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Determining Polycyclic Aromatic Compounds in Bird Feathers Using Pressurized Fluid Extraction

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Abstract: Due to their ease of collecting and transporting from the field and their ability to accumulate pollutants, bird feathers are increasingly being used as a non-invasive biomonitoring tool for environmental monitoring programs. Polycyclic aromatic compounds (PACs) are a diverse class of environmental pollutants, and because of their deleterious impacts on biological species, monitoring these compounds in wildlife is of high importance. Current approaches to measuring PACs in bird feathers involve a time-consuming acid treatment with a concomitant solvent extraction step. Here, a validated method for measuring a suite of PACs in bird feathers using pressurized fluid extraction and identification and quantitation by gas chromatography-tandem mass spectrometry is presented. Chicken (*Gallus domesticus*) feathers were purposely fortified with a suite of 34 PACs separately at three fortification levels and placed inside a pressurized fluid extraction cell containing silica gel/deactivated alumina to provide in situ clean-up of the sample. Except for anthracene and naphthalene, the accuracy of our method ranged for PAHs from 70–120% (irrespective of fortification level), and our intra- and inter-day repeatability was smaller than 28%. For APAHs, our accuracies ranged from 38–158%, and the inter- and intra-day repeatability was less than 35%. Our limits of detection and quantitation for both groups of compounds ranged from 0.5–13 and 1.5–44.3 ng/g, respectively. Overall, the developed method represents an effective and efficient approach for the extraction and quantitation of PACs from bird feathers that negated the need for the time-consuming and potentially harmful acid treatment.

Keywords: polycyclic aromatic compounds; feathers; pressurized fluid extraction

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1. Introduction

Polycyclic aromatic compounds (PACs) are classified as a ubiquitous group of toxic and environmentally persistent organic compounds which can include many polycyclic aromatic hydrocarbons (PAHs) and their derivatives such as alkyl-PAHs (APAHs), nitro-PAHs, oxy-PAHs, halogenated PAHs and hydroxylated PAHs [1,2]. Polycyclic aromatic hydrocarbons can be mostly produced as by-products of incomplete combustion, originating from both natural and anthropogenic combustion sources [3]. Some of these derivative PAHs are known to be more toxic and have a greater potential for accumulation in biological tissues compared to the parent PAHs [1]. In general, PACs are hydrophobic and have a low solubility in water; however, some PAHs can remain in the aqueous phase and contaminate water [4]. In addition to their persistence and bioaccumulation potential, their mutagenicity, carcinogenicity and toxicity make them priority pollutants under the Canadian Environmental Protection Act (1999). As such, sixteen PAHs are classified as toxic and are on the Toxic Substances List [5].

In recent decades, numerous biomonitoring studies have been conducted to assess the fate of environmental contaminants in various ecosystems by measuring their concentrations in living organisms. Targeted compounds include metallic compounds, such as mercury and rare earth elements, as well as organic compounds, such as PACs. Birds have been extensively used as bioindicators of environmental pollution [6,7]. Because of their high trophic position, avian species, such as birds of prey and seabirds play a significant role in the food chain and can biomagnify pollutants from aquatic and terrestrial ecosystems as their level of accumulation is greater than in the lower levels of the food chain and makes it easier to detect and quantify pollutants [8,9]. Moreover, avian species are often long-lived and fairly easy to observe and handle. Thus, birds have routinely been used in many long-term environmental monitoring programs.

In comparison to internal tissues, such as muscle, liver and blood, bird feathers offer a minimally invasive biomonitoring material that are easy to collect, store and transport [10]. Hence, there is a growing interest in using feathers as a sample matrix in avian studies. In Canada, feather examination is also being implemented to determine contaminants bioaccumulation in wild birds. For instance, Miller et al. monitored a range of persistent organic pollutants (POPs) in blood and feather samples of ancient murrelets (*Synthliboramphus antiquus*) along the British Columbia coast during the breeding season [11]. Kardynal et al. assessed total mercury exposure to aerial insectivorous birds across Canada from 2013 to 2018 [12]. In another study, the exposure, uptake and deposition of PACs, including parent and alkylated PAHs, were analyzed in feces and pectoral muscles of tree swallows (*Tachycineta bicolor*) from the Athabasca Oil Sands region [13]. Cruz-Martinez et al. evaluated the natural exposure of tree swallows to air contaminants including PAHs in the Alberta oil sands, and the potential impacts on the health of these birds [14]. Nevertheless, the bioaccumulation of PACs in Canadian wildlife, particularly in wild birds, remains poorly documented and additional investigation is required to fill gaps in the knowledge of PAC environmental dynamics in Canada [15]. The use of feathers as a sampling matrix could improve the temporal and spatial span of PAC biomonitoring [16].

