Trends in Analytical Chemistry 158 (2023) 116853

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Analytical methods for biomonitoring organic chemical hazards in saliva: A systematic review

Jesús Marín-Sáez [a, b, c, 1], Rosalía López-Ruiz [a, b, 1], Madalena Sobral [a], Roberto Romero-González [b], Antonia Garrido Frenich [b], Isabel M.P.L.V.O. Ferreira [a, *]

[a] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal
[b] Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, Agrifood Campus of International Excellence, ceiA3, E-04120, Almeria, Spain
[c] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, E-18071, Granada, Spain

ARTICLE INFO

Article history:
Received 21 October 2022
Received in revised form
22 November 2022
Accepted 22 November 2022
Available online 5 December 2022

Keywords:
Saliva
Sample collection
Extraction
Analytical techniques
Human biomonitoring
Leached materials
Contaminants

ABSTRACT

In the present study, a systematic review from January 1, 2000 to March 15, 2022 is presented, collecting all studies related to the analyses of organic chemical hazards in saliva. The goal was to review saliva sample collection, extraction and analytical techniques, together with the occurrence of the identified compounds. Therefore, the major gaps that limit a wider use of saliva as a valuable human specimen to undertake human biomonitoring of exposure to food chain contaminants were identified. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol was performed via PubMed, SCOPUS, and Web of Science databases. After screening and selection process of primary sources, 46 articles were eligible for full text assessment and data of 30 studies were extracted. All the results and characteristics of those 30 studies were displayed in table format and critically reviewed. The lack of standardized procedures for saliva sampling/collection/storage is a major drawback.

1. Introduction

Oral intake is the main route of exposure for a myriad of organic chemical hazards. Those include environmental pollutants, natural toxins, food processing contaminants, food contact materials released from packaging, residues of pesticides, veterinary medicines, and personal care products [1–5]. Despite the establishment of Regulations by the European Commission that set maximum levels (MLs) for foods consumed regularly [6], or for those which are particularly susceptible for contamination, as infants and toddler foods, food chain contaminants cannot be completely avoided. Moreover, another potential route of oral exposure to unintentional organic compounds is the leaching of monomers from dental composites and orthodontic materials into saliva [7], such as bisphenol A (BPA) and related compounds [8,9] or phthalates from polyvinyl chloride (PVC) materials [10]. Long term exposure to those chemical hazards is recognized as a risk factor in the onset of several non-communicable diseases (e.g., cancer) [2]. However, the establishment of causality between their development and the human exposome to those multiple undesirable chemical compounds is difficult to track since it may take several years or decades of co-exposure at low levels before health-related concerns become visible [11]. Thus, to establish this causality evidences from exposome-wide association studies (ExWAS) are required [12]. ExWAS require repeated analyses of chemicals circulating in the body along time in order to obtain large data sets at different stages of life [13]. In this context, human biomonitoring (HBM) studies are a valuable tool to assess exposure to harmful chemicals or their biological markers in human specimens which could help us to understand the magnitude of the exposure [14,15]. Nevertheless, one of the issues that restrict large-scale HBM is the need for invasive collection of human specimens.

Blood is the most common biospecimen used to identify biomarkers of exposure in HBM studies [16]. It is the golden standard matrix for most contaminants since blood plasma is in contact with

* Corresponding author.
E-mail address: isabel.ferreira@ff.up.pt (I.M.P.L.V.O. Ferreira).
[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.trac.2022.116853

all tissues, being in equilibrium with the organs and tissues where contaminants are distributed [17] or eliminated in faeces if they are not absorbed, in lesser extent [18]. However, the invasive nature of blood sampling limits its applicability [19,20]. Therefore, urine has been used as a non-invasive sampling method and is the second most common biospecimen for HBM, for example, for heavy metals [20]. Nevertheless, urine can only be employed for the analysis of water-soluble and relatively polar chemicals, involving a portion of polar contaminants not absorbed and their metabolites (biomarkers of exposure). For example, polycyclic aromatic hydrocarbon (PAH) metabolites, bisphenols or perfluoroalkyl substances (PFAS) [16,21,22]. Besides, great variations of volume and chemical concentration may occur.

Therefore, saliva can be an alternative biospecimen for biomonitoring the exposure to organic chemical hazards. The continuous exchange of chemicals between saliva and blood plasma makes saliva an interesting biofluid for detection of biomarkers of internal exposition [14]. In addition, saliva has several advantages compared to blood or urine: it is easier to collect and store; it is a less invasive matrix; it requires less manipulation; it is cheaper, safer, and well accepted by the population, which maximises volunteers' participation, being more suitable for large population studies [15], and for continuous monitoring of recent exposure since it causes no/minimum embarrassment or discomfort in comparison with blood or urine tests [15]. In addition, it could be used to analyse a great variety of compounds [23], since saliva metabolome database includes more than 1200 molecules, composed by xenobiotics and endogenous compounds [13]. This is not surprising since the transport of compounds from plasma to oral cavity may occur by different processes, namely, ultrafiltration, intercellular nexus, transudation of plasma compounds into the oral cavity from crevicular fluid or directly from the oral mucosa, passive diffusion through the salivary membranes or active transport [24].

One of the main disadvantages of saliva as HBM matrix is the lower concentrations compared with blood biomarkers. In consequence, only compounds at enough contamination concentrations can be identified in saliva [25]. Nevertheless, due to the latest advances in analytical techniques, namely, miniaturized extraction procedures coupled to gas chromatography (GC) or liquid chromatography (LC) and mass spectrometry detection (MS), which provide high sensitivity and low limits of quantitation (LOQ), the identification and quantification of organic chemical hazards in saliva has been successfully achieved [26,27].

Therefore, the goal of this review was to perform a systematic search of literature focused on organic chemical hazards found in saliva and linked to prevalent food contaminants or leaching materials. Saliva sample collection, treatment, and extraction procedures will be reviewed together with advanced analytical methods and their occurrence. This search also aimed to identify major gaps that can limit the use of saliva as a valuable human specimen to undertake HBM of exposure to organic chemical hazards.

2. Methods

2.1. Search strategy

The methodology applied was the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology, performing a search for publications in the databases PubMed, SCOPUS, and Web of Science using the following keywords: (mycotoxins OR "polycyclic aromatic hydrocarbons" OR plasticizers OR bisphenol OR pesticides OR “food contaminant”) AND (saliva OR spit OR "oral fluids" OR sputum) AND (“chromatography” OR “mass spectrometry”). The collection of papers was done up to March 15th' 2022. A total of 409 publications were identified after compiling all three databases. The compiled articles were inserted in the EndNote library to remove duplicates. Publications prior to 2000, reviews, conference papers, languages other than English, Spanish or Portuguese were also excluded from the list (Supplementary Material Fig. S1).

2.2. Exclusion criteria and results obtained

Four authors of this publication revised independently the title and abstract of each article. Studies involving artificial saliva or organic compounds of intentional use (e.g., drugs) were also excluded. The remaining 46 papers were full text reviewed. Papers with no access to full text, studies where no real samples were analysed (method development only) and those involving artificial saliva and/or spiked saliva were excluded from the list (16 articles). Then, the full text of the selected manuscripts was carefully studied, and the data was collected. From these 30 papers remaining, a higher percentage of them addressed the analysis of plasticizers as BPA and related monomers (45%) and phthalates (11%). More recent papers analyse other compounds, such as PAHs (16%), nicotine (5%), pesticides (8%) or parabens (8%).

In all steps, selection disagreements were solved by meeting with the four authors and deciding together the inclusion or exclusion of the articles according to the previously explained criterion.

2.3. Data extraction

Relevant information of the selected articles was collected including studied compounds, volunteers' features, sample collection method, storage/pre-treatment, extraction methodology, analytical methods, validation parameters, number of analysed saliva samples and detected concentrations of compounds under study (μg/L). The extracted data were collected in Tables 1–3.

3. Results

3.1. Human saliva collection

Although saliva is considered as an easy/non-invasive sampling matrix, its collection must carefully be done and be properly handled to achieve accurate conclusions. Several aspects must be planned: volunteers' selection, sampling collection (i.e., device, place of collection, etc), storage, and handling (i.e., transport and set-up for analysis). According to the articles reviewed in this study, it was verified that a proper volunteer's selection is important when, for instance, an experiment involving saliva sampling for studies of leaching materials is planned. Table 1 summarizes information of volunteer's selection for each type of organic chemical hazard, and leaching materials have a well-stablished exclusion criteria list that was followed in most of the studies included in this review. Lifestyle and health habits are also important data to collect among participants to better understand data results in case of occupational/dietary exposure to these substances (e.g., construction sites, gas station, acryl related works, smoking habits etc). Thus, depending on the study design and goals, the participants selection and an adequate personal questionary may represent a critical step.

