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Review

Hydrophilic interaction liquid chromatography in food analysis

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ABSTRACT

The use of hydrophilic interaction liquid chromatography (HILIC) in food analysis in the last decade is reviewed. The HILIC mechanism is briefly discussed, but main emphasis is put on the use of HILIC for separation of different food matrices. The food matrices are divided into food of animal origin and related products, vegetables, fruits and related compounds, and other food-related matrices. A list on important applications is provided for each category including experimental conditions and a brief summary of the results. The 100 references included will provide the reader a comprehensive overview and insight into HILIC applications to food analysis.

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1. Introduction

Looking back on the history of liquid chromatography, the pioneer liquid chromatographic systems has been operated in normal mode (NPLC), which means that the stationary phase has been polar and elution has been accomplished by non-polar organic solvents. Such stationary phases, however, exhibited heterogeneity that resulted in peak tailing and non-linear retention factors with varying analyte concentration. Later, the development of stationary phases for chromatography, driven by pharmaceutical studies of water/octanol partitioning, offered reversed phase (RP) systems [1,2]. Bonded octadecyl stationary phases allowed efficient separation of analytes within broad range of polarity and fast column equilibration. The lack of retention of highly hydrophilic compounds with functional groups allowing dipolar bonding on RP has been supplemented by separation on ion exchange chromatography [3] or ion paring on RP [4]. However, large group of strongly hydrophilic compounds without the possibility to receive charge in solution has not been able to receive retention on either stationary phases. The problem has been overcome in GC by derivatization, and recently, in LC by developing hydrophilic interaction chromatography (HILIC) [5]. The concept of HILIC is in a way reversed, where polar stationary phase retains polar analytes that are eluted by mixture of organic solvent (usually acetonitrile) and water. HILIC operation mode is friendly for mass spectrometry (MS) analysis with electrospray ionization (ESI) since analytes are ionized in aqueous organic solution, which enhances ESI process [6]. HILIC separation mechanism is opposite to that of RP systems and those two techniques complement [7]. Therefore it has been popular to employ the two mechanisms in tandem for pre-concentration or separation in two-dimensions [8].

Even though the use of HILIC has been extensively growing during the last decade, its mechanism is still today partly unresolved,

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and we discuss this issue later in the text in more detail. HILIC has been used in all application areas of liquid chromatographic separations, ranging from analysis of small molecules [9], pharmaceuticals [10], metabolites [11], drugs of abuse [12], toxins [13], carbohydrates [14], oligosaccharides [15], amino acids [16], peptides [5], and proteins [17].

This review summarizes most of the work carried out on food analysis using HILIC in the last decade. Dedicated food analysis is becoming more important for the society due to the increased demand for human health and thus analysis of allergens, additives, native and exogenous contaminates and determination of the origin of food is of big interest. Although important, discussion on the sample preparation techniques prior to HILIC and the matrix effects are excluded from this review. The HILIC mechanism will briefly be discussed however, the main focus will be on the applicability of HILIC to different food matrices.

2. HILIC mechanism

The term HILIC was firstly mentioned in the literature in 1990 [5], however, the type of LC, i.e. using a hydrophilic stationary phase and a rather hydrophobic eluent mixture, was already utilized in the separation of sugars fifteen years earlier [18,19]. Despite the old history, the HILIC partitioning theory is still today only based on circumstantial evidence based on the well-known facts, that a hydrophilic surface holds water when exposed to mixtures of organic solvent and water. There are several studies suggesting a more multimodal separation mechanism, involving hydrogen bonding as well as dipole–dipole interactions either between the analyte and water bounded on the surface of the stationary phase or between the analyte and stationary phase respectively. The most common HILIC theory states that HILIC retention is caused by partitioning (not by surface adsorption as in normal phase liquid chromatography (NPLC) [5]) of the injected analytes between the mobile phase and a water-enriched layer in the hydrophilic HILIC stationary phase. The higher the hydrophilicity of the analyte is, the more is the partitioning equilibrium shifted towards the immobilized water layer in the stationary phase, and thus, the more is the analyte retained. Further, the second criterion that distinguishes HILIC from other separation mechanism, e.g. NPLC, is that water is the strongest eluting solvent, i.e. increasing the water content in the mobile phase decreases the retention of analytes [5]. In a recent review published by Irgum et al. [20] several studies on HILIC separations were discussed with focus on the mechanism and these results strongly suggest that the original claim of Alpert [5] was correct.

The selectivity and retention in HILIC are mainly affected by adjusting the eluent by varying the type and fraction of organic solvent, the type and concentration of buffer, and the pH value. Typically retention increases with increasing fraction of organic solvent. pH affects the retention by altering the ionization of both the column material and the analytes investigated. An ionized analyte is more hydrophilic than its neutral form, and thus will also have a stronger retention on HILIC columns. It is also recommended to use buffered mobile phases not only to reduce electrostatic interactions between the silanol groups of the stationary phase and the analyte, but also to enhance or suppress ion-exchange or ion exclusion interactions (electrostatic repulsion–hydrophilic interaction chromatography, ERLIC) [21] contributing to the HILIC retention of analytes on charged polar stationary phases.

The effect of column temperature in HILIC separations is often rather small and typically less than in RPLC, indicating that there is small interaction between the stationary phase and the analyte, but eventually this depends on the nature of the retained analyte. However, in some cases the temperature setting may significantly change not only the retention times and bandwidths, but also the selectivity and the order of elution, depending on the type of analyte and stationary phases (see [22] and references therein). No systematic investigation on the thermal stability of various HILIC columns at elevated temperatures has, however, been carried out. Finally, in many cases the peak capacity obtained by HILIC is much higher than by RPLC for the separation of polar and hydrophilic compounds. However, this depends on the application and on the type of analytes.

2.1. Typical columns and solvents

Generally HILIC stationary phases are divided into three different groups, i.e. neutral, charged, and zwitterionic phases. Using neutral stationary phases (e.g. diol and amide phases) there will be no electrostatic interactions, whereas with charged phases (e.g. plain silica and aminopropyl phases) strong electrostatic interactions take place between the analytes and the stationary phase. Zwitterionic phases (e.g. sulfobetaine silica) exhibit only weak electrostatic interactions with analytes. Plane silica is popular in LC–MS since there are no ligands that can possibly bleed and cause high MS background. More discussion about HILIC stationary phases has been reviewed [20,7]. In HILIC water acts as the strong solvent, hence the higher the organic content the greater the retention of the polar analytes will be [22]. For this reason, solvents typically used in HILIC contain 60–90% of organics and are thus suitable for LC–MS analysis using ESI, without significant changes in ionization efficiency during gradient elution. Acetonitrile, as a polar aprotic solvent, has been proven the best organic solvent for HILIC, while methanol, which is a polar protic solvent, produces wider peaks and gives about the same retention. An interesting organic solvent for the mobile phase is tetrahydrofuran, which is a strong hydrogen bond acceptor (typical feature of polar aprotic solvents) and may result in different analyte elution order than acetonitrile. Polar protic solvents, such as methanol, ethanol, or isopropanol can act as both hydrogen bond donors and acceptors and can compete for active polar sites on the HILIC surface. Therefore analytes, which retention is based on strong hydrogen bonding, are poorly retained.

Approaches for method development in HILIC have been extensively discussed in a publication by Dejaegher et al. [23]. In that work, mainly univariate (one-variable-at-a-time, OVAT, approach) method development of HILIC assays was discussed. The most frequently optimized factor was the composition of the mobile phase. The HILIC assays were most often compared to RP-HPLC methods, and usually a different elution order was observed.

3. Application of HILIC in food matrices

In recent years the use of HILIC separations has significantly grown. The increasing use of HILIC can be seen in Fig. 1, where the number of publications related to food analysis during the last ten years is shown. The high number of works published in 2007–2010 is remarkable. These studies, based on the use of HILIC for food analysis, can be grouped according to the nature of the food matrix: animal origin; vegetables, fruits, and related products; other matrices, as drinks, fungi, and baby food. This classification based on the type of matrix, has also been employed in a recent publication [24] where the works related to the HILIC analysis of polar contaminants, such as pharmaceuticals or pesticides, in food and environmental samples were summarized. Moreover, it must be pointed out that 80% of the publications are related to animal or vegetable matrices (see Fig. 2), i.e. meat, fish, and dairy products (Animal) or cereals and fruits (Vegetable).

The use of HILIC stationary phases allows the use of higher concentrations of organic solvent in the mobile phase, which is

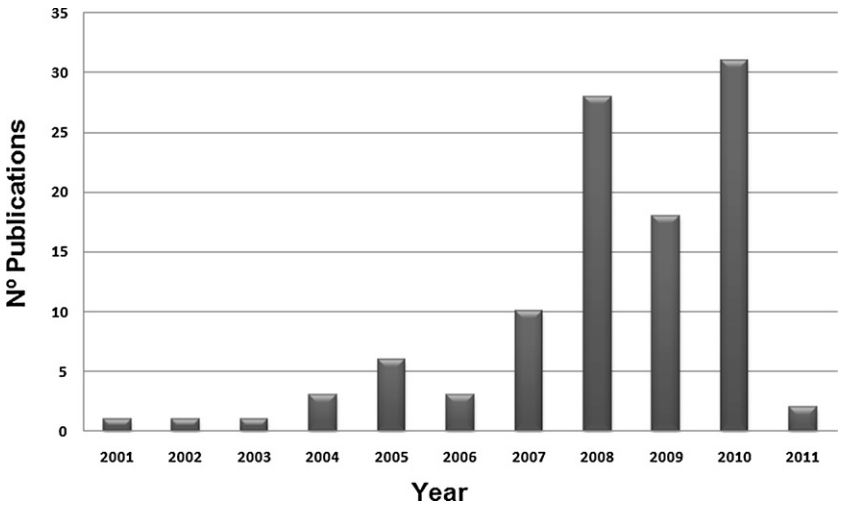

Fig. 1. Evolution of the published works in the last decade in food analysis using HILIC (data up to end of January 2011). The sources of information were the databases ISI-Web of Knowledge, Scirus, and Scopus. The search has been done using as keywords [(Hydrophilic Interaction Chromatography) or (HILIC)] and [(Food) or (Beverages) or (Drinks) or (Vegetables) or (Fruits)].

