



Ultrasound assisted solvent extraction of porous membrane-packed samples followed by liquid chromatography-tandem mass spectrometry for determination of BADGE, BFDGE and their derivatives in packed vegetables

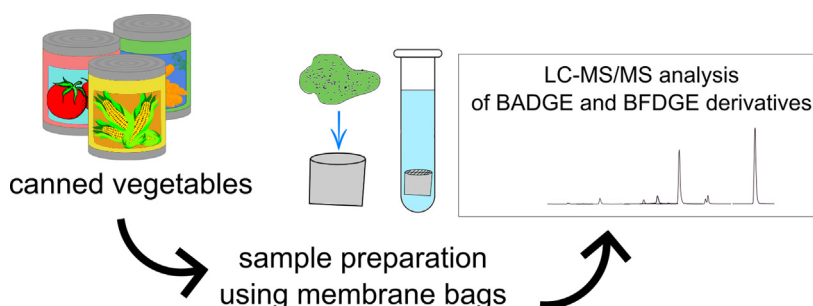
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HIGHLIGHTS

- Novel sample preparation method for determination of endocrine disrupting compounds.
- First use of porous membrane packed sample technique in the food research.
- Determination of BADGE, BFDGE and their derivatives in packaged vegetables.
- Influence of packaging type on the food contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

The problem of the presence of trace organic pollutants in food is of growing importance due to increasing awareness about their impact on newborns, infants and adults of reproductive age. Despite the fact that packaged food products offer many advantages, packaging can be a source of contamination for stored food. Thus, monitoring such pollution in food is of high importance. In this work, a novel methodology based on the solvent extraction of porous membrane-packed samples followed by liquid chromatography-tandem mass spectrometry was applied for the determination of bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives in packed vegetables. Several parameters of the extraction process were optimized, including the volume and type of extraction solvent as well as the sonication time. Due to advantages such as simplicity of use, short analysis time, and a reduction in the required amount solvent, the developed procedure can be considered green. In addition, the developed methodology was characterized by good validation parameters. Limit of quantitation (LOQ) was found to be in the range of 0.8 to 1.5 ng/g. The obtained recoveries varied from 78.3% to 111.2%. The repeatability of the extraction ranged between 0.6% and 5.8% (RSD). The proposed method was successfully applied to determine the presence of BADGE, BFDGE and their derivative compounds in the vegetable samples stored in different types of containers. The obtained data indicate that the majority of investigated samples were contaminated by chlorinated and hydroxyl derivatives of BADGE.

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1. Introduction

In recent years, significant changes have been observed in consumer purchasing preferences. Currently, consumers are increas-

ingly looking for food with long-term shelf-stability and very good quality that also requires little preparation work and low preparation time to prepare a meal (Noureddine et al., 2019). Therefore, an increase in the demand for so-called convenience food and canned/package foods is observed (Scholliers, 2015). Despite the fact that packaged food products offer many advantages, as indicated by numerous data sources, the packaging can unfortunately be a source of contamination for stored food (Sanchis et al., 2017; Szczepańska et al., 2018a). Metallic packaging is commonly coated with a thin polymeric film on the inner surface preventing corrosion and food contamination. The most commonly used materials for coating are epoxy resins or organosols. Epoxy resins are produced from bisphenol A diglycidyl ether (BADGE) and bisphenol A (BPA) or directly from BPA with epichlorohydrin (Grob et al., 2010). Since the polymerization process may be incomplete, there is a high probability that unreacted compounds will be released from the surface of the packaging and will penetrate the food (Arvanitoyannis and Kotsanopoulos, 2014). Issues related to the migration of xenobiotics from packaging surfaces have been of interest to researchers for many years, mainly because many reports have been issued on the subject of the toxic activity of the released compounds. Exposure to such compounds may, therefore, contribute to adverse health effects in consumers. It has been well documented that BADGE and its reaction products show estrogenic and anti-androgenic activity (Gallart-Ayala et al., 2011). Moreover, much information on genotoxic and cytotoxic effects of those chemicals, as well as their developmental and reproductive toxicity, can be found in the literature (Alabi and Rubio, 2014; Xue et al., 2016). Due to possible endocrine disrupting properties, BADGE and its derivative compounds have been regulated in Commission Regulation no. 10/2011 (Szczepańska et al., 2018a). In addition, due to cytotoxic, genotoxic and mutagenic activity, the use of BFDGE has been prohibited in food contact applications in the EU since 2005 (Union, 2005). Considering the above, research must be conducted in order to enable the identification of released compounds and monitor their concentration levels in food samples. In literature, there are many reports confirming the presence of BADGE and its derivative compounds in packaging for meat, fish, vegetables, fruits and beverages (Grob et al., 2006; Sanchis et al., 2017; Szczepańska et al., 2018a). Different methods were applied for the analysis of those compounds in different food samples. Xenobiotics were determined using both gas and liquid chromatography coupled with fluorescence, mass or tandem mass detectors (Sanchis et al., 2017). Before chromatographic separation, a stage of sample preparation involving extraction, purification and concentration of the extracted ingredients must be carried out in order to reduce major interference plausibly present in the sample and to preconcentrate the analytes in order to achieve the desired sensitivity. In the majority of cases, during the sample preparation stage, two classical common extraction techniques are used: extraction with the use of solvent: LSE (liquid solid extraction) and SPE (solid phase extraction). LSE and SPE were applied for the extraction of BADGE and its chlorinated and hydroxy derivatives from vegetables (Leeuwen et al., 2019; Noureddine et al., 2019; Sun et al., 2006), fish (Aristiawan et al., 2015; Miguez et al., 2012; Onekubo and Ayakawa, 2008), fruits (Alabi and Rubio, 2014; Onekubo and Ayakawa, 2008; Zhang et al., 2010) and sauces (Onekubo and Ayakawa, 2008). There is no doubt that these techniques have many advantages, e.g., effective clean-up and good extraction recovery. Unfortunately, in some cases these both of them consume large amounts of toxic organic solvents and require multistep procedures.

