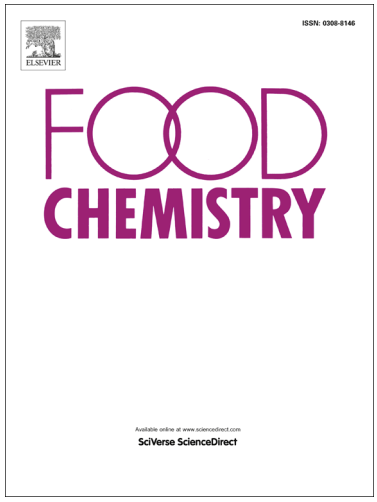

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Hydrophilic interaction chromatography in food matrices analysis: an updated review

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Abstract

This review focuses on the most recent papers (from 2011 to submission date in 2017) dealing with the analysis of different organic components in foods (i.e. nucleobases, nucleosides, nucleotides, uric acid, and creatinine, amino acids and related compounds, choline-related compounds and phospholipids, carbohydrates, artificial sweeteners and polyphenolic compounds), using hydrophilic interaction liquid chromatography (HILIC) combined with different detection techniques. For each compound class, the investigated food matrices are grouped per: foods of animal origin, vegetables, fruits and related products, baby food, and other matrices such as drinks and mushrooms/fungi. Furthermore, the main advantages of HILIC chromatography respect to the other commonly used techniques are discussed.

Keywords: HILIC, food analysis, stationary phases, review

1. Introduction

Hydrophilic interaction liquid chromatography (HILIC) has proven to be a versatile analytical tool for the separation of polar and ionizable compounds, which are features for many analytes of interest in food science. Over the past two decades HILIC has gained in popularity for the separation and analysis of many compounds in foods, including organic nutrients (carbohydrates, phospholipids, amino acids, peptides, and proteins), contaminants, and toxic compounds.

The term HILIC was first introduced by Andrew Alpert in 1990 and refers to the combination of a polar stationary phase (SP) and a mobile phase (MP) containing minimum 2-3% of water and a substantial proportion of water-miscible organic solvent (>60%).

SPs often consist of a silica gel base, but also organic polymers and other type of carriers are used, either functionalized or non-functionalized, and packed into HPLC columns. Non-functionalized supports are typically plain silica and common modifications are amide, aminopropyl, diol, and zwitterionic (sulfoalkylbetaine or phosphorylcholine) (Jandera & Janas, 2017). The polar groups attached to the SPs bind water molecules, leading to the formation of an aqueous layer over the SP surface of thickness depending on the base material, type of functionalization, and the functionalization degree. Acetonitrile is by far the most popular solvent in HILIC mode, being of aprotic character, but depending on the hydrophilicity of the SP (i.e. the column retention capacity) also other protic solvents can be used such as methanol and other lower alcohols (Jandera & Janas, 2017; Zuo et al., 2014).

Buffers are generally recommended and used to improve the robustness for liquid chromatographic (LC) separations of ionizable compounds. Different buffers are also usually added in HILIC MPs, and where the most common choices are volatile salts such as ammonium acetate or ammonium formate. These are used to control the MP pH the MP ionic strength, and thereby the ionization of the analytes and of the SP. The preference for organic buffers is a consequence of the need to improve their solubility in MPs with high organic solvent content, and to ease volatilization with

more modern detection techniques like mass spectrometry (MS), evaporative light scattering detector (ELSD), and charged aerosol detector (CAD).

The pure theoretical HILIC retention mechanism is mainly partitioning of polar compounds between the organic-rich MP and the water-enriched layer partially immobilized on the SP. Several failed attempts have been made to ascribe the retention mechanism essentially to a liquid-liquid partitioning dynamic equilibrium. Instead the HILIC retention has been demonstrated to be the result of more than just simple liquid/liquid partitioning; as other important interaction mechanisms are called into play, namely ionic interactions (of both weak and strong character, depending on the SP choice), adsorption interactions via hydrogen bond, and dipole-dipole interactions (Jandera & Hájek, 2017). All these "secondary" interactions are thought to contribute significantly to HILIC separation depending on the nature of the single components of the HILIC experiment, i.e. SP, MP, analyte, and their mutual interactions (i.e. SP-MP, SP-analyte, MP-analyte, and SP-MP-analyte (Taraji et al., 2017)). HILIC is thus just not HILIC. An excellent overview of the current understanding of the HILIC mechanism has recently been published by McCalley (2017). For HPLC practitioners, however, the "rule of thumb" for predicting broadly whether an analyte will be suitable for HILIC is that the analyte is generally unretained in reversed phase liquid chromatography, being a polar, and of course hydrophilic molecule (i.e. with negative $Logk_{ow}$ and LogD).

On the following pages, we have reviewed papers reporting on the use of "HILIC" in food analysis, published from 2011 to submission date in 2017 searching in the databases PubMed, Scopus, and Google Scholar. The great variety of foods, and their constituents studied posed a major issue in the organization of this text. From the selection of the papers, the different type of HILIC SP's used in methods applied to food science studies have been divided into six categories based on the target analytes investigated, namely 1) nucleobases, nucleosides, nucleotides, uric acid, and creatinine, 2) amino acids, peptides, and proteins, 3) choline-related compounds and phospholipids, 4)

carbohydrates, 5) artificial sweeteners, and 6) polyphenols. Each category was additionally sub-grouped according to the different food matrices following the classification proposed by Bernal: 1) foods of animal origin (meat, fish, milk, dairy products), 2) vegetables, fruits, and related products, 3) baby food, and 4) other matrices such as drinks and mushrooms/fungi (Bernal, Ares, Pol, & Wiedmer, 2011).

Two important and active areas of research have been excluded because of space constraints: studies involving the use of comprehensive and multidimensional techniques in food analysis, and reports aimed at the analysis of food contaminants and toxic compounds.

2. Nucleobases, nucleosides, nucleotides, uric acid, and creatinine

Nucleobases (NuBs), nucleosides (NuSs) and nucleotides (NuTs) are bioactive compounds involved in the regulation and modulation of several physiological processes such as the stimulation of axon growth in vitro, in the adult central nervous system, the maintenance of the immune response, the growth and differentiation of the gastro intestinal tract, the antioxidant, anti-viral, anti-neoplastic, anti-arrhythmic, and anti-seizure activities. They are also useful for understanding cellular energy metabolism and for assessing oxidative stress in human body. Furthermore, it is well known that their concentration is related to the immune response in oncologic patients (Choi & Berdi, 2012).

Uric acid (UA) and creatinine (CR) are physiologic components of human body fluids that need to be controlled to prevent several diseases (gouty arthritis, type 2 diabetes, hypertension, and cardiovascular diseases as well as kidney diseases). The diet may significantly alter the UA and CR levels, and therefore the assessment of their levels in foods can be matter of interest for clinicians and patients who need to select with care the diet to prevent the progress or the deterioration of chronic diseases.

Considering what was reported above, the quantification of NuBs, NuSs, NuTs, UA, and CR in foods may be of great interest. NuBs, NuSs, NuTs, UA and CR are hydrophilic, polar, non-volatile

organic molecules and their separations using RPLC methods are often characterized by poor selectivity. All these molecules are easily converted to anions under mild MP conditions, and therefore both ion-pair (IP) and ion-exchange (IE) mode in RPLC have been used historically (Indyk & Woollard, 2004; Yang, Li, Feng, Hu, & Li, 2010). Several LC and capillary electrophoresis (CE) (Mateos-Vivas, Dominguez-Alvarez, Rodrìguez-Gonzalo, & Carabias-Martinez, 2017) techniques have been used, in particular for separating NuBs, NuSs, and NuTs (Dominguez-Alvarez et al., 2017), including high performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC) (Yao et al., 2015; Zhou et al., 2014), and nano-liquid chromatography (Mateos-Vivas, Fanali, Rodriguez-Gonzalo, Carabias-Martinez, & Aturki, 2016). Mobile phase incompatibility with ion sensitive detection techniques, such as mass spectrometry (MS) hampers the latter techniques. HILIC overcomes many of the limitations and has proven useful for the identification and quantification of NuBs, NuSs, NuTs, UA, and CR in herbal- and animal-derived products (Table 1).

2.1. Foods of animal origin

2.1.1. Tais clavigera.

Nutritional and functional values were assessed and compared between samples of *Tais clavigera*, a taupe shuttle-like conch widespread along the coast of China and Japan (Ge et al., 2015). All of its parts (shell, flesh and operculum) could be used as source of proteins, fatty acids, sterols, and mineral elements. Samples of smashed flesh of the mussel were freeze-dried and extracted in water at room temperature for 60 min in an ultrasonic bath. An amide column was used in gradient mode using a binary eluent system initially consisting of 10% A (5 mM ammonium formate, 5 mM ammonium acetate, and 0.2% formic acid in aqueous solution), and 90% B (1 mM ammonium formate, 1 mM ammonium acetate, and 0.2% formic acid in acetonitrile). Samples from 19 different habitats in China were analyzed, and the results showed concentrations of NuBs and NuSs ranging from 119.41 mg/g to 317.10 mg/g depending on the collection area. It was also demonstrated that

amino acids (see dedicated section) could be used as quality markers to classify the origin of the samples.

