detector is more common [22,24,26,27,30,37–39,41,45,51–53,55,58,64,69].

An internally standardized HPLC method for the simultaneous assays of paraquat and diquat in well water at levels as low as 0.5 ppb has been described [21,23]. Detection was by two independent methods: direct UV absorbance and UV absorbance following post column reaction with sodium hydrosulfite. Using this strategy paraquat and diquat were analyzed and confirmed at levels appropriate for environmental monitoring.

The HPLC method developed by the EPA for diquat and paraquat employs diode-array detection and has minimum detection limits of 0.44 $\mu g\ kg^{-1}$ for diquat and 0.80 $\mu g\ kg^{-1}$ for paraquat using 250 ml of sample. The EPA method depends on UV-spectral scanning for qualitative confirmation [56–58].

An inherent disadvantage of these methods is the lack of analytical specificity, which may result in identification and quantification difficulties, especially in complex matrices. The US EPA recommends methods in which identity is confirmed by MS. Coupling ion-pair chromatography with MS is not a good approach because of the high concentration of non-volatile conventional ion-pair reagents. Systems containing only volatile buffer such as ammonium acetate [18,66,67,74] and heptafluorobutyric acid [28,60] have recently been developed. The chromatographic and mass spectrometry conditions have been established and mass spectra in particle beam (PB) [18], thermospray (TSP) [74], electrospray (ES) [28,66,67], and atmospheric pressure chemical ionization (APCI) [28] have been obtained. A method for the determination of the herbicides diquat and paraquat in water by HPLC (C_1 column) with post

column addition of propionic acid/methanol followed by ES–MS has also been reported [50]. This last technology is transferable to quadrupole and ion trap mass spectrometers.

A useful approach has been designed in the recent years to increase the effectiveness of SPE. It is based on the on-line enrichment of the ammonium quaternary herbicides from aqueous samples on small cartridges filled with a suitable sorbent and subsequent direct determination utilizing switching to analytical HPLC columns. The advantages of this approach are savings in time and a high potential for automation.

Selective on-line coupling of SPE and HPLC–UV determination for diquat, paraquat and difenzoquat from environmental water samples has been accomplished with GCB [45], octadecylsilice [52] or silica [26] using both extraction and analytical columns packing with the same materials. The advantage of the on-line system over the off-line method is that it provides rapid access to information on water quality and allows a relatively high frequency of sampling. The system can be used for monitoring purposes.

A possibility for increasing the selectivity of this approach would be coupling SPE and HPLC–MS. Castro et al. [60] reported the use of Empore extraction disk in a preconcentration system on-line with liquid chromatography–atmospheric pressure ionization mass spectrometry (LC–API–MS) detection, allowing low detection limits in full scan mode [60]. SPE was based on the ion-pair formation by adding to the water samples a volatile counter ion such as formiate or heptafluorobutyric acid.

These on-line methods constitute an alternative procedure for analyzing ammonium quaternary herbicides with a potential for application to monitoring pesticide residues in water.

5.3. Capillary electrophoresis

CE is undergoing a period of rapid expansion, and several instruments are now on the market. It is a promising new general method for the analytical separation of ionic species, which migrate down the column under the influence of an applied voltage or current. A CE system coupled with on-column UV detection has been used to analyze diquat and paraquat in potatoes [35] and in serum [65]. The CE separation technique demonstrated the absence of interferences from coextractives, low migration times and a high separation efficiency; it generated over $4 \cdot 10^5$ theoretical plates per meter and gave total resolution of paraquat and diquat peaks in the electroferogram.

Thus, CE overcomes most of the problems encountered using the spectrophotometric and HPLC techniques and offers an attractive alternative for the determination of paraquat and diquat residues from several matrices after SPE with either C_{18} and silica [35]. The only disadvantage of CE in the case of analysis of biological samples, which require extraction and clean-up processes, lies in the coexistence of ions in a very small volume of samples necessary for injection that can affect analyte retention time.

