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Determination of Ochratoxin A in Brewed Coffee by Dispersive Liquid-Liquid Microextraction-High-Performance Liquid Chromatography-Fluorescence Detection

(Penentuan Okratoksin A di dalam Bancuhan Kopi dengan Pengekstrakan Mikro Cecair-Cecair Berserak-Kromatografi Cecair Berprestasi Tinggi-Pengesanan Pendarfluor)

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ABSTRACT

Coffee, the most commercialised food product and one of the top three most consumed beverages globally, faces significant challenges due to the contamination of ochratoxin A (OTA), a mycotoxin found in various foods. This presents a serious health concern requiring a rapid and sensitive method for detecting OTA. The study examined the analytical performance of dispersive liquid-liquid microextraction (DLLME) for extracting OTA from brewed coffee before analysis with high-performance liquid chromatography and fluorescence detection (HPLC-FLD). Various parameters influencing the extraction efficiency of DLLME, including the volume of extraction solvent and dispersive solvent, exposing time, centrifugation, and the addition of acid, were investigated and optimised. Under the optimised conditions (100 µL of 1-octanol as the extraction solvent, 200 µL of acetonitrile as the dispersive solvent, 1-minute exposing time, with centrifugation, and the addition of 5 µL of glacial acetic acid), the relative recoveries of OTA in spiked brewed coffee samples at 1 µg L^{-1} ranged from 82.0% to 110.4%. The method demonstrated excellent linearity (r=0.9966) and acceptable limits of detection (0.06 µg L^{-1}) and quantification (0.19 µg L^{-1}). OTA levels in locally sourced brewed coffee samples ranged from 0.77-0.90 µg L^{-1}, remaining below the European Commission regulatory limit of 3 µg kg^{-1}. The developed DLLME-HPLC-FLD method achieved a high score of 8.63 using the sample preparation metric of sustainability (SPMS), underscoring its environmental compatibility. The optimised DLLME-HPLC-FLD method provides a reliable, rapid, and environmentally friendly approach for OTA detection in brewed coffee, thereby enabling continuous monitoring of OTA in brewed coffee to ensure consumer safety.

Keywords: Coffee; DLLME; HPLC-FLD; mycotoxin

ABSTRAK

Kopi, sebagai produk makanan yang paling banyak dikomersialkan dan salah satu daripada tiga minuman teratas yang paling banyak diambil di seluruh dunia, menghadapi cabaran besar sebagai akibat pencemaran oleh okratoksin A (OTA), sejenis mikotoksin yang terdapat di dalam pelbagai makanan. Ini merupakan satu isu kesihatan yang serius yang memerlukan satu kaedah yang pantas dan sensitif untuk mengesan OTA. Penyelidikan ini mengkaji prestasi analitikal pengekstrakan cecair-cecair berserak (DLLME) dalam mengekstrak OTA daripada kopi yang telah dibancuh. Pelbagai parameter yang mempengaruhi kecekapan pengekstrakan DLLME, termasuk isi padu pelarut pengekstrak dan pelarut penyerak, masa pendedahan, pengemparan dan penambahan asid telah dikaji dan dioptimumkan. Di bawah keadaan optimum (100 µL 1-oktanol sebagai pelarut pengekstrak, 200 µL asetonitril sebagai pelarut penyerak, masa pendedahan selama 1 minit dengan pengemparan serta penambahan 5 µL asid asetik glasial), perolehan semula relatif OTA daripada sampel kopi bancuhan yang terpaku pada 1 µg L^{-1} adalah dalam julat 82.0% hingga 110.4%. Kaedah ini menunjukkan kelinearan yang sangat baik (r=0.9966) serta had pengesanan (0.06 µg L^{-1}) dan had penguantitian (0.19 µg L^{-1}) yang boleh diterima. Tahap OTA di dalam sampel kopi bancuhan yang diperoleh secara tempatan berada dalam julat 0.77-0.90 µg L^{-1} masih di bawah had peraturan Suruhanjaya Eropah 3 µg kg^{-1}. Kaedah DLLME-HPLC-FLD turut mencapai skor tinggi dalam metrik kelestarian penyediaan sampel (SPMS) iaitu 8.63 yang menunjukkan keserasian kaedah ini dengan prinsip kelestarian alam sekitar. Kaedah DLLME-HPLC-FLD yang dioptimumkan ini menyediakan pendekatan yang boleh dipercayai, pantas dan mesra alam untuk pengesanan OTA di dalam kopi bancuhan, dengan itu membolehkan pemantauan berterusan terhadap OTA di dalam kopi bancuhan untuk memastikan keselamatan pengguna.

