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Simultaneous determination of thirteen polycyclic aromatic hydrocarbons and twelve aldehydes in cooked food by an automated on-line solid phase extraction ultra high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

An on-line solid phase extraction (SPE) ultra high performance liquid chromatography tandem mass spectrometry method has been developed for the simultaneous identification and determination of thirteen polycyclic aromatic hydrocarbons (PAHs) and twelve aldehydes (derivatized with 2,4-dinitrophenylhydrazine). The chromatographic conditions have been optimized to obtain the maximum of sensitivity and resolution taking into account the different retention interactions and the different ionization conditions of PAHs and derivatized aldehydes. LOD values ranging from 0.028 to 0.768 $\mu g\,L^{-1}$ for PAHs and from 0.002 to 0.125 $\mu g\,L^{-1}$ for aldehydes were obtained. The resolution permitted the separation of four couples of PAH isomers. Sample pre-treatment and SPE were optimized in order to apply the whole methodology to the analysis of different food matrices as salmon, frankfurter, steak, and pork chop, subjected to different cooking modes (smoked, grilled, cooked in oil or in butter). Particular attention was devoted to the evaluation of matrix effect that was significantly reduced through the on-line SPE treatment. For each food matrix the method detection limits, the method quantitation limits, and the recovery *R* were evaluated. *R* was shown not to depend on analyte concentration in the explored concentration range (LOQ – 50.000 $\mu g\,L^{-1}$): the average *R* percent ranges from 70.6% to 120.0%.

1. Introduction

Alarm and awareness of possible health hazards associated with cooking processes is recently increasing [1–3]. In 2004 the Environmental Protection Agency (EPA) published a list of hazardous air pollutants emitted from cooking processes; the list includes EPA priority polycyclic aromatic hydrocarbons (PAHs) and linear aldehydes like formaldehyde, acetaldehyde and propionaldehyde [4]. In the last years, attention has been devoted in particular to kitchen fume emissions, mainly from commercial restaurants or households, since during the cooking processes, especially frying processes, PAHs and aliphatic aldehydes can form and pass to cooking fumes [1–3,5–7]. In particular, PAHs, widespread in the environment, mainly originate from intense thermal processes and from many kinds of cooking as smoking, roasting, baking, frying, and grilling [8]. Some amounts of aldehydes, like formaldehyde, acetaldehyde, acrolein and benzaldehyde are naturally present in fruits, vegetables, cheese, alcoholic beverages, eggs, fish, and meat [9], but their concentration increases at high temperatures, due to the degradation of sugars, proteins and fats present in food [10]. Aldehydes can especially form during the frying, due to the degradation of hydroperoxides [11–13].

The toxicity of PAHs is well known [14–21]. PAHs can be originated by the thermal degradation of food components as triglycerides, fatty acids, steroids and amino acids and their formation can be influenced by other ingredients present or added during the cooking process [22]. Also the kind of cooking can play an important role, for instance in determining the amount of PAHs formed in meat and fish [23–25].

In Europe, the maximum admitted content of PAHs in food only concerns benzo[a]pyrene (BaP), and corresponds to 2.0 μg/kg (wet weight) in oils and fats for direct human consumption and to 5.0 μg/kg (wet weight) in smoked fish and smoked fishery products [26].

For what regard the possible toxicity of aldehydes, they are reported to form adducts with thiols and amines of cellular proteins: the protein adducts can be reversible or undergo degradation of products that cross-link other proteins, that in turn can progressively accumulate and disrupt the cellular function [27]. Aldehydes are highly reactive substances and can modify proteins,

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nucleic acids, and other biomolecules in vivo [28–30]. In addition, formaldehyde and acrolein produce local irritation in the respiratory tract [10,31].

Up to now the interest in PAHs and aldehydes formed during food cooking has principally regarded the analysis of cooking fumes, but during cooking PAHs and aldehydes also form in the food itself. In this work we concentrated our attention in the analysis of cooked food, also comparing the results obtained for the same matrix after different cooking processes (grilled, cooked in butter, cooked in oil), that can likely affect the kind and the amounts of the species formed.

Several analytical techniques were used to identify and quantify PAHs and aldehydes in different matrices, but at our knowledge, no method for their simultaneous determination is up to now present in the literature. Only Daher et al. considered these compounds in cigarette emission, but PAHs were determined by gas chromatography with mass spectrometry detection (GC–MS) and aldehydes by high performance liquid chromatography with UV spectroscopy and MS detection (HPLC–UV–MS) [32].

GC–MS [33,34], HPLC with fluorescence detection (HPLC-FD) [15,18,19,21], HPLC-UV [19], and HPLC–MS using atmospheric pressure photo ionization (APPI) [20,35,36] atmospheric pressure chemical ionization (APCI) [16] and electrospray ionization (ESI) with a derivatization step with tropylium [14,17] or silver nitrate [37,38] were employed in PAH determination.

Aldehydes were determined by HPLC-DAD and/or HPLC–MS/MS after derivatization; the most employed derivatization agent being 2,4-dinitrophenylhydrazine (DNPH) that assure a fast, quantitative and irreversible reaction in which the carbonyl group forms a hydrazone derivate [31,39–42]. Recently, also ultra high performance liquid chromatography with MS detection (UHPLC–MS) methods were used for PAHs [20] and carbonyl determination [43]. LC/MS techniques generally allow better sensitivities but on the other hand are affected by matrix effect and require in real sample analysis a sample pre-treatment that depends on the analytes and on the kind of matrix. For PAHs and aldehydes the pre-treatment techniques more frequently employed are solid liquid extraction [19], off-line solid phase extraction (SPE) [8,15,16,18,21,22,40] and on-line SPE [44].

This paper reports a new on-line SPE UHPLC–MS/MS method for the simultaneous determination of thirteen PAHs and twelve aldehydes, previously derivatized with 2,4-dinitrophenylhydrazine, in food. The list of PAH includes 11 hydrocarbons considered by EPA as priority pollutants and four couples of isomers, namely anthracene and phenanthrene, benzo[e]pyrene and benzo[k]fluoranthene, dibenzo[a,c]anthracene and dibenzo[a,h]anthracene, fluoranthene and pyrene.

To increase the method sensitivity, mass spectrometer worked in selected reaction monitoring (SRM), observing for each analyte the two most intensive mass spectrometry transitions. The principal advantage of the method is the relatively low analysis time required for the simultaneous determination of PAHs and aldehydes in cooked food, while on-line SPE, already employed by the authors improves method sensitivity and shorten pre-treatment time [45–47]. The whole methodology is suitable for routine analysis. The method has been applied here to the following food samples: smoked salmon, grilled frankfurter, grilled steak, grilled pork chop, steak cooked in butter or in olive oil.

2. Experimental

2.1. Apparatus

The chromatographic analyses were performed using a Dionex (Sunnyvale, USA) Ultimate 3000 UHPLC system equipped by an Ultimate 3000 Degasser, an Ultimate 3000 Pump, an Ultimate 3000 RS Autosampler and an Ultimate 3000 RS column compartment. The system was interfaced with a 3200 QTrap™ LC–MS/MS system (Applied Biosystems, Foster City, USA) by a Turbo V™ interface equipped with an APCI probe. The data were processed by Analyst 1.5.1 software (Toronto, Canada). A homogenizer Ultra-Turrax T25 (IKA-Werke, Staufen, Germany) and an IEC CL31R multispeed centrifuge (Thermo Electron Corporation, Waltham, USA) were employed in sample preparation.