The way saliva is collected represents a major critical step because its collection may involve several actions that can greatly affect the results. Firstly, to avoid contamination of sample some instructions must be taken prior to collection, like abstain to drink, eat, or brush teeth from a certain period of time among others

Table 1
Summary of volunteers' features and procedures taken to collect and handle saliva in previous studies to analyse organic chemical hazards.[a]

Analyte	Volunteers' features	Instructions prior to sampling	Sampling	Storage and pre-treatment	Ref
Leaching materials					
BPA, TEGDMA, UDMA and BisGMA	Healthy volunteers after dental treatment. Exclusion of smokers, drug abusers, dental splint users, dental protheses and patients with current orthodontic treatment. Questionnaires on lifestyle, food consumption and hygiene habits.	Abstain to drink, eat, brush teeth from 2 to 10 h prior to sampling. Only tap water was allowed for drinking.	Collection was done in a dental clinic before and 1h-1 month after dental treatment. Diverse type of collection: - Spitting in sterilized falcon - Active cheek and tongue movement for 60s (2–5 mL) - Collection by using saliva collection cotton plugs on which the participant chewed to actively induce and collect saliva	Direct analysis or storage at −20°C or at −80°C Diverse types of pre-treatments: - Using whole saliva: Thawing saliva at RT and vortexed (used whole saliva) - Using saliva supernatant: Separation of emulsion and mucin aggregates from aqueous phase by centrifugation - Thawing cotton swab containing the saliva followed by centrifugation	[28–34]
Contaminants from internal exposition					
Bisphenols, parabens, benzophenones and triclocarban	Healthy volunteers	Fasting periods of 30 min to 1 h before saliva collection and rinsing mouth with water	Plastic devices avoided for collection. Diverse type of collection: - Saliva drop into glass tube (5 mL) - Spitting - Passive drool collection over a glass funnel connected with a glass centrifuge tube. - Collection by slowly drain off the lower lip into the conical tubes without spitting	Storage at −20°C or at −80°C. Pre-treatment was centrifugation, to remove suspended	[35–40]
Pesticides and insecticides	Healthy volunteers Occupational exposure was taken into consideration for selection. Exclusion of smokers, alcohol or drug abusers, pregnant or breastfeeding women, etc.	Questionnaire survey to collect sociodemographic characteristics and lifestyle habits.	Collection was done 1h-7 days after pesticide application. Diverse type of collection: - Collection with a cotton swab - Active buccal and tongue movements	Storage at −20°C or at −80°C. Pre-treatment was centrifugation of cotton swab to collect saliva	[41,42]
PAHs and their derivatives (nitro- and oxo-)	Heavy smokers (more than 20 cg/day), light-smokers and non-smokers and firefighters Information about smoking habits/ firefight activities was recorded	Abstain to eat, drink at least 1 h prior to collection.	Collection was done before and 1 h after smoking and immediately-48 h after activity for firefighters. Diverse type of collection: - Expectorate to a plastic container for 30 min - Spitting directly into glass vial - Cotton swab collection	Direct analysis or storage at −20°C or at −80°C. Pre-treatment was performed to obtain saliva supernatant of fresh or thawed saliva by centrifugation	[27,43–46]
Nicotine, anabsine and cotinine	Healthy volunteers	–	Collection in Salisoft tubes containing a polypropylene-polyethylene sponge	Direct analysis or storage refrigerated. Pre-treatment was filtration and centrifugation	[47,48]

[a] Abbreviations: BisGMA: Bisphenol A-glycidyl methacrylate; BPA: Bisphenol A; PAH: Polycyclic aromatic hydrocarbon; RT: Room temperature; TEGDMA: Triethylene glycol dimethacrylate; UDMA: Urethane dimethacrylate.

Table 2
Occurrence of organic chemical hazards in real samples for the reviewed articles.[a]

Compounds	Number of samples	Number of positive samples	Concentrations obtained (μg/L)	Ref.
Leaching materials				
BPA	40 samples before and 1 and 3 h and 1, 3 and 5 days after sealant placement	Only saliva samples after 1 and 3 h were positive	5.80–105.6	[28]
BPA, TEGDMA, bisDMA and bisGMA	8 samples of patients 1 h after dental treatment	All samples were positive in BPA, TEGDMA and bis-GMA	14.20–198.2	[9]
BPA	14 samples before and immediately, 1 and 24 h after dental sealants placement	Positive samples were immediately and 1 h after placement	0.17–96.2	[29]
DEHP	3 samples of volunteer who chewed PVC	It estimated the migration into saliva	Migration of 1.51 μg/cm^3 in 30 min	[35]
BPA	22 samples before and 30 min, 1 day, 1 week, and 1 month after lingual retainers placement	Concentration increase only at 30 min after placement	0.85–20.9	[30]
BPA, BPAHPE, TEGDMA, BADGE, bisDMA and bisGMA	151 samples before and immediately, 1–9 h and 9–30 h after composite restorations	Concentrations decreased to baseline in 1 h for almost all the compounds	0.34–304.5	[49]
TEGDMA, bisEMA, bisGMA, HEMA, UDMA	10 samples at different times before and after dental restoration	Bis-GMA), (HEMA), and (UDMA) quatified and TEGDMA detected 10 min after treatment	Before: [a] 10 min after: 4-9650 1 day after: HEMA detected 7 days after:	[50]
MMA	30 samples after 1 h, 1 and 3 days after denture insertion	Significant increasing at 1 day after insertion	0.03–0.40	[51]
BPA	20 samples at different times before and after bracket bonding	Increasing only 30 min after treatment	Before: 0.49–0.73 30 min after: 0.74–1.82 1 day after: 0.50–1.34 7 days after: 0.50–1.52 30 days after: 0.49–1.23	[52]
Free and conjugated BPA	40 samples (20 for composite group and 20 for control)	8 for composite group and 3 for control	0.11–0.57 Concentrations slightly higher in the composite group	[33]
BPA	40 samples before and 30 min, 1 day, 1 week, and 1 month after orthodontic bonding	Higher concentrations 30 min after orthodontic bonding and for chemically cured resin	0.45–36.20	[53]
8 bisphenols, 2 phthalates, 3 chlorobenzenes, 4-Nonylphenol and triclosan	5 samples from patients undergoing orthodontic treatment	All samples were positive in at least 1 compound. Higher concentrations of BPA, BADGE and phthalates	10.19–8101	[32]
Free and conjugated BPA	20 samples at different times before and after dental treatment	High increase just after treatment (from 0.11 to 385). Then exponential decreasing		[31]
BPA, TEGDMA, UDMA and bisGMA	Samples with a joint research project with Dentistry Department	-[a]	–	[34]
Contaminants from internal exposition				
nicotine, cotinine, and related alkaloids	74 samples	74 samples	Nicotine 0.00–300000 Cotinine: 0.00–20000	[48]
15 PAHs, 8 nitro-PAHs and 8 oxo-PAHs	–	–	PAHs: 2.0 Nitro-PAH and oxo-PAHs: 0.1	[27]
16 PAHs	55 samples	Naphtalene: 4 samples Acenaphthylene: 13 samples Fluorene: 55 samples Phenanthrene: 36 samples Anthracene: 6 samples Fluoranthene: 17 samples Pyrene: 13 samples	Naphtalene: 0.27–0.43 Acenaphthylene: 0.05–1.45 Fluorene: 0.13–1.48 Phenanthrene: 0.15–3.3 Anthracene: 0.19–0.38 Fluoranthene: 0.19–1.1 Pyrene: 0.17–1.5	[45]
15 PAHs	9 samples	1 sample	Phenanthrene: 0.015	[46]
8 PAHs	20 samples (10 smokers and 10 non-smokers)	20 samples	**Non-Smokers (ng/L):** Naphthalene: 55-114 Acenaphthylene: 1-3 Acenaphthene: 2-6 Fluorene: 3-11 Phenanthrene/ Anthracene: 18-44 Fluoranthene: 2-7 **Smokers (ng/L):** Naphthalene: 70-365 Acenaphthylene: 5-105 Acenaphthene: 5-25 Fluorene: 12-77 Phenanthrene/ Anthracene: 40-233 Fluoranthene: 4-38	[43]

13 PAHs	6 non-smoker and 5 smoker subjects (2 light smokers and 3 heavy smokers)	3 heavy smokers	Pyrene: 1-5 **After smoking:** Naphthalene: 0.70−4.10 1-methylnaphthalene: 0.53−1.38 2-methylnaphthalene: 0.42−1.51 Biphenyl: 0.12−0.39 4-phenyltoluene: 0.08−0.17 Phenanthrene: 0.26−0.40 Acenaphthene: 0.17 Fluorene: 0.32−0.80 3-Phenyltoluene: 0.90−0.26 Acenaphthylene: 0.25−1.30	Pyrene: 5-29 **1 h later smoking:** Naphthalene: 0.15−0.75 1-methylnaphthalene: 0.07−0.60 2-methylnaphthalene: 0.10−0.64 Biphenyl: 0.08−0.15 4-phenyltoluene: 0.06 Phenanthrene: 0.19−0.31 Fluorene: 0.22−0.29 3-Phenyltoluene: 0.13 Acenaphthylene: 0.16−0.27	[44]
6 PAHs	6 samples + 1 control	6 samples	Anthracene 0.09−0.30 Chrysene 0.10−0.55 Fluoranthene 0.20−0.55 Fluorene 0.28−0.70 Naphthalene 0.30−1.00 Phenanthrene 0.12−0.60		[54]
Permethrin and 8 metabolites	6 samples	6 samples	From 0.00 to 0.003 Concentrations higher at the beginning of the study		[42]
10 Pesticides	4 samples + 1 control	4 samples	Diazinon from 0.0 to 0.20 Bromopropylate from 0.01 to 0.35		[41]
9 Neonicotinoids insecticides	188 samples	Acetamiprid: 176 samples Imidacloprid: 157 samples Clothianidin: 170 samples Thiacloprid: 154 samples Thiamethoxan: 156 samples Dinoterfuran: 170 samples 5-Hydroxy-imidacloprid: 140 samples N-Desmethyl-1-acetamiprid: 173 samples 1-methyl-3-(tetrahydro-3-furylmethyl)urea: 176 samples	Acetamiprid: 0.01−70.4 Imidacloprid: 0.01−55.5 Clothianidin: 0.02−1.56 Thiacloprid: 0.002−61.6 Thiamethoxan: 0.01−5.9 Dinoterfuran: 0.01−13.10 5-Hydroxy-imidacloprid: 0.01−17.50 N-Desmethyl-1-acetamiprid: 2.00 (ng/L)-2.64 1-methyl-3-(tetrahydro-3-furylmethyl) urea: 0.01−78.8		[26]
12 bisphenols and 6 parabens	10 samples	All parabens except iButPB positives. 5 bisphenols including BPA positives	0.40−48.70		[37]
13 bisphenols	13 samples	BPA was found in all samples and BPF, BPS and BPAF 46%, 62% and 8% of the samples	0.03−0.80		[40]
6 bisphenols, 5 parabens, 5 benzophenones and triclocarban	10 samples	10 samples	BPA from 1.0 to 6.0 Benzophenone from 1.0 to 3.0 Methyl-paraben highest level of 18		[38]
13 phthalates metabolites and BPA	32 samples (16 with oral squamous cell carcinoma and 16 without)	Almost all the compounds were positive	3.62−1351		[36]
3 bisphenols, 7 phthalates, 4 parabens and triclosan	11 samples	11 compounds positive	0.02−43.5		[39]