Kata kunci: DLLME; HPLC-FLD; kopi; mikotoksin

INTRODUCTION

Coffee belongs to the Coffea genus and the Rubiaceae family of plants, and more than 80 coffee species have been identified worldwide (Ballis 2019). For decades, coffee has been the most commercialised food product and one of the most widely consumed beverages worldwide (Lafferty 2025), prized for its distinctive taste, rich aroma, and potential health benefits (Humaid, Alghalibi & Al-Khalqi 2019). Coffee beans contain approximately 1,500 chemical constituents, and coffee is considered a beneficial beverage due to its healthy compounds, including chlorogenic acids, caffeine, trigonelline, diterpenes, and tocopherols (Pakshir et al. 2021). Coffee has been shown to prevent a variety of illnesses, including Parkinson's disease, liver disease, diabetes mellitus, as well as aiding in fat loss, increasing physical activity, improving mood, and lowering the risk of depression, suicide, dementia, stroke, colorectal, and prostate cancer (Bedaso & Jilo 2022). However, coffee is susceptible to a variety of pests and fungi. Ochratoxin A contamination is the most critical factor hindering the global coffee trade (Gonzalez et al. 2020).

Ochratoxin A (OTA) was first reported in 1965, and nine years later, in 1974, it was detected in coffee (Drunday & Pacin 2013). OTA is a mycotoxin present in cereals, coffee, spices, dried fruits, and alcoholic beverages. It was reported that the OTA levels vary widely based on food type, with concentrations reaching up to 32.4 μg kg^{-1} in cereals, 0.1-6070 μg kg^{-1} in dried fruits, 0.0034-0.05066 μg kg^{-1} in spices, and up to 22.28 μg kg^{-1} in wine and 1170.75 μg kg^{-1} in beer, emphasising the widespread presence of this toxin in the food supply (Banahene et al. 2024; Fakhri et al. 2024; Freire et al. 2020; González-Curbelo & Kabak 2023).

OTA was classified as a carcinogen metabolite (Group 2B) generated mostly by *Penicillium verrucosum* and *Aspergillus carbonarius* by the International Agency for Research on Cancer (IARC) (IARC 1993). It is one of the most common and dangerous mycotoxins due to its hepatotoxic, immunosuppressive, nephrotoxic, teratogenic, and nephrocarcinogenic effects in humans (Maham et al. 2013). The presence of OTA has been detected in high-consumption beverages such as coffee and tea at levels of 31.077 μg L^{-1} and 56.7 μg L^{-1}, respectively (Pakshir et al. 2020; Yazdanfar et al. 2022). Extensive research has shown that approximately 10% of coffee is contaminated with OTA. Coffee beans can become contaminated with OTA during storage and shipping, where temperature and humidity, as well as processing phases, can increase OTA production (Gonzalez et al. 2020).

The consumption of this pleasant, functional food, due to its taste and aroma, should be taken with caution because of the presence of OTA, especially among high coffee consumers, where intake of more than 4 cups of coffee a day is a possible scenario. Current estimates indicate that coffee accounts for approximately 12% of total dietary intake of OTA, ranking it as the third most significant source of exposure to this mycotoxin (Schrenk et al. 2020). Exposure to OTA can lead to chronic health problems, and even low levels of exposure have been associated with an increased risk of kidney disease and cancer (Leitão 2019). Therefore, regular monitoring and testing of coffee beans for OTA contamination, along with the establishment of regulatory limits for OTA levels in coffee products, are essential to minimise the risks associated with OTA and to ensure the safety and quality of coffee for consumers. The European Commission establishes permissible OTA limits for food intended for human consumption, such as 3 μg kg^{-1} for roasted coffee beans and 5 μg kg^{-1} for soluble coffee, as a guideline to help consumers understand their exposure to OTA (Commission Regulation (EU) 2022/1370, 2022).

High-performance liquid chromatography with fluorescence detection (HPLC-FLD) is highly effective for the quantitative analysis of mycotoxins, particularly OTA. It is recognised by authoritative bodies such as the Association of Official Analytical Chemists (AOAC) for measuring OTA levels in cereals; however, it requires extensive sample clean-up to ensure accurate detection (Banahene et al. 2024). Solid phase extraction (SPE) (Sargazi et al. 2017) and liquid-liquid extraction (LLE) (Mariño-Repizo et al. 2018) are among the common techniques for OTA clean-up. However, these techniques consume a considerable volume of organic solvents, which are toxic and environmentally unfriendly, and the process is labour-intensive (Tan, Chai & Wong 2018).

Dispersive liquid-liquid microextraction (DLLME) developed in 2006, is a technique that miniaturises traditional liquid-liquid extraction and has gained popularity due to its high extraction efficiency, low solvent volume, affordability, and simple procedure (Faraji 2024). In the early stages of DLLME, most of the extractants used were chlorinated organic compounds, such as chloroform, 1,1,2,2-tetrachloroethane, and trichloroethylene, which are highly toxic and volatile. These solvents can easily cause significant toxicity to operators and the environment (Wang et al. 2019). Recently, due to increased environmental awareness, the development of sensitive, precise, low-cost, and eco-friendly analytical techniques has become a common trend in analytical chemistry (Cina et al. 2022). Therefore, DLLME meets these requirements and aligns with the 12 principles of green analytical chemistry (GAC).

