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 of 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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Contents

1. Introduction .................................................................................................................. 7438
2. HILIC mechanism ....................................................................................................... 7439
   2.1. Typical columns and solvents .................................................................................. 7439
3. Application of HILIC in food matrices ......................................................................... 7439
   3.1. Food of animal origin and related products .............................................................. 7440
   3.2. Food and beverages of plant origin ......................................................................... 7446
   3.3. Other matrices ......................................................................................................... 7450
4. Conclusions .................................................................................................................. 7451
References ......................................................................................................................... 7451

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,
2. HIILC mechanism

The term HIILC 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 HIILC 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 HIILC theory states that HIILC 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 HIILC 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 HIILC 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 HIILC 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 HIILC 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 HIILC 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 HIILC retention of analytes on charged polar stationary phases.

The effect of column temperature in HIILC 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 HIILC columns at elevated temperatures has, however, been carried out. Finally, in many cases the peak capacity obtained by HIILC 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 HIILC 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 HIILC stationary phases has been reviewed [20,7]. In HIILC 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 HIILC 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 HIILC, 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 HIILC surface. Therefore, analytes, which retention is based on strong hydrogen bonding, are poorly retained. Approaches for method development in HIILC 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 HIILC assays was discussed. The most frequently optimized factor was the composition of the mobile phase. The HIILC assays were most often compared to RP-HPLC methods, and usually a different elution order was observed.

3. Application of HIILC in food matrices

In recent years the use of HIILC separations has significantly grown. The increasing use of HIILC 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 HIILC 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 HIILC 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 were 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 HIILC stationary phases allows the use of higher concentrations of organic solvent in the mobile phase, which is