[a] - = Non specified; Abbreviations: BADGE: BPA diglycidyl ether; BisDMA: Bisphenol A dimethacrylate; BisEMA: Ethoxylated bisphenol A dimethacrylate; BisGMA: Bisphenol A glycidyl methacrylate; BPA: Bisphenol; BPAF: Bisphenol AF; BPAHPE: BPA bis(2,3-hydroxyphenyl) ether; BPF: Bisphenol F; BPS: Bisphenol S; DEHP: Di(2-ethylhexyl) phthalate; HEMA: 2-hydroxyethyl methacrylate; iButPB: Isobutylparaben; MMA: Monomeric methyl methacrylate; PAH: Polycyclic aromatic hydrocarbon; TEGDMA: Triethylene glycol dimethacrylate; UDMA: Urethane dimethacrylate.

Table 3
Analytical methodology for the reviewed studies.[a]

Compounds	Extraction technique	Recoveries/ RSD (%)	Analysis technique Column Mobile phases	Detection technique Analysis time	LODs/LOQs (μg/L)	Ref.
Leaching materials						
BPA	SPE with C_{18} cartridges	82-97/2-4	HPLC Supelcosil LC-18 (300 × 4.0 mm, 5 μm) Acetonitrile:water 50:50 (*v/v*)	DAD (λ = 190 −800 nm) –	5.00/-	[28]
BPA, TEGDMA, bisDMA and bisGMA	Deproteinization with acetonitrile and evaporation	-/5-7	GC HP1-MS fused silica capillary (30 m × 0.25 mm x 0.25 μm)	EI-Q-MS SIM mode 19 min	0.30−15.00/ 1.00−50.00	[9]
BPA	Centrifugation, incubation with β-glucuronidase, SPE with C_{18} cartridges and derivatization	–/–	GC DB-5 (30 m × 0.25 mm x 0.25 μm)	EI-HRMS-DFS Magnetic Sector 27 min	0.10/-	[29]
BPA	LLE with MTBE in acid conditions and evaporation	–/–	HPLC Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm, 5 μm) Water 0.1% acetic acid:acetonitrile 60:40 (*v/v*) (isocratic mode)	ESI-Q-MS SIM mode –	0.50/-	[30]
BPA, BPAHPE, TEGDMA, BADGE, bisDMA and bisGMA	–	–	LC – –	MS –	0.40−2.70/-	[49]
TEGDMA, bisEMA, bisGMA, HEMA, UDMA	LLE with ethyl acetate and evaporation	–/–	[a]TEGDMA and HEMA: GC CP-SIL 8 CB WCOT (30 m × 0.25 mm x 0.25 μm) [a]bisEMA, bisGMA and UDMA: HPLC Zorbax XDB-C_8 (250 × 4.6 mm, 5 μm) Methanol:water 80:20 (*v/v*) 10 mM ammonium acetate (isocratic elution)	[a]TEGDMA and HEMA: Q-MS SIM mode 17.5 min [a]bisEMA, bisGMA and UDMA: ESI-TRAP MRM	–/–	[50]
BPA	LLE with MTBE and derivatization with BSTFA + TMCS	–/–	GC NST-05MS (30 m x 0.25 mm × 0.25 μm)	EI-Q-MS SIM 15 min	–/–	[52]
Free and conjugated BPA	Digestion with glucuronidase and precipitation with acetonitrile	-/14-42	UHPLC VisionHT C_{18} (50 × 2.1 mm, 1.5 μm) Water and methanol	QTRAP-MS/MS SRM 9.6 min	0.10/-	[33]
8 bisphenols, 2 phthalates, 3 chlorobenzenes, 4-Nonylphenol and triclosan	Enzymatic digestion with β-glucuronidase, deproteinization and LLME with ethyl acetate:n-hexane 50:50 (*v/v*)	77-97/<11	HPLC Kinetex phenyl-hexyl (150 × 4.6 mm, 5.0 μm) Water and methanol both with 0.1% formic acid	UV (λ = 220 nm) and fluorescence (λ = 263 −305 nm) 80 min	0.07−1.03/ 2.28−6.29	[32]
BPA	LLE with methanol and MTBE in acid conditions and evaporation	–/–	HPLC – Water 0.1% acetic acid:acetonitrile 60:40 (*v/v*)	ESI-Q-MS SIM mode –	0.50/-	[53]
Free and conjugated BPA	Digestion with glucuronidase and precipitation with acetonitrile	-/14-42	UHPLC VisionHT C_{18} (50 × 2.1 mm, 1.5 μm) Water and methanol	QTRAP-MS/MS SRM 9.6 min	0.10/-	[31]
BPA, TEGDMA, UDMA and bisGMA	Centrifugation and FPSE with sol-gel PTHF	90-107/1-12	HPLC Perfect Sil 120 ODS-2 C_{18} (250 × 4.0 mm, 5 μm) Acetonitrile:water (70:30, *v/v*) (isocratic elution)	UV (λ = 220 nm) 11 min	75.00/ 250.00	[34]
DEHP	LLE with dichloromethane	–/–	GC DB-1 (30 m × 0.25 mm x 0.25 μm)	FID –	–/–	[35]
MMA	–	-/20-28	HPLC Nucleosil C_{18} (150 × 4.6 mm, 5 μm) –	UV (λ = 230 nm) –	–/–	[51]
Contaminants from internal exposition						
12 bisphenols and 6 parabens	Deproteinization, one UAE with acetone and another with ethanol and evaporation	86-114/1-15	UHPLC	ESI-QqQ-MS/MS MRM mode 21 min	0.10−0.40/ 0.30−1.00	[37]

Table 3 (*continued*)

Compounds	Extraction technique	Recoveries/ RSD (%)	Analysis technique Column Mobile phases	Detection technique Analysis time	LODs/LOQs (μg/L)	Ref.
			BEH C_{18} (100 × 2.1 mm, 1.7 μm) Water and methanol, both 2 mM ammonium acetate			
13 bisphenols	SUPRASs with hexanol in THF	95-106/ <16	HPLC ACE $3C_{18}$-PFP (150 × 3.0 mm, 3 μm) Water and methanol	TVIS-QTRAP MRM mode 31.5 min	0.01−0.05/ 0.02−0.10	[40]
6 bisphenols, 5 parabens, 5 benzophenones and triclocarban	DLLME with acetone:trichlorometane 3:1	–	LC Atlantis® T3 dC_{18} (75 × 2.1 mm, 3.0 μm) Water and methanol	ESI-QqQ-MS/MS 10 min	0.01−0.15/ 0.05−0.40	[38]
13 phthalates metabolites and BPA	HF-LPME with octanol:ethyl octanoate 1:1 (*v/v*)	83-120/ <19	GC HP-5 MS (30 m × 0.25 mm x 0.25 μm)	EI-TRAP-MS 27 min	0.03−0.53/ 0.09−1.78	[36]
3 bisphenols, 7 phthalates, 4 parabens and triclosan	Enzymatic digestion with β-glucuronidase, DLLME with ethyl acetate and acetonitrile and derivatization with MTBSTFA	84-120/2-14	GC HP-5 MS (60 m × 0.25 mm x 0.25 μm)	EI-Q-MS full scan 31 min	0.02−3.00/ 0.05−10.00	[39]
Nicotine, cotinine, and related alkaloids	On-line SPME	83-98/<7	HPLC Synergi 4u POLAR-RP (150 × 4.6 mm, 2.5 μm) Water 5 mM ammonium formate:methanol 55:45 (*v/v*) (isocratic mode)	ESI-Q-MS 10 min	0.02−0.04/-	[48]
Nicotine, anabasine, and cotinine	SDME	71-111/<9	GC BP-21 (25 m × 0.32 mm x 0.5 μm)	FID 11 min	330.00 −450.00/-	[47]
15 PAHs, 8 nitro-PAHs and 8 oxo-PAHs	–	–	–	ASAP-APCI-QTOF 3 min	5.00 −20.00/-	[27]
16 PAHs	LLE with methanol and ethyl acetate	91-104/11-19	GC HP-5MS (30 m × 0.25 mm x 0.25 μm)	EI-MS 8.25 min	0.06/-	[45]
15 PAHs	Centrifugation and MEPS	78-123/10-17	GC HP-5MS (30 m × 0.25 mm x 0.25 μm)	EI-MS 8.25 min	0.05−0.08/	[46]
8 PAHs	HS-SPME	79-117/ <18	GC VF-Xms (30 m × 0.25 mm x 0.10 μm)	EI-QqQ-MS/MS 32 min	0.70−22.20/ 0.80−26.40 (ng/L)	[43]
13 PAHs	Multi-HS	-/<15%	GC HP-5MS (30 m × 0.25 mm x 0.25 μm)	EI-MS 8.25 min	1.40−43/ 5.20 −143.00 (ng/L)	[44]
6 PAHs	LLME with DES based ferrofluid	61-84/<9	GC HP-5MS (30 m × 0.25 mm x 0.5 μm)	EI-MS 33 min	0.02−0.06/ 0.06−0.22	[54]
10 Pesticides	LLME with DES with menthol:phenylacetic acid by solidification	79-97/<8	GC HP-1 (30 m × 0.25 mm x 0.25 μm)	EI-MS 26 min	2-17/10−85 (ng/L)	[41]
Permethrin and 8 metabolites	–	–	LC Onyx monolithic C_{18} (100 × 3.0 mm, 2 μm) Water and acetonitrile:water 90:10 (*v/v*) both with TFA (pH = 2.2)	AMS 200 min	–	[42]
9 Neonicotinoids insecticides	LLE with ethyl acetate	71-107/2-17	LC Zorbax SB-C_{18} (100 × 2.1 mm, 3.5 μm) Water formic acid and acetonitrile	ESI-QqQ-MS/MS 12 min	-/2−50 (ng/L)	[26]

