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Fast analysis of nine PAHs in beer by Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction coupled with Gas Chromatography-Ion Trap Mass Spectrometry

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Abstract

An Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction (USVADLLME) procedure coupled with Gas Chromatography-Ion Trap Mass Spectrometry (GC-IT/MS) is proposed for fast analysis of nine Polycyclic Aromatic Hydrocarbons (Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo(b)fluoranthene, Benzo(a)pyrene and Benzoperylene) in beer (alcohol by volume ≤ 7 %). Among 5 possible extraction solvents tested, dichloromethane, density 1.325 g mL^{-1} at 25 °C, was selected for the method development. Parameters such as extraction solvent type and volume, extraction time and pH, and NaCl concentration were optimized. Under optimal conditions, the enrichment factors of the nine analytes range between 100 and 200 fold, the recoveries from 83 % to 99 % and the correlation coefficients from 0.9982 to 0.9999. The limit of detection (LOD) and limit of quantification (LOQ) are ≥ 3.8 pg μL^{-1} and ≥ 9.8 pg μL^{-1}, respectively. The precision expressed as relative standard deviation (RSD), is ≤ 4.0 %. The whole proposed methodology has demonstrated to be simple, reproducible and sensible for the determination of trace PAHs in beer samples.

Key-words

Polycyclic Aromatic Hydrocarbons; Beer; Beverage; Contaminant; DLLME; GC-IT/MS

Introduction

Nowadays, food security plays a fundamental key in our society. In particular, the quality plays a predominant role in the characteristics of a foodstuff. Beer is the world's most widely consumed alcoholic beverage,[1] and is the third-most popular drink overall, after water and tea.[2] It is thought by some to be the oldest fermented beverage.[3-5] The brewing beer, subjected to legislation in many countries (starting in 1963 from the UK government), is a process allowing to convert the starch source into a sugary liquid called wort and to convert the worth into the beer in a fermentation process effected by yeast. About the main composition, beer contains phenolic acids, 4-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid.[6]

Among the different contaminants present in the environment, the Polycyclic Aromatic Hydrocarbons (PAHs) are recognized as a global problem due to their chemical stability and carcinogenic effects.[7,8]

Basically, raw foods should not usually contain high PAH levels. On the other hand, processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are the major PAH sources.[9,10] Since PAHs are constituents of the particulate phase of the smoke, their possible presence in processed foods is attributable to the different treatments that food undergoes to increase the shelf life or to give it particular color, flavor and aroma.[11-13] For instance, levels as high as 200 μg kg^{-1} have been found in smoked fish and meat samples for individual PAH.[14-16] In barbecued meat, levels of 130 μg kg^{-1} have also been reported whereas the average background values are usually in the range of 0.01-1 μg kg^{-1} in uncooked foods.[17] Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during technological processes such as direct fire drying, where combustion products may come into contact with the oil seeds or oil.[18-20] Similar situation can occur for the beer making process, i.e. brewing.

However, it should be noted that the estimation to the PAH exposure has only an indicative value:

this is due to the many uncertainties inherent the extrapolation of a biological phenomenon as complex as carcinogenesis at low doses. For example, it could be mentioned the individual susceptibility to genotoxic PAH effects, the possible synergistic or antagonistic effects between carcinogenic and no-carcenogenic PAHs and the involvement in toxicity and cell proliferation phenomena at high doses. Because of these uncertainties, and in the absence of more information on the mechanisms of carcinogenesis at low doses, the Scientific Committee on Food (SCF) of the European Commission declines to indicate a reference value for the presence of carcinogenic PAHs in foods but continuously recalls the precautionary principle "ALARA" (As Low As Reasonably Achievable), i.e. recommends that exposure is as low as reasonably achievable. This statement is based on the assumption that genotoxic carcinogens such as PAHs do not have a dose threshold and thus cannot be indicated human exposure levels completely free of risk.

In any case, despite the very low concentrations found in foods (ng g^{-1}) PAHs are one of the major factors contributing to the onset of cancer in humans. Hence, it is important to develop rapid, easy and reliable analytical methods for their accurate determination in foods (or beverages as well) in order to assess human exposure to these contaminants.[21]

Since many years our research group have dealt the determination of different ubiquitous pollutants present in environment and food by means of Solid Phase Extraction[22-28] or Dispersive Liquid-Liquid Micro-Extraction[29-31] coupled with Gas Chromatography-Ion Trap Mass Spectrometry (GC-IT/MS).