2.2. Reagents

HPLC grade acetonitrile (ACN) and ethanol (>99.8%) were purchased from Merck (Darmstadt, Germany), dichloromethane Chromasolv (>99.9%), methanol Chromasolv (>99.9%) and isopropanol Chromasolv (>99.9%) from Sigma–Aldrich (Milwaukee, USA). Ammonium acetate (99%), 2,4-dinitrophenylhydrazine (DNPH) (>99.0%), sulfuric acid (96–98%) were acquired from Fluka (Buchs, Switzerland). Ultrapure water was produced by a Millipore Milli-Q system (Milford, USA).

Paraformaldehyde (95%) and the other aldehydes (98–99%) were acquired from Fluka (Buchs, Switzerland) and all the PAHs (97–99%) from Sigma–Aldrich (Milwaukee, USA). The analytes studied are reported in Table 1.

The standard stock solutions of PAHs were prepared in ACN and diluted as required with a CH_3OH/H_2O 50/50 (v/v) mixture. The stock solutions were preserved in dark conditions (4 °C) and were stable for three months. For the preparation of DNPH–aldehyde stock solutions see the following paragraph.

2.3. Aldehyde derivatization

The DNPH derivatizing solution was prepared as follows: 0.90 g of DNPH was dissolved in 3.5 mL of H_2SO_4 and then 27.0 mL of a mixture CH_3CH_2OH/H_2O 80/20 (v/v) was slowly added. The resulting solution was carefully filtered on paper filter. The DNPH–aldehyde derivatives were prepared by reaction of 3.5 mL of DNPH solution with an equimolar concentration of the corresponding carbonyl compounds. The DNPH–aldehyde precipitate formed was filtered through a Buchner funnel, recrystallized in hot ACN and dried in desiccator. The stock solutions were prepared by dissolving each DNPH–aldehyde derivative in ACN and diluted as required with a CH_3OH/H_2O 50/50 (v/v) mixture. The solutions were stored in a refrigerator at 4 °C and were stable for a month. LC–MS analysis showed no impurity peak.

2.4. Sample pre-treatment

Smoked salmon, frankfurter, steak, and pork chop purchased in a supermarket were the food samples considered. Two millilitres of DNPH was added on the surface of all the samples that with the exception of the smoked salmon, were cooked at 250 °C for about 5 min. Frankfurter, steak and pork chop were grilled; in addition steak was also cooked in butter and in olive oil. The cooked samples are therefore: (i) grilled frankfurter, (ii) grilled steak, (iii) grilled pork chop, (iv) steak cooked in butter and (v) steak cooked in olive oil. All the samples were undergone to the sample pre-treatment and for the samples (iii), (iv) and (v), also the gravy in which pork chop has been cooked, the butter and the olive oil in which steak has been cooked were also pre-treated and analyzed.

About 10 g of cooked sample was cut into small pieces, homogenized with 10.0 mL of ACN/CH_2Cl_2 80/20 (v/v) mixture in a Falcon tube at 13,500 rpm for 5 min and then centrifuged at 5000 rpm for 5 min. The supernatant was filtered on 0.2 μm PTFE filter, diluted

Table 1
Molecular weight (all the aldehydes reacted with DNPH), time windows during the chromatographic run, SRM transitions (Q1 and Q3 masses) and mass spectrometry parameters: DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential. For each species, the first transition is the most sensitive and was used for quantification and the second one was used for confirmation.

Analyte	Molecular weight (u)	Time window	Q1 mass (*m*/*z*)	Q3 mass (*m*/*z*)	Dwell time (ms)	DP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)
Acenaphthene	154.21	2	154.0	153.0	5	40	7.00	12.85	30	2.26
	154.21	2	154.0	152.0	5	40	7.00	12.85	48	2.43
Acetaldehyde	224.05	1	223.1	151.0	20	−24	−2.50	−20.43	−16	−1.00
	224.05	1	223.1	122.0	20	−24	−2.50	−20.43	−28	−2.83
Acrolein	236.06	1	235.0	158.0	20	−32	−2.50	−20.87	−19	−1.00
	236.06	1	235.0	162.9	20	−32	−2.50	−20.87	−20	−1.00
Anthracene and	178.23	2	178.0	152.1	5	73	10.00	13.64	47	2.31
phenanthrene	178.23	2	178.0	176.0	5	73	10.00	13.64	51	2.44
Benzo[a]pyrene	252.31	2	253.2	252.2	5	70	4.40	16.12	45	3.30
	252.31	2	253.2	250.2	5	70	4.40	16.12	73	3.00
Benzaldehyde	286.13	2	285.0	181.0	80	−37	−4.00	−22.73	−30	−1.00
	286.13	2	285.0	120.0	80	−37	−4.00	−22.73	−35	−1.00
Benzo[e]pyrene and	252.31	2	252.2	250.2	5	100	7.00	16.09	60	2.90
benzo[k]fluoranthene	252.31	2	252.2	248.2	5	100	7.00	16.09	89	3.00
Benzo[ghi]perylene	276.33	2	276.2	274.1	5	117	10.00	16.88	74	3.04
	276.33	2	276.2	272.1	5	117	10.00	16.88	101	3.26
Butanal	252.11	2	251.0	181.0	80	−31	−2.76	−21.46	−25	−1.00
	252.11	2	251.0	191.0	80	−31	−2.76	−21.46	−35	−1.00
Crysene	228.29	2	228.2	226.2	5	69	9.00	15.30	55	2.91
	228.29	2	228.2	202.2	5	69	9.00	15.30	46	2.78
Dibenzo[a,c]anthracene and	278.35	2	278.2	276.1	5	83	10.00	16.95	58	3.12
dibenzo[a,h]anthracene	278.35	2	278.2	274.1	5	83	10.00	16.95	82	2.91
Decanal	336.27	3	335.1	152.0	50	−36	−4.70	−24.57	−36	−1.00
	336.27	3	335.1	122.0	50	−36	−4.70	−24.57	−54	−1.00
Heptanal	294.19	2	293.1	152.0	10	−36	−2.00	−23.02	−31	−1.00
	294.19	2	293.1	122.0	10	−36	−2.00	−23.02	−43	−1.00
Hexanal	280.16	2	279.1	152.1	10	−34	−2.00	−22.50	−27	−1.00
	280.16	2	279.1	122.0	10	−34	−2.00	−22.50	−45	0.00
Fluoranthene and	202.25	2	202.2	200.1	5	78	11.00	14.44	60	2.59
pyrene	202.25	2	202.2	201.1	5	78	11.00	14.44	45	2.82
Fluorene	166.22	2	166.2	165.2	5	48	4.40	13.25	35	2.47
	166.22	2	166.2	164.2	5	48	4.40	13.25	55	2.29
Formaldehyde	210.03	1	208.9	151.0	20	−24	−2.60	−19.90	−13	−1.00
	210.03	1	208.9	76.0	20	−24	−2.60	−19.90	−22	−1.00
Nonanal	322.24	2	321.1	152.0	10	−40	−3.80	−24.06	−37	−1.00
	322.24	2	321.1	163.0	10	−40	−3.80	−24.06	−22	−1.00
Octanal	308.21	2	307.1	152.0	10	−38	−3.00	−23.54	−32	−1.00
	308.21	2	307.1	122.0	10	−38	−3.00	−23.54	−47	−1.00
Pentanal	266.13	2	265.0	152.0	10	−34	−2.00	−21.98	−26	−1.00
	266.13	2	265.0	122.0	10	−34	−2.00	−21.98	−42	−1.00
Propanal	238.08	1	237.1	152.0	20	−20	−3.00	−20.95	−22	−1.00
	238.08	1	237.1	122.0	20	−20	−3.00	−20.95	−31	−1.00

1/2000 (v/v) in a mixture of CH_3OH/H_2O 50/50 (v/v) and then subjected to on-line SPE UHPLC–MS/MS analysis.

2.5. On-line SPE conditions

Sample purification was performed through an on-line SPE method: the SPE column was a Strata C18-E column (2.0 mm × 20.0 mm, 20 μm) (Phenomenex, Milan, Italy).

