frontiers | Frontiers in Sustainable Food Systems

TYPE Methods
PUBLISHED 13 June 2023
DOI 10.3389/fsufs.2023.1159002

OPEN ACCESS

EDITED BY
Milena Corredig,
Aarhus University, Denmark

REVIEWED BY
Fátima Poças,
Escola Superior de Biotecnologia - Universidade Católica Portuguesa, Portugal
Olivier Vitrac,
INRA Centre Versailles-Grignon, France

*CORRESPONDENCE
Chrysoula Kanakaki
✉ ckanakaki@chem.uoa.gr
Nikolaos S. Thomaidis
✉ ntho@chem.uoa.gr

RECEIVED 04 February 2023
ACCEPTED 18 May 2023
PUBLISHED 13 June 2023

CITATION
Kanakaki C, Traka T and Thomaidis NS (2023) Development and validation of multi-analyte methods for the determination of migrating substances from plastic food contact materials by GC-EI-QqQ-MS and GC-APCI-QTOF-MS. *Front. Sustain. Food Syst.* 7:1159002. doi: 10.3389/fsufs.2023.1159002

Development and validation of multi-analyte methods for the determination of migrating substances from plastic food contact materials by GC-EI-QqQ-MS and GC-APCI-QTOF-MS

Chrysoula Kanakaki*, Theodora Traka and Nikolaos S. Thomaidis*

Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Athens, Greece

The packaging has proven to be a source of some serious food contaminants, with several chemicals migrating from the food contact material into the food. Therefore, efficient means of control of the migration extend and identification of the migrating substances must be established. The necessity of migration tests has been underlined by the European Union (EU) Regulation No. 10/2011, requiring the evaluation of the presence of intentionally and non-intentionally added substances. To facilitate this purpose, highly sensitive, multi-analyte methods are required. Targeting a wide range of volatile migrating compounds, we developed and validated a GC-EI-QqQ-MS and a GC-APCI-QTOF-MS method for the simultaneous determination of 131 and 126 food packaging substances, respectively. Even though the GC-EI-QqQ-MS method presented increased sensitivity for several of the investigated compounds, covering the existing EU requirements and specific migration limits (SMLs) for all targeted analytes; the complementary high-resolution method inherently enables the possibility of further utilization of the obtained raw data among others for retrospective analysis. The applicability of both methods was tested using 95% v/v aqueous ethanol food simulant, representative for the worst foreseeable conditions of intended use for many food contact material applications. Real food packaging samples belonging to different types of plastic materials were tested toward chemical migration, utilizing different migration protocols, depending on the sample characteristics. The favorable analytical features of both methods enable their use for the direct analysis of the investigated food simulant, overcoming the need for sample preparation. Thus, labor intensive and/or time-consuming pre-concentration procedures, which would furthermore restrict the applicability of the methods to certain groups of analytes and add to the uncertainty of the overall results, could be eliminated.

KEYWORDS

food contact materials, IAS, NIAS, GC-EI-QqQ-MS, GC-APCI-QTOF-MS

1. Introduction

The packaging has become an indispensable element in the food manufacturing process, as it protects the food, e.g., from light, foreign aroma compounds and microbial contaminations; improving the self-life and stability of products during storage. However, it has been found to represent a serious source of contamination itself through the migration of substances from the packaging into food. The identification and control of these substances is an issue that deserves great attention to assure food quality and consumer safety.

Many of the substances leaching into the food are intentionally added (IAS) to the packaging materials, in order to enhance their properties (Lahimer et al., 2017; Hahladakis et al., 2018). Nevertheless, non-intentionally added substances (NIAS) can also be present at the food packaging materials, with impurities, side or break-down products and process contaminants being the main sources of such compounds. When these substances find their way to the food, unexpected changes in its composition may occur (Muncke, 2009). Therefore, European regulations have been established, dictating the control of FCMs against chemical migration. In particular, European Regulation (EC) 1935/2004 (European Commission, 2004) underlines that any material or article intended to come into contact either directly or indirectly with food must be sufficiently inert to preclude substances from being transferred to food in quantities large enough to endanger human health; or to bring about unacceptable changes in the composition of the food or a deterioration in its organoleptic properties. Additionally, the European Commission (EC) has established with the European Union (EU) Regulation No. 10/2011 (European Commission, 2011) a positive list of more than 1,000 substances or mixtures that are allowed to be used as additives in plastic food contact materials (FCM), providing specific migration limits (SMLs) or other restrictions for their application. However, about half of these substances are not commercially available, and when it comes to their identification, only a limited number of analytical standard methods exist, focusing mainly on a limited number of substances (Silva et al., 2006). The restricted availability of validated multi-analyte methods can be attributed, among others, to the many existing chemical classes of IAS and NIAS, the lack of analytical standards and the fact that many of these substances are not included in chemical or spectral databases, with further issues being extensively discussed in a recent publication (Tsochatzis et al., 2020).

Concerning the analysis of migrant substances from plastic FCMs, several works have been published in the recent analytical literature, employing different hyphenated analytical techniques (Wrona and Nerín, 2020). Some of the most significant contributions in the field of FCMs are the determination of 41 food contact related contaminants in fatty food by HPLC-MS/MS (Vavrouš et al., 2019); the determination of 48 contaminant residues in by microwave-assisted extraction and UPLC-Q Orbitrap HRMS (Zhang et al., 2018) and the determination of 63 photoinitiators and amine synergists by QuEChERS and UPLC-MS/MS (Jung and Simat, 2014). Respectively, on the side of volatile substances, 60 migrant substances were simultaneously analyzed by vortex-assisted liquid–liquid extraction (VA-LLE) followed by GC-Q-Orbitrap HRMS (Miralles et al., 2021); while 84 migrants were determined by LLE-GC–MS (Tsochatzis et al., 2020) and 75 migrants by salt-assisted liquid–liquid extraction (SALLE)-GC–MS/MS (Tsochatzis et al., 2021).

With the wide range of substances potentially migrating from the FCM, the selection of a universal sample preparation procedure for the extraction and pre-concentration of all migrant species present at a FCM sample seems to be an almost unlikely task. Therefore, highly sensitive analytical techniques, in combination with wide-scope sample preparation protocols, need to be applied in the controls of food packaging materials. When the targeted analytes involve exclusively IAS, or some few NIAS compounds, such protocols can be established. However, there are always matrix effects and selectivity restrictions that reduce the applicability of such protocols to different groups of migrating substances. Particularly, when further NIAS compounds need to be introduced into such screening methods, problems related to the sample preparation need to be eliminated. Since these limitations cannot be easily overcome, studies on NIAS identification have so far been restricted to a small number of substances (Pack et al., 2021; Yusà et al., 2021) or have been based on non-target screening approaches (Sapozhnikova et al., 2021; Canellas et al., 2022; Tisler and Christensen, 2022). Therefore, the direct analysis of the food simulant appears to be the best practice, whenever applicable, for the development of wide-scope methodologies.

In order to enhance further the group of target IAS and NIAS migrants that can be analyzed simultaneously, we developed and validated a GC-EI-QqQ-MS (Gas Chromatography-Electron Ionization-Triple Quadrupole Mass Spectrometry) and a GC-APCI-QTOF-MS (Gas Chromatography-Atmospheric Pressure Chemical Ionization-Quadrupole-Time-of-Flight Mass Spectrometry) method for the direct analysis of 95% v/v aqueous ethanol extracts. This high organic content food simulant was selected in order to represent the worst-case scenario of migration for many FCM applications. Direct analysis of this simulant utilizing techniques of high sensitivity liberates us from the need for laborious and/or time-consuming sample preparation methods that would pose an additional source of uncertainty to the overall results.

2. Materials and equipment

2.1. Chemicals

For the applied food simulant (95% v/v aqueous ethanol) and solutions, ethanol of Chromasolv grade purity was purchased from Merck (Darmstadt, Germany), while Ultrapure water (18.2 MΩ) was obtained by a Milli-Q purification system (Millipore Direct-Q UV, Bedford, MA, United States). All the analytical standards were obtained either from Sigma-Aldrich (Steinheim, Germany) or from TCI Chemicals (Tokyo, Japan). All relevant information regarding the target substances are presented in Table 1, including FCM numbers extracted from the positive list of the EU Regulation No. 10/2011 (European Commission, 2011), CAS numbers, molecular formulas, purity (as stated by the supplier) and Cramer Class (provided by the toxtree software). To the group of IAS investigated, several NIAS were added, due to their previously established presence in migration experiments (e.g., as degradation products of certain substances intentionally added to FCMs) (Zhiqing et al., 2016; García Ibarra et al., 2019; Tsochatzis et al., 2020) and the associated interest in their assessment (Silano et al., 2019).

10.3389/fsufs.2023.1159002

TABLE 1 Characteristics of the analyzed substances.