2.1.2 Bovine milk

UA and CR were determined rapidly with a very simple method using isocratic conditions on an amino HILIC column followed by UV detection at 205 nm (Zuo, Zhou, Zuo & Deng, 2015). The method measured the contents of the target analytes in four samples of bovine milk. The levels of UA and CR were in the range 24.1-86 µg/mL and 5.07-11.15 µg/mL, respectively. The method reported by Zuo (2015) appears to be a valuable alternative to conventional IP-RPLC and RPLC-based methods.

2.2. Vegetables, fruits, and related products

Ginkgo biloba seeds and leaves have been widely studied for their use as dietary supplements. Zhou et al. (2014) and Yao et al. (2015) applied rapid, simple, and sensitive HILIC-MS/MS methods in multiple reaction monitoring (MRM) mode for the determination of nucleobases and nucleosides in Ginkgo seeds and leaves, respectively. Both groups used the same sample processing procedure, column, and mobile phase composition. Twenty nucleosides and eleven nucleobases were detected and quantified in approximately ten minutes. Linearity, precision (both intra-day and inter-day), accuracy, and sensitivity were assessed and found appropriate for the purposes of the methods and at least comparable with those obtained using other techniques (Yao et al., 2015; Zhou et al., 2014). The authors underlined the potential of HILIC and tandem MS methods for assessing the quality of medicinal and food products, as well as beverages containing ginkgo seeds or leaves extracts. By using nucleosides and nucleobases as markers Yao (2015) could discriminate twenty-two samples of *G. biloba* leaves into two clusters of products evidencing their regional origin.

2.3. Baby food

HILIC has been proposed for the separation of NuBs derivatives in infant foods (Mateos-Vivas, Rodrìguez-Gonzalo, Domìnguez-Álvarez, Garcìa-Gómez, & Carabias-Martìnez, 2016; Sfakianaki

& Stalikas, 2015). Sfakianaki and Stalikas (2015) used the technique with diode array UV detection for the determination of 5'-mononucleotides in human breast milk and infant milk formulas, after sample pretreatment using alumina and stannia hollow fibers. The method was validated and exhibited good performance in determining cytidine, adenosine, uridine, inosine, guanosine 5'-monophosphate from infant milk after protein removal. Three columns with different stationary phases (amide, amine and diol) were tested, and the amide phase in combination with a mobile phase consisting of acetonitrile-water 70:30 (v/v) proved most useful. The separation and detection of twenty NuSs and NuTs in sixteen dairy and non-dairy baby foods have been carried out via the ion pair formation of hydrophilic ions and HILIC-MS/MS after dilution, ultracentrifugation and further washing of samples (Mateos-Vivas et al., 2016). The mobile phase used consisted of a mixture between acetonitrile and water with 100 mM hexafluoro-2-propanol/50 mM diethylammonium as a cationic ion-pairing reagent. Gradient elution allowed the separation and detection of NuSs, NuT mono-, di-, and tri-phosphates in less than nine minutes. The method was also validated in compliance with 2002/657/EC decision. No NuT di- and triphosphates were detected though in the samples, probably due to degradation (i.e. had been already processed) and all NuT diphosphates and triphosphates had naturally converted into NuTs monophasphates. The LOQ values were lower than those previously reported for similar samples, albeit analyzed by CE and electrospray ionization (ESI)-MS (Mateos-Vivas et al., 2017) and nano-LC-UV (Mateos-Vivas et al., 2016).

2.4. Other food matrices

Ganoderma genus mushrooms are nutritious fungi used as functional food in Asia. The simultaneous determination of sixteen NuSs and NuBs for the quality evaluation of eight batches of *Ganoderma* from different species and origins has been reported (Chen, Bicker, Wu, Xie, & Lindner, 2012). The analytes were isolated from the mushroom samples using ultrasonic-assisted extraction in water at room temperature for ten minutes. Acetonitrile was added to the samples after

the centrifugation and filtration steps, to obtain a final mixture of ACN-water (50:50; v/v) before the analysis. The authors investigated the behavior of the analytes by varying the MP composition, the salt type, the salt concentration, pH, the column temperature, and the injection solvent using the one-factor-at-time approach. Gradient elution at 25 °C with a MP containing initially 15 mM ammonium acetate in ACN-water (95:5%, v/v) provided the best separation and was suitable for both MS and UV detection (254 nm). The method was finally validated and applied to real *Ganoderma* samples. Data cluster analysis showed that the HILIC profiles could be used to discriminate samples for both origin and species.

3. Amino acids, peptides, and proteins

Amino acids (AAs) and nutritionally relevant peptides, also called bioactive peptides, are obtained *in vivo* from the digestion of food proteins, phosphoproteins, and glycoproteins through the action of different proteases. The enzymatic hydrolysis of proteins gives rise to a wide range of AAs and peptides of different sizes and polarity. The nutritional value and food quality related to protein content is determined by amino acidic building blocks of the proteins, the ratio of essential amino acids, the susceptibility to hydrolysis of proteins during digestion, and the effects of food treatment (e.g. cooking) prior to ingestion. Following protein digestion, the released peptides available for absorption and metabolism have small, medium or large molecular sizes, i.e. primary structures containing less than 6-7 AAs, between 7-25 AAs, or more than 25 AAs, respectively (Lahrichi, Affolter, Zolezzi, & Panchaud, 2013).

Intact proteins, phosphoproteins, and glycoproteins can be separated using HILIC or electrostatic repulsion liquid chromatography (Alpert, 2008). A fundamental problem to solve is the solubility miss-match. HILIC require a mobile phase with high percentage of organic solvent which in most cases are incompatible with efficient protein solvation and rather apparent risk of protein precipitation instead. At present, these approaches appear to be confined to fundamental research in

the separation sciences. Proteomic and peptidomics techniques are used to study food protein content, bioactive peptides, and the free AAs contained in food of animal origin. However, since the subject is too complex and wide to find space in the present review, the interested reader is referred to specific references (Gaso-Sokac, Kovac, & Josic, 2011; Lahrichi et al., 2013).

In 2015 Periat published a comprehensive review by covering the overall applicability of HILIC for amino acids, peptides and proteins. To prevent any overlap with the present review, we narrowed to the publications herein to those only dealing with the determination of free AAs and bioactive peptides in food.

Amino acids are both the basic structural units of proteins and the precursors useful in the biosynthesis of neurotransmitters, polyamines, and nitric oxide. The determination thereof is important in the evaluation of nutritional food quality, and several analytical approaches are well established, including direct methods without derivatization (Zhou et al., 2013; Zhou, Wang, Li, Peng, & Li, 2015) and indirect methods involving isolation, purification, and derivatization (Dai, Wu, Jia, & Wu, 2014).

Table 2 summarizes studies on free AAs and peptides in foods published during the last five years.

HILIC followed by MS detection is the most common set-up in fundamental research for the qualitative-quantitative analysis of free AAs and bioactive peptides in foods.

3.1. Foods of animal origin

Meat, seafood, milk, and dairy products are the main sources of protein in food. Those of animal origin are generally more complex than vegetable proteins. The former contain a greater number of components/compounds, with virtually unpredictable number of small proteins and bioactive peptides ranging from high concentration levels (in the percentage order of magnitude) to low levels that may be in the sub-ppm range.

3.1.1. Chicken broth

Chicken broth has been studied for its content of carnosine and anserine (antioxidant dipeptides active in the prevention neuron death due to oxidative stress). The analytical separation was carried out on a column packed with non-functionalized ethylene bridged hybrid (BEH) particles and the results were compared with those from two C18 columns of same physical format as the HILIC column. Sample preparation consisted of an off-line solid phase extraction (SPE) and a micro-SPE purification to remove interferences from the broth. The chromatographic analysis took approximately only ten minutes with the HILIC column, with a method accuracy measured by extraction recoveries, higher than 80% (Macia et al., 2012). Overall, the method performance appeared superior in throughput and sensitivity to those based on RPLC and ionic exchange RPLC (Macia et al., 2012).