6. Applications

There is a large body of literature concerning the SPE of ammonium quaternary herbicides in water, soil, plant and biological materials. The performance of this methodology has been tested with spiked samples. Interest in establishing which of these methodologies are suitable for determining these compounds arises from the possibility of applying them to assessing the environmental pollution level by ammonium quaternary compounds, and coupling them with the monitoring programs established by regulations.

6.1. Water samples

The SPE methods described have been used on real-world environmental water samples.

The methods based on silica extraction and elution with acidic solutions containing tetramethylammonium and ammonium ion have been applied by different authors to monitor diquat and paraquat residues in different kinds of water. Simon [21] estimated consistent recoveries of paraquat and diquat from well water; but neither analyte was found in any of 21 samples collected from the field. Ibáñez et al. [24] showed the suitability of the method in routine analysis of natural water. Diquat was found in two samples at concentrations of 6 μg

l^{-1} and 13 μg l^{-1}. The levels and distribution of diquat, paraquat and difenzoquat were determined in water samples from irrigation channels, rivers and lagoons taken during one year from three different marsh areas of the Valencian Community [27]. All three compounds were detected. The average concentration found for diquat was 0.09 μg l^{-1}, for paraquat 0.01 μg l^{-1}, and difenzoquat was only detected in one sample at a concentration of 1.75 μg l^{-1}. Using the on-line approach the presence of diquat and paraquat was detected in surface water samples [26]. Although the concentration was lower than 1 μg l^{-1} they had a real environmental impact. Diquat, paraquat and difenzoquat were not detected in drinking water.

Rai et al. [33] applied a method for paraquat based on silica concentration and spectrophotometric determination to water samples and found a maximum concentration of 26.0 μg l^{-1}.

Existing methods of concentrations by direct adsorption on non polar support have been also applied. Surface and drinking water samples, representative of typical Ontario water quality parameters were selected to assess SPE with ENVI-8 disk combined with LC–ES–MS. Waters having a wide range of pH, alkalinity, conductivity, total solids, and total organic carbon composition were analyzed in duplicate. Neither diquat nor paraquat were detected in drinking water samples from lake Ontario [50]. On-line SPE with GBC and HPLC determination of a hypersil column was applied to carry out set of measurements in natural samples. Diquat and paraquat were found in three samples at levels between 0.1 and 0.25 μg g^{-1} [45].

To demonstrate the use of ion-pair chromatography on C_{18} Sep-Pak using inorganic ions pair reagent, Vermilion River water was analyzed and the results showed no paraquat and difenzoquat in the samples [51,53].

The method proposed by the EPA has been applied to measure diquat distribution in water after application to submersed weeds. Diquat herbicide and rhodamine WT dye were applied, either with polymer, which reportedly aids in sinking and confinement of aquatic herbicides, or without a polymer to three plots in a lake. Forty-six percent of the samples collected at the edges of the plots did not contain detectable diquat residues and only 66% of those samples with detectable diquat contained more than the level tolerated in potable water (10 ppb).

6.2. Soil and plant materials

A flow-through spectrophotometric sensor for the determination of paraquat at the ng per ml level that combined preconcentration on Dowex resin, reaction and detection in the flow-cell was used to study the adsorption of paraquat in different types of soil [17]. Several interesting conclusions can be drawn from the results. In all cases, a virtually exponential adsorption–time curve defined the behavior of the sediments obtained by mixing the soil and the pesticide aqueous solution. The total amount of clay in the soils played a major role in the adsorption process. The adsorption efficiency increased with the clay content. The amount of organic matter in the soil played a decisive role, as is shown by the results.

Rai et al. [33] applied a method based on silica concentration and spectrophotometric determination of paraquat to soil, rice, apple, sugarcane and potato samples. Maximum concentrations were 0.11, 0.13, 0.12, 0.14 and 0.12 for these samples, respectively.

