Trends in Analytical Chemistry 92 (2017) 12–31

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Determination of nucleosides and nucleotides in food samples by using liquid chromatography and capillary electrophoresis

Javier Domínguez-Álvarez [a, *], María Mateos-Vivas [a], Encarnación Rodríguez-Gonzalo [a], Diego García-Gómez [b], Myriam Bustamante-Rangel [a], María-Milagros Delgado Zamarreño [a], Rita Carabias-Martínez [a]

[a] Department of Analytical Chemistry, Nutrition and Food Science, University of Salamanca, 37008, Salamanca, Spain
[b] Department of Analytical Chemistry, University of Córdoba, 14071, Córdoba, Spain

ARTICLE INFO

Article history:
Available online 18 April 2017

Keywords:
Capillary electrophoresis
Food analysis
Infant foods
Liquid chromatography
Nucleosides
Nucleotides
Sample treatment

ABSTRACT

Nucleosides and nucleotides are intracellular compounds that are precursors of nucleic acids and which take part in various regulatory and metabolic functions. Although they can be synthesized endogenously, a dietary supplement is beneficial to the organism under certain conditions when the demand for these semi-essential nutrients is high. The analysis of these compounds in food is, therefore, of great importance if we are to improve and ensure food quality. This review summarizes the chromatographic and capillary electrophoresis analytical methods described for the analysis of nucleosides and nucleotides in foodstuffs, including sample treatment steps used for isolation and cleanup. In addition, the future prospects in the analysis of these compounds in food matrices have also been included.

1. Introduction

Nucleosides and nucleotides are essential in a large number of biological processes. All mammal, bacteria, and plant cells naturally contain these compounds, which are the precursors of the nucleic acids that make up DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) and, therefore, play a vital role in the storage, transfer, and expression of genetic information. Moreover, nucleosides and nucleotides participate in other metabolic functions as they form part of biosynthetic routes, operate in the transfer of chemical energy, are components of some co-enzymes, and play an important role as biological regulators.

The human body is capable of synthesising these molecules endogenously, which means that they are constantly forming and being broken down in the organism (Fig. 1). Formation may, therefore, occur in two different ways: by *de novo* synthesis in new cells from precursor amino acids; or by alternative recovery routes known as salvage pathways on which the intermediaries generated

Abbreviations: A, adenosine; ACN, acetonitrile; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; BGE, background electrolyte; C, cytidine; CDP, cytidine 5′-diphosphate; CE, capillary electrophoresis; CEC, capillary electrochromatography; CMP, cytidine 5′-monophosphate; CRM, certified reference material; CUF, centrifugal ultrafiltration; CZE, capillary zone electrophoresis; DAD, diode array detector; DBAA, dibutylammonium acetate; DEA, diethylamine; DHAA, dihexylammonium acetate; DMHA, *N*, *N*-dimethylhexylamine; DNA, deoxyribonucleic acid; DSPE, dispersive solid-phase extraction; EDTA, ethylene diamine tetraacetic acid; EOF, electroosmotic flow; ESI, electrospray ionization; EUC, equivalent *umami* concentration; G, guanosine; GDP, guanosine 5′-diphosphate; GMP, guanosine 5′-monophosphate; GTP, guanosine 5′-triphosphate; HFIP, hexafluoro-2-propanol; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; Hx, hypoxanthine; I, inosine; ICP, inductively coupled plasma; IE-LC, ion-exchange liquid chromatography; IMP, inosine 5′-monophosphate; IP-RP-LC, ion-pair reversed-phase liquid chromatography; MEKC, micellar electrokinetic chromatography; MIPs, molecularly imprinted polymers; MS, mass spectrometry; MSPD, matrix solid-phase dispersion; NAD^+, nicotinamide adenine dinucleotide; NDPs, 5′-nucleotide di-phosphates; NMPs, 5′-nucleotide mono-phosphates; NTPs, 5′-nucleotide tri-phosphates; OT, open-tubular; OTCEC, open-tubular capillary electrochromatography; PDFOA, pentadecafluorooctanoic acid; PEEK, polyether ether ketone; *p*HILIC, organic polymer hydrophilic interaction liquid chromatography; PLE, pressurized liquid extraction; QqQ, triple-quadrupole; RNA, ribonucleic acid; RP-LC, reversed-phase liquid chromatography; SPE, solid-phase extraction; TBA, tributylamine; TBAOH, tetrabutylammonium hydroxide; TOF, time of flight; TPAN, total potentially available nucleos(t)ides; U, uridine; UFLC, ultra-fast liquid chromatography; UHPLC, ultra-high performance liquid chromatography; UMP, uridine 5′-monophosphate; UV, ultraviolet; ZIC, zwitterionic.

* Corresponding author.
E-mail address: hamelin@usal.es (J. Domínguez-Álvarez).

http://dx.doi.org/10.1016/j.trac.2017.04.005
0165-9936/

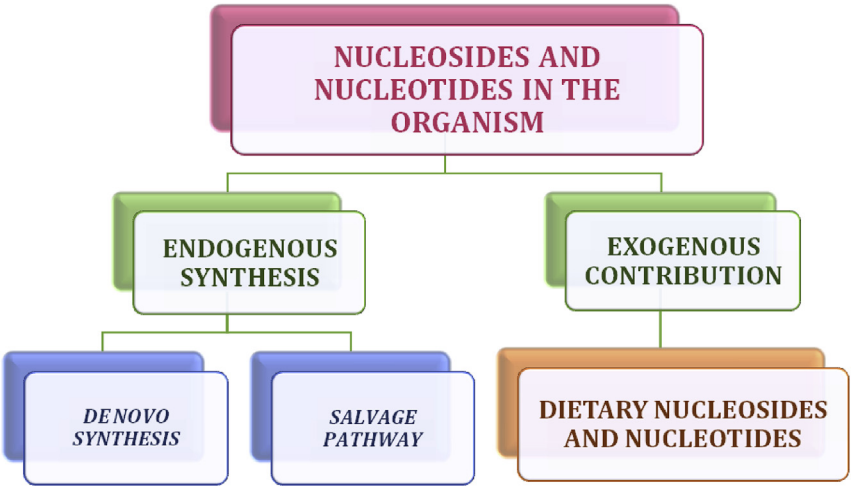


Fig. 1. Pathways leading to the synthesis and incorporation of nucleosides and nucleotides in the body.

in the breaking down of DNA and RNA such as bases and nucleosides are used to synthesise new nucleotides [1,2].

Nucleosides and nucleotides can also enter the organism exogenously through food; the human body is capable of absorbing them and making use of them [3]. Under certain conditions the demand for nucleosides and nucleotides increases, the endogenous supplement becomes insufficient, and the ingestion of these compounds becomes essential for the normal development of the functions of the organism. This may occur during certain periods of illness such as intestinal lesions, if the ingestion of proteins is reduced, or if the immune system is activated during stages of rapid growth or in the presence of regulatory or development factors that interfere with the full expression of the capacity for endogenous synthesis [3]. Many studies have shown that the presence of these compounds in the diet has beneficial effects on the immune response, the absorption of iron, lipid metabolism, gut flora and both intestinal and hepatic morphology and function [2].

Owing to their important functions, the determination of nucleosides and nucleotides is of great interest in different scientific fields such as chemistry, biochemistry, medicine, genetics, metabolomics, and the environment [4].

The methods for the analysis of nucleosides and nucleotides in milk and pediatric formulas were reviewed by Gill and Indyk in 2007 [5]; the determination of nucleotides and nucleosides by capillary electrophoresis in food materials was also reviewed by Chen et al. in 2010 [6]. Taking these bibliographical references as a starting point, this study revises the current status of the analysis of nucleosides and nucleotides in dairy and non-dairy foods including the new analysis methods that have been developed since 2007 based on high performance liquid chromatography (HPLC) and those developed since 2010 based on capillary electrophoresis (CE). Sample treatment, HPLC, and CE analysis are critically discussed and summarized (Tables 2–4); their contents have been arranged according to their separation type (chromatographic or electrophoretic) and to the food type analysed. Fig. 2 shows the distribution of the applications found which were carried out as part of the analysis of nucleosides and nucleotides in foods using different types of HPLC and CE. The number of applications using reversed-phase liquid chromatography (RP-LC)

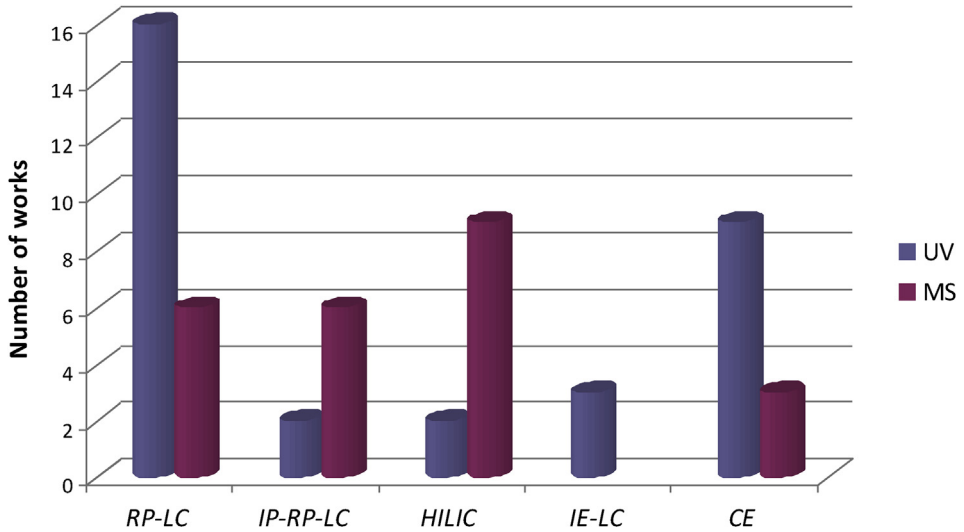
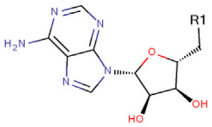
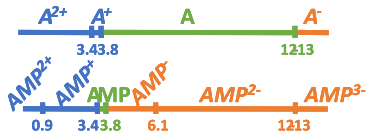
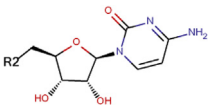
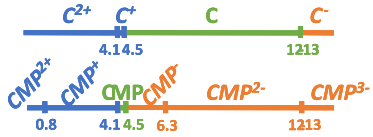
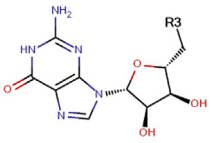
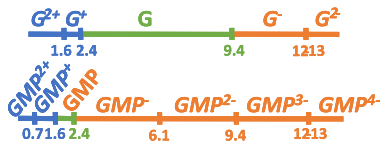
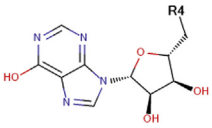
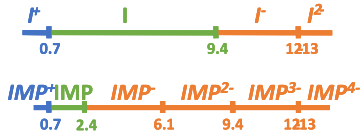
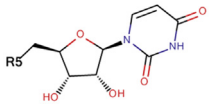
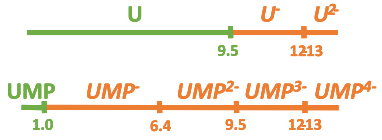


Fig. 2. Classification of the articles found in the literature dealing with the use of HPLC and CE in the detection of nucleosides and nucleotides in food. RP, reversed-phase; IP, ion-pair; HILIC, hydrophilic interaction liquid chromatography; IE, ion-exchange. *Legend*: UV, spectrophotometric detection; MS, mass spectrometry detection.

Table 1
The structures and properties of the main nucleosides and nucleotides analysed in foods.

Nucleosides and Nucleotides	Mw	log D[a] pH = 9	Structure	pKas
Adenosine (**A**)	267	−0.75		
			R1: OH	
Adenosine 5′-monophosphate (**AMP**)	347	−7.68	**R1**: Phosphate	
Adenosine 5′-diphosphate (**ADP**)	427	−8.16	**R1**: Diphosphate	
Adenosine 5′-triphosphate (**ATP**)	507	−10.12	**R1**: Triphosphate	
Cytidine (**C**)	243	−1.81		
			R2: OH	
Cytidine 5′-monophosphate (**CMP**)	323	−9.63	**R2**: Phosphate	
Cytidine 5′-diphosphate (**CDP**)	403	−10.1	**R2**: Diphosphate	
Cytidine 5′-triphosphate (**CTP**)	483	−12.07	**R2**: Triphosphate	
Guanosine (**G**)	283	−1.47		
			R3: OH	
Guanosine 5′-monophosphate (**GMP**)	363	−8.64	**R3**: Phosphate	
Guanosine 5′-diphosphate (**GDP**)	443	−9.12	**R3**: Diphosphate	
Guanosine 5′-triphosphate (**GTP**)	523	−11.09	**R3**: Triphosphate	
Inosine (**I**)	268	−1.97		
			R4: OH	
Inosine 5′-monophosphate (**IMP**)	348	−9.01	**R4**: Phosphate	
Inosine 5′-diphosphate (**IDP**)	428	−9.49	**R4**: Diphosphate	
Inosine 5′-triphosphate (**ITP**)	508	−11.46	**R4**: Triphosphate	
Uridine (**U**)	244	−1.76		
			R5: OH	
Uridine 5′-monophosphate (**UMP**)	324	−8.1	**R5:** Phosphate	
Uridine 5′-diphosphate (**UDP**)	404	−8.55	**R5**: Diphosphate	
Uridine 5′-triphosphate (**UTP**)	484	−10.51	**R5**: Triphosphate	

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2014 ACD/Labs).

coupled to ultraviolet (UV) detection is noteworthy, although the types of ion-pair reversed-phase liquid chromatography (IP-RP-LC) and hydrophilic interaction liquid chromatography (HILIC) have a higher number of applications coupled to mass spectrometry (MS) detectors.

2. Properties of nucleosides and nucleotides

2.1. Chemical structure and properties

A nucleoside consists of the covalent union between a nitrogenised base and a pentose that may be ribose (in RNA) or deoxyribose (in DNA). The nitrogenised base may be either puric or pyrimidinic and, in both cases, its atoms originate mainly from amino acids. The pyrimidinic bases consist of rings of six members and include: uracil, cytosine, and thymine. The puric bases, adenine, guanine, hypoxanthine, and xanthine also have a second ring of five members. A nucleotide is an organophosphate formed by the union of the carbon 5′ of the pentose of the nucleoside with a mono-, di- or tri-phosphate group. Table 1 shows the structures and some of the properties of the main nucleosides and nucleotide mono-, di- and tri-phosphates (NMPs, NDPs and NTPs) analysed in food samples.

Nucleosides and nucleotides are highly polar compounds with distribution coefficient logarithm values (log D) varying between −0.75 and −12.07 at pH 9 (Table 1). Moreover, each of the phosphate groups of nucleotides has two ionisable hydroxyl groups with pK_a values of around 1 and 6, owing to which at pH values of over 6 (which includes physiological pH) all nucleotides have a negative net charge. The amino groups of the rings of adenine, guanine, and cytosine behave as extremely weak bases liable to protonation, with pK_a values of between 2 and 4.5. Because of all

Table 2
Application of liquid chromatography in the analysis of nucleosides and nucleotides in non-infant foods.