This paper would like to set up a simple and reproducible analytical method in order to determine PAHs in alcoholic drinks, with a focus on beer produced industrially.

Materials and Methods

Materials

Among the PAHs, we have investigated 9 PAHs (Sigma Aldrich, Milan, Italy): Fluorene (Abbreviation F; CAS number 86-73-7; Chemical Formula $C_{13}H_{10}$; Molecular Weight 166.222; Median Lethal Dose, DL_{50}, N/A), Phenanthrene (P; 85-01-8; $C_{14}H_{10}$; 178.233; 700 mg kg^{-1} oral), Anthracene (Ant; 120-12-7; $C_{14}H_{10}$; 178.233; 3200 mg kg^{-1} oral), Fluoranthene (Fl; 206-44-0; $C_{16}H_{10}$; 202.255; 2000 mg kg^{-1} oral), Pyrene (Pyr; 129-00-0; $C_{16}H_{10}$; 202.255; > 16000 mg kg^{-1} oral), Chrysene (Chr; 218-01-9; $C_{18}H_{12}$; 228.1928; -), Benzo(b)fluoranthene (BbFl; 205-99-2; $C_{20}H_{12}$; 252.315; -), Benzo(a)pyrene (BaPyr; 50-32-8; $C_{20}H_{12}$; 252.3148; 50 mg kg^{-1} subcutanea) and Benzoperylene (BghiPer; 191-24-2; $C_{22}H_{12}$; 276.337; -). The standard solutions of each nine PAHs (concentration of 5 mg mL^{-1}) were prepared by dissolving the compounds in acetone. Each solution was further diluted with acetone to prepare final solutions (400 µg mL^{-1} and 20 µg mL^{-1}) for spiking both the hydroalcoholic solutions and the real samples; five mix standard solutions (1, 5, 10, 15 and 20 µg mL^{-1} with the addition of 5 µL of I.S.) were prepared for studying the analytical parameters. Octacosane ($C_{28}H_{58}$) was used as Internal Standard (I.S.): 5 mg was dissolved in acetone/*iso*-octane (9+1 v/v) and after the solution was diluted 10-times by acetone (0.5 mg mL^{-1}).

USVADLLME Procedure

A 10 mL aliquot of each sample (standard solution or real/ spiked beverage sample) was transferred into a 10 mL screw cap glass tube with a conical bottom. 50 µL mixture containing NaCl 25 g L^{-1}, octacosane 0.5 mg mL^{-1} (5 µL) and extraction solvent was added. Different extraction solvents were tested at different volumes. The mixture should be freshly prepared every day otherwise the interphase between the two solutions became cloudy because of the presence of NaCl. In this protocol the dispersive solvent was not used: the dispersion was performed by means of 1 min of vortex followed by mechanical rotation by means of 2 min of ultrasound bath. This procedure was

repeated three times, i.e. three minutes of vortex and 6 minutes of ultrasounds: this protocol allows to have a stable emulsion. The solution was centrifugated at 4000 rpm for 10 min: in this way the micro-drop is formed and transferred into a vial. Finally, after sodium sulphate addition for eliminating water residual, the final "solution" was injected in GC-FID or GC-IT/MS for determining PAHs.

GC-IT/MS or GC-FID Analysis and Quantification

A gas chromatograph Finnigan Trace GC Ultra equipped with an Ion Trap Mass Spectrometry detector Polaris Q (Thermo Fisher Scientific, Waltham, MA), a Programmed Temperature Vaporizer (PTV) injector and a PC with a chromatography station Xcalibur (Thermo Fisher Scientific), was used.

A fused-silica capillary column with chemically bonded phase (SE-54, 5% phenyl-95% dimethylpolisiloxane) was prepared in our laboratory[32-35] with the following characteristics: 30 m×250 μm i.d., N (theoretical plate number) 125,000 for *n*-dodecane at 90 °C; K' (capacity factor) 6.9; d_f (film thickness) 0.24 μm; u_{opt} (optimum linear velocity of carrier gas, hydrogen) 38.0 cm s^{-1}, and UTE% (utilization of theoretical efficiency) 92 %. The fused-silica capillary column used is very similar to commercial ones showing very good chromatographic efficiency and being more convenient from an economic point of view.