To assess the greenness of sample preparation methods without taking into account the aspects of the initial sampling and the final detection technique, González-Martín et al. (2023) demonstrated the sample preparation metric of sustainability (SPMS), which includes a clock-like diagram with a central greenness score and nine surrounding parameters (amount, extractant nature and reusability, number of steps, extraction time, additional steps after extraction, sample throughput, energy consumption, and total waste) with each of these parameters is color-coded to indicate sustainability levels: green (successful), yellow (acceptable), orange (tolerable), and red (inadequate).

In this study, the coupling of DLLME with HPLC-FLD was investigated for the analysis of OTA in brewed coffee, aiming to achieve efficient extraction of OTA from this complex beverage matrix. Additionally, the environmental sustainability of the DLLME technique was evaluated using the SPMS to support environmentally friendly practices, thereby providing a reliable, sustainable approach for routine monitoring of OTA in coffee products.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Methanol (chromatography grade), 1-octanol (analytical grade), acetonitrile (chromatography grade), and glacial acetic acid were purchased from Merck (Darmstadt, Germany). The OTA reference standard at a concentration of 100 µg mL^{-1} in methanol was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The serial standard solution was prepared daily by diluting the 100 µg mL^{-1} stock solution with methanol before use. The solutions were stored in the freezer (-20 °C) when not in use.

PREPARATION OF BREWED COFFEE

Two types of coffee samples were used in this study: freshly roasted coffee beans purchased from a local roastery, ground immediately after roasting, and commercially available ground coffee powders from local retail markets. The freshly ground coffee bean samples were primarily used during optimisation because they were expected to contain negligible or non-detectable levels of OTA due to their recent roasting and handling, ensuring that optimisation studies were conducted without background contamination. The commercially available coffee powders were used in the relative recovery experiments and sample analysis, as these represent real consumer products that may contain naturally occurring OTA.

The preparation of brewed coffee was adopted from Cina et al. (2022) with minor modifications. Approximately 1 g of ground coffee powder was weighed, mixed with 10 mL of 80 °C deionised water, vortexed for 1 min, and centrifuged at 5000 rpm for 6 min. Next, the supernatant was withdrawn for DLLME.

DLLME OF OCHRATOXIN A FROM BREWED COFFEE

A volume of 5 µL of acetic acid was added to 5 mL of the sample solution, and the mixture was vortexed for a few seconds using a vortex mixer (WiseStir, Daihan Scientific, South Korea). Subsequently, a premixed solvent consisting of 200 µL of acetonitrile (dispersive solvent) and 100 µL of 1-octanol (extraction solvent) was rapidly injected into the sample solution using a 1-mL disposable syringe. A cloudy emulsion was immediately formed and allowed to stand at room temperature for 1 min to facilitate dispersion. The mixture was then centrifuged at 4000 rpm for 2 min. Following centrifugation, the upper organic phase (1-octanol) containing the extracted OTA was carefully collected and analysed quantitatively by HPLC-FLD. The DLLME procedure is illustrated in Figure 1.

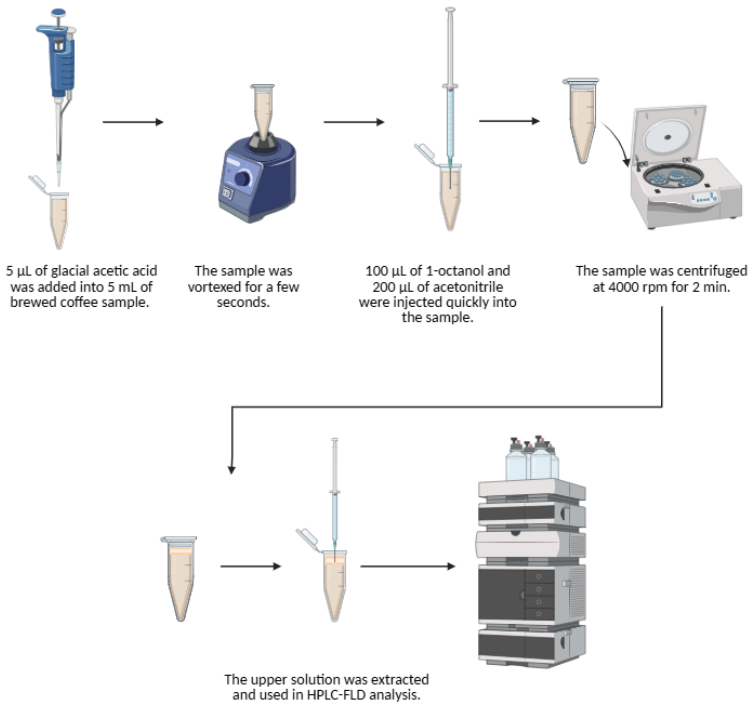


FIGURE 1. DLLME procedure for the extraction of OTA from brewed coffee

OPTIMISATION OF DLLME

The DLLME procedure was optimised using a one-variable-at-a-time approach to enhance the extraction sensitivity. Several extraction variables, namely volume of 1-octanol, volume of acetonitrile, exposing time, effect of centrifugation, and addition of acetic acid, were investigated using spiked brewed coffee samples prepared at 20 μg L^{-1}. Each extraction was performed in triplicate to ensure consistent selection of the best variable.