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
C, **U**, **2′dU**, **I**, **G**, **T**, **A**, **3′dA**	Fungus *Cordyceps* cultures	PLE (0.2 g/0.2 g diatomaceous earth, MeOH, 160°C, 5 min, 1 static cycle)	addition 10 mL H_2O filtration 0.22 μm	RP-UPLC 50 × 2.1 mm, 1.7 μm, C18 Gradient 0.5 mM AcOH// ACN 5 min	UV 254 nm	External calibration. LODs (μg/L) NUs 12-72 dNUs 21-50	mean contents (mg/kg) **C** 716; **U** 1770; **2′dU** 76.3; **I** 80; **G** 1313; **T** 115; **A** 1615; **3′dA** 10917	[33]
G, **A**, **2′dA**, **3′dA//U**	Fungus *Cordyceps* cultures and natural	SLE (H_2O, ultrasonication, 60 min) centrifugation (50°C, 4000*g*)	filtration 0.45 μm	RP-HPLC 250 × 4.6 mm, 5 μm, C18 Isocratic 5% (vol.) ACN 24 min	UV 260 nm	External calibration. LODs (μg/L) NUs 10-13 dNUs 15-16	mean contents (mg/kg) **G** 1865; **A** 1840; **2′dA** 60; **3′dA** 14; **U** 2095 uridine determined separately	[23]
U, **A**, **G**, **I**	Fungus Truffle and *Tuber* fermentation mycelia	SLE(H_2O, ultrasonication 2 min) centrifugation (13000 rpm, 10 min) supernatant dilution with MeOH	DSPE(D3520 resin, 1 min) centrifugation(13000 rpm, 10 min) evaporation N_2 & reconstitution with mobile phase; filtration 0.45 μm	RP-HPLC 250 × 2 mm, 5 μm, C18 Isocratic 99%(vol.) 5 mM $AcONH_4$ pH 2.0 with HCOOH: 1% MeOH 16 min	MS Q ESI+, $[M+H]^+$ SIM mode	I.S. (Thymine). LODs (μg/L) NUs 100-500	mean contents(mg/kg) *natural truffle* **U** 250, **A** 260, **G** 300, **I** 110 *fermentation mycelia* **U** 1300, **A** 2100, **G** 2700, **I** 69 U, A and G in fermentation micelia 5−9 times higher than in natural fruitting-bodies	[61]
C, **U**, **I**, **G**, **T**, **A**	Mushrooms *Ganoderma L.*	SLE (H_2O, ultrasonication, 45 min) evap. under N_2	filtration 0.45 μm	RP-HPLC 150 × 4.6 mm, 3.5 μm, C18 Gradient 5 mM $AcONH_4$// MeOH 16 min	MS Ion-Trap ESI+, $[M+H]^+$, $[M+Na]^+$, different product ions Scan mode, 50−400 u	External calibration. LODs NUs 100−300 μg/L	mean contents (mg/kg) **C** 57; **U** 157; **I** 25;**G** 54; **T** 19; **A** 51 quantification by UV at 254 nm	[22]
CMP, **UMP**, **XMP**, **GMP**, **IMP**, **C**, **U**, **AMP**, **X**, **G**, **A**	Mushrooms *Boletus*	SLE (H_2O, water bath, 1 min)	filtration 0.2 μm	RP-HPLC 150 × 3 mm, 4 μm Gradient 50 mM phosphate buffer pH 5.8//MeOH 14 min	UV 254 nm	External calibration. LODs (μg/L) NUs 3-20 NMPs 6-20	mean contents(mg/kg) **CMP** 390, **UMP** 1300, **XMP** 250, **GMP** 470, **IMP** 35, **C** 120, **U** 510, **AMP** 1200, **X** 520, **G** 570, **A** 600 several RP columns and one HILIC tested	[8]
AMP, **CMP**, **GMP**, **IMP**, **UMP**, **XMP** *elution order not described*	Mushrooms	SLE(H_2O, 100°C, 1 min) centrifugation (4000 rpm, 30 min)	supernatant filtration 0.22 μm	RP-HPLC 250 × 4.6 mm, 5 μm, C18 Isocratic 95%(vol.) 0.05% phosphoric acid: 5% MeOH	MS Ion-Trap ESI- Scan mode, 50−2000 u	not mentioned. Quantification by UV and fluorescence	mean contents(mg/kg) **AMP** 4500, **CMP** 6000, **IMP** 2600, **UMP** 2300, **XMP** 870	[10]
U, **A**, **I**, **G**	Pork meat raw, dry-cured ham and cooked ham	SLE (H_2O, ultrasonication, water bath, 30 min) centrifugation (20°C, 39200*g*)	supernatant filtration hexane washing centrifugation (5000*g*) evap. under N_2	RP-UPLC 150 × 1.0 mm, 1.8 μm, C18 Gradient 0.1% formic acid// ACN 8.5 min	MS/MS QqQ ESI-, two transitions MRM	Matrix matched calibration & I.S.$^{15}N_2$-xanthine. LODs NUs 40−150 μg/kg	mean contents(mg/kg) *Raw pork meat* U 69; A 2.7; I 1750; G 29 *Dry-cured ham* U 266; A 7.7; I 1348; G 91 *Cooked ham* U 114; A 87; I 2297; G 85	[24]
G, **I**, **AMP**, **IMP** *elution order not described*	Pork meat dry-cured ham	SLE(0.6 M $HClO_4$, 4°C, 10 min) centrifugation(10000*g*, 4°C, 20 min), supernatant filtration & neutralization K_2CO_3	centrifugation(10000*g*, 4°C, 20 min) supernatant storage (−20°C), centrifugation(10000*g*, 4°C, 5 min)	RP-HPLC 150 × 4.6 mm, 4 μm, C18 Gradient 50 mM potassium phosphate buffer pH 4.0// MeOH 25 min	UV 250 nm	not mentioned	mean contents(aprox., mg/kg) **G** 650, **I** 2000, **AMP** 100, **IMP** 750 salting and post-salting processing have a significant effect on the degradation of nucleotides	[45]

(*continued on next page*)

Table 2 (*continued*)

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
U, **I**, **G**, **2′dG**, **2′dI**, **T**, **A**	Mollusk *Mactra Veneriformi*	SLE (50% MeOH, ultrasonication 120 min) centrifugation (12000 rpm, 10 min)	supernatant filtration 0.22 μm	RP-HPLC 250 × 4.6 mm, 5 μm Gradient MeOH//H_2O 35 min	MS QTOF ESI+, $[M+H]^+$ Scan mode 100 −1000 u and UV 260 nm	External calibracion. LODs (μg/L) NUs 35-101 dNUs 36-61	mean contents(mg/kg) **U** 1485, **I** 668, **G** 178, **2′dG** 263, **2′dI** 701, **T** 715, **A** 88 quantification by UV 260 nm	[27]
I	Hake	SPE(MIP, hypoxanthine recognition sites; washing *n*-hexane; elution 5.55 μM NH_4OH pH 9.0)		RP-UPLC 50 × 2.1 mm, 1.7 μm, C18 Gradient 0.1%(vol.) TFA pH 2.2//MeOH 1 min	UV 250 nm	Standard additions. LODs (μg/L) I 1800	mean contents(mg/kg) **I** 230	[63]
CMP, **GMP**, **IMP**, **I**, **AMP**, **ADP**, **A**, **ATP**	Squid Seaweeds Crab canned Mushroom	SLE (1 M $HClO_4$,10 min) centrifugation (1500*g*, 10 min) & supernatant precipit. ClO_4^- & neutralization (1 M KOH, 30 min, cold)	centrifugation (1500*g*, 10 min) supernatant water dilution & filtration 0.45 μm & dilution phosphate buffer pH 7.5	RP-HPLC 250 × 4.6 mm, 5 μm, C18 Gradient 95%(vol.) 950:10:5 (vol.) H_2O:TEA:H_3PO_4//90:10 (vol.) H_2O:ACN 37 min	UV 270 nm	External calibracion. LODs (μg/L) NUs 403-979 NMPs 614-1750 ADP 893 ATP 437	mean contents(mg/kg) *squid* **CMP** 27.8, **GMP** 7.4, **IMP** 9.0, **I** 352, **AMP** 59, **ADP** 32, **A** 0.8; *seaweeds* **CMP** 174, **GMP** 42, **IMP** 70, **I** 416, **AMP** 595, **ADP** 608, **A** 92, **ATP** 1220; *canned crab* **GMP** 79, **IMP** 99, **AMP** 5.7; *mushroom* **CMP** 7.5, **GMP** 91, **I** 45, **AMP** 11, **ADP** 19, **A** 4.2; comparison between high- and low-freshness samples	[9]
A	Royal jelly	SLE (90%(vol.) EtOH, ultrasonication, 15 min)	filtration 0.45 μm	RP-HPLC, 250 × 4.6 mm, 5 μm, C18 Gradient 0.4% H_3PO_4// MeOH 25 min	UV 257 nm	External calibracion. LOD (μg/L) A 17	mean content(mg/kg) *commercial royal jelly* **A** 293 45 samples. Median 70 mg/kg	[35]
IMP, **ATP**, **ADP**, **AMP**, **I**	Royal jelly	SLE (5% $HClO_4$, -5°C) centrifugation (0°C, 12000*g*)	supernatant precipit. ClO_4^- & neutralization (KOH/ K_2CO_3) centrifugation (0°C, 12000*g*), supernatant water dilution, filtration 0.22 μm	RP-UPLC, 100 × 2.1 mm, 1.7 μm, C18 Gradient 50 mM phosphate buffer pH 6.5//ACN 3 min	UV 257 nm	External calibration. LODs (μg/L) I 520 NMPs 420-460 ADP 680 ATP 430	mean contents(mg/kg) *fresh royal jelly* **IMP** 627, **ATP** 51, **ADP** 251, **AMP** 1144, **I** 23.8 *commercial royal jelly* **IMP** 593, **ATP** 5.4, **ADP** 51, **AMP** 887, **I** 44 extractions with boiling water or boiling $MgSO_4$ solution give lower recoveries	[20]
ATP, **IMP**, **ADP**, **C**, **AMP**, **U**, **I**, **G**, **2′dU**, **T**, **A**	Royal jelly	SLE (50%(vol.) EtOH, acetone, ultrasonication, 50°C, 25 min) centrifugation (20°C, 12000*g*)	supernatant decantation, water dilution filtration 0.22 μm	RP-HPLC, 250 × 4.6 mm, 5 μm, C18 Gradient 10 mM phosphate buffer pH 6.5//ACN 40 min	UV 257 nm	External calibration. LODs (μg/L) NUs 24-87 2′dU 27 NMPs 20-64 ADP 65 ATP 47	mean contents(mg/kg) *fresh royal jelly* **ATP** 19, **IMP** 290, **ADP** 150, **C** 11, **AMP** 1500, **U** 36, **I** 117, **G** 178, **2′dU** 2.2, **T** 15, **A** 331 *commercial royal jelly* **IMP** 555, **ADP** 57, **C** 19, **AMP** 1104, **U** 87, **I** 143, **G** 353, **2′dU** 2.5, **T** 31, **A** 720	[37]
C, **G**, **A**	Fungus *Cordyceps* cultures and natural	SLE ($HClO_4$ commercial, 1 h water bath) neutralization NaOH	filtration & water dilution filtration 0.45 μm	HPLC 250 × 4.6 mm, 5 μm, alkyl RP Gradient 5 mM TEA//MeOH 16 min	UV 254 nm	External calibration. LODs (μg/L) NUs 20-60	mean contents(mg/kg) *Natural cordyceps* **C** 460, **G** 610, **A** 490 *Culture cordyceps* **C** 650, **G** 1400, **A** 2500	[41]

U, **I**, **G**, **A**, **3′dA**	Fungus *Cordyceps* cultures and natural	Three methods: PLE(MeOH)//SLE (H_2O, 100°C, 30 min)//SLE (H_2O, room temp., 30 min) for SLE, centrifugation (12000 rpm, 5 min)	filtration 0.45 μm	HPLC 250 × 4.6 mm, 5 μm, alkyl RP Gradient 10 mM TEA// MeOH 25 min	UV 254 nm	External calibration. LODs (μg/L) NUs 30-120	mean contents(mg/kg) *Natural cordyceps* **U** 1100, **I** 970, **G** 510, **A** 280 *Culture cordyceps* **U** 2200, **I** 550, **G** 1600, **A** 1700, **3′dA** 580 extraction methods greatly influence the NUs contents	[34]
IMP, **GMP**, **ADP**, **AMP**, **I** *elution order not described*	Duck meat Nanjing cooked	SLE (5 %$HClO_4$, ice bath, macerat.) centrifugation (4 °C, 10000*g*)	supernatant collection filtration Whatman#54 pH 6.5 with KOH	HPLC 250 × 4.6 mm, 5.0 μm, C18 Gradient 0.72%(vol.) TEA, 0.35% phosphate buffer pH 6.5//MeOH 20 min	UV 254 nm	Not mentioned	mean contents (mg/kg) **IMP** 2650, **GMP** 92, **ADP** 394, **AMP** 72, **I** 2510 different processing stages of food	[42]
CMP, **UMP**, **GMP/IMP**, **AMP**, **cAMP**, **CDP/GDP**, **UDP**, **IDP**, **ADP**, **CTP**, **UTP**, **GTP/ITP**, **ATP**	Beer yeast *S. Cerevisiae* culture	centrifugation (−20°C, 5000*g*) SLE (EtOH/H_2O, 95°C) evap. under N_2	water dilution centrifugation (13000*g*)	IP-RP-HPLC 100 × 1 mm, 3.5 μm, C18 Gradient 10 mM DBAA, 5%(vol.) ACN pH 6.7// 10 mM DBAA, 84%(vol.) ACN 15 min	MS/MS QqQ ESI-, $[M-H]^-$ > [nitrogenous base]$^-$ SRM	^{13}C Isotopic dilution. LODs NMPs 2−45 μg/L NDPs 3−10 μg/L NTPs 6−12 μg/L	*s. cerevisiae* (mg/kg) **AMP** 300; **ADP** 660; **ATP** 2600 column effluent mixed with 50% ACN prior ESI	[36]
NAD^+, **AMP**, **NADH**, **$NADP^+$**, **ADP**, **ATP**, **NADPH**	Beer yeast *S. Cerevisiae* culture	SLE (1:2 vol. MeOH/$CHCl_3$, 4°C, 60 min) centrifugation (4°C, 5000*g*)	water phases combined lyophilization redissolved in 10 mM TBA, 10 mM AcOH	IP-RP-UPLC 100 × 2.1 mm, 2.7 μm, phenyl-hexyl Gradient 10 mM TBA, 10 mM AcOH//10 mM TBA, 10 mM AcOH in 90:10 (vol.) MeOH/H_2O 14 min	MS/MS QqQ ESI-, $[M-H]^-$ or $[N-2H]^-$ > two product ions MRM	I.S.^{13}C,^{15}N isotope-labelled. LODs (ng) AMP 0.3, ADP 0.5, ATP 1.2 NADs (0.5−1)x10^3	(mg/kg) **NAD^+** 4.2, **AMP** 0.11, **NADH** 0.08, **$NADP^+$** 0.53, **ADP** 0.39, **ATP** 4.6, **NADPH** 0.05	[38]
C/UMP, **GMP**, **U**, **AMP**, **I**, **G**, **T**, **A**, **3′dA**	Fungus *Cordyceps* cultures and natural	SLE (H_2O, 100°C, reflux 30 min) or (H_2O 25°C, 24−56 h, reflux 5 min)	supernatant filtration 0.45 μm	IP-RP-HPLC 250 × 4.6 mm, 5 μm Gradient 0.25 mM PDFOA// ACN 60 min	MS Ion-Trap ESI+, $[M+H]^+$, $[2\ M+H]^+$, different product ions SRM	I.S.(5-CCA). LODs (μg/L) NUs 110-240 3′dA 40 NMPs 180-410	mean contents(mg/kg) *Natural cordyceps* **C** 124, **UMP** 343, **GMP** 525, **U** 846, **AMP** 452, **I** 633, **G** 679, **T** < LOQ, **A** 308, **3′dA** < LOQ *Culture cordyceps* **C** 90, **UMP** 420, **GMP** 470, **U** 2800, **AMP** 300, **I** 130, **G** 1900, **T** 150, **A** 2400, **3′dA** 2100 sample preparation methods had significant effects on the amounts	[16]
CMP, cGMP, dCMP, UMP, GMP, IMP/dUMP/ cAMP, dGMP/TMP, AMP, GDP/CDP/OMP/ UDP/XMP, ADP, CTP/ dCTP/GTP, dGTP/UTP, ATP/TTP, dATP	Curdle bacterium *L. Lactis*	Suspension culture freeze-thaw cycles (x3) (−80°C//icewater bath) centrifugation(4248*g*)	DSPE(activated charcoal, ice-bath) centrifugation(4248*g*, 4°C, 20 min) ice-cold water washing x2 extraction 2 %NH_3, 50 % ACN, ice-bath reconstitution 10 mM TBA, 10 mM AcOH	IP-RP-UPLC 100 × 2.1 mm, 2.7 μm, phenyl-hexyl Gradient 10 mM TBA, 10 mM AcOH pH 5.5// 10 mM TBA, 10 mM AcOH in 90:10 (vol.) MeOH/H_2O 26 min	MS/MS QqQ ESI-, $[M-H]^-$ > one product ion SRM	I.S.^{13}C,^{15}N isotope-labelled. LODs (ng) NMPs 1-30 dNMPs 2-15 cNMPs 1 NDPs 3-9 NTPs 1-4 dNTPs 1-8	mean contents(mg/kg) **CMP** < LOQ, **dCMP** 0.01, **UMP** 0.16, **GMP** 0.04, **IMP** 0.21, **TMP** 0.10, **AMP** 0.14, **CDP** 0.01, **UDP** 0.08, **XMP** 0.18, **GDP** < LOQ, **ADP** 0.73, **CTP** 0.48, **dCTP** 0.65, **GTP** 5.2, **dGTP** 0.02, **UTP** 2.4, **ATP** 8.1, **TTP** 0.39, **dATP** 0.20	[62]