Helium was used as carrier gas at constant flow rate of 1 mL min^{-1} and as dumping gas in the ion trap at 0.3 mL min^{-1}. The column was kept at 100 °C for 30 s and then the temperature was programmed from 100 °C to 150 °C (isotherm for 120 s) and 320 °C (isotherm for 300 s) at 20 °C min^{-1}, respectively. The PTV injector was performed in splitless mode. Ten seconds after injection the vaporizer was heated from 110 °C to 320 °C at 800 °C min^{-1} and cooled after 120 s; the splitter valve was closed for 150 s. The transfer line and ion source were held at 270 °C and 250 °C, respectively. Scan acquisition in positive chemical ionization was from m/z 100 up to 450 a.m.u. at 1.68 scan s^{-1} and 70 eV.

The GC-FID analysis was carried out by means of a gas chromatograph DANI (Monza, Italy) equipped with an electronic flow control system, a PTV and a FID detector. The capillary column was the same used in the analysis with GC-IT/MS as well as the experimental conditions adopted with the FID temperature at 320 °C.

In both cases the PAH concentrations were obtained by calibration graphs of the ratio $Area_{(PAH)}/Area_{(IS,C28)}$ plotted versus each PAH concentration (pg μL^{-1}). All the samples were quantified in triplicate.

Results and Discussion

Among the 16 PAHs present in the list of the "priority pollutants" published by US EPA[36] some of them are in Group 2A ("probably carcinogenic to humans") and Group 2B ("possibly carcinogenic to humans") according to the criteria established by the International Agency for Research on Cancer (IARC). Among them, we focused our attention on 9 PAHs: Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Chrysene, Benzo(b)fluoranthene, Benzo(a)pyrene and Benzoperylene. Our study was addressed in two directions: firstly, we set up the entire analytical procedure using a hydroalcoholic solution simulating the "beer" matrix and after we applied the methodology to real samples.

For the preparation of the hydroalcoholic solution we considered the pH value and the alcoholic grade to be important parameters. According to the beers available on the Italian market, the pH averagely ranges between 3.20 and 4.80 whereas the alcoholic grade between 4 and 6.5 alc vol^{-1}. Actually, there are also beers showing alcoholic grade up to 10 alc vol^{-1} but they are a little portion (< 5 %) of the beers sold in Italy.[37] So, we prepared hydroalcoholic solutions with 5 alc vol^{-1} by ethanol and pH 4 by HCl 0.001 M.

Following our experience we decided to apply the Dispersive Liquid-Liquid MicroExtraction

(DLLME) procedure[38] which is based on the important role played by the dispersive solvent. In fact, this favors the action of the extraction solvent[38] which is finely dispersed in the sample solution. Actually, in this protocol the dispersive solvent was not added: the dispersion was obtained, and increased as well, by means of endothermic energy furnished by vortex and ultrasounds, i.e. the Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid MicroExtraction, USVADDLME. The extraction solvent, such as micro-droplets, makes the opalescent solution. After centrifugation, the extraction solvent can be separated (depending on the different density respect to the water) and the solution injected in the GC instrument. For this study such scheme was followed.

Preliminarily, we tested 5 extraction solvents: dichloromethane, chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1,2,2-tetrachloroethane. Table 1 shows the main recoveries obtained using dichloromethane, chloroform and 1,2-dichloroethane whereas in Tables 1-5 of the Supplementary Material all the measurements are reported. In particular, carbon tetrachloride and 1,1,2,2-tetrachloroethane do not show significant recoveries and they were not evaluated as extraction solvents; on the other hand, dichloromethane, chloroform and 1,2-dichloroethane show good recoveries ranging between 88.7-102.0 %, 80.2-104.6 % and 80.7-104.0 %, respectively.

For investigating the reproducibility of the analytical measurements experiments (five replicates) on the recoveries using the above-cited extraction solvents and relative volumes were performed. The data reported in Table 2 show different reproducibility using the three solvents. The recoveries range between 88.1 and 101.9 % with a Relative Standard Deviation (RSD) $\leq$ 4.0 using dichloromethane; between 73.6 and 90.6 % with a RSD $\leq$ 17.2 using chloroform; between 76.0 and 106.5 % with a RSD $\leq$ 9.9 using 1,2-dichloroethane. So, dichloromethane (250 μL) is the best extraction solvent in such analytical conditions.