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 (dифunctionally bonded ODS) are the most commonly used and are classified as neutral (no electrostatic interactions).
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 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
<table>
<thead>
<tr>
<th>Matrix</th>
<th>Compounds of interest</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parmesan cheese</td>
<td>Proteins, peptides, amino acids, and related compounds; Organic acids</td>
<td>TSKgel Amide-80 (250 mm × 1.5 mm, 5 μm)</td>
<td>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</td>
<td>ESI-MS/MS</td>
<td>[6]</td>
</tr>
<tr>
<td>Pork raw meat, cooked ham, dry cured ham, beef, wild boar, roe deer tissues, Chicken broth tissues</td>
<td>Carbohydrates, glycosides, and related compounds; Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine; Lipids</td>
<td>Atlantis HILIC Silica (150 mm × 4.6 mm, 3 μm), ZIC-HILIC (150 mm × 4.6 mm, 5 μm), LiChroCART Li Chropro S6 60 (250 mm × 4.0 mm, 10 μm), TSK gel Amide-80 (300 mm × 7.8 mm, 5 μm)</td>
<td>Different combinations of ammonium acetate, water and ACN depending on each column</td>
<td>ESI-MS/MS, DAD</td>
<td>[26]</td>
</tr>
<tr>
<td>Porcine muscles [27], pork loin and chicken breast [28], Cooked ham [54]</td>
<td>Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine</td>
<td>Atlantis HILIC Silica (150 mm × 4.6 mm, 3 μm)</td>
<td>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)</td>
<td>DAD</td>
<td>[27, 28, 54]</td>
</tr>
<tr>
<td>Chicken broth</td>
<td>Proteins, peptides, amino acids, and related compounds; Carbohydrates, glycosides, and related compounds</td>
<td>TSKgel Amide-80 (300 mm × 7.8 mm, 5 μm)</td>
<td>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)</td>
<td>ESI-MS/MS, NMR</td>
<td>[29]</td>
</tr>
<tr>
<td>Stewed beef juice</td>
<td>Proteins, peptides, amino acids, and related compounds; Carbohydrates, glycosides, and related compounds; Creatine and Creatinine</td>
<td>TSKgel Amide-80 (300 mm × 21.5 mm, 10 μm)</td>
<td>A: TFA (0.1% in water), B: ACN containing 0.1% TFA</td>
<td>ESI-MS/MS, NMR</td>
<td>[30]</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine</td>
<td>TSKgel Amide-80 (250 mm × 1.0 mm, 5 μm) and ZIC-HILIC (150 mm × 1.0 mm, 3.5 μm)</td>
<td>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)</td>
<td>ESI-MS/MS</td>
<td>[31]</td>
</tr>
<tr>
<td>Milk</td>
<td>Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine</td>
<td>Atlantis HILIC Silica (300 mm × 4.6 mm, 5 μm)</td>
<td>1% formic acid</td>
<td>DAD</td>
<td>[32]</td>
</tr>
<tr>
<td>Gouda Cheese</td>
<td>Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine</td>
<td>TSKgel Amide-80 (300 mm × 7.8 mm, 10 μm)</td>
<td>A: ACN, B: water; both components with 0.1% ammonium acetate (pH 3.0)</td>
<td>ESI-MS/MS, NMR</td>
<td>[34]</td>
</tr>
<tr>
<td>Milk hydrolysates</td>
<td>Proteins, peptides, amino acids, and related compounds; Creatine and Creatinine</td>
<td>Atlantis HILIC Silica (150 mm × 2.1 mm, 3 μm) for the second dimension</td>
<td>A: 0.1% formic acid in ACN, B: 10 mM ammonium acetate and 0.1% formic acid</td>
<td>ESI-MS/MS</td>
<td>[35]</td>
</tr>
<tr>
<td>Human milk</td>
<td>Glycoproteins</td>
<td>ZIC-HILIC 200 A (10 μm) only for enrichment</td>
<td>ACN/water; formic acid (50:49:1, v/v/v)</td>
<td>MALDI-TOF-MS, ESI-MS/MS</td>
<td>[36]</td>
</tr>
<tr>
<td>Chicken muscle [37], Veal muscle simple [41]</td>
<td>Veterinary antibiotics</td>
<td>ZIC–HILIC (100 mm × 2.1 mm, 3.5 μm) [37], ZIC–HILIC (50 mm × 2.1 mm, 5 μm) [41]</td>
<td>A: 50 mM ammonium formate in water at pH 2.5, B: ACN [37], A: 0.4% formic acid in water, B: ACN [41]</td>
