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Zeolite Linde Type L as micro-solid phase extraction sorbent for the high performance liquid chromatography determination of ochratoxin A in coffee and cereal

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ABSTRACT

Zeolite Linde Type L (LTL) crystals with different length, diameter and particle size (nanosized LTL, rod LTL, cylinder LTL and needle LTL) were synthesized, characterized and were used as sorbent in the micro-solid phase extraction of ochratoxin A (OTA) before the high performance liquid chromatography detection. Under the optimized conditions, the detection limits of OTA for coffee and cereal were 0.09 ng g^{-1} and 0.03 ng g^{-1}, respectively, while the quantification limits were 0.28 ng g^{-1} and 0.08 ng g^{-1}, respectively. The recoveries of OTA of coffee and cereal spiked at 0.5, 10 and 25 ng g^{-1} ranged from 91.7 to 101.0%. The proposed method was applied to forty-five samples of coffee and cereal. The presence of OTA was found in twenty-five samples, ranging from 0.28 to 9.33 ng g^{-1}.

1. Introduction

Ochratoxin A (OTA) (Fig. 1), a mycotoxin that is produced by fungi of the genera *Aspergillus* and *Penicillium*, is one of the most widespread and hazardous mycotoxins. OTA can be found in several food products such as beer, cacao, spices, dried fruits, wine and grape juice. Several countries have reported the presence of OTA in coffee and cereal products [1–4]. Considering that cereals contribute to 50% with the OTA intake [5], the Commission Regulations (EU) has established the maximum levels for OTA in cereals of 5 μg kg^{-1}, all products derived from cereals of 3 μg kg^{-1}, processed cereal-based foods and baby-foods of 0.5 μg kg^{-1}. The limits for OTA in roasted coffee and instant coffee are 5 μg kg^{-1} and 10 μg kg^{-1}, respectively [6].

Carry over effects from contaminated feed may lead to the occurrence of residual OTA in the liver, kidney and other tissue of livestock and humans. In humans, OTA has been reported to have teratogenic, nephrotoxic, genotoxic, immunotoxic and carcinogenic effects [7,8]. Besides that, OTA is also suspected to cause the Balkan Endemic Nephropathy in rural areas in South-Eastern Europe and urinary tract tumour.

Due to health concerns, there is an increasing need for fast, reliable and low-cost analytical methods for monitoring OTA in foodstuffs. Several methods for the determination of OTA in food have been reported, including enzyme-linked immunosorbent assay (ELISA) [9], gas chromatography (GC) [10], thin-layer chromatography (TLC) [11], capillary electrophoresis (CE) [12] and liquid chromatography (LC) [13]. High performance liquid chromatography (HPLC) with fluorescence detection remains the most commonly used method at the moment, although liquid chromatography–mass spectrometry (LC–MS) is indispensable for positive confirmation.

The most widely used methods for the extraction and clean-up of OTA are liquid–liquid extraction (LLE) or solid-phase extraction (SPE). Recent trends have been directed toward developing more simplified, economical, and miniaturized sample preparation methods. Solid phase microextraction (SPME) [14], hollow fiber liquid phase microextraction (HF-LPME) [15,16], supramolecule liquid phase microextraction (SM-LPME) [17], liquid microextraction (DLLME) [18] and more recently in-tube solid-phase microextraction [19] have been reported for the analysis of OTA in food. Micro-solid phase extraction (μ-SPE) is a promising alternative to the multi-step SPE method for the preconcentration of analytes in the complex samples [20]. Generally, with the exception

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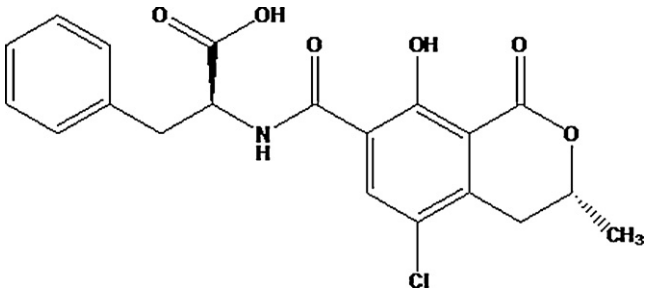

Fig. 1. Chemical structure of ochratoxin A (OTA).

of the molecularly imprinted polymer (MIP) sorbent [21,22], the sorbents used (e.g., C_2, C_8, C_{18}, etc.) are of low selectivity.

Zeolites are crystalline microporous aluminosilicates consisting open framework structures and exchangeable cations which generate a system of pores and cavities having molecular dimensions (3–10 Å) [23]. Unlike other sorbents, zeolites have specific pore shape and size which can suitably be used for the distinction and controlling the access of molecules with different size, shape and polarity. Particularly, the zeolite known as Linde Type L (LTL), with one dimensional 12-membered ring channel system has been shown as promising material in environmental and industrial applications [24]. Control of the morphology and size of the zeolite crystals allowed them to be filled with a variety of guest species such as metal complexes, organic dyes, contaminants or clusters [25–28].