(*continued on next page*)

Table 2 (*continued*)

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
							compounds without I.S. showed different concentrations by external calibration *vs.* standard addition method	
C, **U**, **I**, **G**, **T**, **A**, **X**, **CMP**, **UMP**, **GMP**, **IMP**, **AMP**, **XMP**, **CDP**, **IDP**/**GDP**, **UDP**, **ADP**, **CTP**, **GTP**, **UTP**, **ATP**, **TTP**	Soup Cauliflower heated & raw Beer low-malt	CUF(12000*g*, 20 min) filtrate diluted with mobile phase	filtration 0.22 μm	IP-RP-UPLC 100 × 2.1 mm, 1.8 μm, C18 Gradient 1.25 mM DHAA, 10 mM $HCOONH_4$ pH 5.0// 1.25 mM DHAA in ACN 45 min	MS Q NUs ESI+, $[M+H]^+$, the rest ESI-, $[M-H]^-$ SIM mode	External calibration. LODs (μg/L) NUs 8.1–179 NMPs 11-33 NDPs 77-157 NTPs 157-167	mean contents (mg/kg) *beer* **C** 190, **U** 42, **I** 4.5, **G** 86, **T** 7.3, **A** 6.9, **CMP** 0.032, **UMP** 0.097, **AMP** 0.035 *soup* **I** 560, **A** 51, **UMP** 30, **GMP** 170, **IMP** 1.4×10^4, **AMP** 99 *heated cauliflower* **C** 5.9, **U** 4.7, **G** 5.2, **A** 23, **CMP** 15, **UMP** 14, **GMP** 4.4, **IMP** 0.035, **AMP** 74, **CDP** 13, **IDP** 0.51, **GDP** 20, **UDP** 9.9, **ADP** 95, **CTP** 15, **GTP** 30, **UTP** 22, **ATP** 100, **TTP** 0.63. *Raw cauliflower* NUs only	[54]
AMP, **UMP**, **GMP**, **XMP**, **CMP**, **IMP**, **I**, **G**, **A**, **cAMP** elution order not described	Chicken broth	SLE(H_2O, 24 h 90°C) *n*-pentane defatted broth collection by filtration	filtration 0.45 μm water dilution 1:100	HILIC 300 × 7.8 mm, 5 μm, amide Gradient 1% formic acid in ACN//1% formic acid 50 min	MS/MS Ion-Trap ESI+, $[M+H]^+$ > one product ion SRM	External calibration	contents (mg/L) **AMP** 42, **UMP** 3.2, **GMP** 7.3, **XMP** 7.3, **CMP** 3.2, **IMP** 120, **I** 210, **G** 0.85, **A** 1.1, **cAMP** 1.0	[19]
I, **AMP**, **NAD**$^+$, **IMP**, **ADP**, **ATP**	Pork meat (*L. dorsi* muscles)	freezing (liquid N_2) SLE (0.6 M $HClO_4$, 4°C) centrifugation (4°C, 24500*g*)	supernatant filtration and neutralization (K_2CO_3) centrifugation (4°C, 24500*g*) supernatant deproteiniz. (ACN)	ZIC-*p*HILIC 150 × 4.6 mm, 5.0 μm Gradient ACN//100 mM $AcONH_4$ pH 7//150 mM AcNH4 pH 3.5//H_2O 35 min	UV 254 nm	External calibration. LODs (μg/L) NUs 160 NMPs 50-160	mean contents (mg/kg) **I** 370, **AMP** 87, **IMP** 1900, **ADP** 210, **ATP** 400, **NAD** 220	[44]
T, **2′dU**, **2′dA**, **U**, **A**/**2′dI**, **I**, **2′dC**, **2′dG**, **C**, **G**	Clams commercial	vacuum drying 3 days & pulverization SLE (H_2O, ultrasonication, 30°C, 60 min). Three times centrifugation (10000 rpm, 15 min)	dilution with 10% MeOH filtration 0.22 μm	HILIC-UPLC 100 × 2.1 mm, 1.7 μm, amide Gradient ACN//0.1% formic acid 7 min	MS/MS Ion-Trap positive ionization, $[M+H]^+$ > one product ion SRM	External calibration. LODs (μg/L) NUs 0.10–4.35 dNUs 0.42–9.20	mean contents(mg/kg) **T** 140, **2′dU** 55, **2′dA** 20, **U** 164, **2′dI** 588, **A** 22, **I** 637, **2′dC** 18, **2′dG** 184, **C** 55, **G** 347	[32]
T, **2′dA**, **U**, **A**, **I**, **2′dC**, **2′dG**, **C**, **G**	Marine orgamisms	SLE (60% vol. MeOH, ultrasonication, 15 minx3) rotary evaporation	dissolved in 90% (vol.) ACN filtration 0.45 μm	HILIC 250 × 4.6 mm, 5 μm Gradient ACN//0.2% formic acid 20 mM $AcONH_4$ 45 min	MS TOF ESI+, $[M+H]^+$ Scan mode 60–500 u	External calibration. LODs (μg/L) NUs 1-130 dNUs 0.7–22	mean contents (mg/kg) **T** 178; **2′dA** 112; **U** 250; **A** 42; **I** 73; **2′dC** 17; **2′dG** 100; **C** 71, **G** 365	[26]
T, **2′dU**, **2′dA**, **U**, **A**/**2′dI**, **I**, **2′dC**, **2′dG**, **C**, **G**, **CMP**	Seaweeds	SLE (H_2O, ultrasonication, 30 min) centrifugation (15000*g*, 10 min)	supernatant storage 4°C filtration 0.22 μm	HILIC-UPLC 100 × 2.1 mm, 1.7 μm, amide Gradient 0.05% AcOH in ACN//0.8% AcOH, 10 mM $AcONH_4$ 11.5 min	MS/MS QqQ ESI+, $[M+H]^+$ > one product ion SRM	External calibration. LODs (μg/L) NUs 0.04–3.4 dNUs 0.06–1.33 CMP 1.22	mean contents(mg/kg) **T** 10, **2′dU** 16, **2′dA** 7.2, **U** 779, **A** 86, **2′dI** 9.7, **I** 16, **2′dC** 1.2, **2′dG** 14.5, **C** 12.4, **G** 44, **CMP** 9.8	[29]
T, **2′dU**, **2′dA**, **U**, **A**, **2′dI**, **2′dC**, **I**, **2′dG**, **C**, **G**, **cAMP**, **cGMP**, **2′dAMP**, **CMP**	Fruits and leafs *Ziziphus*	SLE (H_2O, ultrasonication 30 min) centrifugation (15000*g*, 10 min)	supernatant filtration 0.22 μm	HILIC-UPLC 100 × 2.1 mm, 1.7 μm, amide Gradient 0.1% AcOH in ACN//0.8% AcOH, 10 mM $AcONH_4$ 10 min	MS/MS QqQ ESI+, $[M+H]^+$> one production SRM	External calibracion. LODs (μg/L) NUs 0.11–0.84 dNUs 0.12–1.70 CMP 2.44	mean content(mg/kg) *fruits* **T** 2.0, **2′dA** 0.7, **U** 122, **A** 77, **2′dI** 0.5, **I** 16, **C** 33, **G** 67, **cAMP** 330, **cGMP** 95, **2′dAMP** 0.45, **CMP**	[18]

						cNMPs 0.6–0.7 2′dAMP 1.57	5.3 *leafs* **T** 10.8, **2′dU** 2.2, **2′dA** 11, **U** 205, **A** 124, **2′dI** 0.8, **2′dC** 6.2, **I** 11, **2′dG** 7.4, **C** 204, **G** 140, **2′ dAMP** 0.5, **CMP** 5.4	
T, **U**, **A**, **I**, **C**, **G**, **cAMP**, **cGMP**	Fruits *Ziziphus*	SLE (H_2O, ultrasonication 60 min) centrifugation (15000g, 10 min)	supernatant collection	HILIC-UPLC 100 × 2.1 mm, 1.7 μm, amide Gradient 2 mM $AcONH_4$ in ACN//10 mM $AcONH_4$ 9 min	MS/MS QqQ ESI+, $[M+H]^+$> one product ion SRM	External calibracion. LODs (μg/L) NUs 0.25–8.44 dNUs 9.12–9.33	mean contents(mg/kg) **T** 0.38, **U** 156, **A** 4.0, **I** 0.54, **C** 3.0, **G** 32, **cAMP** 9.0, **cGMP** 5.6	[31]

ACN, Acetonitrile; **AcO**, Acetate; ***c(prefix)***, cyclic; **5-CCA**, Chloro-cytosine arabinoside; **CUF**, Centrifugal ultrafiltration, ***d(prefix)***, deoxy; **DBAA**, Dibutylammonium acetate; **DHAA**, Dihexylammonium acetate; ***DP(sufix)***, Diphosphate; **DSPE**, dispersive solid-phase extraction; **ESI**, Electrospray ionization; **MeOH**, Methanol; **NAD**, Nicotinamide adenine dinucleotide; **NADH**, Nicotinamide adenine dinucleotide reduced; **NADP**, Nicotinamide adenine dinucleotide phosphate; **NADPH**, Nicotinamide adenine dinucleotide phosphate reduced; **HILIC**, Hydrophilic interaction liquid chromatography; **IE**, Ion exchange; **IP**, Ion pair; **I.S.**, Internal standard calibration; **LODs**, Limits of detection; **MIP**, molecularly imprinted polymer; ***MP(sufix)***, Monophosphate; **MRM**, Multiple reaction monitoring; **MSPD**, matrix solid-phase dispersion; **NDPs**, nucleotide di-phosphates; **NMPs**, Nucleotide mono-phosphates; **NTPs**, Nucleotide tri-phosphates; **NUs**, Nucleosides; **OMP**, orotidine-5′-monophosphate; **PDFOA**, Pentadecafluorooctanoic acid; ***p*HILIC**, Organic polymer HILIC; **PLE**, pressurized liquid extraction; **Q**, quadrupole; **QqQ**, Triple quadrupole; **RP**, Reversed phase; **SLE**, Solid-liquid extraction; **SRM**, Single reaction monitoring; **T**, Thymidine; **TBA**, tributylamine; **TEA**, Triethylamine; **TFA**, trifluoroacetic acid; **TOF**, Time of flight; ***TP(sufix)***, Triphosphate; **UPLC**, Ultra performance liquid chromatography; **X**, Xanthosine; **ZIC**, Zwitterionic.

[a] In elution order.

[b] Final time of separation. Equilibration times not considered.

this, the net charges vary considerably (Table 1) depending on the species and the pH.

2.2. Sources of nucleosides and nucleotides. Digestion and absorption

Nucleosides and nucleotides are present in any foodstuff composed of living cells; the largest concentrations are found in foods with a greater cell density or which come from metabolically active tissue. This means that, in general, foods of animal origin contain a larger amount than those deriving from plants, with the exception of beans and other pulses, which have relatively high amounts in their seeds. Various studies have shown that the internal organs of animals such as the liver, the kidney, or the heart and small fish, and shellfish are rich in nucleosides and nucleotides. The latter are also found to a lesser extent in pulses, mushrooms, and vegetables [7].

Some nucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) are used as flavour enhancers. The *umami* or "fifth taste" [8–10] includes substances such as IMP and glutamic acid and is also associated with foods rich in proteins. The effect of nucleotides as flavour enhancers implies their presence in a wide variety of prepared foods and beverages, which increases the level of ingestion of these compounds. Although no recommendations exist as to the amount of nucleosides and nucleotides that may be beneficial in the diet, it has been suggested that it is better to take a certain amount than none [7].

2.3. Their importance in infant foods

The analysis of nucleosides and nucleotides in infant foods constitutes an area of application of great interest, given that the exogenous contribution of these compounds is particularly important in the stages of rapid growth of both babies and children [5] and also has a positive effect on the regulation of the immune response.

Human milk is the best source of nucleotides and the latter form part of the non-proteic nitrogen content of milk. In the case of cow's milk however non-proteic nitrogen represents only 2% of total nitrogen [11]. For these reasons, the European Scientific Committee on Food (SCF, 1993) recommends supplementing infant formulas with nucleotides in concentrations similar to those found in human milk. The free forms of cytidine 5′-monophosphate (CMP), uridine 5′-monophosphate (UMP), adenosine 5′-monophosphate (AMP), GMP and IMP are those appearing in a higher concentration in human milk, although some authors have found that cytidine 5′-diphosphate (CDP) is also a predominant nucleotide in some human milk [11]; guanosine di-phosphate mannose and uridine di-phosphate hexose have also been found at lower concentrations [12].

Infant formulas are only supplemented with the five free NMPs as these are the main forms of intestinal absorption. Human milk contains, however, other forms of nucleotides which are also nutritionally available to babies: polymeric ribonucleic acids and nucleoside and nucleotide adducts. In some cases it is interesting to determine the total potentially available nucleos(t)ides (TPAN) [13,14]. Over 80% of the TPAN is present in the form of polymeric nucleotides (43.3%) and free nucleotides (39.9%) and the remainder in that of nucleoside and nucleotide adducts.

Non-dairy food complements must have a suitable composition as far as energy and nutrients are concerned, as in some cases they represent the only source of nutrition for babies aged between 6 and 12 months [15]. Although no information is currently available on the levels of nucleotides that are desirable in this kind of food,

Table 3
Application of liquid chromatography in the analysis of nucleosides and nucleotides in infant foods.