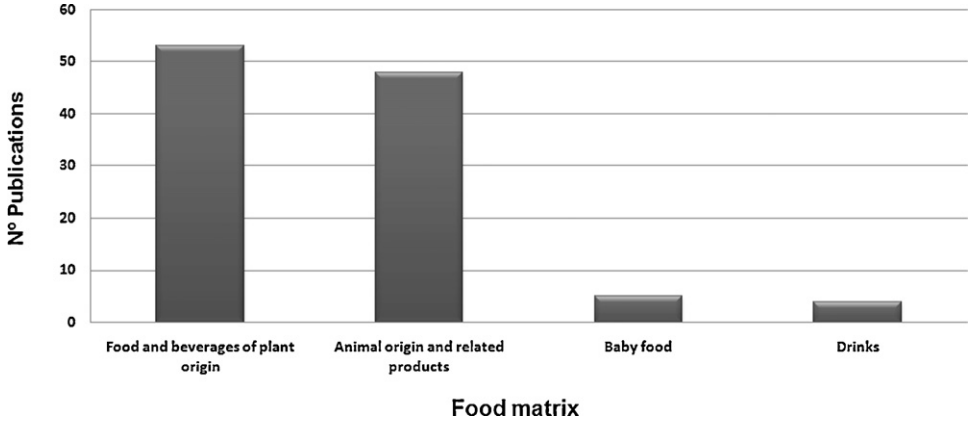

Fig. 2. Summary of food matrices analyzed with HILIC in the last decade.

favorable for mass spectrometric ionization, especially ESI. Meanwhile water or aqueous solvent mixtures play the role of a stronger eluting solvent. The most employed organic solvent is acetonitrile (ACN), and the aqueous solution typically contains salts of ammonium, formate, or acetate. Several stationary phases have been employed (see Fig. 3). The HILIC columns TSK-gel Amide-80 (carbamoyl-functionalized silica stationary phase), ZIC-HILIC (zwitterionic, sulfobetaine-functionalized silica), and Atlantis HILIC Silica (difunctionally bonded ODS) are the most commonly used and are classified as neutral (no electrostatic interactions),

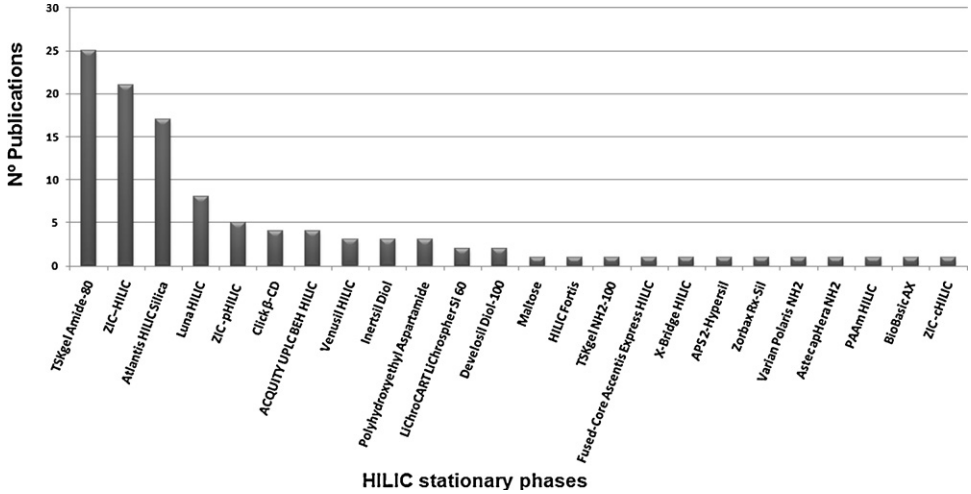

Fig. 3. HILIC stationary phases used in food analysis from 2001 to 2011.

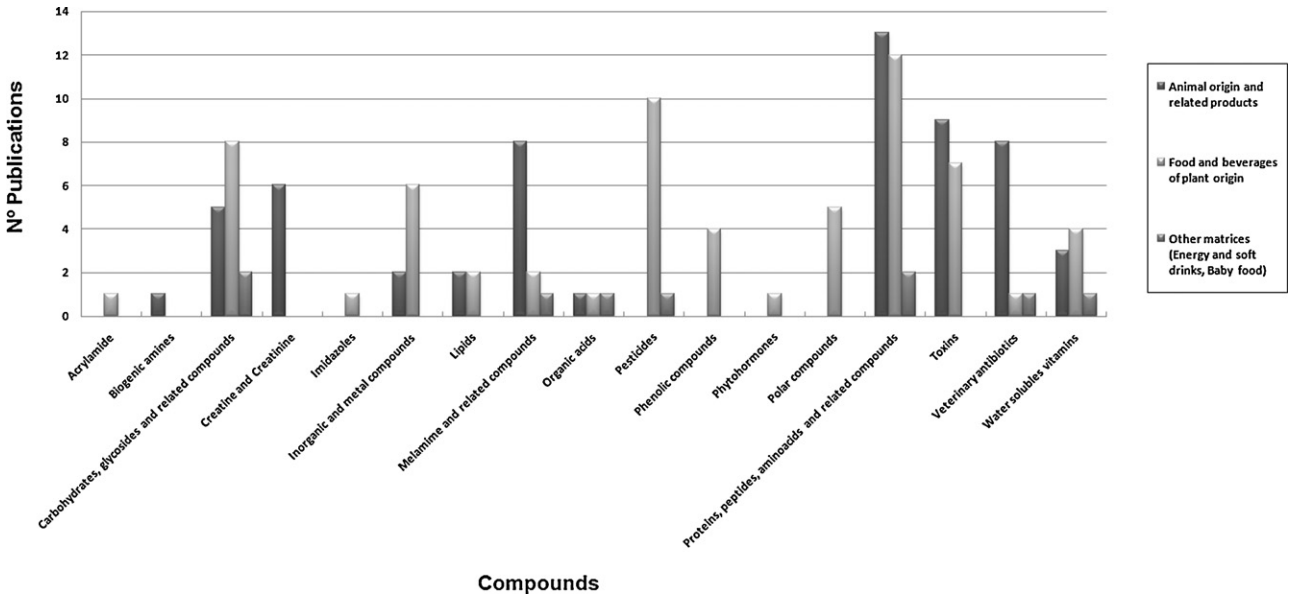

Fig. 4. Summary of the compounds analyzed and their matrix of origin with HILIC.

zwitterionic (weak electrostatic interactions), or charged (strong electrostatic interactions) columns.

A large variety of compounds have been separated by HILIC, including nutritional compounds (proteins, carbohydrates, lipids, or water soluble vitamins) or other types of compounds, such as pesticides or toxins (Fig. 4).

Regarding detection techniques, mass spectrometric and diode array (DAD) detection have been employed the most (Fig. 5). The use of electrospray ionization in mass spectrometry has gained much attention in the last years. The use of low aqueous/high polar organic solvents in HILIC mobile phases is highly compatible with ESI-MS detection, and has in many cases resulted in increased sensitivity [25].

As the aim of this review is to present the applicability of HILIC to food analysis, this section is divided according to the types of food matrices, with special focus on ~~to~~ the compounds analyzed, the HILIC stationary phases, and the detection technique employed.

3.1. Food of animal origin and related products

Food of animal origin is an important source of nutritional compounds for human beings. Animal products are important in the diet as a source of long chain fatty acids – essential elements which cannot be metabolically obtained. Accordingly, food of animal origin improves the diet in terms of essential nutrients.

Proteins are the compounds typically analyzed in this type of foods, as can be seen in Fig. 4, where different types of compounds analyzed in food matrices are summarized. The samples analyzed were composed mainly of different classes of meat (pork, chicken, beef), dairy (milk, eggs, cheese), and fish products (see Table 1).

Dipeptides derived from β-alanine and histidine, like carnosine and its methylated analogues anserine and balenine, which are widely distributed in vertebrate animal tissues, heart, and the central nervous system, have been analyzed by HILIC [26–30]. In most of these works, the matrix was meat (pork, beef, wild boar, chicken) [26–28] and in all the cases the same type of silica HILIC column (Atlantis HILIC Silica) was used. DAD was used in all cases, except for one of the works [26] in which MS/MS with ESI was employed. In the work by Mora et al. [28] a simple and fast method directly applied to the study of meat components, without the need for complex clean-up or sample derivatization, was developed. That study allowed analysis of carnosine, anserine, balenine, creatine, and creatinine in pork and chicken (Fig. 6) using a silica HILIC column.

Also other columns have been used for the determination of similar types of compounds. For example, Dunkel et al. [29] characterized several β-alanyl dipeptides in chicken broth or in stewed beef juice [30] using a carbamoyl-functionalized silica phase and

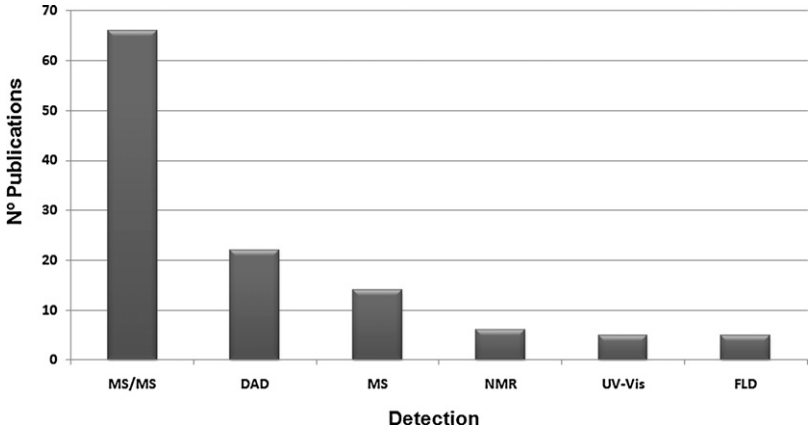

Fig. 5. Detection techniques employed in HILIC food analysis.