In this paper, LTL zeolites were used as sorbents in μ-SPE to extract OTA in coffee and cereal. To the best of our knowledge, this is the first paper utilizing zeolite LTL to extract OTA in foodstuffs.

2. Experimental

2.1. Chemicals and materials

Ochratoxin A (≥98%), potassium hydroxide (≥85%), Ludox (HS-40 colloidal silica, 40 wt.%), sodium chloride (≥99%), sodium hydrogencarbonate (≥99.5%) were purchased from Sigma (St. Louis, MO, USA). HPLC-grades acetonitrile (99.99%) and methanol (≥99.96%), glacial acetic acid (≥99.5%), aluminum sulfate octadecahydrate ($Al_2(SO_4)_3{\cdot}18H_2O$) and hydrochloric acid (37%, w/w) were purchased from Merck (Darmstadt, Germany). C_8, C_{18}, C_{30} sorbent were purchased from United Chemical Technologies (Bristol, PA, USA). Molecularly imprinted polymer crushed monolith and bead (AFFINIMIP™ OTA) were provided by Polyintell (Val de Reuil, France). Accurel polypropylene flat membrane sheet (200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, USA) and was used throughout for the preparation of solutions.

2.2. Preparation of zeolite LTL

Four samples of K^+-LTL with different morphologies were synthesized using the molar gel compositions and synthesis conditions as follows [29,30]:

Nanosized LTL: 10.0 K_2O:1 Al_2O_3:4 SiO_2:100 H_2O (180 °C, 1 day)
Rod LTL: 10.0 K_2O:1 Al_2O_3:20 SiO_2:800 H_2O (180 °C, 3 days)
Cylinder LTL: 10.2 K_2O:1 Al_2O_3:20 SiO_2:1030 H_2O (180 °C, 3 days)
Needle LTL: 10.2 K_2O:1 Al_2O_3:20 SiO_2:1200 H_2O (180 °C, 3 days)

To prepare rod LTL, $Al_2(SO_4)_3{\cdot}18H_2O$ (1.73 g) was dissolved in an aqueous KOH solution consisting KOH (3.44 g) and distilled water (22.14 g) followed by stirring for 10 min until a clear solution was obtained. A silica solution was prepared separately by mixing Ludox (HS-40 colloidal silica) (7.82 g) with distilled water (9.80 g). The silica solution was then added slowly to the alumina solution under vigorous stirring. The gel was then stirred for 18 h at room temperature and then transferred into a Teflon-lined stainless steel autoclave. The crystallization process was allowed to take place at 180 °C for 3 days, after which the reaction was quenched by plunging the autoclave into cold water. The resulting crystals were filtered and purified with distilled water until pH ~ 7 and dried at 80 °C overnight. The other LTL zeolites with different molar gel compositions were prepared using the similar procedure as described above.

2.3. Characterizations of LTL zeolites

The X-ray diffraction (XRD) patterns were recorded with Siemens D5000 diffractometer with Cu-K_α radiation (λ = 1.5406 Å). Scanning electron micrographs were taken on a Philips XL 30 LaB_6 scanning electron microscope (SEM) and elemental analysis was done by energy dispersive spectrometry, EDX (Edax Falcon System). Infrared spectra were recorded on Spectrum One Perkin Elmer spectrometer using the KBr pellet technique (KBr:sample ratio = 200:1).

2.4. Standard solutions

Stock solution of OTA (200 μg mL^{-1}) was prepared by dissolving the solid standard in methanol and stored at −18 °C and protected from light. Standard solutions were prepared from appropriate dilutions of the stock solution with methanol:water (80:20, v/v).

2.5. Sample pretreatment

Twenty coffee samples and twenty-five cereal samples were purchased from local supermarkets and were stored at ambient temperature. The samples (10 g of coffee/5 g of cereal) were mixed with 100 mL of 1% $NaHCO_3$ solution. The suspension was shaken at 200 rpm for 30 min and passed through a Whatman No. 4 paper filter. The pH for 10 mL of the filtrate was adjusted to 1.5 by using 1.0 M HCl before performing the μ-SPE procedure.

For fortification, blank ground coffee (10 g) and cereal samples (5 g) were weighed into a conical flask (250 mL). The blank

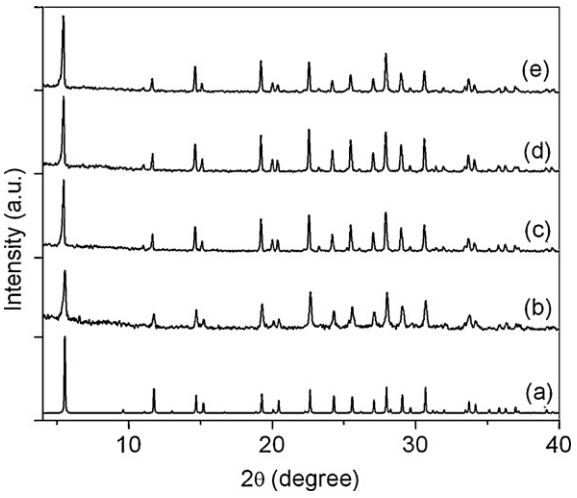

Fig. 2. X-ray diffraction patterns for (a) simulated LTL, (b) nanosized LTL, (c) rod LTL, (d) cylinder LTL and (e) needle LTL.

