Hydrophilic interaction liquid chromatography coupled to mass spectrometry-based detection to determine emerging organic contaminants in environmental samples

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Abstract

Hydrophilic interaction liquid chromatography (HILIC) has gained widespread acceptance as an alternative to reversed-phase liquid chromatography (RPLC) for the retention and separation of polar compounds. Because a great number of emerging organic contaminants are polar, this represents progress in terms of solving most of the problems and limitations encountered in the conventional methods developed for their determination. In this review, recent HILIC applications developed in the environmental field are described, which are normally coupled with mass spectrometry, in order to reach the limits required to quantify contaminants present at trace levels and benefit from its capacity for confirmation. Special attention is paid to the stationary and mobile phases commonly used in terms of the advantages that they offer compared to reversed-phase columns and the high-water content mobile phases traditionally used in RPLC. The most important features related to the matrices and contaminants normally studied are also discussed, as well as the contribution of the HILIC mode towards reducing the matrix effect.

Keywords: Hydrophilic interaction liquid chromatography; Mass spectrometry; Emerging organic contaminants; Environmental samples

1 Introduction

Hydrophilic interaction liquid chromatography (HILIC) is a relatively new chromatographic mode developed in response to the lack of retention of polar compounds in reversed-phase liquid chromatography (RPLC) [1]. When using liquid chromatography (LC), RPLC is the preferred mode and the C_{18} columns are the most commonly used stationary phases, due to their versatility and robustness. However, sometimes the separation of polar compounds is difficult to achieve and they also tend to elute close to the void volume, even when a high content of water is used in the mobile phase. This is a significant drawback when coupling LC to mass spectrometry (MS)-based detection, because high contents of water in the flow that enters the instrument hinder ionization. In addition, the salts and polar interferences that co-elute with the analytes at the beginning of the chromatogram can cause suppression or enhancement of the response, known as matrix effect (ME) [2,3]. To overcome these problems, Alpert [4] introduced the term HILIC to describe a chromatographic mode based on the combination of a polar stationary phase with a mobile phase containing water and an organic solvent, in which the aqueous phase was the stronger eluting solvent.

In his study, Alpert proposed that the mechanism of HILIC consisted of partitioning between a water-enriched layer partially immobilized on the stationary phase and the mobile phase being mainly polar organic solvent. Since then, a large part of the research dedicated to HILIC has focused on its mechanism [2,5–7]. Recently, Guo [8] reviewed the progress made to date in terms of understanding the HILIC mechanism, focusing on fundamental aspects such as the water layer absorbed onto the stationary phase, the selectivity of several stationary phases and the kinetic performance of the technique. Frequently, the predominant mechanism of HILIC is hydrophilic partitioning, as mentioned before, and the presence of the water-enriched layer onto the surface of the stationary phases has been demonstrated experimentally. However, other mechanisms such as surface adsorption or electrostatic interactions also play an important role in the retention, which gain relevance depending on several factors, such as the organic solvent content. In general, retention in HILIC is very complex and depends on the type of stationary phase, mobile phase composition and the properties of the analytes.

In fact, as several interactions can contribute to retention in HILIC, the selection of the stationary phase might not be as straightforward as in RPLC, where partition is the main interaction and retention can be reasonably well predicted on phases like C_{18} and C_8 according to the polarity of the compounds. Each of the stationary phases available for HILIC promotes specific interactions that can result in different retention ~~behaviour~~ behavior and selectivity for a specific group of compounds. Over the last decade, several authors have reviewed the different HILIC stationary phases used since the introduction of this technique, focusing on the properties and applications of bare silica, bonded-silica with polar groups such as amide, diol, zwitterionic and macromolecules, ionic-exchangers and mixed-mode phases, among others [6,9–11]. Recently, Qiao *et al.* [12] discussed the updates and recent progress made in the development and characterization of HILIC stationary phases, mostly focusing on zwitterionic, mixed-mode, monolithic and macromolecule-bonded phases.

The mobile phase conditions, such as the organic solvent, pH and salt concentration, also have a great influence on the retention of the analytes [7]. Frequently, the preferred organic solvent is acetonitrile (ACN) and the aqueous phase contains additives such as salts and acids, which are used to control the pH and ionic strength during the analysis, and should be volatile to avoid problems in the interface with MS-based detectors [11]. The fundamental aspects, influence and trends of the parameters affecting the mobile phase to be considered when optimizing a method in HILIC have been discussed extensively [7,13,14].

HILIC was initially developed for the separation of carbohydrates, peptides, nucleic acids or proteins. However, this chromatographic mode has since been applied to determine several polar and hydrophilic compounds in different matrices, such as biological, foodstuff or environmental samples, contributing to the fields of metabolomics, proteomics and the pharmaceutical, environmental and food industries [7,15]. A large number of emerging organic contaminants (EOCs) found in environmental samples have polar properties, because their solubility in water facilitates their transportation through wastewaters into the environment. In 2011, van Nuijs *et al.* [1] reviewed several methods based on HILIC applied to food and environmental samples. The methods related to the environmental field focused on pharmaceuticals (estrogens, cytostatic drugs, antibiotics, metformin, contrast agents, etc.), drugs of abuse and pesticides, mainly in river, surface and drinking water and wastewater samples. Also in 2011, Li *et al.* [16] reviewed the HILIC methods available to determine several contaminants in environmental samples, paying special attention to the sample preparation. Since then, new methods with environmental applications have been developed that have shown advantages of using the HILIC technique. The aim of the present review is to discuss recent applications of HILIC to environmental matrices, paying particular attention to the advantages and contribution that this chromatographic mode has offered in the environmental field.