QUANTIFICATION OF OCHRATOXIN A WITH HPLC-FLD

The detection of OTA was conducted using a Shimadzu HPLC system (Kyoto, Japan). The system was equipped with a solvent delivery module (LC40D XS), a degassing unit (DGU-405), a system controller (CBM-40), an autosampler (SIL-40C XS), and a FLD (RF-20A XS). Chromatographic separation was achieved using a ShimPack GIST C18 column (4.6 mm × 150 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 2% (v/v) acetic acid in a 55:45 ratio (isocratic mode). The column temperature was maintained at 40 °C. The flow rate was set at 1.0 mL min^{-1}, with an injection volume of 10 μL. Fluorescence detection was carried out at excitation and emission wavelengths of 333 nm and 460 nm, respectively.

VALIDATION OF DLLME-HPLC-FLD FOR THE DETERMINATION OF OCHRATOXIN A IN BREWED COFFEE

The DLLME-HPLC-FLD method for analysing OTA in brewed coffee was validated by assessing linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). A seven-point calibration curve was created using OTA standard solutions at concentrations of 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, and 20.0 μg L^{-1}. Linear least-squares regression was utilised to develop the calibration curve over the studied concentration range. Accuracy was evaluated through relative recovery experiments with spiked samples (n=3) from five different brands of brewed coffee, fortified at a single concentration level of 1.0 μg L^{-1}. The LOD and LOQ were determined based on the signal-to-noise ratio of 3:1 and 10:1, respectively (Karami-Osboo et al. 2015).

ASSESSMENT OF ENVIRONMENTAL FRIENDLINESS OF DLLME WITH SAMPLE PREPARATION METRIC OF SUSTAINABILITY

The environmental sustainability of the DLLME process for extracting OTA in brewed coffee was then evaluated using the SPMS. The SPMS is a multidimensional assessment tool that considers sample preparation parameters, including sample amount, extractant volume and type, procedural complexity, energy use, and waste generation (González-Martín et al. 2023).

RESULTS AND DISCUSSION

OPTIMISATION OF DLLME

In this study, 1-octanol was chosen as the extraction solvent because of its lower density than that of the sample solution. This facilitated the removal of the extract, which floated on the top layer of the sample solution. Acetonitrile was selected as the dispersive solvent because it is less viscous (0.369 cP), facilitating rapid dispersion of the extraction solvent (Gomes et al. 2025; Mousavi et al. 2025). The impact of various extraction variables on the efficiency of DLLME for extracting OTA was examined using spiked brewed coffee samples rather than spiked deionised water to eliminate the matrix effect, since coffee is extremely complex and contains numerous compounds that can influence sensory properties.

The effect of the extraction solvent or 1-octanol's volume on the extraction efficiency for the DLLME of OTA was examined through rapid injections of solutions containing a fixed volume of acetonitrile (200 μL) as the dispersive solvent, along with varying volumes of 1-octanol. As shown in Figure 2(a), the extraction efficiency increased when the volume of 1-octanol was decreased from 300 μL to 50 μL. This is because a smaller volume of extraction solvent leads to greater analyte enrichment (Loh et al. 2016). However, the 50 μL droplet was too small and difficult to withdraw, resulting in poorer repeatability in triplicate extractions at this volume compared to other volumes. Consequently, 100 μL of 1-octanol was used for further optimisation studies.