The direction followed by modern analytical chemistry postulates that procedures used are to be characterized by appropriate metrological parameters, whereby their environmental noxious impact should be negligible. An increasing amount of information

can be therefore found in the literature concerning new extraction methods characterized by miniaturization, simplification and automation (Namieśnik et al., 2015; Płotka-Wasyłka et al., 2015). Up to this point, many different techniques have been developed and successfully implemented for the extraction of various types of analytes from samples with a complex and often variable matrix composition.

Unfortunately, as of yet, research in the use of novel microextraction techniques for the isolation of BADGE and its derivatives from food samples is still in an early stage. Very few reports of the use of modern sample preparation techniques can be found in the literature. Solid phase microextraction (SPME) has been successfully employed to isolate and enrich BADGE, BFDGE and their derivatives from aqueous canned food (Nerín et al., 2002). In another work, authors used the QuEChERS approach for the isolation and determination of BADGE compounds from milk, milk beverages and yogurts (Cheng et al., 2017). Moreover, molecularly imprinted polymers and supramolecular solvents have been applied for the extraction of these compounds from canned energy drinks (Gallo et al., 2017) and different categories of canned food (Alabi and Rubio, 2014).

Porous membrane protected microsolid-phase extraction (μ -SPE) is one of many green sample preparation techniques. This technique was developed in 2006 as an alternative to multistep SPE. In this technique, small sorbent bags (1–4 cm²) made of a porous membrane filled with a small amount of sorbent are used for the extraction of analytes (Płotka-Wasyłka et al., 2015). The membranes are typically made of polypropylene. The extraction procedure uses a μ -SPE device, as in the case of SPE, which includes the conditioning of the sorbent, extraction of the analytes and desorption into a suitable solvent. Due to the advantages of this technique – including low cost, rapidity, ease of fabrication, efficiency in the extraction of analytes in complex sample matrix, and the use of very small amounts of organic solvents – μ -SPE has been applied for isolation and quantitative determination of a wide spectrum of analytes from environmental (Huang and Kee, 2015; Ling et al., 2016; Nyi et al., 2016), food (Huang et al., 2012; Sajid et al., 2016) and biological samples (Sajid et al., 2017, 2015; Sánchez-gonzález et al., 2016). In 2019, Sajid and coworkers proposed a modification of the previously developed approach consisting of packing a solid sample inside a porous membrane bag which was subject to solvent extraction under ultrasonication. With the applied procedure, it was possible to shorten the time of the analysis (elimination of many sample preparation steps) as well as lower its costs (elimination of the need to use sorbents). Moreover, the procedure presented low LOD values.

Without any doubt, analytical laboratories have an essential role to play in the protection of environmental and human life through monitoring pollutants in air, water or soil as well as toxic and harmful compounds in food products. On the other hand, analytical activities involve the use of many chemicals, thus generating toxic residues. This was the reason for introducing green analytical chemistry (GAC) in 2000. GAC practices are performed to reduce or remove the side effects of analytical practices on operators and the environment. The lack of information regarding the use of modern extraction techniques in research concerning the identification and quantification of BADGEs in food samples became a driving force for undertaking this research. The main objective of this work was to develop a simple sample preparation method for the determination of BADGE and its derivatives in samples of vegetables: green peas, beans, tomatoes, carrots, corn and chickpeas (both fresh and stored in jars, cans and multilayer packages). In this study, vegetable samples were packaged inside porous membrane bags and subjected to solvent extraction under ultrasonication. The applicability of the approach was evaluated by the determination of BADGEs in vegetable samples stored in different types of containers by using HPLC-ESI-MS/MS analysis. To

present the green character of the developed procedures, the Analytical Eco-scale and Green Analytical Procedure Index (GAPI) were applied and discussed. Developed methodologies seem to be eco-friendly given our analysis. To the best of our knowledge, this is the first report where protected membrane bags were employed for the isolation and enrichment of BADGE, BFDGE and their derivatives in food samples.

2. Materials and methods

2.1. Chemicals

All standards investigated in the study were obtained from Sigma-Aldrich (St. Louis, USA): Bisphenol A diglycidyl ether (CAS no. 1675-54-3), bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) ether (CAS no. 227947-06-0), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (CAS no. 76002-91-0), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (CAS no. 13836-48-1), bisphenol A bis (2,3-dihydroxypropyl) ether (CAS no. 5581-32-8), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (CAS no. 4809-35-2), bisphenol F diglycidyl ether (mixture of isomers) (CAS no. 2095-03-06), bisphenol F bis(2,3-dihydroxypropyl) (CAS no. 72406-26-9), ether, bisphenol F bis (3-chloro-2-hydroxypropyl) ether (CAS no. 4809-35-2), bisphenol A dimethacrylate (CAS no. 3253-39-2), and 3-ring novolac glycidyl ether (mixture of isomers) (CAS no. 158163-01-0). Internal standard d_{10} -labeled BADGE was supplied by Cambridge Isotope Laboratories Inc. (UK). Methanol (MeOH) (CAS no. 67-56-1), acetonitrile (CAS no. 75-05-8), ethyl acetate (CAS no. 141-78-6), dichloromethane (CAS 75-09-2), and n-hexane (CAS no. 110-54-3) were of LC-MS hypergrade purity and obtained from Merck KGaA (Darmstadt, Germany). Ammonium formate (CAS no. 540-69-2) was purchased from Sigma-Aldrich (St. Louis, USA). All reagents were of analytical purity grade. Ultrapure water was produced by a Milli-Q Gradient A10 system equipped with an EDS-Pak cartridge (Merck-Millipore, Darmstadt, Germany). Polypropylene (PP) flat membrane sheets (pore size 0.1 μm , wall thickness 100 μm) were obtained from GVS Filter Technology (Roma, Italy).