[a] Abbreviations: AMS: Accelerator mass spectrometry; APCI: Atmospheric pressure chemical ionization; ASAP: Atmospheric-pressure solid analysis probe; BADGE: BPA diglycidyl ether; BisDMA: Bisphenol A dimethacrylate; BisEMA: Ethoxylated bisphenol A dimethacrylate; BisGMA: Bisphenol A glycidyl methacrylate; BPA: Bisphenol; BPAF: Bisphenol AF; BPAHPE: BPA bis(2,3-hydroxyphenyl) ether; BPF: Bisphenol F; BPS: Bisphenol S; BSTFA: N,O-bis(trimethylsilyl) trifluoroacetamide; DAD: Diodes array detector; DEHP: Di(2-ethylhexyl) phthalate; DES: Deep eutectic solvent; DLLME: Dispersive liquid-liquid microextraction; EI: Electron impact; ESI: Electrospray ionization; FID: Flame ionization detector; FPSE: Fabric phase sorptive extraction; GC: Gas chromatography; HEMA: 2-hydroxyethyl methacrylate; HF-LPME: Hollow fiber liquid phase microextraction; HPLC: High pressure liquid chromatography; HRMS: High resolution mass spectrometry; HS: Head space; LC: Liquid chromatography; LLE: Liquid-liquid extraction; LLME: Liquid-liquid microextraction; LOD: Limit of detection; LOQ: Limit of quantification; MEPS: Microextraction by packed sorbents; MMA: Monomeric methyl methacrylate; MRM: Multiple reaction monitoring; MS: Mass spectrometry; MTBE: Methyl tertiary-butyl ether; MTBSTFA: N-methyl-N-(tert-bultyl dimethyl silyl) trifluoroacetamide; PAH: Polycyclic aromatic hydrocarbon; PTHF: Polytetrahydrofuran; Q: Quadrupole; QqQ: Triple quadrupole; QTOF: Quadrupole-time of flight; QTRAP: Quadrupole-ion trap; RSD: Relative standard deviation; SDME: Single-drop microextraction; SIM: Selected ion monitoring; SPE: Solid-phase extraction; SPME: Solid-phase microextraction; SRM: Selective reaction monitoring; SUPRASs: In situ formation of supramolecular solvents; TEGDMA: Triethylene glycol dimethacrylate; TFA: Trifluoroacetic acid; THF: Tetrahydrofuran; TMCS: Trimethylchlorosilane; TRAP: Ion trap; TVIS: Turbo V ion source; UAE: Ultrasonic assisted extraction; UDMA: Urethane dimethacrylate; UHPLC: Ultrahigh pressure liquid chromatography; UV: Ultraviolet−visible; WCOT: Wall-coated open tubular.

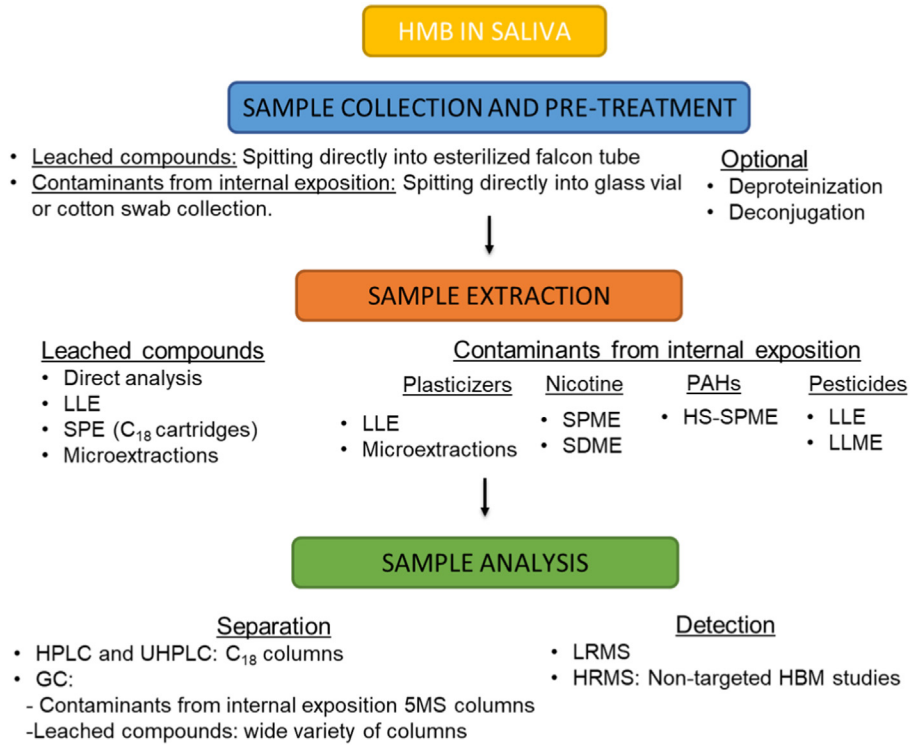

Fig. 1. Recommended methodology for human biomonitoring studies in saliva.

(Table 1). The choice of the collection device is also of paramount importance, since it may strongly affect the capability to recover the analytes and determines the volume of saliva required for analysis. Saliva collection can be performed by different methods (Fig. 1), including: i) passive drooling of unstimulated whole saliva into plastic tubes, often referred as the gold standard for biological assays; ii) spitting it into a pre-weighed or graduated container, which might have some stimulatory effects, and iii) swab-based sampling of unstimulated whole saliva. Different devices are commercially available for collecting saliva. Salivette® is widely used and its main advantages are being simple and hygienic for saliva collection, decrease the viscosity of the saliva, and to eliminate the dead cells. However, the resulting saliva is different from whole unstimulated saliva, and levels of the analytes under study may be affected because the analytes contained in the saliva sample are in contact with the cotton swab [43]. So, depending on target analyte, different saliva collection may be chosen. For instance, Martin Santos et al. [43] reported lower recoveries of PAHs in saliva collected with Salivette® system since compounds were retained in the cotton swab, concluding that spitting method was preferred for PAHs determination. Moreover, saliva can be collected in different sampling period depending on study design. This is widely used in studies involving leaching materials from dental composites and orthodontic materials [28–31]. These studies performed the sample collection in a dental clinic to keep the same conditions in each collection period. Finally, saliva container must be carefully selected since it could contain some compounds, as bisphenols or phthalates, that could interfere during the analytical stage.

After saliva collection, different handling procedures may be followed: i) direct analysis or ii) storage at 4, -20 or −80°C until analysis. During storage, saliva proteins and mucins tend to precipitate which leads to the presence of a non-homogenised solution. Thus, after sample thawing, saliva can be analysed as a whole (after homogenization) or subject to a centrifugation step (Table 1), and only the supernatant is analysed. However, this additional pre-treatment may have a significant impact on results since target analytes may be lost together with the salivary proteins, being no longer available for further detection [43].

Altogether, a proper study design containing saliva sampling must carefully address the selection of participants (e.g., define exclusion criteria if applicable and making questionaries about life habits), set-up instructions prior to sampling to avoid contaminations, selecting the most appropriate sampling collection procedure for target analytes, together with storage conditions and handling. However, up to now, there are no standard operation procedures for collecting and/or handling saliva. The selection of the best condition must be performed either by literature search of previous works or by designing a pre-study comparing different collection/handling conditions to define which one suits best the analysis of the targeted analytes. For instance, there are already several studies published for leaching materials (Table 1), however, for other chemical hazards it could not be possible to use the mentioned methodology since their concentrations are considerably lower and fewer studies have been found, being saliva collection/handling not even mentioned [41,44,47].

3.2. Biomonitoring of leaching compounds to saliva

Dental materials, as for example dental composites and orthodontic materials can leach monomers from their structure. The most studied ones are BPA and related compounds as bisphenol S (BPS) or bisphenol F (BSF). Besides, there are other compounds which are normally used in these dental materials as bisphenol A glycidyl methacrylate (bis-GMA), and bisphenol A dimethacrylate (bis-DMA) [9]. Phthalates are also compounds of interest because they are found in PVC materials [32] in contact with oral fluids.