A mixture of H_2O/CH_3OH 90/10 (v/v) solution (indicated as A in Table 2a) was the loading solution, while the eluting agent had the same initial composition (time = 0 min) of the mobile phase used in the chromatographic separation. To minimize the carryover effects, the autosampler syringe was washed for 22.4 min (medium rate 0.540 mL min^{-1}) with a mixture of ACN/CH_3OH 60/40 (v/v), indicated as B in Table 2a.

The system setup for the on-line SPE consists of three steps. In the loading step 1200 μL of the extract of the real samples is loaded onto the cartridge through the Dionex 3000 autosampler. The trap cartridge is fitted into the loading position of the Valco 6-port switching valve [45–47]. Through the Dionex Ultimate 3000 RS Dual pump the sample is loaded at flow rate of 3.000 mL min^{-1} onto the trapping cartridge (left pump). While the analytes are retained on the SPE column and the matrix is flushed to waste, the analytical LC column is equilibrated by the mobile phase (right pump). In the injection step, at 1.0 min, the valve is switched to injection position that couples the SPE cartridge with the chromatographic column, into which the analytes are transferred. The Dionex right pump is used to provide the gradient elution. In the separation step the analytes are separated in the analytical column. After 25.0 min the valve is switched back to the loading position to equilibrate the on-line SPE cartridge with the loading phase flowing at 4.000 mL min^{-1} for 2.0 min, prior to go back to the initial conditions and inject the next sample. The on-line SPE conditions are reported in detail in Table 2a.

2.6. UHPLC–MS/MS conditions

The stationary phase was an Acquity UPLC HSS T3 column (2.1 × 150 mm, 1.8 μm) purchased from Waters (Milford, USA). The mobile phase was a mixture of 10.0 mM ammonium acetate solution (component C), ACN/H_2O 85/15 (v/v) solution (component D), and $ACN/CH_3OH/(CH_3)_2CHOH$ 49/49/2 (v/v/v) (component E) eluting at flow rate 0.250 mL min^{-1} and under the UHPLC gradient conditions shown in Table 2b. The chromatographic column was re-equilibrated for 5 min between consecutive analyses. The temperatures of the autosampler and of the column oven were set at 5 °C and 40 °C, respectively. All the connection tubes must be in

Table 2
Mobile phase gradient for on-line SPE system and UHPLC. (a) On-line SPE conditions, percentage of: (A) H_2O/CH_3OH 90/10 (v/v); (B) ACN/CH_3OH 60/40 (v/v). (b) UHPLC conditions, percentage of: (C) ammonium acetate 10.0 mM; (D) ACN/H_2O 85/15 (v/v); (E) $ACN/CH_3OH/(CH_3)_2CHOH$ 49/49/2 (v/v/v).

Left pump on-line SPE				
Time (min)	Flow ($mL\,min^{-1}$)	Valve position	A%	B%
(a)				
0.0	3.000	Loading	100	0
1.0	3.000	Injection	100	0
1.5	0.100	Injection	100	0
4.6	0.100	Injection	0	100
24.0	0.100	Injection	0	100
25.0	4.000	Loading	0	100
27.0	4.000	Loading	0	100
27.1	3.000	Loading	100	0

Right pump UHPLC					
Time (min)	Flow ($mL\,min^{-1}$)	Valve position	C%	D%	E%
(b)					
0.0	0.250	Loading	100	0	0
1.0	0.250	Injection	100	0	0
16.7	0.250	Injection	0	100	0
22.2	0.250	Injection	0	100	0
22.3	0.250	Injection	0	0	100
25.0	0.250	Loading	0	0	100
25.1	0.250	Loading	100	0	0
30.0	0.250	Loading	100	0	0

stainless steel, because peek connections could interact in the long run with the DNPH reagent.

The APCI was obtained using the Turbo V™ interface working both in positive ion mode (PI) for PAH compounds and in negative ion mode (NI) for DNPH–aldehyde compounds. The instrumental parameters were set as follows: Curtain Gas (N_2) at 20 psig, Nebulizer Gas GS1 and GS2 at 40 and 0 psig respectively, desolvation temperature (TEM) at 450 °C, collision activated dissociation gas (CAD) at 6 units of the arbitrary scale of the instrument and nebulized current (NC) at ±4.5 μA depending on the working polarity (PI or NI). Unit mass resolution was established and maintained in each mass-resolving quadrupole by keeping a full width at half maximum (FWHM) of about 0.7 μm.

3. Results and discussion

3.1. Mass spectrometry characterization

Since PAHs and DNPH–aldehydes present different physical–chemical properties, to perform their simultaneous separation in mass spectrometry detection, ionization conditions suitable for all the analytes must be chosen. This means that best compromise must be searched for kind of source and polarity ionization, kind of the solvent and temperature of the ionization source. The first important choice to be done is the kind of source and the ionization polarity. Underivatized PAHs are ionized only by APCI source in PI mode and give no *m*/*z* signal in ESI. In turn, DNPH–aldehydes give the best *m*/*z* signals in ESI NI, but they can be ionized, although with less sensitivity, also in APCI NI. The need to use NI mode is due to the presence in DNPH–aldehyde structure of nitro groups that stabilize by resonance the negative charge on the aromatic ring. Therefore, to simultaneously ionize PAHs and DNPH–aldehydes, APCI source must be used, switching the polarity from PI (suitable for PAHs) to NI (suitable for DNPH–aldehydes).

The second condition to choose is the organic solvent able to favor the ionization process. While the use of CH_3OH allows to obtain better DNPH–aldehyde signals than with ACN, the opposite effect is observed for PAHs. With the only exception of benzo[a]pyrene, for PAHs the precursor ion is a radical cation, whose formation is disadvantaged by the protic solvent CH_3OH. The use of the aprotic solvent ACN allowed the ionization of all the analytes, included DNPH–aldehydes that anyway give suitable signals.

The third condition is the temperature of the ionization source, since temperature plays opposite effects on the chemicals. A temperature increase from 350 to 600 °C (step of 50 °C) caused, together with an increased PAH ionization, a progressive ionization decrease of DNPH–aldehydes, until to signal disappearance for DNPH–formaldehyde and DNPH–acetaldehyde. The best compromise was reached for a temperature of 450 °C. As concerns the nebulized current, the optimum value for all the analytes was 4.5 μA.

Under these conditions, the analytes were then subjected to a MS/MS characterization study in APCI source in PI mode for PAHs and in NI mode for DNPH–aldehydes, with the double purpose to identify the successive fragments formed under increasing collision energy and to optimize the instrumental potential values. The characterization experiments were carried out for direct infusion of 1.0 $mg\,L^{-1}$ ACN solutions of each analyte connected through a T valve to the syringe pump (syringe flow-rate: 20.0 $\mu L\,min^{-1}$, chromatographic pump flow-rate 200 $\mu L\,min^{-1}$).

All the analytes presented many transitions: for each of them the most intense was used for the quantitative analysis and referred as "quantifier" transition, while the second one (the "qualifier" transition) was employed to confirm the identification. The "quantifier" and "qualifier" transitions are reported for all the analytes in Table 1, together with the instrumental potential values.

3.2. Development and optimization of the on-line SPE UHPLC–MS/MS method

Taking into account that as mentioned, PAHs and DNPH–aldehydes ionize in PI and NI mode respectively, experiments have been performed with the ambitious aim to find, if possible, chromatographic conditions able to split all the compounds along the chromatographic run and to elute the two classes of congeners in two different time windows. The target was to switch the ionization polarity only once, so increasing the dwell time of the monitored transitions and improving sensitivity. In this optimization step of the chromatographic conditions mainly devoted to analyte separation, we did not introduce the on-line SPE process yet.