2 Stationary phases

There are several phases that can be used for HILIC applications that have the capacity to promote different interactions, such as hydrophilic partition, cation or anion-exchange or hydrogen bonding, yielding different retention ~~behaviour~~behavior, elution order and selectivity. In RPLC, the C_{18} stationary phase is often suitable for a wide range of compounds, so it is considered quite versatile, whereas, in HILIC, there is no such universal column [6]. Manufacturers have developed phases with different chemistry to promote particular mechanisms, ranging from materials that can behave as RP or HILIC phases (depending on the mobile phase) to columns with ionic functionalities. As previously stated, the properties of the different HILIC stationary phases and their applications have been extensively reviewed [6,9–12]. In this section, the stationary phases most frequently used for environmental applications will be discussed, as well as relevant observations related to their performance. It should be mentioned that the most commonly used HILIC stationary phases in the environmental field are the bare silica, zwitterionic, amide and diol phases, as shown in Table 1.

Table 1 Stationary phases, mobile phases and type of elution used in environmental applications under HILIC conditions.

alt-text: Table 1

Stationary phase	Column	Mobile phase	Elution	Ref
Bare silica	Ascentis Express HILIC	A: ACN, B: 15 mM CH_3COONH_4/CH_3COOH aqueous buffer (pH 4.5)	Gradient	[17]
	Atlantis HILIC	A: ACN, B: H_2O (both 0.1% HCOOH)	Gradient	[18]
	Altima HP	A: ACN, B: H_2O (both 0.1% HCOOH)	Isocratic	[19]
	ZORBAX Rx-SIL HILIC	A: ACN, B: 2 mM CH_3COONH_4/CH_3COOH aqueous buffer (pH 4.5)	Gradient	[20–22]
	Atlantis HILIC	A: ACN, B: isopropanol, C: 200 mM $HCOONH_4$/HCOOH aqueous buffer (pH 3)	Isocratic	[23]
	Atlantis HILIC	A: ACN, B: aqueous 20 mM $HCOONH_4$	Gradient	[24]
	Kinetex HILIC	15:10:75 $HCOONH_4$ (pH 3.5):MeOH:ACN	Isocratic	[25]
	Atlantis HILIC	A: ACN, B:H_2O	Gradient	[26]

	Kinetex HILIC	A: ACN, B: H_2O (both 0.1% HCOOH and 2 mM $HCOONH_4$)	Gradient	[27]
Zwitterionic	ZIC-HILIC	A: ACN, B: aqueous 60 mM HCOOH	Isocratic	[28]
	ZIC-HILIC	A: ACN, B: H_2O (both 0.1% HCOOH)	Gradient	[29]
	Nucleodur HILIC	A: ACN, B: MeOH, C: 20 mM CH_3COONH_4/CH_3COOH aqueous buffer	Pseudo isocratic	[30]
	ZIC-HILIC	A: ACN, B: 10 mM $HCOONH_4$/HCOOH aqueous buffer (pH 3)	Gradient	[31]
	ZIC-HILIC	A: ACN, B: 2:3 aqueous 30 mM CH_3COONH_4:ACN (pH not adjusted)	Gradient	[32]
	ZIC-HILIC	A: ACN, B: H_2O (both 5% CH_3COONH_4 and 0.01% HCOOH)	Gradient	[33]
	Luna C18 and ZIC-HILIC	A: 5% aqueous 5 mM CH_3COONH_4 (pH 6.8) in ACN, B: 25% aqueous 5 mM CH_3COONH_4 (pH 6.8) in ACN	Gradient	[34]
	Syncronis	A: ACN, B: 100 mM $HCOONH_4$/HCOOH aqueous buffer (pH 3.75)	Gradient	[35]
	ZIC-HILIC	A: ACN, B: 2 mM $HCOONH_4$/HCOOH aqueous buffer (pH 3)	Gradient	[36]
	Nucleoshell HILIC	A: 95:5 ACN:2 mM CH_3COONH_4, B: 97:3 2 mM CH_3COONH_4:ACN (both 0.05% HCOOH)	Gradient	[37]
	Poroshell 120 EC-C18 and ZIC-HILIC (in series)	A: ACN, B: H_2O, C: RPLC mobile phase (aqueous 10 mM CH_3COONH_4/ACN)	Gradient	[38]
	ZIC-HILIC	A: ACN, B: H_2O (both 0.1% HCOOH)	Gradient	[39]
	ZIC-HILIC	A: ACN, B: H_2O (both 0.1% HCOOH)	Gradient	[40]
	ZIC-cHILIC	ACN/CH_3COONH_4/CH_3COOH aqueous buffer (pH 5)	Isocratic	[41]
	ZIC-HILIC	A: 5% aqueous 20 mM $HCOONH_4$ (pH 4) in n-propanol, B: 50% aqueous 20 mM $HCOONH_4$ (pH 4) in n-propanol, C: 100 mM aqueous $HCOONH_4$ (pH 4)	Gradient	[42]
Amide	TSK-gel Amide-80	A: ACN, B: aqueous 0.05% TFA	Isocratic	[28]
	XBridge amide	A: ACN, B: H_2O	Isocratic	[43]
	TSK-gel Amide-80	A: 5% solvent B in ACN, B: 4 mM $HCOONH_4$ (both pH 3.5 using HCOOH)	Isocratic	[44]
	TSK-gel Amide-80	A: ACN, B: H_2O (both 50 mM HCOOH)	Gradient	[45]
	TSK-gel Amide-80	A: 5% H_2O in ACN, B: H_2O (both 5 mM $HCOONH_4$ and 3.6 HCOOH, pH 3.5)	Gradient	[46]
	Acquity UPLC BEH Amide	A: 5% H_2O in ACN, B: H_2O (both 1 mM $HCOONH_4$ and 0.01% HCOOH)	Gradient	[47]
Diol	Luna HILIC	A: 5% H_2O in ACN, B: H_2O (both adding 5 mM CH_3COONH_4 at pH 3.5)	Gradient	[48]
	Luna HILIC	A: ACN, B: aqueous 10 mM CH_3COONH_4	Gradient	[49]
	Luna HILIC	A: ACN, B: aqueous 5 mM CH_3COONH_4	Gradient	[50]
	Luna HILIC	A: ACN:MeOH 87.5:12.5, B: aqueous 5 mM CH_3COONH_5	Gradient	[51]
Other	RestekUltra IBD phase (polar-embedded alkyl)	A: 0.1% HCOOH in ACN, B: aqueous 10 mM $HCOONH_4$ (2.9)	Gradient	[52]