Another critical point regards the salting-out effect, i.e. the variation of the PAH solubility in presence of different NaCl concentrations. In particular, we studied 5 different NaCl concentrations (0, 10, 25, 50, 100 g L^{-1}): Table 6 of the Supplementary Material shows the NaCl effect for a

hydroalcoholic solution (pH 4 and 5 alc vol^{-1}) spiked with 20 μg mL^{-1} of each PAH and 5 μL of I.S. (500 μg mL^{-1}): NaCl decreases the PAH solubility (salting out) for concentration above 25 g L^{-1} whereas the results are almost similar below this concentration. This occurrence is in agreement with previous studies.[30,39]

Finally, the pH influence on the analyte recoveries was studied, i.e. pH 4 and pH 9. The pH 9 is reached by adding NaOH 1 M: at such pH a gel formation is detected. For verifying the effect of this gel on the analyte recoveries, a comparison between the recoveries at pH 4 and pH 9 was performed. Two real beer samples were spiked with 5 ppm of each PAH: recoveries obtained at pH 9 (ranging between 23.5 and 59.0 %) are definitely lower than those obtained at pH 4 (ranging between 85.3 and 102.7 %). This occurrence means that the gel, formed adding the strong alkaline compound, adsorbs the analytes and thus the recoveries are poor.

We tested our entire procedure both on blank sample, i.e., a hydroalcoholic solution (pH 4 and 5 alc vol^{-1}) with NaCl (25 g L^{-1}) and I.S., and real sample, i.e., stout beer (4.2 alc vol^{-1}) sample added with NaCl (25 g L^{-1}) and I.S., before and after the fortification with a PAH standard solution, 10 μg mL^{-1}. The solutions were separately processed according to the USVADLLME procedure and GC-FID analysis. Figure 1a shows the chromatogram obtained analyzing the blank sample. After the entire methodology application only the I.S. (octacosane) peak is detected. Figure 1 shows also the chromatograms of the real sample (German Weiss beer sample, 4.2 alc vol^{-1}) before (Figure 1b) and after (Figure 1c) the spiking with PAHs: the peaks result well-separated and well-solved. Finally, Figure 2 shows the same real sample spiked with 10 μg mL^{-1} of each PAH and analyzed by GC-IT/MS: also in this case the procedure evidences no contamination problem.

Table 3 reports the correlation coefficients (R^2) calculated in the range 0.1-100 pg μL^{-1}, the LODs and the LOQs and the inter- and intra-day repeatability of each compounds investigated in this study. The linearity ranges for all the PAHs calculated by GC-IT/MS are above 0.9982. The LODs and LOQs of each compound analyzed in the beer matrix, were determined by means of GC-IT/MS (in SIM mode): they are very good and allow an estimation of such compounds in the beer samples.

In particular, the LODs range between 1.2 and 3.8 pg μL^{-1} with relative standard deviation (RSD) ≤ 3.9 whereas LOQs range between 3.0 and 9.8 pg μL^{-1} with RSD ≤ 6.1. These values were determined according to the Knoll's definition,[40] i.e., an analyte concentration that produces a chromatographic peak equals to three times (LOD) and seven times (LOQ) the standard deviation of the baseline noise. Furthermore, Table 3 shows also the recoveries (%) with the RSD of each PAH calculated on alcoholic and beer samples fortified with 1 and 50 μg mL^{-1} of each PAH, respectively. It can be noted a very slight difference between the two determinations (blank sample and real sample) due to the matrix effect. For the beer matrix the PAH recoveries are good ranging between 85.3-102.7 % (RSD ≤ 9.0 %) for the sample fortified with low PAH concentration whereas they vary between 83.6 % and 98.5 % (RSD ≤ 8.8 %) for the sample fortified with 50 μg mL^{-1} of each PAH.