The current approach for extraction of PACs from feathers was adapted from the seminal work on POPs in human hair [16]. Feathers are first washed with either water or a surfactant solution, air dried, cut into small pieces, and then immersed in an acidic solution for 24 h at elevated temperatures. After this time, POPs are back-extracted using a hydrophobic extraction solvent. Typically, a clean-up step using either an in-house adsorption chromatography column or solid-phase extraction column is used to purify extractions prior to gas chromatographic analysis [17–26]. For example, Acampora et al. immersed common tern (*Sterna hirundo*) feathers into 15 mL of 37% HCl and 20 mL of a solvent mixture of two parts of hexane and one part of acetone. Samples were fortified with internal standards (PAHs) and put in the oven at 37 °C overnight [27]. In another study, raptors' feathers were placed in a test tube with a 10 mL mixture of nitric acid and extraction solvent and spiked with internal standards [9]. Meyer et al. digested feathers in 10 mL HNO_3 (69%) for 48 h at room temperature for full digestion. Extraction was performed by solid-phase extraction using Oasis HLB Plus SPE cartridges [28]. In one study, PAHs were extracted from the feathers of European storm petrels (*Hydrobates pelagicus*) using 15 mL of 37% HCl with an extraction solvent in an oven for 15 h [29]. Gonzalez-Gomez et al. assessed PAHs in body feather samples of feral pigeons (*Columba livia domestica*) using ultrasound-assisted solvent extraction at 40 °C with a mixture of hexane-dichloromethane and HCl [30]. Zhao et al. extracted PAHs from little egret (*Egretta garzetta*) chicks' feathers using 10 mL HNO_3 (69%) for digesting. Extraction was conducted by solid-phase extraction, and extracts were analyzed by high performance liquid chromatography (HPLC) [31].

Pressurized fluid extraction (PFE) is another analytical technique which involves using solvents at high temperatures and pressures to facilitate penetration of solid or semi-solid matrix being extracted, increasing extraction efficiency and reducing processing time [32]. Several studies have employed PFE for the extraction of PACs from different matrices. Pissinatti et al. optimized and validated a method for the determination of PAHs

in roasted coffee by isotope dilution gas chromatography coupled to mass spectrometry using PFE, followed by a clean-up step [33]. In another study, extraction of oxy-PAHs in mussels was performed by PFE and followed by clean-up on silica. Extracts were analyzed by HPLC-mass spectrometry (MS) [34]. Harris et al. used PFE to measure metabolites of benzo(a)pyrene from tissue samples. Pressurized fluid extraction followed by HPLC-fluorimetry was employed to quantify separated analytes [35]. In the study of Kacmaz et al., a method was developed for the determination of four PAHs (benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene) in some cereal-based foods, such as cornflakes and breads by using PFE coupled to GC-MS [36]. Merlo et al. used an alternative method of pressurized liquid extraction using a hard cap espresso machine followed by HPLC-MS/MS to determine PAHs in smoked bacon [37].

To our knowledge, only one study has used PFE for the determination of PAHs in feathers [38]. This study focused on a suite of POPs including 16 PAHs and no full validation on the method was described. Here we hypothesize that PFE and an in situ adsorption step can be used to exhaustively extract PAHs, including alkyl-PAHs from feathers. Here we provide details of the full validation study and empirically derived performance characteristics as described in the Eurachem analytical guidelines [39]. Target analyte detection and quantitation was performed using GC-MS/MS with electron ionization and in the multiple reaction monitoring ion (MRM) mode.