Playing on the different chemical properties of the compounds to be separated, we searched for the conditions able to retain PAHs mainly through hydrophobic interactions and DNPH–aldehydes through prevailing anionic interactions, suggested by the low pK_a value of DNPH [48,49]. At this purpose we chose an Acclaim Trinity P1 stationary phase (3.0 mm × 100 mm, 3 μm, Dionex, Sunnyvale, USA) suitable for multiple retention mechanisms including reversed-phase, anion-exchange and cation-exchange. Both the variation of ammonium acetate concentration (from 0 to 100 mM) and the variation of mobile phase pH (for addition of formic or acetic acid) exert the same effect on DNPH–aldehydes and PAHs.

To favor the anionic interactions of DNPH–aldehydes, we coupled the on-line SPE with UHPLC–MS/MS system and experimented the use of different stationary phase packings, taking into account the tolerated back-pressures of the SPE cartridge and of the chromatographic column. Also with the use of both weak and strong anion exchange cartridges (Poros PI 2.1 mm × 30 mm, 10 μm, Applied Biosystems, Foster City, USA and Poros HQ 2.1 mm × 30 mm, 10 μm, Applied Biosystems, Foster City, USA, respectively) and different pH conditions (from addition of 1%

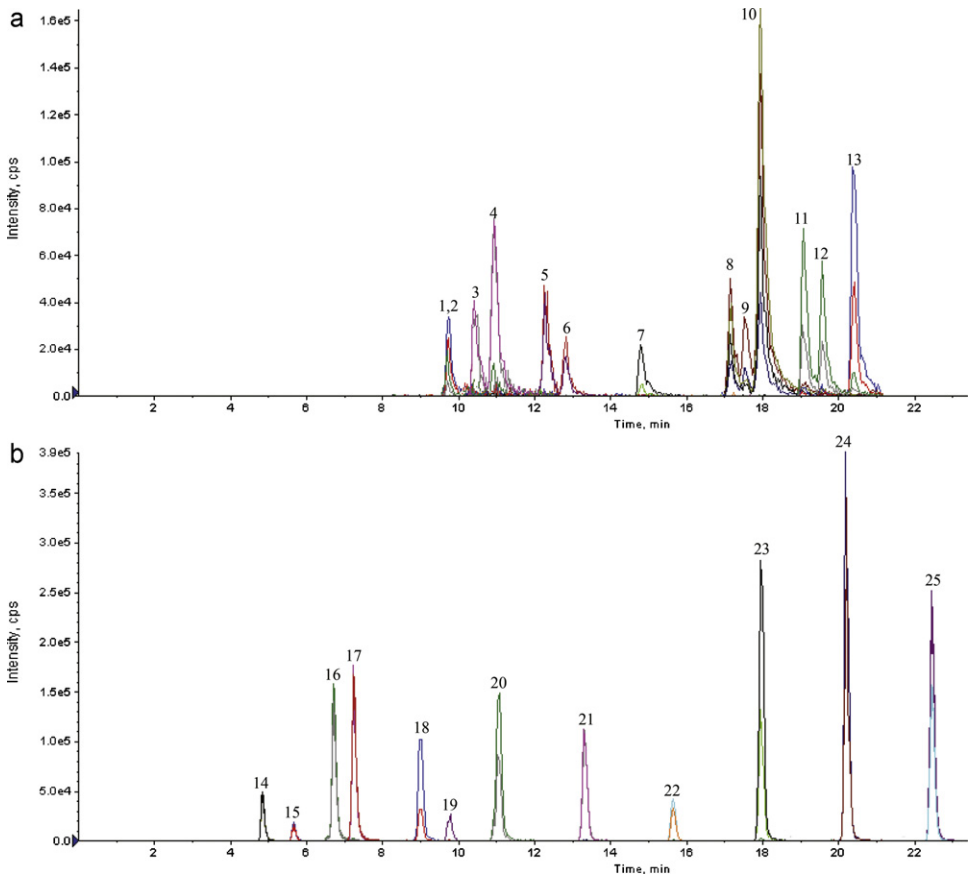

Fig. 1. A typical on-line SPE UHPLC–MS/MS chromatogram of a standard mixture of the analytes (50.000 μg L^{-1} each). The chromatographic conditions are reported in Section 2. (a) PAH separation in APCI PI mode. (b) DNPH–aldehyde separation in APCI NI mode. Chromatographic peaks: (1 and 2) acenaphthene and fluorene; (3) phenantrene; (4) anthracene; (5) fluoranthene; (6) pyrene; (7) crysene; (8) benzo[e]pyrene; (9) benzo[k]fluoranthene; (10) benzo[a]pyrene; (11) dibenzo[a,c]anthracene; (12) dibenzo[a,h]anthracene; (13) benzo[g,h,i]perylene; (14) formaldehyde; (15) acetaldehyde; (16) acrolein; (17) propanal; (18) butanal; (19) benzaldehyde; (20) pentanal; (21) hexanal; (22) heptanal; (23) octanal; (24) nonanal; (25) decanal.

acetic acid to addition of 1% ammonia), the DNPH–aldehydes preferentially undergo hydrophobic interactions with respect to anionic ones.

Therefore, we changed the SPE cartridge with a Strata C18-E (2.0 mm × 20 mm, 20 μm, Phenomenex, Milan, Italy) and tested other stationary phases as Poroshell 120 SB-C18 column (2.1 mm × 50 mm, 2.7 μm, Agilent Technologies, Milan, Italy) and Acquity UPLC BEH Phenyl column (2.1 mm × 100.0 mm, 1.7 μm, Waters Milford, USA). In all cases a resolution decrease was observed. The best resolution was obtained by the use of an Acquity UPLC HSS T3 column (2.1 mm × 30.0 mm, 1.8 μm, Waters Milford, USA), in which, unlike the other C18 columns, the T3 bonding utilizes a trifunctional C18 alkyl phase bonded at a ligand density that promotes polar compounds retention. Actually, the T3 endcapping process is much more effective than the traditional trimethyl silane endcapping. The optimization of the UHPLC mobile phase indicated that the use of 10.0 mM ammonium acetate and ACN/H_2O 85/15 (v/v) favored the complete desorption of all the analytes (including the isomeric PAHs) from the SPE cartridge and gave the best separation. A post-column addition of ACN with or without ammonia (0.2% v/v) did not improved the *m*/*z* signals of DNPH–aldehydes.

The use of a mixture ACN/CH_3OH/$(CH_3)_2CHOH$ 49/49/2 (v/v/v) for 2.7 min assured a complete elution of possible matrix interfering compounds retained on SPE cartridge or on the chromatographic column. The increase of column temperature from 30 to 60 °C, experimented to decrease the chromatographic run time, showed that for temperatures greater than 40 °C the resolution between the isomeric PAHs was lost.

The maximum organic percentage in the loading solution that guarantees a good retention of all the analytes on the SPE cartridge was 10% (v/v) CH_3OH.

The linearity of the response as a function of the total injected amount was evaluated by injecting larger injection volumes (namely 300, 600, 1200 and 1600 μL): for all the analytes the response increases proportionally with the injected volume up to the injection volume of 1200 μL.

To maximize the sensitivity of the method and increase as much as possible the dwell time of the *m*/*z* transitions, the chromatographic run was separated in three different time windows (Table 1). The first window ranged from 0 to 8 min, the second from 8 to 21 min and the third from 21 to 28 min.

Fig. 1 reports a typical chromatogram of the standard mixture of the analytes (50.000 μg L^{-1} each), recorded under the optimized conditions described.