Table 4 reports a comparison between LOD and LOQ values determined by GC-FID and GC-IT/MS after the performance of the same preconcentration procedure (USVADLLM). The GC-FID values were obtained by the same authors using the same analytical conditions. The LODs and LOQs determined by both the procedures are really different: in particular, levels determined by GC-IT/MS are lower than GC-FID, up to 127-times in case of Chrysene. Even if the limits reached by GC-FID are quite good, the very low limits reached by USVADLLM-GC-IT/MS demonstrate the strength of this methodological approach. It should be underlined that at authors' knowledge there are very few studies focused on the PAH determination in the beer matrix so data are very scarce.[41] In fact, in literature there are only two papers regarding the determination of EPA PAHs, conducted on both domestic and imported beers and reporting analytical methods based on HPLC analysis.[42,43] Joe et al.[42] developed a HPLC method for determining 4 PAHs, i.e. Benz(a)anthracene, Benzo(a)pyrene, Benzo(g,h,i)perylene, and Dibenzo(a,i)pyrene, in beer. The method is based on the PAH extraction with isooctane from a beer sample to which sodium hydroxide, ethanol, and acetone had been added. The isooctane extract is washed and purified by chromatography; the eluate is concentrated to dryness and the resulting residue is dissolved in methanol-acetonitrile (1+1) and

analysed by reverse phase HPLC analysis. Both ultraviolet (UV) and fluorescence detectors are used to monitor the HPLC column effluent. Using this methods, the authors achieved recoveries ranging between 77-108 % by UV measurement and between 73-97 % by fluorescence measurement. Moret et al.[43] carried a clean-up procedure based on SPE extraction (using Superclean ENVI-18 cartridges), and, after concentrated with nitrogen, re-dissolved in acetonitrile and enriched with benzophenone, analyzed the eluate by means of HPLC coupled with two different detectors, UV/Vis and fluorimetric detectors: inn this way they analysed 16 EPA PAHs. Their results show poor resolution for naphthalene and vary from 57 % to 103 % for the other PAHs (the fluorimetric detector is less sensitive for the most volatiles PAHs and more selective for the "heavy" PAHs, besides the recoveries are better than 57 % for fortified samples). These authors found minimum detectable amounts (MDA; defined as the amount capable of producing a peak three times greater than the background noise) ranging between 60 and 250 pg for PAHs determined by HPLC-UV/Vis and between 20 and 70 pg for PAHs determined by HPLC-Fluorescence (in this case, the MDA of Fluorene, Phenanthrene and Chrysene were not determined).

The analytical procedure above reported was applied to 9 commercial beer samples packed both in glass bottles (4) and tin (5) with alcohol content around 5 alc vol^{-1}, and to 2 special beers flavored with Tequila and Mojito, respectively, with alcohol content around 6 alc vol^{-1}. All the samples are available in the Italian market. The determinations were performed by GC-IT/MS and the results are reported in Table 5. It is interesting to note that all the beers investigated show the presence of one PAH, at least: in particular, Chrysene is present in all the samples. The PAHs are not systematically present in all the samples, scilicet dark beers made using roasted malt or roasted barley. On the other hand, it could be noted that benzoperylene is present in only the two Polish beers whereas benzo(a)pyrene, the most significant PAH, is found in two samples, both Stout (Italian and German) beers: this presence is probably due to processes carried out for obtaining such beverage. According to the total PAHs the two special beers show the maximum content, 3.79 and

3.10 μg mL^{-1} for Pils flavored with Tequila and Mojito, respectively. The analysis does not give information about the source of each PAH but it evidences the importance of this measurement also considering the toxic effects of PAHs on the human health.

Finally, the authors would like to point out a speculative effort aimed to investigate the PAH content ingested during a day or event (e.g., dinner, party). Although beer consumption in the world is increasing 6 % annually,[44] a study shows that a daily consumption of 3 cups by 250 mL beer (Pils) reduces by 24.7 % the risk of cardiovascular disease.[45] This means a daily intake of total PAHs ranging between 360 μg and 2115 μg for regular beers whereas for special beers investigated in this study, i.e. Pils flavoured with Tequila or Mojito, the content can reach levels around 2500 μg (2842 μg and 2325 μg, respectively) of total PAHs. Further, it should be considered that sometimes beer is a favourite beverage during barbecue party/dinner: in such case the PAH content in food is just relatively high due to the grill cooking operations (average of 17.6 μg of BaP per kg of meat).[16,17,46-49]