No.	Substance name	CAS no.	Purity	FCM no.*	Cramer class	Molecular formula
1	Ethyl methacrylate	97-63-2	>99%	181	I	C6H10O2
2	Butyl acetate	123-86-4	>99%	300	–	C6H12O2
3	tert-Butyl methacrylate	585-07-9	>98%	342	I	C8H14O2
4	Allyl methacrylate	96-05-9	>99%	175	II	C7H10O2
5	Styrene	100-42-5	>99%	193	I	C8H8
6	n-Butyl acrylate	141-32-2	>99%	325	I	C7H12O2
7	Triethylphosphite	122-52-1	>98%	293	III	C6H15O3P
8	α-Pinene	80-56-8	>98%	155	I	C10H16
9	Isobutyl methacrylate	97-86-9	>98%	183	I	C8H14O2
10	2-hydroxyethyl acrylate	818-61-1	>95%	371	–	C5H8O3
11	Vinyltriethoxysilane	78-08-0	≥98%	142	III	C8H18O3Si
12	Dimethyl isophthalate	1459-93-4	>99%	420	I	C10H10O4
13	Benzaldehyde	100-52-7	>98%	195	I	C7H6O
14	Butyl methacrylate	97-88-1	>99%	184	–	C8H14O2
15	β-Pinene	18172-67-3	>94%	–	I	C10H16
16	Aniline	62-53-3	>98%	–	III	C6H7N
17	Alpha-methylstyrene	98-83-9	>99%	187	I	C9H10
18	Butyl lactate	138-22-7	>98%	322	–	C7H14O3
19	2-Hydroxyethyl methacrylate	868-77-9	>95%	374	I	C6H10O3
20	1,4-Dichlorobenzene	106-46-7	>99%	217	III	C6H4Cl2
21	2-Ethyl-1-hexanol	104-76-7	>99.5%	209	I	C8H18O
22	Benzyl alcohol	100-51-6	>99%	194	I	C7H8O
23	N-nitroso-N-methylaniline	614-00-6	>98%	–	III	C7H8N2O
24	Glycidyl methacrylate	106-91-2	>95%	220	III	C7H10O3
25	Acetylbenzene	98-86-2	>98%	–	I	C8H8O
26	4-Methylphenol	106-44-5	>99%	216	I	C7H8O
27	Methyl benzoate	93-58-3	≥99%	171	I	C8H8O2
28	Nonyl aldehyde	124-19-6	>95%	–	I	C9H18O
29	Divinylbenzene	1321-74-0	>50%	405	I	C10H10
30	ε-Caprolactone	502-44-3	>99%	342	I	C6H10O2
31	Camphor	76-22-2	≥95%	136	III	C10H16O
32	2,6-Dimethylaniline	87-62-7	>99%	–	III	C8H11N
33	Ethyl benzoate	93-89-0	>99%	172	I	C9H10O2
34	Tributylamine	102-82-9	>98%	–	I	C12H27N
35	Diethylene glycol monobutyl ether	112-34-5	>99%	–	I	C8H18O3
36	Methyl salicylate	119-36-8	>99%	284	–	C8H8O3
37	Triethylene glycol	112-27-6	>99%	266	I	C6H14O4
38	Cyclohexyl methacrylate	101-43-9	>98%	197	I	C10H16O2
39	4-Methoxyphenol	150-76-5	>99%	–	I	C7H8O2
40	2-Ethylhexyl acrylate	103-11-7	>99%	206	I	C11H20O2
41	1-Phenyl-2-butanone	1007-32-5	>95%	–	–	C10H12O
42	Phenyl methacrylate	2177-70-0	>97%	439	–	C10H10O2
43	Butylated hydroxytoluene	128-37-0	>99%	315	–	C15H24O

(Continued)

TABLE 1 (Continued)

No.	Substance name	CAS no.	Purity	FCM no.*	Cramer class	Molecular formula
44	Propyl benzoate	2315-68-6	>99%	441	I	C10H12O2
45	2-Methoxy-5-methylaniline	120-71-8	>98%	–	I	C8H11NO
46	ε-Caprolactam	105-60-2	>99%	212	III	C6H11NO
47	n-Octyl acrylate	2499-59-4	>98%	448	–	C11H20O2
48	2-Hydroxy-2-methylpropiophenone	7473-98-5	>96%	–	I	C10H12O2
49	4-Tert-butylphenol	98–54-4	>98%	186	I	C10H14O
50	1,3-Diaminobenzene	108-45-2	>98%	236	III	C6H8N2
51	Ethylene glycol dimethacrylate	97-90-5	>97%	185	–	C10H14O4
52	N,N-dibutylformamide	761-65-9	>98%	–	III	C9H19NO
53	2,4,6-Trimethylbenzaldehyde	487-68-3	>95%	–	I	C10H12O
54	Benzyl methacrylate	2495-37-6	>98%	447	I	C11H12O2
55	Glycerol triacetate	102-76-1	>99%	–	I	C9H14O6
56	Toluene 2,4 diisocyanate	584-84-9	>98%	354	III	C9H6N2O2
57	Diethylene glycol monobutyl ether acetate	124-17-4	>98%	–	I	C10H20O4
58	Biphenyl	92-52-4	>99%	–	III	C12H10
59	5-Chloro-2-methoxyaniline	95-03-4	>98%	–	III	C7H8ClNO
60	3-(Trimethoxysilyl) propyl methacrylate	2530-85-0	>98%	788	III	C10H20O5Si
61	4-(Methylthio) benzaldehyde	3446-89-7	>97%	–	I	C8H8OS
62	Methyl- 4-hydroxybenzoate	99-76-3	>99%	189	–	C8H8O3
63	2,6-Di-tert-butyl-1,4-benzoquinone	719-22-2	>98%	–	II	C14H20O2
64	Dimethyl terephthalate	120-61-6	>99%	288	I	C10H10O4
65	2,4-Di-tert-butyl-phenol	96-76-4	>97%	–	I	C14H22O
66	Methyl laurate	111-82-0	>98%	–	I	C13H26O2
67	Ethyl 4-hydroxybenzoate	120-47-8	>99%	287	I	C9H10O3
68	1,1-Diphenylethylene	530-48-3	>98%	–	III	C14H12
69	Dibutyl maleate	105-76-0	>95%	–	I	C12H20O4
70	Tetramethylene glycol dimethacrylate	2082-81-7	>97%	434	I	C12H18O4
71	Lauric acid	143-07-7	>98%	330	I	C12H24O2
72	Vinyl laurate	2146-71-6	>99%	436	–	C14H26O2
73	2-Naphthylamine	91-59-8	>95%	–	III	C10H7NH2
74	1,6-Bis (acryloyloxy) hexane	13048-33-4	>85%	–	I	C12H18O4
75	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	>98.5%	497	I	C16H30O4
76	4,4′-Difluorobenzophenonen	345-92-6	>99%	337	I	C13H8F2O
77	Diethyl phtalate	84-66-2	>99%	–	I	C12H14O4
78	2-Aminobiphenyl	90-41-5	>99%	–	III	C12H11N
79	Propyl-4-hydroxybenzoate	94-13-3	≥99%	173		C10H12O3
80	Diphenylmethanol	91-01-0	>99%	–	III	C13H12O
81	Benzophenone	119-61-9	>99%	286		C13H10O
82	Triethyl citrate	77-93-0	≥99%	140	III	C12H20O7
83	4-Methoxy phenyl isocyanate	5416-93-3	>98%	–	–	C8H7NO2
84	Dodecyl acrylate	2156-97-0	>98%	437	–	C15H28O2

(Continued)

10.3389/fsufs.2023.1159002

TABLE 1 (Continued)