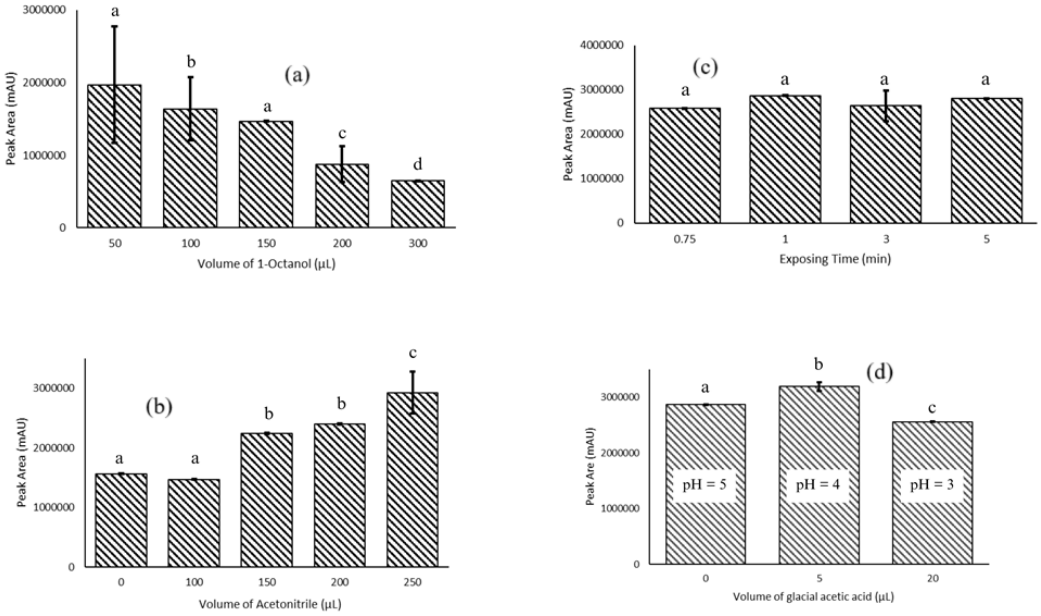
To examine the effect of dispersive solvent or acetonitrile volume on the recovery of OTA using DLLME, different volumes of acetonitrile premixed with 100 μL of 1-octanol were separately added into 5 mL of spiked brewed coffee samples. The variation in the OTA peak areas with the acetonitrile volume is shown in Figure 2(b). The extraction efficiency was improved by increasing the volume of acetonitrile. This was most likely due to an increase in the solubility of 1-octanol in the acetonitrile as the volume of acetonitrile increased (Ferrone et al. 2018). As a consequence, the dispersibility of the 1-octanol in the aqueous sample solution was also expanded to accelerate the mass transfer of OTA into the extraction solvent. It was observed that 250 μL of acetonitrile improved the OTA recovery. However, the repeatability of using 250 μL of acetonitrile was poorer. The ratio of extraction solvent to dispersive solvent plays a critical role in the efficiency of the DLLME process. An excessive volume of dispersive solvent can increase the solubility of the target analytes in the aqueous phase, thereby reducing extraction efficiency. On the other hand, an insufficient volume may hinder the formation of a stable cloudy solution, as the extraction solvent may not disperse effectively throughout the sample matrix (Sharifi, Abbasi & Nosrati 2016). To ensure optimal dispersion and extraction efficiency, a combination of

100 µL of 1-octanol (extraction solvent) and 200 µL of acetonitrile (dispersive solvent) was selected for the subsequent optimisation experiments.

Exposing time is the period during which the sample solution is exposed to the extraction solvent. The time is measured from the injection of the solvent mixture containing the extraction and disperser solvents into the sample solution until just before centrifugation for phase separation. In this study, the sample solutions were exposed to the solvent mixture for 45 s to 5 min to examine the effect of exposure time. The results indicated that exposing time had no significant impact on DLLME extraction efficiency (Figure 2(c)). The dispersive concept applied in the DLLME accelerated the dispersion of the extraction solvent throughout the sample solution, thereby speeding up the mass transfer of OTA into the extraction solvent within a minute of exposure. The large contact surface between the extraction solvent and the aqueous sample solution in the emulsion system enabled rapid attainment of the extraction equilibrium. When the exposure time was further increased to 3 and 5 min, the peak areas declined slightly but insignificantly. Nevertheless, the optimal exposing time at 1 min was still chosen to standardise the extraction condition for better repeatability.

Centrifugation is necessary in DLLME for phase separation following extraction. Once phase separation occurs, the lighter extraction solvent, which floats on top of the sample solution, is easier to withdraw. Results showed that OTA recovery was 53.0% higher when centrifugation was used for phase separation than without it. The extract was withdrawn 1 min after exposing the sample solution to the solvent mixture, when the centrifugation was not applied. Some extraction solvent was not fully separated from the disperser solvent and sample solution, resulting in lower OTA recovery. Consequently, DLLME was conducted with centrifugation because this process is crucial for obtaining three distinct phases in the extraction tubes, facilitating the collection and withdrawal of the target analyte in the extraction solvent (Karami-Osboo et al. 2015).

Acetic acid was added to the sample to evaluate the effect of pH on the extraction efficiency of DLLME of OTA from spiked brewed coffee. Two volumes of acetic acid, 5 µL and 20 µL, were tested and compared to a control sample with no pH adjustment (original pH ~5). The resulting sample pH values were approximately pH 4 and pH 3 for the 5 µL and 20 µL additions, respectively. The 10 µL volume was not investigated, as preliminary testing showed it did not further lower the pH beyond that achieved with 5 µL (remaining at pH 4). As illustrated in Figure 2(d), the extraction efficiency increased with 5 µL of acetic acid, but declined at 20 µL. OTA is a weak acid with two pKa values: approximately 4.4 (carboxylic group) and 7.05-7.30 (phenolic group). At pH 4, the carboxylic acid group exists predominantly in its neutral form (non-dissociated -COOH), which enhances its solubility in organic solvents such as 1-octanol due to the absence of charge (Elik et al. 2023). However, under strongly acidic conditions, OTA may undergo partial hydrolysis or structural degradation, decreasing its stability and extractability (Antep & Bozkurt 2015). Based on these findings, adding 5 µL of glacial acetic acid was selected as the optimal condition for subsequent experiments.