	Restek Viva PFPP	A: ACN, B: H_2O (both 0.1% HCOOH)	Gradient	[53]
	ZORBAX SB-C18 and Venusil HILIC (in series)	A: ACN, B: aqueous 10 mM CH_3COONH_4 (both 0.1% HCOOH)	Gradient	[54]

Unmodified bare silica gel under the brands Atlantis HILIC from Waters and the Kinetex HILIC from Phenomenex are the most frequently used columns. Columns with zwitterionic functionalities are silica or polymer-based and frequently bonded with sulfoalkylbetaine moieties that contain sulfonic acid and quaternary amine groups separated by a short alkyl chain [9]. There are several columns with zwitterionic properties commercially available, but, as can be seen in Table 1, the most widely used is the ZIC-HILIC phase manufactured by Merck. This commercial supplier also developed the ZIC-cHILIC phase with phosphorylcholine moieties bearing phosphoric acid groups instead of sulfonic acid groups and interchanging the order of the charges in the ligand. Because this column is relatively new, environmental applications using it are less common [41]. Other zwitterionic columns with similar functionalities that are less frequently used but commercially available are Nucleodur, Syncronis and Nucleoshell. Amide and cross-linked diol phases are also frequently used, with the TSK(It should be a dash in between: "TSK-gel")gel Amide-80 (Tosoh Bioscience) and the Luna HILIC (Phenomenex) being the most commonly used.

Several HILIC studies discuss the comparison between different polar stationary phases to identify the most suitable one for a group of analytes [50,53]. In the study by van Nuijs *et al.* [50], a Luna HILIC (cross-linked diol) column was selected for the separation of 9 drugs of abuse because it provided better sensitivity and robustness when compared to a Zorbax RX-Sil silica phase, obtaining good retention and satisfactory separation within 8 min of analysis in the LC-triple quadrupole (QqQ) system. The authors compared their results with a previous study where the Zorbax RX-Sil silica column showed late elution for ecgonine methyl ester causing a higher matrix effect in the ion trap MS detector, as ionic interferences also eluting last can affect the ionization [21]. Therefore, the limit of quantification (LOQ) for this compound was improved in the method using the cross-linked diol phase. Bisceglia *et al.* [53] published an interesting study in which 13 stationary phases were compared for the separation of 23 drugs of abuse including three HILIC phases, RP phases with embedded polar functionalities and even phases that can be operated in both RPLC and HILIC modes. They observed enhanced retention of the compounds on the HILIC phases Luna HILIC (cross-linked diol), Obelisc-N (zwitterionic) and Ultra IBD (embedded polar group, proprietary), especially in the case of highly polar compounds such as ecgonine and anhydroecgonine. However, resolution was poor, so it was concluded that HILIC might be an alternative for RPLC when retention problems cannot be addressed, but might not be suitable for the separation of a large number of analytes.

Another interesting comparison can be drawn from three methods describing the separation of artificial sweeteners using three different stationary phases: zwitterionic, bare silica and cross-linked diol [25,35,55]. Even when the mobile phase conditions and flow-rate used were different in each case some differences were clear. For instance, the zwitterionic stationary phase gave higher retention for four of the compounds ((Add the whole name of the compounds instead of the acronym. That is (cyclamate, sucralose, neohesperidine and aspartame))CYC, SUC, NHDC, ASP) even when a higher flow-rate was used. This lead to better selectivity and separation of the peaks. Interestingly, the elution order was the same in all cases. Fig. 1 compares the separation obtained for the bare silica and zwitterionic stationary phases during the optimization step of one of the studies above [35], showing how the zwitterionic phase gave better separation.

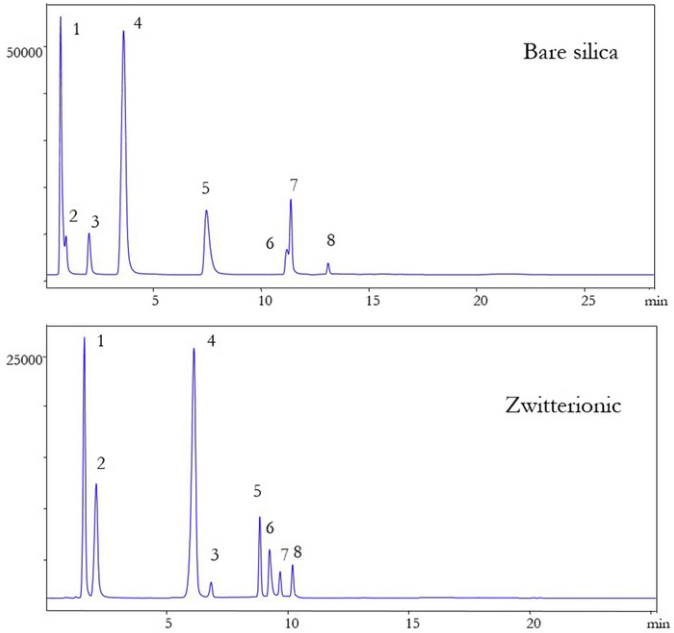

Fig. 1 Chromatographic separation of a group of artificial sweeteners on bare silica and zwitterionic stationary phases obtained during the HILIC optimization step [35]. Peak identities: (1) acesulfame, (2) saccharine, (3) sucralose, (4) cyclamate, (5) neohesperidine, (6) aspartame, (7) stevioside, (8) glycyrrhizic acid.