No.	Substance name	CAS no.	Purity	FCM no.*	Cramer class	Molecular formula
85	1-Hydroxycyclohexyl-phenyl-ketone	947-19-3	>98%	–	I	C13H16O2
86	Ethyl 4-dimethylaminobenzoate	10287-53-3	>98%	–	I	C11H15NO2
87	Trans-1,2-diphenylethylene	103-30-0	>98%	–	III	C14H12
88	2-Hydroxybenzophenone	117-99-7	>95%	–	III	C13H10O2
89	Diallyl phthalate	131-17-9	>98%	316	II	C14H14O4
90	4-Aminobiphenyl	92-67-1	>98%	–	III	C12H11N
91	4-Methylbenzophenone	134-84-9	>95%	–	III	C14H12O
92	3,5-Ditert-butyl-4-hydroxybenzaldehyde	1620-98-0	>98%	–	II	C15H22O2
93	2-n-Octyl-4-isothiazolin-3-one	26530-20-1	>98%	–	III	C11H19NOS
94	Diisobutyl phthalate	84-69-5	>98%	–	I	C16H22O4
95	4-Cumylphenol	599-64-4	>98%	358	III	C15H16O
96	2,2-Dimethoxy-2-phenyl acetophenone	24650-42-8	>98%	–	III	C16H16O3
97	Hexadecyltrimethylammonium bromide	57-09-0	≥98%	104	III	C19H42BrN
98	Hexadecanoic acid	57-10-3	>99.5%	105	I	C16H32O2
99	Methyl 3-(3,5-di-tertbutyl-4-hydroxyphenyl) propanoate	6386-38-5	>98%	–	–	C18H28O3
100	Methyl-2-benzoylbenzoate	606-28-0	>98%	–	III	C15H12O3
101	Dibutyl phthalate	84-74-2	≥97%	157	I	C16H22O4
102	Trimethylolpropane trimethacrylate	3290-92-4	>90%	463	I	C18H26O6
103	2,2-Bis (4-hydroxyphenyl) hexafluoropropane	1478-61-1	>98%	–	III	C15H10F6O2
104	2,4-Dihydroxybenzophenone	131-56-6	>98%	318	I	C13H10O3
105	4,4′-Ethylidenebisphenol	2081-08-5	>98%	–	III	C14H14O2
106	Dibutyl sebacate	109-43-3	>98%	242	I	C18H34O4
107	Ethyl 2-cyano-3,3-diphenylacrylate	5232-99-5	>98%	487	III	C18H15NO2
108	N-(2-hydroxyethyl) phthalimide	3891-07-4	>98%	–	III	C10H9NO3
109	4,4′-Dichlorophenyl sulphone	80-07-9	≥98%	152	III	C12H8Cl2O2S
110	Tri-n-butyl acetyl citrate	77-90-7	≥97%	138		C20H34O8
111	Isophorone diisocyanate	4098-71-9	>99%	475	III	C12H18N2O2
112	4-(Dimethylamino) benzophenone	530-44-9	>98%	–	III	C15H15NO
113	2-Ethylhexyl-4- dimethylamino benzoate	21245-02-3	>98%	–	I	C17H27NO2
114	2,2-Bis (4-hydroxy-3-methylphenyl) propane	79-97-0	>98%	–	III	C17H20O2
115	o-Toluene-azo-o-toluidine	97-56-3	>97%	–	III	C14H15N3
116	2-Methyl-4′-(methylthio)-2-morpholinopropiophenone	71868-10-5	>98%	–	III	C15H21NO2S
117	Benzyl butyl phthalate	85-68-7	≥97%	159	I	C19H20O4
118	Bis (2,6-diisopropylphenyl)-carbodiimide	2162-74-5	>98%	438	–	C25H34N2
119	Bis (2-ethylhexyl) adipate	103-23-1	>98%	207	I	C22H42O4
120	Butyl stearate	123-95-5	>97%	301	–	C22H44O2
121	2,2′-methylene bis (4-methyl-6-tert-butylphenol)	119-47-1	>99%	285	III	C23H32O2

(Continued)

TABLE 1 (Continued)

No.	Substance name	CAS no.	Purity	FCM no.*	Cramer class	Molecular formula
122	4-Phenylbenzophenone	2128-93-0	>98%	–	III	C19H14O
123	4,4′-Methylene-bis (2-chloroaniline)	101-14-4	>90%	–	III	C13H12Cl2N2
124	2,2′-methylene bis(4-ethyl-6-tert-butylphenol)	88-24-4	>98%	163	III	C25H36O2
125	Bis (2-ethylhexyl) phthalate	117-81-7	>98%	283	I	C24H38O4
126	Diphenyl phthalate	84-62-8	>98%	–	I	C20H14O4
127	1,1-Bis (4-hydroxyphenyl) cyclohexane	843-55-0	>98%	–	III	C18H20O2
128	2-Ethylhexyl 2-cyano-3,3-diphenylacrylate	6197-30-4	>98%	492	III	C24H27NO2
129	Bis (2-ethylhexyl) terephthalate	6422-86-2	≥97%	798	I	C24H38O4
130	2-Hydroxy-4-n-octyloxybenzophenone	1843-05-6	>98%	431	–	C21H26O3
131	Erucamide (cis-13-docosenamide)	112-84-5	>85%	271	III	C22H43NO
IS	3-(4-Isopropylphenyl) isobutyraldehyde	103-95-7	>92%	–	–	C13H18O

*According to Table 1 of Annex I of Reg. (EU) No. 10/2011 (European Commission, 2011).

2.2. Preparation of standard solutions

For each analyte and internal standard (IS), stock solutions containing 10 mg mL^{-1} were prepared, using ethanol as solvent. Appropriate working solution mixtures were prepared by diluting the stock standard solutions with ethanol. All stock solutions were stored at −18°C, while working solutions were stored at 4°C and prepared on a weekly basis. In order to prevent any light-induced degradation or isomeric conversion of the substances, all standard solutions were prepared and stored in amber vials.

2.3. Analysis of real FCM samples

The applicability of the developed methods to real samples was tested using 6 FCM products provided by EU plastic producers. Before the analysis, all samples were stored in wrapped aluminum foil at room temperature (20 ± 5°C). The samples tested were general purpose FCM materials and the migration experiments were performed according to the type of each material, using the 95% ethanol simulant for the prediction of migration under a worst-case scenario. For the flexible film samples, pouches were prepared, using a 2 dm^2 material surface in contact with 100 mL food simulant. For the bottles and trays, total immersion experiments were performed, using parts of the samples that were previously cut into rectangular pieces (approximately 0.2 dm^2; 4 × 5 cm). The time and temperature conditions applied were in compliance with the Regulation (EU) No. 10/2011 (European Commission, 2011). All relevant information concerning the material types, the type of migration experiment, the amount of food simulant and the contact time and temperature, are presented in Table 2.

2.4. GC-EI-QqQ-MS

The GC-EI-QqQ-MS system used consisted of a Bruker 456 GC, a CTC PAL3 RSI Autosampler and a Triple Quadruple mass spectrometer (EVOQ-GC TQ, Bruker Daltonics) with an EI source. Single taper liners and a Restek Rxi-5Sil MS column of 30 m (0.25 mm i.d. × 0.25 µm film thickness) were used, with helium as carrier gas, at a constant flow rate of 1.5 mL min^{-1}. The injection volume was 1 µL and pulsed pressure splitless injection was applied, in order to improve the sensitivity and reproducibility of the analysis of ethanolic extracts. The pulse pressure was set at 2.76 bar, with a pulse duration of 0.8 min, while the spitless purge valve was activated 1 min after injection. The GC oven was programmed as follow: 55°C initial hold for 3 min, increased at a rate of 15°C min^{-1} to 180°C, then increase with a step of 6.5°C min^{-1} to 280°C and hold for 5 min, followed by an increase of 10°C min^{-1} to 300°C and hold for 3 min. The injector port, transfer line and MS source temperature was maintained at 280, 290, and 250°C, respectively.

In order to be able to proceed with the method development for the target screening, all analytes investigated were incorporated into a database, containing all the required information, such as retention time, precursor ions, etc. The method development started analyzing small groups of the compounds in Full Scan mode and identifying their retention times and most abundant, as well as characteristic, fragment ions. Then, Product Scans were performed at different collision energies. After careful control of the provided data, the most abundant product ions for every compound and for every parent ion and collision energy were selected, creating the Multiple Reaction Monitoring (MRM) method. However, for some analytes with relatively small molecular weight, the additional fragmentation step was not applicable. Consequently, the Single Ion Monitoring (SIM) scan mode was also employed, where no MRMs could be established. Thus, the final method was a combination of SIM and MRM segments (Table 3). This method was able to simultaneously monitor 131 IAS and NIAS (Figure 1) at the low ppb level. Since, for 111 out of the 131 compounds investigated, characteristic MRMs were applied, for the majority of the analytes there was no requirement for peak resolution, reducing significantly the analysis time.

2.5. GC-APCI-QTOF-MS

The GC-APCI-QTOF system consisted of a Bruker 456 GC, a CP-8400 Autosampler and a hybrid quadrupole time of flight mass

TABLE 2 Description of the analyzed FCM samples, type of migration experiment, the applied volume of food simulant (95% v/v aqueous EtOH), and the contact time and temperature conditions.

Sample code	Material type	Migration experiment	Amount of food simulant* (mL)	Time–temperature conditions
S1A	Multilayer PET/PE film	Pouch, 2 dm^2	100	60°C × 10 d
S1B	Multilayer PP copolymer film	Pouch, 2 dm^2	100	60°C × 10 d
S1C	Multilayer BOPP copolymer film	Pouch, 2 dm^2	100	60°C × 10 d
S2	Monolayer PET bottle	Immersion, 1 dm^2	100	60°C × 10 d
S3A	Monolayer PET tray	Immersion, 1 dm^2	100	60°C × 10 d
S3B	Monolayer PET tray	Immersion, 1 dm^2	100	60°C × 10 d

*For all generic use plastic FCMs investigated, 95% v/v aqueous EtOH was used, simulating a worst-case scenario of chemical migration.

spectrometer (QTOF-MS) (Maxis Impact, Bruker Daltonics) with an APCI ion source. An equivalent analytical column and single taper liners were used, with pulsed pressure injection being again applied, following the same pressure, flow and temperature conditions as for the aforementioned GC-EI-QqQ-MS method.