3.1.2. Meat

HILIC-MS/MS analysis has been reported for hydrophilic low-molecular weight compounds having antioxidant properties (free AAs, di- and tripeptides, together with carnitine, carnosine, anserine, taurine, and several minor components in traces such as creatine or valine) in fermented chorizo sausages meat extracts (Broncano, Otte, Petrón, Parra, & Timón, 2012). The target analytes were separated with gradient elution on a zwitterionic alkylsulfobetaine stationary phase using different proportions of 6.5 mM ammonium acetate buffer and acetonitrile as mobile phase system. Free AAs and bioactive peptides were detected and identified after a complex sample extraction procedure (essential for the complexity of the starting food matrix and the challenging chemical properties of the target analytes). Frozen samples were homogenized with perchloric acid at 4°C, followed by centrifugation, neutralization of the supernatant, elimination of salt precipitate, ultrafiltration with a 3 kDa cut-off filter, and preliminary fractionation/purification by RPLC. The need for such complex sample preparation procedures seriously hamper and could likely be a limitation this HILIC method applicability for meat product analysis .

3.1.3. Seafood

Freeze-dried mussels (*Mytilus galloprovincialis)* were tested for twenty-one AAs. The content was reported both as free AAs content after a simple extraction procedure, and the total AAs were quantified after hydrolysis. The proteins isolated by solubilization, were submitted to acidic hydrolysis using 4% v/v thioglycolic acid as the reducing agent. The mixture was separated on a column with amide modified BEH particles and detection carried out by MS/MS. The method was validated for all the analytes in terms of linearity, precision, recovery, and sensitivity (LOD and LOQ). Accuracy and precision were also assessed and found suitable for the purpose of the study (Tsochatzis, Begou, Gika, Karayannakidis, & Kalogiannis, 2017).

The identification of arginyl dipeptides (demonstrated to be salt taste enhancers) is an interesting HILIC application and can be performed on an amide column. The quantification of these analytes was carried out in different laboratory-made and commercial hydrolysates samples, including those obtained from fermented fish sauces (Schindler et al., 2011).

These applications demonstrate that HILIC is a viable option to RPLC for the analysis of free AAs in seafood. The strongpoint with HILIC is the unique ability to separate very polar and hydrophilic compounds which otherwise would require more laborious sample pretreatment, including derivatization and further purification.

3.2. Vegetables, fruits, seeds, and related products

When analyzing fruits and seeds, free AAs are often extracted with water from dried sample material using ultrasound-assisted extraction, as reported for *Ziziphus jujuba* fruits (Guo et al., 2013), *Siraitia grosvenorii* fruits (an experimental design for method optimization was applied) (Zhou et al., 2015), and *Ginkgo biloba* seeds (Zhou et al., 2013).

Peach seeds (*Prunus persica L. Batsch*) were studied to assess their content of peptides with antioxidant properties (Vásquez-Villanueva, Marina, & García, 2016). The sample preparation required an initial crushing and defatting of the peaches seeds. Ground and defatted material was extracted for one minute with a mixture of 0.5% SDS and 0.5% dithiothreitol (DTT) in Tris-HCl

buffer at pH 7.5, using an ultrasound probe. Proteins were precipitated from the supernatant solution by adding cold acetone and keeping the samples in fridge for fifteen minutes. The isolated proteins were digested using four enzymes (Alcalase, Thermolysin, Flavourzyme, and Protease P) and the peptide extracts were fractionated by ultrafiltration. Peptide sequences were identified by ESI-time of flight (TOF) mass spectrometry after the separation by two separation modes: HILIC (with a non-functionalized solid-core silica column) and RP (with an octadecyl-functionalized solid-core silica column). The combined approach provided the best separation of short chain antioxidant peptides obtained after enzymatic digestion of proteins present in peach by-products and their fractionation by ultrafiltration using 5 and 3 kDa cut-off filters. Eighteen peptides characterized by a high percentage of hydrophobic amino acids were detected and the results indicated a synergic antioxidant effect among the different sequences identified. Other HILIC columns used for the separation of small peptides and their metabolites were the BEH non-functionalized silica column, the amide-80, and an amide modified BEH column.

These sample preparation procedures appear rather laborious and provide aqueous extracts which, as such, are difficult to analyze with HILIC. The aqueous content causes peak broadening and/or poor peak shape and impair the chromatographic separation. Two possible measures to alleviate the problem with injection of aqueous extracts onto HILIC columns: 1) dilution of the aqueous sample in an appropriate organic solvent (usually acetonitrile) prior to injection, and 2) use the smallest possible sample injection volume to ascertain satisfactory peak shape and not to impair the retention time repeatability.

3.3 Other liquid foods and beverages

Twenty-two AAs were monitored in liquid foodstuffs, such as beer, fruit juices (peach, apricot, orange, sour cherry, apple), wine, tea, and honey using HILIC and Orbitrap-MS detection with about six minutes analysis (Gokmen, Serpen, & Mogol, 2012). The analytes were determined after simple sample dilution with a water-acetonitrile mixture (50:50 v/v) and filtration. A binary mobile

phase system consisting of acetonitrile and 0.1% formic acid in water was used in gradient mode on a non-functionalized silica column demonstrating a high throughput and efficacy.

4. Choline-related compounds and phospholipids

Adequate choline intake is essential for the normal function of cells, for fetal development, in preventing liver and kidney failure, memory dysfunction, and other various diseases (Bekdash, 2016; Zeisel & da Costa, 2009). Choline, in different forms, is present in food and biological tissues, including phospatidylcholine (PC) and lysophosphatidylcholine (LPC), together with other glycerophospholipids, i.e. phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphatidylinositol (PI), sphingolyelin (SM), and phosphatidylglycerol (PG). Phospholipids possess not only positive nutritional values, but provide also cardiovascular beneficial effects and anti-inflammatory activity (Kullenberg, Taylor, Schneider, & Massing, 2012). During the last seven years, analytical methods have been developed for the detection and quantification of choline-related compounds and phospholipids, due to their importance in food. Phosphorus-31 Nuclear Magnetic Resonance has shown an impressive performance as analytical detection technique in the assessment of phosphorus-containing compounds in food, and as far as phospholipids congeners are concerned (Garcia et al., 2012; Kaffarnik, Ehlers, Grobner, Schleucher, & Vetter, 2013; Noack, Smernik, McBeath, Armstrong, & McLaughlin, 2014). LC coupled with fluorescence, MS, or evaporative light scattering (ELS), and gas chromatography (GC) with MS have been reported for such analyses (Andrieux, Kilinc, Perrin, & Campos-Gimenez, 2008), but none of these techniques could simultaneously quantify all choline derivatives and phospholipids. NP chromatography coupled with MS has also been used (Verardo, Gomez-Caravaca, Gori, Losi, & Caboni, 2013), but even if different phospholipids classes and choline-related compounds can be well retained and resolved, the non-polar mobile phases applied are not ideal for ESI-MS. RPLC-MS has also been extensively used, but only specific classes of

phosphatidylcholines (i.e., compounds with different acyl chain lengths and degree of unsaturation) can be well retained and resolved. Table 3 summarizes recent papers demonstrating the applicability and appropriateness of HILIC as an analytical separation tool for choline-related compounds and phospholipids.

4.1. Foods of animal origin

4.1.1. Egg yolk

The identification and quantification of choline-related compounds in egg yolks has been performed using a non-functionalized solid-core silica column and a mobile phase system with acetonitrile and aqueous ammonium formate. In comparison with RPLC elution, it offered greater retention of the more polar and hydrophilic analytes, such as choline and betaine, and greater resolution of peak-clusters eluted over a narrow retention time window (about fourteen minutes per analysis). Furthermore, it was possible to differentiate the different species of the same class of compounds by MS detection, considering their molecular weight and specific fragmentation (Zhao, Xiong, & Curtis, 2011). The same research group also developed a simple approach for the extraction from food and biological samples of eight phospholipid congeners and six aqueous choline-related compounds in a single homogeneous phase that could be directly analyzed in MS. The study focused both on the choice of the buffer solvent (ammonium formate buffer in organic solvent or in water could affect the ion suppression of some analytes differently) and of the optimal pH value (which could influence retention, selectivity, and sensitivity especially of PI and PS). Using a binary mobile phase consisting of acetonitrile and ammonium formate buffer at $^{s/w}$pH 3, optimal conditions in terms of sensitivity and resolution were achieved (Xiong et al., 2012).

4.1.2. Seafood

The phospholipid composition of shrimp waste was investigated by HILIC-QTOF-MS after SPE extraction of the analytes on an in-house made cartridge containing TiO_2-coated silica core-shell composites. Sixty-nine phospholipids were separated on a diol column using gradient elution with

mobile phase consisting of acetonitrile acidified with 53 mM formic acid at pH ca. 4.0-4.5, and 60 mM ammonium formate and 53 mM formic acid in water at pH ca. 3.6 (Shen & Cheung, 2014). The same column was also successfully used to separate 50 phospholipid congeners isolated by chloroform extraction and purified by SPE from five species of dried male seahorses; the analytes were eluted in gradient at 50 °C using a binary MP (10 mM ammonium formate and 0.2% formic acid aqueous solution, eluent A; acetonitrile with 0.2% formic acid, eluent B) (Shen, Dai, Huang, & Cheung, 2016). To the best of the authors knowledge, no equivalent study has been published using other HPLC modes.