Conclusion

Among drinks spread on the market, the beer, as also wine, has long been opposed as alcoholic drink, and with its damaging effects. Actually, beer is aqueous beverage with alcohol in small quantities, which not only are not harmful to the human health, but are even beneficial, expecially for the cardiovascular system, as evidenced by all the scientific literature on mortality linked to heart attack and stroke. On the other hand, a serious problem regards the raw material for brewing. This paper shows a new protocol for analyzing nine carcinogenic PAHs in the beer beverage. The analytical methodology provides a protocol based on the ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction coupled with gas chromatography-ion trap mass spectrometry. The method shows very good LOQs (3.0-9.8 ng mL^{-1}) with recoveries between 83.6 and 102.7 %. The

method was compared with the same protocol anazyed by GC-FID (LOQs between 135-621 ng mL^{-1}) whereas no paper about such determination and based on GC analysis is present in literature for comparing the analytical performance. The other analytical parameters obtained (linearity range, LODs and recoveries) are also very good and allow the PAH determination in such matrix. The entire methodology was applied to 11 real samples (9 commercial beer samples packed by bottle and tin, and 2 special commercial beers), available in the Italian market. The only PAH present in all the samples is the Chrysene which is not relevant from a human health point of view. On the other hand, a systematic PAH presence in the samples has not been found: the only interesting issue regards the Benzo(a)pyrene level in two samples. Even if it is not possible to identify the source (this should be just possible analyzing the raw materials of these two beer samples), this analysis confirms the importance of having a fast, valuable and reliable protocol for analyzing such compounds.

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Table 1. PAH average recoveries (%) obtained using 250 µL of dichloromethane, 150 µL of chloroform and 250 µL of 1,2-dichloroethane, respectively, determined in hydroalcoholic solutions (pH 4 and 5 alc vol^{-1}) spiked with 50 µL of a PAH mix (20 µg mL^{-1} each) and 5 µL of I.S. (500 µg mL^{-1}).

PAH	Recovery		
	dichloromethane 250 µL	chloroform 150 µL	1,2-dichloroethane 250 µL
Fluorene	94.6	102.5	102.2
Phenanthrene	97.9	104.6	99.8
Anthracene	96.7	100.7	89.9
Fluoranthene	100.7	98.7	96.4
Pyrene	102.0	96.3	104.0
Chrysene	97.6	90.2	101.5
Benzo(b)fluoranthene	90.8	92.9	91.8
Benzo(a)pyrene	88.7	86.3	82.5
Benzoperylene	89.7	80.2	80.7

Table 2. Precision and accuracy using dichloromethane (250 μL), chloroform (150 μL) and 1,2-dichloroethane (250 μL), calculated as recoveries of the PAHs in hydroalcoholic solutions (pH 4 and 5 alc vol^{-1}) spiked with 50 μL of a PAH mix (20 μg mL^{-1} each) and 5 μL of I.S. (500 μg mL^{-1}). In parentheses the RSDs are reported among five experiment replicates.

PAH	dichloromethane	chloroform	1,2-dichloroethane
Fluorene	97.4 (4.0)	89.4 (15.0)	97.5 (4.5)
Phenanthrene	98.3 (1.9)	90.6 (16.4)	96.4 (3.3)
Anthracene	94.2 (2.0)	88.9 (15.4)	91.9 (3.9)
Fluoranthene	98.5 (1.8)	87.1 (14.3)	94.4 (3.2)
Pyrene	101.9 (0.3)	89.5 (11.9)	106.5 (4.3)
Chrysene	95.9 (2.2)	82.4 (12.6)	94.7 (8.7)
Benzo(b)fluoranthene	89.3 (0.7)	80.4 (16.6)	83.5 (9.9)
Benzo(a)pyrene	89.9 (1.2)	76.0 (15.2)	83.2 (8.6)
Benzoperylene	88.1 (2.8)	73.6 (17.2)	76.0 (7.2)

Table 3. Correlation coefficients (R^2) calculated in the range 0.5-100 pg μL^{-1}, LOD (expressed as pg μL^{-1}) and LOQ (pg μL^{-1}) and inter- and intra-day repeatability (RSD, expressed as %) of each PAH determined by GC-IT/MS, and recoveries (%) calculated in alcoholic and beer samples containing NaCl 25 g L^{-1} and fortified with 1 µg mL^{-1} (a) and 50 µg mL^{-1} (b) of each PAH, respectively. In brackets the RSDs are reported