2.2. Preparation of standard solutions

Individual stock solutions (approximately 0.5 mg/mL) of all analytes were prepared separately by dissolving weighed amounts

of analytical standards in MeOH. A working solution was employed to prepare calibration solutions and spiked samples. All solutions were stored at $-20\text{ }^{\circ}\text{C}$. BADGE d_{10} was used as the internal standard (IS) and prepared separately by dissolving the proper amount to obtain a stock solution at a concentration of 2.5 $\mu\text{g/mL}$.

2.3. Chromatographic conditions

Details of chromatographic analyses are presented in (Szczepańska et al., 2019), although in the electronic [supplementary material](#), we present basic information on the studies performed.

2.4. Samples, sample preparation and spiking procedures

Twenty different vegetable samples packaged in cans, jars and multilayer containers were purchased at local supermarkets in Gdańsk, Poland. The samples were divided into the categories of green peas, beans, tomatoes, carrots, corn and chickpeas. After removal from packages, vegetables were milled (Knife Mill GRINDOMIX GM 200, Retsch, Verder, Poland). To obtain homogeneity, samples were homogenized (3 min at 30,000 rpm, VWR VDI12 homogenizer, VWR International, Poland) and then placed in an ultrasonic bath (10 min, 60 W, HTW). Samples after homogenization and sonication were stored at $-20\text{ }^{\circ}\text{C}$, except those analyzed immediately after preparation.

For the extraction procedure, the membrane bag was prepared by heat-sealing the edges of a PP membrane sheet. One end of bag was kept open to fill the sample. Approximately 0.1 g of sample was placed in the bag, and afterwards the open end was heat-sealed. The dimensions of the bag were 1.5 \times 1.5 cm. The membrane bag was placed in a 10 mL vial and 8 mL MeOH was added. The vial with the bag was placed in an ultrasonic bath (25 min, 60 W). After 25 min passed, the bag was removed from the vial, and the solvent was dried under a gentle nitrogen stream. MeOH (1 mL) was added to reconstitute the analytes. The residue was vortexed, filtered using syringe filters and transferred to chromatographic vials for further analysis. A general scheme of the applied extraction procedure is given in Fig. 1.

To ensure repeatability, accuracy and precision for different kinds of matrices, it was necessary to prepare spiked samples. Fresh beans, peas, tomatoes and carrots were bought at a local

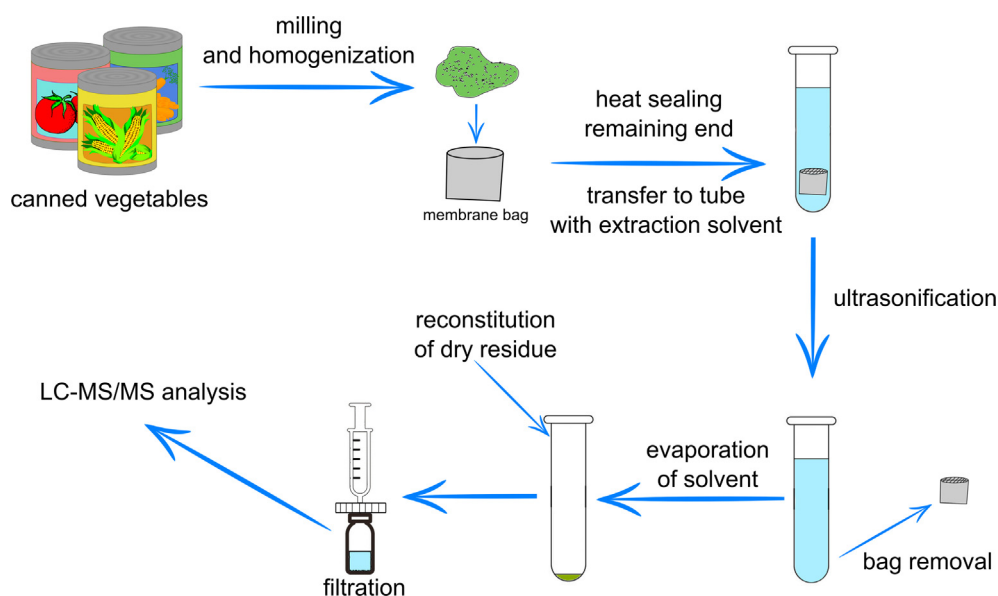


Fig. 1. Schematic presentation of the extraction procedure used.