4.1.3. Milk

Due to the increasing interest in the nutritional and technological properties of polar lipids, the major classes of phospholids were studied in milk of four different mammalians, namely human, cow, goat, and donkey. A binary mobile phase at pH 5.5 was used and quantitative data were obtained for PI, PE, PS, PC, LPC, and SM after separation on a non-functionalized solid-core silica column connected to ELSD. The same separation method was also used with ion trap-time-of-flight (IT-TOF) mass-spectrometric detection for the identification of the different analytes in each class (Russo et al., 2013).

A comprehensive investigation on 70 different polar lipids, of which 22 never previously reported, in 32 cow milk samples, was reported using a validated HILIC-MS method (Liu, Moate, Cocks, & Rochfort, 2015). Combining the HILIC selectivity of a diol column with high resolution quantitation power of the Orbitrap detector, a method with good performance was developed.

4.2. Vegetable, fruits, and related products

Pulses (beans, lentils, peas, soybeans) commercially available in North America were studied, for their content of free choline and choline-relate compounds (glycerophosphocholine, phosphocholine, SM, PC, and LPC), and an extensive comparison between the nutritional phospholipid content before and after cooking was reported. Using a non-functionalized solid-core

silica column, tandem MS detection, and a binary gradient MP (acetonitrile and 10 mM ammonium formate in water, pH adjusted with formic acid to 3.0), the analytes were analyzed in 30 minutes. The reported method seem time-consuming, not so easy to perform, and quite expensive. However, the method appears well justified, considering the target analytes, and by the difficulty of the separation not so easily accomplished with other HPLC modes (Lewis et al., 2014). Phospholipids and phospholipid loss in veiled virgin olive oil after the storage at 4 °C for 12 h was studied. The analytes were extracted using both n-hexane and a diol SPE cartridge, and their separation was carried out on a non-functionalized solid-core silica HILIC column, using ESI-QTOF-MS detection. The final method was validated for the determination of phosphatidylglycerol, PE, PC, PI, and LPC and for the characterization of the phospholipid profile of olive oil (Verardo et al., 2013). Despite the complexity and costs of the method reported, HILIC provided a unique solution to the analytical challenge of separating highly polar and hydrophilic compounds in complex matrices.

5. Carbohydrates

Saccharides are the most abundant compounds in nature, the most important sources of energy in living organisms, and commonly used as sweeteners in food. Saccharides have also been extensively used as functional ingredients to improve food quality for many years (Bekers, Marauska, Grube, Karklina, & Duma, 2004). Despite the positive effects of some saccharides, there is a general awareness of health problems associated with a chronic high intake of sugars. For these reasons sugar analysis, has been, and will always be important for food products. At present, HILIC together with different detection techniques is considered as one of the best analytical tools for sugar analysis (Table 4), together with the official titrimetric method (AOAC 1995, method 942.15) and direct methods based on spectroscopic techniques combined with chemometric data analysis (Ackermann et al., 2017); Marrubini, Papetti, Genorini, & Ulrici, 2017; Ozbalci, Boyaci, Topcu, Kadilar, & Tamer, 2013; Xu, Qi, Sun, Fu, & Ying, 2012). Other analytical techniques (NMR, CE,

and GC) have limitations such as low sensitivity, poor repeatability, and need of derivatization steps.

5.1. Food of animal origin

5.1.1. Milk and colostrum

Oligosaccharides (OSs) are major components of human milk (7-12 g/L) and they have important biological functions especially for infant health. Sugars stimulate Bifidobacteria growth in the colon, prevent infections binding, neutralize pathogens, and are essential for brain development (Jantscher-Krenn, 2012). Human milk contains higher amounts and more complex OSs than other mammals milk (Warren et al., 2001). Cow milk is like human milk in terms of sugar composition and amounts, and it is the most common ingredient in infant formula as well as the basic material for industrial dairy products.

A HILIC-Orbitrap-MS method using a diol column, allowed the simultaneous detection of thirteen OSs in bovine milk. The method was validated using stachyose and verbascose as neutral OSs analogues, and three acidic OSs model, namely 3'-sialyllactose, 6'-sialyllactose and 6'-sialyl-lactosamine as reference standard compounds. The method was applied to 32 different milk samples from cows fed with three different types of diets, and the obtained data evidenced the variation of the OSs profile between the cows, as well as specific correlations between the major OSs measured in the milk samples (Liu, Moate, Cocks, & Rochfort, 2014).

Thermal treatments of milk in food production can lead to the formation of lactulose and epilactose which are non-digestible lactose derivatives with prebiotic properties. For this reason, lactulose can be used as an indicator of heat-induced modification in milk. Lactose, lactulose and epilactose were determined combining two chromatographic techniques in assaying commercial milk drinks (Rentschler et al., 2016). An IP-RPLC separation system coupled with a UV detector provided the separation and quantification of lactose and epilactose derivatives, while an HILIC/ion exchange (IEX) chromatography system coupled to an ELSD was used to determine lactulose. A mixed-mode

stationary phase was used with a mixture of acetonitrile and ammonium formate (80:20 v/v), pH 3.65. Under these conditions, it was possible to establish baseline separation of underivatized lactulose, derivatized lactose and epilactose, as well as residual derivatization reagents and other matrix interfering ionic compounds. The method was validated and could be used to analyze lactose, lactulose and epilactose in milk.

Goat colostrum was investigated to find new natural sources of bioactive OSs. A HILIC-MS method was developed for the separation and quantification of the main OSs using an amide functionalized stationary phase. Different gradient programs, operating temperatures, and mobile phase mixtures of acetonitrile-water modified with several additives were tested. The best separation could be achieved using a mobile phase with acetonitrile and water containing 0.1% ammonium hydroxide at 40 °C. For the first time, seventy eight OSs were identified by MS in goat colostrum, and it was concluded that the OSs profile of goat was closer to human than to bovine. This finding suggested the potential use of goat colostrum as an efficient source of naturally-occurring bioactive OSs (Martín-Ortiz et al., 2016).

Equine colostrum OSs composition showed over 60% structural overlap with human milk. The characterization and quantification of sixteen analytes in colostrum, of four different horse breeds, was performed combining CE with laser-induced fluorescence and MS detectors, and HILIC-MS^n followed by an exoglycosidase degradation (Difilippo et al., 2015). An amide functionalized column was used and the analytes eluted in gradient mode using a ternary mobile phase made of (A) water-acetonitrile (99:1%,v/v), (B) acetonitrile, and (C) 200 mM ammonium formate, pH 3.0. The relative abundance of the different OSs varied depending on the horse's breed, but differences were highlighted within the same breed also due to the intrinsic variation in composition of the colostrum sample obtained from every animal. Horse-specific OSs structures were identified and ten of the identified OSs were common to milk and colostrum of other domestic animals.

5.2. Vegetables, fruits, and related products

5.2.1. Dates

An efficient and highly sensitive UHPLC-MS method was developed for the simultaneous determination of fructose, glucose, sucrose, kestose, and nystose in date extracts (Ghfar et al., 2015). The chromatographic conditions were optimized and the best separation was achieved in less than seven min using gradient elution on an amide functionalized column. Acetonitrile and 0.1% (v/v) ammonia aqueous solution was used at constant flow rate of 0.4 mL/min.

5.2.2. Rehmannia glutinosa Libosch

One of the most used herbs in traditional Chinese dietary supplements, is *Rehmannia glutinosa Libosch.* Parts of this flowering plant, including the roots, are marketed for the purpose, and the quality control of the products and of their final preparations is an active area of research since years. Different HPLC columns packed with various stationary phases, both functionalized and non-functionalized (including aminopropyl-silica, plain silica, amide modified silica, and zwitterionic sulfoalkylbetaine), have been tested for the purpose. An amide functionalized silica column was chosen by Liu (2013) because it offered a higher resistance to degradation at 50 °C and tolerated the required basic pH conditions for suppressing sugar mutarotation. The neutral saccharides extractable from crude and dried roots were detected by TOF-MS (Liu et al., 2013).

The zwitterionic sulfoalkylbetaine stationary phase was also used in two systems, with either ELSD or ESI-MS detection, for investigating the composition in α-galactosides and oligosaccharides of the raffinose family of dried roots extracts (Kim, Kim, & Sung, 2012). Three model compounds (*viz* raffinose, stachyose, and verbascose) not easily analyzed using RPLC or any other HPLC mode, were studied. Not all the peaks reported in the chromatograms appeared sharp, but all the target analytes could be well separated within about 10 min.