A C_{18} Sep-Pak cartridge for purifying was applied to authentic samples of sunflower seeds, which had been dessicated with paraquat before they were harvested. The samples were dehulled and the hulls were analyzed. Substantial amounts of paraquat remained on outer portions of the seeds (1.4–2.7 μg g^{-1}) [37]. In the same way, twelve virgin olive oil samples from olive crops sprayed with diquat and paraquat were analyzed [39]. Only two of the analyzed oils presented traces of diquat.

A method based on clean-up using C_{18} cartridge, HPLC and specific detection and quantification by liquid chromatography–tandem mass spectrometry (LC–MS–MS) was applied first to chlormequat residue analysis on grain [66]. In Denmark the primary use of chlormequat is for winter cereals, and 11 such winter wheat samples from the Danish National Pesticide Survey were analyzed. Residue contents ranged from below 0.01 up to 0.45 mg kg^{-1}, and were thus below the EU maximum residue level of 2.0 mg kg^{-1} for wheat. The method was extended to establish information on chlormequat and mepiquat residues in grain for human consumption [67]. Of 77 samples analyzed, 51 contained chlormequat and 11 contained mepiquat. The highest

levels of chlormequat were found in samples of oatmeal (3.756 mg kg^{-1}) and rye (1.08 mg kg^{-1}). In nine rye grain samples containing chlormequat, five also contained mepiquat. However, in all samples analyzed, the residues of chlormequat and mepiquat were below maximum residue limits.

6.3. Biological samples

Rai et al. [33] satisfactorily applied a method based on silica concentration and spectrophotometric determination of paraquat to blood serum, urine and mother's milk samples. They found a maximum concentration of between 1.05 –2.08 μg 10 ml^{-1}.

The paraquat concentrations in the psoas muscle, liver, lung and kidneys of a swine dosed with 0.16 g kg^{-1} of paraquat were investigated using ion-pair chromatography in conjunction with spectrophotometry [61]. Concentrations of paraquat in the tissues ranged from 5.0 to 296.6 mg kg^{-1}.

An ion-pair reversed-phase HPLC method with UV detection was developed to measure paraquat in brain extracts [68]. The method was used to determine paraquat concentrations in discrete brain areas at different times after its systemic administration in rats. In addition, the method was employed in the determination of paraquat levels in whole-brain samples from rats of various ages systemically treated with several doses of the herbicide [69]. Age-dependent paraquat brain concentrations were detected in rats, with the highest herbicide brain levels being obtained in very young and older animals.

7. Conclusions

Analysis for ammonium quaternary herbicides is a demanding task of trace analysis of complex samples. Spectrometric or HPLC determinations are not enough sensitive to allow direct determination of these compounds in the samples, and liquid–liquid extraction and concentration methods with an organic solvent are unsuitable. SPE, which combines efficient isolation and preconcentration, is best suited for the purpose.

SPE extraction of diquat and paraquat is now well developed as is evidenced by the inclusion of this method in the standard procedures used, for example, by the EPA (Method 549.1). The sample preparation technique in this method involves SPE using ion-pair reagent. SPE has been increasingly combined with other techniques. Recent trends to improve SPE methodology are based on on-line enrichment followed by HPLC, HPLC–MS and other hyphenated techniques.

The present review clearly indicates that there are two main drawbacks to these methods that still remain. First, SPE of cationic compounds is always complicated by their ionic nature and implicates, in one or other way, the use of ion-exchange mechanisms, which are strongly affected by the ionic strength. However, the studies on the sample matrix effects are extremely scarce. Second, SPE methodologies for the most recently developed ammonium quaternary compounds, difenzoquat, mepiquat and chlormequat, have been presented only in a few papers. It is important emphasized the need to extend the existing methodology to include these three compounds and to point out that although insufficient, an important effort has been made.

In this evaluation of the published methodology for ammonium quaternary herbicides SPE, it should be noted that some problems still remain (accuracy and/or precision were poor and the methods were often too time-consuming and tedious) which make it difficult to optimize the methods for monitoring large numbers of samples. However, the enormous growth in techniques and ideas gives strong evidence that experience and inventive imagination can solve the difficult analytical problems related to SPE of ammonium quaternary herbicides.

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