<td>ESI-MS/MS, ESI-MS/MS</td>
<td>[37, 41]</td>
</tr>
<tr>
<td>Chicken muscle, eggs</td>
<td>Veterinary antibiotics</td>
<td>Fused-Core TM Ascentis Express HILIC (100 mm × 2.1 mm, 2.7 μm)</td>
<td>A: ACN, B: 50 mM formic acid/ammonium formate buffer (pH 4)</td>
<td>ESI-MS/MS</td>
<td>[38]</td>
</tr>
<tr>
<td>Kidney and muscle tissues</td>
<td>Veterinary antibiotics</td>
<td>ZIC–HILIC (100 mm × 2.1 mm, 5 μm)</td>
<td>A: 1% formic acid/150 mM ammonium acetate, B: ACN</td>
<td>ESI-MS/MS</td>
<td>[39]</td>
</tr>
<tr>
<td>Poultry, seafood samples</td>
<td>Veterinary antibiotics</td>
<td>TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)</td>
<td>A: ACN, B: water containing 0.2% formic acid</td>
<td>ESI-MS/MS</td>
<td>[40]</td>
</tr>
<tr>
<td>Milk</td>
<td>Veterinary antibiotics</td>
<td>TSKgel Amide-80 (150 mm × 2.0 mm, 3 μm)</td>
<td>A: 50 mM ammonium acetate in water (pH 4.0), B: ACN</td>
<td>ESI-MS/MS</td>
<td>[42]</td>
</tr>
<tr>
<td>Milk</td>
<td>Veterinary antibiotics</td>
<td>HILIC Fortis (100 mm × 3.0 mm, 3 μm)</td>
<td>A: 150 mM ammonium formate (pH 4.5), B: ACN</td>
<td>ESI-MS</td>
<td>[43]</td>
</tr>
<tr>
<td>Milk and eggs</td>
<td>Veterinary antibiotics</td>
<td>Luna HILIC (150 mm × 2.0 mm, 3 μm)</td>
<td>A: ACN containing 0.05% formic acid, B: water</td>
<td>ESI-MS</td>
<td>[44]</td>
</tr>
<tr>
<td>Eggs, pork, liver and kidney and pork, shrimp, sausage casing, honey and dairy product [45]</td>
<td>Melamine and related compounds</td>
<td>Atlantis HILIC Silica (150 mm × 2.1 mm, 5 μm) [45], Atlantis HILIC Silica (50 mm × 3.0 mm, 3 μm) [50]</td>
<td>A: 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]</td>
<td>ESI-MS/MS</td>
<td>[45, 50]</td>
</tr>
<tr>
<td>Milk</td>
<td>Melamine and related compounds</td>
<td>Varian Polaris TM NH2 (150 mm × 3 mm, 5 μm)</td>
<td>A: ACN, B: Ammonium acetate 10 mM and 0.1% glacial acetic acid in water</td>
<td>ESI-MS/MS</td>
<td>[46]</td>
</tr>
<tr>
<td>Matrix</td>
<td>Compounds of interest</td>
<td>Stationary phase</td>
<td>Mobile phase</td>
<td>Detection</td>
<td>Reference</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------</td>
<td>------------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Royal jelly and royal jelly lyophilized powder</td>
<td>Melamine and related compounds</td>
<td>Zorbax Rx-Sil (150 mm × 2.1 mm, 5 μm)</td>
<td>A: ACN; B: 5mM ammonium acetate buffer</td>
<td>ESI-MS/MS</td>
<td>[47]</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>Melamine and related compounds</td>
<td>ACQUITY UPLC BEH HILIC (100 mm × 2.1 mm, 1.7 μm)</td>
<td>A: 10 mM ammonium acetate in ACN, B: water</td>
<td>ESI-MS/MS</td>
<td>[48]</td>
</tr>
<tr>
<td>Pork, chicken</td>
<td>Melamine and related compounds</td>
<td>BioBasic AX (150 mm × 2.1 mm, 5 μm)</td>
<td>A: ACN, B: isopropanol, C: 50 mM aqueous ammonium acetate</td>
<td>ESI-MS/MS</td>
<td>[49]</td>
</tr>
<tr>
<td>Milk powders</td>
<td>Melamine and related compounds</td>
<td>ZIC-HILIC (150 mm × 4.6 and 250 mm × 4.6 mm, 5 μm)</td>
<td>A: ACN; B: 25 mM ammonium acetate, pH 6.8</td>
<td>UV</td>
<td>[51]</td>
</tr>
<tr>
<td>Milk, yoghurt, ice cream, milk powder and egg</td>
<td>Melamine and related compounds</td>
<td>Venusil HILIC (250 mm × 4.6 mm, 5 μm)</td>
<td>A: ACN; B: 10 mM ammonium formate buffer solution at pH 3.5</td>
<td>ESI-MS</td>
<td>[52]</td>
</tr>
<tr>
<td>Ham</td>
<td></td>
<td></td>
<td></td>
<td>DAD</td>
<td>[53]</td>
</tr>
<tr>
<td>Ham</td>
<td>Water solubles vitamins,</td>
<td>TSKgel Amide-80 (100 mm × 4.6 mm, 5 μm)</td>