The QTOF mass spectrometer was calibrated with Perfluorotributylamine (PFTBA or FC43) prior to each injection. The system was operating in broadband collision-induced dissociation (bbCiD) acquisition mode and recorded spectra over the range m/z 50–1,000 with a scan rate of 2 Hz. The Bruker bbCiD mode provides MS and MS/MS spectra at the same time, working at two different collision energies. At the low collision energy of 4 eV, MS spectra are acquired, with all of the ions from the pre-selected mass range passing through the flight tube without isolation at the quadrupole and without any collision-induced dissociation at the collision cell. At the high collision energy of 25 eV, isolation is taking place at the quadrupole, while the ions from the preselected mass range being fragmented at the collision cell. This is a Data Independent Acquisition (DIA) mode, resulting in better sensitivity, since more time is dedicated to every spectrum recorded, and is generally used for structure elucidation and retrospective analysis. However, during the database creation, the Data Dependent Acquisition (DDA) mode was also implemented for analysis of the standard mixture, recording MS/MS data that correspond only to the five most abundant masses. The application of DDA results in clearer spectra, due to sample matrix elimination, with the obtained spectra being used for compound identification and verification purposes, ensuring that specific fragments belong unambiguously to a particular analyte. Both MS and MS/MS spectra, in both DIA and DDA acquisition modes were recorded and utilized for the development of the equivalent positive HRMS database for the analytes of interest, while sample analysis was performed under DIA.

In contrast to the electron impact ionization, which is considered to be a "hard" ionization technique, APCI is a soft source that generally imparts less energy to an analyte molecule than the EI ionization, resulting in less fragmentation and usually a simpler spectrum, while it allows the determination of the molecular mass. The developed GC-APCI-QTOF-MS method was able to simultaneously monitor 126 IAS and NIAS (Table 4 and Figure 2), with only five from the targeted analytes not being successfully ionized under APCI conditions. However, the advantage of high-resolution enables again the determination of all analytes without the requirement for peak resolution, maintaining reduced analysis time.

3. Methods

Both methods developed were validated in terms of linearity, precision, trueness, limit of detection (LOD) and quantification (LOQ), taking into consideration the international guidelines for performance criteria and validation procedures (Thompson et al., 2002; Bratinova et al., 2009; European Commission, Directorate General for Health and Food Safety, 2017). The linearity for every analyte was assessed by analyzing standard solution mixtures at six concentration levels for each of the target analytes and calculating the linear regression coefficients (R^2) and residuals plots. The linear regression coefficients were above 0.99 for all studied substances with the GC-EI-QqQ-MS/MS method, indicating a good linearity of the calibration curves. The same requirement was applied for the GC-APCI-QTOF-MS method, where 100 compounds had regression coefficients above 0.99 and only for 26 substances those values were above 0.98, with the residuals plots indicating no particular trend for the concentration ranges investigated. The upper linear limit was calculated by consecutive injections of standard mixtures, where signal suppression was observed due to high concentrations and overlapping of peaks. Even though the upper linear limit for many compounds can be considered high, no effects on linearity were identified.

The calibration curves were constructed with the ratio of the analyte peak area to the IS peak area. The performance of two different internal standards was studied, namely the 3-(4-Isopropylphenyl) isobutylaldehyde (IS1), which has also been used as IS in previous studies (Tsochatzis et al., 2020), and the deuterated diethyl phthalate-d_4 (IS2). Starting with the GC-EI-QqQ-MS method, for each internal standard we constructed the corresponding calibration curve for every investigated analyte and noticed that the appropriate standard, improving the analyte's correlation coefficient for 129 out of the 131 compounds investigated was the IS1. Diethyl phthalate-d_4 proved to perform better only for 2,2′-methylene bis(4-ethyl-6-tert-butylphenol) (FCM 163) and 2,2′-methylene bis(4-methyl-6-tert-butylphenol) (FCM 185), with differences in the correlation coefficients that were statistically insignificant. Thus, only the IS1 was further used, and will be from now on referred to as IS. In addition, since no sample preparation steps are involved in the overall analytical procedure, the use of IS compensates for variations related to sample injection. Thus,

10.3389/fsufs.2023.1159002

TABLE 3 Characteristic MS detection parameters, retention times and analytical features of the validated GC-EI-QqQ-MS method.

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT (min)	Parent (m/z)	Product (m/z)	Collision Energy (EV)	LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
2	300	No	4.53	73	45.1	10	4.3	13.0	1,000
4	175	50	5.26	81	79	10	4.8	14.6	1,000
7	293	ND	5.81	111	83	10	6.4	19.3	1,000
8	155	No	5.91	93	77	10	5.0	15.0	1,000
9	183	6,000	6.11	87	45.1	10	4.1	12.4	1,000
12	420	50	6.29	163	134.9	10	9.10	27.3	1,000
13	195	No	6.31	106	105	10	1.8	5.3	1,000
14	184	6,000	6.62	87	59.1	10	2.9	8.7	1,000
15	–	n/a	6.65	93	77	20	1.4	4.3	1,000
16	–	n/a	6.66	93	93	10	9.7	29.4	1,000
17	187	No	6.69	118	117	10	3.3	9.9	1,000
20	217	No	7.09	146	110.9	10	0.5	1.4	1,000
21	209	30,000	7.21	83	55.1	10	14.8	44.8	800
22	194	No	7.32	108	79	10	12.8	38.8	1,000
23	–	n/a	7.69	106	77	10	5.9	17.9	1,000
24	220	20	7.69	69	41.2	10	2.6	7.8	1,000
25	–	n/a	7.73	105	51.1	30	10.2	31.0	1,000
26	216	No	7.75	105	77	10	8.4	25.5	1,000
27	171	No	8.05	83	55.1	10	10.1	30.7	1,000
28	–	n/a	8.09	98	41.2	20	8.8	26.7	1,000
29	405	ND	8.20	130	115	10	7.1	21.5	1,000
31	136	No	8.65	108	93	10	2.8	8.5	1,000
32	–	n/a	8.82	121	106.4	10	15.7	47.6	1,000
33	172	No	8.85	105	77	10	10.0	30.3	1,000
36	284	30,000	9.14	120	92	10	11.8	35.9	1,000
38	197	50	9.19	87	59	20	3.8	11.4	1,000
39	–	n/a	9.31	124	109	10	15.6	47.1	800
41	–	n/a	9.45	148	53	30	11.6	35.1	1,000
43	315	3,000	9.84	219	205	10	4.0	12.1	800
44	441	No	9.86	123	77	30	2.6	7.9	1,000
45	–	n/a	9.91	122	94	10	6.4	19.3	1,000
46	212	15,000	9.97	113	56.2	10	28.4	86.2	1,000
47	448	6,000	9.99	84	55.1	10	13.3	40.3	1,000
48	–	n/a	10.01	105	51	30	17.4	52.6	1,000
49	186	50	10.04	135	107	10	11.9	36.0	1,000
50	236	ND	10.14	108	79	10	12.5	37.8	1,000
52	–	n/a	10.17	114	85	10	9.7	29.3	1,000
53	–	n/a	10.20	147	91.4	20	9.3	28.3	1,000
54	447	6,000	10.45	131	91	10	3.5	10.5	1,000
55	–	n/a	10.50	103	43.1	10	2.4	7.3	1,000
57	–	n/a	10.65	87	43	10	19.2	58.0	1,000
58	–	n/a	10.92	154	152	20	2.5	7.7	1,000
59	–	n/a	11.10	142	113.9	10	4.9	14.9	1,000

(Continued)

10.3389/fsufs.2023.1159002

TABLE 3 (Continued)

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT (min)	Parent (m/z)	Product (m/z)	Collision Energy (EV)	LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
60	788	50	11.24	216	68.7	20	12.6	37.8	1,000
61	–	n/a	11.39	152	151	10	19.5	59.0	1,000
62	189	No	11.62	121	93	10	34.8	105.4	1,000
63	–	n/a	11.65	135	90.9	10	6.1	42.3	1,000
64	288	No	11.97	163	134.9	10	10.7	32.6	1,000
65	–	n/a	11.98	191	57	10	5.6	17.0	1,000
67	287	No	12.12	121	93	10	5.6	17.1	1,000
68	–	n/a	12.16	180	165	10	1.7	5.0	1,000
69	–	n/a	12.21	99	62.6	10	12.2	37.1	1,000
70	434	50	12.22	69	41.2	10	9.3	28.0	1,000
71	330	No	12.36	129	86.9	10	6.8	20.5	1,000
72	436	No	12.41	183	57	10	14.4	43.7	1,000
73	–	n/a	12.57	143	115	20	5.0	15.2	600
74	–	n/a	12.66	82	67.1	10	17.4	52.3	1,000
75	497	5,000	12.82	98	55.1	10	11.4	34.6	1,000
76	337	50	12.85	123	95	10	5.0	15.0	1,000
77	–	n/a	12.85	149	65	20	5.8	17.6	1,000
78	–	n/a	12.92	169	168	10	21.9	66.5	400
79	173	No	13.18	138	120.9	10	6.5	19.6	1,000
80	–	n/a	13.29	182	76.9	20	9.5	28.8	1,000
81	286	600	13.32	105	77	10	10.1	30.7	1,000
82	140	60,000	13.44	157	68.9	20	7.8	23.5	1,000
83	–	n/a	13.70	149	133.9	10	7.2	21.9	1,000
85	–	n/a	13.97	99	81	10	20.5	62.3	1,000
86	–	n/a	14.12	148	42.1	10	5.0	15.2	1,000
87	–	n/a	14.15	180	179.1	10	12.9	39.2	1,000
88	–	n/a	14.38	121	65	10	12.0	36.2	1,000
89	316	ND	14.45	149	65	20	4.6	14.0	1,000
90	–	n/a	14.63	169	169	10	9.8	29.8	400
91	–	n/a	14.69	196	118.9	10	11.9	36.1	1,000
92	–	n/a	14.73	219	191	10	5.2	15.7	1,000
93	–	n/a	15.69	114	86.9	10	15.3	46.4	1,000
94	–	n/a	15.81	149	65	20	10.3	31.3	1,000
95	358	No	15.82	197	103	10	4.4	13.4	600
96	–	n/a	16.14	105	77	10	8.7	26.5	1,000
98	105	No	16.70	129	87	10	2.5	7.4	600
99	–	n/a	16.71	277	146.9	10	13.1	39.6	1,000
100	–	n/a	16.79	105	77	10	8.8	26.5	1,000
101	157	300	16.95	149	65	20	9.1	27.5	1,000
102	463	50	17.65	253	68.9	10	6.9	20.8	1,000
103	–	n/a	17.96	336	267.3	10	7.6	22.9	1,000
104	318	6,000	19.01	137	81	10	19.4	58.9	1,000