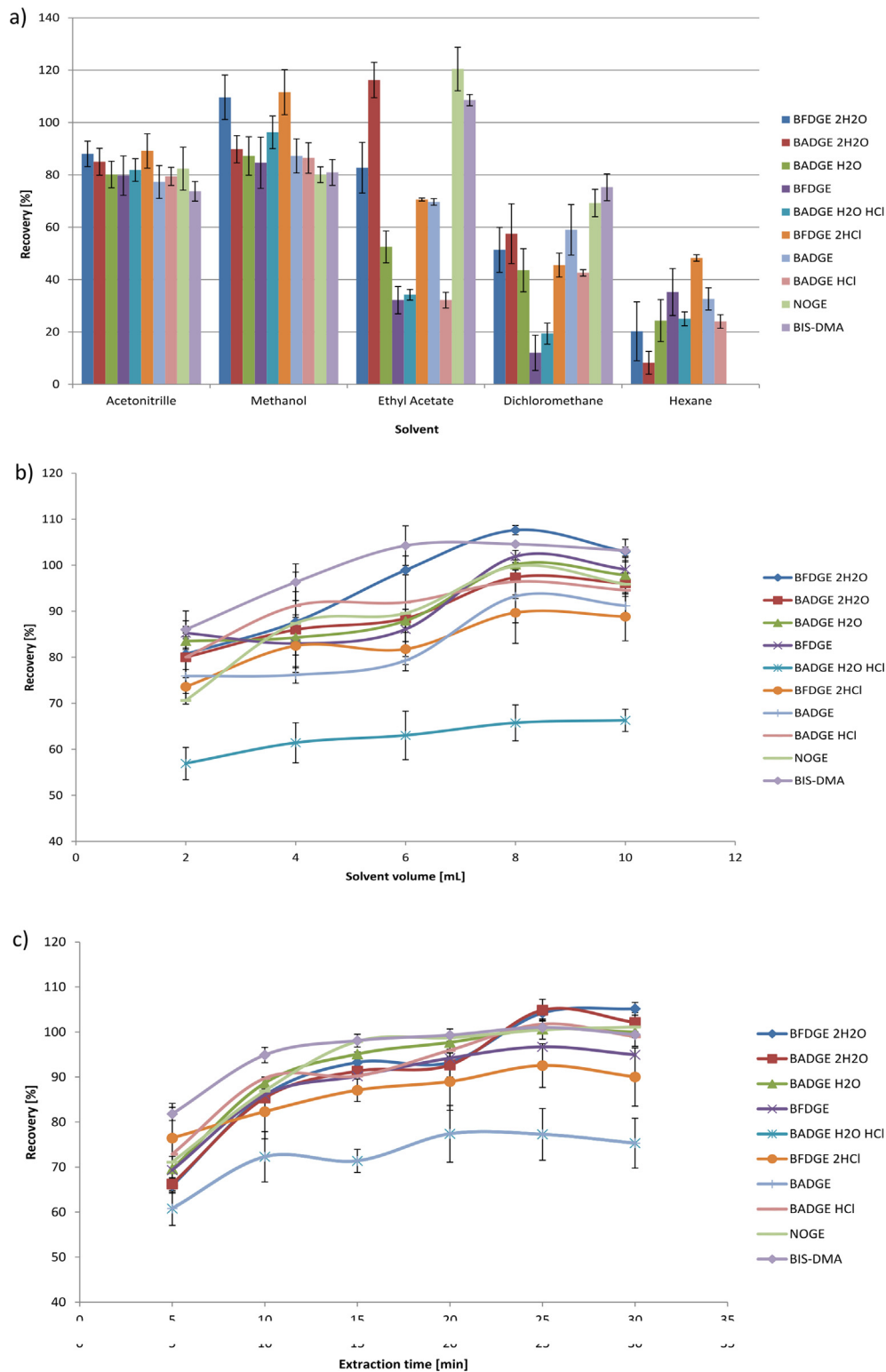


Fig. 2. Graphical presentation of: a) the extraction efficiencies of different solvents, b) solvent volume impact on recovery values, c) extraction time impact on recovery values.

market (Gdańsk, Poland). Approximately 100 g of each vegetable was cooked, milled and homogenized in a similar manner to the samples of packed vegetables. Samples were divided into 10 g aliquot portions and to each of them the working solution was added to obtain different concentrations of particular analytes (0.5, 1, 5, 10, 25, 50, 100 ng/g). These spiked samples were used

to prepare respective calibration curves. In each sample, the IS concentration was kept at 100 ng/g. In a similar manner, spiked samples were made to evaluate accuracy and precision. Spiked samples of cooked vegetables were prepared in the same way as packed vegetables. Fresh calibration samples were prepared for each batch of samples.

Table 1
Values of method linearity, limits of detection (LODs) and quantitation (LOQs) and recoveries.

