Evaluation of estrogenic chemicals in capsule and French press coffee using ultra-performance liquid chromatography with tandem mass spectrometry

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ABSTRACT
The objective of this study was to examine exposure to estrogenic chemicals (ECs) via capsule coffee. Twenty-two brands of capsule coffee and 15 brands of French press coffee for comparison were brewed, and their contents of ECs were identified and quantified using ultra-performance liquid chromatography with tandem mass spectrometry. Exposure to ECs in coffee were compared to tolerable daily intake guidelines to assess potential hazard to health. Benzophenone was the most frequently detected EC in capsule coffee (mean concentration ± SD: 20.37 ± 20.37 ng/mL, n = 6), followed by bisphenol A (BPA, 0.31 ± 0.71, n = 4), dibutyl phthalate (1.41 ± 3.58, n = 3), 4-nonylphenol (0.67 ± 1.82, n = 3) and bisphenol F (BPF, 0.49 ± 1.54, n = 2). BPA and BPF were each detected in 3 French press coffee samples (0.29 ± 0.58 and 0.85 ± 1.75 ng/mL, respectively). Two French press coffee brands purchased as ground coffee rather than whole bean were positive for ECs (BPA in one and BPF in both). Hazard indexes were below 1.0 for each EC for both coffee types. These results indicate that there is EC contamination in capsule and French press coffee, but the quantities of ECs are low relative to established safety guidelines.

1. Introduction

Coffee is a popular beverage in the US, with 49 % of US adults consuming coffee daily [1]. While the most common method of brewing coffee in the US is drip coffee, single-serve coffee makers utilizing a pre-packed capsule or pod (capsule coffee) have recently become popular. Indeed, 28 % of US adults reported drinking coffee prepared from a single-serve coffee maker compared to 50 % from a drip coffee maker [2].

Capsule coffee is popular because it is quick to prepare and convenient; however, the brewing process requires high temperature and pressure which can cause harmful endocrine disruptors to leach from the plastic in the capsules and machine into the coffee [3]. Endocrine disruptors interfere with the hormonal system by inhibiting or mimicking certain hormones such as the sex hormones estrogen and androgens, causing deleterious health effects [4]. Xenoestrogens, estrogenic chemicals (ECs) that mimic the effects of estrogen, can pass from mothers’ breast milk to infants, potentially causing developmental issues such as shorter gestation, low birth weight, low rate of weight gain, and shorter head and abdominal circumferences [5]. In men, EC exposure can reduce fertility and sperm count and increase the incidence of congenital malformations [6,7].

Due to their utility in improving qualities of plastic compounds, certain types of ECs are highly prevalent in everyday items. For example, phthalates, with demonstrated capacity to disrupt sex hormones [8–11], enhance the properties of polystyrene polystyrene, a synthetic substance commonly used in food packaging. Similarly, bisphenol A (BPA) is used in the production of many plastic-based items such as water bottles, food containers and epoxy resins, and has been rigorously studied as a xenoestrogen [12]. Consequently, there have been calls to reduce its usage and production. However, bisphenol analogues such as bisphenol S (BPS) and bisphenol F (BPF), which impose similar health risks, are still

Abbreviations: -, not detected; 4-NP, 4-nonylphenol; BP, benzophenone; BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DMTP, dimethyl terephthalate; EC, estrogenic chemical; EDI, estimated daily intake; HI, hazard index; HPLC, high-performance liquid chromatography; MDL, method detection limit; MQL, method quantification limit; SD, standard deviation; TDI, tolerable daily intake; UPLC-MS/MS, ultra-performance liquid chromatography with tandem mass spectrometry.

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frequently used as BPA replacements [12,13].

Given the popularity of capsule coffee and its potential to increase exposure to ECs over traditional coffee brewing methods, it is critical to examine the level of EC exposure associated with capsule coffee consumption. The current evidence regarding EC contents of capsule coffee is fairly limited. Two studies reported the EC contents of coffee brewed from Italian espresso capsules [4,14], although espressos are less common in the US. Therefore, the objective of this study was to identify and quantify EC concentrations in popular US capsule coffees and evaluate the exposure in relation to established safety guidelines.