3.2.1. Occurrence in saliva samples

Leaching material studies are mainly focused on dental materials after different medical interventions, as the release of

bisphenols after dental treatment (dental sealant placement, lingual retainer placement, composite restorations, denture insertion and bracket bonding). Therefore, most of the studied samples contained the compounds under study (Table 2). Moreover, samples were also analysed before treatment because exposure to BPA and related compounds can be produced by several exposition routes such as food, air, dust, water, etc [55]. Indeed, in the reviewed studies most of all analysed samples contained some compounds before treatment, although at lower concentrations than after treatments. Most articles analysed BPA [28,29,31,52,53] or BPA with other related monomers (triethylene glycol dimethacrylate (TEGDMA), bisphenol A dimethacrylate (bisDMA), bisphenol A-glycidyl methacrylate (bisGMA), ethoxylated bisphenol A dimethacrylate (bisEMA), BPA bis(2,3-hydroxyphenyl) ether (BPAHPE), BPA diglycidyl ether (BADGE), 2-hydroxyethyl methacrylate (HEMA) and urethane dimethacrylate (UDMA)) [9,49,50]. These studies clearly state that although concentrations increased after dental treatment, they rapidly decreased in 1–3 h, concluding that dental treatments do not imply a higher health risk than from other contamination routes [51].

In other studies, the BPA concentrations between two groups of people, one submitted to composite restoration and another as control were compared [33], obtaining a slight increment in the treated group 30 min after treatment. Russo et al. [32] analysed 8 bisphenols, 2 phthalates, 3 chlorobenzenes, 4-nonylphenol and triclosan in saliva samples of patients submitted to an orthodontic treatment, observing that all samples were positive in at least 1 compound, reaching the highest concentrations for the phthalate DEHP (8.10 mg/L). The phthalate DEHP has been studied after chewing PVC pieces to simulate what can happen when someone introduces a material made with it in its mouth (e.g., children who introduce a toy in their mouth) obtaining a migration of 6.04 μg/cm^3 per hour [35], being the daily received dose of 18 μg/kg. These results are considerably lower than the maximum daily phthalate intake (50 mg/kg per day) recommended by the Scientific Committee on Toxicity, Ecotoxicity, and Environment cited by this article, concluding that this route of exposure does not seem to be a health risk.

3.2.2. Extraction methods for leached compounds

Most studies are focused on the analyses of BPA and related compounds in saliva from patients that were submitted to dental procedures (Table 3). Therefore, the compounds under study were usually found in saliva samples at enough concentration to be quantified. The simplest extraction methods include direct analysis [51], or protein precipitation with organic solvents [9]. An extra deconjugation step with β-glucuronidase can be included for the analysis of both free and conjugated BPA [31,33].

Liquid-liquid extraction (LLE) methods have also been widely employed for the analysis of BPA and related compounds in saliva samples [30,50,52,53]. Unfortunately, none of these studies evaluated the method performance, so accuracy and repeatability could not be discussed [49]. More exhaustive extraction methods are employed as a strategy to reduce matrix effect and/or improve method sensitivity. This is the case of solid-phase extraction (SPE), with C_{18} cartridges, employed by Fung et al. [28] and Joskow et al. [29] for the analysis of BPA or fabric phase sorptive extraction (FPSE) for the extraction of BPA and related monomers, being considered a greener extraction method [34].

3.2.3. Analytical methods for leached compounds

LC and GC have been widely employed as separation techniques for these compounds (Table 3). Bisphenols and phthalates are more easily separated by LC [28,30–34,50,51,53], whereas other specific BPA related monomers as TEGDMA and HEMA and some phthalates can be separated by GC [9,35,50]. Besides, derivatization can be carried out to improve the analytical performance for BPA by GC, employing N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) [52].

MS is the predominant technique for the detection of these compounds, employing all of them electrospray ionization (ESI), although LC has also been coupled to ultraviolet–visible (UV) or fluorescence detectors [28]. MS methods comprised a wide variety of analysers. Quadrupole-MS analyzers (Q-MS), working in selected ion monitoring mode (SIM), provided LODs lower than 15 μg/L and LOQs lower than 50 μg/L in all cases [9,30,50,52,53]. If more sensitive methods are needed, other alternatives need to be used as ion trap analysers (TRAP) or the hybrid Q-TRAP, working in multiple reaction monitoring (MRM) (LODs<0.049 μg/L and LOQs<0.098 μg/L) [50] or selective reaction monitoring (SRM) (LODs<0.1 μg/L) [31,33]. Only the study of Joskow et al. [29] employed a HRMS instrument, DFS Magnetic Sector, with a LOD of 0.10 μg/L.

3.3. Biomonitoring of external exposition to organic chemical hazards

Exposure to bisphenols and phthalates can come from other materials different than orthodontic products that are present as daily use products, such as bottles, microwave ovenware, storage containers, internal protective lining for food and beverage cans, etc [55]. For that reason, the next studies will be reviewed in this section, although they could also have been included in the Section 3.3 (Table 3). Together with bisphenols and phthalates commented above, other compounds could leach from these daily products. Parabens for example are compounds present in personal care products, pharmaceuticals and food or beverages that, like bisphenols, have adverse health effects as diseases as obesity or diabetes mellitus [56,57].

3.3.1. Occurrence in saliva samples

The occurrence of organic chemical hazards from internal exposition in saliva samples has been scarcely studied (Table 2). Moreover, the focus changed over the years, from chemical substances derived from exposure to smoke, as nicotine, cotinine, and PAHs to the exposure to pesticides and plastic materials.

In the study of Kataoka et al. [58] the presence of nicotine, cotinine, and related alkaloids was evaluated in 74 samples of saliva and urine of 52 non-smoking volunteers and 22 smoking volunteers. The compounds nicotine and cotinine were found in all samples, but concentrations were higher in smoking volunteers (Table 3).

PAHs were analysed in the saliva of smokers and non-smokers [43,44]. Naphthalene was detected at high concentration (from 0.07 to 4.10 μg/L) in smokers' saliva followed by phenanthrene (from 0.04 to 0.60 μg/L). In addition, one study [44] evaluated the concentration of PAHs right after smoking and 1 h after to check how their concentration decreased after this time. Another study evaluated the presence of PAHs, nitro-PAHs and oxo-PAHs in saliva samples [27], detecting PAHs at 2 μg/L and nitro-PAHs and oxo-PAHs at 0.1 μg/L. Finally, Martin-Santos et al. monitored the presence of 16 PAHs in 55 saliva samples [45] to follow occupational exposure of firefighters, and 15 PAHs [46] in non-exposed individuals (9 samples). Fluorene was found in all samples at concentrations ranging from 0.13 to 1.48 μg/L.

There are only three articles focused on the detection of pesticides in saliva samples [41,42,59], and all of them were published in recent years, which reflects that saliva has recently begun to be consider for contaminant biomonitoring. The first one monitored permethrin and 8 metabolites, which were detected at concentrations from <LOD to 3 ng/L in 6 samples of saliva of individuals that

do not report any use of permethrin in the previous 6 months. In addition, the experiment was performed during a monitoring study from 1 h to 7 days, and the concentration was higher at the beginning of it [42]. The second was focused on the analysis of pesticides in saliva of four farmers exposed during the spraying procedure [41]. Among the ten pesticides included in the study, two, diazinon and bromopropylate, were detected at concentrations ranging from <LOD to 0.35 μg/L. In the third study, 9 neonicotinoids were monitored in 188 samples of saliva, being acetamiprid and 1-methyl-3-(tetrahydro-3-furylmethyl)urea the neonicotinoids most detected (176 samples) at concentrations ranging from 0.01 to 80 μg/L, whereas the other compounds were detected from 2 ng/L to 61.6 μg/L [26].

Finally, compounds as bisphenols, parabens or phthalates were detected in saliva samples (Table 2). Parabens were studied by Moscoso-Ruiz et al. [37] (6 parabens in 10 samples), de Oliveira et al. [38] (5 parabens in 10 samples) and by Vu et al. [39] (4 parabens in 11 samples). All the analysed samples were positive in at least one the compounds. Besides, some parabens appeared more frequently in female saliva samples [38]. This can be explained since parabens are compounds frequently present in personal care products [60] which are generally more used by women. Methylparaben (MetPB) was the most detected compound and at the highest concentration in two of the articles [37,38], whereas buthylparaben (ButPB) was the most abundant in the Vu et al. study [39]. Concentrations ranged from 5.7 to 48.7 μg/L in all cases.

Bisphenols were analysed by Moscoso-Ruiz et al. [37] (12 bisphenols in 10 samples), Romera-Garcia et al. [40] (13 bisphenols in 13 samples) de Oliveira et al. [38] (6 bisphenols in 10 samples) Messias Gomes et al. [36] (BPA in 32 samples) and by Vu et al. [39] (3 bisphenols in 11 samples). BPA was the compound most detected and at a higher concentration (0.057−19.9 μg/L). BPAF and BPS were also detected in a high percentage of samples. Almost all the samples were positive in at least one compound. Concentrations were lower than for parabens, ranging from 0.057 to 20.5 μg/L, except in the study of Messias Gomes et al. [36] in where concentrations ranged from 12.77 to 83.96 μg/L.

Phthalates were studied by Messias Gomes et al. [36] (13 phthalates in 32 samples) and by Vu et al. [39] (7 phthalates in 11 samples). Monomethyl phthalate (MMP) and monoethyl phthalate (MEP) [36], and monobenzyl phthalate (MBzP) and mono-(2-ethyl-5-oxohexyl) phthalate (MeOHP) [39] were the monomers most detected with concentrations ranged from 3.62 to 1351.2 μg/L [36] and from 1.71 to 26.9 μg/L [39].