matrix	analyte	Cal. curve equation $y = ax + b$	S_a	S_b	r	LOD [ng/g]	LOQ [ng/g]	Recovery [%] (RSD)		
								5 [ng/g]	25 [ng/g]	50 [ng/g]
peas	BFDGE-2H ₂ O	$y = 0.001952x + 0.00700$	0.000038	0.00029	0.998	0.49	1.5	86.0 (0.5)	81.9 (0.1)	111.2(1.1)
	BADGE-2H ₂ O	$y = 0.002715x + 0.01112$	0.000046	0.00035	0.998	0.42	1.3	110.3 (2.4)	93.8 (2.8)	112.6 (2.5)
	BADGE-H ₂ O	$y = 0.003027x + 0.00331$	0.000044	0.00033	0.997	0.36	1.1	98.8 (0.7)	89.9 (1.3)	105.6 (1.9)
	BFDGE	$y = 0.001096x + 0.00212$	0.000018	0.00013	0.998	0.40	1.2	85.5 (0.7)	84.1 (0.7)	102.2 (5.7)
	BADGE-H ₂ OHCl	$y = 0.00534x + 0.02170$	0.00011	0.00080	0.997	0.49	1.5	105.2 (2.7)	84.0 (1.7)	83.9 (0.3)
	BFDGE-2HCl	$y = 0.000748x + 0.0018658$	0.000011	0.000084	0.998	0.37	1.1	100.9 (1.7)	97.6 (1.4)	104.9 (4.3)
	BADGE	$y = 0.00736x + 0.0192$	0.00013	0.0010	0.997	0.45	1.3	108.0 (1.9)	98.5 (1.9)	100.5 (4.9)
	BADGE-HCl	$y = 0.001605x + 0.00323$	0.000019	0.00015	0.997	0.30	0.91	106.5 (2.4)	100.8 (2.4)	107.4 (0.5)
	3-Ring NOGE	$y = 0.00700x + 0.03288$	0.00011	0.00085	0.999	0.40	1.2	97.3 (5.6)	95.6 (2.3)	94.2 (2.1)
	BIS-DMA	$y = 0.0862x + 0.0638$	0.0013	0.0095	0.998	0.36	1.1	106.89 (0.04)	100.6 (2.7)	108.1 (4.5)
tomato	BFDGE-2H ₂ O	$y = 0.001058x + 0.001183$	0.000013	0.000097	0.999	0.30	0.91	89.9 (5.9)	84.9 (5.3)	97.1 (1.8)
	BADGE-2H ₂ O	$y = 0.002473x + 0.000155$	0.000030	0.00022	0.999	0.30	0.90	86.5 (4.8)	79.3 (2.1)	91.6 (1.1)
	BADGE-H ₂ O	$y = 0.002473x + 0.000092$	0.000026	0.00020	0.999	0.26	0.78	80.6 (5.7)	84.9 (1.8)	96.2 (2.4)
	BFDGE	$y = 0.001096x + 0.00104$	0.000017	0.00013	0.998	0.38	1.2	80.3 (0.7)	84.0 (1.1)	91.6 (6.3)
	BADGE-H ₂ OHCl	$y = 0.000906x + 0.00855$	0.000017	0.00013	0.998	0.47	1.4	110.3 (1.8)	103.1 (5.9)	99.3 (0.7)
	BFDGE-2HCl	$y = 0.0003288x + 0.000349$	0.0000058	0.000044	0.998	0.44	1.3	109.7 (6.8)	101.4 (5.9)	102.7 (2.9)
	BADGE	$y = 0.00674x - 0.00070$	0.00011	0.00081	0.998	0.39	1.2	78.9 (3.6)	85.7 (1.4)	96.9 (1.9)
	BADGE-HCl	$y = 0.001293x - 0.00043$	0.00017	0.00013	0.998	0.33	0.99	84.1 (3.5)	79.7 (2.8)	92.7 (3.4)
	3-Ring NOGE	$y = 0.005585x - 0.00041$	0.000093	0.00068	0.998	0.41	1.2	86.1 (1.2)	83.1 (2.1)	96.8 (2.4)
	BIS-DMA	$y = 0.06662x - 0.0048$	0.00073	0.0055	0.998	0.27	0.81	80.4 (2.4)	83.1 (0.7)	91.6 (1.6)
bean	BFDGE-2H ₂ O	$y = 0.021108x + 0.00118$	0.000022	0.00016	0.997	0.49	1.47	87.6 (5.6)	82.5 (3.6)	97.9 (2.8)
	BADGE-2H ₂ O	$y = 0.002473x + 0.00015$	0.000029	0.00022	0.999	0.30	0.90	106.8 (5.8)	86.2 (0.3)	95.3 (2.0)
	BADGE-H ₂ O	$y = 0.00273x + 0.00009$	0.000028	0.00020	0.999	0.29	0.86	78.3 (2.9)	80.4 (1.5)	98.9 (2.7)
	BFDGE	$y = 0.001091x + 0.00105$	0.000016	0.00012	0.999	0.36	1.1	82.2 (4.7)	82.7 (2.2)	95.6 (8.5)
	BADGE-H ₂ OHCl	$y = 0.000856x + 0.00842$	0.000015	0.00013	0.998	0.44	1.3	108.4 (5.5)	85.1 (3.6)	84.7 (6.4)
	BFDGE-2HCl	$y = 0.0003199x + 0.000660$	0.0000059	0.000045	0.998	0.46	1.4	99.8 (5.4)	93.9 (0.6)	95.5 (2.8)
	BADGE	$y = 0.00684x - 0.00078$	0.000085	0.00064	0.998	0.31	0.93	78.3 (3.1)	86.4 (2.9)	97.6 (1.9)
	BADGE-HCl	$y = 0.001271x + 0.00044$	0.000020	0.00015	0.998	0.40	1.2	104.9 (0.7)	87.3 (0.8)	100.9 (2.2)
	3-Ring NOGE	$y = 0.005492x - 0.00027$	0.000067	0.00051	0.998	0.31	0.92	80.3 (0.6)	83.7 (3.1)	98.9 (3.3)
	BIS-DMA	$y = 0.06794x - 0.0061$	0.00074	0.0056	0.999	0.27	0.82	85.6 (2.6)	78.6 (1.1)	100.8 (1.2)
carrot	BFDGE-2H ₂ O	$y = 0.001059x + 0.00029$	0.000011	0.00011	0.998	0.34	1.0	82.4 (5.1)	86.3 (2.3)	83.3 (0.2)
	BADGE-2H ₂ O	$y = 0.001817x + 0.00117$	0.000031	0.00023	0.998	0.42	1.3	84.8 (1.7)	85.2 (5.5)	84.0 (1.4)
	BADGE-H ₂ O	$y = 0.002370x - 0.00088$	0.000042	0.00032	0.996	0.44	1.3	81.9 (5.5)	80.7 (0.7)	88.7 (1.8)
	BFDGE	$y = 0.001102x + 0.00104$	0.000011	0.00013	0.998	0.38	1.3	87.6 (0.7)	81.9 (2.8)	88.7 (2.0)
	BADGE-H ₂ OHCl	$y = 0.000887x + 0.00861$	0.000014	0.00011	0.998	0.41	1.2	101.2 (2.8)	95.5 (3.9)	93.2 (1.7)
	BFDGE-2HCl	$y = 0.0003165x + 0.008069$	0.0000059	0.000044	0.997	0.46	1.4	100.9 (1.7)	97.6 (1.4)	104.9 (4.3)
	BADGE	$y = 0.00677x - 0.00074$	0.00011	0.00081	0.998	0.39	1.2	93.8 (5.7)	103.5 (2.3)	106.7 (1.2)
	BADGE-HCl	$y = 0.001379x + 0.00042$	0.000017	0.00013	0.999	0.32	0.97	100.5 (5.5)	96.4 (2.1)	93.9 (1.7)
	3-Ring NOGE	$y = 0.005657x - 0.00042$	0.000091	0.00068	0.999	0.40	1.2	82.2 (0.7)	86.6 (3.8)	96.3 (4.4)
	BIS-DMA	$y = 0.06767x - 0.0058$	0.00071	0.0054	0.999	0.26	0.78	84.8 (0.6)	88.8 (3.9)	91.6 (1.1)

3. Results and discussions

3.1. Extraction conditions

To optimize the extraction process, a series of experiments were carried out using fresh and cooked green pea samples spiked at the level of 50 ng/g (green pea was selected as reference material for the experiments on extraction optimization due to the fact that it has the most complicated matrix in comparison to the rest objects of interest). Such optimized extraction procedure was afterwards checked and used for determination of given analytes in the fortified samples of carrots, beans and tomatoes. Results of repeatability and intermediate precision are presented in the Table S2 (supplementary material). The type of extraction solvent, sonication time and volume of solvent were optimized as parameters that affect extraction efficiency. Each set of experiments was performed in triplicates.