2. Methods

2.1. Coffee brewing equipment and samples

Twenty-two popular brands of plastic capsule coffee were selected, which were each designed to be brewed in one of four different capsule coffee brewing machines. Additionally, a stainless-steel French press coffee maker (Model SFP-34DS, Secura Inc., Brookfield, WI) and coffee grinder (Model BCG111OB, KitchenAid, St. Joseph, MI) were purchased and used to brew French press coffees, which were used for comparison to capsule coffees. French press coffees were selected to match the coffee brands. Of the 22 capsule coffee brands, 15 were available as either whole bean (13 brands) or ground coffee (2 brands) and were used in the French press. Whole bean coffees were selected over ground whenever available as the lower surface area was believed to limit exposure in relation to established safety guidelines.

French press coffee samples were prepared as follows: first, the coffee grinder was filled to the 4-cup fill line with whole beans and ground for 14 s to achieve a coarse grind. Then, 11 g of coffee was transferred to the French press coffee maker. Then, 500 mL of hot (but not boiling) HPLC-grade water was poured into the French press and brewed for four minutes. Finally, the coffee was poured out and mixed, and 110 mL was subsequently saved and used for analysis. These steps were repeated for each French press coffee brand, rinsing in between brands. For the two coffee brands that were purchased as ground, the first step was skipped and 11 g were directly added to the French press. All glassware and equipment used for coffee preparation were washed and rinsed in distilled water then autoclaved for 45 min at 150 °C. Extra care was taken to avoid any contact with plastic throughout the preparation and handling processes.

2.3. Extraction of ECs from coffee samples

The extraction of ECs from capsule and French press coffee samples was performed with liquid-liquid extraction. Twenty-five mL of coffee was spiked with 50 μL of the surrogate 4-hydroxy-biphenyl-d9 to a final concentration of 125 ng/mL. Meanwhile, the lab control sample and matrix spike sample were spiked with the target ECs to a final concentration of 500 ng/mL for each EC. Twenty mL of ethyl acetate was added to each sample and the mixture was centrifuged (2500 rpm for 5 min), removing the top ethyl acetate layer with a pipette. The remaining emulsion was then evaporated in an EZ-2 Genevac evaporator (Genevac Ltd, Ipswich, UK) at the low boiling point setting for 60 min. Then, 950 μL methanol was added followed by 50 μL of the internal standard 1-naphthol-d3 to a final concentration of 500 ng/mL. Glass equipment including syringes, vials and pipettes were used in order to avoid contact with plastic.

2.4. Identification and quantification of ECs

EC contents of capsule and French press coffee samples were identified and quantified using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS). LC-MS/MS methods are highly selective and sensitive, demonstrating a limit of detection of 0.4 ng/mL for BPA [15]. They are also advantageous compared to gas chromatography-mass spectrometry methods because they utilize a simple sample preparation without the need for derivatization [16]. Identification and characterization of unknown ECs were conducted based on retention time and m/z values of compounds/fragments or signature ion fragments of a peak generated by EC standards. Nine ECs...
were selected for target analysis: caprolactam, BPA, BPS, BPF, benzophenone (BP), 4-nonylphenol (4-NP), dimethyl terephthalate (DMTP), dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP).

2.5. UPLC and mass spectrometric conditions

Quality control and coffee samples were analyzed using a Waters Acquity™ UPLC® coupled with an Acquity™ TQD™ tandem mass spectrometer (Waters Co., Milford, MA). Analytic conditions were modified from Langer et al. [17] and are outlined in Table 1. The detection and quantification of analytes, surrogate, and internal standard compounds were performed in negative ESI-MS/MS mode (MRM) using the Waters, Inc. IntelliStart™ software for analyte signal optimization. Statistical analysis for obtaining calibration and quantification results for all compounds were performed using Waters QuanLynx™, which was included in the MassLynx software v.4.2. Parameters for the mass spectrometer were set as follows: capillary voltage, 3.2 kV; variable cone voltage and collision energy; desolvation temperature, 350 °C; source temperature, 145 °C; desolvation gas flow, 600 L/h; collision gas flow, 0.2 mL/min.