alt-text: Fig. 1

The use of polar stationary phases in the HILIC mode has had significant advantages in the separation of several pollutants. For instance, Scheurer *et al.* [31] used a zwitterionic phase (ZIC-HILIC) for the separation of the antidiabetic pharmaceutical metformin, which shows almost no retention in RPLC phases, due to the strong basic properties of this compound. Using this LC mode, they reported the first results on metformin occurrence in environmental waters in Germany. Apart from poor retention on RP stationary phases, basic compounds can also exhibit peak tailing on silica-based RP phases due to the additional retention that negatively charged residual silanols provide through ion-exchange interactions [56]. In this respect, HILIC has contributed towards resolving the problems associated to this type of compounds [57]. For instance, the determination of the antibiotics spectinomycin and lincomycin in environmental samples was improved in terms of retention, separation and peak shape using a bare silica stationary phase under HILIC conditions [19].

Several of the studies using HILIC for environmental applications have compared HILIC methods with RPLC methods [19,21,36,43,50,53,55]. For example, Chao *et al.* [43] compared the performance of the RP column XBridge C_{18} and the HILIC phase XBridge Amide to separate fullerols (hydroxylated fullerenes), finding poor retention in the C_{18} phase when compared to the amide phase. They attributed this ~~behaviour~~ behavior to the inability of the hydrophobic core of the analytes to interact with the RP phase due to the presence of the hydroxyl groups on the surface of the fullerols. Meanwhile, the amide phase exhibited efficient retention of the analytes using 90% ACN in the mobile phase. In another study [36], three RP stationary phases (Ascentis Express C_{18}, Zorbax Eclipse XDB-C_{18} and the Ascentis Express RP-amide with polar groups embedded) were compared with the ZIC-HILIC column for the separation of five iodinated X-ray contrast media, being highly polar compounds. In their comparison, the authors found poor retention of the analytes in the RP phases, requiring high water content in the mobile phase in order to provide some retention, which strongly affected the ionization in the LC-MS instrument. In contrast, the HILIC method offered higher retention and enhanced MS response. However, the separation of isomers that was observed in some of the RP phases was not possible in the HILIC column. Ordoñez *et al.* [55] also compared RPLC and HILIC methods for a group of artificial sweeteners using the Luna C_{18} and Luna HILIC (diol) columns. The chromatograms comparing both methods are shown in Fig. 2 where the different selectivity and retention between both separations can be observed. They found higher response which lead to better LOQ for the HILIC method but a higher matrix effect and lower reproducibility when compared to RPLC separation. For this reason, they preferred the RPLC method over the HILIC one. Although there is no general rule to know which analytes of environmental interest are better separated in HILIC considering the studies published so far, we can attempt to say that basic analytes such as illicit drugs and those with high content of hydroxyl groups often show good results when separated by HILIC.

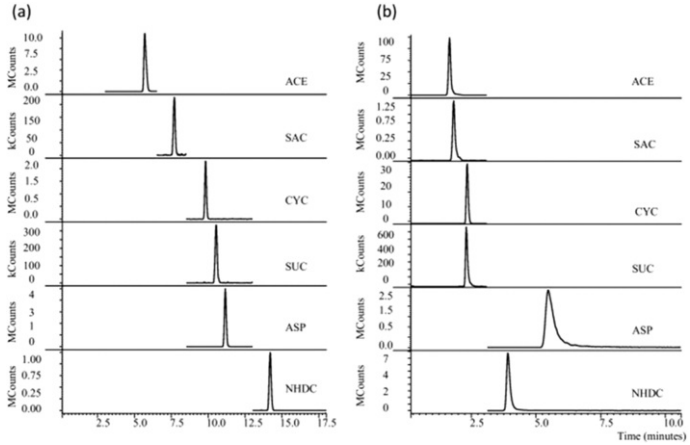

Fig. 2 Typical LC–MS/MS chromatograms of a 500 µg/L standard obtained with (a) the RPLC and (b) the HILIC column [48].

alt-text: Fig. 2

Because they exhibit alternative selectivity, the use of RPLC and HILIC in a single method is frequent, either coupling columns in series [34,38,54], injecting the sample in two different methods (HILIC and RPLC) [37,46,52], or comprehensive two-dimensional chromatography [58,59]. However, to the best of our knowledge, there are no recent environmental applications using the latter strategy. The approach of injecting the sample separately in HILIC and RPLC methods has been widely used in recent years, thanks to the additional information provided by separating peaks that are not resolved by using just a single LC mode. For example, Lajeunesse *et al.* [46] performed the chromatographic separation of a list of cyanotoxins in two groups, the polar ones on an amide phase and the less polar ones on a C_{18} phase, solving problems of low retention and distortion of peak shapes. Bisceglia *et al.* [52] also used a reversed phase (pentafluorophenyl-propyl) for the separation of cocaine and cocaine metabolites, and a polar-embedded alkyl phase under HILIC conditions specifically to analyze ecgonine and anhydroecgonine, because these two analytes showed no retention under RP conditions. Very recently, Christophoridis *et al.* [47] used a HILIC phase (amide) and an RPLC phase (C_{18}) to separate isomeric transformation products (TPs) obtained during the ozonation of ranitidine, which co-eluted when using the RPLC method only. Injecting the sample in two different chromatographic methods may double the analysis time, whereas the coupling of columns might deliver a faster analysis, depending on the conditions used.