2. Experimental Section

2.1. Materials

All organic solvents used in this study were of high purity (optima grade) and purchased from Fisher Chemicals (Ottawa, Ontario, Canada). Eighteen (18) individual APAHs, sixteen (16) unsubstituted PAHs as a native mix and isotope dilution internal standards were >98% purity and purchased from AccuStandard Inc. (New Haven, CT, USA) and Caledon Laboratory Chemicals (Georgetown, ON, Canada). The isotope dilution internal standards consist of 15 of the 16 deuterated PAHs, used for recovery internal standard (RIS) and the labelled compound (d_{10}-anthracene), used as the instrument performance internal standard (IPIS). Furthermore, silica gel (923 grade, 100–200 mesh), alumina (60–325 mesh), diatomaceous earth (DE) dispersant, Ottawa sand and anhydrous sodium sulphate were all purchased from Fisher Chemical. Chicken (*Gallus domesticus*) feathers were collected from a local farmer in Manitoba (Oakbank, MB, Canada).

2.2. Sample Processing by Pressurized Fluid Extraction (PFE)

Feather samples were washed with pure water (HPLC grade) and air-dried for 24 h before extracting by PFE. Cleaned whole feathers were cut into smaller pieces, then weighed accurately (0.01 g) and mixed with DE dispersant (1.5 g, baked at 600 °C for 6 h). The mixture was transferred to a 34 mL PFE extraction cell, which was fitted with two glass fiber filters and filled with silica gel/deactivated alumina (5 g/4.5 g) to clean up the sample matrix. Finally, PFE cells were spiked with a mixture of PAHs and APAHs at three dosing levels (10, 50, 200 pg/µL (n = 8 in each case)), and RIS (0.5 ng/µL, 20 µL). Ottawa sand (baked at 600 °C for 6 h) was added to fill the dead volume of the PFE cell. Method blanks (n = 12) were prepared by only using the dispersant. Dichloromethane (DCM) was used as the extraction solvent at a temperature > 100 °C under high pressure (1500–1700 psi). Our conditions for the PFE system (Thermo Scientific, Waltham, MA) were as follows: oven heat-up time: 5 min; two static cycles of 5 min each; followed by 60% volume flush of extraction cell and nitrogen purge of 150 psi for 60 s. After extraction, 5 g of sodium sulfate was added to each extract vial to remove excess moisture and transferred to another collection vial. The extract volume was then reduced to approximately 2 mL under high purity (UHP) nitrogen gas. Obtained extracts were then transferred to a conical glass test tube and reduced again to 100 µL under a gentle stream of UHP nitrogen gas. The final extracts were fortified with IPIS (0.5 ng/µL, 20 µL) and transferred to GC vials.

To compare our PFE method with the traditional acid digestion technique, we carefully fortified small cut pieces of chicken feathers with a mixture of PAHs and APAHs (medium level: 50 pg/µL). This was performed by spiking feathers directly with the mixture and immersing them into a mixture of 15 mL HCl 37% and 20 mL solvent (DCM: Hex, 1:4). Vials (n = 4) were filled to the top and sealed to reduce the head-space and then placed in a water-bath overnight at 40 °C. An additional gel permeation chromatography step was needed as we observed an oily substance in our solvent. Oil-free extracts were then transferred into the conical test tubes and reduced to 100 µL under a gentle stream of UHP nitrogen gas.

2.3. GC-MS/MS Conditions

An Agilent 7890 GC coupled with a triple quadrupole mass spectrometer fitted with an electron ionization (EI) source was used for the MS/MS acquisition. An Agilent J&W DB-5 ms ultra inert column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a constant flow rate of 1.2 mL/min was used. An amount of 1 µL of the sample was injected with a PAL RSI 85 autosampler at 250 °C in splitless mode. The oven temperature was held at 60 °C for 1 min, then raised to 120 °C at 35 °C/min, further ramped up to 220 °C at 14 °C/min, 260 °C at 5 °C/min and held for 5 min, to 300 °C at 10 °C/min and finally to 310 °C at 50 °C/min. Both the transfer line and source temperature were set at 320 °C. The quantification and confirmation ions and the MRM ion transitions for PAHs and APAHs can be found in Idowu et al. [40].

2.4. Method Performance Characteristics

The method limit of detection (LOD) and limit of quantification (LOQ) for our pressurized solvent extraction study were determined by extracting eight replicates (n = 8) of feather matrices fortified with 10 pg/µL PAHs. Procedural blanks (n_b = 12) were prepared by only using the dispersant spiked with only the suite of deuterated PAH internal standards. The adjusted standard deviation (s_0') was calculated from the results of replicate measurements by the ratio defined in the Eurachem Guide [23].

$$s_0\prime = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

where s_0 is the estimated standard deviation of single results for each target analyte, $s_{0'}$ is the adjusted standard deviation used for determining the LOD and LOQ values. Finally, LODs were calculated as 3 × s_0', and LOQs were calculated as 10 × s_0'.