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
CMP, **UMP**, **GMP**, **IMP**, **AMP**	Infant formulas	dispersion in 1 M NaCl, 5 mm EDTA SPE (strong anion exchange)	washing 0.3 M KBr elution 0.5 M KH_2PO_4 pH 3.0 filtration 0.2 μm	RP-HPLC, 250 × 4.6 mm, 5 μm, C18 Gradient 0.1 M KH_2PO_4 pH 5.6//MeOH 30 min	UV 260 nm	I.S. TMP. LODs (μg/L) NMPs 0.8–1.9	mean contents(mg/kg) *bobine-milk based* **CMP** 81, **UMP** 33, **GMP** 29, **IMP** 10, **AMP** 40 *caprine-milk based* **CMP** 40, **UMP** 82, **GMP** 64, **IMP** 3.0, **AMP** 23 *soy based* **CMP** 1.0, **UMP** 3.0, **GMP** 3.0, **AMP**5.0 caprine-milk and soy not supplement.	[56]
CMP, **UMP**, **GMP**, **IMP**, **AMP**	Infant formulas & adult/pediatric nutritional formulas	SPE (strong anion exchange)	filtration 0.2 μm	RP-HPLC 250 × 4.6 mm, 5 μm, C18 Gradient 10 mM KH_2PO_4 pH 5.6//MeOH 45 min	UV 250, 260, 270 nm	I.S. TMP. LODs NMPs 60–190 μg/kg	AOAC official method	[57]
CMP, **UMP**, **GMP**, **IMP**, **C**, **U**, **AMP**, **G**, **I**, **A**	Infant formulas	dispersion in H_2O CUF (3500*g* 60 min)	analysis of filtrate	RP-HPLC 250 × 4.6 mm, 5 μm, C18 Gradient 10 mM $AcONH_4$, 5 mM NH_4HCO_3 pH 5.6//MeOH 16 min	MS/MS Ion-Trap ESI+, $[M+H]^+$ > [nitrogenous base]$^+$ $[UMP + H]^+ > 97$ SRM	I.S.^{13}C,^{15}N isotope-labelled. LODs (μg/kg) NUs 100-1200 NMPs 100-1300	concentration levels not mentioned accuracy validated against CRM. Recoveries in spiked samples, 80–115%	[48]
CMP, **AMP**, **UMP**, **GMP**, **IMP**	Infant formulas	dispersion in H_2O, 50°C pH 4.6 with 1 %HCOOH SLE(ultrasonication, room temp., 10 min) centrifugation (15000 rpm, 15 min)	supernatant filtration 0.22 μm	HPLC 150 × 2.1 mm, 3.5 μm, polar embedded C18 Gradient 0.1% formic acid//ACN 8 min	MS/MS QqQ ESI-, $[M-H]^-$ > two product ions MRM	External calibration. LODs (μg/L) NMPs 9-15	mean contents (mg/kg) **CMP** 103, **AMP** 53, **UMP** 47, **GMP** 30, **IMP** 29	[25]
C, **U**, **G**, **A**	Bovine milk	1 M NaOH, 30 min neutralization, HCl & water dilution enzymatic hydrolysis SPE(phenylboronate, high pH)	washing twice at high pH elution, 0.25 M H_3PO_4 filtration	RP-HPLC 150 × 4.6 mm, 5 μm, C18 Gradient 50 mM AcONa pH 4.8//MeOH 18 min	UV 260 nm	I.S. (5 mC). LODs not mentioned	mean contents (0–30 days of lactation) (mg/L) **C** 6.8, **U** 64, **G** 2.0, **A** 0.25 (μmol/dL) monomeric, adducts and polymeric nucleotides: **C** 7.5, **U** 22, **G** 2.3, **A** 2.8 nucleotides hydrolysed to nucleosides before analysis	[13]
C, **U**, **G**, **A**	Bovine, caprine and ovine milks	1 M NaOH, 30 min neutralization, HCl & water dilution enzymatic hydrolysis SPE(phenylboronate, high pH)	washing twice at high pH elution, 0.25 M H_3PO_4 filtration	RP-HPLC 150 × 4.6 mm, 5 μm, C18 Gradient 50 mM AcONa pH 4.8//MeOH 18 min	UV 260 nm	I.S. (5 mC). LODs not mentioned	mean contents (ovine) (mg/L) **C** 5.6, **U** 36, **G** 1.7 (μmol/dL) monomeric, adducts and polymeric nucleotides: **C** 7.1, **U** 292, **G** 30, **A** 28. Highest concentration of TPAN in ovine milk	[14]
C, **U**, **A**, **G**, **I**, **CMP**, **UMP**, **AMP**, **GMP**, **IMP**, **CDP** *elution order not described*	Human milk	protein precipitation, 10%(w/v) TCA, 30min centrifugation (30000*g*, 15 min) TCA elimination (diethyl ether)	lyophilization & storage −30°C dissolved in H_2O filtration 0.45 μm	HPLC 500 × 4.6 mm, C18, polymer silica coating Isocratic 25 mM $TBAHSO_4$ 50 mM K_3PO_4 pH 3.5	UV 254 nm	not mentioned	mean contents(mg/L) **C** 2.2, **U** 1.5, **A** 0.05, **G** 0.10, **I** 0.06, **CMP** 16, **UMP** 1.8, **AMP** 1.0, **GMP** 0.30, **IMP** 8.8, **CDP** 52 taiwanese breast milk with higher free nucleotides regarding US, Europe and Southeast Asia	[11]

CMP, **UMP**, **GMP**, **IMP**, **AMP**	Infant formulas	dispersion in H_2O centrifugation (15 min, 2800g) CUF (2800g, 30 min)	analysis of filtrate	nano-IP-RP-HPLC 150 × 75 μm, 3.6 μm, C18 core-shell Isocratic 95%(vol.) 100 mM $HCOONH_4$, 20 mM TBAOH pH 8.0: 5% MeOH 5 min	UV 260 nm	External calibration. LODs (μg/L) NMPs 250	mean contents (mg/kg) **CMP** 96, **UMP** 48, **GMP** 12, **IMP** 12, **AMP** 25	[51]
AMP, **CMP**, **GMP**, **IMP**, **UMP** *elution order not described*	Infant formulas	dispersion in H_2O, 50°C 6.5%(vol.) $HClO_4$ SLE(room temp., 15 min) centrifugation (4000g, 20 min)	supernatant neutralization (KOH) & dilution ice-bath 1 h, decantation filtration 0.45 μm	IP-RP-HPLC 150 × 4.6 mm, 3.0 μm, C18 Isocratic 100 mM potassium phosphate, 4 mM TBA, pH 6.0 30 min	UV 260 nm, 278 nm	External calibration. LODs not mentioned	not mentioned	[43]
CMP, **UMP**, **GMP**, **IMP**, **AMP**	Baby foods	dispersion in H_2O protein precipitation, 3% TCA 3°C, 15 min centrifugation (6000 rpm, 10 min)	supernatant collection filtration 0.45 μm	IP-RP-HPLC 150 × 4.6 mm, 5 μm, C18 Isocratic 95%(vol.) 0.1 M formate buffer, 0.01 M DMHA pH 5.5: 5% MeOH 17 min	MS TOF ESI- & APCI-, $[M-H]^-$ Scan 50—600 u	External calibration. LODs (μg/L) NMPs 11-19	mean contents(mg/kg) *meat based* **CMP** 0.43, **UMP** 1.4, **GMP** 0.28, **IMP** 1.1, **AMP** 1.6 *fish based* **CMP** 1.7, **UMP** 2.2, **GMP** 1.0, **IMP** 2.2, **AMP** 3.2 *vegetables* **CMP** 1.0, **UMP** 2.2, **GMP** 1.0, **IMP** 1.1, **AMP** 2.4 *fruit based* **CMP** 1.0, **UMP** 2.3, **GMP** 0.59, **IMP** 0.21, **AMP** 1.0 *cereals* **CMP** 0.22, **UMP** 0.15, **GMP** 0.20	[40]
CMP, **UMP**, **AMP**, **IMP**, **GMP**	Infant formulas	dispersion in H_2O, stored 4°C, 10 min centrifugation (2328g, 15 min) CUF (supernatant, 2328g, 15 min)	analysis of filtrate	IE(anion)-HPLC, 250 × 4.6 mm, 5 μm, NH_2 Isocratic 50 mM $(NH_4)_2HPO_4$ pH 4 with H_3PO_4. 30 min	UV 254 nm	External calibration	mean contents (mg/kg) **CMP** 73, **UMP** 23, **AMP** 23, **IMP** 29, **GMP** 31 CMP eluted with front sample matrixes. pH critical to avoid interferences	[46]
CMP, **AMP**, **UMP**, **GMP**	Infant formulas	dispersion in H_2O, 60°C pH acidic with 1 M HCl centrifugation (11000g, 20 min) water dilution & neutralization NaOH	SPE(strong anion exchange) elution 0.05 M HCl & pH adjustment 2.5 water dilution filtration 0.2 μm	IE(dendritic)-HPLC 150 × 2.1 mm, silica based Isocratic 75 mM KH_2HPO_4/H_3PO_4 pH 2.5 7.5 min	UV 254 nm	External calibracion. LODs (μg/L) NMPs 130-170	mean contents(mg/L) **CMP** 11, **AMP** 1.6, **UMP** 3.1, **GMP** 1.4 different dendritic stationary phases	[58]
CMP, **AMP**, **UMP**, **GMP**	Baby foods	dispersion in H_2O ultrasonication 5min protein precipitation, 3% TCA, 15 min centrifugation (6000 rpm, 10 min)	supernatant collection & water dilution filtration 0.2 μm	IE(anion)-HPLC, 150 × 4.0 mm, 5 μm, SAX Isocratic 10 mM phosphate buffer pH 3.5 10 min	UV 252, 260, 271 nm	External calibration. LODs (μg/L) NMPs 20-40	contents(mg/kg) *dairy foods (mean)* **CMP** 12, **AMP** 3.1, **UMP** 4.8, **GMP** 6.3 *chicken* puree **CMP** 2.5, **AMP** 4.4, **UMP** 3.7, **GMP** 6.6 *vegetables* puree **CMP** 2.7, **AMP** 6.2, **UMP** 4.1, **GMP** 6.5 cereals **CMP** 7.5, **AMP** 10, **UMP** 12, **GMP** 2.7 *fruits lyophilized (mean)* **CMP** 14, **AMP** 20, **UMP** 15, **GMP** 13	[39]
AMP/UMP, **IMP**, **GMP**/ **CMP**	Infant formulas	dispersion in H_2O, 5 min mixed CUF(2328g, 15 min)	analysis of filtrate diluted with MeOH	HILIC 150 × 2 mm, 3 μm, amino Gradient MeOH// 30 mM $HCOONH_4$ pH	MS/MS QqQ ESI+, $[M+H]^+$ > two product ions MRM	I.S.^{13}C,^{15}N isotope-labelled. LODs (μg/L) NMPs 5-10	mean contents (mg/kg) **AMP** 34, **UMP** 43, **IMP** 17, **GMP** 36, **CMP** 110 unlike [46] this could be applied	[47]

(*continued on next page*)

Table 3 (*continued*)

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
				2.5 35 min			to hypoallergenic infant formulas. Has led to an AOAC method [65]	
CMP, **AMP**, **UMP**, **IMP**, **GMP**	Infant formulas Human breast milk	powders: dispersion AcOH 3%(vol.) centrifugation (4000 rpm, 10 min) hollow fibre in supernatant (40°C) desorption by ultrasonication 1% NH_3	evap. under N_2 redissolved in mobile phase filtration PTFE	HILIC 250 × 4.6 mm, 5 μm, amine, amide, diol, three columns Isocratic 70%(vol.) ACN: 30% formic acid pH 2.5 42 min	UV 280, 260, 250, 254 nm	External calibration. LODs (μg/L) NMPs 30-90	mean contents(mg/L) *infant milk powders* **CMP** 1.9, **AMP** 2.0, **UMP** 2.3, **IMP** 4.8, **GMP** 4.5 *human breast milk* **CMP** 3.5, **AMP** 1.4, **UMP** 1.6, **IMP** 4.0, **GMP** 2.1	[64]
A/U/I, **G**, **C**, **AMP**, **ADP**, **UMP**, **IMP**, **UDP**, **IDP**/ **ATP**, **CMP**, **CDP**, **UTP**/ **GMP**, **GDP**/**ITP**, **CTP**, **GTP**	Baby foods	dispersion in H_2O centrifugation (15 min, 2800g) CUF (2800g, 30 min)	washing CUF filter with H_2O analysis of filtrate diluted with ACN	HILIC 100 × 2.1 mm, 3.5 μm, amide Gradient ACN//100 mM HFIP, 50 mM DEA 9 min	MS/MS QqQ NUs except U ESI+, $[M+H]^+$> one product ion; the rest ESI-, $[M-H]^-$ > one product ion SRM	External calibration & addition of standards. LODs (μg/kg) NUs 200-2700 NMPs 300-500	mean contents(mg/kg) *fish based* **A** 11, **U** 4.7, **I** 82, **G** 8.0, **C** 1.9, **AMP** 27, **UMP** 15, **IMP** 31, **CMP** 2.1, **GMP** 3.2 *meat based* **A** 13, **U** 5.5, **I** 102, **G** 9.0, **C** 3.5, **AMP** 22, **UMP** 8.7, **IMP** 45, **CMP** 1.2, **GMP** 2.1 *fruit based* **A** 12, **U** 1.8, **G** 3.0, **C** 1.6	[52]

5 mC, 5′-methylcytidine; **APCI**, Atmospheric pressure chemical ionization; **CRM**, certified reference material; **DEA**, Diethylamine; **DMHA**, *N*,*N*-Dimethylhexylamine; **PTFE**, Polytetrafluoroethylene; **SAX**, Strong anion exchange; **TCA**, Trichloroacetic acid; **TBAOH**, Tetrabutylammonium hydroxide; **TPAN**, Total potentially available nucleos(t)ides. Other abbreviations as in Table 2.

[a] In elution order.

[b] Final time of separation, equilibration times not considered.

their study is arousing interest as they are including more and more different ingredients.

3. Analytical techniques

3.1. Sample treatment

Owing to the high polarity of nucleosides and nucleotides, the most frequently used method for extracting these analytes from different samples of food was liquid-liquid or solid-liquid extraction, using mainly water or other polar solvent [16–18]. It is also common to carry out extraction assisted by temperature [8,10,16,19–21], ultrasounds [18,22–32] and pressurized liquid extraction (PLE) [33,34]. The use of different mixtures of solvents such as water-ethanol [35,36], water-methanol [26,27,30,32], water-ethanol-acetone [37], or methanol-chloroform [38] has also been recommended. It has been found that the extraction methods employing PLE and water extraction, at high or room temperature, could greatly influence the quantitation of nucleosides [34]. Nucleosides, monomeric nucleotides, polymeric nucleotides and nucleotide adducts have been determined in milk samples employing parallel enzymatic treatments and obtaining each contribution by means of the difference [13,14]: i) measurement of innate nucleosides; ii) a treatment involved phosphatase (pH 8.5, 3 h) to dephosphorylate monomeric nucleotides to nucleosides; iii) a treatment involved nuclease (pH 5.1, 16 h) and phosphatase to hydrolyse polymeric nucleotides to monomeric nucleotides and dephosphorylate them to nucleosides; iv) a treatment consisted of nuclease, pyrophosphatase and phosphatase to convert polymeric, monomeric nucleotides, and nucleotide adducts to free nucleosides.

In sample treatments, one of the most widely used strategies was the addition of acid mediums to precipitate the proteins and thus eliminate any possible interference caused in the macromolecules present in food matrices. Some of the most frequently used acids were trichloroacetic acid [11,39,40], formic acid [25], and perchloric acid [9,20,41–45]. Owing to the instability of nucleotides at a low pH, an additional stage of the neutralization of the acid is required before the analysis is carried out [20,42,44]. Unlike the acid treatment, boiling water or saline solution/heating methods (to inactivate enzymes) do not require this neutralization, but significantly lower recoveries have been found [20] for adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP), and recoveries higher than 100% for IMP when the three treatments were compared. In the analysis of samples of milk and baby foods, highly satisfactory results have been obtained with the use of centrifugal ultrafiltration (CUF) [46–52]. This is a simple and efficient procedure for the cleaning of complex samples such as those of food matrices, in the case of both foodstuffs in general [53–55] and baby foods; it is based on the retention by ultrafiltration of interfering macromolecular species. Fig. 3 shows the total ion electropherograms (TIE) and extracted ion electropherograms (XIE) of a powdered infant formula (**A**) and a human milk (**B**) samples analysed by CUF followed by CE-ESI-MS. These results revealed that this is a satisfactory method to carry out the analysis of NMPs in infant foods.

Depending on the levels of nucleosides and nucleotides in the samples, and also on the separation-detection technique to be used, the implementing of a stage of the preconcentration of the analytes may be recommendable or necessary. Solid phase extraction (SPE) is one of the most frequently used procedures for the cleaning and the preconcentration of samples. SPE has been carried out with anionic exchange sorbents [56–58], metal oxide affinity chromatography materials [59], and boronate sorbents [13,14]. It has been found that trapping of the nucleosides on boronate affinity columns

Table 4
Application of capillary electrophoresis in the analysis of nucleosides and nucleotides in foods.