3.3.2. Extraction and concentration techniques

As compounds from internal exposition are found in saliva at lower concentrations than those released from orthodontic products, direct analysis are not possible, and several extraction techniques must be used to preconcentrate the targeted compounds. Thus, ultrasonic assisted extraction (UAE) with acetone followed by another UAE with ethanol has been employed for the extraction of 12 bisphenols and 6 parabens with recoveries from 86 to 114% and RSD lower than 15% [37]. *In situ* formation of supramolecular solvents (SUPRASs) is considered a greener method, and has been applied for the extraction of 13 bisphenols [40], achieving excellent recoveries, from 95 to 106% and RSD<16%.

Microextraction methods can be another alternative, which employs low amount of extraction solvents and allow the preconcentration of the analytes. Thereby, dispersive liquid−liquid microextraction (DLLME) [61] has also been optimized for the extraction of different classes of compounds as bisphenols, parabens, and phthalates, employing acetone and trichloromethane as dispersant and extraction solvent respectively (method performance not detailed) [38] or ethyl acetate and acetonitrile as dispersant and extraction solvent (recoveries = 84−120% and RSD<14%) [39]. Hollow fiber-liquid phase microextraction (HF-LPME) was also employed to extract phthalates and BPA [36] using octanol and ethyl octanoate as extraction solvents (recoveries from 83 to 120% and RSD<19%).

Other organic compounds were also monitored in saliva (Table 3), as uptake of nicotine and tobacco-related toxicants by smokers [47,48], PAHs monitored for the first time in the saliva of smokers and firefighters [43,44,46,54] and pesticides, including neonicotinoids, pyrethroids and organophosphates, and their metabolites [26,41,42]. LLE was used to determine 9 neonicotinoid insecticides [26] and 16 PAHs [45] in saliva samples. In both cases recoveries and RSD values were between 70-105% and 2−20%, respectively. However, LLE induced to a high dilution of the samples being a problem in terms of the minimum concentration detected in samples.

Microextractions as microextraction by packed sorbent (MEPS), single-drop microextraction (SDME), solid-phase microextraction (SPME) and head space (HS) can be used as an alternative and a greener method to determine organic compounds in saliva. MEPS was employed to determine 15 PAHs using as sorbent a C_{18} cartridge, obtaining recoveries between 78 and 123% and RSD between 10 and 17% [46]. SDME was used for the determination of nicotine, anabasine, and cotinine and it was based on the sample exposure to a drop of chloroform and agitation during 30 min. For this method, recoveries were from 71 to 111% and RSD <9% [47]. Finally, HS-SPME or HS alone were employed for the determination of PAHs (8 and 13, respectively [43,44]). HS-SPME method was based on the use of a fiber of polydimethylsiloxane (PDMS). Satisfactory results were achieved during the determination of 8 PAHs in saliva samples, obtaining recoveries between 79 and 117% and RSD<18%. On the other hand, HS was employed by Peña et al. [44] to extract 13 PAHs, providing RSD values lower than 15%.

As summarized in Fig. 1, for leached compounds, present at high concentrations direct analysis or LLE methods can be applied, while SPE or microextraction methods are required for biomonitoring of internal exposition to organic chemical hazards.

3.3.3. Analytical techniques

LC-MS, LC coupled to accelerator mass spectrometry (AMS), GC−MS, GC coupled to flame ionization detector (FID) and atmospheric pressure solid analysis probe (ASAP) coupled to HRMS are the analytical techniques employed to detect organic chemical hazards in saliva (Table 3).

LC-MS was used for bisphenols [40], bisphenols and parabens [37] and bisphenols, parabens, benzophenones and triclocarban [38], all of them employing C_{18} columns. Triple quadrupole (QqQ) [37,38] or QTRAP [40] presented similar detection/quantification limits (LOD between 0.01 μg/L and 0.40 μg/L and LOQs from 0.024 μg/L and 1 μg/L). GC-MS was used for phthalates and BPA [36], as well as for bisphenols, phthalates, parabens and triclosan [39]. This last one employed a derivatization step with N-methyl-N-(tert-bultyl dimethyl silyl) trifluoroacetamide (MTBSTFA) to obtain a better performance of the selected analytes. TRAP-MS (LODs = 0.03−0.53 μg/L and LOQs = 0.09−1.78 μg/L) and Q-MS (LODs = 0.02−3.00 μg/L and LOQs = 0.05−10.00 μg/L), respectively.

GC-FID was used for the determination of nicotine, cotinine, and related compounds [47]. The main disadvantage of this methodology was the high LOD, ranging between 330 and 450 μg/L. In contrast, lower LOD 15−40 ng/L) can be achieved by using electrospray ionization (ESI) Q-MS [48] (Table 2).

PAHs have been analysed by GC-MS (Table 3). Non-target techniques were also used in the case of PAHs analysis. With this methodology nitro-PAHs and oxo-PAHs analyses were performed using ASAP coupled to high resolution mass analysers quadrupole-

time of flight (QTOF) [27]. A screening is obtained in 3 min of analysis time, but the main disadvantage is the higher LOD compared to conventional GC-MS (e.g., LOD = 5–20 μg/L, for nitro-PAHs and oxo-PAHs determinations). ASAP can be useful for rapid screening and semi-quantitative simultaneous analysis of contaminants.

Pesticides were analysed by both, LC and GC. LC was used to assess internal exposure to pesticides as permethrin and metabolites [42] and neonicotinoids insecticides [26] while GC was used for the analyses of 10 non-polar to medium polar pesticides [41]. Both separation techniques were coupled to MS analysers. In the case of permethrin and metabolites [42], a graphite AMS was employed. To analyse neonicotinoids insecticides LC-QqQ-ESI-MS was performed [26]. The analysis time was the lowest of the three exposed methods (12 min) and the LOQ ranged from 2 to 50 ng/L.

As summarized in Fig. 1, the most suitable separation technique was GC for non-polar to medium compounds as PAHs, pesticides and phthalates and LC for the rest, bisphenols, nicotine and related compounds and pesticides.

4. Conclusions and future perspectives

In recent years, an increased number of studies used saliva as a specimen for biomonitoring exposure to different organic chemical hazards. This can be explained by the improvements observed on sample pre-treatment and extraction procedures, which are critical issues, since they limit the quantification of analytes that are found at very low concentrations. Traditional techniques like LLE, and SPE are being replaced by miniaturized extraction methods, using “green” and high-sensitivity analytical approaches. These improvements on the extraction methods coupled to the latest GC and LC separation methods and sensitive mass detection instruments enabled the analyses of saliva biomarkers, on small sample volumes with good precision and trueness. Therefore, saliva has potential to be used as a biospecimen to track exposure to organic chemical hazards.

Concerning the biomonitoring of leaching compounds to saliva, bisphenols, parabens or phthalates were in general found in the studied saliva samples. Although their concentrations increase after dental treatments, a rapid decrease is observed in 1–3 h. Therefore, dental treatments do not imply a higher health risk than those from other contamination routes. On the other hand, the articles that were not focused on leaching of dental materials, address saliva monitoring of smoking and occupational exposure biomarkers. The analysis of PAHs and pesticides in saliva of exposed volunteers reported the presence of those compounds (or their metabolites), although non-exposed individuals also contained a variable concentration of them, which may be explained by the intake from dietary exposure. Analysis of mycotoxins in saliva were not reported in the literature.

In the future, a wider use of saliva as a specimen for biomonitoring exposure to food chain hazards or their biological markers requires that several limitations identified in this systematic review are overcome. The lack of standardized sampling procedures for saliva collection makes it difficult to compare results from literature concerning the magnitude of exposure. Another issue that needs standardization is the selection of the most appropriate storage conditions, that assure matrix stability, and the analysis. Therefore, the lack of those standardized procedures makes data concerning the occurrence of organic chemical hazards in saliva is difficult to compare.

Funding

This research was supported by National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) in the framework of the project DIETxPOSOME project (PTDC/SAU-NUT/6061/2020).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

European Union through FEDER funds (NORTE-01-0145-FEDER-000052) and UIDB/50006/2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trac.2022.116853.