Repeatability of our method validation study was calculated by extracting and quantifying PACs from feathers in replicate over three consecutive days (inter-day, n = 4) and over a 24 h period (intra-day, n = 8). Accuracy was determined by analyzing eight replicates of feathers at each dosing level of PACs.

The working range for PAHs and APAHs was based on a seven-point calibration curve (4–1000/2–500 pg/µL, respectively). The IPIS was added to each calibration point at a constant concentration (100 pg/µL) to account for any small fluctuations in the signal of the instrument between injections. Calibration standards were injected randomly and in triplicate. The peak area obtained for each PACs analyte was normalized to d_{10}-anthracene (IPIS) and plotted as a function of concentration. The linearity was evaluated by the magnitude of R^2 (correlation coefficient) value and the level of significance (p-value). Residual plots were also generated and examined to ensure the random distribution about zero to confirm linearity.

The ruggedness of our method was evaluated by purposely making small differences in the method and comparing whether the performance characteristics remained unaffected. The following changes were examined for our method: (i) double the mass of feathers (0.02 g) and, (ii) vary the mass of silica gel and alumina (6, 5.5 g/4, 3.5 g, respectively)

used in the PFE extraction step. A single fortification level (50 pg/μL) was used in the ruggedness test.

3. Results and Discussion

The performance characteristics of our PFE method are presented in Table 1. The average recoveries of PACs were determined by comparing the calculated values with the fortified amounts found in our in-house chicken feather reference material, which was created by spiking feathers with known amounts of PACs at three dosing levels (10, 50, 200 pg/μL).

Table 1. Method performance characteristics of our method for the analysis of PACs in the chicken feathers using pressurized fluid extraction and GC-EI-MS/MS detection and quantitation [a].

Compound	Spiking Level						Inter-Day Precision RSD (%)	LOD ng g^{-1}	LOQ ng g^{-1}
	10		50		200				
	pg μL^{-1}		pg μL^{-1}		pg μL^{-1}				
	Accuracy (%)[a]	Precision RSD (%)	Accuracy (%)	Precision RSD (%)	Accuracy (%)	Precision RSD (%)			
PAHs									
Ace	72.97 (71.90)	13.00	69.87 (66.63)	5.47	79.44 (79.33)	16.69	10.52	1.20	4.01
Acy	94.52 (105.79)	21.40	84.18 (82.20)	3.58	87.27 (86.70)	8.71	10.53	2.59	8.64
Ant	22.5 (24.45)	75.35	14.09 (13.98)	46.84	26.29 (25.98)	31.48	72.15	4.51	15.03
B[a]A	96.12 (101.20)	20.25	73.30 (72.18)	2.23	76.07 (79.30)	8.31	13.36	2.22	7.41
B[a]P	96.07 (101.92)	28.03	83.27 (81.34)	8.40	113.48 (110.76)	19.28	11.67	3.96	13.20
B[b]F	92.62 (91.94)	19.92	73.84 (73.03)	1.06	77.93 (75.45)	6.37	8.37	2.36	7.88
B[ghi]P	87.78 (87.81)	13.15	75.64 (75.64)	0.92	75.56 (75.65)	6.40	5.84	1.48	4.93
B[k]F	103.21 (96.29)	21.98	93.85 (93.09)	1.40	84.28 (83.31)	3.54	6.08	2.32	7.72
Chr	110.04 (104.76)	19.40	88.02 (87.62)	0.98	79.55 (79.15)	4.27	7.51	2.71	9.02
D[a,h]A	83.69 (84.69)	4.33	79.93 (80.05)	1.53	75.99 (76.87)	4.88	5.19	0.46	1.55
Flt	105.26 (95.82)	25.36	76.31 (76.98)	3.64	75.39 (73.55)	7.36	6.53	4.14	13.79
Flu	102.93 (92.99)	25.96	80.77 (81.11)	0.52	76.16 (76.02)	3.68	3.48	1.32	4.40
Ind	86.76 (84.82)	15.24	74.72 (73.61)	1.39	70.50 (70.31)	3.13	5.88	1.69	5.64
Nap	128.82 (131.08)	18.63	100.20 (98.65)	3.94	79.10 (77.56)	3.27	10.22	6.2	20.66
Phen	110.32 (109.47)	16.42	82.77 (79.92)	3.00	77.86 (76.38)	6.48	4.54	2.22	7.40
Pyr	116.98 (104.36)	25.11	71.16 (67.52)	3.99	90.42 (91.09)	14.61	16.13	1.92	6.40
APAHs									
1,7-Me2-Phen	106.12 (106.95)	4.03	97.68 (94.78)	5.01	83.81 (85.04)	7.27	11.43	0.55	1.83
1,8-Me2-Phen	88.37 (85.19)	8.33	91.63 (89.13)	5.49	87.89 (79.79)	27.59	11.97	0.94	3.14
1-Me-Nap	89.07 (85.31)	15.62	49.88 (48.08)	15.16	41.38 (41.19)	12.23	16.41	1.16	3.88
1-Me-Phen	94.49 (94.44)	5.67	80.05 (80.83)	3.85	70.26 (71.55)	7.59	10.00	0.69	2.29
2,6-Me2-Phen	104.6 (104.27)	3.78	94.29 (94.52)	3.43	82.23 (81.74)	7.62	9.75	0.51	1.69
2-Me-Nap	74.11 (73.23)	25.40	64.50 (61.70)	13.98	48.57 (48.20)	10.57	20.82	2.39	7.96