<td>A: ACN, B: 0.1% TFA</td>
<td>DAD</td>
<td>[55]</td>
</tr>
<tr>
<td>Redfish tissues from</td>
<td>Water solubles vitamins</td>
<td>APS-2 Hypersil (50 mm × 2.1 mm, 3 μm)</td>
<td>A: ACN, B: ammonium acetate</td>
<td>UV, ESI-MS/MS</td>
<td>[56]</td>
</tr>
<tr>
<td>Spanish dry-cured sausages</td>
<td>Water solubles vitamins</td>
<td>Luna HILIC (150 mm × 3.0 mm, 3 μm)</td>
<td>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)</td>
<td>DAD</td>
<td>[57]</td>
</tr>
<tr>
<td>Milk</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>ZIC-HILIC (150 mm × 2.1 mm, 5 μm)</td>
<td>A: MeOH, B: water containing ammonium acetate5 mM</td>
<td>ESI-MS</td>
<td>[58]</td>
</tr>
<tr>
<td>Pork loin muscle</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>ZIC-pHILIC (150 mm × 4.6 mm, 5 μm)</td>
<td>A: ammonium acetate 150 mM pH 3.5, B: ammonium acetate 100 mM pH 7, C: ACN, D: water</td>
<td>DAD</td>
<td>[65]</td>
</tr>
<tr>
<td>Bivalves</td>
<td>Toxins</td>
<td>Luna HILIC (150 mm × 2 mm, 3 μm)</td>
<td>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</td>
<td>ESI-MS</td>
<td>[61]</td>
</tr>
<tr>
<td>Mussels (Mytilus edulis)</td>
<td>Toxins</td>
<td>TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm) and ZIC-HILIC (150 mm × 2.1 mm, 3.5 μm)</td>
<td>A: water, B: 95% ACN/water solution (both containing 2mM ammonium formate and 3.6 mM formic acid)</td>
<td>APCI-MS/MS</td>
<td>[62]</td>
</tr>
<tr>
<td>Mussel (Mytilus galloprovincialis)</td>
<td>Toxins</td>
<td>TSKgel Amide-80 (250 mm × 2 mm, 5 μm)</td>
<td>A: water, B: 95% ACN/water solution (both containing 2mM ammonium formate and 3.6 mM formic acid)</td>
<td>ESI-MS/MS</td>
<td>[13,63,65]</td>
</tr>
<tr>
<td>Mussels (Mytilus edulis and M. trossulus)</td>
<td>Toxins</td>
<td>TSKgel Amide-80 (250 mm × 7.8 mm, 5 μm)</td>
<td>A: water, B: ACN/water (95:5) (both components containing 2.0 mM ammonium formate and 3.6 mM formic acid (pH 3.5))</td>
<td>ESI-MS/MS, NMR</td>
<td>[64]</td>
</tr>
<tr>
<td>Mussel and cockle flesh</td>
<td>Toxins</td>
<td>TSKgel Amide-80 (200 mm × 0.05 mm, 5 μm)</td>
<td>A: ACN/water (95:5, v/v), B: water/ACN (95:5, v/v)</td>
<td>ESI-MS</td>
<td>[66]</td>
</tr>
<tr>
<td>Puffer fish</td>
<td>Toxins</td>
<td>ZIC-HILIC (150 mm × 2.1 mm, 5 μm)</td>
<td>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.</td>
<td>ESI-MS/MS</td>
<td>[67]</td>
</tr>
<tr>
<td>Puffer fish</td>
<td>Toxins</td>
<td>TSK gel Amide-80 (150 mm × 2.0 mm, 5 μm)</td>
<td>A: 16mM ammonium formate (pH 5.5), B: ACN</td>
<td>ESI-MS</td>
<td>[68]</td>
</tr>
<tr>
<td>Pig, beef, wild boar and roe deer</td>
<td>Lipids</td>
<td>LiChroCART LiChrospher Si 60 (250 mm × 4.0 mm, 10 μm)</td>
<td>A: water, B: ACN; Both components containing 1% formic acid</td>
<td>ESI-MS/MS</td>
<td>[69]</td>
</tr>
<tr>
<td>Milk powder and milk</td>
<td>Inorganic and metal compounds</td>
<td>Inertsil Diol (150 mm × 3.0 mm, 3.5 μm)</td>
<td>A: MeOH, B: 0.1% formic acid (60:40, v/v)</td>
<td>ESI-MS/MS</td>
<td>[70]</td>
</tr>
<tr>
<td>Cheese</td>
<td>Biogenic amines</td>
<td>Atlantis HILIC (150 mm × 2.1 mm, 3 μm)</td>
<td>A: ACN, B: ammonium formate 50.0 mM (pH 4)</td>
<td>APCI-MS/MS</td>
<td>[71]</td>
</tr>
<tr>
<td>Dogfish muscle</td>
<td>Inorganic and metal compounds</td>
<td>ZIC-HILIC (150 mm × 2.1 mm, 3.5 μm) or (50 mm × 2.1 mm, 3.5 μm)</td>
<td>78–70% ACN and ammonium acetate 125 mM pH 8.3</td>
<td>ICP-MS, ESI-MS/MS</td>
<td>[72]</td>
</tr>
</tbody>
</table>

A and B relate to the mobile phase components; ACN: acetonitrile; MeOH: methanol; TFA: trifluoroacetic acid.