References

[1] I.A. Rather, W.Y. Koh, W.K. Paek, J. Lim, The sources of chemical contaminants in food and their health implications, Front. Pharmacol. 8 (2017). https://doi.org/10.3389/fphar.2017.00830.
[2] Y. Huang, M. Fang, Nutritional and environmental contaminant exposure: a tale of two Co-existing factors for disease risks, Environ. Sci. Technol. 54 (2020) 14793–14796. https://doi.org/10.1021/acs.est.0c05658.
[3] M. Silva, O. Viegas, A. Melo, D. Finteiro, O. Pinho, I.M.P.L.V.O. Ferreira, Fast and reliable extraction of polycyclic aromatic hydrocarbons from grilled and smoked muscle foods, Food Anal. Methods 11 (2018) 3495–3504. https://doi.org/10.1007/s12161-018-1325-8.
[4] O. Viegas, P.S. Moreira, I.M.P.L.V.O. Ferreira, Influence of beer marinades on the reduction of carcinogenic heterocyclic aromatic amines in charcoal-grilled pork meat, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess 32 (2015) 315–323. https://doi.org/10.1080/19440049.2015.1010607.
[5] C. Mansilha, P. Silva, S. Rocha, P. Gameiro, V. Domingues, C. Pinho, I.M.P.L.V.O. Ferreira, Bisphenol A migration from plastic materials: direct insight of ecotoxicity in Daphnia magna, Environ. Sci. Pollut. Control Ser. 20 (2013) 6007–6018. https://doi.org/10.1007/s11356-013-1614-0.
[6] European Commission, EU Pesticides data base. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN, 2016. (Accessed 23 June 2022).
[7] J.L. Ferracane, Elution of leachable components from composites, J. Oral Rehabil. 21 (1994) 441–452.
[8] C.M. McDonough, H.S. Xu, T.L. Guo, Toxicity of bisphenol analogues on the reproductive, nervous, and immune systems, and their relationships to gut microbiome and metabolism: insights from a multi-species comparison, Crit. Rev. Toxicol. 51 (2021) 283–300. https://doi.org/10.1080/10408444.2021.1908224.
[9] A. Zafra, M. del Olmo, R. Pulgar, A. Navalón, J.L. Vílchez, Determination of bisphenol-A and related compounds in human saliva by gas chromatography-mass spectrometry, Chromatographia 56 (2002) 213–218.
[10] I. Mesquita, M. Lorigo, E. Cairrao, Update about the disrupting-effects of phthalates on the human reproductive system, Mol. Reprod. Dev. 88 (2021) 650–672. https://doi.org/10.1002/mrd.23541.
[11] M. Eskola, C.T. Elliott, J. Hajšlová, D. Steiner, R. Krska, Towards a dietary-exposome assessment of chemicals in food: an update on the chronic health risks for the European consumer, Crit. Rev. Food Sci. Nutr. 60 (2020) 1890–1911. https://doi.org/10.1080/10408398.2019.1612320.
[12] V. Siroux, L. Agier, R. Slama, The exposome concept: a challenge and a potential driver for environmental health research, Eur. Respir. Rev. 25 (2016) 124–129. https://doi.org/10.1183/16000617.0034-2016.
[13] V. Bessonneau, J. Pawliszyn, S.M. Rappaport, The saliva exposome for monitoring of individuals' health trajectories, Environ. Health Perspect. 125 (2017). https://doi.org/10.1289/EHP1011.
[14] N. Steckling, A. Gotti, S. Bose-O'Reilly, D. Chapizanis, D. Costopoulou, F. de Vocht, M. Garí, J.O. Grimalt, E. Heath, R. Hiscock, M. Jagodic, S.P. Karakitsios, K. Kedikoglou, T. Kosjek, L. Leondiadis, T. Maggos, D. Mazej, K. Polańska,

A. Povey, J. Rovira, J. Schoierer, M. Schuhmacher, Z. Špirić, A. Stajnko, R. Sierum, J.S. Tratnik, I. Vassiliadou, I. Annesi-Maesano, M. Horvat, D.A. Sarigiannis, Biomarkers of exposure in environment-wide association studies – opportunities to decode the exposome using human biomonitoring data, Environ. Res. 164 (2018) 597–624. https://doi.org/10.1016/j.envres.2018.02.041.
[15] B. Michalke, B. Rossbach, T. Göen, A. Schäferhenrich, G. Scherer, Saliva as a matrix for human biomonitoring in occupational and environmental medicine, Int. Arch. Occup. Environ. Health 88 (2015) 1–44. https://doi.org/10.1007/s00420-014-0938-5.
[16] K. Vorkamp, A. Castaño, J.P. Antignac, L.D. Boada, E. Cequier, A. Covaci, M. Esteban López, L.S. Haug, M. Kasper-Sonnenberg, H.M. Koch, O. Pérez Luzardo, A. Osite, L. Rambaud, M.T. Pinorini, G. Sabbioni, C. Thomsen, Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative, Environ. Int. 146 (2021). https://doi.org/10.1016/j.envint.2020.106082.
[17] M.M. Plassmann, W. Brack, M. Krauss, Extending analysis of environmental pollutants in human urine towards screening for suspected compounds, J. Chromatogr. A 1394 (2015) 18–25. https://doi.org/10.1016/j.chroma.2015.03.040.
[18] D.B. Barr, R.Y. Wang, L.L. Needham, Biologic monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's Study, Environ. Health Perspect. 113 (2005) 1083–1091. https://doi.org/10.1289/ehp.7617.
[19] K. Vorkamp, A. Castaño, J.P. Antignac, L.D. Boada, E. Cequier, A. Covaci, M. Esteban López, L.S. Haug, M. Kasper-Sonnenberg, H.M. Koch, O. Pérez Luzardo, A. Osite, L. Rambaud, M.T. Pinorini, G. Sabbioni, C. Thomsen, Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative, Environ. Int. 146 (2021). https://doi.org/10.1016/j.envint.2020.106082.
[20] M. Esteban, A. Castaño, Non-invasive matrices in human biomonitoring: a review, Environ. Int. 35 (2009) 438–449. https://doi.org/10.1016/j.envint.2008.09.003.
[21] Ž. Tkalec, T. Kosjek, J. Snoj Tratnik, A. Stajnko, A.A. Runkel, M. Sykiotou, D. Mazej, M. Horvat, Exposure of Slovenian children and adolescents to bisphenols, parabens and triclosan: urinary levels, exposure patterns, determinants of exposure and susceptibility, Environ. Int. 146 (2021). https://doi.org/10.1016/j.envint.2020.106172.
[22] P. Martín Santos, M. del Nogal Sánchez, J.L. Pérez Pavón, B. Moreno Cordero, Determination of polycyclic aromatic hydrocarbons in human biological samples: a critical review, TrAC, Trends Anal. Chem. 113 (2019) 194–209. https://doi.org/10.1016/j.trac.2019.02.010.
[23] K.K. Dennis, E. Marder, D.M. Balshaw, Y. Cui, M.A. Lynes, G.J. Patti, S.M. Rappaport, D.T. Shaughnessy, M. Vrijheid, D.B. Barr, Biomonitoring in the era of the exposome, Environ. Health Perspect. 125 (2017) 502–510. https://doi.org/10.1289/EHP474.
[24] F.G. Bellagambi, T. Lomonaco, P. Salvo, F. Vivaldi, M. Hangouët, S. Ghimenti, D. Biagini, F. di Francesco, R. Fuoco, A. Errachid, Saliva sampling: methods and devices. An overview, TrAC, Trends Anal. Chem. 124 (2020). https://doi.org/10.1016/j.trac.2019.115781.
[25] World Health Organization, Human biomonitoring: facts and figures. http://www.euro.who.int/pubrequest, 2015.
[26] H. Zhang, N. Zhang, W. Zhou, X. Zeng, X. Wang, M. Zhan, W. Xu, Y. Huang, L. Lu, Z. Li, Y. Gao, Profiles of neonicotinoid insecticides and their metabolites in paired saliva and periodontal blood samples in human from South China: association with oxidative stress markers, Ecotoxicol. Environ. Saf. 212 (2021), 112001. https://doi.org/10.1016/j.ecoenv.2021.112001.
[27] D. Carrizo, C. Domeño, I. Nerín, P. Alfaro, C. Nerín, Atmospheric pressure solid analysis probe coupled to quadrupole-time of flight mass spectrometry as a tool for screening and semi-quantitative approach of polycyclic aromatic hydrocarbons, nitro-polycyclic aromatic hydrocarbons and oxo-polycyclic aromatic hydrocarbons in complex matrices, Talanta 131 (2015) 175–184. https://doi.org/10.1016/j.talanta.2014.07.034.
[28] E.Y.K. Fung, N.O. Ewoldsen, H.A. St Germain JR, D.B. Marx, C.-L. Miaw, C. Siew, H.-N. Chou, S.E. Gruninger, D.M. Meyer, Pharmacokinetics of bisphenol a released from a dental sealant, J. Am. Dental Assoc. 131 (2000) 51–58.
[29] R. Joskow, D.B. Barr, J.R. Barr, A.M. Calafat, L.L. Needham, C. Rubin, Exposure to bisphenol A from bis-glycidyldimethacrylate–based dental sealants, J. Am. Dental Assoc. 137 (2006) 353–362.
[30] Y.-G. Kang, J.-Y. Kim, J. Kim, P.-J. Won, J.-H. Nam, Release of bisphenol A from resin composite used to bond orthodontic lingual retainers, Am. J. Orthod. Dentofacial Orthop. 140 (2011) 779–789. https://doi.org/10.1016/j.ajodo.2011.04.022.
[31] T.L.L. Berge, G.B. Lygre, S.A. Lie, C.H. Lindh, L. Björkman, Bisphenol A in human saliva and urine before and after treatment with dental polymer-based restorative materials, Eur. J. Oral Sci. 127 (2019) 435–444. https://doi.org/10.1111/eos.12647.
[32] G. Russo, F. Barbato, D.G. Mita, L. Grumetto, Simultaneous determination of fifteen multiclass organic pollutants in human saliva and serum by liquid chromatography–tandem ultraviolet/fluorescence detection: a validated method, Biomed. Chromatogr. 33 (2019). https://doi.org/10.1002/bmc.4427.
[33] T.L.L. Berge, G.B. Lygre, B.A.G. Jönsson, C.H. Lindh, L. Björkman, Bisphenol A concentration in human saliva related to dental polymer-based fillings, Clin. Oral Invest. 21 (2017) 2561–2568. https://doi.org/10.1007/s00784-017-2055-9.
[34] E. Andreasidou, P. Mourouzis, L. Daktylidi, A. Kabir, K.G. Furton, V. Samanidou, A fabric phase sorptive extraction method for the LC-UV determination of bisphenol A and leaching monomers from dental materials in human saliva, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1188 (2022), 123073. https://doi.org/10.1016/j.jchromb.2021.123073.
[35] K.S. Corea-Téllez, P. Bustamante-Montes, M. García-Fábila, M.A. Hernández-Valero, F. Vázquez-Moreno, Estimated risks of water and saliva contamination by phthalate diffusion from plasticized polyvinyl chloride, J. Environ. Health 71 (2008) 34–39. https://doi.org/10.2307/26327739.
[36] J. Messias Gomes, T.F. Araujo Almeida, T. Aparecida Da Silva, Z. de L. Cardeal, H. Costa Menezes, Saliva biomonitoring using LPME-GC/MS method to assess dentistry exposure to plasticizers, Anal. Bioanal. Chem. 412 (2020) 7799–7810. https://doi.org/10.1007/s00216-020-02908-x/Published.
[37] I. Moscoso-Ruiz, Y. Gálvez-Ontiveros, S. Cantarero-Malagón, A. Rivas, A. Zafra-Gómez, Optimization of an ultrasound-assisted extraction method for the determination of parabens and bisphenol homologues in human saliva by liquid chromatography-tandem mass spectrometry, Microchem. J. 175 (2022), 107122. https://doi.org/10.1016/j.microc.2021.107122.
[38] M.L. de Oliveira, B.A. Rocha, V.C. de Oliveira Souza, F. Barbosa, Determination of 17 potential endocrine-disrupting chemicals in human saliva by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry, Talanta 196 (2019) 271–276. https://doi.org/10.1016/j.talanta.2018.12.067.
[39] T.H. van Vu, H.-H. Lim, H.-S. Shin, Determination of 15 biomarkers of endocrine disrupting chemicals in human saliva by gas chromatography–mass spectrometry, Bull. Kor. Chem. Soc. 41 (2020) 424–432. https://doi.org/10.1002/bkcs.11986.
[40] E. Romera-García, N. Caballero-Casero, S. Rubio, Saliva-induced coacervation of inverted aggregates of hexanol for simplifying human biomonitoring: application to the determination of free bisphenols, Talanta 204 (2019) 465–474. https://doi.org/10.1016/j.talanta.2019.06.043.
[41] A. Jouyban, M.A. Farajzadeh, M. Khoubnasabjafari, V. Jouyban-Gharamaleki, M.R. Afshar Mogaddam, Development of deep eutectic solvent based solidification of organic droplets-liquid phase microextraction; Application to determination of some pesticides in farmers saliva and exhaled breath condensate samples, Anal. Methods 11 (2019) 1530–1540. https://doi.org/10.1039/c8ay02279h.
[42] B.A. Buchholz, K.C. Ahn, H. Huang, S.J. Gee, B.J. Stewart, T.J. Ognibene, B.D. Hammock, Pharmacokinetics, metabolite measurement, and biomarker identification of dermal exposure to permethrin using accelerator mass spectrometry, Toxicol. Sci. 183 (2021) 49–59. https://doi.org/10.1093/toxsci/kfab082.
[43] P. Martín Santos, L. Campo, L. Olgiati, E. Polledri, M. del Nogal Sánchez, S. Fustinoni, Development of a method to profile 2- to 4-ring polycyclic aromatic hydrocarbons in saliva samples from smokers and non-smokers by headspace-solid-phase microextraction-gas chromatography-triple quadrupole tandem mass spectrometry, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1152 (2020), 122273. https://doi.org/10.1016/j.jchromb.2020.122273.
[44] J. Peña, M.E. Fernández Laespada, C. García Pinto, J.L. Pérez Pavón, Multiple headspace sampling coupled to a programmed temperature vaporizer – gas chromatograph-mass spectrometer for the determination of polycyclic aromatic hydrocarbons in water and saliva, Microchem. J. 161 (2021), 105822. https://doi.org/10.1016/j.microc.2020.105822.
[45] P. Martin Santos, M. del Nogal Sánchez, J.L. Pérez Pavón, B.M. Cordero, R.V. Fernández, Liquid-liquid extraction-programmed temperature vaporizer-gas chromatography-mass spectrometry for the determination of polycyclic aromatic hydrocarbons in saliva samples. Application to the occupational exposure of firefighters, Talanta 192 (2019) 69–78. https://doi.org/10.1016/j.talanta.2018.09.030.
[46] P. Martín Santos, C. Jiménez Carracedo, M. del Nogal Sánchez, J.L. Pérez Pavón, B. Moreno Cordero, A sensitive and automatic method based on microextraction by packed sorbents for the determination of polycyclic aromatic hydrocarbons in saliva samples, Microchem. J. 152 (2020), 104274. https://doi.org/10.1016/j.microc.2019.104274.
[47] F. Kardani, A. Daneshfar, R. Sahrai, Determination of nicotine, anabasine, and cotinine in urine and saliva samples using single-drop microextraction, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 878 (2010) 2857–2862. https://doi.org/10.1016/j.jchromb.2010.08.041.
[48] H. Kataoka, R. Inoue, K. Yagi, K. Saito, Determination of nicotine, cotinine, and related alkaloids in human urine and saliva by automated in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 108–114. https://doi.org/10.1016/j.jpba.2008.09.044.
[49] A. Kingman, J. Hyman, S.A. Masten, B. Jayaram, C. Smith, F. Eichmiller, M.C. Arnold, P.A. Wong, J.M. Schaeffer, S. Solanki, W.J. Dunn, Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations, J. Am. Dental Assoc. 143 (2012) 1292–1302.
[50] V.B. Michelsen, H.B.M. Kopperud, G.B. Lygre, L. Björkman, E. Jensen, I.S. Kleven, J. Svahn, H. Lygre, Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo, Eur. J. Oral Sci. 120 (2012) 89–95. https://doi.org/10.1111/j.1600-0722.2011.00897.x.
[51] R.D. Singh, R. Gautam, R. Siddhartha, B.P. Singh, P. Chand, V.P. Sharma, S.K. Jurel, High performance liquid chromatographic determination of residual monomer released from heat-cured acrylic resin: an in vivo study,