Table 1
HILIC applications in the analysis of food of animal origin.

Matrix	Compounds of interest	Stationary phase	Mobile phase	Detection	Reference
Parmesan cheese	Proteins, peptides, amino acids, and related compounds; Organic acids	TSKgel Amide-80 (250 mm × 1.5 mm, 5 μm)	A: Ammonium acetate buffer (0.65 mM, pH 5.5) in 90% ACN, B: ammonium acetate buffer (2.6 mM, pH 5.5) in 60% ACN	ESI-MS/MS	[6]
Pork raw meat, cooked ham, dry cured ham, beef, wild boar, roe deer tissues, Chicken broth tissues	Carbohydrates, glycosides, and related compounds; Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine; Lipids	Atlantis HILIC Silica (150 mm × 4.6 mm, 3 μm), ZIC-pHILIC (150 mm × 4.6 mm, 5 μm), LiChroCART Li Chrospher Si 60 (250 mm × 4.0 mm, 10 μm), TSK gel Amide-80 (300 mm × 7.8 mm, 5 μm)	Different combinations of ammonium acetate, water and ACN depending on each column	ESI-MS/MS, DAD	[26]
Porcine muscles [27], pork loin and chicken breast [28], Cooked ham [54]	Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine	Atlantis HILIC Silica (150 mm × 4.6 mm, 3 μm)	A: 0.65 mM ammonium acetate, pH 5.5, in water/ACN (25:75), B: 4.55 mM ammonium acetate, pH 5.5, in water/ACN (70:30)	DAD	[27,28,54]
Chicken broth	Proteins, peptides, amino acids, and related compounds; Carbohydrates, glycosides, and related compounds	TSKgel Amide-80 (300 mm × 7.8 mm, 5 μm)	*Peptides*: A: (95:5; v/v) of ACN and ammonium acetate (10 mM, pH 6.5), B: ammonium acetate (10 mM, pH 6.5). *Nucleotides and nucleosides*: A: ACN containing 1% formic acid, B: formic acid (1% in water)	ESI-MS/MS, NMR	[29]
Stewed beef juice	Proteins, peptides, amino acids, and related compounds; Carbohydrates, glycosides, and related compounds; Creatine and Creatinine	TSKgel Amide-80 (300 mm × 21.5 mm, 10 μm)	A: TFA (0.1% in water), B: ACN containing 0.1% TFA	ESI-MS/MS, NMR	[30]
Cheddar cheese	Proteins, peptides, amino acids, and related compounds	TSKgel Amide-80 (250 mm × 1.0 mm, 5 μm) and ZIC-HILIC (150 mm × 1.0 mm, 3.5 μm)	A: ammonium acetate buffer (0.50 g/L, pH 5.5) in ACN/water (90:10), B: ammonium acetate buffer (0.50 g/L, pH 5.5) in ACN/ water (60:40)	ESI-MS/MS	[31]
Milk	Proteins, peptides, amino acids, and related compounds	Atlantis HILIC Silica (300 mm × 4.6 mm, 5 μm)	1% formic acid	DAD	[32]
Gouda Cheese	Proteins, peptides, amino acids, and related compounds	TSKgel Amide-80 (300 mm × 7.8 mm, 10 μm)	A: ACN, B: water; both components with 0.1% ammonium acetate (pH 3.0)	ESI-MS/MS, NMR	[34]
Milk hydrolysates	Proteins, peptides, amino acids, and related compounds	Atlantis HILIC Silica (150 mm × 2,1 mm, 3 μm) for the second dimension	A: 0.1% formic acid in ACN, B: B of 10 mM ammonium acetate and 0.1% formic acid	ESI-MS/MS	[35]
Human milk	Glycoproteins	ZIC-HILIC 200 Å (10 μm) only for enrichment	ACN:water;formic acid (50:49:1, v/v/v)	MALDI-TOF-MS, ESI-MS/MS	[36]
Chicken muscle [37], Veal muscle simple [41]	Veterinary antibiotics	ZIC–HILIC (100 mm × 2.1 mm, 3.5 μm) [37] ZIC-HILIC (50 mm × 2.1 mm, 5 μm) [41]	A: 50 mM ammonium formate in water at pH 2.5, B: ACN [37], A: 0.4% formic acid in water, B: ACN [41]	ESI-MS/MS	[37,41]
Chicken muscle, eggs	Veterinary antibiotics	Fused-Core TM Ascentis Express HILIC (100 mm × 2.1 mm, 2.7 μm)	A: ACN, B: 50 mM formic acid/ammonium formate buffer (pH 4)	ESI-MS/MS	[38]
Kidney and muscle tissues	Veterinary antibiotics	ZIC-HILIC (100 mm × 2.1 mm, 5 μm)	A: 1% formic acid/150 mM ammonium acetate, B: ACN	ESI-MS/MS	[39]
Poultry, seafood samples	Veterinary antibiotics	TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)	A: ACN, B: water containing 0.2% formic acid	ESI-MS/MS	[40]
Milk	Veterinary antibiotics	TSKgel Amide-80 (150 mm × 2.0 mm, 3 μm)	A: 50 mM ammonium acetate in water (pH 4.0), B: ACN	ESI-MS/MS	[42]
Milk	Veterinary antibiotics	HILIC Fortis (100 mm × 3.0 mm, 3 μm)	A: 150 mM ammonium formate (pH 4.5), B: ACN	ESI-MS	[43]
Milk and eggs	Veterinary antibiotics	Luna HILIC (150 mm × 2.0 mm, 3 μm)	A: ACN containing 0.05% formic acid, B: water	ESI-MS	[44]
Egg, pork, liver, kidney and pork, shrimp, sausage casing, honey and dairy product [45] Fish filets, trout, salmon, catfish, tilapia and shrimp [50]	Melamime and related compounds	Atlantis HILIC Silica (150 mm × 2.1 mm, 5 μm) [45], Atlantis HILIC Silica (50 mm × 3.0 mm, 3 μm) [50]	A: 10% 100 mM ammonium formate in ACN with pH 3.2, B: 0.1% formic acid in ACN [45] A: ACN, B: 20 mM ammonium formate [50]	ESI-MS/MS	[45,50]
Milk	Melamime and related compounds	Varian Polaris TM NH2 (150 mm × 3 mm, 5 μm)	A: ACN, B: Ammonium acetate 10 mM and 0,1%glacial acetic acid in water	ESI-MS/MS	[46]

Table 1 (*Continued*)

Matrix	Compounds of interest	Stationary phase	Mobile phase	Detection	Reference
Royal jelly and royal jelly lyophilized powder	Melamime and related compounds	Zorbax Rx-Sil (150 mm × 2.1 mm, 5 μm)	A: ACN, B: 5mM ammonium acetate buffer	ESI-MS/MS	[47]
Milk, milk products	Melamime and related compounds	ACQUITY UPLC BEH HILIC (100 mm × 2.1 mm, 1.7 μm)	A: 10 mM ammonium acetate in ACN, B: water	ESI-MS/MS	[48]
Pork, chicken	Melamime and related compounds	BioBasic AX (150 mm × 2.1 mm, 5 μm)	A: ACN, B: isopropanol, C: 50 mM aqueous ammonium acetate	ESI-MS/MS	[49]
Milk powders	Melamime and related compounds	ZIC-HILIC (150 mm × 4.6 and 250 mm × 4.6 mm, 5 μm)	A: ACN, B: 25 mM ammonium acetate, pH 6.8	UV	[51]
Milk, yoghurt, ice cream, milk powder and egg	Melamime and related compounds	Venusil HILIC (250 mm × 4.6 mm, 5 μm)	A: ACN, B: 10 mM ammonium formate buffer solution at pH 3.5.	ESI-MS	[52]
Ham	Creatine and Creatinine	ZIC-pHILIC (150 mm × 4.6 mm, 5 μm)	A: 1 mM of ammonium acetate, pH 5.5, in water/ACN (20:80), B: 1 mM of ammonium acetate, pH 5.5, in water/ACN (70:30).	DAD	[53]
Ham	Water solubles vitamins,	TSKgel Amide-80 (100 mm × 4.6 mm, 5 μm)	A: ACN, B: 0.1% TFA	DAD	[55]
Redfish tissues from	Water solubles vitamins	APS-2 Hypersil (50 mm × 2.1 mm, 3 μm)	A: ACN, B: ammonium acetate	UV, ESI-MS/MS	[56]
Spanish dry-cured sausages	Water solubles vitamins	Luna HILIC (150 mm × 3.0 mm, 3 μm)	A: ACN: 50 mM ammonium acetate pH 5.8 (90:10, v/v), B: ACN: 10 mM ammonium acetate pH 5.8 (50:50, v/v)	DAD	[57]
Milk	Carbohydrates, glycosides, and related compounds	ZIC-HILIC (150 mm × 2.1 mm, 5 μm)	A: MeOH, B: water containing ammonium acetate5 mM	ESI-MS	[58]
Pork loin muscle	Carbohydrates, glycosides, and related compounds	ZIC-pHILIC (150 mm × 4.6 mm, 5 μm)	A: ammonium acetate 150 mM pH 3.5, B: ammonium acetate 100 mM pH 7, C: ACN, D: water	DAD	[65]
Bivalves	Toxins	Luna HILIC (150 mm × 2 mm, 3 μm)	A: 95% ACN with 3.6 mM formic acid and 0.5 mM ammonium formate, B: water with 3.6 mM formic acid and 5 mM ammonium formate	ESI-MS	[61]
Mussels (*Mytilus edulis*)	Toxins	TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm) and ZIC-HILIC (150 mm × 2.1 mm, 3.5 μm)	A: water, B: 95% ACN/water solution (both containing 2mM ammonium formate and 3.6 mM formic acid)	APCI-MS/MS	[62]
Mussel *(Mytilus galloprovincialis*)	Toxins	TSKgel Amide-80 (250 mm × 2 mm, 5 μm)	A: water, B: 95% ACN/water solution (both containing 2mM ammonium formate and 3.6 mM formic acid)	ESI-MS/MS	[13,63,65]
Mussels (*Mytilus edulis* and *M. trossuls*)	Toxins	TSKgel Amide-80 (250 mm × 7.8 mm, 5 μm)	A: water, B: ACN/water (95:5) (both components containing 2.0 mM ammonium formate and 3.6 mM formic acid (pH 3.5))	ESI-MS/MS, NMR	[64]
Mussel and cockle flesh	Toxins	TSKgel Amide-80 (200 mm × 0.05 mm, 5 μm)	A: ACN/water (95:5; v/v), B: water/ACN (95:5; v/v)	ESI-MS	[66]
Puffer fish	Toxins	ZIC-HILIC (150 mm × 2.1 mm, 5 μm)	A: 10 mM ammonium formate and 10 mM formic acid in water. B: 80% ACN and 20% water with a final concentration of 5 mM ammonium formate and 2 mM formic acid.	ESI-MS/MS	[67]
Puffer fish	Toxins	TSK gel Amide-80 (150 mm × 2.0 mm, 5 μm)	A: 16mM ammonium formate (pH 5.5), B: ACN	ESI-MS	[68]
Pig, beef, wild boar and roe deer	Lipids	LiChroCART LiChrospher Si 60 (250 mm × 4.0 mm, 10 μm)	A: water, B: ACN; Both components containing 1% formic acid	ESI-MS/MS	[69]
Milk powder and milk	Inorganic and metal compounds	Inertsil Diol (150 mm × 3.0 mm, 3.5 μm)	A: MeOH, B: 0.1% formic acid (60:40, v/v)	ESI-MS/MS	[70]
Cheese	Biogenic amines	Atlantis HILIC (150 mm × 2.1 mm, 3 μm)	A: ACN, B: ammonium formate 50.0 mM (pH 4)	APCI-MS/MS	[71]
Dogfish muscle	Inorganic and metal compounds	ZIC-HILIC (150 mm × 2.1 mm, 3.5 μm) or (50 mm × 2.1 mm, 3.5 μm)	78–70% ACN and ammonium acetate 125 mM pH 8.3	ICP-MS, ESI-MS/MS	[72]