Table 1. *Cont.*

Compound	Spiking Level						Inter-Day Precision RSD (%)	LOD ng g^{-1}	LOQ ng g^{-1}
	10		50		200				
	pg μL^{-1}		pg μL^{-1}		pg μL^{-1}				
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision			
	(%)[a]	RSD (%)	(%)	RSD (%)	(%)	RSD (%)			
2-Me-Phen	85.16 (81.31)	13.31	84.98 (85.39)	3.50	70.31 (71.00)	7.90	12.50	1.45	4.84
3,6-Me2-Phen	100.72 (99.97)	10.15	95.11 (96.45)	3.01	84.86 (84.24)	8.55	9.38	1.31	4.36
3-Me-Phen	105.84 (104.92)	8.10	88.90 (89.13)	3.02	76.31 (77.63)	8.64	13.74	1.10	3.66
9/4-Me-Phen	102.72 (102.08)	6.12	89.09 (89.38)	7.95	74.46 (75.43)	7.53	10.42	0.81	2.69
1,4-Me2-Nap	53.18 (53.77)	10.44	47.40 (45.88)	11.37	43.62 (43.78)	10.19	10.96	0.71	2.37
1,3-Me2-Phen	103.47 (103.18)	5.25	93.79 (94.46)	4.92	81.83 (82.54)	7.15	8.29	0.70	2.32
2,3,5-Me3-Nap	56.60 (56.74)	10.83	56.65 (56.50)	6.89	51.81 (51.34)	8.49	9.83	0.79	2.62
1,2,6-Me3-Phen	108.04 (104.31)	9.56	123.41 (120.86)	11.05	103.73 (100.92)	7.33	9.58	1.32	4.41
6-n-Bu-Chr	74.32 (69.89)	34.64	111.09 (110.86)	20.39	94.44 (94.72)	7.66	12.59	13.28	44.26
1.4,6,7-Me4-Nap	38.51 (40.23)	14.24	40.68 (38.09)	16.69	48.62 (47.86)	12.13	10.9	0.70	2.34
1,2,6,9-Me4-Phen	115.37 (113.19)	12.10	132.56 (129.03)	7.10	113.25 (110.10)	5.85	20.60	1.79	5.96
Retene	120.13 (116.98)	6.88	115.77 (111.98)	8.63	99.65 (96.29)	8.84	11.93	1.06	3.53

Note: Ace = Acenaphthene; Acy = Acenaphthylene; Ant = Anthracene; B[a]A = Benzo[a]Anthracene; B[a]P = Benzo[a]Pyrene; B[b]F = Benzo[b]Fluoranthene; B[g,h,i]P = Benzo[g,h,i]Pyrene; B[k]F = Benzo[k]Fluoranthene; Chr = Chrysene; D[a,h]A = Dibenzo[a,h]Anthracene; Flt = Fluoranthene; Flu = Fluorene; Ind = Indeno[1,2,3-c,d]pyrene; Nap = Naphthalene; Phen = Phenanthrene; Pyr = Pyrene. [a] Values reported as the arithmetic mean and median (in brackets). Note: Me = Methyl; Et = Ethyl; Pr = Propyl; Bu = Butyl.