3.3. Validation of the analytical method

For each analyte a calibration plot reporting the peak area of the "quantifier" transition signal (*y*) versus standard concentration (*x*) was built: eleven concentration levels in the range between the LOQ value and 50.000 μg L^{-1} were considered. To overcome possible memory effects, the standard solutions were injected in randomized order. For all the analytes a linear regression fit was used with

Table 3
Regression coefficient (R^2), LOD, LOQ, intra- and inter-day RSD (%) on concentration for all the analytes considered.

Analyte	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	R^2	Intra-day RSD (%) ($n=5$)	Inter-day RSD (%) ($n=35$)
Acenaphthene	0.265	1.000	0.9952	2.2	7.7
Acetaldehyde	0.006	0.020	0.9994	3.4	6.0
Acrolein	0.002	0.006	0.9998	1.6	3.7
Anthracene	0.064	0.250	0.9990	4.0	5.8
Benzo[a]pyrene	0.028	0.100	0.9982	2.5	9.7
Benzaldehyde	0.125	0.371	0.9980	2.4	7.1
Benzo[e]pyrene	0.315	1.000	0.9988	1.1	6.7
Benzo[ghi]perylene	0.143	0.500	0.9978	1.2	2.6
Benzo[k]fluoranthene	0.768	2.500	0.9932	3.9	8.8
Butanal	0.047	0.143	0.9990	3.6	2.2
Crysene	0.073	0.250	0.9952	3.2	7.5
Dibenzo[a,c]anthracene	0.347	1.000	0.9986	3.7	4.5
Dibenzo[a,h]anthracene	0.339	1.250	0.9992	1.7	2.0
Decanal	0.004	0.012	0.9998	3.0	5.0
Heptanal	0.059	0.194	0.9934	2.3	9.5
Hexanal	0.012	0.036	0.9936	1.6	1.8
Fluoranthene	0.291	1.000	0.9962	1.9	8.3
Fluorene	0.291	1.000	0.9922	1.4	4.9
Formaldehyde	0.002	0.007	0.9994	2.0	3.0
Nonanal	0.008	0.022	0.9956	3.4	8.0
Octanal	0.006	0.021	0.9956	4.0	4.2
Pentanal	0.002	0.008	0.9922	4.0	1.5
Phenanthrene	0.137	0.500	0.9974	2.0	4.1
Propanal	0.002	0.006	0.9996	2.4	4.2
Pyrene	0.262	1.000	0.9984	2.2	7.7

a weighting factor $1/x$ and for all the calibration plots a good linearity with regression coefficients (R^2) always greater than 0.9922 was obtained.

The limit of detection (LOD) is calculated as the concentration of the analyte that gives a signal (peak area) equal to the average background (S_{blank}) plus three times the standard deviation s_{blank} of the blank ($LOD = S_{blank} + 3s_{blank}$), while the limit of quantification LOQ is given as $LOQ = S_{blank} + 10s_{blank}$ [50]. R^2 of the calibration plots, LOD and LOQ is reported in Table 3 for all the analytes. LOD values for PAHs range from 0.028 to 0.768 $\mu g\,L^{-1}$ and for aldehydes from 0.002 to 0.125 $\mu g\,L^{-1}$. These values are comparable with those obtained in other studies just devoted to the only determination of PAHs [19,21,23,37] or aldehydes [31,40].

The method detection limit (MDL) and the method quantitation limit (MQL) of each analyte in the matrix were determined by using a statistical approach [51,52]. The procedure involves the spiking of seven replicates of each blank matrix with each analyte at a concentration that gives an instrumental signal to noise ratio between 2.5 and 5. The MDL was then calculated as $MDL = t_{(n-1,1-\alpha=0.99)} \times S_d$ where $t = 3.14$ that corresponds to the Student's value appropriate for a 99% confidence level and 6 degrees of freedom, whereas S_d is the standard deviation of the replicate analyses. The MDL values ranged from 0.001 to 1.833 μg/kg as reported in Table 4, MQLs, evaluated as 3 MDLs, ranged from 0.003 to 5.499 μg/kg.

The intra- and inter-day precisions on retention time and on concentration were evaluated by analyzing a standard mixture of all the analytes (20.000 $\mu g\,L^{-1}$ each) every day (five replicates) for a week. The results show that intra-day precision of retention time ranges from 0.2% to 0.3% and inter-day precision from 0.6% to 4.0%. The intra-day and inter-day relative standard deviation (RSD%) of concentration ranges from 1.1% to 4.0% and from 1.5% to 9.7%, respectively (Table 3).

To check the stability of the system as it concerns the quantitative response, at random intervals along the analyses, two standard quality control (QC) solutions of the mixture of the analytes at concentration of 5.000 and 10.000 $\mu g\,L^{-1}$ were injected. All the results obtained for the QC solutions lay within the $\pm 3\sigma$ control limits of the calibration plots.

No memory effect was evidenced, likely due to the optimized washing process of the SPE sorbent during the loading step.

3.4. Optimization of the extraction solvent of the sample pre-treatment

In order to maximize the analyte extraction, we tested several solvents and mixtures of solvents. The use of the only ACN did not allow a complete extraction, in particular of PAHs and of the more lipophilic DNPH–aldehydes and, in addition, did not help in clarifying the supernatant solution. Therefore, mixtures of ACN with more apolar solvents were investigated. The mixture ACN/dioxane 50/50 (v/v) did not improve the extraction process and did not remove solution turbidity. The use of ACN/toluene 50/50 (v/v) permitted to clarify the supernatant solution and to increase PAH extraction, but the DNPH–aldehyde extraction worsened.

The best results were obtained by using ACN/CH_2Cl_2 80/20 (v/v).

3.5. UHPLC–MS/MS analysis of real samples and evaluation of recovery

The whole UHPLC–MS/MS methodology developed was applied to the analysis of nine different matrices, namely: (i) smoked salmon, (ii) grilled frankfurter, (iii) grilled steak, (iv) grilled pork chop, (v) grilled pork chop gravy, (vi) steak cooked in butter, (vii) the butter in which steak has been cooked, (viii) steak cooked in olive oil and (ix) the olive oil in which steak has been cooked.

Taking into account the performance criteria suggested by the EU Commission [53], also the SRM ratio between the abundances of the two selected transitions (qualifier transition to quantifier transition) was used to confirm the analyte identification: in the presence of the analyte the calculated SRM ratio in the real sample must be within ±20% of the average SRM ratio calculated for the standard. For each concentration level considered in the calibration plot, the SRM ratio was calculated. The quantification data obtained by standard addition method (at concentrations 2-, 3- and 4-folds the native concentration) are reported in Table 5.

To evaluate the recovery R of each analyte in each sample matrix and to verify its possible dependence on the concentration, mixtures of analyte standard solutions at three different concentration levels were added to the food samples. The solutions were prepared taking into account that samples are diluted 1/2000 (v/v) in a 50/50 (v/v) mixture of H_2O/CH_3OH solution prior to injection, in order

Table 4
Method detection limit (MDL) for all the analytes considered in each sample.