A and B relate to the mobile phase components; ACN: acetonitrile; MeOH: methanol; TFA: trifluoroacetic acid.

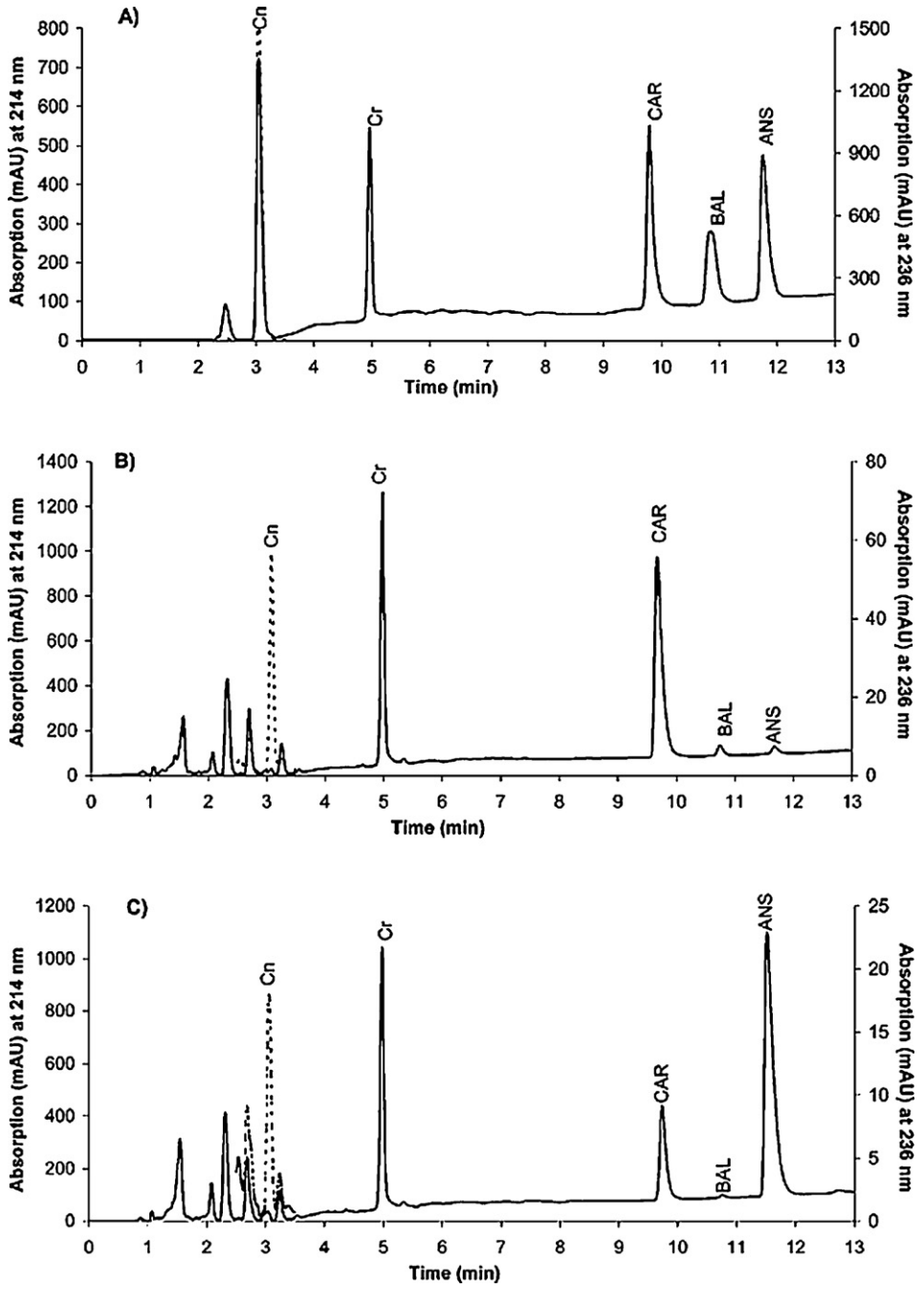

Fig. 6. (A) Chromatogram of the HILIC separation of carnosine (CAR), anserine (ANS), balenine (BAL), creatine (Cr), and creatinine (Cn). (B) Chromatogram corresponding to a pork loin sample. (C) Chromatogram corresponding to a chicken breast sample. In all cases CAR, ANS, balenine BAL, and Cr were detected at 214 nm (solid line), whereas Cn was detected at 236 nm (dotted line). Reprinted with permission from [28]. Copyright 2007 American Chemical Society.

ESI-MS/MS detection. To note is that in these works nuclear magnetic resonance (NMR) was used a complementary identification tool. The same type of column was used for ESI-MS identification of glutamyl dipeptides in Parmesan cheese [6], and for the separation and identification of glycoconjugates between amino acids and reducing sugars (so called Amadori products, APs). Glutamyl di- and tripeptides have also been analyzed in Cheddar cheese with the same column (TSKgel Amide-80) using ESI-MS/MS detection [31], and the conclusion of that study was that glutamyl peptides are not a precondition for the savoury flavor. A zwitterionic HILIC column was also employed in that work [31], aiming at separating two dipeptides. Determination of sugars have been carried out in some other works as well. For example, furosine, which is considered as a suitable marker of the thermal process, was analyzed in milk samples with a silica HILIC column connected to DAD [32]. Other compounds like glycine, diglycine, triglycine, and their APs, which can be found in cooking meat, were separated and determined in standard solutions with the same HILIC stationary phase (Atlantis HILIC Silica) by ESI-MS/MS detection [33].

The potential role of peptides for inducing the bitter taste to cheese was studied. For this purpose a HILIC-ESI-MS/MS method (TSKgel Amide-80) was developed to isolate and identify the most intensely bitter metabolites by using sensory directed fractionation and a total of 16 bitter peptides formed by proteolysis of caseins in Gouda cheese could be identified [34].

Multidimensional approaches have also been employed, for example, a two-dimensional chromatographic method (2D-RPLCxHILIC) with ESI-MS/MS detection was developed for the identification of hydrophilic angiotensin I-inhibiting peptides in enzymatically hydrolyzed milk proteins [35]. A silica HILIC column used in the second dimension enabled further separation of the poorly retained hydrophilic fraction from a RP column. However, the HILIC stationary phases have not only been used as analytical columns, e.g. in one work [36], micro-columns packed with zwitterionic-HILIC material were used to perform glycopeptide enrichment prior to the HPLC-ESI-MS/MS analysis. Veterinary antibiotics (aminoglycosides, β-lactams, coccidiostats, lincosamides, macrolides, quinolones, sulfonamides, and tetracyclines) are commonly used for therapeutic, prophylactic, and/or as feed additives to promote growth in livestock, and as a consequence, monitoring of veterinary drug residues is an important issue to ensure the safety of food. HILIC tandem-mass spectrometry (MS/MS) has become the predominant technique for analyzing their residues in foods [37–42] (see Table 1). However, different HILIC stationary phases have been employed. In some of these works, a zwitterionic ZIC-HILIC column that allowed the determination of 24 multi-class veterinary drugs in chicken muscle [37] or 39 drug residues, including amynoglycosides, in veal muscle was selected [41]. This column has also been employed for the identification of seven kinds of aminoglycoside antibiotics in kidney and muscle [39], while the analysis of *Coccidiostast amprolium* in eggs and chicken muscle was performed with a Fused-Core Ascentis Express HILIC column [38]. Besides, a carbamoyl-functionalized silica HILIC column (TSKgel Amide-80) was used with success for analyzing food additives (azodicarbamides) in poultry and seafood samples by MS/MS detection [40]. HILIC-ESI-MS has been also employed for determination of streptomycin and dihydrostreptomycin in milk samples [43] and antibacterial sulfonamides in milk and eggs [44]. In the first of these works [43], a HILIC Fortis column was employed, whereas the separation of the thirteen sulfonamides was carried out on an amino column (Luna HILIC) [44] in combination with microextraction on a polymer monolith.