The coupling of RPLC and HILIC stationary phases in series benefits from the increasing percentage of organic solvent in the usual gradient of RPLC, because HILIC separations start with a high proportion of organic solvent. Chen *et al.* [54] and Rajab *et al.* [38] reported methods using serial coupling to determine toxins [54] and TPs of diclofenac [38] in environmental samples. In the first case, the separation of 19 toxins with lipophilic and hydrophilic properties was achieved on a ZORBAX SB-C_{18} column coupled to a Venusil HILIC (amide) column. The authors observed that the C_{18} column retained the 8 hydrophobic toxins but did not retain the 11 toxins with hydrophilic properties, which were retained and resolved in the HILIC column. Likewise, in the HILIC phase, the hydrophobic toxins eluted at void volume, except for yessotoxin, which eluted last due to its enhanced retention in both columns [54]. In the other study, the diclofenac TPs obtained after oxidation with a boron-doped diamond electrode were separated in a single injection coupling Poroshell 120 EC-C_{18} and ZIC-HILIC columns, providing a more efficient, faster and easier approach for studying TPs in complex samples [38].

3 Mobile phases

The selection of the mobile phase conditions in HILIC separations is very important for obtaining satisfactory results, so a section discussing their optimization is often included in several publications. At the beginning of the analysis, HILIC separations are performed at a high content of organic solvent, in contrast to RPLC, in which high contents of water are needed to retain polar analytes. This represents an advantage when using MS-based detection, as ionization and desolvation processes in the ion sources are favored when using high proportions of organic solvent. Gradient profiles (when needed) in HILIC often start at ~5% water increasing it up to ~40%. A minimum percent of water (*i.e.*, 2%) is always needed to ensure the formation of the water layer on the surface of the stationary phase. In addition, the use of salts and buffer solutions is more common in HILIC, as it is more sensitive to pH changes and the presence of salts can influence retention and selectivity [6,7]. This ~~behaviour~~ behavior is related to the different retention mechanisms involved in HILIC and the influence of mobile phase parameters on the water layer immobilized on the polar stationary phase. These effects are more evident when ionic interactions are present, as the analytes can change their charge state from charged to neutral improving or decreasing retention. It has also been proven that an increase in salt concentration increases the thickness of the immobilized water layer, which delivers higher retention if hydrophilic partitioning is predominant in the overall mechanism [5,8].

Table 1 shows that the organic solvent commonly used in environmental applications is ACN, as is commonly the case for HILIC approaches in general. The reason is that polar protic solvents such as MeOH tend to compete with water for active sites on the surface of the stationary phase affecting the water layer formed on the phase surface, which leads to lower retention of polar analytes [14]. In addition, the mobile phases usually contained ammonium acetate or ammonium formate in concentrations up to 200 mM, and they were normally adjusted to acidic pH values between 3 and 4.5 using formic acid (preferred acid). These buffered solutions enabled pH and ionic strength to be controlled during separation. These additives were placed either in the aqueous phase or in both the organic and aqueous phase. For example, Halme *et al.* [44] tested different organic/aqueous proportions, three pH values (3, 3.5 and 4) and three salt concentrations (2, 4 and 10 mM) for a mobile phase containing ACN and an aqueous $HCOONH_4$/HCOOH buffer solution to separate a group of toxins. The final conditions were 4 mM of the buffered aqueous phase and ACN (with 5% of the aqueous phase), both adjusted to pH 3.5 using HCOOH at 40:60 (v/v) in isocratic mode, because it gave a better peak shape, resolution and faster analysis. For the separation of artificial sweeteners, the HILIC mobile phase was optimized(Add a space here, it should be read: "optimized on")on a Luna HILIC (cross-linked diol) column by testing different salt additives (CH_3COONH_4 or $HCOONH_4$) at 5 mM adjusted either with CH_3COOH or HCOOH to different pH values from 3.5 to 6.5. In this study, the retention of the analytes increased as the pH was raised but one of the analytes (aspartame) displayed peak broadening. For this reason, and because $HCOONH_4$ caused peak splitting in the case of acesulfame, the optimal salt was CH_3COONH_4 adjusted to pH 3.5 [48].

The addition of a small percentage of MeOH to the mobile phase can improve the sensitivity in some cases, as demonstrated in a method for the determination of 19 acidic herbicides and metabolites in river water samples, where the response of some analytes was increased by a factor of between 2 and 10 [30]. This fact was attributed to better ionization in the ESI source due to the protic character of MeOH. In this study, different salt concentrations (20–200 mM) in a CH_3COONH_4 buffered aqueous phase were tested observing that this increase in ionic strength did not lead to any substantial change in retention, so 20 mM was selected. The authors also obtained lower instrumental detection limits (LOD) when compared to RPLC methods, demonstrating the advantages of HILIC with regard to enhanced ionization. The high amount of organic modifier contributes to the formation of smaller droplets in the ion source, facilitating ionization and desolvation [3]. Similar to the previous study, Hayama *et al.* [23] also observed an increase in sensitivity when adding isopropanol to the ACN/buffered aqueous $HCOONH_4$/HCOOH (pH 3) mobile phase.