5.2.3. Sugar beet and Rosa canina L.

Pectin is widely used in the production of jams and jellies, and marketed in many countries as a safe food additive. The analysis of pectin is interesting for the food industry for assessing the value of

starting materials and to predict functional properties of food products. The oligomers released after extended enzymatic degradation of methylesterified and acetylated sugar beet pectins were studied using a HILIC-ELSD-ESI-IT-MS^n method (Remoroza et al., 2012). The separation of the analytes was obtained on an amide modified column using a binary mobile phase with acetonitrile and water containing 0.01M ammonium formate and 0.05M formic acid. The acquired results demonstrated that the HILIC-based approach could provide essential and detailed information on the composition of pectin structure. The same stationary and mobile phases, and detection methods allowed the isolation and characterization of *Rosa canina* L. (rose hip fruit) pectin derivatives starting from an extraction from the dry fruits with aqueous citric acid (1%, v/v) (Ognyanov et al., 2016).

5.2.4. Selenosugars in plants

Another amide stationary phase provided the required separation of selenosugars, identified by electrospray linear trap/orbitrap tandem mass spectrometry, for the first time in extracts of staple crops such as wheat, rice, and maize grown on soils rich in selenium. The same unusual analytes were also studied in more details in black mustard seeds and other vegetables of the *Brassica* genus (Ouerdane et al., 2013).

5.3 Other food matrices

5.3.1. Wine, beer, fruit juices, vegetable extracts

HILIC coupled to MS has gained popularity in the analysis of complex mixtures of monosaccharides, disaccharides, and oligosaccharides in liquid samples such as wines, beers, milks, fruit juices or plant extracts (Kotoni, Ciogli, Villani, Bell, & Gasparrini, 2014; Liu et al., 2014) (Table 4). Neutral and acidic OSs, as well as neutral OSs rich in arabinose originating from pectin polysaccharides of grape cell walls have been characterized in Carignan red wine for the first time. After removing phenolic compounds and polysaccharides, OSs were fractionated by anion exchange and size-exclusion chromatography. Each fraction was analyzed using MS with ESI and an ion trap mass analyzer, after separation on an ammonium sulfonic acid modified silica column.

The OSs composition is relevant for wine production because of the technological and sensory properties associated with the presence of the sugars profile (Doco, Williams, Meudec, Cheynier, & Sommerer, 2015).

The analysis of complex mixtures of sugars in beer samples, including mono-, disaccharides, and maltose oligomers was reported using a new HILIC stationary phase bearing both bridged bis-ureido and free amino groups (Kotoni et al., 2014). The stationary phase was made from 2.5µm totally porous silica particles prepared following a one-pot procedure and by an end-capping reaction. Compared to other commercially available columns such as the amide functionalized ethylene bridged hybrid material and solid core plain silica, the new stationary phase named US-HILIC-NH_2-2.5SP showed very good performances and better peak shape that the authors attributed to the free amino groups present on the bidentate urea-type silica phase.

6. Artificial sweeteners

Sugar substitutes are commonly added to foods and beverages because they have high sweetening power yet low content of calories, and extend the shelf-life of food products. Due to the widespread use of sugar substitutes as food additives, their potential adverse effects on human health have been studied and EU regulations defined concentration limits in foods and beverages (Commission Regulation EU No 1131/2011; Commission Implementing Regulation EU No 872/2012). The most popular artificial sweeteners are: acesulfame-K, saccharin, cyclamates, aspartame, sucralose, alitame, neohesperidin dihydrochalcone, and neotame. In addition to synthetic sweeteners, also natural compounds are available such as xylitol, glycyrrhizin, phyllodulcin, mogrosides, thaumatin, and stevioside.

Steviol glycosides are natural sweeteners isolated from *Stevia rebaudiana* Bertoni, a perennial plant native to Paraguay and Brazil. Steviol glycosides were recognized as a new class of sweeteners in 2014 by the European Union, and the most abundant compounds are stevioside and rebaudioside A.

Other minor components are dulcoside A, steviolbioside, rubusoside, and rebaudioside C, D, E, and F. Considering the concentration levels allowed by the regulatory authorities, sensitive analytical methods are needed to quantify these compounds in foods and beverages. Table 4 summarizes recent methods reported for artificial sweeteners analysis.

The major steviol glycosides in dried *Stevia* leaves were analyzed with HILIC and isotopic dilution MS/MS detection with the deuterated 16,17-dihydrosteviol glycosides as internal standards (Well, Frank, & Hofmann, 2013). All the analytes, including the isobaric pairs steviolbioside/dulcoside A, stevioside/rebaudioside B, and rebaudioside E/rebaudioside A were separated using an amine functionalized column. More recently, HILIC coupled with tandem mass spectrometry was used for the determination of eight artificial sweeteners and steviol glycosides in popular beverages (Kubica, Namiesnik, & Wasik, 2016). Three columns were evaluated: a stationary phase with polymer amine ligands that combines hydrophilic and ionic properties, a solid-core non-functionalized silica phase, and a mixed mode phase. The analytes were eluted in gradient mode using MP consisting of mixtures of methanol, acetonitrile, ammonium acetate, and acetic acid in water. The mixed mode column together with a mobile phase consisting of 40 mM ammonium acetate pH 6.8 and acetonitrile (20 minutes run time) offered a separation of fourteen different compounds. The authors concluded that the new method allowed complete resolution of the steviol glycosides, and with better efficiency in comparison with RPLC mode.

7. Polyphenolic compounds

Polyphenols are secondary metabolites present in fruits and vegetables. Their intake with food has been put in relation since years and linked with the reduction of risk of some chronic diseases (Abate, Marziano, Rungratanawanich, Memo, & Uberti, 2017; Vazahappilly, Dellaire, & Rupasinghe, 2017). A great effort has been deployed in biomedical research to assess the best

natural sources of polyphenols in foodstuff and beverages. In the last years, many HILIC applications have been published on the topic (Table 5).

The effect of many operating experimental parameters on the separation of fifteen flavonoids was investigated using a zwitterionic sulfobetaine functionalized methacrylate stationary phase (Sentkowska, Biesaga, & Pyrzynska, 2013). Acetonitrile and methanol were compared as organic modifiers in the mobile phase. Both solvents provided the same elution order of the target flavonoids. However, increasing the content of organic solvent, methanol gave higher retention factors and allowed the SP to retain more strongly the analytes in comparison with acetonitrile. As mentioned in the introduction, methanol and/or other low molecular weight alcohols-based mobile phases can be used under isocratic conditions but in this case methanol could not help to resolve the critical couples of peaks represented by kaempferol and luteolin, and by hesperidin and quercetin. No significant changes in the retention were registered when pH was increased from 2.8 to 9.0. This was attributed to the low net negative surface charge at the boundary between the flowing and the static streams of mobile phase flowing over the SP, were only little affected by pH. Considering the effect of salt in the mobile phase, it was concluded that the ion-exchange mechanism did not contribute significantly to the overall retention of the more hydrophobic aglycons (*viz*, rhamnetin, kaempferol, and quercetin). For quercetin glycosides, only high salt concentrations resulted in a decrease in retention. The column temperature was also investigated for the flavonoids exhibiting sufficient retention under isocratic conditions (methanol or acetonitrile-10 mM ammonium formate, pH 7, 95:5, v/v), and only a slight decrease in retention was registered with the increasing of temperature. The selected temperature for the analysis of hesperidin and naringin in orange juices was 30 °C.

Willemse et al. (2013) used HILIC for the analysis of anthocyanins. After an evaluation of several HILIC stationary and mobile phases, the authors concluded that an amide functionalized stationary phase provided the best performance when a mobile phase of water-acetonitrile containing TFA

0.4% (v/v) was used. An acidic mobile phase was essential for ensuring satisfactory peak shapes and the prevalence of the flavilium cationic species of these molecules. The interesting mechanism underlying this observation is that the flavilium ion at neutral to basic pH through hydration could easily lead to the formation of the corresponding carbinol pseudo-base, which in turn undergoes further ring opening to yield the chalcone. In this case, further improvement in chromatographic performances may be obtained by increasing the column temperature to 50 °C. The retention behavior of anthocyanins resulted qualitatively predictable; in fact, higher is the degree of glycosylation, and thus higher is the hydrophilic character of the molecule, stronger is the retention, as already observed by many other groups for other classes of compounds (e.g., carbohydrates or nucleosides/nucleotides). HILIC was successfully used for the analysis of anthocyanins as demonstrated in the assessment of anthocyanin profiles of five different food matrices, namely blueberries, red cabbage, red radish, grape skins, and black beans.