Melamine has been the subject of study in many dairy products because of the possible addition of melamine to give a false impression of high protein content. After checking the published literature (see Table 1), it could be stated that melamine and related compounds (ammeline; the hydrolysis product of melamine, ammelide; the hydrolysis product of ammeline, and cyanuric acid) have usually been identified by ESI-MS/MS [45–50]. Due to the low UV absorbance of melamine, DAD detection has been employed only once, and in that study melamine was analyzed in milk with a zwitterionic HILIC column [50]. Since the Chinese infant formula powder contamination incident, as a result of adulteration with melamine for increasing the apparent protein levels, and due to its risk to human health according to the animal toxicological test, the determination of melamine in milk and infant formulas has become necessary. The use of a HILIC amino-bonded column (Varian Polaris NH_2) allowed the determination and confirmation of melamine and its metabolites in an infant milk-based formula by ESI-MS/MS [46]. A new HILIC-ESI-MS method, using a hydrophilic neutral Venusil HILIC column, was recently developed for the simultaneous detection and quantification of melamine and related compounds in milk, yoghurt, ice cream, milk powder, and egg [52]. The effects of different mobile phases were also studied in this work, and it was shown that ESI-MS responses of the analytes with HILIC mobile phases were better than with conventional RP-HPLC mobile phases. The determination of melamine in milk has also been carried out with a bridged ethyl hybrid (BEH) HILIC column [48] and an amino-bonded HILIC column (Varian Polaris NH_2) [46]. Other HILIC stationary phases, like silica, have been employed as well for determining melamine in eggs, pork, shrimp, honey, and dairy products [39] or fish [50]. In addition, melamine residues have been characterized in pork and chicken [49] or in royal jelly [47] with anionic exchange (BioBasic AX) or silica columns (Zorbax Rx-Sil), respectively.

HILIC with DAD detector is the analytical technique of choice for determining creatine and creatinine [26–28,53,54], which play important roles in the energy metabolism of skeletal muscle, taking part in the post-mortem biochemical processes occurring immediately after slaughter. HILIC silica columns have been employed in the analysis of creatine and creatinine in various meat and processed meat samples [26], in porcine muscles [27], in pork loin and chicken breast [28], and in cooked ham [54]. The same compounds were determined in ham using a zwitterionic (ZIC-pHILIC) column [53].

Vitamin C, which is widely used as a food additive because of its antioxidant activity, was analyzed in ham [55] and redfish [56] by HILIC-UV or DAD. Moreover, Drivelos et al. [56] compared the effect of the mobile phase and the column temperature on the mechanism of HILIC retention for the separation of ascorbic and isoascorbic acids. An APS-2 Hypersil column that involved mainly electrostatic interactions due to the positively charged functional groups of the stationary phase, was finally chosen. Not only vitamin C has been determined by HILIC, but also a method to quantify thiamine (vitamin B1) in Spanish dry-cured sausages [57] using an amino HILIC column and DAD as a detector has been developed.

Carbohydrates play numerous essential roles in living beings. Thus, monosaccharides are the major source of energy for metabolism, while polysaccharides serve for the storage of energy and can act as structural components. Galactooligosaccharides, used in novel treatment strategies as functional mimics for the cell-surface toxin receptor, were analyzed by Sinclair et al. [58] using a zwitterionic HILIC column and ESI-MS detection. This column has usually been used to separate nucleosides, nucleotides, and their derivatives [26,59] and detection has been made by DAD or ESI-MS/MS. In the first of these studies [59] comparison between the concentrations of the compounds measured by HILIC and ion-pair reversed-phase chromatography was done. Very good correlation between the data sets was obtained. However, in another publication a different column, a carbamoyl-functionalized silica HILIC column [30], was chosen for the determination of nucleosides in chicken broth.

Paralytic shellfish poisoning (PSP) toxins are potent neurotoxins produced by marine dinoflagellates (*Alexandrium*, *Pyrodinium*, and *Gymnodiniumgenera*) and can accumulate to highly toxic concentrations in shellfish and other filter-feeding organisms, being potentially lethal health hazards to seafood consumers. The most common PSP toxins have been studied, and the principal toxins associated with PSP were saxitoxin and its hydrophilic derivatives [60]. MS/MS is effective for their detection of the compounds in mussels, and the HILIC column mostly used is the carbamoyl-functionalized silica HILIC column, TSKgel Amide-80, even though zwitterionic (ZIC-HILIC) and amino columns (Luna HILIC) have been used as well [61] (see Table 1). It must be noted that ESI was employed in all of the studies, with the exception of one work [62] in which the optimized method for separation, detection, and confirmation of a wide range of PSP toxins comprised a ZIC-HILIC column and detection was made by atmospheric pressure chemical ionization (APCI). This column was also employed in the study of domoic acid [63] and some lipophilic toxins [65] in mussels, as well as PSP toxins, using ESI-MS/MS detection.

The potential of coupling technologies, still on increase, have been used for studying these toxins. Marchesini et al. [66] employed for the first time the combination of biosensor-based bioanalysis with nano-HILIC-TOF-MS and a carbamoyl-functionalized silica HILIC column for determining PSP toxins in mussels and cockle flesh. The samples were screened by using a chip in the PSP inhibi-

tion biosensor immunoassay, then the samples suspected of being non-compliant were reinjected over the recovery chip, and finally injected into a loop-type interface to be separated and analyzed with nano-HILIC-TOF-MS.

Not only PSP toxins have been analyzed, but also tetrodotoxin and related compounds, which induce dangerous marine intoxications, have been determined in puffer fish samples with a zwitterionic HILIC [67] or a carbamoyl-functionalized silica HILIC column [68] and detected by ESI-MS.

Sphingolipids, which are considered protective against carcinogenesis, could also be found in meat matrices (pig, beef, wild boar, roe, deer tissues) [26,69] employing ESI-MS/MS and LiChroCART LiChrospher Si 60 as a HILIC stationary phase.

Finally, also other compounds have been analyzed to some content. For example, organic acids were separated and identified without derivatization in Parmesan cheese using a carbamoyl-functionalized silica column and ESI-MS/MS detection [6]. Quantification of perchlorate in milk powder and milk has been performed with a dihydroxypropyl bonded HILIC column, which provided the shortest separation time, and detection was made by ESI-MS/MS [70]. A HILIC-APCI MS/MS method (in positive ionization mode), using a silica column, was developed for the determination of seven biogenic amines in cheese [71]. Organoarsenic compounds are used as insecticides, herbicides, and fungicides, and their high toxicity and impact on human health has resulted in much research on the quantification and speciation of these in various matrices. Successful separation of nine organoarsenic compounds in dogfish muscle was carried out with a zwitterionic HILIC column, using ICP-MS and ESI-MS/MS detection [72].

3.2. Food and beverages of plant origin

Vegetables and fruits are essential for the balance of the human diet. Their contribution to the maintenance of the correct level of fibers, vitamins, and minerals in the body, which can help to prevent chronic diseases, is especially relevant. Several published studies demonstrated that people who eat enough vegetables and fruits have less cardiovascular disease, cancer, and risk of stroke than those who consume minimal amounts [73–75]. As previously mentioned, this food group is the second most commonly analyzed by HILIC techniques (see Fig. 2).

Proteins and carbohydrates have been thoroughly studied in this type of foods (see Fig. 4). Table 2 summarizes different types of matrices, such as vegetables (broccoli, tomato, cucumbers, soybeans), fruits (apples, pears, grapes), and cereals (wheat gluten, bread, maize), analyzed by HILIC.

Furosine, has been analyzed not only in animal food but also in processed cereals and crisp bread with a silica HILIC column and DAD [32]. Free amino acids and many glutamyl di- and tripeptides were identified in wheat gluten hydrolysate utilizing MS/MS detection and a TSK gel Amide-80 column [6]. Besides, these compounds have also been determined in beans with the help of NMR [76] and in three different kinds of wheat gluten [77], where in all cases [6,76,81], the polar fraction was studied after gel permeation chromatography.

Other compounds like glycine, diglycine, triglycine, and their APs, were separated with a silica HILIC column, using ESI-MS/MS detection [33]. This is of practical usefulness in the analysis of products in roasting of coffee beans, baking of bread, or toasting of cereals. Moreover, γ-aminobutyric acid (GABA), the chief inhibitory neurotransmitter in the mammalian central nervous system and the compound directly responsible for the regulation of muscle tone, was quantified without derivatization in apple, orange juice, potato, and soybeans employing a zwitterionic HILIC stationary phase and MS/MS detection [78]. Meanwhile, this compound has been also studied in morel mushrooms, which are used as tasty ingredients, by using a carbamoyl-bonded column in combination with ESI-MS and NMR detection [79]. In another application, betaine and free choline were analyzed in cereals using a bridged ethyl hybrid UHPLC HILIC column and ESI-MS/MS detection [80]. The separation and detection of carbohydrates is very complicated due to chirality, the possible presence of anomers, the existence of structures containing branching, crosslinking, and several differences in their three-dimensional structures. The use of carbamoyl-functionalized silica HILIC columns and MS or MS/MS detection have been employed for the analysis of highly polar compounds, such as amino acids, carbohydrates such us glycosides, oligosaccharides, and nucleotides, in *Curcubita maxima* pumpkin [81] or in plant metabolomics studies [82], where the HILIC results were compared to RPLC. It was concluded in this work [82], that both approaches could be useful in plant metabolomic, as they performed equally well with regard to retentive capacities, but they perform complementary instead of comprehensive in the perspective of LC–MS based metabolomics. This means, that the use of HILIC next to RPLC could provide an extra value in the coverage of plant metabolome by detecting potential biomarkers that are not retained by RPLC. It must be pointed out, that Spagou et al. [83] have described in a recent publication the applications and the utility of HILIC-MS in metabolomic/metabonomic studies and certain characteristic examples in plant-food sciences are highlighted. Other glucosinolates, i.e. organic compounds containing sulfur and nitrogen, derived from glucose and an amino acid, have been determined by using DAD as a detector, and a zwitterionic HILIC column in plant extracts [84] and in Broccoli [85]. In the first of these works [84], the use of polyhydroxyethyl aspartamide columns was discarded due to degradation observed after 100 injections, and separations were successfully performed in columns with zwitterionic stationary phases composed of sulfoalkylbetaine functional groups by simple adjustment of ionic strength and pH.