2.6. Method validation

The UPLC-MS/MS analytic method was first validated to demonstrate the accuracy, precision, method detection limit (MDL) and method quantification limit (MQL) for each EC, according to Environmental Protection Agency guidelines [18]. Briefly, accuracy was calculated as the mean calculated concentration of the analyte relative to the nominal concentration of the spike while precision was calculated as the relative standard deviation. MDL was determined as 3.143 (the Student’s t-value for a single-tailed 99th percentile for seven replicates) times the standard deviation (SD) of the replicate analysis. MQL was determined as 10 times the SD.

2.7. Data analysis

In order to succinctly present the results, the EC concentrations in coffee samples were averaged (mean ± SD) for each brewing method. The range of ECs detected and the number (%) of positive coffee samples were also presented. To evaluate exposure to ECs from consuming coffee, the estimated daily intake (EDI) and hazard index (HI) for each EC was calculated [19]. EDI was determined by multiplying the average amount of coffee consumed by US adult coffee consumers (417 mL) [1] by the mean (expected estimate of EDI) or highest (least conservative estimate of EDI) EC concentration and standardizing by the weight of an adult (70 kg). The HI for each EC was calculated by dividing the EDI by its tolerable daily intake (TDI), established by external guidelines and references [8,12,20–22].

3. Results

3.1. Method validation

The UPLC-MS/MS analytic method for determining EC content was highly accurate and precise, with an overall recovery of 94.9 % (range: 80–105 %) and precision of 2.0 % (range: 0.7–3.8 %) for the nine measured ECs (Table 2). This method was also highly sensitive, as indicated by the low MDLs and MQLs of the ECs. The high accuracy, precision and sensitivity for detecting ECs make this method suitable for identifying and quantifying ECs in coffee samples.

MDL and MQL were determined using a 2 ng/mL spike of the estrogenic chemical mixture. MDL = 3.14*SD. MQL = 10*SD. Abbreviations: 4-NP, 4-nonylphenol; BP, benzophenone; BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DMTP, dimethyl terephthalate; EC, estrogenic chemical; MDL, method detection limit; MQL, method quantification limit; SD, standard deviation.

3.2. EC concentration in capsule and French press coffee

Of the nine ECs targeted, five were detected in capsule coffee. BP was the most prevalent, detected in six of the 22 capsule coffee samples, followed by BPA (four), 4-NP (three), DBP (three), and BPF (two) (Table 3). There was wide variation in the concentrations of BP in the samples, ranging from 0 to 149 ng/mL. BPA and BPF were the only ECs found in French press coffee (detected in three of 15 samples for each). Notably, for the two French press coffee brands purchased as ground coffee, one was positive for BPA and both were positive for BPF. This suggests that French press coffee prepared from pre-packed ground coffee may contain more ECs than French press coffee prepared from freshly ground whole beans. BP, 4-NP and DBP were not detected in any French press coffee, and DMTP, BPS, and caprolactam were not detected in either type of coffee. Trace amounts of DEHP were detected (in amounts below the MDL) in one capsule and three French press coffee samples. There were no ECs detected in sample blanks for each capsule machine and the French press, suggesting no detectable contamination was introduced through the brewing machines, equipment, or analytical procedures. Due to the low number of positive samples (especially in the French press coffee), it was not possible to meaningfully compare the average EC concentrations between capsule and French press coffee.

3.3. Estimated daily intake and hazard indexes of ECs

EDIs of ECs were calculated using mean and highest EC concentration in coffee samples, and then used to calculate HI (Table 4). Overall, for both capsule and French press coffee, the HI for each EC using the mean concentration was very low (BP, the highest estimate: 4 × 10⁻³), suggesting minimal exposure. Estimates using the highest concentration increased HIs approximately ten-fold in all samples but HIs remained low. In capsule coffee samples, the HIs for BPA, BPF, 4-NP and DBP were comparable to one another, while the HI for BP was approximately ten-fold higher.

None of the samples had detectable amounts of DMTP, BPS, or caprolactam. DEHP was detected in quantities below the method detection limit in one capsule and three French press samples. EDI was calculated by multiplying the concentration of the chemical by 417 mL.