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 performed to use 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 Coccidiostat amprolium in eggs and chicken muscle was performed with a Fused-Core Ascenx Express HILIC column [38]. Besides, a carbamoyl-functionalized silica HILIC column (TSKgel Amide-80) was used with success for analyzing food additives (azodicarbonamides) 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 melanine and related compounds (amline; the hydrolysis product of melamine, ammelide; the hydrolysis product of amline, 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 NH2) 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 hydrophobic 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 NH2) [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 antioxidative 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 in 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-
ution 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, which 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 retention 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 metabolic/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 HILIC×HILIC–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 HILIC×RP-LC method for the analysis of phenolic derivatives [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 derivatives) 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.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Compounds of interest</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed cereals and crisp bread</td>
<td>Proteins, peptides, amino acids, and related compounds</td>
<td>Atlantis HILIC silica (300 mm x 4.0 mm, 5 μm)</td>
<td>1% formic acid</td>
<td>DAD</td>
<td>[32]</td>
</tr>
<tr>
<td>Wheat gluten hydrolysates [6], dried beans [76]</td>
<td>Proteins, peptides, amino acids, and related compounds</td>
<td>TSKgel Amide 80 (250 mm x 1.5 mm, 5 μm) [6] TSKgel Amide-80 semipreparative (300 mm x 7.8 mm, 5 μm) or preparative scale (300 mm x 21.5 mm, 10 μm) [76]</td>
<td>A: 0.1% TFA in water B: ACN containing 0.1% TFA [76]</td>
<td>ESI-MS/MS, NMR</td>
<td>[6,76]</td>
</tr>
<tr>
<td>Three different wheat gluten hydrolysates</td>
<td>Proteins, peptides, amino acids, and related compounds</td>
<td>TSKgel Amide-80</td>
<td>A: Ammonium acetate buffer (6.5 mM, pH 5.5) in 90% ACN, B: Ammonium acetate buffer (2.6 mM, pH 5.5) in 60% ACN.</td>
<td>ESI-MS/MS</td>
<td>[77]</td>
</tr>
<tr>
<td>Apple, orange juice, potato and soybeans</td>
<td>Proteins, peptides, amino acids, and related compounds</td>
<td>ZIC-HILIC (150 mm x 2.1 mm, 3.5 μm)</td>
<td>A: 40 mM ammonium bicarbonate (pH 7) with formic acid, B: MeOH.</td>
<td>ESI-MS/MS</td>
<td>[78]</td>
</tr>
<tr>
<td>Morel mushrooms</td>
<td>Carbohydrates, glycosides and related compounds; Proteins, peptides, amino acids and related compounds</td>
<td>TSKgel Amide-80 (300 mm x 7.8 mm)</td>
<td>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)</td>
<td>ESI-MS, NMR</td>
<td>[79]</td>
</tr>
<tr>
<td>Cereals flour and cereals products</td>
<td>Proteins, peptides, amino acids, and related compounds</td>
<td>ACQUITY UPLC BEH HILIC (150 mm x 2.1 mm, 1.7 μm)</td>
<td>A: 10 mM ammonium acetate containing 0.005% acetic acid, B: ACN</td>
<td>ESI-MS/MS</td>
<td>[80]</td>
</tr>
<tr>
<td>Curcubita maxima pumpkin</td>
<td>Carbohydrates, glycosides, and related compounds; Proteins, peptides, amino acids and related compounds</td>
<td>TSKgel Amide-80 (250 mm x 2.0 mm, 5 μm)</td>
<td>A: ACN, B: 6.5 mM ammonium acetate pH 5.3.</td>
<td>ESI-MS/MS</td>
<td>[81]</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Proteins, peptides, amino acids, and related compounds; Lipids; Phenolic compounds; Carbohydrates, glycosides and related compounds</td>