Similar letters that are directly above the error bars indicate no significant difference according to the Tukey test ($p > 0.05$)

FIGURE 2. Effect of extraction solvent volume (a), dispersive solvent volume (b), exposing time (c), and acid addition (d) on the extraction efficiency of DLLME of OTA from spiked brewed coffee prepared at 20 µg L^{-1}

VALIDATION AND APPLICATION OF DLLME-HPLC-FLD FOR THE DETERMINATION OF OCHRATOXIN A IN BREWED COFFEE

The selected parameters for minor method validation were examined, which included linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. The calibration curve was generated using linear least-squares regression of peak area versus concentration. The linearity for OTA was satisfactory within the range of 0.25 to 20.0 µg L^{-1}, as shown by the linear correlation coefficients (r) of 0.9935 and 0.9966 for DLLME of OTA in spiked deionised (DI) water and brewed coffee samples, respectively. The regression results (Table 1) indicated matrix effects in the spiked brewed coffee, confirming the need to establish a matrix-matched calibration curve for quantifying OTA in brewed coffee.

The LOD and LOQ were determined using the signal-to-noise ratio method, where LOD and LOQ were estimated from signal-to-noise ratios of 3:1 and 10:1, respectively. The LOQ and LOD determined in brewed coffee were 0.19 µg L^{-1} and 0.06 µg L^{-1}, respectively, both supporting that the proposed DLLME-HPLC-FLD was capable of detecting ultra-trace of OTA in brewed coffee.

The accuracy of the DLLME-HPLC-FLD was assessed using a relative recovery study. Different brands of brewed coffee samples spiked to 1.0 µg L^{-1}, and their blank samples were extracted using DLLME before HPLC-FLD analysis. The relative recovery results ranged from 82.0 to 110.4%, with acceptable precision as measured by relative standard deviation (RSD), indicating that matrix effects were insignificant when matrix-matched calibration was used for the quantification of OTA in brewed coffee. Table 2 shows the relative recovery results.

All 10 samples of brewed coffee purchased from the local market were analysed, and 2 brands of coffee contained detectable levels of OTA, ranging from 0.77 to 0.90 µgL^{-1}, which is still below the accepted limit for roasted coffee (3 µg kg^{-1}). In contrast, the others were lower than LOD.

ASSESSMENT OF ENVIRONMENTAL FRIENDLINESS OF DLLME WITH SAMPLE PREPARATION METRIC OF SUSTAINABILITY

Figure 3 summarises the SPMS clock diagram and shows the sustainability score obtained in the study, 8.63, indicating that the proposed DLLME for OTA has high eco-compatibility. The amounts of sample and extractant applied in DLLME were 5 mL and ≤0.1 mL, respectively, which then contributed to the 'successful' SPMS scores. While the nature of the 1-octanol as the extractant was 'alternative degradable', which was considered 'acceptable'. In addition, the DLLME procedure involved 3 steps, namely dispersion, centrifugation, and extraction, which was then allocated an 'acceptable' score. Lastly, the DLLME extraction took only 1 min and required no additional steps after extraction, which contributed to the 'successful' score again. However, this method used a single sample throughput, which reduced the SPMS score.

Energy consumption was minimised by using vortex mixing for dispersion and centrifugation for phase separation, both are common and energy-efficient laboratory techniques. Furthermore, the entire extraction process was conducted at room temperature, eliminating the need for external heating. The total waste generated per sample was estimated to be less than 10 mL, consisting primarily of aqueous and small amounts of organic solvent

TABLE 1. Results of linearity, LOD, and LOQ

Samples	Range, µg L^{-1}	Regression	r	LOD, µg L^{-1}	LOQ, µg L^{-1}
Spiked deionised water	0.25-20.0	y=136979x - 16472	0.9935	0.10	0.34
Spiked brewed coffee	0.25-20.0	y=102879x + 21260	0.9966	0.06	0.19

TABLE 2. Relative recovery of OTA in different spiked local brewed coffee samples

Sample	Blank sample concentration, µg L^{-1}	Spiked concentration, µg L^{-1}	Concentration found, µg L^{-1}	Average relative recovery, % ± RSD, %, n=3
Brand A	0.77	1.0	1.87	110.4 ± 1.4
Brand B	0.90	1.0	1.94	104.0 ± 1.5
Brand C	Not detected	1.0	0.93	93.1 ± 4.2
Brand D	Not detected	1.0	0.87	86.6 ± 7.9
Brand E	Not detected	1.0	0.82	82.0 ± 0.7

*Not detected = Less than LOD, 0.06 µg L^{-1}

FIGURE 3. SPMS clock diagram of the DLLME of OTA in brewed coffee

residues, meeting the criteria for minimal waste generation. All the above criteria have achieved the 'successful' green scores. However, the extractant used in this study can be used only once after HPLC analysis, so it cannot be reused for another extraction. Therefore, no score was assigned for the extractant's reusability.

An SPMS score of 8.63 (on a scale from 0 to 10) places the developed DLLME of the OTA procedure in the 'green' category, indicating a sustainable sample preparation approach. This aligns with the findings of the SPMS pioneers, who reported a score for the DLLME technique ranging from 6.21 to 8.63 (González-Martín et al. 2023). The high score emphasises the method's adherence to green analytical chemistry principles, particularly in terms of sample economy, energy efficiency, and procedural simplicity. Overall, the developed DLLME-HPLC-FLD method demonstrates a strong balance between analytical performance and environmental responsibility, making it a suitable approach for the routine determination of OTA in brewed coffee.