3.1.1. Extraction solvent and volume

Selection of an appropriate extraction solvent is a crucial step to achieve extraction efficiency. The extraction solvent should be selected based on its affinity to target compounds. The selected solvent should effectively desorb analytes from the matrix but should also properly dilate the pores of the PP membrane for effec-

tive transfer of the analytes through the membrane. In this research, five solvents of varying polarity index (acetonitrile, methanol, ethyl acetate, dichloromethane and n-hexane) were investigated as extraction agents. Methanol was found to be the most effective extraction solvent compared to other examined solvents and was selected for further analysis (Fig. 2a). To conduct the extraction step in a reproducible manner, the applied volume should be sufficient enough to completely immerse the membrane bag (Sajid, 2017). On the other hand, the volume of organic solvents used should be reduced as much as possible according to green analytical chemistry principles. Volumes of MeOH were tested in the range of 2–10 mL (Fig. 2b). The highest recovery values of analytes were obtained using 8 mL of MeOH. The use of larger volumes of solvent resulted in slightly lower recovery probably because of the dilution factor. Lower than 6 mL of MeOH resulted in poor reproducibility. Thus, a volume of extraction solvent of 8 mL was selected as the optimum.

3.1.2. Extraction time

Extraction was examined in the range of 5–30 min. Based on the data presented in Fig. 2c, the extraction efficiency increased with increased sonication time. The equilibrium state was achieved at 25 min. Beyond this time, no further increase in response of analytes was observed. Hence, an extraction time of 25 min was selected as the optimal one.

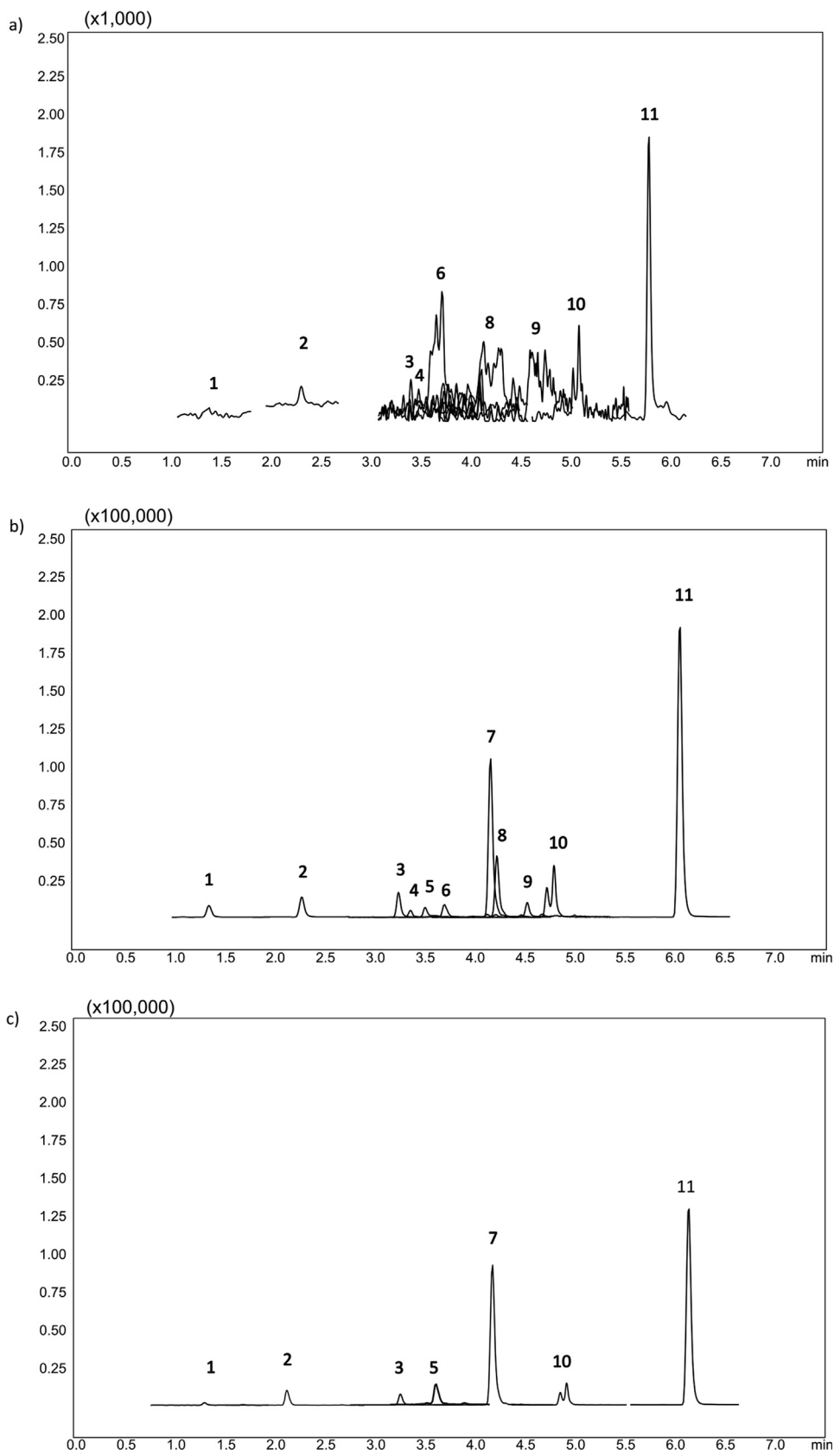


Fig. 3. Chromatogram: blank unspiked, spiked, real samples.

Table 2
Results of quantitative analyses of samples studied.