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
cAMP, **AMP**, **ADP**, **ATP**	Beer yeast *S. Cerevisiae*	lysis frozen yeast (boiling H_2O, 10 min) centrifugation (4°C, 13000 rpm)	supernatant collection	CE 44 cm × 50 μm, uncoated, −20 kV 60 mM borate buffer pH 9.5, 1%(w/v) PEG 38 min	UV 210 nm	External calibration. LODs (μg/L) cAMP 1.76; AMP 0.76; ADP 1.22; ATP 1.11	contents in extract (mg/L) **cAMP** 2.96; **AMP** 41.67; **ADP** 26.50; **ATP** 9.44	[21]
A, **G**, **U**, **I**, **AMP**, **GMP**, **UMP**	Fungus Mushrooms	SLE (MeOH, ultrasonication, 60 min) Filtration, evaporation	dissolved in H_2O filtration 0.45 μm	CE 42 cm × 50 μm, uncoated, 15 kV 125 mM borate pH 9.1 10 min	UV 260 nm	External calibration. LODs (mg/L) NUs 20-30 NMPs 38-80	mean contents (mg/kg) **A** 884, **G** < LOQ, **U** 1320, **I** < LOQ, **GMP** < LOQ	[28]
AMP, **GMP**, **IMP**, **XMP** *migration order not described*	Mushrooms *Lentinus edodes*	SLE(H_2O, 22−70°C, 30 −60 min) filtration (Whatman#1) CUF(3000*g*, 30 min)	analysis of filtrate	CE 56 cm × 75 μm, uncoated, 20 kV 50 mbar 20 mM phosphate-borate buffer pH:9.2 30 min	UV 254 nm	I.S. purine. LODs not mentioned	mean contents (70°C, 360 min, mg/kg extract) **AMP** 1380, **GMP** 3260, **XMP** 55 5′-phosphodiesterase breaks down RNA in the mushrooms into 5′-nucleotides at higher temperatures	[55]
AMP, **CMP**, **GMP**	Glucoside and peptide capsule			CE 30 kV 15 mM $NaBO_3$, 35 mM Na_2CO_3, 60 mM HP-β-CD	UV 254 nm	External calibration. LODs (μg/L) NMPs 2000-3000		[100]
AMP, **GMP**, **IMP**, **XMP** *migration order not described*	Beef meat minced	SLE(0.01 M HCl, vortexing, 2 min) centrifugation (10000*g*, 4°C, 30 min) supernatant filtration (Whatman#1) CUF(3000*g*, 30 min)	analysis of filtrate	CE 56 cm × 75 μm, uncoated, 20 kV 50 mbar 20 mM phosphate-borate buffer pH:9.2 30 min	UV 254 nm	I.S. purine. LODs not mentioned	mean contents (mg/kg) **AMP** 81.2, **GMP** 9.2, **IMP** 2.1	[55]
I, **IMP**, **AMP**, **GMP** *migration order not described*	Goat meat raw & cooked	SLE (H_2O cold, vortexing, 1 min) centrifugation (4°C, 20 min, 10000*g*) filtration (Whatman#1) CUF(5000*g*, 4°C, 4 h)		CE 56 cm × 75 μm, uncoated, 20 kV 50 mbar 20 mM phosphate-borate buffer pH:9.2 30 min	UV 254 nm	I.S. purine. LODs not mentioned	mean contents(mg/kg) *raw meat* (mg/kg) **I** 1520, **IMP** 1050, **AMP** 5, **GMP** 25 *cooked meat* (mg/kg) **I** 1130, **IMP** 690, **AMP** 87, **GMP** 16 I, IMP and GMP decreased by about one third and AMP increased by 17-fold during cooking	[53]
C/**A**/**3′dA**, **T**, **G**/**2′dG**, **U**, **I**	Marine organisms	SLE (60% vol. MeOH, ultrasonication, 15 min × 3) rotary evaporation	dissolved in 2% (vol.) NH_3, 4%(vol.) MeOH centrifugation (6000*g*, 10 min) filtration 0.45 μm	CE 80.5 cm × 50 μm, uncoated, 25 kV 30 mM $AcONH_4$ pH 9.9 11 min	MS TOF ESI+, $[M+H]^+$ Sheath liquid 0.25% formic acid, 50% (vol.) MeOH Scan 110−500 u	External calibration. LODs (μg/L) NUs 30-1000	only nitrogenous bases found in two of the three samples studied identifying known nucleobases and nucleotides in different natural products (dereplication)	[30]
GMP, **IMP**	Soy sauce			CE 50 cm × 50 μm, uncoated 30 mM $NaBO_3$, 30 mM Na_2CO_3, 60 mM HP-β-CD	UV 254 nm	LODs (μg/L) NMPs 2000	sample medium 50 mM AcOH	[101]
AMP, **CMP**, **GMP**, **UMP**, **IMP**	Human milk	dispersion in H_2O centrifugation (15 min, 2800*g*) CUF (3500*g*, 60 min)	analysis of filtrate	CE 87.5 cm × 50 μm, uncoated, 30 kV 30 mM $HCOONH_4$ pH:9.6 12 min	MS Q ESI-, $[M-H]^-$ Sheath liquid 50% (vol.) isopropanol SIM mode	External calibration & addition of standards. LODs (μg/L) NMPs 80-130	*1-month human milk* (mg/L) **AMP** 1.6, **CMP** 6.5, **GMP** 0.8, **UMP** 2.1 *human milk bank* (high press., mg/L) **AMP** 0.8, **CMP** 3.8, **UMP** 0.7 pasteurization exhibit higher levels than high pressure preservation	[50]
AMP, **CMP**, **GMP**, **IMP**, **UMP**	Infant formulas	dispersion in 50 mM AcOH aqueous solution centrifugation (9000*g*)	supernatant filtration	CE 40 cm × 75 μm, uncoated, 20 kV 30 mM borate buffer, 80 mM HP-β-CD pH 10 23 min	UV 254 nm	External calibration. LODs (μg/L) NMPs 800-1000	mean contents (mg/kg) **AMP** 34; **CMP** 103; **GMP** 41; **IMP** 14; **UMP** 58	[17]

(*continued on next page*)

Table 4 (*continued*)

Analytes[a]	Sample	Extraction	Clean-up	Separation[b]	Detection	Quantification	Concentrations & observations	Ref.
AMP, **CMP**, **GMP**, **UMP**, **IMP**	Infant formulas	dispersion in H_2O centrifugation (15 min, 2800g) CUF (3500g, 60 min)	analysis of filtrate	CE 87.5 cm × 50 μm, uncoated, 30 kV 30 mM $HCOONH_4$ pH:9.6 12 min	MS Q ESI-, $[M-H]^-$ Sheath liquid 50% (vol.) isopropanol SIM mode	Addition of standards. LODs (μg/kg) NMPs 800-1800	mean contents (mg/kg) **AMP** 30, **CMP** 100, **GMP** 15, **UMP** 41, **IMP** 14	[49]
C, **A**, **3′dA**, **T**, **U**, **G**, **2′dG**, **I**	Marine organism	SLE (60% vol. MeOH, ultrasonication, 15 min x 3) rotary evaporation	dissolved in 2% (vol.) NH_3, 4%(vol.) MeOH centrifugation (6000g, 10 min) filtration 0.45 μm	MEKC 56 cm × 75 μm, uncoated, 20 kV 25 mM Na_2HPO4, 70 mM SDS, pH 8.7 8 min	UV 260 nm	External calibration. LODs (μg/L) NUs 500-750 dNUs 500	mean contents (mg/kg) **C** 400, **T** 1100, **U** 2300, **G** 1800, **2′dG** 900, **I** 3100	[94]

CE, Capillary electrophoresis; **CEC**, Capillary electrochomatography; **HP-β-CD**, Hydroxypropyl-β-cyclodextrin; **MEKC** Micellar electrokinetic capillary chromatography; **PEG**, Polyethylene glycol. **SDS**, sodium dodecyl sulfate. Other abbreviations as in Tables 3 and 2

[a] In migration order.

[b] Final time of separation. Flushing/conditioning times not considered.

is possible only in 100% acetonitrile (ACN) conditions and this retention is not specific to *cis*-diol-containing compounds [60]. Dispersive solid-phase extraction (DSPE) has also been used, adding the sorbent material to the extract to separate the compounds of interest from the matrix component and then, removing it from the extract by centrifugation. The materials used as DSPE materials to retain nucleosides and nucleotides with complex matrices were resins [61] and activate charcoal [62]. Furthermore, molecularly imprinted polymer-matrix solid-phase dispersion (MIP-MSPD) has been prepared by using hypoxanthine as a template so as to achieve the selective retention of inosine (I) and hypoxanthine (Hx) [63].

A novel procedure has been described in the extraction and preconcentration of nucleotides from samples of infant formulas and human milk; in which selective micro-extraction is carried out using alumina and stannia hollow fibres, previously precipitating the proteins in an acidic medium [64]. Alumina- and stannia-based hollow fibres were synthesized via sol–gel procedures and the micro-extraction of NMPs takes place owing to the high affinity of phosphorylated species for metal oxides.

Owing to the great importance of the analysis of nucleosides and nucleotides in infant formulas, in collaboration with 12 laboratories from different countries, B. D. Gill and H. E. Indyk have developed a method of the AOAC [57]. This method is applicable to the analysis of *5′*-NMPs in infant and pediatric nutritional formulas and, also, formulas for adults. The extraction of analytes is carried out with anionic exchange SPE sorbents, and a C_{18} stationary phase with an elution gradient is used for chromatographic separation. UV detection and quantification is carried out with the internal standard technique (*5′*-monophosphate thymidine). Another method of the AOAC [65], based on CUF and HILIC-MS/MS, had also been proposed in 2012 to carry out this same determination.

3.2. Liquid chromatography

LC has been the most frequently used technique for the analysis of these compounds; however, the simultaneous chromatographic determination of nucleosides and nucleotides is no easy task. They are highly polar and ionisable compounds, which means that their retention on C_{18} conventional columns, that are normally used in reversed-phase liquid chromatography (RP-LC), is limited. However, at the appropriate mobile phase pH, polar nucleotides can be retained on a reversed-phase column [10,20,45,56] and an organic solvent gradient is able to remove late-eluting nucleosides [8,9,13,14,22,24,27,33,37,48,61]. An attempt has been made to improve retention by the use of stationary phases with polar modifiers, the so-called polar embedded and polar end-capped phases [4,25]. IP-RP-LC has currently become one of the most commonly used modes, as when working at a suitable pH the ionic nature of the organophosphate of the nucleotides allows interaction with cationic reagents capable of forming ionic pairs [16,36,38,40,43,54,62]. The use of ion-exchange liquid chromatography (IE-LC) with stationary phases of anion exchange has also been proposed [39,46,58]. Knowledge of the type of interaction of nucleotides with novel stationary phases [66] is arousing interest: is the case of the mixed-mode stationary phases [67,68]; affinity chromatography monolithic columns for specific *cis*-diol interactions of nucleosides [69,70]; or counter-current chromatography using aqueous potassium phosphate buffer [71] or ammonium sulfate buffer [72] as mobile phases, respectively.

In recent years, the use of HILIC [73–75] has become very important in the separation of these polar analytes [19,29,31,42,47,64,65]. Partitioning between the aqueous layer associated with the stationary phase and the organic component of the mobile phase is the main retention mechanism in HILIC,

although secondary interactions such as dipole-dipole, hydrogen bonding, and ion exchange may play a role in separation. Novel materials with different functional groups – charged, non-charged and zwitterionic (ZIC) – are being developed and the separation mechanisms are being investigated [68,76,77]. It is possible that the use of HILIC in the analysis of compounds as polar as nucleosides and nucleotides has the limitation that the analytes must be extracted in aqueous mediums and that injection, in an HILIC system, should be carried out with a high content of organic solvent (generally ACN). For this reason, it is always necessary to change the solvent of the aqueous extract before its injection into the chromatographic system, and/or to inject small volumes of the sample.

One of the most frequently used detectors for coupling with HPLC has been the UV-spectrophotometer with diode array detection (DAD) [8,9,11,13,14,20,23,33–35,37,39,41–46,51,56–58,63,64,76,77]. Currently however, hyphenation with mass spectrometry (MS) gives greater sensitivity and selectivity [10,16,18,19,22,24–27,29,31,32,36,38,40,47,48,52,54,61,62]. In the MS detection of nucleotides, nucleosides and nucleobases it is very important to obtain a good chromatographic separation, as the fragmentations that may occur at the source may cause interference with the MS signals [78]. With the use of MS detection, those mobile phases with non volatile components are not compatible. The high salt concentrations used in IE-LC, therefore, mean that its coupling to electrospray ionization-mass spectrometry (ESI-MS) may be inadvisable and that, for those LC modes based on ion-pairing, the use of volatile reagents is required. The ion-pairing reagents used include *N*, *N*-dimethylhexylamine (DMHA) [40,79], diethylamine (DEA) [52,80], tributylamine (TBA) [38,62], dibutylammonium acetate (DBAA) [36], tetrabutylammonium hydroxide (TBAOH) [51], and dihexylammonium acetate (DHAA) [54]; all of these interact with the phosphate groups of nucleotides. Also, ion-pairing reagents that interact with the amino groups, instead of the phosphate groups (such as pentadecafluorooctanoic acid or PDFOA) could be used at very low concentrations (0.25 mM) [16].

Although the use of HILIC in the analysis of nucleosides and nucleotides in foods is currently rather limited, we believe that it is the most suitable chromatographic mode for the simultaneous determination of a large number of nucleotides and their metabolites. It should be pointed out that, in the analysis of a subset of analytes, other chromatographic modes may also be suitable. The efficiency in the separation of NMPs, nucleosides and nucleobases by three different separation methods (RP-HPLC, IP-RP-HPLC and HILIC), all of which coupled to ESI-MS/MS has been investigated [78]. The results obtained indicated that RP-HPLC, with stationary phases tolerating the use of mobile phases with a high aqueous content, was the most suitable separation mode for the 5′-monophosphates regarding the 2′- and 3′-phosphorylated isomers. Those authors also pointed out that the combination of RP-HPLC and inductively coupled plasma (ICP)-MS detection was straightforward, and detection limits were lower than one order of magnitude.

Very satisfactory results have been obtained in the separation of 15 nucleosides and nucleotides within 10 min with the use of an amide HILIC column, in ultra-high performance liquid chromatography coupled to triple-quadrupole tandem mass spectrometry (HILIC-UHPLC -MS/MS) [18]. More recently, ultra-fast liquid chromatography coupled with tandem mass spectrometry (HILIC-UFLC-MS/MS) has also been applied in the analysis of nucleosides using an amide type column of 100 × 2.1 mm (1.7 μm); this allows the separation of 17 nucleosides and nucleobases and 23 free amino acids in clam samples in a single run of 15 min [32].

In the analysis of phosphorylated compounds (as is the case of NMPs, NDPs and NTPs) by LC-MS, the analytes may interact with specific parts of the instrumental configuration [81]. These interactions occur in two different ways, either by the absorption of the analytes on the silanol groups of the stationary phases when one is working in an acid medium, or by means of the formation of metallic complexes of the phosphorylated waste with metallic ions generated in different parts of the chromatographic system [82]. Both processes lead to the reduction of the chromatographic signals and to the appearance of peak tailing. Various strategies have been proposed to prevent the formation of links by hydrogen bridges between the silanol groups and the phosphorylated compounds, such as the derivatization of the free silanol groups or the deactivation of the silica with trimethylchlorosilane [83].

The interaction between the phosphorylated compounds and the metals, which mainly come from the stainless steel of the HPLC-ESI-MS systems or from the solvents themselves, affects detection owing to the formation of complexes of the phosphorylated compounds with Fe(III) [84]. In order to prevent this interaction, strategies have been proposed such as replacing the stainless steel with polyether ether ketone (PEEK), the use of mobile phases with a high pH [81], the pre-treatment of the chromatographic system with phosphoric acid [82], or the use of mobile phases containing carbonate anions [48,85] or chelating agents such as ethylene diamine tetraacetic acid (EDTA) [84]. However, some of these treatments are not very compatible with MS and it is, therefore, necessary to work at low ionic strength in the mobile phase with the aim of avoiding a loss of sensitivity and obstructions in the detector [48]. A method was recently validated for the simultaneous determination of 5 nucleosides and 15 mono-, di- and tri-phosphate nucleotides by HILIC [80]. In order to prevent the adsorption of nucleotides on the LC-MS setup and to enhance their retention on the HILIC stationary phase, a mobile phase containing hexafluoro-2-propanol (HFIP), and DEA as hydrophilic cationic ion-pairing reagent, was used. The results revealed the formation of an ionic-association compound between the phosphorylated analytes and the cationic ion-pairing reagent and the improvement of peak symmetry due to HFIP [80,86].

3.2.1. Analysis of nucleosides and nucleotides in non-infant foods by liquid chromatography

New HPLC methods from 2007 to date applied to the analysis of non-infant food samples are summarized in Table 2.