J. Prosthodont. 22 (2013) 358–361. https://doi.org/10.1111/jopr.12004.
[52] M.R. Moreira, L.G. Matos, I.D. de Souza, T.A.V. Brigante, M.E.C. Queiroz, F.L. Romano, P. Nelson-Filho, M.A.N. Matsumoto, Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography, Am. J. Orthod. Dentofacial Orthop. 151 (2017) 477–483. https://doi.org/10.1016/j.ajodo.2016.07.019.
[53] M.K. Manoj, R. Ramakrishnan, S. Babjee, R. Nasim, High-performance liquid chromatography analysis of salivary bisphenol A levels from light-cured and chemically cured orthodontic adhesives, Am. J. Orthod. Dentofacial Orthop. 154 (2018) 803–808. https://doi.org/10.1016/j.ajodo.2018.02.008.
[54] A. Jouyban, M.A. Farajzadeh, M. Nemati, A.A. Alizadeh Nabil, M.R. Afshar Mogaddam, Preparation of ferrofluid from toner powder and deep eutectic solvent used in air-assisted liquid-liquid microextraction: application in analysis of sixteen polycyclic aromatic hydrocarbons in urine and saliva samples of tobacco smokers, Microchem. J. 154 (2020), 104631. https://doi.org/10.1016/j.microc.2020.104631.
[55] A. Ballesteros-Gómez, S. Rubio, D. Pérez-Bendito, Analytical methods for the determination of bisphenol A in food, J. Chromatogr. A 1216 (2009) 449–469. https://doi.org/10.1016/j.chroma.2008.06.037.
[56] F. Vela-Soria, O. Ballesteros, A. Zafra-Gómez, L. Ballesteros, A. Navalón, A multiclass method for the analysis of endocrine disrupting chemicals in human urine samples. Sample treatment by dispersive liquid-liquid microextraction, Talanta 129 (2014) 209–218. https://doi.org/10.1016/j.talanta.2014.05.016.
[57] I. Lee, Y.J. Park, M.J. Kim, S. Kim, S. Choi, J. Park, Y.H. Cho, S. Hong, J. Yoo, H. Park, G.J. Cheon, K. Choi, M.K. Moon, Associations of urinary concentrations of phthalate metabolites, bisphenol A, and parabens with obesity and diabetes mellitus in a Korean adult population: Korean National Environmental Health Survey (KoNEHS) 2015–2017, Environ. Int. 146 (2021). https://doi.org/10.1016/j.envint.2020.106227.
[58] H. Kataoka, R. Inoue, K. Yagi, K. Saito, Determination of nicotine, cotinine, and related alkaloids in human urine and saliva by automated in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 108–114. https://doi.org/10.1016/j.jpba.2008.09.044.
[59] H. Zhang, N. Zhang, W. Zhou, X. Zeng, X. Wang, M. Zhan, W. Xu, Y. Huang, L. Lu, Z. Li, Y. Gao, Profiles of neonicotinoid insecticides and their metabolites in paired saliva and periodontal blood samples in human from South China: association with oxidative stress markers, Ecotoxicol. Environ. Saf. 212 (2021), 112001. https://doi.org/10.1016/j.ecoenv.2021.112001.
[60] H.M. Koch, A.M. Calafat, Human body burdens of chemicals used in plastic manufacture, Phil. Trans. Biol. Sci. 364 (2009) 2063–2078. https://doi.org/10.1098/rstb.2008.0208.
[61] M. Rezaee, Y. Assadi, M.-R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction, J. Chromatogr. A 1116 (2006) 1–9. https://doi.org/10.1016/j.chroma.2006.03.007.