Analysis of carbohydrate-related metabolites (ranging from neutral sugars and sugar alcohols to negatively charged sugar phosphates) in *Arabidopsis thaliana* has been carried out using a ZIC-HILIC column and ESI-MS/MS [86], while a monolithic silica capillary column modified with polyacrylamide was developed and tested for highly efficient separation and sensitive detection of non-derivatized carbohydrates with the same detector [87]. As a special case, a HILICxHILIC-QTOF-MS system was developed for identifying saponins from *Quillaja saponaria* using a carbamoyl-functionalized silica HILIC column for the first dimension and a short neutral, polar column (PolyHydroxyethyl Aspartamide) in the second dimension [88].

There are more than 4000 compounds under the denomination of “phenolic compounds”, which have been associated with several health promoting activities like decreasing blood sugar levels, reducing bodyweight, antioxidant, anticarcinogenic, and anti-inflammatory effects [89]. Flavonoids from licorice extracts, that co-eluted on RP columns, were well separated with a silica HILIC column, while a cyclodextrin (CD)-based column was useful for the separation of other isoflavones in a kudzu extract sample, using DAD detection in both separations [90]. This result suggests that well-retained compounds on RP columns can be successfully separated with HILIC columns. The combination of both techniques offers great potential in the development of 2D methods for analysis of complex samples. For example, Kalili et al. have developed an off-line 2D HILICxRPLC method for the analysis of phenolic derivates [91,92]. They employed a zwitterionic HILIC column for procyanidins in apples and cocoa beans [91], and phenolic compounds (proanthocyanidins, phenolic acids, flavonols and flavonol derivates) in green tea [92]. Detection was done by fluorescence (FLD), DAD, and ESI-MS/MS detection. It must be pointed out that these compounds cannot be separated in a single analysis by conventional 1D HPLC methods.

Table 2
HILIC applications in the analysis of food and beverages of plant origin.

Matrix	Compounds of interest	Stationary phase	Mobile phase	Detection	Reference
Processed cereals and crisp bread	Proteins, peptides, amino acids, and related compounds	Atlantis HILIC silica (300 mm × 4.0 mm, 5 μm)	1% formic acid	DAD	[32]
Wheat gluten hydrolysate [6], dried beans [76]	Proteins, peptides, amino acids, and related compounds	TSKgel Amide 80 (250 mm × 1.5 mm, 5 μm) [6] TSKgel Amide-80 semipreparative (300 mm × 7.8 mm, 5 μm) or preparative scale (300 mm × 21.5 mm, 10 μm)[76]	A: 0.1% TFA in water B: ACN containing 0.1% TFA [76] *Positive mode*: A: Ammonium acetate buffer (0.65 mM, pH 5.5) in 90% ACN, B: ammonium acetate buffer (2.6 mM, pH 5.5) in 60% ACN. *Negative ionization mode* pH is 7.0 [6]	ESI-MS/MS, NMR [76]	[6,76]
Three different wheat gluten hydrolysates	Proteins, peptides, amino acids, and related compounds;	TSKgel Amide-80	A: Ammonium acetate buffer (6,5mM, pH = 5,5 in 90% ACN), B: Ammonium acetate buffer (7.5 mM, pH = 5,5 in 60% ACN)	ESI-MS/MS	[77]
Apple, orange juice, potato and soybeans	Proteins, peptides, amino acids, and related compounds	ZIC-HILIC (150 mm × 2.1 mm, 3.5 μm)	A: 40 mM ammonium bicarbonate (pH 7) with formic acid, B: MeOH.	ESI-MS/MS	[78]
Morel mushrooms	Carbohydrates, glycosides and related compounds; Proteins, peptides, amino acids and related compounds	TSKgel Amide-80 (300 mm × 7.8 mm)	A: 80/20 of ACN and aqueous ammonium formate (7 mM; pH 5.5), B: 20/80, of ACN and aqueous ammonium formate (7 mM; pH 5.5)	ESI-MS, NMR	[79]
Cereals flour and cereals products	Proteins, peptides, amino acids, and related compounds	ACQUITY UPLC BEH HILIC (150 mm × 2.1 mm, 1.7 μm)	A: 10 mM ammonium acetate containing 0.005% acetic acid, B: ACN	ESI-MS/MS	[80]
Curcubita maxima pumpkin	Carbohydrates, glycosides, and related compounds; Proteins, peptides, amino acids, and related compounds	TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)	A: ACN, B: 6.5 mM ammonium acetate pH 5.5.	ESI-MS/MS	[81]
Arabidopsis thaliana	Proteins, peptides, amino acids, and related compounds; Lipids; Phenolic compounds; Carbohydrates, glycosides and related compounds	TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)	A: water containing 0.1% v/v formic acid, B: 90:10 ACN/water containing 0.1% v/v formic acid	ESI-MS	[82]
Plant extracts [84], *Arabidopsis thaliana* [86]	Carbohydrates, glycosides, and related compounds	ZIC-HILIC (150 mm × 4.6 mm, 5 μm) [84], (150 mm × 2.1 mm, 3.5 μm) [86]	15 mM ammonium formate pH 4.5 in ACN:H_2O [84] A: ACN modified with 0.1% (v/v) formic acid, B: 5mM ammonium acetate modified with 0.1% (v/v) formic acid (pH 4) [86]	DAD [84], ESI-MS/MS [86]	[84,86]
Broccoli and other vegetables	Carbohydrates, glycosides, and related compounds	Polyhydroxyethyl Aspartamide (100 mm × 4.6 mm, 3 μm)	A: ACN, B: water with 30 mM ammonium formate pH 5.4.	DAD	[85]
Soybean, corn and *Arabidopsis thaliana*	Carbohydrates, glycosides, and related compounds	200T-PAAm HILIC (285 mm × 0.2 mm)	A: ACN, B: buffer solution of 13 mM ammonium acetate (pH = 5.5).	UV–vis, ESI-MS/MS	[87]
Quillaja saponaria	Carbohydrates, glycosides, and related compounds	First dimension: TSKgel Amide-80 column (150 mm × 2.0 mm, 3 μm), second dimension: a laboratory-prepared PolyHydroxyethyl A (p-OH-Et A) (35 mm × 2.1 mm, 5 μm)	A: ACN, B: 1% formic acid for the first dimension. A: ACN, B: 10 mM ammonium acetate and 1% acetic acid for the second dimension	ESI-MS/MS	[88]
Licorice and kudzu extracts	Phenolic compounds	Atlantis HILIC Silica (250 mm × 64.6 mm, 5 μm) and CD-column (150 mm × 64.6 mm, 5 μm)	A: water with 0.1% formic acid, B: ACN with 0.1% formic acid	DAD	[90]
Green tea, apple and cocoa beans	Phenolic compounds	Develosil Diol-100 (250 mm × 1 mm, 5 μm)	A: ACN and acetic acid (99:1, v/v), B: MeOH, water and acetic acid (94.05: 4.95:1, %v/v/v)	DAD, FLD, ESI-MS/MS	[91,92]

Carthamus tinctorius Linn.	Polar compounds; Phenolic compounds; Lipids; Carbohydrates, glycosides, and related compounds	Maltose (150 mm × 4.6 mm, 5 μm) as the first dimension and TSK gel Amide-80 (150 mm × 4.6 mm, 3 μm) or Click β-CD (150 mm × 2.1 mm, 5 μm) as the second dimension	*First dimension* A: ACN, B: water; both components containing 0.1% formic acid *Second dimension* A: ACN/water (95:5, v/v), B: water; both components containing 10 mM ammonium acetate.	DAD	[93]
Ligusticum chuanxiong, Chinese herb	Polar compounds; Phenolic compounds; Lipids Carbohydrates, glycosides, and related compounds	Venusil HILIC (250 mm × 4.6 mm, 5 μm)	A: water containing 0.1% formic acid, B: MeOH, C: ACN	DAD	[94]
Carthamus tinctorius Linn.	Polar compounds; Phenolic compounds; Lipids; Carbohydrates, glycosides, and related compounds	β-cyclodextrin (150 mm × 4.6 mm, 5 μm)	A: ACN with 2.5% 200 mM ammonium acetate aqueous solution and 0.1% acetic acid/ H_2O with 2.5% 200 mM ammonium acetate aqueous solution, B:0.1% acetic (93:7, v/v)	DAD	[95]
Carthamus tinctorius Linn.	Polar compounds; Phenolic compounds; Lipids; Carbohydrates, glycosides, and related compounds	Click β-CD column (150 mm × 4.6 mm, 5 μm) as the second dimension	A: water, B: ACN; both components containing 0.1% formic acid.	DAD	[96]
Lonicera japonica	Hydrophilic and hydrophobic solutes	Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm)	A: ACN, B: 100 mM ammonium formate	UV–vis	[97]
Tomato [98], Fruits, tomatoes, cucumbers, tamarillos, papaya, and broccoli [99]	Pesticides	ZIC-pHILIC (150 mm × 2.1 mm, 5 μm)[98], (150 mm × 4.6 mm, 5 μm) [99]	A: 5 mM ammonium acetate, B: ACN [98] A: ACN, B: 10 mM aqueous ammonia [99]	ESI-MS/MS	[98,99]
Watermelon, spinach, potato, tomato, and radish-root plants	Pesticides	Venusil HILIC column (250 mm × 4.6 mm, 5 μm)	A: water containing 0.1% formic acid, B: ACN	DAD, ESI-MS/MS	[100]
Apple, vegetables, wheat, tea, dried mushrooms and black pepper powder.	Pesticides	ACQUITY UPLC BEH HILIC (50 mm × 2.1 mm, 1.7 μm)	A1: MeOH, A2: ACN, B: Water, in different composition	ESI-MS/MS	[101]
Leek, cucumber, mushroom	Pesticides	Luna HILIC (100 mm × 2.0 mm, 3 μm)	A: water containing 0.1% formic acid, B: ACN	ESI-MS/MS	[102]
Mellow apple	Pesticides	Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm)	A: water, B: ACN; both components containing 0.05% formic	ESI-MS/MS	[110]
Fresh fruits, juices, beer, bread, mushrooms, and coffee powder	Pesticides	Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm)	A: ACN, B: Formic acid-ammonium formate buffer (pH 3.75)	ESI-MS/MS	[111]
Blue-green algae	Toxins	TSKgel Amide-80 (150 mm × 2.0 mm)	A: 0.05% TFA, B: ACN	ESI-MS	[103]
Cycad seed and cyanobacterias [104], algae [108]	Toxins	ZIC-HILIC (50 mm × 2.1 or 150 mm × 2.1 mm, 5 μm)[104], (250 mm × 64.6 mm, 5 μm)[108]	A: ACN, B: 60 mM formic acid [104] A: 10 mM ammonium formate and 10 mM formic acid, B: 80% ACN and 20% water with 5 mM ammonium formate and 2 mM formic acid [108]	ESI-MS/MS	[104,108]
Maize Plants	Toxins	ZIC-HILIC (150 mm × 4.6 mm, 3.5 μm)	A: ACN, B: water buffered with 100 mM ammonium formate (pH 6.4)	DAD and ESI-MS	[105]
Panax plant species	Toxins	Atlantis HILIC Silica (50 mm × 2.1 mm, 3 μm)	A: 200mM ammonium formate (pH 3.0), B: ACN	ESI-MS/MS	[106]
Potato tuber	Toxins	Polyhydroxyethyl A HILIC (100 mm × 2.1 mm, 3 μm)	A: 5 mM ammonium acetate (pH 5.5 with acetic acid), B: ACN	ESI-MS/MS	[107]
Microalgae	Toxins	Luna HILIC (150 mm × 2.0 mm, 3 μm)	A: ACN, B: water; both components containing 3.6 mM formic acid and 5 mM ammonium formate.	FLD, ESI-MS	[109]
Chestnut, fruit juices	Water solubles vitamins	TSK gel Amide-80 (100 mm × 4.6 mm, 5 μm)	A: ACN, B: 0.1% TFA	DAD	[55]
Dried fruit, tea drinks	Water solubles vitamins	Inertsil Diol (250 mm × 4.6 mm, 5 μm)	A: ACN, B: water, C: formic acid	UV	[112]
Soybean milk, soybean powder	Melamime and related compounds	Atlantis HILIC Silica (150 mm × 2.1 mm, 5 μm)	A: 10% 100mM ammonium formate in ACN at pH 3.2, B: 0.1% formic acid in ACN	ESI-MS/MS	[45]
Bakery goods and flour	Melamime and related compounds	ACQUITY UPLC BEH HILIC (100 mm × 2.1 mm, 1.7 μm)	A: 10 mM ammonium acetate in ACN, B: water	ESI-MS/MS	[48]