It is worth noting that, in the field of food technology, there is great interest in assessing both the freshness of fish and meat samples and the efficiency of the various technologies used in food preservation such as salting, dry-curing, freezing, high pressure treatments, atmosphere/vacuum packaging and irradiation. It is known that, during postmortem storage, the small portion of free forms of nucleotides present in animal muscle are degraded in a series of stages due to endogenous biochemical changes; the level of major adenine nucleotides and their related compounds has been used extensively as an index of freshness of, mainly, fish muscle. Different freshness indices (*K*, *Ki*, *P*, log*G* and *H*) have been commonly used [87], all of which provide similar information. In order to determine these indices, most of the methods are based on or are variants of the old RP-HPLC-UV method developed by J. M. Ryder in 1985 [88] or that of M. Aliani in 2005 [89]. Food technology, therefore, includes a multitude of applications orientated in this sense, mainly for fish muscle samples [90] and also in other foods. These methods assess the ATP content together with some/all of its breakdown/degradation products – ADP, AMP, IMP, I and Hx. In some cases, co-elution with matrix components and/or other different nucleosides, nucleotides and nucleobases has been found, which means that these methods should be used with care [9,88]. For this reason, new methods have now been developed that not only avoid the interferences indicated but also allow the

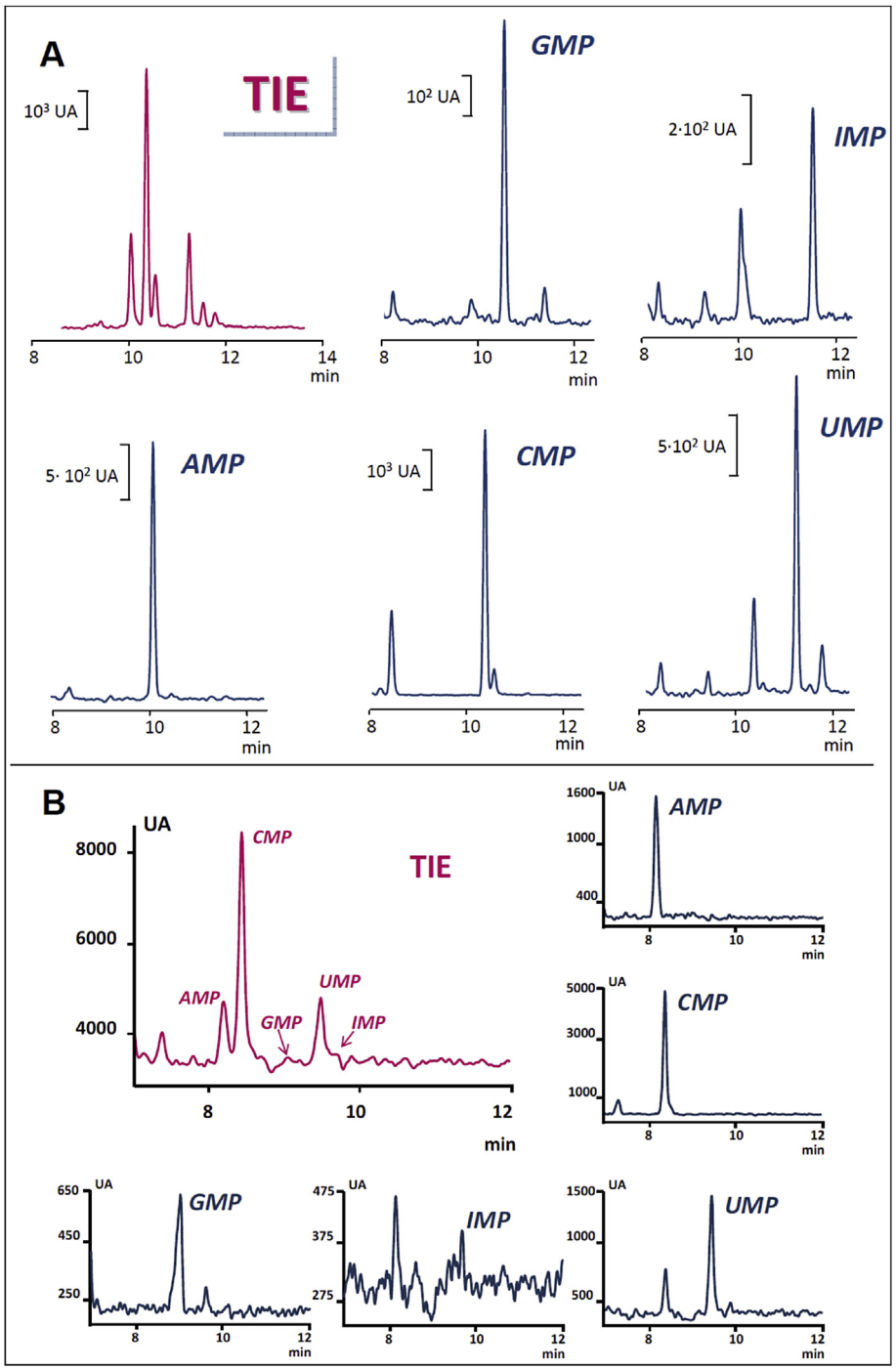


Fig. 3. Total ion electropherograms (TIE) and extracted ion electropherograms (XIE) of powdered infant formula (**A**) and human milk (**B**) samples treated by CUF and analysed by CE-ESI-MS. *Separation conditions*: 30 mM ammonium formate-ammonia medium (pH 9.6); 30 kV/87.5 cm (positive CE mode), 25°C; sheat liquid 50:50, v/v isopropanol:UHQ water mixture, split 1:100, ESI(−) 4000 V, $[M\text{-}H]^-$. *Legend* as in the text. Adapted with permission from Ref. [49]. Reproduced from Ref. [50].

assessment of other purine and pyrimidine compounds which are also associated with the freshness of marine foods [9].

There is a large number of new methods concentrating on the analysis of different fungi and mushrooms by RP-HPLC [8–10,22,23,33,34,41,61] and IP-RP-LC [16] (Table 2). This type of matrix has been the subject of various studies because the ingredients responsible for the *umami* taste and their concentrations in mushrooms are influenced by the species type, the maturity stage, the part of the mushroom, the quality grade, and the storage time [91]. The *umami* taste in mushroom extracts has recently been analysed [10], with the result that the equivalent *umami* concentrations (EUC) obtained by LC-MS have a strong correlation with the results obtained by the electronic tongue system and sensory evaluation.

The analysis of marine organisms including fish, molluscs, crabs, squid, and seaweeds by liquid chromatography [9,26,27,29,32,63] has also attracted great attention. Marine products of the bivalve species type are foods with a high nutritional value, and have been studied in order to determine their contents in nucleosides, nucleobases and free amino acids. Their determination using an RP column coupled to MS has been satisfactorily used in the separation

of ten nucleosides and nucleobases in 35 min [27]. Also, a highly efficient separation of 17 nucleosides and nucleobases and 23 free amino acids in clam samples in a single run within 15 min has been achieved by using ultra-fast LC employing an amide column. This last method has been using to monitor the quality of food products from aquacultural areas and in ascertaining the time of year when the contents of nucleosides, nucleobases and free amino acids in clams are the highest [32].

New methods for determining freshness or assessing the influence of different treatments on the quality of meat products have also been developed [19,24,42,44,45]. The use of UPLC-MS/MS allowed the simultaneous determination of the content of four nucleosides and two nucleobases and uric acid in pork meat, dry cured ham and cooked ham. The matrix effects were variable and depended on the analyte type and on the matrix; for this reason, it is recommendable to carry out the quantification by using matrix matched calibration [24]. The use of HILIC for the simultaneous separation of AMP, ADP, ATP, IMP, I, nicotinamide adenine dinucleotide (NAD^+), and a nucleobase in pork meat samples has been proposed [44]. Fig. 4 shows the chromatograms corresponding to standard (**A**) and 5 h post-mortem pork loin sample (**B**) analysis employing a HILIC column. Pork meat samples at different post-mortem times were analysed using both a zwitterionic-organic polymer HILIC (ZIC-*p*HILIC) column and a Zorbax© Eclipse© XDB-C18 column. The results obtained with both modes were significantly equal, although the HILIC method allowed the additional separation of the family of guanosine, GMP, guanosine 5′-diphosphate (GDP) and guanosine 5′-triphosphate (GTP), together with creatinine and uric acid in a standard mixture.

In royal jelly, which is the food of honeybee larvae, a method for the determination of adenosine (A) in 45 samples has been proposed [35]. ATP and its degradation metabolites (ADP, AMP, IMP, I and Hx) have been determined by RP-UPLC [20] with relatively short analysis times. On the other hand, the assessment of ATP and its metabolites, together with a large number of nucleosides and nucleobases, requires the increasing of the time of chromatographic separation to 40 min [37].

Other foodstuffs have been studied such as curdle bacterium [62]; beer and beer yeast [36,38,54]; and cauliflower and certain types of soups enriched with nucleotides as flavour enhancers [54]. The presence of nucleosides and nucleotides in the fruits and leaves [18,31] of *Ziziphus* – a food and medicinal plant used in China – has also been determined. These matrices turned out to be rich in nucleosides and nucleotides and could be selected as healthy foods; as far as the nucleosides analysed are concerned, the highest contents in both fruits and leaves correspond to uridine (U) and A while the highest nucleotide contents are found in the fruits.

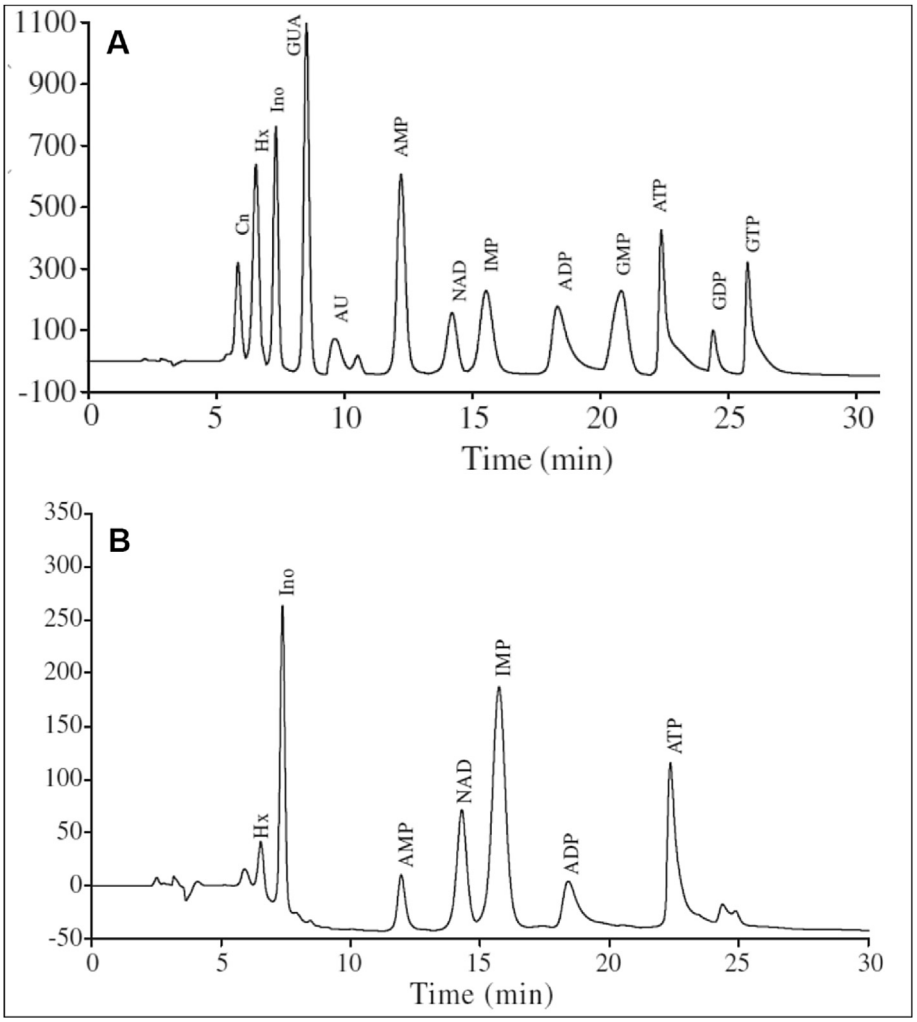


Fig. 4. Chromatograms corresponding to standard (**A**) and 5 h post-mortem pork loin sample (**B**) analysis. *Separation conditions*: ZIC-*p*HILIC column (150 × 4.6 mm, 5 μm); flow rate: 0.5 mL/min; 28°C; gradient elution: (1)ammonium acetate 150 mM pH 3.5; (2)ammonium acetate 100 mM pH 7; (3) ACN and (4)bidistilled water; UV detection 254 nm. *Legend*: creatinine (Cn), guanosine (GUA), Inosine (Ino), uric acid (AU), other acronyms as in the text. Reproduced from Ref. [44].

3.2.2. Analysis of nucleosides and nucleotides in infant foods by liquid chromatography

Table 3 summarizes the applications of LC in the analysis of nucleosides and nucleotides in dairy and non-dairy infant foods. Analytical determinations in baby foods [39,40,52], bovine, caprine and ovine milk [13,14], human milk [11,64], and infant formulas have been carried out.

Infant formulas supplemented with 5′-NMPs have been analysed by RP-HPLC-UV [56,57], IP-RP-HPLC-UV [43,51], IE-HPLC-UV [46,58], and HILIC-UV [64]. Novel dendrimeric IE stationary phases have been proposed, in which nucleotide retention was determined by both the increase in the number of NR_3^+ groups and the ability to penetrate stationary phase layers [58]. In the IP-RP-HPLC-UV AOAC method [57], intended for infant formulas, the interferences were removed by anion-exchange SPE and the quantification was carried out by internal standard calibration. Methods based on MS have also been developed that could be applied to support the more frequently used HPLC-UV methods for the determination of 5′-NMPs [25,47] and the simultaneous determination of 5′-NMPs and nucleosides [48] in infant formulas. The latter has been validated against the NIST 1849a certified reference material (CRM). It is worth nothing that, when developing methods for routine analysis, their validation is crucial; the NIST 1849a CRM provides reference mass fraction values for the nucleotide monophosphates AMP, CMP, GMP and UMP, and also information regarding mass fraction values for the sum of each NMP with its homologous nucleoside – AMP plus A, CMP plus cytidine (C), GMP plus guanosine (G), and UMP plus U. Most of all these applications have been used to study nucleosides or nucleotides separately, which may be due to the difficulties of the joint determination of these compounds [54] and to the loss of sensitivity deriving from the problems inherent to the analysis of phosphorylated compounds [48].

For babies aged between 6 and 12 months complementary feeding should have an appropriate composition as regards energy and nutrients, since it is sometimes their main source of nutrition. Analytical methods for the determination of 5′-NMPs in infant foods by IP-RP-HPLC-Time of flight (TOF)-MS [40] and IE-HPLC-UV [39] have been proposed. The sensitivity, together with the mass accuracy of the TOF-MS method, allowed the correct identification of compounds despite the complexity of the sample matrix. A recent development has been an analytical method based on HILIC-MS/MS, in the presence of DEA as a hydrophilic ion-pairing reagent, for the simultaneous separation of nucleosides and nucleotide mono-, di- and tri-phosphates within 9 min [80] which was successfully applied to infant food samples [52].

Regarding CRM for non-dairy infant foods, NIST 2383a should be cited which is a CRM for an infant food composite – a mixture of fruits, vegetables, macaroni, rice flour, and milk powder – intended, primarily, for use in validating methods for determining calories, vitamins, carotenoids, and other elements in food matrices. However, it does not offer reference values for nucleotides or nucleosides and no CRM has been described for the joint determination of nucleosides and nucleotides in non-dairy infant foods; neither has an official method been proposed for that purpose.

3.3. Capillary electrophoresis

CE is presented as a suitable, efficient, and versatile option for the separation of nucleosides and nucleotides, as they are highly polar compounds and are also charged on a wide pH interval [92]. To determine these compounds, micellar electrokinetic chromatography (MEKC) [93,94] and mixed-mode hydrophilic/strong anion-exchange interactions in pressurized capillary electrochromatography (CEC) [95] have been used. In addition, multifunctional open-tubular (OT) columns covalently modified with either [3-(phenylamino)propyl] trimethoxysilane [96] or 3-[2-(2-aminoethylamino)ethylamino] propyl-trimethoxysilane [97] have been developed for the separation, among others, of nucleosides and NMPs, respectively; open-tubular capillary electrochromatography (OT-CEC) does not require the fabrication of any frits or blending of monomeric reagents with suitable porogens in precise proportions for monoliths. MIPs having a phthalocyanine-based recognition centre have also been developed as receptors for nucleosides for their use as selective separation matrices in typical CEC [98]. The use of CEC in food analysis in its different modalities: packed-colum, monolithic and open tubular-column has recently been reviewed [99].