CONCLUSIONS

This study successfully optimised the DLLME technique for extracting OTA from brewed coffee and quantifying it using HPLC-FLD. The method provides rapid, sensitive, and selective analysis of OTA in brewed coffee, making it useful for routine testing. The use of organic extractant is minimal, and sample throughput can be increased due to the very short extraction time. Additionally, the HPLC-FLD effectively separates OTA from complex coffee matrices, enabling selective detection. All locally sourced coffee samples analysed were well below the regulatory limit of 3 µg L^{-1} for roasted coffee. This indicates that OTA contamination remains within safe consumption levels, although it is present. Given the potential health risks of long-term OTA exposure, ongoing monitoring and strict regulatory enforcement are essential to protect consumers. The SPMS score assigned in this study places the DLLME within the green category, reflecting a sustainable sample preparation method that offers ecological benefits while reducing environmental impacts.

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REFERENCES

Antep, H.M. & Bozkurt, S.S. 2015. Enrichment of ochratoxin A in cereals using ionic liquid based dispersive liquid-liquid microextraction. *Hacettepe Journal of Biology and Chemistry* 43(2): 99-104.

Ballis, A.S. 2019. Coffee a worldwide trend with health benefits. *Acta Scientific Nutritional Health* 3(3): 70-73.

Banahene, J.C.M., Ofosu, I.W., Odai, B.T., Lutterodt, H.E., Agyemang, P.A. & Ellis, W.O. 2024. Ochratoxin A in food commodities: A review of occurrence, toxicity, and management strategies. *Heliyon* 10(20): e39313.

Bedaso, Z.A. & Jilo, K.R. 2022. Coffee consumption benefits and risks on human health. *Journal of Medicine and Biology* 4(1): 41-52.

Cina, M., del Valle Ponce, M., Fernandez, L. & Cerutti, S. 2022. A green approach for ochratoxin A determination in coffee infusions. *Journal of Food Composition and Analysis* 114: 104777.

Commission Regulation (EU) 2022/1370 2022. *Official Journal of the European Union* 206, 8.8.2022. pp. 11–14. EUR-Lex.

Drunday, V. & Pacin, A. 2013. Occurrence of ochratoxin A in coffee beans, ground roasted coffee and soluble coffee and method validation. *Food Control* 30(2): 675-678.

Elik, A., Ablak, Ö., Haq, H.U., Boczkaj, G. & Altunay, N. 2023. Combination of homogeneous liquid–liquid extraction and vortex assisted dispersive liquid–liquid microextraction for the extraction and analysis of ochratoxin A in dried fruit samples: Central composite design optimization. *Journal of Food Composition and Analysis* 124: 105656.

Fakhri, Y., Ranaei, V., Pilevar, Z., Belaia, O.F., Kolaeva, N.V., Sarafraz, M. & Mousavi Khaneghah, A. 2024. Prevalence and concentration of ochratoxin A in beer: A global systematic review, meta-analysis, and health risk assessment. *Food Science and Nutrition* 12(11): 8503-8514.

Faraji, H. 2024. Advancements in overcoming challenges in dispersive liquid-liquid microextraction: An overview of advanced strategies. *Trends in Analytical Chemistry* 170: 117429.

Ferrone, V., Genovese, S., Carlucci, M., Tiecco, M., Germani, R., Preziuso, F., Epifano, F., Carlucci, G. & Taddeo, V.A. 2018. A green deep eutectic solvent dispersive liquid-liquid micro-extraction (DES-DLLME) for the UHPLC-PDA determination of oxyprenylated phenylpropanoids in olive, soy, peanuts, corn, and sunflower oil. *Food Chemistry* 245: 578-585.

Freire, L., Braga, P.A.C., Furtado, M.M., Delafiori, J., Dias-Audibert, F.L., Pereira, G.E., Reyes, F.G., Catharino, R.R. & Sant'Ana, A.S. 2020. From grape to wine: Fate of ochratoxin A during red, rose, and white winemaking process and the presence of ochratoxin derivatives in the final products. *Food Control* 113: 107167.

Gomes, A.L., Sherudillo, A., Shagurin, A., Antina, L., Moskaieva, O., Dubois, J., Kalugin, O.N., Sauvage, F., Miannay, F.A. & Idrissi, A. 2025. Photophysical modulation of D205 dye in acetonitrile-butanol mixtures: interplay of solvent polarity, hydrogen bonding, and conformational dynamics. *Journal of Molecular Liquids* 435: 128109.

Gonzalez, A.L., Lozano, V.A., Escandar, G.M. & Bravo, M.A. 2020. Determination of ochratoxin A in coffee and tea samples by coupling second-order multivariate calibration and fluorescence spectroscopy. *Talanta* 219: 121288.