(Continued)

10.3389/fsufs.2023.1159002

TABLE 3 (Continued)

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT (min)	Parent (m/z)	Product (m/z)	Collision Energy (EV)	LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
105	–	n/a	19.14	199	152	30	7.0	21.3	1,000
106	242	60,000	19.41	241	115.3	10	7.3	22.2	600
107	487	50	19.75	204	175.9	20	12.4	37.6	1,000
108	–	n/a	20.06	160	77	10	10.4	31.5	1,000
109	152	50	20.49	159	74.9	20	10.2	30.8	1,000
110	138	60,000	20.60	185	110.9	10	19.6	59.3	1,000
111	475	No	20.60	123	81	10	5.0	15.2	1,000
112	–	n/a	20.77	225	148	10	7.6	23.1	1,000
113	–	n/a	20.80	277	164.9	10	10.3	31.3	1,000
114	–	n/a	20.89	241	132.9	10	17.1	51.8	1,000
115	–	n/a	20.89	106	77	10	3.4	10.4	1,000
116	–	n/a	21.02	128	42	20	24.2	73.2	1,000
117	159	30,000	21.80	149	65	20	14.5	44.1	1,000
118	438	50	21.96	347	185.9	10	10.27	30.8	1,000
119	207	18,000	22.20	129	55.1	10	18.7	56.7	1,000
121	285	1,500	22.59	149	121	10	2.7	8.3	1,000
122	–	n/a	23.15	181	152	20	14.0	42.4	600
123	–	n/a	23.42	231	194.9	10	5.4	16.4	600
124	163	1,500	23.68	163	134.9	10	6.3	19.1	600
125	283	1,500	24.01	167	148.9	10	14.7	44.4	1,000
126	–	n/a	24.18	225	76.9	20	5.7	17.4	1,000
127	–	n/a	24.88	225	103	30	12.1	36.5	600
128	492	50	25.53	204	117.1	20	11.4	34.1	1,000
129	798	60,000	26.08	149	65	10	3.7	11.2	1,000
130	431	6,000	26.42	213	76.9	10	4.1	12.6	1,000
131	271	No	26.43	126	83	10	16.6	50.2	600
IS	–	–	11.62	133	105	10			
				Quan	**Qual1**	**Qual2**			
1	181	6,000	4.15	69	99	86	4.7	14.4	1,000
3	342	6,000	5.03	57	87	69	1.0	3.0	800
5	193	No	5.54	104	78	51	4.1	12.4	1,000
6	325	6,000	5.59	55	56	73	4.6	13.9	1,000
10	371	6,000	6.12	55	73	86	21.1	63.9	1,000
11	142	50	6.27	145	135	175	7.8	23.5	1,000
18	322	No	7.17	57	45	41	24.9	75.5	800
19	374	6,000	7.02	69	41	87	2.0	6.2	800
30	342	50	8.61	55	84	114	3.2	9.8	1,000
34	–	n/a	9.01	100	142	58	9.2	28.0	1,000
35	–	n/a	9.12	75	57	45	19.0	57.6	1,000
37	266	No	9.17	58	89	45	7.9	23.8	800
40	206	50	9.38	55	77	83	10.5	31.7	1,000
42	439	6,000	9.54	94	69	162	11.5	34.9	1,000

(Continued)

10.3389/fsufs.2023.1159002

TABLE 3 (Continued)

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT (min)	Parent (m/z)	Product (m/z)	Collision Energy (EV)	LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
				Quan	**Qual1**	**Qual2**			
51	185	50	10.16	69	41	113	10.1	30.2	1,000
56	354	No	10.62	174	145	132	14.0	42.3	1,000
66	–	n/a	12.05	87	74	55	7.3	22.0	1,000
84	437	50	13.77	55	83	97	10.20	30.60	1,000
97	104	6,000	16.30	58	57	43	3.9	12.0	1,000
120	301	No	22.32	56	43	60	8.7	26.5	1,000

*According to Table 1 of Annex I of Reg. (EU) No. 10/2011 (European Commission, 2011).

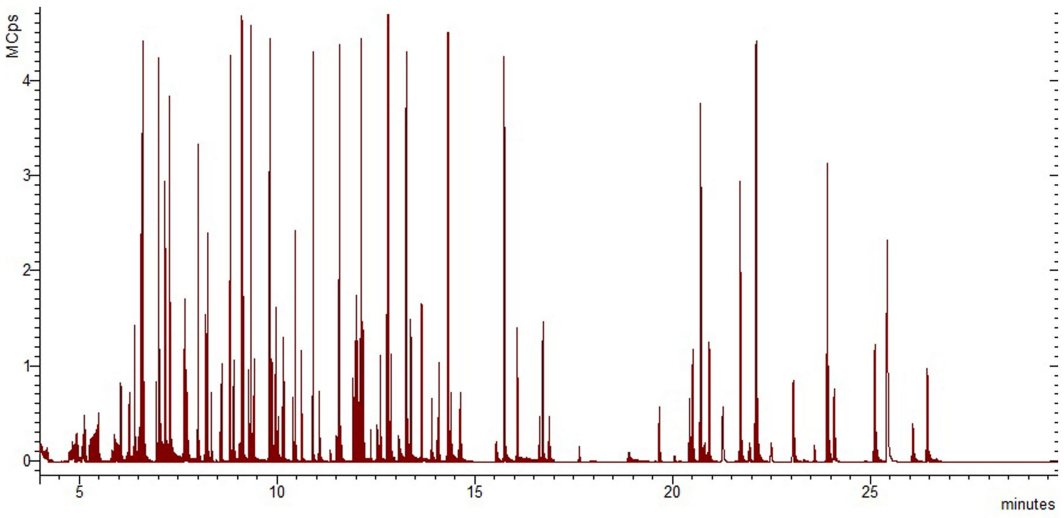

FIGURE 1
GC-EI-QqQ-MS extracted ion and MRM chromatograms of spiked 95% v/v aqueous EtOH.

the selection of additional standards, applied for particular groups of compounds, was not considered necessary.

The estimation of target analytes' LODs and LOQs was based on the data from regression analysis, using the equations $LOD = 3 \times S_b/a$, where S_b is the standard deviation of the intercept and a is the slope of the curves; and $LOQ = 3 \times LOD$. The method's LOQ had the additional requirement of covering the existing EU requirements and limits (SMLs). When the LOQ values were compared with the SMLs as listed in the Regulation, all LOQs were below the proposed SMLs for the GC-EI-QqQ-MS method. For the GC-APCI-QTOF-MS method, the LOQs of 13 substances were higher than the provided SMLs (Table 4). Thus, for quantification of these particular substances, namely FCM 142, 185, 197, 206, 220, 337, 342, 420, 438, 434, 463, 487, 788; with SMLs equal to 50 ng g^{-1}, the GC-EI-QqQ-MS method should be applied. In addition, the use of the APCI source at the HRMS system prevented the fragmentation for 5 of the targeted analytes (FCM 104, 105, 189, 266, 330), reducing the applicability range of the method from 131 to 126 compounds.

Precision and trueness were assessed by analyzing fortified simulant (95% EtOH) with all the selected analytes at several mass fraction levels. For the GC-EI-QqQ-MS method the levels used were 100, 250, and 500 µg kg^{-1}, while for the complementary GC-APCI-QTOF-MS method the 250, 400, and 800 µg kg^{-1} were selected. For intra-day precision or repeatability, six replicates of the 250 µg kg^{-1} sample were analyzed during 1 day, while for intermediate precision 3 replicates of all investigated levels were analyzed over two consecutive days. The trueness of the analytical method was based on the calculation of the recovery (%), expressed as the amount found in the fortified food simulant over the known amount added × 100. For the GC-EI-QqQ-MS method, reported results show good precision of the method, with RSDs for the repeatability and intermediate precision being lower that 20% for all studied substances. Recoveries for all studied substances were also between 70 and 120%. For the GC-APCI-QTOF-MS method, the recoveries of the analytes investigated were again between 70 and 120%. However, 86% of the analytes had RSDs below 20% for the repeatability measurements, with the remaining 14% ranging from 20.4 to 29.6%. Concerning the intermediate precision, 77% of the analytes had RSDs below 20%, while for 29 analytes the RSD values were between 20.1 and 37.3%.