<td>TSKgel Amide-80 (250 mm x 2.0 mm, 5 μm)</td>
<td>A: water containing 0.1% v/v formic acid, B: 90:10 ACN/water containing 0.1% v/v formic acid</td>
<td>ESI-MS</td>
<td>[82]</td>
</tr>
<tr>
<td>Plant extracts [84], Arabidopsis thaliana [86]</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>ZIC-HILIC (150 mm x 4.6 mm, 5 μm) [84], (150 mm x 2.1 mm, 3.5 μm) [86]</td>
<td>15 mM ammonium formate pH 4.5 in ACN:H₂O [84]</td>
<td>DAD [84], ESI-MS/MS [86]</td>
<td>[84,86]</td>
</tr>
<tr>
<td>Broccoli and other vegetables</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>Polyhydroxyethyl Aspartamide (100 mm x 4.6 mm, 3 μm)</td>
<td>A: ACN, B: water with 30 mM ammonium formate pH 5.4.</td>
<td>DAD</td>
<td>[85]</td>
</tr>
<tr>
<td>Soybean, corn and Arabidopsis thaliana</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>200T-PAAm HILIC (285 mm x 0.2 mm)</td>
<td>A: ACN, B: buffer solution of 13 mM ammonium acetate (pH=5.5).</td>
<td>UV−vis, ESI-MS/MS</td>
<td>[87]</td>
</tr>
<tr>
<td>Quillaja saponaria</td>
<td>Carbohydrates, glycosides, and related compounds</td>
<td>First dimension: TSKgel Amide-80 column (150 mm x 2.0 mm, 3 μm), second dimension: a laboratory-prepared Polyhydroxyethyl A (p-OH-Et A) (35 mm x 2.1 mm, 5 μm)</td>
<td>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</td>
<td>ESI-MS/MS</td>
<td>[88]</td>
</tr>
<tr>
<td>Licorice and kudzu extracts</td>
<td>Phenolic compounds</td>
<td>Atlantis HILIC Silica (250 mm x 64.6 mm, 5 μm) and CD-column (150 mm x 64.6 mm, 5 μm)</td>
<td>A: water with 0.1% formic acid, B: ACN with 0.1% formic acid</td>
<td>DAD</td>
<td>[90]</td>
</tr>
<tr>
<td>Green tea, apple and cocoa beans</td>
<td>Phenolic compounds</td>
<td>Developol Diol-100 (250 mm x 1 mm, 5 μm)</td>
<td>A: ACN and acetic acid (99:1, v/v), B: MeOH, water and acetic acid (94.05: 4.95:1, 3v/v)</td>
<td>DAD, FLD, ESI-MS/MS</td>
<td>[91,92]</td>
</tr>
</tbody>
</table>
**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  
DAD [93]

**Ligusticum chaussong**, 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-cycloexetrin (150 mm × 4.6 mm, 5 μm)  
A: ACN with 2.5% 200 mM ammonium acetate aqueous solution and 0.1% acetic acid/H₂O 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, 5 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 [98,99]  
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)  
A: 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 acid  
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 cyanobacteria** [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]  
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  
Polyhydroxethyl 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**  
Melamine and related compounds  
Atlantis HILIC Silica (150 mm × 2.1 mm, 5 μm)  
A: 10% 100 mM ammonium formate in ACN at pH 3.2, B: 0.1% formic acid in ACN  
ESI-MS/MS [45]

**Bakery goods and flour**  
Melamine 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)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Compounds of interest</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae and phytochemical samples</td>
<td>Inorganic and metal compounds</td>
<td>ESI-MS/MS</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana, wheat plant</td>
<td>Inorganic and metal compounds</td>
<td>ESI-MS/MS</td>
</tr>
<tr>
<td></td>
<td>S. sieva yeast</td>
<td>Inorganic and metal compounds</td>
<td>ESI-MS/MS</td>
</tr>
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<td>S. sieva yeast</td>
<td>Inorganic and metal compounds</td>
<td>ESI-MS/MS</td>
</tr>
<tr>
<td></td>
<td>Coffee grounds</td>
<td>Acrylamide</td>
<td>ESI-MS/MS</td>
</tr>
<tr>
<td></td>
<td>Oryza sativa, Arabidopsis thaliana and oilseed tissue</td>