With a few exceptions, our accuracies at the medium and high fortification levels were well within the criteria of 70–120% as established by the Association of Official Agricultural Chemists (AOAC). The notable exception are recoveries we observed for anthracene which was low at all fortification levels. The uniformly poor accuracy for anthracene implies that there is a systematic error in our method. In addition, the high intra- and inter-day variability for anthracene also suggests that this compound is susceptible to random error. It remains unclear why anthracene behaved so differently from the other PAHs studied especially when similar observations were not made in our earlier work [41].

Based on the Student *t*-test, there were no statistically significant differences ($p < 0.05$) in accuracies for PAHs at all fortification levels. Precision (relative standard deviation, RSD) was assessed using inter-day and intra-day repeatability. For PAHs, RSD percentages as the intra- and inter-day repeatability were all smaller than 30% except for anthracene.

For the 18 APAHs, accuracies fell in the range of 38–120, 41–133 and 41–113% at the low, medium, and high fortification levels, respectively. Except for 1-methyl naphthalene, there were no statistical differences (Student *t*-test, $p < 0.05$) in APAHs recoveries at the three (3) fortification. The mean recoveries of 1-methyl naphthalene at the low dose were statistically greater ($p > 0.05$) than those at the medium and high fortification levels. Recoveries of 6-n-butyl-chrysene were greater at the medium dose relative to that observed at the low and high fortification levels. Our data also show that alkylated naphthalenes showed the smallest recoveries of all the APAHs (range: 44–89%) and this may be related to the fact that these compounds are more volatile than the other APAHs studied. Finally, our intra-day repeatability for all APAHs, irrespective of the fortification level, was smaller than 30% except for 6-n-butyl-chrysene which had a %RSD of 35. The inter-day repeatability for APAHs were all smaller than 25%.

The LODs/LOQs, expressed on a mass/mass basis, for PAHs ranged from 0.5 to 6.2 ng/g and 1.5 to 21 ng/g, respectively. The LODs/LOQs for APAHs were all below 2.5/8 ng/g, except for 6-n-butyl-chrysene (13.3/44.3 ng/g).

Ruggedness was assessed using the two variables that could affect the performance of our method, including feather masses and the mass of silica gel/alumina. There were no significant differences ($p < 0.05$) in the performance of our method because of these purposeful changes implying that our method is robust.

We also compared the performance of our method against the conventional acid treatment approach at the medium fortification level for PAHs (see Figure 1). There were no significant differences ($p < 0.05$) in the recoveries at the stated spiking level except for anthracene (14.09%). Overall, this implies that our method performs equally well to the established acid digestion approach.