10.3389/fsufs.2023.1159002

TABLE 4 Characteristic MS detection parameters, retention times and analytical features of the validated GC-APCI-QTOF-MS method.

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT	Exact mass (m/z)		LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
1	181	6,000	4.5	114.067531	M+	4.8	14.4	1,000
2	300	No	4.95	117.091006	[M+H]+	51.1	153.2	1,000
3	342	6,000	5.56	142.098831	M+	3.7	11.1	800
4	175	50	5.73	127.075356	[M+H]+	4.3	12.9	1,000
5	193	No	6.04	105.069877	[M+H]+	21.9	66.4	1,000
6	325	6,000	6.02	129.091006	[M+H]+	56.9	172.3	1,000
7	293	ND	6.38	167.083157	[M+H]+	14.8	44.4	1,000
8	155	No	6.51	137.132477	[M+H]+	38.7	117.4	1,000
9	183	6,000	6.52	142.098831	M+	7.3	21.9	800
10	371	6,000	6.7	117.054621	[M+H]+	51.0	154.6	1,000
11	142	50	6.7	191.109797	[M+H]+	21.9	66.2	1,000
12	420	50	6.82	195.065185	[M+H]+	67.3	201.9	1,000
13	195	No	6.87	107.049141	[M+H]+	5.4	16.2	1,000
14	184	6,000	6.99	143.106656	[M+H]+	4.3	12.9	1,000
15	–	n/a	7.04	137.132477	[M+H]+	24.2	73.4	1,000
16	–	n/a	7.08	94.065126	[M+H]+	7.5	22.5	1,000
17	187	No	7.09	119.085527	[M+H]+	3.7	11.1	800
18	322	No	7.38	147.101571	[M+H]+	12.1	36.3	800
19	374	6,000	7.53	131.070271	[M+H]+	21.6	65.3	800
20	217	No	7.47	145.968457	M+	70.9	214.8	1,000
21	209	30,000	7.72	130.135217	M+	58.5	177.2	1,000
22	194	No	7.86	108.056966	M+	16.6	49.9	1,000
23	–	n/a	8.13	137.070939	[M+H]+	16.1	48.3	1,000
24	220	20	8.05	143.070271	[M+H]+	8.3	24.9	1,000
25	–	n/a	8.13	121.064791	[M+H]+	11.2	33.6	800
26	216	No	8.38	109.064791	[M+H]+	41.5	125.8	1,000
27	171	No	8.44	137.059706	[M+H]+	9.9	29.6	800
28	–	n/a	8.46	143.143042	[M+H]+	39.8	120.5	1,000
29	405	ND	8.65	131.085527	[M+H]+	18.5	56.1	1,000
30	342	50	8.97	115.075356	[M+H]+	24.1	72.9	1,000
31	136	No	9.02	153.127392	[M+H]+	30.9	93.8	1,000
32	–	n/a	9.25	122.096426	[M+H]+	2.6	7.7	800
33	172	No	9.23	151.075356	[M+H]+	19.2	57.6	1,000
34	–	n/a	9.35	186.221626	[M+H]+	50.6	153.3	1,000
35	–	n/a	9.6	163.132871	[M+H]+	33.1	100.2	1,000
36	284	30,000	9.53	153.054621	[M+H]+	20.9	63.2	300
38	197	50	9.55	168.114481	M+	20.8	62.9	600
39	–	n/a	9.88	125.059706	[M+H]+	4.5	13.5	600
40	206	50	9.73	184.145781	M+	23.1	69.9	1,000
41	–	n/a	9.81	149.096091	[M+H]+	23.7	71.1	1,000
42	439	6,000	9.82	163.075356	[M+H]+	41.7	126.4	1,000
43	315	3,000	10.12	220.182167	M+	21.6	65.5	1,000
44	441	No	10.22	165.091006	[M+H]+	36.2	109.8	1,000

(Continued)

10.3389/fsufs.2023.1159002

TABLE 4 (Continued)

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT	Exact mass (m/z)		LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
45	–	n/a	10.3	138.09134	[M+H]+	7.1	21.3	800
46	212	15,000	10.5	114.09134	[M+H]+	44.6	135.0	1,000
47	448	6,000	10.33	185.153606	[M+H]+	32.7	99.0	1,000
48	–	n/a	10.39	165.091006	[M+H]+	46.8	141.8	1,000
49	186	50	10.58	150.103917	M+	12.7	38.1	800
50	236	ND	10.81	109.076025	[M+H]+	39.5	119.6	1,000
51	185	50	10.54	198.08866	M+	22.4	67.2	1,000
52	–	n/a	10.5	158.153941	[M+H]+	18.7	56.1	1,000
53	–	n/a	10.59	149.096091	[M+H]+	7.2	21.6	800
54	447	6,000	10.8	176.083181	M+	9.2	27.5	800
55	–	n/a	11.54	219.086315	[M+H]+	4.8	14.4	600
56	354	No	11.01	174.042379	M+	14.2	42.6	800
57	–	n/a	10.97	205.143436	[M+H]+	28.3	85.6	1,000
58	–	n/a	11.31	155.085527	[M+H]+	22.3	66.9	1,000
59	–	n/a	11.48	158.036718	[M+H]+	3.5	10.4	1,000
60	788	50	11.55	248.107452	M+	42.6	129.0	1,000
61	–	n/a	11.78	153.036862	[M+H]+	34.4	104.1	1,000
63	–	n/a	12.04	221.153606	[M+H]+	33.2	100.5	1,000
64	288	No	12.35	195.065185	[M+H]+	55.2	167.2	1,000
65	–	n/a	12.42	206.166517	M+	11.4	34.2	1,000
66	–	n/a	12.4	215.200557	[M+H]+	49.7	150.5	1,000
67	287	No	12.74	167.070271	[M+H]+	25.6	76.8	1,000
68	–	n/a	12.55	181.101177	[M+H]+	22.5	67.5	1,000
69	–	n/a	12.56	229.143436	[M+H]+	50.4	152.9	1,000
70	434	50	12.59	226.119961	M+	37.4	113.5	1,000
72	436	No	12.78	227.200557	[M+H]+	35.5	107.6	1,000
73	–	n/a	13.06	144.080776	[M+H]+	3.7	11.1	800
74	–	n/a	13.03	227.127786	[M+H]+	71.7	217.1	1,000
75	497	5,000	13.18	287.221686	[M+H]+	53.0	160.6	1,000
76	337	50	13.26	219.061598	[M+H]+	24.0	72.7	1,000
77	–	n/a	13.23	223.096485	[M+H]+	27.5	82.4	1,000
78	–	n/a	13.36	170.096426	[M+H]+	15.8	47.3	1,000
79	173	No	13.72	181.085921	[M+H]+	13.7	41.1	1,000
80	–	n/a	13.75	184.088266	M+	3.9	11.7	1,000
81	286	600	13.75	183.080441	[M+H]+	19.4	58.3	1,000
82	140	60,000	13.83	277.128179	[M+H]+	30.7	93.0	1,000
83	–	n/a	14.15	150.054955	[M+H]+	7.6	22.8	1,000
84	437	50	14.11	240.208382	M+	14.2	42.9	1,000
85	–	n/a	14.45	205.122306	[M+H]+	15.9	47.6	1,000
86	–	n/a	14.57	194.117555	[M+H]+	29.8	89.3	1,000
87	–	n/a	14.63	181.101177	[M+H]+	4.4	13.2	800
88	–	n/a	14.85	199.075356	[M+H]+	18.7	56.1	1,000
89	316	ND	14.89	247.096485	[M+H]+	12.3	36.9	800

(Continued)

10.3389/fsufs.2023.1159002

TABLE 4 (Continued)