González-Curbelo, M.A. & Kabak, B. 2023. Occurrence of mycotoxins in dried fruits worldwide, with a focus on aflatoxins and ochratoxin A: A review. *Toxins* 15(9): 576.

González-Martín, R., Gutiérrez-Serpa, A., Pino, V. & Sajid, M. 2023. A tool to assess analytical sample preparation procedures: Sample preparation metric of sustainability. *Journal of Chromatography A* 1707: 464291.

Humaid, A.A.H., Alghalibi, S.M.S. & Al-Khalqi, E.A.A. 2019. Aflatoxins and ochratoxin a content of stored yemeni coffee beans and effect of roasting on mycotoxin contamination. *International Journal of Molecular Microbiology* 2(1): 11-21.

International Agency for Research on Cancer (IARC). 1993. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins.

Karami-Osboo, R., Miri, R., Javidnia, K., Kobarfard, F., AliAbadi, M.H.S. & Maham, M. 2015. A validated dispersive liquid-liquid microextraction method for extraction of ochratoxin A from raisin samples. *Journal of Food Science and Technology* 52(4): 2440-2445.

Lafferty, J. 2025. *Most Consumed Beverages in the World: What People Drink Most.* https://mysticdrink.com/most-consumed-beverages-in-the-world-what-people-drink-most/ Accessed 11 December 2025.

Leitão, A.L. 2019. Occurrence of ochratoxin a in coffee: Threads and solutions - A mini-review. *Beverages* 5(2): 36.

Loh, S.H., Chong, Y.T., Afindi, K.N.N. & Kamaruddin, N.A. 2016. Determination of polycyclic aromatic hydrocarbons in beverage by low density solvent based-dispersive liquid-liquid microextraction-high performance liquid chromatography-fluorescence detection. *Sains Malaysiana* 45(10): 1453-1459.

Maham, M., Kiarostami, V., Waqif-Husain, S., Karami-Osboo, R. & Mirabolfathy, M. 2013. Analysis of ochratoxin A in malt beverage samples using dispersive liquid–liquid microextraction coupled with liquid chromatography-fluorescence detection. *Czech Journal of Food Sciences* 31(5): 520-525.

Mariño-Repizo, L., Goicoechea, H., Raba, J. & Cerutti, S. 2018. A simple, rapid and novel method based on salting-out assisted liquid–liquid extraction for ochratoxin A determination in beer samples prior to ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. *Food Additives and Contaminants - Part A* 35(8): 1622-1632.

Mousavi, S.S., Shahsavani, A., Aladaghlo, Z. & Fakhari, A.R. 2025. Solvent-assisted dispersive solid-phase extraction based on salen as a new and efficient sorbent followed by GC-FID for determination of polycyclic aromatic hydrocarbons in water, food, and soil samples. *Journal of the Iranian Chemical Society* 22: 2005-2014.

Pakshir, K., Dehghani, A., Nouraei, H., Zareshahrabadi, Z. & Zomorodian, K. 2021. Evaluation of fungal contamination and ochratoxin A detection in different types of coffee by HPLC-based method. *Journal of Clinical Laboratory Analysis* 35(11): e24001.

Pakshir, K., Mirshekari, Z., Nouraei, H., Zareshahrabadi, Z., Zomorodian, K., Khodadadi, H., & Hadaegh, A. 2020. Mycotoxins detection and fungal contamination in black and green tea by HPLC-based method. *Journal of Toxicology* 2020(1): 2456210.

Sargazi, A., Aliabadi, A., Rahdari, A., Allahdini-Hesaroiyeh, S., Nejati-Yazdinejad, M. & Majd, M.H. 2017. A simple and fast method for magnetic solid phase extraction of ochratoxin A. *Journal of the Brazilian Chemical Society* 28(6): 950-959.

Schrenk, D., Bodin, L., Chipman, J.K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Alexander, J., Dall'Asta, C., Mally, A., Metzler, M., Binaglia, M., Horváth, Z., Steinkellner, H. & Bignami, M. 2020. Risk assessment of ochratoxin A in food. *EFSA Journal* 18(5): e06113.

Sharifi, V., Abbasi, A. & Nosrati, A. 2016. Application of hollow fiber liquid phase microextraction and dispersive liquid-liquid microextraction techniques in analytical toxicology. *Journal of Food and Drug Analysis* 24(2): 264-276.

Tan, Y.H., Chai, M.K. & Wong, L.S. 2018. A review on extraction solvents in the dispersive liquid-liquid microextraction. *Malaysian Journal of Analytical Sciences* 22(2): 166-174.

Wang, Q., Chen, R., Shatner, W., Cao, Y. & Bai, Y. 2019. State-of-the-art on the technique of dispersive liquid-liquid microextraction. *Ultrasonics Sonochemistry* 51: 369-377.

Yazdanfar, N., Mahmudiono, T., Fakhri, Y., Mahvi, A.H., Sadighara, P., Mohammadi, A.A., & Yousefi, M. 2022. Concentration of ochratoxin A in coffee products and probabilistic health risk assessment. *Arabian Journal of Chemistry* 15(12): 104376.

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