PAH	R^2	LOD	LOQ	Intra-day	Inter-day	Recovery[a]		Recovery[b]	
						Alcoholic	Beer	Alcoholic	Beer
Fluorene	0.9982	3.7	8.9	4.6	4.6	96.7 (0.7)	97.6 (7.4)	93.9 (0.9)	93.6 (6.9)
Phenanthrene	0.9989	1.5	3.5	2.6	2.6	88.0 (0.7)	85.3 (8.4)	89.0 (0.8)	86.7 (6.0)
Anthracene	0.9986	3.0	7.3	1.0	1.3	95.2 (1.1)	91.2 (8.3)	96.3 (1.6)	92.4 (7.2)
Fluoranthene	0.9996	1.4	3.3	4.6	4.5	89.8 (0.2)	86.1 (6.8)	94.9 (1.2)	91.1 (7.5)
Pyrene	0.9996	1.4	3.5	4.0	3.6	100.3 (3.7)	98.2 (6.3)	99.7 (3.0)	98.5 (7.3)
Chrysene	0.9997	1.2	3.0	4.6	4.3	107.4 (4.7)	102.7 (9.0)	94.7 (4.4)	95.7 (8.8)
Benzo(b)fluoranthene	0.9996	3.8	8.8	2.5	2.6	97.7 (1.9)	91.6 (7.7)	98.9 (1.5)	91.4 (5.5)
Benzo(a)pyrene	0.9998	3.5	8.1	3.0	2.9	89.8 (3.0)	87.8 (6.9)	99.1 (2.3)	85.5 (8.3)
Benzoperylene	0.9999	3.5	9.8	1.8	1.6	89.0 (1.9)	86.4 (8.4)	84.1 (2.3)	83.6 (7.5)

Table 4. Comparison between LODs and LOQs determined by GC/FID and GC-IT/MS (pg μL^{-1}).

	GC-FID		GC-IT/MS	
	LOD	LOQ	LOD	LOQ
Fluorene	123	269	3.7	8.9
Phenanthrene	94	213	1.5	3.5
Anthracene	78	135	3.0	7.3
Fluoranthene	124	310	1.4	3.3
Pyrene	58	259	1.4	3.5
Chrysene	110	380	1.2	3.0
Benzo(b)fluoranthene	250	523	3.8	8.8
Benzo(a)pyrene	276	621	3.5	8.1
Benzoperylene	75	216	3.5	9.8

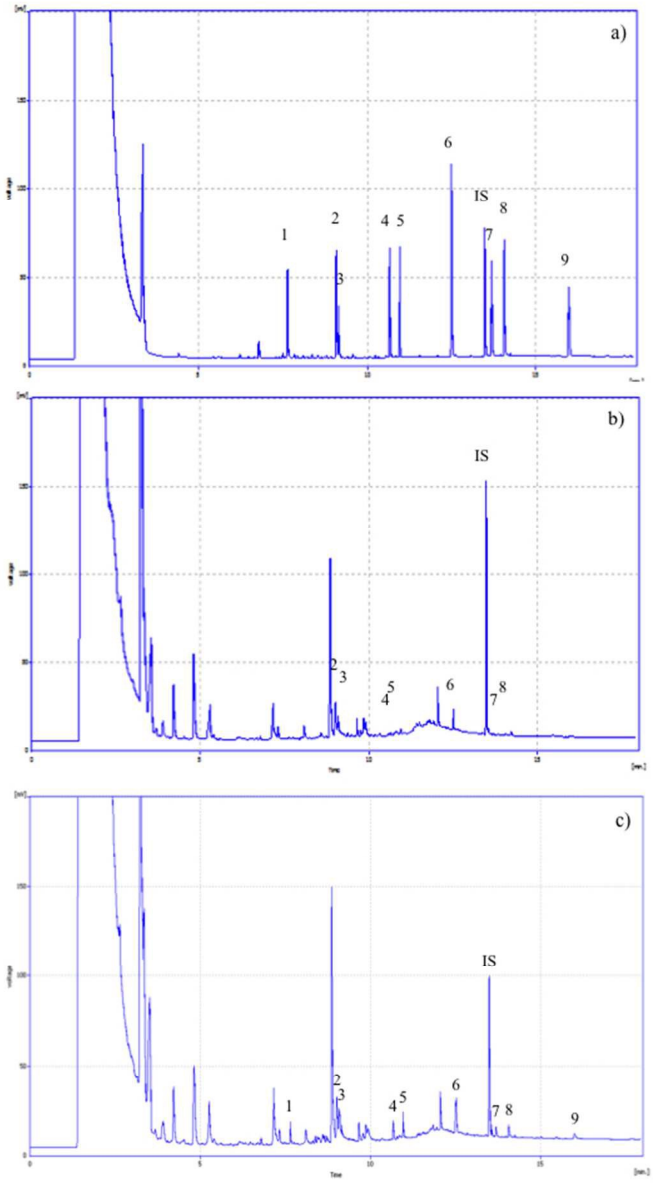
Table 5. PAHs concentration levels (μg mL^{-1}) determined in beers and special beers (c) packed in glass bottles (a) or tin (b). All beers are available on the Italian market.