Analyte no.	FCM no.*	SML [ngg^{-1}]	RT	Exact mass (m/z)		LOD [ngg^{-1}]	LOQ [ngg^{-1}]	Upper limit [ngg^{-1}]
90	–	n/a	15.16	170.096426	[M+H]+	31.1	94.3	1,000
91	–	n/a	15.15	197.096091	[M+H]+	10.8	32.4	800
92	–	n/a	15.18	235.169256	[M+H]+	22.4	67.2	800
93	–	n/a	16.08	214.126012	[M+H]+	16.4	49.2	1,000
94	–	n/a	16.26	279.159086	[M+H]+	34.0	102.0	1,000
95	358	No	16.44	213.127392	[M+H]+	56.2	170.3	1,000
96	–	n/a	16.59	257.117221	[M+H]+	83.4	250.3	1,000
99	–	n/a	17.16	292.203296	M+	27.9	83.7	800
100	–	n/a	17.28	241.085921	[M+H]+	12.1	36.6	800
101	157	300	17.41	279.159086	[M+H]+	28.8	87.1	1,000
102	463	50	18.16	338.17239	M+	36.9	111.8	1,000
103	–	n/a	18.91	336.05795	M+	12.6	37.8	800
104	318	6,000	19.84	215.070271	[M+H]+	24.3	72.9	1,000
105	–	n/a	19.95	214.098831	M+	68.0	206.1	1,000
106	242	60,000	19.87	315.252986	[M+H]+	46.8	141.8	1,000
107	487	50	20.22	277.10973	M+	28.0	84.8	1,000
108	–	n/a	15.19	192.06552	[M+H]+	31.7	96.1	1,000
109	152	50	21.1	286.969482	[M+H]+	8.9	26.8	1,000
110	138	60,000	21.02	403.232644	[M+H]+	52.4	157.2	1,000
111	475	No	20.2	223.144104	[M+H]+	44.4	134.4	1,000
112	–	n/a	21.23	225.114816	M+	10.1	30.2	800
113	–	n/a	21.3	278.211456	[M+H]+	16.4	49.1	1,000
114	–	n/a	21.63	256.145781	M+	68.9	208.8	1,000
115	–	n/a	21.49	226.133874	[M+H]+	4.1	12.3	800
116	–	n/a	21.53	280.136576	[M+H]+	13.5	40.4	800
117	159	30,000	22.29	313.143436	[M+H]+	41.3	125.2	1,000
118	438	50	22.48	362.271651	M+	45.3	137.3	1,000
119	207	18,000	22.65	371.315586	[M+H]+	44.6	135.1	1,000
120	301	No	22.46	340.333582	M+	15.8	48.0	1,000
121	285	1,500	23.1	340.239682	M+	6.2	18.6	800
122	–	n/a	23.69	258.103917	M+	7.9	23.6	800
123	–	n/a	24.02	266.037205	M+	18.9	56.7	1,000
124	163	1,500	24.16	368.270982	M+	6.1	18.4	800
125	283	1,500	24.44	391.284286	[M+H]+	82.5	250.1	1,000
126	–	n/a	24.69	319.096485	[M+H]+	38.2	115.8	1,000
127	–	n/a	25.79	268.145781	M+	13.2	39.7	1,000
128	492	50	25.97	361.2036	M+	4.2	12.6	600
129	798	60,000	26.64	391.284286	[M+H]+	25.1	75.3	1,000
130	431	6,000	27.03	326.187646	M+	44.6	135.3	1,000
131	271	No	27.04	338.341741	[M+H]+	72.0	218.3	1,000
IS	–	–	11.97	190.135217	M+			

*According to Table 1 of Annex I of Reg. (EU) No. 10/2011 (European Commission, 2011).

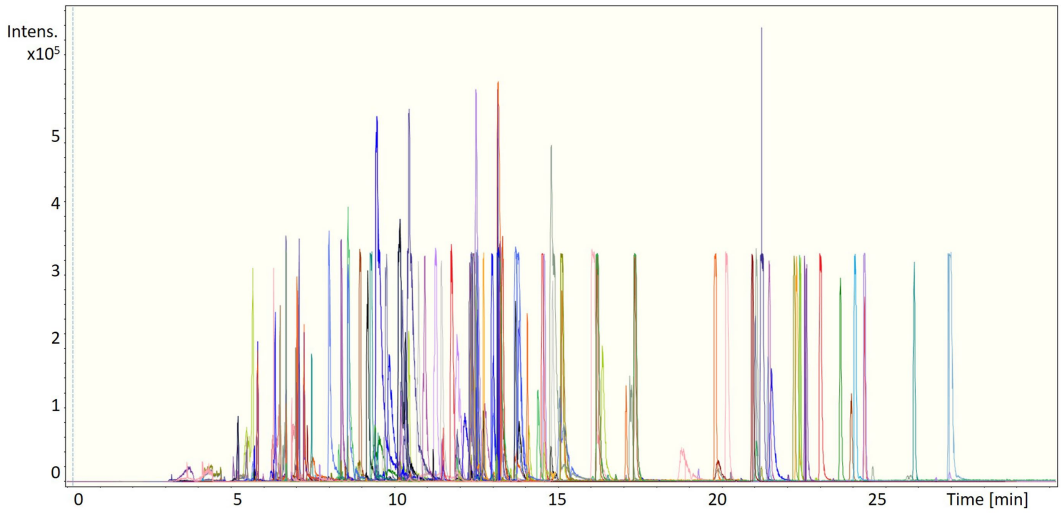

FIGURE 2
GC-APCI-QTOF-MS extracted ion chromatograms of spiked 95% v/v aqueous EtOH.

4. Results

The applicability of the method was evaluated by studying six commercial FCM polymeric samples comprised of different materials (Table 2). It is important to mention that the FCM samples to be examined toward chemical migration should be tested before coming into contact with the respective food product. This ensures that any compounds detected (at the applied food simulant) originate from the packaging material itself and are not constituents of the packaged food. Removal of the food product and subsequent analysis of the packaging material cannot provide reliable results, since, food contaminations could remain at the food contact material and be falsely detected as migrating substances, while other actual migrating compounds may have already be transferred to the packaged food and consequently removed without being detected. Thus, testing commercially available packaged food products could significantly compromise the integrity of the results in terms of compound identification as well as migration rate and corresponding material inertness. Therefore, such evaluations should only be performed at original materials and not at final, commercially available products. However, original FCM samples are not easily accessible and obtaining them requires the collaboration with companies that either produce the materials themselves or use them for the packaging of their products. Such a co-operation is hard to establish, since the non-legislative nature of the EU regulation allows the companies to perform the bare minimum of migration tests necessary for compliance, avoiding the application of highly sensitive and quantitative methods for the identification of migrating substances. In addition, even when a FCM is provided for analysis, the recipe of the material is rarely disclosed, since either the manufacturer is not willing to share these information for reasons of confidentiality or competitiveness, or the retailer or collaborating food company may also not have access to them.

Even though finding original, intact samples of food contact materials is a difficult task, having performed an open call for co-operation through the national association of manufacturers of packaging materials, we managed to collect six representative samples of polymeric FCMs. The migration experiments were designed according to the type of each product, using the 95% EtOH food simulant, while keeping the samples for 10 days at 60°C. This time and temperature combination was selected, since it testing under these conditions shall cover long term storage above 6 months at room temperature and below including heating up to 70°C for up to 2 h, or heating up to 100°C for up to 15 min, according to the EU Regulation 10/2011. In particular, pouches were formed for the flexible films examined, while the tray and bottle samples undergo total immersion experiments. The results of the experiments in terms of detected substances and corresponding concentrations, are presented in Table 5. Two out of the six samples investigated did not release any of the method's target analytes under the defined test conditions. These were the multilayer PP copolymer film and the monolayer PET bottle, with the other PET samples showing higher migration rates. In particular, low levels of Butyl acetate (FCM 300) processing aid were detected for sample S3A, while the antioxidant Butylated hydroxytoluene (FCM 315) and the UV inhibitor 4-Methoxyphenol were also detected at both PET trays. The presence of 4-Methoxyphenol, even though not included in the positive list of substances at the EU Regulation 10/2011, has already been reported in previous work (Lago and Ackerman, 2016) and was, thus, added to our list of target analytes. From the remaining FCMs, only sample S1A released caprolactam (FCM 177) monomer at a concentration level of 2.3 mg kg^{-1}, as calculated with both applied methods. This concentration was more than six times lower than the SML provided for the particular substance and was calculated taking into consideration the area which was in contact with the food simulant and the standard surface-to-food mass ratio of 6 dm^2 kg^{-1} food. Only traces of tri-n-butyl acetyl citrate (FCM 138) plasticizer were also detected in

TABLE 5 Identification and quantification data for the substances migrating from the tested FCM plastic samples into food simulant 95% EtOH.

Sample code	Material type	FCM No.*	FCM substances*	SML (ngg^{-1})	Detected amounts (ngg^{-1})	
					GC-EI-QqQ-MS	GC-APCI-QTOF-MS
S1A	Multilayer PET/PE film	177	Caprolactam	15,000	2,322	2,284
S1B	Multilayer PP copolymer film	–	–	–	–	–
S1C	Multilayer BOPP copolymer film	138	Tri-n-butyl acetyl citrate	60,000	76	59
S2	Monolayer PET bottle	–	–	–	–	–
S3A	Monolayer PET tray	315	Butylated hydroxytoluene	3,000	820	837
		300	Butyl acetate	no	475	493
		–	4-Methoxyphenol	n/a	180	185
S3B	Monolayer PET tray	315	Butylated hydroxytoluene	3,000	552	558
		–	4-Methoxyphenol	n/a	85	93

*According to Table 1 of Annex I of Reg. (EU) No. 10/2011 (European Commission, 2011).

sample S1C, proving that all investigated copolymers were highly inert, according to the migration tests performed.

Overall, the analysis of real FCM samples showed that the migrated amounts of the regulated substances of all tested materials were compliant with the requirements in the corresponding Regulation, with only one NIAS compound, belonging to Cramer Class I being additionally detected at the low mg kg^{-1} range.