Proanthocyanidins, also commonly known as condensed tannins, are oligomeric or polymeric compounds composed of flavan-3-ol (the most represented being catechin) and flavan-3,4-diol units. Their separation and determination is generally problematic because these molecules exist as complex mixtures of monomers, oligomers, and polymers which are present in vegetables and fruits to a degree of polymerization still not well defined. In recent years, HILIC has been successfully applied for the separation of condensed tannins in wild vegetables such as the sea buckthorn berries (*Hippophae rhamnoides*) (Kallio, Yang, Liu, & Yang, 2014; Yang, Laaksonen, Kallio, & Yang, 2016, 2017) used both for nutritional and medicinal purposes in Asia. After extraction of the soluble fraction from dried material in acetone-water-acetic acid (90:19.5:0.5, v/v/v), proanthocyanidins were isolated from flavonols and other impurities using a column with hydroxypropylated dextran beads crosslinked to yield a polysaccharide network. Differently from RPLC and NPLC, HILIC mode provided the separation of proanthocyanidins based on the degree of polymerization and molecular size, with early eluting compounds having lower degree of polymerization (starting from

monomers, and dimers) up to a degree of polymerization as high as eleven. All the condensed tannins observed were B-type with (epi)gallocatechin being the monomeric unit more frequently observed. The quantification of dimers, trimers, and tetramers was carried out with ESI-MS-SIR (Kallio et al., 2014). Using the same purification steps, column, chromatographic and ESI-MS conditions, the proanthocyanidins profile and content were investigated in three different subspecies of wild and cultivated sea buckthorn from China, Finland, and Canada (Yang et al., 2016). The results indicated that the berries grown in northern Finland were the richest in B-type proanthocyanidins, evidencing also a strong influence of genetic background and interaction with growth location on proanthocyanidins composition and content. The same research group, using the same method, reported the effects of growth environment on Finnish sea buckthorn berries from northern and southern Finland (Yang et al., 2017).

8. Conclusions

Food analysis requires specific, robust, and sensitive methods for the determination of nutrients and bioactive components. The number of applications reported in literature has vertiginously increased in the last decade. Many studies reported on the use of several chromatographic techniques coupled with detectors of consolidated use such as spectrophotometric, spectroscopic, and of course MS, MS/MS and MS^n. Generally, in food analysis LC is preferred over GC because the target compounds should be analyzed under mild conditions (*viz*, direct injection in non-denaturing solvents, at room temperature) and that laborious sample clean-up and derivatization steps can be omitted. Among liquid chromatography, RP-mode is the most common in food analysis. However, in the last years many HILIC methods have been reported for polar, hydrophilic and ionic analytes in foods. HILIC for food analysis offers many advantages over RP and appears to be an orthogonal technique that brings new and complementary information to the analysts.

In this review, we present an up-date overview of the use of HILIC starting from 2011 to the submission data in 2017, focusing the attention to mono-dimensional methods applied to the determination of major food components avoiding the discussion of food toxins and contaminants which will be considered in a future review. The high number of different applications discussed herein evidence that HILIC can be considered as an established LC mode, widely recognized as a useful tool in food quality control analysis.

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Analysis of food components using hydrophilic interaction chromatography is reviewed.

Advantages/disadvantages of HILIC in vegetable and animal food analysis is discussed.

The reviewed HILIC methods focus on the papers from 2011 to submission date in 2017.

Table 1. HILIC applications in the analysis of nucleobases, nucleosides, nucleotides, uric acid, and creatinine in food.

Sample	Number, type of analytes	Column	Chromatographic conditions	Elution mode	Detector	LODs LOQs	Reference
Thais clavigera extract	41, nucleosides and nucleobases, and amino acids	Amide functionalized ethylene bridged hybrid (2.1x100mm, 1.7µm)	5mM ammonium formate, 5mM ammonium acetate, 0.2% formic acid aqueous solution (solvent A) and 1mM ammonium formate, 1mM ammonium acetate, 0.2% formic acid in acetonitrile (solvent B); flow rate 0.4 mL/min; 35 °C.	Gradient	QqQ with ESI	0.003-0.112 µg/mL 0.008-0.352µg/mL	Ge et al. (2015)
Bovine milk	3, uric acid, creatinine and ascorbic acid	Amine (4.6x250mm, 5µm)	Acetonitrile – aqueous 10mM sodium phosphate pH 4.75 (50:50%, v/v); flow rate 1.2 mL/min; injection volume 20 µL	Isocratic	UV at 205 nm	0.045-0.18 µg/mL 0.15-0.60 µg/mL	Zuo et al. (2015)
Ginkgo biloba seed aqueous extract	20, nucleobases and nucleosides	Amide functionalized ethylene bridged hybrid (2.1x100mm, 1.7µm)	0.8% acetic acid and 10mM ammonium acetate in aqueous solution (solvent A) and 0.1% acetic acid in acetonitrile (solvent B); flow rate 0.4 mL/min; 35 °C; injection volume 1 µL	Gradient	QqQ with ESI	0.02-42.54 ng/mL 0.05-98.18 ng/mL	Zhou et al. (2014)
Ginkgo biloba leave aqueous extract	11, nucleobases and nucleosides	Amide functionalized ethylene bridged hybrid (2.1x100mm, 1.7µm)	0.8% acetic acid and 10mM ammonium acetate in aqueous solution (solvent A) and 0.1% acetic acid in acetonitrile (solvent B); flow rate 0.4 mL/min; 35 °C; injection volume 1 µL	Gradient	QqQ with ESI	0.24-41.36 ng/mL 0.75-101.33 ng/mL	Yao et al. (2015)
Infant milk formulas	5, 5'-mononucleotides (AMP, CMP, GMP, IMP,	Amide functionalized silica	acetonitrile (solvent A) and water (solvent B), pH 2.5 (with formic acid), 70:30, v/v; flow rate 0.8 mL/min; 25 °C; injection volume 20	Isocratic	DAD	0.03-0.09 µg/mL	Sfakianaki & Stalikas (2015)

	UMP)	(150x4.6mm, 5µm)	µL				
Baby foods	20, nucleosides and nucleotides mono-, di-, triphosphates	Amide functionalized silica (100x2.1mm, 3.5µm)	ACN (solvent A) and water (solvent B) with 100mM hexafluoro-2-propanol and 50mM diethylammonium; flow rate 0.35mL/min; 20 °C	Gradient	QqQ with ESI	5-60 ng/mL 15-200 ng/mL	Mateos-Vivas et al. (2016)
Ganoderma species fungi	16, nucleosides and nucleobases	Sulfobetaine functionalized silica (100 x4.6mm, 3.5µm)	acetonitrile with 3mM ammonium acetate (solvent A) and 15mM ammonium acetate in H_2O, pH=6 (solvent B); flow rate 1 mL/min; 25 °C; injection volume 2 µL	Gradient	DAD	0.005-0.029 µg/mL 0.018-0.096 µg/mL	Chen et al. (2012)

QqQ: Triple Qudrupole; ESI: Electrospray Ionization; UV: Ultraviolet; DAD: Diode Array Detector.

Table 2. HILIC applications in the analysis of amino acids and peptides in food.

Sample	**Number, type of analytes**	**Column**	**Chromatographic conditions**	**Elution mode**	**Detector**	**LODs LOQs**	**References**
Chicken broth	2, carnosine and anserine	Non-functionalized ethylene bridged hydride silica (100 x 2.1 mm, 1.7 µm)	0.65 mM ammonium acetate in water-acetonitrile (25:75, v/v) (solvent A) and 4.55 mM ammonium acetate in water-acetonitrile (70:30, v/v) (solvent B); flow rate 0.4 mL/min; 30 °C; injection volume 10µL	Gradient	QqQ with ESI	0.6-1.8 µg/L 2-6 µg/L	Macià e al. (2012)
Chorizo sausages	22, amino acids, di-and tri-	Sulfobetaine functionalized silica	6.5mM ammonium acetate in acetonitrile-water (90:10, v/v)	Gradient	IT with	- -	Broncano et al.