However, the electrophoretic mode that has been used, as a preference, in the analysis of these compounds in foods was capillary zone electrophoresis (CZE) [17,21,28,30,49,50,53,55,100,101]. With this mode, great flexibility can be achieved in the selectivity of the separation merely by changing the composition of the background electrolyte (BGE) [17,100–102]. The use of separation media in a basic medium (a median pH of 9.5) and, also, in the presence of organic modifiers [21] is frequent.

As has previously been mentioned, these phosphorylated compounds tend to be adsorbed on surfaces of silica such as the inner wall of the CE capillaries. One approach [102] consisted of masking silanols with phosphates to prevent nucleotides from interacting with the capillary wall, and of using pressurized-assisted CE to assist the ESI-MS coupling.

The mostly frequently used detector was the spectrophotometric detector with DAD operating at wavelengths of between 200 and 260 nm [17,21,28,53,55,94,100,101], although coupling to MS detection could improve the specificity of the analysis [30,49,50]. Despite the fact that different types of interfaces have been developed such as those that do not need additional liquid, those of liquid union, or those of direct electrodes, systems using additional liquid continue to be the most frequently used couplings. Finally, CE to ICP-MS interfacing [103] has also been proposed, in which the separation and quantification of phosphorus in deoxy-nucleotide mono-phosphates has been achieved with low detection limits.

As for the food samples analysed, it is worth pointing out that although a wide variety of foods may act as a potential source of nucleosides and nucleotides, there are, however, less specific applications for the determination of these compounds in food by CE compared with those based on LC. The application of CZE to the analysis of AMP, ADP, and ATP for the study of the cell metabolism of the yeast *Saccharomyces cerevisiae* [21], which is widely used in the processes of the industrial fermentation of beer, wine, and bread, has recently been described. Some recent applications also exist for the determination of these analytes in edible mushrooms and fungi. The results showed that CZE is a simple and effective method for the simultaneous analysis of nucleosides and nucleotides, although some of the analytes could not be quantified due to their very low contents in the samples [28]. The analysis by CZE of mushroom extracts allowed the proposal of mixtures of concentrated extracts with foods – such as minced beef steak – in order to achieve substantial changes in the *umami* taste of foods, without significantly changing the flavour attributes of the final product [55]. AMP, CMP and GMP have also been analysed in health-care capsules containing glucoside and peptide [100].

GMP, IMP and AMP influence the taste of *umami*; the taste intensity of GMP is stronger than that of IMP or monosodium L-glutamate. GMP and IMP were analysed in commercial soy sauces by CZE using β-cyclodextrins [101] and the quantification results closely coincided with those obtained by HPLC.

Methods based on CZE have also been applied to the analysis of NMPs and I in goat meat samples [53]. The results showed that the

relative contents of the nucleotides and inosine varied significantly during the cooking of the meat samples.

Some recent applications exist for the analysis of edible marine organisms [30,94]. The first of them, which is non quantitative in type, is based on the use of CZE hyphenated to high resolution MS so as to carry out studies of dereplication. This is a crucial process thanks to which the reisolation of previously known compounds is avoided, discriminating between known versus new compounds as early as possible. The use of CE-ESI-TOF turned out to be a rapid and effective method for dereplicating known nucleoside compounds in marine organisms. The second application mentioned above, of a quantitative nature, uses MEKC to determine nucleosides among other analytes; micelle formation in MEKC contributed to increase resolution of nucleosides and nucleobases in the crude extracts of the sample.

In the field of infant foods, determinations of NMPs in infant formulas by CE-UV have been described [17]. The addition of hydroxypropyl-β-cyclodextrin as additive in the running buffer could successfully improve the resolution of the five nucleotides in infant formulae with a shorter run time than HPLC. On the other hand, coupling CE to MS has allowed the development of a simple and highly efficient method for the determination of five ribonucleotide monophosphates in pediatric formulas [49]. CE-ESI-MS has also been used in the analysis of samples from a human milk bank [50]. The effects on nucleotide preservation by Holder pasteurization and high-pressure processing were compared, with the result that the use of high pressure could be a suitable alternative to traditional pasteurization in the preservation of human milk, because it did not reveal any modification in the nucleotide contents.

4. Conclusions and perspectives

The assessment of NMPs in infant foods, together with the relation between the contents of the various NTPs and metabolites (NDPs, NMPs, and nucleosides) and the freshness and the origin of certain foods, has generated a growing interest in the development of methods validated for the simultaneous analysis of a large number of nucleotides and nucleosides in foods. In these methods, the separation times must be relatively short, avoiding the co-elution of interferences of the matrix or among the analytes themselves. In this field new efforts must be devoted to the preparation of CRMs for nucleos(t)ides in foods compared with those to validate the methods developed.

With the aim of eliminating undesired interactions of the phosphorylated compounds (NMPs, NDPs, and NTPs) with different parts of the instrumental equipment, both on the silica of the columns/capillaries of separation and with metallic areas of the MS devices, there is a tendency to use physical or chemical coatings of silica or the use of additives within the separation media themselves to avoid, or to compete, with said undesired interactions of the analytes. In the analysis in foods, the use of HILIC coupled to mass spectrometry with the use of new stationary phases stands out as the best approach. On the other hand, the high polarity of the analytes, and the level of concentration found in the foodstuffs, means that capillary electrophoresis coupled to mass spectrometry is a very good alternative for the joint determination of nucleosides and nucleotides; it should, however, be pointed out that its use is still not very widespread.

Finally, it should be indicated that, the progress achieved in the methods of determination that are constantly being proposed in food materials may be exportable (and vice versa) to the field of biochemistry; in which nucleosides, nucleotides and their deoxy-metabolites play an important role as biomarkers or in the follow-up of intracell reactions, among others.

Acknowledgements

The authors acknowledge financial support from Universidad de Salamanca “Programa Propio de Financiación de Grupos de Investigación”.

References

[1] S. Yamamoto, M.F. Wang, A.A. Adjei, C.K. Ameho, Role of nucleosides and nucleotides in the immune system, gut reparation after injury, and brain function, Nutrition 13 (1997) 372–374.
[2] G. Hu, F.Q. Yang, Biological activities of nucleosides and their analogues in dietary foods, Chem. Rapid Comm. 2 (2014) 22–28.
[3] C.T. Van-Buren, F. Rudolph, Dietary nucleotides, a conditional requirement, Nutrition 13 (1997) 470–472.
[4] S. Studzińska, B. Buszewski, Effect of mobile phase pH on the retention of nucleotides on different stationary phases for high-performance liquid chromatography, Anal. Bioanal. Chem. 405 (2013) 1663–1672.
[5] B.D. Gill, H.E. Indyk, Determination of nucleotides and nucleosides in milks and pediatric formulas, a review, J. AOAC Int. 90 (2007) 1354–1364.
[6] X.J. Chen, F.Q. Yang, Y.T. Wang, S.P. Li, CE and CEC of nucleosides and nucleotides in food materials, Electrophoresis 31 (2010) 2092–2105.
[7] R. Verkerk, Nucleotides, speculation on lifestyle-induced essentiality, NHD Clin. 64 (2011) 29–32.
[8] A. Ranogajec, S. Beluhan, Z. Smit, Analysis of nucleosides and monophosphate nucleotides from mushrooms with reversed phase HPLC, J. Sep. Sci. 33 (2010) 1024–1033.
[9] M. Ishimaru, M. Haraoka, H. Hatate, R. Tanaka, Simultaneous analysis of purine and pyrimidine compounds associated with the freshness and taste of marine foods, Food Anal. Method 9 (2016) 1606–1615.
[10] C. Phat, B. Moon, C. Lee, Evaluation of *umami* taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system, Food Chem. 192 (2016) 1068–1077.
[11] K.Y. Liao, T.C. Wu, C.F. Huang, C.C. Lin, I.F. Huang, L. Wu, Profile of nucleotides and nucleosides in Taiwanese human milk, Pediatr. Neonatol. 52 (2011) 93–97.
[12] A. Gil, F. Sánchez-Medina, Acid-soluble nucleotides of human milk at different stages of lactation, J. Dairy Res. 49 (1982) 301–307.
[13] B.D. Gill, H.E. Indyk, M. Manley-Harris, Determination of total potentially available nucleosides in bovine milk, Int. Dairy J. 21 (2011) 34–41.
[14] B.D. Gill, H.E. Indyk, M. Manley-Harris, Determination of total potentially available nucleosides in bovine, caprine, and ovine milk, Int. Dairy J. 24 (2012) 40–43.
[15] A. Mir-Marqués, A. González-Masó, M.L. Cervera, M. Guardia, Mineral profile of Spanish commercial baby food, Food Chem. 172 (2015) 238–244.
[16] F.Q. Yang, D.Q. Li, K. Feng, D.J. Hu, S.P. Li, Determination of nucleotides, nucleosides and their transformation products in by ion-pairing reversed-phase liquid chromatography–mass spectrometry, J. Chromatogr. A 1217 (2010) 5501–5510.
[17] X. Ding, Y. Li, B. Song, S. Zhao, Z. Wang, Determination of 5′-mononucleotides in infant formula by capillary electrophoresis with ultraviolet detection, Dairy Sci. Technol. 91 (2011) 759–770.
[18] S. Guo, J. Duan, D. Qian, H. Wang, Y. Tang, Y. Qian, D. Wu, S. Su, E. Shang, Hydrophilic interaction ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry for determination of nucleotides, nucleosides and nucleobases in Ziziphus plants, J. Chromatogr. A 1301 (2013) 147–155.
[19] A. Dunkel, T. Hofmann, Sensory-directed identification of β-alanyl dipeptides as contributors to the thick-sour and white-meaty orosensation induced by chicken broth, J. Agric. Food Chem. 57 (2009) 9867–9877.
[20] L. Zhou, X.F. Xue, J.H. Zhou, Y. Li, J. Zhao, L.M. Wu, Fast determination of adenosine 5′-triphosphate (ATP) and its catabolites in royal jelly using ultraperformance liquid chromatography, J. Agri. Food Chem. 60 (2012) 8994–8999.
[21] P. Zhu, S. Wang, J. Wang, L. Zhou, P. Shi, A capillary zone electrophoresis method for adenine nucleotides analysis in Saccharomyces cerevisiae, J. Chromatogr. B 1008 (2016) 156–163.
[22] J.L. Gao, K.S.Y. Leung, Y.T. Wang, C.M. Lai, S.P. Li, L.F. Hu, G.H. Lu, Z.H. Jiang, Z.L. Yu, Qualitative and quantitative analyses of nucleosides and nucleobases in *Ganoderma* spp. by HPLC–DAD-MS, J. Pharm. Biomed. 44 (2007) 807–811.
[23] R. Ikeda, M. Nishimura, Y. Sun, M. Wada, K. Nakashima, Simple HPLC-UV determination of nucleosides and its application to the authentication of *Cordyceps* and its allies, Biomed. Chromatogr. 22 (2008) 630–636.
[24] M. Clariana, M. Gratacós-Cubarsí, M. Hortós, J.A. García-Regueiro, M. Castellari, Analysis of seven purines and pyrimidines in pork meat products by ultra high performance liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 4294–4299.
[25] Y. Ren, J. Zhang, X. Song, X. Chen, D. Li, Simultaneous determination of 5′-monophosphate nucleotides in infant formulas by HPLC-MS, J. Chromatogr. Sci. 49 (2011) 332–337.
[26] H. Zhao, J. Chen, Q. Shi, X. Li, W. Zhou, D. Zhang, L. Zheng, W. Cao, X. Wang, F.S.-C. Lee, Simultaneous determination nucleosides in marine organisms