Table 1

| Column | Acquity™ UPLC CSH Phenyl-Hexyl (1.7 μm, 2.1 x 100 mm) |
|--------|-----------------------------------------------------|
| Column temp. (°C) | 55 |
| Solvent A | 2 mM ammonium acetate in 95 % water/5 % methanol |
| Solvent B | 2 mM ammonium acetate in 100 % methanol |
| Time (min) | 0 0.2 6 7.8 8.8 |
| Solvent A (%) | 50 50 0 0 50 |
| Total run time (min) | 9 |
| Injection volume (μL) | 5 |
| Flow rate (mL/min) | 0.2 |

Abbreviation: UPLC-MS/MS, ultra-performance liquid chromatography with tandem mass spectrometry.

Table 2

| EC | Recovery (%) | Precision (%) | MDL (ng/mL) | MQL (ng/mL) |
|----|--------------|---------------|-------------|-------------|
| Caprolactam | 80.3 | 2.1 | 0.19 | 0.6 |
| BPA | 81.5 | 3.1 | 0.34 | 1.1 |
| BPS | 100.0 | 1.0 | 0.23 | 0.7 |
| BPF | 90.6 | 1.5 | 0.35 | 1.1 |
| BP | 100.6 | 0.7 | 0.25 | 0.8 |
| 4-NP | 96.4 | 2.0 | 0.18 | 0.6 |
| DMTP | 96.6 | 3.8 | 0.67 | 2.1 |
| DBP | 105.1 | 1.1 | 0.26 | 0.8 |
| DEHP | 102.8 | 2.7 | 0.29 | 0.9 |
Table 3
Concentration of ECs in coffee brewed from capsules and French press (ng/mL).

| Samples          | Capsules (n = 22) | Capsule mean ± SD | French press (n = 15) | French press mean ± SD | Coffee samples | Coffee sample mean ± SD |
|------------------|-------------------|------------------|----------------------|------------------------|---------------|-------------------------|
|                  | Positive sample, n (%) | 4 (18.2 %) 0.31 ± 0.71 0.29 ± 0.58 | Positive sample, n (%) | 3 (20.0 %) 3 (20.0 %) 0 | | |
|                  | Mean ± SD         | 6 (27.3 %) 20.37 ± 47.07 0.85 ± 1.75 | Mean ± SD            | 0 0 0 | | |
|                  | Range             | 3 (13.6 %) 0.67 ± 1.82 | Range                | 0–7.39 0–12.34 | | |
|                  | 3 (13.6 %) 1.41 ± 3.58 | 0–149.0 | 0–149.0 | 0–149.0 | 0–149.0 | 0–149.0 |

None of the samples had detectable amounts of DMF, BPS, or caprolactam. DEHP was detected in quantities below the method detection limit in one capsule and three whole bean samples. Abbreviations: 4-NP, 4-nonylphenol; BPA, bisphenol A; BPF, bisphenol F; BP, benzophenone; DPB, dibutyl phthalate; n.d., not detected; SD, standard deviation.

Table 4
Estimated daily intakes (ng/kg body weight) and hazard indexes of estrogenic chemicals from coffee prepared from capsules and French press.

| Coffee samples | BPA | BP | 4-NP | DBP |
|----------------|-----|----|------|-----|
| MEAN CONCENTRATION |     |    |      |     |
| Capsule         | 1.85 | 5 × 10⁻⁴ | 2.92 | 3 × 10⁻⁴ | 121.35 | 4 × 10⁻³ | 3.99 | 8 × 10⁻⁴ | 8.40 | 8 × 10⁻⁴ | 8.40 | 8 × 10⁻⁴ |
| French press    | 1.73 | 4 × 10⁻⁴ | 5.06 | 5 × 10⁻⁴ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| HIGHEST CONCENTRATION |     |    |      |     |
| Capsule         | 14.42 | 4 × 10⁻³ | 23.18 | 3 × 10⁻³ | 887.61 | 3 × 10⁻² | 44.02 | 9 × 10⁻³ | 73.51 | 7 × 10⁻³ | 73.51 | 7 × 10⁻³ |
| French press    | 10.13 | 3 × 10⁻³ | 31.22 | 3 × 10⁻³ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