	peptides, L-carnitine, and creatinine	(150 x 2.1 mm, 3.5 µm)	(solvent A) and 6.5mM ammonium acetate in acetonitrile-water (40:60, v/v) (solvent B); flow rate 0.05mL/min; injection volume 10µL		ESI		(2012)
Mussels	21, amino acids	Amide functionalized ethylene bridged hybrid (150 x 2.1 mm, 1.7µm)	Acetonitrile with 5mM ammonium formate, pH 3.0 (95:5, v/v) (solvent A) and acetonitrile with 5mM ammonium formate, pH 3.0 (40:60, v/v) (solvent B); 40 °C; injection volume 5µL	Gradient	QqQ with API	0.001-0.034 g/100g 0.003-0.104g/100g	Tsochatzis et al. (2016)
Enzymatic digest of fish protein	13, arginine-containing dipeptides	Amide-80 (150 x 4.6 mm, 3µm; 300 x 7.8 mm, 10µm)	0.1% formic acid in acetonitrile (solvent A) and 0.1% aqueous formic acid solution (solvent B); flow rate 1.0 mL/min or 6.0 mL/min; 40 °C	Gradient	ELSD	- -	Schindler et al. (2011)
		Amide-80 (150 x 2.0 mm, 3µm)	5.0 mM aqueous ammonium acetate, pH 6.5 (solvent A) and acetonitrile-water (95:5, v/v) containing 5 mM ammonium acetate (solvent B); flow rate 0.2 mL/min; injection volume 1µL	Gradient	TOF	- -	
Fruit	23, amino acids	Amide functionalized ethylene bridged hybrid (100 x 2.1 mm, 1.7µm)	Water, 10mM ammonium formate, 0.15% formic acid, pH 3.0 (solvent A) and acetonitrile, 2 mM ammonium formate, 0.15% formic acid (solvent B); flow rate 0.4mL/min; 35 °C; injection volume 2µL	Gradient	QqQ with ESI	0.17-79.25 ng/mL 0.68-294.00 ng/mL	Guo et al. (2013)
Fruit	27, amino acids, dipeptides, and tripeptides	Amide functionalized ethylene bridged hybrid (100 x 2.1 mm, 1.7µm)	Water, 10mM ammonium acetate, 0.5% acetic acid (solvent A) and acetonitrile in 1mM ammonium acetate, 0.1% acetic acid (solvent	Gradient	QTRAP® with ESI	0.15-63.66 ng/mL 0.45-190.85 ng/mL	Zhou et al. (2015)

			B); flow rate 0.4mL/min; 35 °C; injection volume 3µL				
Gingko seeds extract	24, amino acids	Amide functionalized ethylene bridged hybrid (100 x 2.1 mm, 1.7µm)	Water, 5mM ammonium formate, 5mM ammonium acetate, 0.15% formic acid (solvent A) and acetonitrile in 1mM ammonium formate, 1mM ammonium acetate, 0.15% formic acid (solvent B); flow rate 0.4mL/min; 35 °C; injection volume 1µL	Gradient	QqQ with ESI	0.15-78.66 ng/mL 0.56-270.12 ng/mL	Zhou et al. (2013)
Peach by-product (seeds)	6, peptides	Non-functionalized solid-core silica (100 x 2.1, 2.7µm)	65 mM ammonium acetate in acetonitrile (solvent A) and 65 mM ammonium acetate in water (solvent B); flow rate 0.3 mL/min; 25 °C; injection volume 10µL	Gradient	Q-TOF with ESI	- -	Vásquez-Villanueva et al. (2016)
Fruit juices, beer, wine, and honey	22, amino acids	Non-functionalized silica (150 x 2.1 mm, 3□m)	Acetonitrile (solvent A) and 0.1% formic acid aqueous solution (solvent B); flow rate 0.4mL/min; 30 °C; injection volume 5µL	Gradient	Orbitrap with ESI	0.5-47 ng/mL 1.5-15.5 ng/mL	Gökmen et al. (2012)

QqQ: Triple quadrupole; ESI: Electrospray Ionization; IT: Ion Trap; API: Atmospheric Pressure Ionization; ELSD: Evaporative Light Scattering Detector; TOF: Time Of Flight; QTRAP®: hybrid triple–Quadrupole Linear Ion Trap; Q-TOF: Quadrupole-Time Of Flight.

Table 3. HILIC applications in the analysis of choline-related compounds and phospholipids in food.

Sample	**Number, type of analytes**	**Column**	**Chromatographic conditions**	**Elution mode**	**Detector**	**LODs LOQs**	**References**
Egg yolks	11, choline-related	Non-functionalized Solid core silica	Acetonitrile (solvent A) and 10mM aqueous ammonium formate, pH	Gradient	QTRAP® with	- 0.02-0.25 µg/mL	Zhao et al.

	compounds	(150 x 2.1 mm, 2.7µm)	3.0 (solvent B); flow rate 0.2 mL/min; injection volume 2µL		Turbospray Ion Source		(2011)
Homogenated meals and egg yolks	14, choline-related compounds, phospholipids	Non-functionalized Solid core silica (150 x 2.1 mm, 2.7µm)	Acetonitrile (solvent A) and 10mM aqueous ammonium formate, pH 3.0 (solvent B); flow rate 0.2 mL/min and 0.4 mL/min; 25 °C; injection volume 2µL	Gradient	QTRAP® with ESI	0.004-0.1 µg/mL 0.01-0.5 µg/mL	Xiong et al. (2012)
Shrimp waste	69, phospholipids	Diol HILIC (250 x 4.6 mm, 3µm)	Acetonitrile in 53 mM formic acid (solvent A, pH 4.0-4.5) and aqueous solution 60 mM ammonium formate-53 mM formic acid (solvent B, pH 3.6); flow rate 0.2 mL/min; 50 °C; injection volume 5µL	Gradient	Q-TOF with ESI	0.12-0.25 µg/mL 0.37-0.78 µg/mL	Shen et al. (2014)
Dried seahorse	50, phospholipids	Diol HILIC (250 x 4.6 mm, 3µm)	10 mM ammonium formate in 0.2% formic acid (solvent A) and acetonitrile in 0.2% formic acid (solvent B); flow rate 0.2 mL/min; 50 °C; injection volume 5µL	Gradient	Q-TOF with ESI	0.12-0.52 ng/mL 0.37-1.57 ng/mL	Shen et al. (2016)
Milk	6, phospholipids	Non-functionalized Solid core silica (150 x 4.6 mm, 2.7µm)	Acetonitrile-ammonium formate (90:10, v/v, pH 5.5) (solvent A) and acetonitrile-methanol-ammonium formate (55:35:10, v/v/v, pH 5.5); flow rate 0.7 mL/min; injection volume 5µL	Gradient	ELSD IT-TOF with ESI	3.97-4.17 mg/L 4.35-4.59 mg/L - -	Russo et al. (2013)
Milk	8, phospholipids	Diol HILIC (250 x 4.6 mm, 5µm)	5 mM aqueous ammonium acetate (solvent A) and 0.1 % formic acid in acetonitrile (solvent B); flow rate 0.6 mL/min; 30 °C; injection volume 5µL	Gradient	Orbitrap with HESI	0.01-0.1 ng/mL 0.05-0.5 ng/mL	Liu et al. (2015)
Pulses	5, choline and choline-related	Non-functionalized Solid core silica	acetonitrile (solvent A) and 10 mM ammonium formate in water	Gradient at two	QTRAP®	3.97-4.17 mg/L	Lewis et al.

	compounds	(150 x 2.1, 2.7 µm)	(solvent B) (adjusted with formic acid to pH 3.0; flow rate from 0.2 to 0.4 mL/min; 25 °C; injection volume 2 µL	flow rates	with ESI	4.35-4.59 mg/L	(2014)
Virgin olive oil	5, phospholipids	Non-functionalized Solid core silica (150 x 2.1 mm, 2.6µm)	Chloroform-methanol-ammonium formate buffer 1 M (pH 3), (87.5:12:0.5, v/v/v) (solvent A) and chloroform-methanol-ammonium formate buffer 1M (pH 3) (28:60:12, v/v/v) (solvent B); flow rate 0.25mL/min; 25 °C; injection volume 5µL	Gradient	Q-TOF with ESI	0.010-0.123 µg/mL 0.160-0.493 µgm/L	Verardo et al. (2013)

QTRAP®: hybrid triple–Quadrupole Linear Ion Trap; ESI: Electrospray Ionization; Q-TOF:Quadrupole-Time Of Flight; ELSD: Evaporative Light Scattering Detector; IT: Ion Trap; IT-TOF: Ion Trap-Time Of Flight; HESI: Heated Electrospray Ionization.

Table 4. HILIC applications in the analysis of carbohydrates and artificial sweeteners in food.