using ultrasound assisted extraction followed by hydrophilic interaction liquid chromatography–electrospray ionization time-of-flight mass spectrometry, J. Sep. Sci. 34 (2011) 2594–2601.
[27] R. Liu, J. Ji, L. Wang, S. Chen, S. Guo, H. Wu, Characterisation of nucleosides and nucleobases in *Mactra veneriformis* by high performance liquid chromatography coupled with diode array detector-mass spectrometry (HPLC–DAD–MS), Food Chem. 135 (2012) 548–554.
[28] F.-Q. Yang, R. Lv, Y.-l. Zhang, Z.-N. Xi, Comparison study on nucleosides and nucleotides in edible mushroom species by capillary zone electrophoresis, Anal. Methods 4 (2012) 546–549.
[29] Y. Cao, J. Duan, J. Guo, S. Guo, I. Zhao, Rapid determination of nucleosides, nucleobases and free amino acids in brown seaweeds using ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry, J. Appl. Phycol. 26 (2014) 675–686.
[30] J. Chen, Q. Shi, Y. Wang, Z. Li, S. Wang, Dereplication of known nucleobase and nucleoside compounds in natural product extracts by capillary electrophoresis-high resolution mass spectrometry, Molecules 20 (2015) 5423–5437.
[31] S. Guo, J.-A. Duan, D. Qian, Y. Tang, D. Wu, S. Su, H. Wang, Y. Zhao, Content variations of triterpenic acid, nucleoside, nucleobase and sugar in jujube (*Ziziphus jujuba*) fruit during ripening, Food Chem. 167 (2015) 468–474.
[32] X.-Z. Wang, H. Wu, N. Li, Y. Cheng, H.-M. Wen, R. Liu, C. Chai, Rapid determination of free amino acids, nucleosides and nucleobases in commercial clam species harvested at different seasons in Jiangsu, China, using UFLC-MS/MS, Food Anal. Method 9 (2016) 1520–1531.
[33] F.Q. Yang, J. Guan, S.P. Li, Fast simultaneous determination of 14 nucleosides and nucleobases in cultured *Cordyceps* using ultra-performance liquid chromatography, Talanta 73 (2007) 269–273.
[34] F.Q. Yang, S.P. Li, Effects of sample preparation methods on the quantification of nucleosides in natural and cultured *Cordyceps*, J. Pharm. Biomed. 48 (2008) 231–235.
[35] X.F. Xue, J.H. Zhou, L.M. Wu, L.H. Fu, J. Zhao, HPLC determination of adenosine in royal jelly, Food Chem. 115 (2009) 715–719.
[36] R.M. Seifar, C. Ras, J.C. van-Dam, W.M. van-Gulik, J.J. Heijnen, W.A. van-Winden, Simultaneous quantification of free nucleotides in complex biological samples using ion pair reversed phase liquid chromatography isotope dilution tandem mass spectrometry, Anal. Biochem. 388 (2009) 213–219.
[37] L. Wu, L. Chen, J.N. Selvaraj, Y. Wei, Y. Wang, Y. Li, J. Zhao, X. Xue, Identification of the distribution of adenosine phosphates, nucleosides and nucleobases in royal jelly, Food Chem. 173 (2015) 1111–1118.
[38] O. Magdenoska, P.B. Knudsen, D.K. Svenssen, K.F. Nielsen, Quantifying intracellular metabolites in yeast using a matrix with minimal interference from naturally occurring analytes, Anal. Biochem. 487 (2015) 17–26.
[39] P. Viñas, N. Campillo, I. López-García, S. Martínez-López, M.I. Vasallo, M. Hernández-Córdoba, Anion exchange liquid chromatography for the determination of nucleotides in baby and/or functional foods, J. Agri. Food Chem. 57 (2009) 7245–7249.
[40] P. Viñas, N. Campillo, G. Férez-Melgarejo, M.I. Vasallo, I. López-García, M. Hernández-Córdoba, Ion-pair high-performance liquid chromatography with diode array detection coupled to dual electrospray atmospheric pressure chemical ionization time-of-flight mass spectrometry for the determination of nucleotides in baby foods, J. Chromatogr. A 1217 (2010) 5197–5203.
[41] H. Fan, F.Q. Yang, S.P. Li, Determination of purine and pyrimidine bases in natural and cultured *Cordyceps* using optimum acid hydrolysis followed by high performance liquid chromatography, J. Pharm. Biomed. 45 (2007) 141–144.
[42] Y. Liu, X.-L. Xu, G.H. Zhou, Changes in taste compounds of duck during processing, Food Chem. 102 (2007) 22–26.
[43] M. Krpan, N. Vahčić, M. Hruškar, Validation of an HPLC method for the determination of nucleotides in infant formulae, J. Liq. Chromatogr. R. T 32 (2009) 2747–2755.
[44] L. Mora, A.S. Hernández-Cázares, M.-C. Aristoy, F. Toldrá, Hydrophilic interaction chromatographic determination of adenosine triphosphate and its metabolites, Food Chem. 123 (2010) 1282–1288.
[45] A.S. Hernández-Cázares, M.-C. Aristoy, F. Toldrá, Nucleotides and their degradation products during processing of dry-cured ham, measured by HPLC and an enzyme sensor, Meat Sci. 87 (2011) 125–129.
[46] K. Inoue, R. Obara, T. Akiba, T. Hino, H. Oka, Determination of nucleotides in infant formula by ion-exchange liquid chromatography, J. Agri. Food Chem. 56 (2008) 6863–6867.
[47] K. Inoue, R. Obara, T. Akiba, T. Hino, H. Oka, Development and application of an HILIC-MS/MS method for the quantitation of nucleotides in infant formula, J. Agri. Food Chem. 58 (2010) 9918–9924.
[48] B.D. Gill, H.E. Indyk, M. Manley-Harris, Analysis of nucleosides and nucleotides in infant formula by liquid chromatography–tandem mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 5311–5319.
[49] E. Rodríguez-Gonzalo, J. Domínguez-Álvarez, M. Mateos-Vivas, D. García-Gómez, R. Carabias-Martínez, A validated method for the determination of nucleotides in infant formulas by capillary electrophoresis coupled to mass spectrometry, Electrophoresis 35 (2014) 1677–1684.
[50] M. Mateos-Vivas, E. Rodríguez-Gonzalo, J. Domínguez-Álvarez, D. García-Gómez, R. Ramírez-Bernabé, R. Carabias-Martínez, Analysis of free nucleotide monophosphates in human milk and effect of pasteurisation or high-pressure processing on their contents by capillary electrophoresis coupled to mass spectrometry, Food Chem. 174 (2015) 348–355.
[51] M. Mateos-Vivas, S. Fanali, E. Rodríguez-Gonzalo, R. Carabias-Martínez, Z. Aturki, Rapid determination of nucleotides in infant formula by means of nano-liquid chromatography, Electrophoresis 37 (2016) 1873–1880.
[52] M. Mateos-Vivas, E. Rodríguez-Gonzalo, J. Domínguez-Álvarez, D. García-Gómez, R. Carabias-Martínez, Determination of nucleosides and nucleotides in baby foods by hydrophilic interaction chromatography coupled to tandem mass spectrometry in the presence of hydrophilic ion-pairing reagents, Food Chem. 211 (2016) 827–835.
[53] M.S. Madruga, J.S. Elmore, M.J. Oruna-Concha, D. Balagiannis, D.S. Mottram, Determination of some water-soluble aroma precursors in goat meat and their enrolment on flavour profile of goat meat, Food Chem. 123 (2010) 513–520.
[54] N. Yamaoka, Y. Kudo, K. Inazawa, S. Inagawa, M. Yasuda, K.I. Mawatari, K. Nakagomi, K. Kaneko, Simultaneous determination of nucleosides and nucleotides in dietary foods and beverages using ion-pairing liquid chromatography–electrospray ionization-mass spectrometry, J. Chromatogr. B 878 (2010) 2054–2060.
[55] M. Dermiki, N. Phanphensophon, D.S. Mottram, L. Methven, Contributions of non-volatile and volatile compounds to the *umami* taste and overall flavour of *shiitake* mushroom extracts and their application as flavour enhancers in cooked minced meat, Food Chem. 141 (2013) 77–83.
[56] B.D. Gill, H.E. Indyk, M.C. Kumar, N.K. Sievwright, M. Manley-Harris, A liquid chromatographic method for routine analysis of 5′-mononucleotides in pediatric formulas, J. AOAC Int. 93 (2010) 966–973.
[57] B.D. Gill, H.E. Indyk, Analysis of nucleotide 5′-monophosphates in infant formulas by HPLC-UV, Collaborative study, Final Action 2011.20, J. AOAC Int. 98 (2015) 971–979.
[58] S. Studzińska, R. Rola, B. Buszewski, Determination of nucleotides in infant milk formulas using novel dendrimer ion-exchangers, J. Chromatogr. B 949–950 (2014) 87–93.
[59] S.-T. Wang, W. Huang, Y.-F. Deng, Q. Gao, B.-F. Yuan, Y.-Q. Feng, "Old" metal oxide affinity chromatography as "novel" strategy for specific capture of *cis*-diol-containing compounds, J. Chromatogr. A 1361 (2014) 100–107.
[60] R. Tuytten, F. Lemière, E.L. Esmans, W.A. Herrebout, B.J. van-der-Veken, B.U.W. Maes, E. Witters, R.P. Newton, E. Dudley, Role of nitrogen lewis basicity in boronate affinity chromatography of nucleosides, Anal. Chem. 79 (2007) 6662–6669.
[61] P. Liu, Y.-Y. Li, H.-M. Li, D.-J. Wan, Y.-J. Tang, Determination of the nucleosides and nucleobases in *Tuber* samples by dispersive solid-phase extraction combined with liquid chromatography–mass spectrometry, Anal. Chim. Acta 687 (2011) 159–167.
[62] O. Magdenoska, J. Martinussen, J. Thykaer, K. Fog Nielsen, Dispersive solid phase extraction combined with ion-pair ultra high-performance liquid chromatography tandem mass spectrometry for quantification of nucleotides in *Lactococcus lactis*, Anal. Biochem. 440 (2013) 166–177.
[63] M.C. Cela-Pérez, L. Barbosa-Pereira, X. Vecino, M. Pérez-Ameneiro, A. Lasagabaster-Latorre, J.M. López-Vilariño, M.V. González-Rodríguez, A.B. Moldes, J.M. Cruz, Selective removal of ATP degradation products from food matrices II, rapid screening of hypoxanthine and inosine by molecularly imprinted matrix solid-phase dispersion for evaluation of fish freshness, Talanta 135 (2015) 58–66.
[64] A.G. Sfakianaki, C.D. Stalikas, Selective microextraction of mononucleotides from milk using alumina and stannia hollow fibers prior to their determination by hydrophilic interaction liquid chromatography-diode array detection, Food Chem. 184 (2015) 188–195.
[65] K. Inoue, D. Dowell, HILIC-MS/MS method for the quantitation of nucleotides in infant formula and adult nutritional formula, first action 2011.21, J. AOAC Int. 95 (2012) 603–605.
[66] M. Pabst, J. Grass, R. Fischl, R. Léonard, C. Jin, G. Hinterkörner, N. Borth, F. Altmann, Nucleotide and nucleotide sugar analysis by liquid chromatography-electrospray ionization-mass spectrometry on surface-conditioned porous graphitic carbon, Anal. Chem. 82 (2010) 9782–9788.
[67] Y. Li, J. Yang, J. Jin, X. Sun, L. Wang, J. Chen, New reversed-phase/anion-exchange/hydrophilic interaction mixed-mode stationary phase based on dendritic polymer-modified porous silica, J. Chromatogr. A 1337 (2014) 133–139.
[68] T. Aral, H. Aral, B. Ziyadanoğulları, R. Ziyadanoğulları, Synthesis of a mixed-model stationary phase derived from glutamine for HPLC separation of structurally different biologically active compounds, HILIC and reversed-phase applications, Talanta 131 (2015) 64–73.
[69] H. Li, Z. Liu, Recent advances in monolithic column-based boronate-affinity chromatography, Trend. Anal. Chem. 37 (2012) 148–161.
[70] H. Li, X. Zhang, L. Zhang, X. Wang, F. Kong, D. Fan, L. Li, W. Wang, Preparation of a boronate affinity silica stationary phase with enhanced binding properties towards *cis*-diol compounds, J. Chromatogr. A 1473 (2016) 90–98.
[71] Y. Shibusawa, A. Shoji, C. Suzuka, A. Yanagida, Y. Ito, Counter-current chromatographic separation of nucleic acid constituents with a hydrophilic solvent system, J. Chromatogr. A 1217 (2010) 3457–3460.
[72] Y. Shibusawa, A. Yanagida, A. Ogihara, Y. Ma, X. Chen, Y. Ito, Separation of nucleobases and their derivatives with organic-high ionic strength aqueous phase systems by spiral high-speed counter-current chromatography, J. Chromatogr. B 891–892 (2012) 94–97.
[73] J. Bernal, A.M. Ares, J. Pól, S.K. Wiedmer, Hydrophilic interaction liquid chromatography in food analysis, J. Chromatogr. A 1218 (2011) 7438–7452.
[74] M. Roberto-Gama, R. Gomes-da-Costa-Silva, C.H. Collins, C.B.G. Bottoli, Hydrophilic interaction chromatography, Trend. Anal. Chem. 37 (2012) 48–60.

[75] D. García-Gómez, E. Rodríguez-Gonzalo, R. Carabias-Martínez, Stationary phases for separation of nucleosides and nucleotides by hydrophilic interaction liquid chromatography, Trend. Anal. Chem. 47 (2013) 111–128.
[76] T. Zhou, C.A. Lucy, Hydrophilic interaction chromatography of nucleotides and their pathway intermediates on titania, J. Chromatogr. A 1187 (2008) 87–93.
[77] G. Marrubini, B.E.C. Mendoza, G. Massolini, Separation of purine and pyrimidine bases and nucleosides by hydrophilic interaction chromatography, J. Sep. Sci. 33 (2010) 803–816.
[78] S. Neubauer, A. Rugova, D.B. Chu, H. Drexler, A. Ganner, M. Sauer, D. Mattanovich, S. Hann, G. Koellensperger, Mass spectrometry based analysis of nucleotides, nucleosides, and nucleobases—application to feed supplements, Anal. Bioanal. Chem. 404 (2012) 799–808.
[79] R.L. Cordell, S.J. Hill, C.A. Ortori, D.A. Barrett, Quantitative profiling of nucleotides and related phosphate-containing metabolites in cultured mammalian cells by liquid chromatography tandem electrospray mass spectrometry, J. Chromatogr. B 871 (2008) 115–124.
[80] M. Mateos-Vivas, E. Rodríguez-Gonzalo, D. García-Gómez, R. Carabias-Martínez, Hydrophilic interaction chromatography coupled to tandem mass spectrometry in the presence of hydrophilic ion-pairing reagents for the separation of nucleosides and nucleotide mono-, di- and triphosphates, J. Chromatogr. A 1414 (2015) 129–137.
[81] R. Tuytten, F. Lemière, E. Witters, W. Van-Dongen, H. Slegers, R.P. Newton, H. Van-Onckelen, E.L. Esmans, Stainless steel electrospray probe, A dead end for phosphorylated organic compounds, J. Chromatogr. A 1104 (2006) 209–221.
[82] A. Wakamatsu, K. Morimoto, M. Shimizu, S. Kudoh, A severe peak tailing of phosphate compounds caused by interaction with stainless steel used for liquid chromatography and electrospray mass spectrometry, J. Sep. Sci. 28 (2005) 1823–1830.
[83] F. Gritti, Y.V. Kazakevich, G. Guiochon, Effect of the surface coverage of endcapped C18-silica on the excess adsorption isotherms of commonly used organic solvents from water in reversed phase liquid chromatography, J. Chromatogr. A 1169 (2007) 111–124.
[84] S. Liu, C. Zhang, J.L. Campbell, H. Zhang, K.K.C. Yeung, V.K.M. Han, G.A. Lajoie, Formation of phosphopeptide-metal ion complexes in liquid chromatography/electrospray mass spectrometry and their influence on phosphopeptide detection, Rapid Commun. Mass Sp. 19 (2005) 2747–2756.
[85] Y. Asakawa, N. Tokida, C. Ozawa, M. Ishiba, O. Tagaya, N. Asakawa, Suppression effects of carbonate on the interaction between stainless steel and phosphate groups of phosphate compounds in high-performance liquid chromatography and electrospray ionization mass spectrometry, J. Chromatogr. A 1198–1199 (2008) 80–86.
[86] G. Zhang, A.D. Walker, Z. Lin, X. Han, M. Blatnik, R.C. Steenwyk, E.A. Groeber, Strategies for quantitation of endogenous adenine nucleotides inhuman plasma using novel ion-pair hydrophilic interaction chromatography coupled with tandem mass spectrometry, J. Chromatogr. A 1325 (2014) 129–136.
[87] G. ElMasry, N. Nakazawa, E. Okazaki, S. Nakauchi, Non-invasive sensing of freshness indices of frozen fish and fillets using pretreated excitation–emission matrices, Sens. Actuat. B 228 (2016) 237–250.
[88] J.M. Ryder, Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography, J. Agric. Food Chem. 33 (1985) 678–680.
[89] M. Aliani, L.J. Farmer, Precursors of chicken flavor. I. Determination of some flavor precursors in chicken muscle, J. Agric. Food Chem. 53 (2005) 6067–6072.
[90] J. Lee, J.W. Park, Pacific whiting frozen fillets as affected by postharvest processing and storage conditions, Food Chem. 201 (2016) 177–184.
[91] Y. Zhang, C. Venkitasamy, Z. Pan, W. Wang, Recent developments on *umami* ingredients of edible mushrooms-a review, Trends Food Sci. Tech. 33 (2013) 78–92.
[92] W.F. Smyth, V. Rodriguez, Recent studies of the electrospray ionisation behaviour of selected drugs and their application in capillary electrophoresis–mass spectrometry and liquid chromatography–mass spectrometry, J. Chromatogr. A 1159 (2007) 159–174.
[93] J. Iqbal, C.E. Müller, High-sensitivity capillary electrophoresis method for monitoring purine nucleoside phosphorylase and adenosine deaminase reactions by a reversed electrode polarity switching mode, J. Chromatogr. A 1218 (2011) 4764–4771.
[94] Q. Shi, J.H. Chen, H.Q. Zhao, X. Li, L. Zheng, X.R. Wang, J.Y. Zang, Rapid simultaneous determination of 15 nucleosides and nucleobases in marine medicinal organism *Anthopleura lanthogrammica* Berkly by micellar electrokinetic capillary chromatography, J. Anal. Chem. 69 (2014) 169–178.
[95] X. Wang, Y. Zheng, C. Zhang, Y. Yang, X. Lin, G. Huang, Z. Xie, Preparation and characterization of hybrid-silica monolithic column with mixed-mode of hydrophilic and strong anion-exchange interactions for pressurized capillary electrochromatography, J. Chromatogr. A 1239 (2012) 56–63.
[96] X. Lin, J. Tan, S. Xu, Z. Xie, Phenylaminopropyl-functionalized stationary phase for open-tubular capillary electrochromatography of alkaloids and aromatic acids, J. Sep. Sci. 34 (2011) 2337–2344.
[97] X. Zhang, X. Lin, Z. Chen, X. Wang, X. Wu, Z. Xie, Triamine-bonded stationary phase for open tubular capillary electrochromatography, J. Sep. Sci. 33 (2010) 3184–3193.
[98] L. Longo, S. Scorrano, G. Vasapollo, RNA nucleoside recognition by phthalocyanine-based molecularly imprinted polymers, J. Polym. Res. 17 (2010) 683–687.
[99] G. D'Orazio, M. Asensio-Ramos, C. Fanali, J. Hernández-Borges, S. Fanali, Capillary electrochromatography in food analysis, Trend. Anal. Chem. 82 (2016) 250–267.
[100] Y. Li, X.-J. Ding, Y.-Y. Yang, S. Zhao, Z. Wang, Capillary electrophoretic determination of 3 nucleotides in G-P health-care capsule, Phys. Testing Chem. Ana. Part B, Chem. Anal. 2010-11.
[101] J. Li, Y. Li, B.H. Song, X.-J. Ding, S. Zhao, Z. Wang, Determination of 5′-monophosphate nucleotides in soy sauce by high performance capillary electrophoresis, Food Sci. 2011-22.
[102] T. Soga, T. Ishikawa, S. Igarashi, K. Sugawara, Y. Kakazu, M. Tomita, Analysis of nucleotides by pressure-assisted capillary electrophoresis–mass spectrometry using silanol mask technique, J. Chromatogr. A 1159 (2007) 125–133.
[103] S.-I. Fujii, K. Inagaki, A. Takatsu, T. Yarita, K. Chiba, Determination of phosphorus using capillary electrophoresis and micro-high-performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry for the quantification of nucleotides, J. Chromatogr. A 1216 (2009) 7488–7492.