5. Discussion

In the frame of this study, two methods were developed and validated for the target screening of a large group of IAS and NIAS migrating from plastic food contact materials, covering all different types of virgin or recycled plastics. Both methods were used for the direct analysis of sample migrants, where the 95% EtOH food simulant was applied. The GC-EI-QqQ-MS method was used for the determination of more volatile analytes, with 131 compounds being simultaneously analyzed, over the 126 compounds being successfully identified and quantified by the complementary GC-APCI-QTOF-MS method. Additionally, the low-resolution method maintained the sensitivity advantage, while presenting wider linearity ranges for several of the targeted species. These features are mandatory for the analysis of substances with significantly different SMLs. In particular, the GC-EI-QqQ-MS method was able to reach the appropriate LOQs and cover the legislative limits for all investigated substances, without the requirement of any pre-concentration step. For the respective GC-APCI-QTOF-MS method, only for 13 out of the 126 investigated compounds the obtained LOQs were higher than the proposed SMLs. At the same time, both methods are still able to identify at the low ppb level targeted NIAS, for which such limits have not been yet established, but considering that some of them belong to Cramer Class III, low SMLs have to be expected. Furthermore, the proposed GC-APCI-QTOF-MS method provides the significant advantage for retrospective analysis. Building up target screening databases, this is an important property, allowing further compounds to be added to the controls as the list of analytes of interest is expanding with more and more NIAS compounds being detected.

When both methods were applied for the analysis of real FCM samples, the results obtained proved the efficiency of both methods. They also showed that all investigated samples, in their examined form, are highly inert toward the investigated group of substances. Additionally, the calculated concentrations of the detected migrants illustrated the complementarity of both methods, which could in most of the cases also be used interchangeably, since they provided comparable concentration rates for all detected analytes.

In conclusion, this is the first work addressing the simultaneous determination of over 130 volatile migrants of plastic FCMs, utilizing both low- and high-resolution MS systems. The direct, highly sensitive analysis of such a large group of EU regulated substances and NIAS compounds is of particular importance for both the competent authorities and control laboratories, since it would enable them to perform efficient screening on different instrumentations available and enhance the validity of safety statements for the food contact materials tested.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CK and NT contributed to the conception and design of the study. NT provided the resources. CK performed the experiments and development of both analytical methods and wrote the first draft of the manuscript. CK and TT performed the data evaluation and statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This study was partially funded by a Scholarship (scholarship number 2019–050–0503-18008), provided by the State Scholarships Foundation (IKY), within the framework of the project "Reinforcement of Postdoctoral Researchers—2nd Cycle" (MIS-5033021) of the operational program "Human Resource Development, Education and Lifelong Learning," co-funded by Greece and the European Union (European Social Fund).

Acknowledgments

We would like to thank Emmanouil D. Tsochatzis for all the discussions and useful suggestions during the course of this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Bratinova, S., Raffael, B., and Simoneau, C. (2009). Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. EUR 24105 EN. Luxembourg: Publications Office of the European Union, JRC53034.

Canellas, E., Vera, P., Nerin, C., Dreolin, N., and Goshawk, J. (2022). The detection and elucidation of oligomers migrating from biodegradable multilayer teacups using liquid chromatography coupled to ion mobility time-of-flight mass spectrometry and gas chromatography-mass spectrometry. *Food Chem.* 374:131777. doi: 10.1016/j.foodchem.2021.131777

European Commission, Council of the European Union. (2004). Regulation (EC) no 1935/2004 of the European Parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. *OJ L* 338, 4–17.

European Commission. (2011). Commission regulation (EU) no 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *OJ L* 12, 1–89.

European Commission, Directorate General for Health and Food Safety (DG SANTE). (2017). SANTE/11813/2017, Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.

García Ibarra, V., Bernaldo, R., de Quirós, A., Paseiro Losada, P., and Sendón, R. (2019). Non-target analysis of intentionally and non-intentionally added substances from plastic packaging materials and their migration into food simulants. *Food Packag. Shelf Life* 21:100325. doi: 10.1016/j.fpsl.2019.100325

Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., and Purnell, P. (2018). An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* 344, 179–199. doi: 10.1016/j.jhazmat.2017.10.014

Jung, T., and Simat, T. J. (2014). Multi-analyte methods for the detection of photoinitiators and amine synergists in food contact materials and foodstuffs – part II: UHPLC-MS/ MS analysis of materials and dry foods. *Food Addit. Contam. A* 31, 743–766. doi: 10.1080/19440049.2014.882519

Lago, M. A., and Ackerman, L. K. (2016). Identification of print-related contaminants in food packaging. *Food Addit. Contam. A* 33, 518–529. doi: 10.1080/19440049.2015.1136435

Lahimer, M. C., Ayed, N., Horriche, J., and Belgaied, S. (2017). Characterization of plastic packaging additives: food contact, stability and toxicity. *Arab. J. Chem.* 10, S1938–S1954. doi: 10.1016/j.arabjc.2013.07.022

Miralles, P., Yusà, V., Sanchís, Y., and Coscollà, C. (2021). Determination of 60 migrant substances in plastic food contact materials by vortex-assisted liquid-liquid extraction and GC-Q-Orbitrap HRMS. *Molecules* 26:7640. doi: 10.3390/molecules26247640

Muncke, J. (2009). Exposure to endocrine disrupting compounds via the food chain: is packaging a relevant source? *Sci. Total Environ.* 407, 4549–4559. doi: 10.1016/j.scitotenv.2009.05.006

Pack, E. C., Lee, K. Y., Jung, J. S., Jang, D. Y., Kim, H. S., Koo, Y. J., et al. (2021). Determination of the migration of plastic additives and non-intentionally added substances into food simulants and the assessment of health risks from convenience food packaging. *Food Packag. Shelf Life* 30:100736. doi: 10.1016/j.fpsl.2021.100736

Sapozhnikova, Y., Nuñez, A., and Johnston, J. (2021). Screening of chemicals migrating from plastic food contact materials for oven and microwave applications by liquid and gas chromatography—orbitrap mass spectrometry. *J. Chromatogr.* 1651:462261. doi: 10.1016/j.chroma.2021.462261

Silano, V., Barat Baviera, J. M., Bolognesi, C., Chesson, A., Cocconcelli, P. S., Crebelli, R., et al. (2019). Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2- ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and diisodecylphthalate (DIDP) for use in food contactmaterials. *EFSA J.* 17:5838. doi: 10.2903/j.efsa.2019.5838

Silva, A. S., Sendón García, R., Cooper, I., Franz, R., and Paseiro Losada, P. (2006). Compilation of analytical methods and guidelines for the determination of selected model migrants from plastic packaging. *Trends Food Sci. Technol.* 17, 535–546. doi: 10.1016/j.tifs.2006.04.009

Thompson, M., Ellison, S. L. R., and Wood, R. (2002). Harmonized guidelines for single laboratory validation of methods of analysis. *Pure Appl. Chem.* 74, 835–855. doi: 10.1351/pac200274050835

Tisler, S., and Christensen, J. H. (2022). Non-target screening for the identification of migrating compounds from reusable plastic bottles into drinking water. *J. Hazard. Mater.* 429:128331. doi: 10.1016/j.jhazmat.2022.128331

Tsochatzis, E. D., Alberto Lopes, J., Hoekstra, E., and Emons, H. (2020). Development and validation of a multi-analyte GC-MS method for the determination of 84 substances from plastic food contact materials. *Anal. Bioanal. Chem.* 412, 5419–5434. doi: 10.1007/s00216-020-02758-7

Tsochatzis, E. D., Lopes, J. A., Gika, H., Dalsgaard, T. K., and Theodoridis, G. (2021). A fast SALLE GC-MS/MS multi-analyte method for the determination of 75 food packaging substances in food simulants. *Food Chem.* 361:129998. doi: 10.1016/j.foodchem.2021.129998

Vavrouš, A., Ševčík, V., Dvořáková, M., Čabala, R., Moulisová, A., and Vrbík, K. (2019). Easy and inexpensive method for multiclass analysis of 41 food contact related contaminants in fatty food by liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* 67, 10968–10976. doi: 10.1021/acs.jafc.9b02544

Wrona, M., and Nerín, C. (2020). Analytical approaches for analysis of safety of modern food packaging: a review. *Molecules* 25:752. doi: 10.3390/molecules25030752

Yusà, V., López, A., Dualde, P., Pardo, O., Fochi, I., Miralles, P., et al. (2021). Identification of 24 unknown substances (NIAS/IAS) from food contact polycarbonate by LC-orbitrap tribrid HRMS-DDMS3: safety assessment. *Int. J. Anal. Chem.* 2021, 1–13. doi: 10.1155/2021/6654611

Zhang, X., Zhang, P., Shi, C., Hua, H., Rong, Y., Lu, J., et al. (2018). Rapid determination of 48 contaminant residues in food contact plastic products by ultra performance liquid chromatography-quadrupole/electrostatic field orbitrap high resolution mass spectrometry coupled with microwave-assisted extraction. *Chin. J. Chromatogr.* 36, 634–642. doi: 10.3724/SP.J.1123.2018.01047

Zhiqing, X., Jinfeng, H., Xiu-Ying, L., Senyu, L., Shuang, H., Xindong, G., et al. (2016). Simultaneous determination of seven acrylates in food contact paper products by GC/MS and modified QuEChERS. *Anal. Methods* 8, 3953–3958. doi: 10.1039/C6AY00613B