Sample	**Number, type of analytes**	**Column**	**Chromatographic conditions**	**Elution mode**	**Detector**	**LODs LOQs**	**References**
Bovine milk	13, free oligosaccharides	Diol HILIC (250 x 4.6mm, 5 µm)	0.1% formic acid aqueous solution (solvent A) and 0.1% formic acid in acetonitrile (solvent B); flow rate 0.5 mL/min; 30 °C; injection volume 5µL	Gradient	LTQ Orbitrap with HESI	0.05 ng/mL 0.25 ng/mL	Liu et al. (2014)
Milk	1, lactulose	Mix-mode (100 x 3.0 mm, 3 µm)	Acetonitrile (solvent A) and 100mM ammonium formate, pH=3.65 (solvent B), 80:20, v/v; flow rate 0.5 mL/min; 60 °C;	Isocratic	ELSD	139 mg/L 444 mg/L	Rentschler et al. (2016)

			injection volume 5µL				
Goat colostrum	78, oligosaccharides	Amide-functionalized ethylene bridged hybrid (150 x 4.6mm, 3.5 µm)	0.1% ammonium hydroxide aqueous solution (solvent A) and 0.1% ammonium hydroxide in acetonitrile (solvent B); flow rate 0.4 mL/min; 40°C; injection volume 5µL	Gradient	Q with ESI	3.28-10.94 ng/mL 10.94-535.41 ng/mL	Martín-Ortiz et al. (2016)
Horse colostrum	16, oligosaccharides	Amide-functionalized ethylene bridged hybrid (150 x 2.1mm, 1.7µm)	Water with 1% acetonitrile (solvent A), acetonitrile (solvent B), and 200 mM ammonium formate, pH=3 (solvent C); flow rate 0.6 mL/min; injection volume 5µL	Gradient	LTQ Orbitrap with ESI	- -	Difilippo et al. (2015)
Dates	5, monosaccharides and oligosaccharides	Amide-functionalized ethylene bridged hybrid (50 x 2.1mm, 1.7µm)	Acetonitrile (solvent A) and water with 0.1% ammonia (solvent B); flow rate 0.4mL/min; injection volume 1µL	Gradient	TQ with ESI	0.25-0.69 µg/mL 0.82-3.58 µg/mL	Ghfar et al. (2015)
Rehmannia glutinosa radix	9, mono- and oligosaccharides	Amide-functionalized ethylene bridged hybrid (100 x 2.1mm, 1.7 µm)	8 mM ammonium formate aqueous solution, pH=9.8 (solvent A) and 8 mM ammonium formate in acetonitrile, pH=9.8 (solvent B); flow rate 0.3 mL/min; 50 °C; injection volume 2µL	Gradient	TOF with ESI	1.9-2.9 ng/mL 5.6-9.8 ng/mL	Liu et al. (2013)
Rehmannia glutinosa radix	3, oligosaccharides	Sulfobetaine functionalized silica (100 x 4.6 mm, 5µm)	Acetonitrile (solvent A) and water with 0.03% formic acid (solvent B); flow rate 0.3mL/min;	Gradient	ELSD	<18.9 µg/mL <57.4 µg/mL	Kim et al. (2012)
Sugar beet	23, oligomers derived from pectin	Amide-functionalized ethylene bridged hybrid (150 x 2.1xmm, 1.7 µm)	Acetonitrile-water (80:20, v/v) containing 0.01M ammonium formate and 0.05M formic acid (solvent A) and acetonitrile-water (20:80, v/v) containing 0.01M ammonium formate and 0.05M	Gradient	ELSD with on line IT with ESI	- -	Remoroza et al. (2012)

			formic acid (solvent B); flow rate 0.6 mL/min; 35 °C; injection volume 10µL				
Rose hip fruits (*Rosa canina* L.)	16, pectic polisaccharides	Amide-functionalized ethylene bridged hybrid (150 x 2.1mm, 1.7µm)	Water-acetonitrile (99:1, v/v) (solvent A), acetonitrile (solvent B), and 200mM ammonium formate-50mM formic acid buffer, pH=3 (solvent C); flow rate 0.5 mL/min; 35 °C; injection volume 5µL	Gradient	ELSD with on line LTQ with ESI	- -	Ognyanov et al. (2016)
Mustard seeds	33, selenosugars	Amide 80 (250 x 1mm, 5 µm)	10 mM ammonium formate, pH=5.5 (solvent A) and acetonitrile (solvent B); flow rate 0.05 mL/min; injection volume 3µL	Gradient	ICP Orbitrap with ESI	- -	Ouerdane et al. (2013)
Carignan red wine	25, oligosaccharides	ammonium sulfonic acid modified silica HILIC (100 x 1mm, 1.8 µm)	Acetonitrile-formic acid (99.5:0.5, v/v) (solvent A) and water- formic acid (99.5:0.5, v/v) (solvent B); flow rate 0.3 mL/min; 35°C	Gradient	IT with ESI	- -	Doco et al. (2015)
Beer, sugars standard mixtures	16, polyols, mono-, di- and oligosaccharides	USP-HILIC-NH_2-2.5SP (100 x 3.2mm, 2.5 µm)	Acetonitrile (solvent A) and water (solvent B) + 10mM ammonium acetate (total), 85:15, v/v, (for Polyols); Acetonitrile (solvent A) and water (solvent B) + 10mM ammonium acetate (total), 90:10, v/v (for sugars); flow rate 0.8 mL/min, 25°C; injection volume 1-5µL	Isocratic Gradient	DAD ELSD	- - - -	Kotoni et al. (2014)
Dried *Stevia* leaves	10, stevioside, rebaudiosides A-F, dulcoside, rubososide, steviolbioside	Amino-functionalized (150 x 2.0 mm, 3 µm)	5mM ammonium acetate and 0.05% formic acid in acetonitrile-water (95:5, v/v) (solvent A) and 5mM ammonium acetate and 0.05% formic acid in acetonitrile-water (10:90, v/v) (solvent B); flow rate 0.2mL/min; injection volume	Gradient	QTRAP® with ESI	- -	Well et al. (2013)

			10µL				
Alcoholic and non-alcoholic beverages and instant drink powders	14, artificial sweeteners and steviol glycosides	Acclaim Trinity P2 mixed-mode (100 x 2.1 mm, 3 µm)	40mM ammonium acetate, pH 6.8 (solvent A) and 0.01% acetic acid in acetonitrile (solvent B); flow rate 0.6 mL/min; 30°C; injection volume 2µL	Gradient	QqQ with ESI	0.81-3.30 ng/mL 2.32-9.89 ng/mL	Kubica et al. (2016)

LTQ Orbitrap: Linear Trap Quadrupole Orbitrap; HESI: Heated Electrospray Ionization; ELSD: Evaporative Light Scattering Detector; Q: Quadrupole; ESI: Electrospray Ionization; TQ: Tandem Quadrupole; TOF: Time Of Flight; IT: Ion Trap; ICP: Inductively Coupled Plasma; DAD: Diode Array Detector; QTRAP®: hybrid triple–Quadrupole Linear Ion Trap; QqQ: Triple Quadrupole; DAD: Diode Array Detector.

Table 5. HILIC applications in the analysis of polyphenolic compounds in food.

Sample	**Number, type of analytes**	**Column**	**Chromatographic conditions**	**Elution mode**	**Detector**	**LODs LOQs**	**References**
Orange juices	15, flavonoids	Sulfobetaine funtionalized methacylate (150 x 2.1 mm, 5µm)	10 mM formic acid aqueous solution (pH 2.8) or 10 mM ammonium acetate (pH 7.0 or 9.0) (solvent A) and methanol or acetonitrile (solvent B); flow rate 0.20 mL/min; 95 (A)-5 (B), v/v; 25-40 °C; injection volume 2µL	Gradient or isocratic	QTRAP® with ESI	- -	Sentkowaska et al. (2013)
Blueberries, red cabbage, red radish, grape skins, black beans	23, anthocyanins	Amide functionalized ethylene bridged hybrid (150 x 1.0 mm, 1.7µm)	0.4% TFA in acetonitrile (solvent A) and 0.4% TFA in water (solvent B); flow rate 0.025mL/min; 50 °C; injection volume 2µL	Gradient	Q-TOF with ESI	- -	Willemse et al. (2013)

Wild sea buckthorn berries	12, proanthocyanidins	Diol HILIC (100 x3.0 mm, 3µm)	Acetonitrile (solvent A) and 0.5% formic acid in water (solvent B); flow rate 0.6 mL/min; injection volume 10µL	Gradient	TQ with ESI	- -	Kallio et al. (2014)
Sea buckthorn berries	12, proanthocyanidins	Diol HILIC (100 x3.0 mm, 3µm)	Acetonitrile (solvent A) and 0.5% formic acid in water (solvent B); flow rate 0.6 mL/min; injection volume 10µL	Gradient	TQ with ESI	- -	Yang et al. (2016)
Sea buckthorn berries	12, proanthocyanidins	Diol HILIC (100 x3.0 mm, 3µm)	Acetonitrile (solvent A) and 0.5% formic acid in water (solvent B); flow rate 0.6 mL/min; injection volume 10µL	Gradient	TQ with ESI		Yang et al. (2017)

QTRAP®: hybrid triple–Quadrupole Linear Ion Trap; ESI: Electrospray Ionization; Q-TOF:Quadrupole-Time Of Flight; TQ: Tandem Quadrupole.