Table 2 (*Continued*)

Matrix	Compounds of interest	Stationary phase	Mobile phase	Detection	Reference
Algae and phytoplankton samples	Inorganic and metal compounds	ACQUITY UPLC BEH HILIC (50 mm × 2.1 mm, 1.7 μm)	A: water with 2% ACN, B: ACN	ESI-MS/MS	[113]
Potato Snacks	Inorganic and metal compounds	X-Bridge HILIC (50 mm × 2.1 mm, 3.5 μm)	A: water, B: ACN; Both components containing 0.1% formic acid	ESI-MS/MS	[114]
Arabidopsis thaliana, wheat plant	Inorganic and metal compounds	ZIC-HILIC and ZIC-cHILIC (150 mm × 61.0 mm)	A: 10 mM ammonium acetate/ACN (10/90) at pH 7.3; B: 30 mM ammonium acetate/ACN (80/20) at pH 7.3	ESI-MS	[115]
Se-rich yeast	Inorganic and metal compounds	Atlantis HILIC Silica (150 mm × 2.1 mm, 5 μm)	A: ACN, B: water containing 1mM ammonium acetate and 10mM acetic acid (pH 4.7)	ESI-MS/MS, ICP-MS	[116]
Se-rich yeast	Inorganic and metal compounds	TSKgel Amide-80 (250 mm × 4.6 mm, 5 μm)	A: 0.1% (v/v) TFA in ACN, B: 0.1% (v/v) TFA in water	ESI-MS/MS, ICP-MS	[117,118]
Crisp bread and potato	Acrylamide	ZIC-HILIC (150 mm × 2.1 mm, 5 μm)	Different combinations of water, MeOH and ACN	ESI-MS/MS	[119]
Coffee podwer	Imidazoles	Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm)	A: MeOH, B: 0.01% ammonium formate	ESI-MS	[120]
Oryza sativa, *Arabidopsis thaliana* and oil seed tissue	Phytohormones	Luna HILIC (250 mm × 2.0 mm, 5 μm)	A: 85% ACN, B: 15% water; both components containing 0.01% formic acid	ESI-MS/MS	[121]
Extra-virgin olive oil [122], white wine [123]	Lipids [122], glycoproteins [123]	ZIC–HILIC 200 Å (10 μm) only for extraction of polar compounds [122], or enrichment of glycoproteins [123]	ACN:water (1:1, v/v), and 0.1% formic acid [123]	MALDI-TOF-MS	[122,123]

A and B relate to the mobile phase components; ACN: acetonitrile; MeOH: methanol; TFA: trifluoroacetic acid.

Traditional Chinese medicines (TCMs), which have been used in Chinese medicine for thousands of years, have extensively been analyzed. TCMs are always dissolved in water samples, like tea or coffee, which then can be drunk with therapeutic purposes. Due to the dissolution in water many polar compounds (such us flavonoids, polyacetylenes, serotonin derivatives, steroids, lignans, alkane diols, etc.) are included in decoctions. Detection has typically been done by DAD or UV–vis [93–97]. For their separation, different HILIC columns have been used to produce chemical information that could be overlooked in conventional RPLC analysis. For example, a fingerprint analysis of polar compounds in *Ligusticum chuanxiong* was developed using a Venusil HILIC column [94] and analysis of the compounds in *Carthamus tinctorius* Linn. was achieved with a β-cyclodextrin (β-CD) column [95].

Compounds in *C. tinctorius* Linn. has also been studied by two-dimensional chromatography [93,96,97]. In one of these works [96] a 2D-RPLC/HILIC method was developed for the analysis of compounds and the β-cyclodextrin (β-CD) column in the second dimension resulted in a chromatogram containing 42 peaks. In comparison, when the same sample was injected onto a C18 column, only five peaks appeared (Fig. 7). In other study a silica HILIC column (Atlantis HILIC) column was used in the first dimension [97], while a 2D-HILIC method, with a maltose column in the first dimension and a carbamoyl-functionalized silica HILIC column (TSKgel Amide-80) or a β-CD column in the second dimension, was used in the analysis of very polar components in *C. tinctorius* Linn.

Pesticides have been frequently studied in vegetables, and ESI-MS/MS has been the most commonly employed analytical tool in their determination (see Table 2). Dithiocarbamate (DTC) fungicides are widely used in agriculture and horticulture due to their broad spectrum of activity, low acute mammalian toxicity, and low production costs. This group of fungicides has been analyzed with a ZIC-pHILIC stationary phase in combination with a quick, easy, cheap, effective, rugged, and safe extraction method (QuEChERS) for the extraction of propylenethiourea in tomato [98]. The same column allowed determination of DTC fungicide residues in vegetables and fruits [99]. Glyphosate and glufosinate, which are polar phosphonic herbicides, have been determined in several vegetable matrices and water with a Venusil HILIC column and DAD [100]. Methamidophos, a organophosphate insecticide, has been determined in several types of vegetables and fungi by ESI-MS/MS employing a bridged ethyl hybrid HILIC column [101]. Meanwhile cyromazine, which is a triazine insect growth regulator used as an insecticide and an acaricide, and its metabolite melamine, have been identified in different plant matrices (leek, cucumber, mushrooms) with a Luna HILIC column and ESI-MS/MS detection [102].

The analysis of toxins has been reported in several works (see Table 2), and MS has been the most commonly employed detector for identification. A cyanobacterial neurotoxin has been analyzed in blue-green algae utilizing a carbamoyl-functionalized silica HILIC column [103], while in another work a zwitterionic HILIC column [104] it was selected for its determination in cycad seed. Other toxins, such as moniliformin, a mycotoxin frequently found in cereals and maize, has been analyzed in whole maize plants employing a zwitterionic HILIC column [105], while a neurotoxic agent was found in *Panax* plants when using a HILIC Silica column [106]. In both cases ESI-MS/MS was selected as a detector. The determination of glycoalkaloids, which are of great concern for potato producers due to their toxicity, was carried out with a polar column (PolyHydroxyethyl Aspartamide). This method was compared with a RPLC method [107], and results proved the RPLC procedure to be more precise, accurate, and rugged than the HILIC method. Algae, a potential natural source of vitamins, have been subjected to study in several publications. For example, analyses of saxitoxin (a neurotoxin) and its analogues, which are agents of PSP and naturally synthesized by some algae (marine dinoflagellates

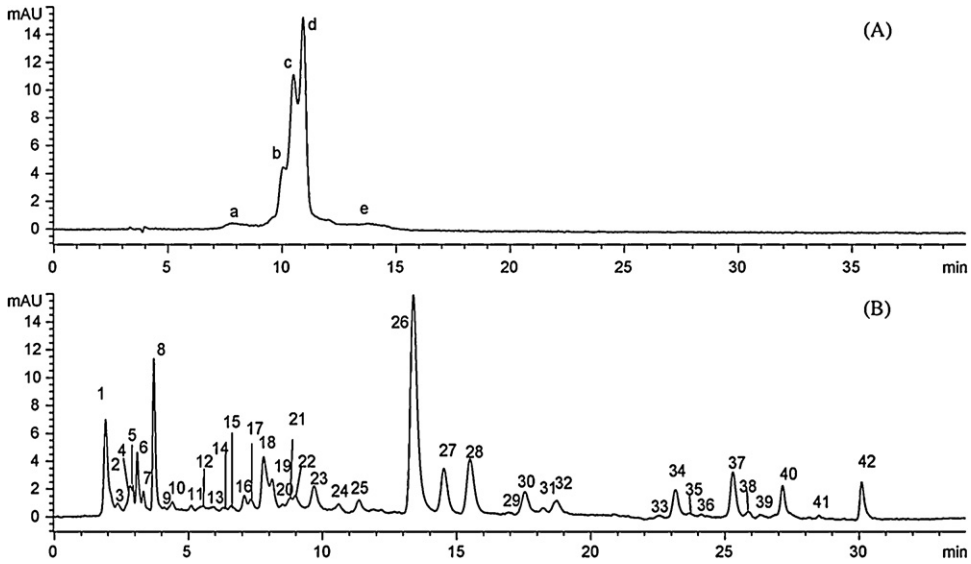

Fig. 7. The HPLC/UV (280 nm) chromatograms of fraction 7 (eluted between 10 and 11 min in Fig. 1B from [96]) on a C18 column (A) and click β-CD column (B). Different sets of peak numbers are marked in the two chromatograms. Reprinted from [96], with permission from Elsevier. Copyright 2008.

of the genus *Alexandrium, Gymnodinium*, *Pyrodinium*) were carried out with zwitterionic [108] or amino [109] HILIC stationary phases, using ESI-MS/MS or FLD.