Salts or acids were sometimes added to both the organic and aqueous phases, especially when gradient elution was used, in order to maintain the ionic strength and pH of the mobile phase constant throughout the entire analysis [18,27,33,37,45]. This fact can be observed in Table 1, in which several methods add the same percentage of HCOOH (for example 0.1%) to both aqueous and organic phases or the same concentration of salt. Furthermore, gradient elution mode was preferred over isocratic elution. In HILIC, the use of isocratic elution is sometimes preferred to avoid the long equilibration times that are frequently needed when using gradient profiles [44] as retention times are often altered if the column is not well equilibrated, affecting the reproducibility of the method. Nevertheless, the use of this elution mode is often not possible if separation is not satisfactory or if complete elution requires even longer times than column stabilization. Most studies do not often address this limitation in detail or they simply add long equilibration times after each analysis. Because this problem might be related to the stationary phase, it should be expected to be reduced in recent stationary phases with enhanced technologies. However, environmental applications have limited to commonly used phases rather than new ones. Future studies in this area could potentially benefit from using the most recent stationary phases that have included advances in phase technology.

As the mobile phase in HILIC contains high proportions of ACN and the aqueous phase is the strong eluting solvent, the injection solvent in HILIC must also contain high proportions of organic solvent or be 100% organic solvent if possible, in order to avoid any distortion of the peak shape. For this reason, the direct injection of extracts from the sample preparation steps is possible when using HILIC separations. In a previous study [35], our group reported the direct injection in the LC-high-resolution MS (HRMS) system of the SPE extracts in the NH_4OH:MeOH:ACN (1:4:15) solution used for the elution of the analytes in the SPE procedure, avoiding evaporation steps, which allowed the simplification of the procedure while achieving similar LOQs. Barbaro *et al.* [39] also injected the methanolic eluting fraction obtained from the SPE performed in an Oasis HLB cartridge directly into the HILIC column. Halme *et al.* [44] treated freeze-dried algae samples using HILIC mobile phase (40:60, 4 mM ammonium formate buffered aqueous phase adjusted to pH 3.5: 5% buffered aqueous phase in ACN) as the extracting solvent, which was directly injected in the LC instrument after spiking with internal standards. The development of an on-line SPE-HILIC method was also possible thanks to the HILIC's compatibility with organic solvents by directly transferring the methanolic fraction eluted from the SPE to the column [17]. The first HILIC method using large-volume injection (LVI) injected 750 µL of the samples containing the analyte (acrylamide) using dichloromethane as the injection solvent, as it proved to focus the analyte better in the head of the column compared to acetone and ethyl acetate, as can be seen in Fig. 3 [18]. Even when several other methods have directly injected organic extracts obtained from sample preparation procedures [23,33,49,54], the change of solvents by evaporating and reconstituting the sample is more common, probably because optimized extracting solvents are incompatible with HILIC or for preconcentrating the sample. In order to benefit fully from all the advantages of this LC mode, the injection of organic extracts is a great option to simplify the method and reduce manipulation of the sample.

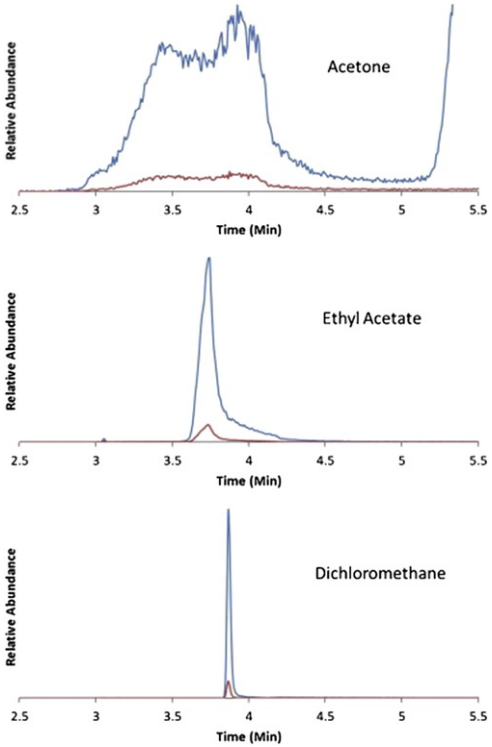

Fig. 3 The effect of three different sample solvents (acetone, ethyl acetate, and dichlormethane) on the peak shape of acrylamide when injecting volumes of 750 µL [18].

alt-text: Fig. 3

4 Application to environmental samples

Within the environmental field, HILIC has been mainly applied to environmental water samples including drinking water, tap water, surface water such as river, creek and lagoon waters, irrigation water and wastewaters from urban treatment plants or hospitals, as can be seen in Table 2.

Table 2 Type of analytes and matrices studied and MS instrumentation used in environmental applications of HILIC.

alt-text: Table 2

	Analytes	Matrix	Column	MS analyzer	Ref
Pharmaceuticals	Pharmaceuticals and illicit drugs	River and wastewaters	Ascentis Express HILIC	ESI-MS	[17]
	Antibiotics	River water, liquid manure and run-off samples	Altima HP	APCI-QqQ	[19]
	Pharmaceuticals	Surface and wastewaters	ZIC-HILIC	ESI-Qtrap	[31]
	Cytostatics	Hospital wastewaters	ZIC-HILIC	ESI-QqQ, ESI-LTQ-Orbitrap MS	[32]
	Pharmaceuticals	Surface and wastewaters	Luna HILIC	ESI-QqQ	[51]
	Antibiotics	Wastewaters	Nucleoshell HILIC	ESI-QqQ	[37]