Analyte	Smoked salmon (μg/kg)	Grilled frankfurter (μg/kg)	Grilled steak (μg/kg)	Grilled pork chop (μg/kg)	Grilled pork chop gravy (μg/kg)	Steak cooked in butter (μg/kg)	Steak-cooked butter (μg/kg)	Steak cooked in olive oil (μg/kg)	Steak-cooked olive oil (μg/kg)
Acenaphthene	0.367	0.240	0.261	0.286	0.300	0.271	0.150	0.270	0.174
Acetaldehyde	0.012	0.008	0.009	0.009	0.010	0.009	0.005	0.009	0.006
Acrolein	0.003	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001
Anthracene	0.122	0.080	0.087	0.095	0.100	0.090	0.050	0.090	0.058
Benzo[a]pyrene	0.049	0.032	0.035	0.038	0.040	0.036	0.020	0.036	0.023
Benzaldehyde	0.272	0.178	0.193	0.212	0.223	0.201	0.111	0.200	0.129
Benzo[e]pyrene	0.367	0.240	0.261	0.286	0.300	0.271	0.150	0.270	0.174
Benzo[ghi]perylene	0.428	0.280	0.304	0.334	0.350	0.317	0.175	0.315	0.203
Benzo[k]fluoranthene	1.833	1.200	1.303	1.431	1.500	1.357	0.750	1.351	0.871
Butanal	0.105	0.069	0.075	0.082	0.086	0.078	0.043	0.077	0.050
Crysene	0.214	0.140	0.152	0.167	0.175	0.158	0.088	0.158	0.102
Dibenzo[a,c]anthracene	0.367	0.240	0.261	0.286	0.300	0.271	0.150	0.270	0.174
Dibenzo[a,h]anthracene	0.611	0.400	0.434	0.477	0.500	0.452	0.250	0.450	0.290
Decanal	0.006	0.004	0.004	0.004	0.005	0.004	0.002	0.004	0.003
Heptanal	0.071	0.047	0.051	0.056	0.058	0.053	0.029	0.052	0.034
Hexanal	0.026	0.017	0.019	0.020	0.021	0.019	0.011	0.019	0.012
Fluoranthene	0.855	0.560	0.608	0.668	0.700	0.633	0.350	0.630	0.406
Fluorene	0.489	0.320	0.348	0.382	0.400	0.362	0.200	0.360	0.232
Formaldehyde	0.005	0.003	0.004	0.004	0.004	0.004	0.002	0.004	0.002
Nonanal	0.016	0.011	0.012	0.013	0.013	0.012	0.007	0.012	0.008
Octanal	0.010	0.007	0.007	0.008	0.008	0.008	0.004	0.007	0.005
Pentanal	0.003	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001
Phenanthrene	0.244	0.160	0.174	0.191	0.200	0.181	0.100	0.180	0.116
Propanal	0.003	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001
Pyrene	0.367	0.240	0.261	0.286	0.300	0.271	0.150	0.270	0.174

Table 5
Quantification data in the real samples (n.d. for not detected). (a) Smoked salmon, grilled frankfurter, grilled steak, grilled pork chop and grilled pork chop gravy. (b) Steak cooked in butter, butter in which steak has been cooked, steak cooked in olive oil and olive oil in which steak has been cooked.

Analyte	Smoked salmon (μg/kg)	Grilled frankfurter (μg/kg)	Grilled steak (μg/kg)	Grilled pork chop (μg/kg)	Grilled pork chop gravy (μg/kg)
(a)					
Acenaphthene	n.d.	n.d.	<LOQ	n.d.	<LOQ
Acetaldehyde	6291 ± 295	10361 ± 747	2261 ± 98	1787 ± 108	2389 ± 92
Acrolein	57 ± 4	93 ± 5	51 ± 3	87 ± 5	62 ± 5
Anthracene	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo[a]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.
Benzaldehyde	n.d.	n.d.	<LOQ	n.d.	n.d.
Benzo[e]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo[ghi]perylene	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo[k]fluoranthene	n.d.	n.d.	n.d.	n.d.	n.d.
Butanal	3750 ± 112	910 ± 66	567 ± 27	<LOQ	<LOQ
Crysene	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,c]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.
Decanal	391 ± 28	n.d.	<LOQ	79.9 ± 0.7	171 ± 7
Heptanal	<LOQ	470 ± 25	404 ± 19	<LOQ	n.d.
Hexanal	615 ± 36	1441 ± 111	278 ± 20	251 ± 18	291 ± 16
Fluoranthene	n.d.	n.d.	n.d.	n.d.	n.d.
Fluorene	n.d.	<LOQ	n.d.	n.d.	n.d.
Formaldehyde	855 ± 56	2988 ± 243	795 ± 67	513 ± 49	272 ± 21
Nonanal	764 ± 62	675 ± 44	252 ± 20	n.d.	n.d.
Octanal	85.2 ± 0.54	n.d.	1107 ± 79	n.d.	268 ± 22
Pentanal	3178 ± 223	893 ± 81	n.d.	984 ± 84	945 ± 58
Phenanthrene	n.d.	n.d.	n.d.	n.d.	2620 ± 180
Propanal	3023 ± 190	3145 ± 217	210 ± 14	206 ± 14	140 ± 10
Pyrene	n.d.	n.d.	<LOQ	n.d.	n.d.

Analyte	Steak cooked in butter (μg/kg)	Steak-cooked butter (μg/kg)	Steak cooked in olive oil (μg/kg)	Steak-cooked olive oil (μg/kg)
(b)				
Acenaphthene	n.d.	n.d.	n.d.	<LOQ
Acetaldehyde	1942 ± 114	n.d.	2955 ± 100	n.d.
Acrolein	n.d.	10.7 ± 0.3	n.d.	n.d.
Anthracene	n.d.	n.d.	n.d.	n.d.
Benzo[a]pyrene	n.d.	n.d.	n.d.	n.d.
Benzaldehyde	n.d.	n.d.	n.d.	n.d.
Benzo[e]pyrene	n.d.	n.d.	n.d.	n.d.
Benzo[ghi]perylene	n.d.	n.d.	n.d.	n.d.
Benzo[k]fluoranthene	n.d.	n.d.	n.d.	n.d.
Butanal	n.d.	n.d.	<LOQ	n.d.
Crysene	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,c]anthracene	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	n.d.
Decanal	n.d.	n.d.	267 ± 13	n.d.
Heptanal	<LOQ	n.d.	n.d.	n.d.
Hexanal	151 ± 12	n.d.	161 ± 13	n.d.
Fluoranthene	n.d.	n.d.	n.d.	n.d.
Fluorene	n.d.	n.d.	<LOQ	n.d.
Formaldehyde	606 ± 39	113 ± 9	789 ± 37	n.d.
Nonanal	n.d	n.d.	335 ± 27	n.d.
Octanal	n.d.	n.d.	n.d.	n.d.
Pentanal	206 ± 12	n.d.	77 ± 5	n.d.
Phenanthrene	n.d.	n.d.	n.d.	n.d.
Propanal	19.3 ± 0.4	n.d.	452 ± 12	n.d.
Pyrene	n.d.	n.d.	n.d.	n.d.

to obtain final concentration values laying in the linearity range (LOQ value, 5.000 and 10.000 $\mu g\,L^{-1}$). Each analysis was repeated three times. The recovery values were calculated as C_{obs}/C_{ref} where C_{obs} is the difference between the concentration determined for the spiked sample and the native concentration in the same sample, and C_{ref} is the spiked concentration. A *t*-test at 95% confidence level showed that for all the analytes the difference among the *R* values obtained for the three concentration levels was not statistically significant and indicated that, in the explored concentration range, recovery does not depend on analyte concentration. For all the analytes and for each sample matrix an average percentage of recovery $\bar{R}$ (%) was therefore calculated and reported in Table 6: as it can be observed all the $\bar{R}$ (%) values range from 70.6% to 120.0%.

As reported in Table 5, no significant difference can be envisaged between the results obtained for the steak sample, when cooked in butter or in olive oil, with the only exception of the greater amount of DNPH–aldehydes (like propanal, nonanal, and decanal) formed during the cooking in oil. In both the samples no PAH was found. Most of the aldehydes were anyway found in the cooked steak, whereas the presence of the aldehydes is much lower both in the butter and in the olive oil in which steak has been cooked.

Also the aldehyde content of the three samples (frankfurter, steak and pork chop) undergone to the grilled cooking process was comparable, while some differences can be observed in the PAH content. We found acenaphthene and pyrene in grilled steak, and fluorene in frankfurter. While no PAH at detectable level was found in pork chop, in the gravy in which pork chop has been cooked, the presence of acenaphthene and phenanthrene has been found.