Vegetable sample	Food container	Analyte [ng/g]						
		BADGE-2H ₂ O	BADGE-H ₂ O	BADGE-H ₂ OHCl	BADGE	BADGE-HCl	3-Ring NOGE	BIS-DMA
peas	Jar	4.6 ± 0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Can 1	14.8 ± 0.7	2.9 ± 0.1	<LOD	<LOD	<LOD	3.1 ± 0.1	<LOD
	Can 2	15.6 ± 0.6	4.9 ± 0.4	30.5 ± 0.1	<LOD	<LOD	14.6 ± 0.7	14.0 ± 0.6
sweet corn	Can 3	30.9 ± 0.1	3.6 ± 0.1	29.5 ± 1.4	<LOD	<LOD	<LOD	<LOD
	Can 1	29.2 ± 1.7	6.4 ± 0.4	23.9 ± 0.2	<LOD	<LOD	3.2 ± 0.1	1.7 ± 0.1
	Can 2	15.6 ± 0.3	4.3 ± 0.5	25.6 ± 1.8	3.8 ± 0.1	<LOD	654.6 ± 33.5*	57.3 ± 3.5
tomato	Can 3	38.1 ± 2.5	12.8 ± 0.6	20.6 ± 0.8	<LOD	<LOD	<LOD	<LOD
	Jar 1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Jar 2	<LOD	<LOD	16.8 ± 0.3	<LOD	<LOD	<LOD	<LOD
bean	Can 1	<LOD	44.6 ± 1.1	55.2 ± 0.6	<LOD	<LOD	<LOD	<LOD
	Can 2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.6 ± 0.2
	Can 3	<LOD	23.7 ± 1.05	52.0 ± 1.6	<LOD	<LOD	<LOD	<LOD
	Multilayer 1	<LOD	<LOD	21.2 ± 0.9	<LOD	<LOD	<LOD	4.1 ± 0.2
	Multilayer 2	<LOD	<LOD	14.0 ± 1.3	<LOD	<LOD	<LOD	<LOD
	Can	74.7 ± 2.8	10.7 ± 1.9	18.8 ± 1.0	<LOD	1.3 ± 0.3	<LOD	<LOD
chickpeas	Can	565.5 ± 21.7*	89.5 ± 3.2	142.6 ± 3.5*	<LOD	5.1 ± 0.1	<LOD	<LOD
	Can	42.0 ± 1.3	4.9 ± 0.6	35.7 ± 1.3	<LOD	2.4 ± 0.4	<LOD	<LOD
carrot	Jar	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.5 ± 0.2
	Can	693.6 ± 48.1*	36.9 ± 2.6	178.0 ± 19.5*	<LOD	2.4 ± 0.5	<LOD	<LOD
carrot	Jar	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.3 ± 0.1
	Can	17.2 ± 2.4	4.1 ± 0.1	30.6 ± 6.2	<LOD	<LOD	89.3 ± 18.7	9.2 ± 1.2

*Original extracts diluted 10 times and re-analyzed.

3.2. Analytical characteristic of the method

The performance of the analytical method was evaluated in terms of linearity, limits of detection (LODs) and quantitation (LOQs), recoveries, precision and accuracy. The obtained results are presented in Table 1. Unspiked blank samples of cooked vegetables were prepared and analyzed. Peaks were reported only for BADGE-H₂O and BADGE-H₂OHCl at their specific retention times; thus, corrections were made in the analysis of real samples, spiked samples and in the calibration data (Fig. 3a). The calibration curves were linear in the studied concentration range between 0.5 and 100 ng/g and the correlation coefficients (*r*) were greater than 0.990 for all compounds. To increase accuracy in the lowest concentration range, a weighing factor 1/*x* was applied to every calibration curve. Quantification and characterization of the analytical method were performed with the use of a matrix-matched calibration curve. The LOD was calculated according to the formula: $LOD = 3 \cdot S_b/a$, where *S_b* is the standard deviation of the intercept of the calibration curve, and *a* is the slope of the calibration curve. The limit of quantitation (LOQ) was defined as $3 \cdot LOD$. LOQ was found to be in the range from 0.8 to 1.5 ng/g. More detailed information on characteristic analytical method parameters is given in Table 1. To assess the recovery of the proposed method, spiked samples at three concentration levels (5, 25 and 50 ng/g) were extracted under optimized conditions. Obtained recoveries varied from 78.3% to 111.2%. Repeatability of the extraction ranged between 0.6% and 5.8% (RSD). Moreover, inter-day precision was evaluated by utilizing six replicates at the abovementioned three concentration levels on three consecutive days. For each sample studied, precision was better than 10% (Table S2).

3.3. Analysis of real samples

The proposed method was successfully applied to determine BADGE, BFDGE and their derivative compounds in the vegetable samples stored in different type of containers. Each experiment was carried out in triplicate. The whole packaging content was

milled and homogenized. Extraction using membrane device was carried out under optimal conditions. The concentration of the analytes in the investigated samples are summarized in Table 2 (only detected compound were shown), and an example of a chromatogram of a real sample is presented in Fig. 3c.

BADGE derivatives (BADGE-H₂O, BADGE-2H₂O, BADGE-H₂OHCl) were detected in all investigated samples (at least one analyte above LOD). The LOD and LOQ values in other studies ranged from 0.15 to 14.92 ng/g and from 0.31 to 30.6 ng/g respectively, while values of LOD and LOQ of this study ranged 0.3–0.5 ng/g and 0.8 – 1.5 ng/g respectively. The concentrations of detected analytes were between 1.6 and 693.6 ng/g for BIS-DMA and BADGE-2H₂O, respectively. Additionally, the samples stored in cans contained significantly higher amount of BADGE derivatives than those stored in jars and multilayer packaging. BFDGE, BFDGE-2H₂O and BFDGE-2HCl were not detected. The obtained data indicated that the majority of investigated samples were contaminated by chlorinated and hydroxyl derivatives of BADGE. Among all the compounds, BADGE-H₂OHCl was found in all canned samples, showing a maximum level of 178.0 ± 19.5 ng/g in samples of chickpeas stored in cans. The presence of this analyte in all samples is probably an effect of the thermal treatment process in canned food preservation (Gallart-Ayala et al., 2011). The mean contamination levels we determined are similar to those reported by other authors (Table 3). BADGE was detected only in canned sweet corn, which may be a result of long storage time and transformation of maternal compounds into hydroxyl derivatives. The absence of BADGE in studied canned samples is most likely due to BADGE transformation into its derivative products (Szczepańska et al., 2019).