A HILIC silica column was successfully used to retain and separate streptomycin (an antibiotic) in mellow apples [110] and two quaternary ammonium growth regulators in fresh fruit, juices, beer, mushrooms, vegetables, bread, and coffee powder [111]. In both of the studies solid phase extraction (SPE) was used for sample clean-up.

HILIC using a carbamoyl-functionalized silica column and DAD as a detector was shown to be a fast, simple, flexible, and robust alternative to conventional ion-pair and RP methods for the determination of vitamin C in chestnuts and fruit juices [55]. In addition, the use of a dihydroxypropyl column and UV–vis detection also enabled simultaneous determination of ascorbic acid and other three related products in dried fruit and tea drinks [112].

The number of studies devoted to the determination of melamine and similar compounds in vegetables, fruits, or related products are shown in Fig. 4. In one of these works, a bridged ethyl hybrid HILIC column and ESI-MS/MS was employed for the analysis of melamine levels in bakery goods and flour [48]. In another study, a silica HILIC column and ESI-MS/MS detection was used for determining melamine and cyanuric acid in soybean milk and powder [45], after sample pretreatment by SPE.

Some metal species have been analyzed in these matrices due to their central role in plants. For example, dimethylsulfonic propionate, which is an osmolyte produced from marine plankton, macro-algae, and higher plants, has been determined with a bridged ethyl hybrid HILIC column using ESI-MS/MS detection [113]. This detector has also been utilized for analyzing traces of bromate, used to enhance the maturing process and baking of flour products, in potato snacks using a X-Bridge HILIC column [114]. Two different zwitterionic stationary phases, a sulfobetaine-type material (ZIC-HILIC) and a phosphatidylcholine-type material (ZIC-cHILIC), were compared under identical conditions and their suitability for separating small, noncovalent metal species, and free ligands in plants was studied [115].

Yeast rich in selenium or mushrooms are the matrices most investigated in products originated from fungi (see Table 3). Selenium, an essential trace element, is known for its antioxidant activities and has been proposed to be an important ingredient at low levels in food due to its implication in cancer prevention. One of the most popular selenium supplements is yeast and selenium is mainly included into yeast proteins in the form of selenomethionine. In these works ICP-MS or MS/MS were chosen for determining selenium metabolites in a water extract of selenium-rich yeast [116–118]. Different types of HILIC columns were used in these studies. In two of the works separation was done with a carbamoyl-functionalized silica HILIC column [117,118], while in another study a silica HILIC column was employed [116], the latter offering better separation than under normal phase conditions.

The applicability of HILIC to other compounds in vegetables, fruits, and related products, has been carried as well and, e.g. comparative retention studies of acrylamide in crisp bread and potato on RP and HILIC columns has been done using ESI-MS/MS detection [119]. In this case the zwitterionic HILIC column resulted in low retention of the analytes and was hence left out of the study. In another study, two imidazoles coffee powders were separated after SPE clean-up or supercritical fluid extraction on a HILIC Silica column connected to ESI-MS [120]. Cytokinins, which are a vital group of phytohormones, have been analyzed in plant matrices and oil seed rape tissues. A comparative study between HILIC and RPLC was carried out, demonstrating higher sensitivity and better separation of the compounds with an amino column than with conventional RP columns [121].

Finally, in recent studies, zwitterionic HILIC columns were used for the extraction and enrichment of the polar fractions of extra virgin olive and hazelnut oils [122] or glycoproteins in white wine [123]. In the first case [122], sample treatment with ZIC-HILIC columns prior to MALDI-TOF-MS analysis sphingolipids was employed in order to detect possible adulteration of the olive oil with hazelnut oil [122]. In the other case [123] a zwitterionic micro-column was employed as a SPE cartridge and identification was done with LTQ-Orbitrap XL MS using electrospray ionization.

3.3. Other matrices

Table 3 summarizes the use of HILIC in the analysis of compounds in other food matrices than the aforementioned over the reviewed period (2001–2011). As shown in Fig. 4, proteins and related compounds along with water soluble vitamins are the compounds that have been mostly analyzed in beverages.

Table 3
HILIC applications in the analysis of drinks and baby foods.

Matrix	Compounds of interest	Stationary phase	Mobile phase	Detection	Reference
Baby food	Veterinary antibiotics	TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)	A: ACN, B: water containing 0.2% formic acid.	ESI-MS/MS	[40]
Infant formula	Melamime and related compounds	Varian Polaris TM NH_2 (150 mm × 3 mm, 5 μm)	A: ACN, B: Ammonium acetate 10 mM and 0,1%glacial acetic acid in water	ESI-MS/MS	[46]
Baby food	Pesticides	Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm)	A: ACN, B: formic acid-ammonium formate buffer (pH 3.75)	ESI-MS/MS	[110]
Energy drink	Proteins, peptides, amino acids and related compounds	Astec apHera NH_2 (150 mm × 4.6 mm, 5 μm)	A: MeOH, B: water	ESI-MS/MS	[124]
Soft drinks	Carbohydrates, glycosides and related compounds	Luna HILIC (250 mm × 4.6 mm)	A: ACN, B: water	DAD and ESI-MS/MS	[125]
Energy drink	Water solubles vitamins	Inertsil Diol (150 mm × 4.6 mm, 5 μm)	A: ammonium acetate (or ammonium formate) at various concentrations and pH, B: ACN–water 90:10 containing ammonium acetate (or ammonium formate), at concentrations equal to those in A	DAD	[126]
Drinking water	Organic acids	Luna HILIC (150 mm × 2,1 mm, 5 μm)	A: ACN, B: 40 mM of ammonium formate	ESI-MS/MS	[127]
Infant formula	Proteins, peptides, amino acids and related compounds	TSKgel Amide-80 (250 mm × 1.5 mm, 5 μm)	A: water pH 2.7, B: ACN pH 2.7	ESI-MS/MS	[128]
Infant formula	Carbohydrates, glycosides and related compounds	TSKgel NH_2-100 (150 mm × 2.0 mm, 3 μm)	A: 30 mM ammonium formate in water (pH 2.5), B: MeOH	ESI-MS/MS	[129]

A and B relate to the mobile phase components; ACN: acetonitrile; MeOH: methanol; TFA: trifluoroacetic acid.

Energy drinks, which have become so popular in the last years, have many physiological and functional effects due to their richness in compounds such as vitamins, carbohydrates, and amino acids. Taurine and methionine, related with the production and maintenance of muscle, have been simultaneous separated using a polymeric gel column containing chemically bonded polyamine groups (Astec apHera NH_2) and identified by ESI-MS/MS detection [124]. Two detection systems (ESI-MS/MS and DAD) have been employed for studying the stability of two steviol glycosides, which have been also analyzed in soft drinks on a Luna HILIC column [125]. In addition, water soluble vitamins (complex-B and vitamin C) have been separated with a column with a dihydroxypropyl bonded phase (Inertsil Diol) and detected by DAD [126] without further clean up. Meanwhile, an amino phase column (Luna HILIC) and ESI-MS/MS detection were used in the separation and quantification of dichloroacetic acid, a common contaminant that causes carcinogenic effects, in drinking water [127].

Quaternary ammonium growth regulators have been found baby food, but in this case a silica HILIC column and ESI-MS/MS detection were chosen [111].

N,N-Dicarbamoylhydrazine (biurea) has also been analyzed in baby food employing a HILIC column with carbamoyl functional groups (TSKgel Amide-80) and MS/MS [40]. Using this method it was possible to eliminate the risk of false results of semicarbazide due to the presence of azodicarbonamide components. The same column (TSKgel Amide-80) was also useful for quantifying a milk oxidation product (dytirosine) in infant formulas, using ESI-MS/MS identification [128]. Another application in the analysis of baby food was the quantification of nucleotides, which have been added to food because of suggested beneficial effects. The analysis was carried out with an amino column (TSKgel NH_2-100) in HILIC mode [129].

4. Conclusions

In this work we present an overview on the use of HILIC in food analysis covering the period 2001–2011. The number of applications in food analysis has continuously increased over the years, and especially the use of comprehensive techniques. HILIC has proven to be a powerful tool for analyzing a wide variety of compounds, including nutritional compounds (proteins, carbohydrates, lipids, or water soluble vitamins) and other types of compounds, such as pesticides and toxins polar compounds, and has afforded increased selectivity, higher sensitivity, and improved efficiency for their quantification of analytes in complex matrices like foods. Studies have mainly focused on food of animal and vegetable origin, although other matrices like drinks, fungi, and baby foods have also been studied to some extent. Despite many commercial HILIC columns the most commonly used stationary phases have been neutral and charged. Meanwhile detection by MS/MS has been most popular, followed by DAD and MS. The on-going developments of new HILIC stationary phases will most probably further increase the use of HILIC to the analysis of food in the future.

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