Particular interest was devoted to the results obtained in the analysis of the smoked salmon, the only food that has not been

Table 6
Average recovery yields $\bar{R}$ (%) and matrix effect (ME) evaluation in the different matrices. (a) Smoked salmon, grilled frankfurter, grilled steak, grilled pork chop and grilled pork chop gravy. (b) Steak cooked in butter, butter in which steak has been cooked, steak cooked in olive oil and olive oil in which steak has been cooked.

Analyte	Smoked salmon		Grilled frankfurter		Grilled steak		Grilled pork chop		Grilled pork chop gravy	
	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)
(a)										
Acenaphthene	108 ± 11	No	103.8 ± 2.2	No	93.90 ± 0.80	No	90.1 ± 1.7	No	95 ± 10	No
Acetaldehyde	107.0 ± 4.1	−24.2	94.4 ± 5.5	−16.7	100.3 ± 8.7	−15.1	103.4 ± 8.5	−19.4	100.2 ± 5.3	−24.2
Acrolein	100.6 ± 4.8	−12.9	105 ± 11	−13.2	97.3 ± 3.1	−15.8	97.7 ± 2.3	−18.5	101.1 ± 7.0	−15.7
Anthracene	95.0 ± 5.0	No	116.3 ± 5.2	No	117.1 ± 5.9	No	89.0 ± 5.3	No	94.8 ± 7.4	No
Benzo[a]pyrene	106.20 ± 0.90	No	92.0 ± 3.7	No	94.2 ± 7.2	No	96.6 ± 5.6	No	101 ± 10	No
Benzaldehyde	115 ± 15	+45.0	84.8 ± 6.3	+49.9	101.8 ± 7.0	No	89.2 ± 9.4	+23.2	90.9 ± 7.3	+16.0
Benzo[e]pyrene	95.2 ± 6.4	+34.2	116.4 ± 7.9	No	109 ± 11	No	117.5 ± 8.6	No	88.54 ± 0.21	No
Benzo[ghi]perylene	79.3 ± 4.9	No	77.9 ± 3.2	No	104 ± 11	−33.7	78.6 ± 5.5	No	74.1 ± 3.5	No
Benzo[k]fluoranthene	93.7 ± 5.9	+62.0	89.3 ± 6.1	+6.7	103.0 ± 8.2	No	119.0 ± 9.9	No	88.8 ± 6.2	No
Butanal	103.0 ± 6.0	No	110 ± 12	No	97.3 ± 5.9	No	117.6 ± 3.0	No	86.5 ± 5.1	+29.0
Crysene	107.1 ± 8.4	No	84.5 ± 8.9	No	116.7 ± 2.4	No	102.4 ± 7.7	No	110.2 ± 3.1	No
Dibenzo[a,c]anthracene	119.4 ± 5.4	+17.2	102.5 ± 5.9	No	91.8 ± 6.4	No	75.5 ± 5.4	No	74.7 ± 3.5	No
Dibenzo[a,h]anthracene	110.0 ± 6.8	+21.0	113.8 ± 5.4	No	91.5 ± 4.5	No	103.5 ± 6.9	No	94.3 ± 4.4	No
Decanal	99.0 ± 7.2	−7.4	105 ± 14	−11.8	93.97 ± 0.22	−21.6	97.81 ± 0.24	−16.9	100.1 ± 3.9	−14.8
Heptanal	103.2 ± 4.2	No	98.4 ± 2.8	No	93.6 ± 7.8	No	93.8 ± 6.2	No	102.8 ± 3.9	No
Hexanal	79.1 ± 3.8	No	98.1 ± 6.5	No	91.9 ± 7.0	No	91.5 ± 2.1	+31.4	93.3 ± 3.9	+32.2
Fluoranthene	94.5 ± 7.4	No	78.7 ± 8.4	No	87.0 ± 8.8	No	99.4 ± 3.6	No	97.2 ± 8.7	No
Fluorene	97.2 ± 4.5	No	101.2 ± 4.1	No	104 ± 12	No	103.1 ± 5.4	No	100 ± 10	No
Formaldehyde	89.9 ± 3.2	−15.2	72.3 ± 2.5	−24.3	94 ± 11	−24.8	92.1 ± 8.4	−30.2	93.1 ± 7.9	−12.4
Nonanal	83.50 ± 0.24	No	81.3 ± 6.4	No	82.4 ± 6.0	No	89.7 ± 7.0	No	110.3 ± 2.3	No
Octanal	84.62 ± 0.27	No	92.8 ± 2.5	No	119.1 ± 5.1	No	119.0 ± 5.6	No	115.7 ± 3.1	No
Pentanal	91.6 ± 5.7	No	104.0 ± 2.8	No	97 ± 10	No	89.3 ± 5.4	No	105.3 ± 7.3	No
Phenanthrene	83.4 ± 8.6	No	82.8 ± 4.1	No	88.9 ± 1.3	No	102.5 ± 8.9	No	83.3 ± 5.0	No
Propanal	103.7 ± 5.2	No	77.1 ± 1.3	−32.6	84.7 ± 6.3	−36.9	82.2 ± 5.3	−38.5	89.1 ± 6.9	−34.0
Pyrene	82.6 ± 2.9	+38.2	105.2 ± 8.4	+28.4	110 ± 10	No	109.3 ± 8.9	No	82.3 ± 3.5	+22.0

Analyte	Steak cooked in butter		Steak-cooked butter		Steak cooked in olive oil		Steak-cooked olive oil	
	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)	$\bar{R}$ (%)	ME (%)
(b)								
Acenaphthene	86.4 ± 5.2	No	80.5 ± 1.7	No	75.8 ± 4.2	No	73.9 ± 5.1	No
Acetaldehyde	92.4 ± 6.2	No	101.2 ± 2.4	−16.0	95.1 ± 6.4	−20.8	99.3 ± 8.0	−23.1
Acrolein	96.7 ± 3.3	−15.4	96.9 ± 1.9	−20.2	99.7 ± 7.7	−18.4	94.7 ± 2.8	−20.2
Anthracene	90.4 ± 3.6	No	70.6 ± 4.2	No	68.2 ± 4.0	No	98.8 ± 6.0	No
Benzo[a]pyrene	95.1 ± 8.5	No	78.5 ± 5.3	No	94.3 ± 9.9	No	96.9 ± 3.3	No
Benzaldehyde	86.9 ± 4.1	No	106 ± 10	No	93.2 ± 7.6	+29.5	72.4 ± 4.2	No
Benzo[e]pyrene	80.5 ± 2.2	No	74.50 ± 0.14	No	96.6 ± 1.9	−30.3	102.3 ± 9.4	−45.4
Benzo[ghi]perylene	78.75 ± 0.10	−33.5	73.9 ± 5.6	No	83.1 ± 1.4	−41.2	91.1 ± 3.8	−34.3
Benzo[k]fluoranthene	112 ± 17	No	92.1 ± 2.3	No	103 ± 11	No	103 ± 10	No
Butanal	120.0 ± 1.3	+28.9	90.6 ± 3.1	+24.3	91.4 ± 3.6	+27.7	110 ± 10	No
Crysene	99.2 ± 5.9	No	77.8 ± 5.6	No	91.4 ± 8.9	No	91.3 ± 8.1	No
Dibenzo[a,c]anthracene	71.6 ± 1.5	−30.1	86.8 ± 7.0	−38.1	85.3 ± 6.7	No	86.7 ± 5.3	No
Dibenzo[a,h]anthracene	92.1 ± 5.1	−13.1	91.5 ± 9.1	−17.4	89.9 ± 6.6	−37.0	101.0 ± 8.2	−24.4
Decanal	86.0 ± 8.4	−23.8	70.9 ± 4.7	−49.6	99.6 ± 3.6	−15.1	92.5 ± 4.7	−20.0
Heptanal	100.7 ± 8.2	No	97.1 ± 4.1	No	108 ± 10	No	115.5 ± 4.0	No
Hexanal	93.6 ± 6.6	+8.8	117.0 ± 8.1	No	116 ± 11	+67.0	119.7 ± 8.8	No
Fluoranthene	73.1 ± 2.7	No	80.5 ± 2.9	No	102.9 ± 3.4	No	102.7 ± 3.6	No
Fluorene	81.4 ± 2.3	No	71.9 ± 2.1	No	86.7 ± 6.7	No	81.0 ± 2.3	No
Formaldehyde	94.0 ± 5.3	−15.2	90.3 ± 3.9	−28.1	98 ± 10	+22.1	84.6 ± 5.3	−35.3
Nonanal	113.4 ± 2.6	No	94.2 ± 2.0	No	88.5 ± 5.1	+37.0	111.5 ± 9.3	No
Octanal	100.4 ± 7.7	No	101.3 ± 2.7	No	119.5 ± 9.8	No	109.3 ± 7.2	No
Pentanal	106.2 ± 8.0	No	104.4 ± 5.1	No	110.7 ± 5.6	No	114.2 ± 5.4	No
Phenanthrene	86.3 ± 1.3	No	71.0 ± 6.2	No	98.7 ± 7.4	No	87.5 ± 6.8	No
Propanal	85.5 ± 1.0	−37.5	87.2 ± 5.6	−40.0	84.3 ± 2.1	−36.1	85.7 ± 2.7	−40.0
Pyrene	91.65 ± 0.35	No	96.3 ± 5.1	No	95.00 ± 0.57	No	74.6 ± 3.4	No