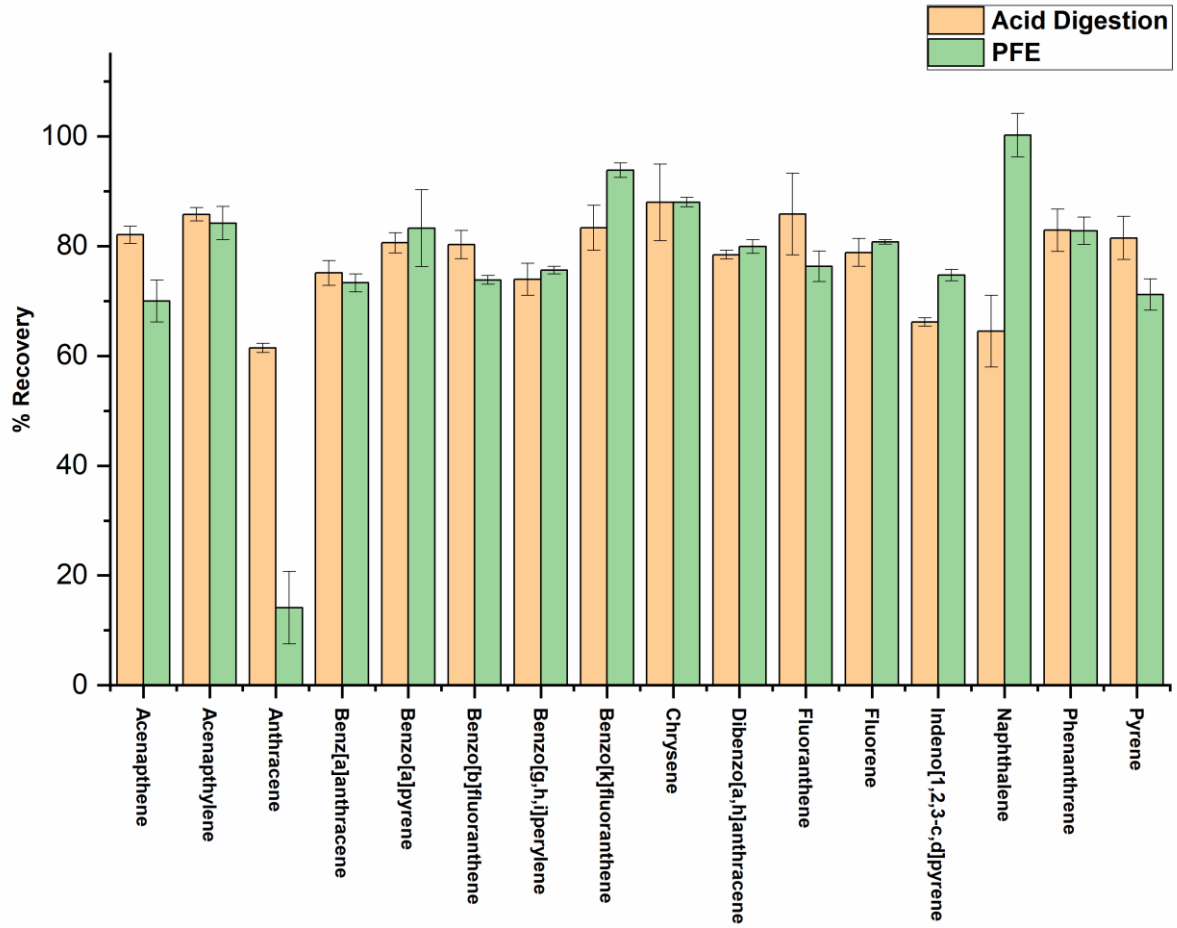

Figure 1. Comparison of the mean recovery percent ± standard deviation of PAHs in chicken feathers using our PFE method and the acid digestion method.

There are a few articles that have used acid digestion for the determination of PAHs in feathers [27,29–31]. However, to our knowledge, there are no fully validated studies similar to ours that we can use to robustly compare data quality objectives. Gonzalez-Gomez reported similar recoveries (75–120%) and relative standard deviations (<20%) for PAHs fortified in pet hair [42]. Significantly smaller LOQs were reported in that study (0.11 to 1.9 ng/g), which may be a result of the different matrices used.

The use of feathers as a sampling matrix for the measure of PACs in wild birds will likely improve our knowledge of how these compounds are distributed after their emission in the environment. In that regard, the present study supports the effort toward a reduced impact of monitoring wildlife. However, one must always consider the pathway leading

the targeted contaminants to the biological tissues when selecting a sampling matrix. The relevance of using feathers was discussed in the literature because chemical compounds follow both endogenic and exogenic pathways to reach feathers, with some integrated within the growing feather and some more recently adsorbed onto the feather surface from the surrounding media and from the endogenic preen oil [43]. In this study, we extracted and quantified all PACs present onto or in the feathers. As such, the method validated here applies well when the objective is to investigate both recent exposure and exposure during the feathers' growth. This is a critical step if countries are going to adopt feathers as the tissue compartment of choice instead of liver, plasma or serum. Perhaps a controlled laboratory study whereby birds are purposely exposed to PACs via their diet and tissues sampled at periodic intervals analyzed will help understand the temporal and tissue distribution of PACs in birds. Furthermore, this scenario would allow us to tease apart method performance of PAC exclusively absorbed to feathers.

4. Conclusions

To our knowledge, this is the first validated approach for the determination of PAHs and APAHs in feathers using PFE. With a few exceptions, the performance characteristics of our method suggest that it performs as well as the acid digestion approach. The overarching advantages of our method relative to acid digestion is that it is fully automated making it significantly less time-consuming, negates the need for any further sample processing and there is also no need for the use or handling of dangerous acids. This method is an important step towards filling the gaps in the knowledge of the environmental distribution of PACs, more specifically, in wild birds, that is currently still scarce.

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