the average daily coffee consumption of US adults ≥20 years old [1], and dividing by 70 kg, the assumed weight of an adult. HI was calculated by dividing EDI by TDI, obtained from references: BPA: TDI = 4 × 10⁶ng/kg BW [12], BPF: TDI = 1.1 × 10⁶ng/kg BW [20], BP: TDI = 3 × 10⁶ng/kg BW [21], 4-NP: TDI = 5 × 10⁶ng/kg BW [8], DBP: TDI = 1 × 10⁶ng/kg BW [22]. EDIs and HIs were calculated using the mean and highest concentrations of estrogenic chemicals detected in coffee extract solutions. Abbreviations: 4-NP, 4-nonylphenol; BPA, bisphenol A; BPF, bisphenol F; BP, benzophenone; DBP, dibutyl phthalate; n.d., not detected; TDI, tolerable daily intake; HI, hazard index; n.d., not detected; TDI, tolerable daily intake.

4. Discussion

The purpose of this study was to determine the content of ECs in capsule coffee and assess the degree of exposure to these chemicals from typical consumption. This first required validation of an analytic method that, to our knowledge, has not yet been used to measure ECs in coffee. The UPLC-MS/MS method was suitable for this type of analysis given its high degree of accuracy, precision and sensitivity for these ECs in our samples. BPA, BPF, BP, 4-NP and DBP were detected in capsule coffee, while only BPA and BP were detected in French press coffee. BP was the most frequently detected EC in capsule coffee samples and had the highest HI, which was still considerably low. While we were unable to make statistical inferences comparing the EC content of capsule coffee to French press coffee, it is evident that ECs may be prevalent in minor, yet detectable concentrations in both types of coffee.

A few studies have similarly evaluated the content of plasticizers and phthalates in different types of coffee. DEHP and DBP have been identified in instant black coffee, with higher concentrations in coffee prepared in plastic compared to coffee prepared in glass [23]. DEHP as well as other phthalates have also been found in Italian espresso [4,14], with higher concentrations when brewed in a plastic capsule versus by a moka pot (plastic-free) [14]. Furthermore, plasticizers including BP were evident in plastic capsule coffee [24], which is consistent with the results of our study. In our study, neither DBP nor BP were detected in French press coffee, suggesting that the content of ECs in coffee may be contingent on the types of material making contact with the coffee. Plasticizers can leach from food packaging, particularly from plastic containers [25] including polyvinyl chloride film (food wrap) and polypropylene capsules [24]. However, even non-plastic food containers such as paper and cardboard can leach ECs [26] which may explain the BPA and BP detected in the few French press coffee samples. There is thus some suggestion that the packaging material may play an integral role in the EC contamination of coffee, but further studies are required for confirmation.

Estimated EC exposure from consuming a typical amount of capsule coffee was below established safety limits [5,12,20–22] for the identified compounds, and therefore unlikely to cause health concerns. Even using the least conservative estimate, HIs remained low. While there were only two studies that evaluated the HIs for ECs in coffee, they reported low HIs for DBP and DEHP, both when prepared in plastic and in metal [4,14]. Comparatively, in our study the HI for DBP was in the same order of magnitude for capsule coffee, and DEHP was not detected in either type of coffee. However, while the HIs for each EC were low, it is important to consider the aggregate effects of EC mixtures found in coffee and other sources, including dietary and non-dietary sources. ECs are ubiquitous and abundant in other commercial food and food products [27–29]. The scope of this study was restricted to assessing the exposure to ECs from two different types of coffee and therefore we are unable to evaluate the contribution of EC from coffee relative to total EC exposure. Based on current evidence, food safety organizations have reported that typical low-level exposure to phthalates [22,30], BPA [12] and BP [21] pose little health risk to most individuals, but warn that certain populations may be more vulnerable, including occupationally exposed workers, women of reproductive age, and children [30]. Studies using biological samples to assess exposure to estrogenic chemicals confirm that exposure is ubiquitous, which may have harmful effects especially in these vulnerable populations. Analyses on hair samples have indicated that children have greater exposure to BPA compared to adults, likely due to the ubiquity of BPA in toys and food products and the fact that children consume more food per body weight than adults [31]. Additionally, women of childbearing age were found to have BPA, phthalates and 4-NP detected in urine, and those with recurrent spontaneous abortion had higher concentrations of some of these estrogenic chemicals [32]. Furthermore, while the typical exposure to ECs from coffee appears to be minimal, coffee consumption is often habitual and typically involves several drinks daily for years or decades. The effects of
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