cooked, because bought already smoked. Fig. 2 reports a typical chromatogram of an extract of smoked salmon previously diluted in 1/200 (v/v) H_2O/CH_3OH mixture. A high amount of aldehydes has been found: aldehydes are already present in the sample acquired that was not further cooked. It can be noticed that, on contrary, no PAH at detectable level was observed, even if their presence was expected as a consequence of the smoking process.

3.6. Matrix effect evaluation

To evaluate the presence of the matrix effect (ME), a *t*-test at 95% confidence level was applied to compare the slopes of the external calibration plot and of the standard addition plot, built for all the real samples.

The samples prepared by adding a mixture of the standard analytes (at concentrations 2-, 3- and 4-folds the native concentration) to the extracts were undergone to the on-line SPE UHPLC–MS/MS analysis and the analysis was repeated three times.

The average matrix effect was estimated for each analyte through the ratio $slope_{add}/slope_{ext}$, where $slope_{add}$ is the slope of the standard addition plot and $slope_{ext}$ is the slope of the external calibration plot [46,54,55]. The percentages of matrix effect were calculated as $ME(\%) = (slope_{add}/slope_{ext}) \times 100 - 100$. When ME (%) is equal to 0 there is no ME, a negative value indicates a signal suppression and a positive one a signal enhancement (Table 6).

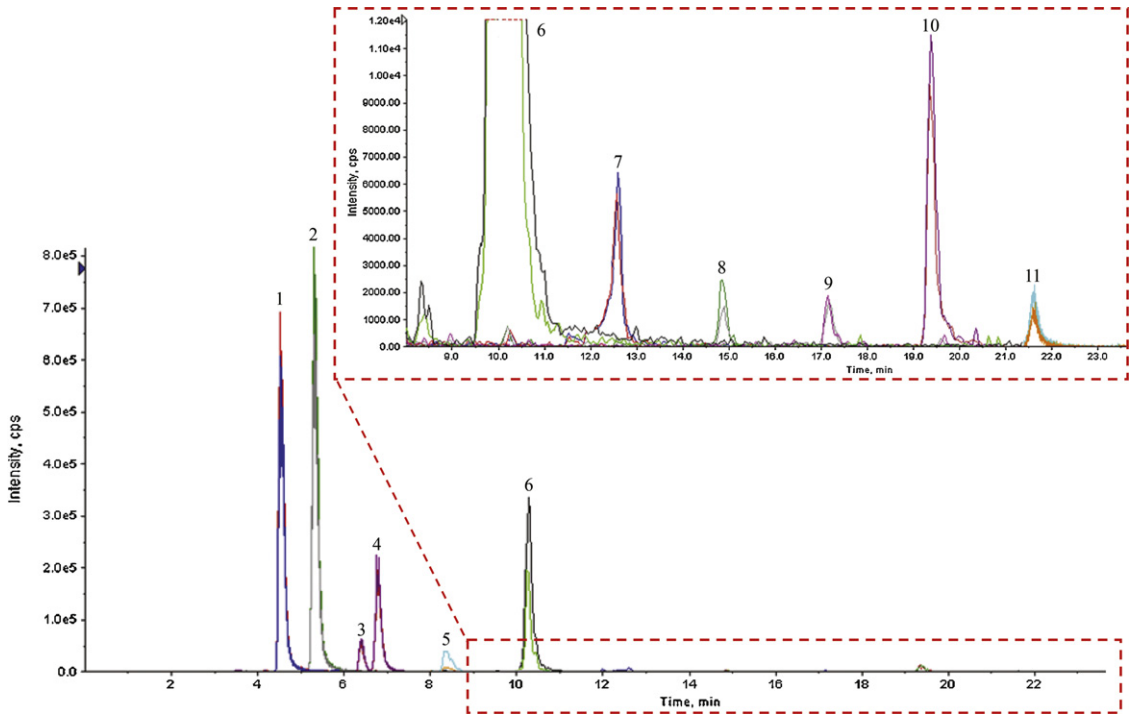

Fig. 2. On-line SPE UHPLC–MS/MS chromatogram of a smoked salmon extract previously diluted 1/200 (v/v) in a mixture H_2O/CH_3OH 50/50 (v/v). The sample pre-treatment and the chromatographic conditions are reported in Section 2. Chromatographic peaks: (1) formaldehyde; (2) acetaldehyde; (3) acrolein; (4) propanal; (5) butanal; (6) pentanal; (7) hexanal; (8) heptanal; (9) octanal; (10) nonanal; (11) decanal.

Taking into account that the food samples analyzed mostly contain fats, it can be said that we have obtained a good lowering of ME, being about 66% of the analytes present in each sample not affected by ME. This result can be likely ascribed to the optimized washing step of SPE sorbent, that employs a washing volume of about 50-folds the column volume.

In general the washing step seems more efficient for PAHs than for DNPH–aldehydes, since seven out of the thirteen PAHs are not affected by ME, whereas only three out of the twelve DNPH–aldehydes are ME free. Moreover the DNPH–aldehydes, in particular the more polar ones, preferentially show signal suppression.

4. Conclusions

An automated on-line SPE UHPLC–MS/MS method for the identification and determination in food of thirteen polycyclic aromatic hydrocarbons (PAHs) and twelve 2,4-dinitrophenylhydrazine–aldehyde derivatives was developed: the innovative aspect of the method proposed here consists in the simultaneous determination of PAHs and aldehydes, indicated as possibly present together in cooked food. Due to the different chemical structures and properties of PAHs and aldehydes, different are also the analysis conditions generally adopted. In this work, in order to obtain their simultaneous determination in cooked food, in which they are often present together, common conditions of on-line extraction, chromatography and mass spectrometry detection have been envisaged. The results are more than satisfactory, as shown by the validation parameters (linearity range, precision, LOD, LOQ, MDL, MQL, and recovery). In addition, the occurrence of the matrix effect was evaluated and overcome. The method has been applied to the analysis of food samples characterized by rather complex matrices.

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