	Italian Superior Blond[a]	Italian Stout Red[a]	German Weiss[a]	German Stout[a]	German Pils[b]	German Lager[b]	Polish Premium[b]	Polish Pils[b]	Dutch Premium[b]	Pils flavored with Tequila[ac]	Pils flavored with Mojito[ac]
Fluorene	< LOQ	0.24	< LOQ	0.36	0.34	< LOQ	< LOQ	< LOQ	< LOQ	0.42	0.13
Phenanthrene	0.18	< LOQ	0.10	< LOQ	< LOQ	0.06	0.92	0.32	0.18	< LOQ	< LOQ
Anthracene	0.21	< LOQ	0.22	< LOQ	0.55	0.33	0.79	< LOQ	0.21	< LOQ	< LOQ
Fluoranthene	< LOQ	< LOQ	0.15	< LOQ	< LOQ	< LOQ	< LOQ	0.13	< LOQ	0.49	< LOQ
Pyrene	< LOQ	0.78	0.18	0.62	0.14	< LOQ	0.40	0.16	< LOQ	0.99	0.87
Chrysene	0.26	0.64	0.08	0.84	0.61	0.09	0.30	0.19	0.10	1.22	2.10
Benzo(b)fluoranthene	< LOQ	0.11	0.12	0.25	0.22	< LOQ	< LOQ	< LOQ	< LOQ	0.34	< LOQ
Benzo(a)pyrene	< LOQ	0.11	0.05	0.24	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.33	< LOQ
Benzoperylene	< LOQ	< LOQ	0.06	< LOQ	< LOQ	< LOQ	0.41	0.53	< LOQ	< LOQ	< LOQ
Total PAHs	*0.65*	*1.88*	*0.96*	*2.31*	*1.86*	*0.48*	*2.82*	*1.33*	*0.49*	*3.79*	*3.10*

Legenda Figure

Figure 1. GC-FID chromatograms of (a) blank sample (i.e., alcoholic solution, 5 % (v/v) ethanol, with NaCl 25 g L^{-1}), (b) German Weiss beer (4.2 alc vol^{-1}) with no PAH and (c) German Weiss beer spiked (10 μg mL^{-1} of each PAH) (NaCl 25 g L^{-1}). For experimental conditions: see text. Peak list: 1: Fluorene; 2: Phenanthrene; 3: Anthracene; 4: Fluoranthene; 5: Pyrene; 6: Chrysene; IS: Octacosane (C_{28}); 7: Benzo(b)fluoranthene; 8: Benzo(a)pyrene; 9: Benzoperylene.

Figure 2. GC-IT/MS chromatograms of (a) blank sample (i.e., alcoholic solution, 5 % (v/v) ethanol, with NaCl 25 g L^{-1}), (b) German Weiss beer (4.2 alc vol^{-1}) with no PAH and (c) German Weiss beer spiked (10 μg mL^{-1} of each PAH) (NaCl 25 g L^{-1}). For experimental conditions: see text. Peak list: 1: Fluorene; 2: Phenanthrene; 3: Anthracene; 4: Fluoranthene; 5: Pyrene; 6: Chrysene; IS: Octacosane (C_{28}); 7: Benzo(b)fluoranthene; 8: Benzo(a)pyrene; 9: Benzoperylene.



254x338mm (72 x 72 DPI)