3.4. Assessment of the green nature of the new analytical procedure used during studies

The calculations that supply an answer as to whether analytical methodology can be considered eco-friendly should be carried out utilizing tools specifically for such an evaluation. It has become a goal to attempt to develop such a tool that would allow for the

Table 3
Comparison of contamination levels with analytes of interest determined in this research and others.

Analytes	Matrix	Sample preparation method	Amount of sample	Extraction time [min]	Solvent volume	Determination method	LOD LOQ	Recovery [%]	Concentrations determined	Ref.
BADGE, BFDGE and derivatives, NOGE, BIS-DMA	Peas, tomato, bean, carrot, sweet corn, chickpeas	Ultrasound assisted solvent extraction of porous membrane packed samples	0.1 g	25	8 mL MeOH	HP LC-ESI-MS/MS	0.3–0.5 0.8–1.5 [ng/g]	78.3–111.2	1.3 ± 0.3–693.6 ± 48.1 [ng/g]	This study
BADGE-2H ₂ O	Bean, chickpeas, okra	LSE, SPE	0.2 g	780	6 mL MeOH, 10 mL ACN	UPLC-FID	0.26–14.92 0.31–30.6 [µg/kg]	70–120	88.62 ± 4.58–500.21 ± 32.17 [µg/kg]	[1]
BADGE and its derivatives	Sweet corn, red pepper, asparagus	LSE, US	3 g	65	6 mL ethyl acetate	LC-ESI-MS/MS	0.15–8 [µg/kg]	60–90	0.9 ± 0.1–675 ± 100 [µg/kg]	[7]
BADGE and its derivatives	Tomato, asparagus, mushroom	LSE SPE	2–5 g	-	15 mL ACN, 10 mL MeOH, 10 mL petroleum ether,	LC-ESI-MS/MS	0.39–0.69 [ng/g]	69–98	0.8 – 106.4 [ng/g]	[15]
BADGE its and derivatives	Peas, baked bean, mushroom, corn	LSE, SPE	5 g	greater than 50	5 mL ethyl acetate 90 mL ACN, 75 mL hexane, 8 mL MeOH 2 mL ethyl acetate	HP LC-FID	4.5–7.9 13.7–24.1 [µg/kg]	86.07–114.06	0.08–0.4 [mg/kg]	[13]

assessment of as much information as possible about a given analytical methodology, thus illustrating the nature of this procedure. The Analytical Eco-scale Green Analytical Procedure Index (GAPI) is one of the most popular tools used for this purpose (Gałuszka et al., 2012; Plotka-Wasyłka, 2018) and this methodology, being semiquantitative one, has been applied to evaluate greenness of the developed procedure.

The Analytical Eco-scale tool is based on assigning penalty points (PPTs) to parameters of analytical methodologies (amount of reagents, hazards, energy and waste) that are not in agreement with an ideal green analysis (electronic supplementary Table 2) (Gałuszka et al., 2012; Nations United, 2011; Raynie and Driver, 2009). The fundamental idea of the Analytical Eco-scale is that the ideal green analysis has a value of 100; thus, the closer an obtained value is to this score, the greener the procedure. The sum of PPTs for the whole evaluated procedure is subtracted from the ideal score of 100 to obtain the Analytical Eco-Scale score. The GAPI tool presents information on the entire analytical protocol in a pictorial form from sampling, through sample preparation, to final determination.

Penalty points for the developed procedure have been calculated in accordance with the principles presented in Table S3. Thus, considering PPTs (Table 4) given for the described procedure for analysis of vegetable samples stored in different type of containers, (11 PPTs), it can be assumed that the developed methodology represents a green analysis. The results of this assessment were partially confirmed based on the analysis of GAPI pictogram (Table 4). However, the GAPI pictogram shows that the procedure has some critical points (potential for improvement includes area of the off-line analysis, the use of nongreen solvents, and moderate waste generation).

4. Conclusions

The results of the studies described in this paper include new, simple and cost-effective sample preparation methods for the determination of BADGE, BFDGE and their derivatives in packed vegetables. To the best of our knowledge, it is the first scientific report in the field of food analysis involving the use of the µ-SPE modification method based on placing the sample inside a porous membrane bag in the extraction step. To obtain high performance extraction, different parameters, such as the type of organic solvent, volume of solvent and sonication time, were optimized. In comparison to results obtained in other studies presented in the literature (Table 3), this methodology presents lower LOD, as low as 0.3 ng/g, for the selected analytes. Further, the time and amount of organic solvent required during sample preparation was significantly reduced, which contributed to an increase in the green character of the proposed approach, as confirmed by the Analytical Eco-scale tool. Low values of LOQ, high recoveries and good repeatability of results make this method suitable for food quality and safety control studies. The developed methodology was successfully applied in the process of determining the presence of BADGE, BFDGE and their derivatives in vegetable samples stored in different types of containers.

The obtained results justify the statement that a low intake of BADGE and its derivatives can be derived from canned vegetables. Synergistic behavior was detected for these compounds, especially at low concentration levels (Szczepańska et al., 2018b). It seems necessary to monitor the concentration of these compounds in food in order to accurately assess health risks and model transformation pathways, thus allowing for the correlation of the content of primary and secondary pollution in food (Szczepańska et al., 2019).

Table 4

Penalty points (PPts) and GAPI given for the developed procedures.

Analytical Eco-scale assessment		GAPI
Reagents	PPts	
Methanol	4	
Ammonium formate	2	
Σ 6		
Instruments	PPts	
Transport	1	
HPLC-ESI-MS/MS	2	
Occupational hazard	0	
Waste	2	
Σ 5		
Total PPts: 11 Score: 89		

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.135178>.

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