<td>Isoflavonoids</td>
<td>ESI-MS/MS</td>
</tr>
<tr>
<td></td>
<td>Extra virgin olive oil</td>
<td>Lipids</td>
<td>ESI-MS/MS</td>
</tr>
<tr>
<td></td>
<td>A &amp; B relate to the mobile phase</td>
<td>Triamterene</td>
<td>ESI-MS/MS</td>
</tr>
</tbody>
</table>

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 as 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 an 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) 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, 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 cyano bacterial 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 (PolyHydroxethyl 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
of the genus Alexandrium, Gymnодinium, 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 melanine 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 melanine levels in bakery goods and flour [48]. In another study, a silica HILIC column and ESI-MS/MS detection was used for determining melanine 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-chHILIC), 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 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]. Cytokins, 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.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Compounds of interest</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby food</td>
<td>Veterinary antibiotics</td>
<td>TSKgel Amide-80 (250 mm × 2.0 mm, 5 μm)</td>
<td>A: ACN, B: water containing 0.2% formic acid.</td>
<td>ESI-MS/MS</td>
<td>[40]</td>
</tr>
<tr>
<td>Infant formula</td>
<td>Melamine and related compounds</td>
<td>Varian Polaris TM NH2 (150 mm × 3.0 mm, 5 μm)</td>
<td>A: ACN, B: Ammonium acetate 10 mM and 0.1%glacial acetic acid in water</td>
<td>ESI-MS/MS</td>
<td>[46]</td>
</tr>
<tr>
<td>Baby food</td>
<td>Pesticides</td>
<td>Atlantis HiPLC Silica (150 mm × 2.1 mm, 3 μm)</td>
<td>A: ACN, B: formic acid-ammonium formate buffer (pH 3.75)</td>
<td>ESI-MS/MS</td>
<td>[110]</td>
</tr>
<tr>
<td>Energy drink</td>
<td>Proteins, peptides, amino acids and related compounds</td>
<td>Astec apflera NH2 (150 mm × 4.6 mm, 5 μm)</td>
<td>A: MeOH, B: water</td>
<td>ESI-MS/MS</td>
<td>[124]</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>Carbohydrates, glycosides and related compounds</td>
<td>Luna HILIC (250 mm × 6.5 mm)</td>
<td>A: ACN, B: water</td>
<td>DAD and ESI-MS/MS</td>
<td>[125]</td>
</tr>
<tr>
<td>Energy drink</td>
<td>Water solubles vitamins</td>
<td>Inertsil Diol (150 mm × 4.6 mm, 5 μm)</td>
<td>A: ammonium acetate (or ammonium formate) at various concentrations and pH, B: ACN–water 90:10 containing ammonium formate, at concentrations equal to those in A</td>
<td>ESI-MS/MS</td>
<td>[126]</td>
</tr>
<tr>
<td>Drinking water</td>
<td>Organic acids</td>
<td>Luna HILIC (150 mm × 2.1 mm, 5 μm)</td>
<td>A: ACN, B: 40mL of ammonium formate</td>
<td>ESI-MS/MS</td>
<td>[127]</td>
</tr>
<tr>
<td>Infant formula</td>
<td>Proteins, peptides, amino acids and related compounds</td>
<td>TSKgel Amide-80 (250 mm × 1.5 mm, 5 μm)</td>
<td>A: water pH 2.7, B: ACN pH 2.7</td>
<td>ESI-MS/MS</td>
<td>[128]</td>
</tr>
<tr>
<td>Infant formula</td>
<td>Carbohydrates, glycosides and related compounds</td>
<td>TSKgel NH2–100 (150 mm × 2.0 mm, 3 μm)</td>
<td>A: 30 mM ammonium formate in water (pH 2.5), B: MeOH</td>
<td>ESI-MS/MS</td>
<td>[129]</td>
</tr>
</tbody>
</table>

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 apflera NH2) 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 in 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 (dityrosine) 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 NH2–100) 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.

### References