	Pharmaceuticals and TPs	Ultrapure water	Acquity UPLC BEH Amide	ESI-QTOF	[47]
	Pharmaceuticals and TPs	Ultrapure water, synthetic hard drinking water and wastewater	Poroshell 120 EC-C18 and ZIC-HILIC (in series)	ESI-TOF	[38]
	Pharmaceuticals	Surface waters	ZIC-HILIC	ESI-QqQ	[40]
	Gadolinium contrast media	Tap water	ZIC-cHILIC	ICP-MS	[41]
	Pharmaceuticals	Hospital wastewaters	ZIC-HILIC	ICP-MS	[42]
	Iodinated X-ray media	Wastewaters	ZIC-HILIC	ESI-QqQ	[36]
Toxins	β-N-methylamino-L-alanine (BMAA, neurotoxin)	Cyanobacteria	ZIC-HILIC	ESI-IT-MS, ESI-QqQ	[28]
	Saxitoxin (neurotoxin)	Algae	TSK-gel Amide-80	ESI-IT-MS/MS	[44]
	β-N-methylamino-L-alanine (BMAA, neurotoxin)	River water, biofilm, cyanobacteria	ZIC-HILIC	ESI-QqQ	[29]
	Saxitoxin analogues (neurotoxins)	Algae and cyanobacteria	TSK-gel Amide-80	ESI-QqQ, ESI-QqTOF	[46]
	Toxins	Algae	ZORBAX SB-C18 and Venusil HILIC (in series)	ESI-TOF	[54]
	Domoic acid (toxin)	Lagoon and sea water	ZIC-HILIC	ESI-QqQ	[39]
	Cylindrospermopsin (toxin)	Aquatic organisms	Kinetex HILIC	ESI-QqQ	[27]
Drugs of abuse	Cocaine and metabolites	Surface and wastewaters	ZORBAX Rx-SIL HILIC	ESI-IT-MS/MS	[20–22]
	Drugs of abuse	Surface and wastewaters	Luna HILIC	ESI-QqQ	[50]
	Cocaine and metabolites	Wastewaters	RestekUltra IBD phase (polar-embedded alkyl)	ESI-QqQ, ESI-MSD	[52]
	Drugs of abuse	Wastewaters	Restek Viva PFPP	ESI-QqQ	[53]
Sweeteners	Artificial sweeteners	wastewaters	Kinetex HILIC	ESI-QqQ	[25]
	Artificial sweeteners	Surface and wastewaters	Luna HILIC	ESI-QqQ	[48]
	Artificial sweeteners	River and wastewaters	Syncronis	ESI-Orbitrap	[35]
Other	Estrogens	River water	Luna C18 and ZIC-HILIC (column switching)	ESI-Qtrap	[34]
	Hydroxylated fullerenes	Ultrapure water	XBridge amide	ESI-QqQ	[43]
	Acrylamide	Drinking and surface waters	Atlantis HILIC	ESI-QqQ	[18]
	Melamine	Fish and shrimp	Atlantis HILIC	ESI-QqQ	[24]
	Melamine	Crop, soil and irrigation water samples	ZIC-HILIC	ESI-QqQ	[33]
	Surfactants	Ultrapure water	Atlantis HILIC	ESI-QTOF	[26]

	Pesticides	River water	Atlantis HILIC	ESI-QqQ	[23]
	Herbicides and metabolites	River water	Nucleodur HILIC	ESI-QqQ	[30]
	Methylphosphonic acid (chemical weapon metabolite)	Well, river and tap water	Luna HILIC	ESI-Qtrap	[49]

Of these samples, urban wastewater and river water samples are the most commonly studied, as they give information about the consumption and transportation of EOCs to sewage, their transformation during the treatment processes and their release into the environment [17,21,25,31,35,36,52]. Other types of environmental matrices analyzed using HILIC include algae [44,46], cyanobacteria [28,45,60] and, to a lesser extent, aquatic organisms [27], fish tissue [24], soil [33] and manure [19].

Table 2 shows that the type of EOCs that have been most frequently determined using HILIC are pharmaceuticals, toxins and drugs of abuse. With respect to pharmaceuticals, the list of analytes includes antibiotics, anti-ulcer treatment and anti-inflammatory drugs, β-blockers, antidiabetics, antidepressants, analgesics and iodinated X-ray contrast media, among others. The developed methods can be multi-analyte [31,37] or focused on a single type of pharmaceutical [17,31,38,47], sometimes studying their TPs or metabolites [38,47]. With regard to toxins, the cyanobacterial neurotoxins β-N-methylamino-L-alanine (BMAA) and saxitoxin are frequently studied using the HILIC approach [28,29,44,54,60]. The determination of hydrophilic saxitoxin analogues and domoic acid in environmental samples has also been reported [39,46]. Meanwhile, the drugs of abuse analyzed by HILIC include cocaine and its metabolites benzoylecgonine, ecgonine methyl ester, codeine, morphine, amphetamine and 6-acetylmorphine, among others [17,20,21,50,52].

Recent applications also include the determination of artificial sweeteners in environmental waters [25,35]. HILIC applications have also been reported for pesticides [23], industrial-related chemicals such as surfactants [26], melamine [24,33] and acrylamide [18], and estrogens [34]. The studies by Chao *et al.* [43] on hydroxylated fullerenes and Rodin *et al.* [49] to determine methylphosphonic acid are examples of less common applications of HILIC but very interesting and promising. In general, analytes containing polar functional groups such as amines, carboxylic acids and hydroxyl groups, seems to be excellent candidates to be separated in HILIC phases.

The preferred technique for the sample preparation of environmental waters when using HILIC approaches is SPE, while algae, cyanobacteria or other samples are commonly extracted with acidic aqueous/organic mixtures using agitation or sonication, which may or not be followed by an SPE clean-up step. Of the SPE sorbents available, the most frequently used is Oasis HLB from Waters. Mixed-mode sorbents were used together with HILIC separation for compounds with ionic properties, due to the ability of these sorbents to retain compounds through hydrophobic interactions as well as ionic interactions [31,50,53]. Other approaches, such as activated carbon [23], sorbents prepared in-house [17] or coupling of cartridges [32], have also been used. As previously stated, the main feature of HILIC is the possibility of injecting extracts obtained during the sample treatment steps directly into the HILIC separation system.

The treatment procedure selected for any sample has a great influence on the ME observed in MS-based detectors, because the properties of the final solution to be injected in the chromatographic instrument will affect the ionization, and the clean-up strategies used should contribute to the elimination of interferences. The type of ionization source has also proven to have an effect on the MS response. It has been claimed that the use of HILIC helps to improve ionization in the interface between the LC instrument and the MS detector because the use of high contents of organic solvents facilitates the desolvation process [2,3,9]. Because other parameters apart from the LC conditions affect the ME commonly observed for complex matrices such as environmental waters, it is difficult to ascertain whether or not the use of HILIC has contributed to its reduction. Similarly, the comparison between the HILIC methods developed can be a challenge as different sample treatment procedures and MS conditions are used. Nevertheless, some discussion on how ME is assessed and the results observed for the studies reviewed can be discussed. Most of the MS detectors coupled to HILIC separations are based on tandem MS and they are also equipped with ESI sources, because this ion source is more suitable for polar compounds. In some cases, APCI was tested, achieving lower ME but also lower sensitivity [18]. Table 2 shows that instruments based on ESI-QqQ are the detectors most commonly coupled with HILIC. However, instruments using ion trap (IT or Q-trap) are also reported. In some cases, HILIC has been coupled to HRMS to take advantage of its powerful identification capacity [32,35,46,47], including instruments based on Orbitrap, linear ion trap (LTQ)-Orbitrap, time of flight (TOF) and Q-TOF analyzers.

With respect to HILIC methods applied to environmental samples, some reported low ME values while others reported a high ME affecting the MS response. In several cases, some discussion was made but data was not shown or the ME was simply not subjected to discussion. For instance, Rodin *et al.* [49] reported no ME (−2% in average) for methylphosphonic acid in surface water samples while van Nuijs *et al.* [50] observed ME values up to −51% for drugs of abuse in a similar matrix. Echeverría *et al.* [36] observed high ME values for iodinated X-ray contrast media in wastewater samples even after testing several strategies to reduce it. They claimed that analytes showed a higher response when working in ultrapure water but the signal decreased when analyzing environmental samples. Different studies determining BMAA in cyanobacteria have observed different ME values, being supposedly low [60] according to the recoveries reported, moderate (−40%) [29] or exhibiting suppression of 5 times the response [28]. When discussing the results, little attention is paid to the advantages that HILIC really offers regarding ME, because the comparison of this LC mode with others such as RPLC is frequently done during optimization with ultrapure water in terms of retention and separation. In order to evaluate HILIC performance properly in this respect, comparison with another LC separation must be done using the same complex matrix. Peru *et al.* [19] observed a very high ME for spectinomycin (up to 100% enhancement) when using a RPLC approach while, in the HILIC approach, it was

negligible, because the lack of retention in RPLC caused co-elution with polar interferences in the matrix. Ordóñez *et al.* [55] compared an RPLC method with a HILIC approach in terms of the ME observed for an influent wastewater sample, finding better results for the RPLC separation (−23%– to 3%) versus HILIC (−93% to −31%) observing high signal suppression for saccharine and sucralose (−93% and −90%, respectively). In summary, HILIC has proven to show excellent results within the environmental field for retaining and separating polar and hydrophilic compounds. However, its advantages in terms of ME are not frequently discussed thoroughly.

5 Conclusions

Within the environmental field, HILIC has proven to be an excellent alternative for the determination of several contaminants whose polar properties caused problems in retention, separation and detection when using conventional RPLC methods. Bare silica, zwitterionic, amide and diol are the stationary phases mostly commonly used for environmental applications using ACN/aqueous mobile phases usually containing ammonium formate and acetate buffer solutions. HILIC is mostly coupled with QqQ analyzers using ESI sources (suitable for the polar character of a great number of contaminants), thanks to their adequate selectivity and sensitivity, achieving low quantification limits for these complex samples.

Selected examples showed less matrix effect and enhanced sensitivity when using HILIC. However, discussion addressing schematic comparison in this regard between both RPLC and HILIC is often missing. Future studies should include detailed comparison of both methods for particular groups of analytes not only in ultrapure water but also in complex matrices discussing the advantages regarding matrix effect and sensitivity.

Recent progress focuses on benefiting from the complementary selectivity of RPLC and HILIC, either by coupling columns in series or injecting the samples in two developed methods.

HILIC has established itself as a promising technique for the determination of polar compounds in environmental samples, which could potentially improve chromatographic separation and sensitivity to quantify the contaminants in these highly complex matrices more effectively. Furthermore, current advances in the HILIC technique are mostly the development of new stationary phases which could be applied in future studies in the environmental field.

Acknowledgments

The authors would like to thank the Ministry of Economy and Competitiveness (CTQ2014-52617-P) for the financial support given. D. Salas also acknowledges the Ministry of Economy and Competitiveness for a grant (BES-2012-057792).

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Highlights

- Recent trends in the applications of HILIC-MS for environmental samples analysis.
- Discussion of commonly used stationary phases and mobile phase conditions.
- Advantages of HILIC vs RPLC. Combination of HILIC and RPLC phases.
- Examples of HILIC-MS in environmental field covering different analytes.

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