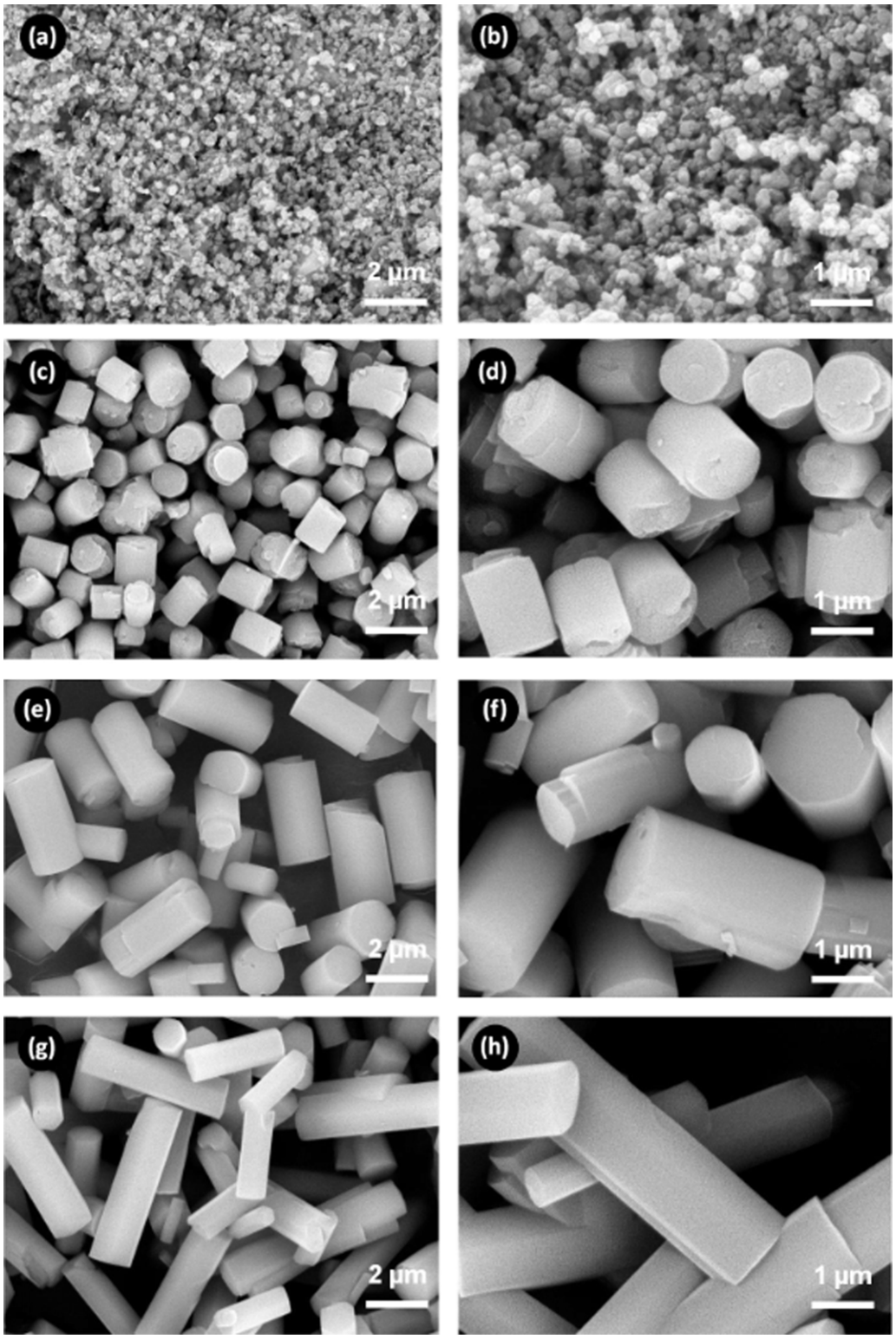

Fig. 3. Scanning electron micrographs of zeolite-L crystals synthesized (a and b) nanosized LTL (c and d) rod LTL (e and f) cylinder LTL (g and h) needle LTL. Scale bar = 1 μm and 2 μm.

coffee and cereal samples were fortified with OTA working solution to achieve different concentration levels and were left in the fume cupboard overnight prior to the μ-SPE procedure (Section 2.6).

2.6. Preparation of the μ-SPE device

The μ-SPE device consisted of zeolite LTL (25 mg) packed within an envelope made from polypropylene membrane sheet of

Table 1
Length, width and elemental analyses for LTL zeolites.

	Nanosized	Rod	Cylinder	Needle
SEM				
Length (μm)	0.2	1.3	3.4	5.7
Width (μm)	0.2	1.2	2.0	1.5
Aspect ratio	1.0	1.1	1.7	3.8
EDX				
Si (at.%)	18.0	17.5	16.0	16.2
Al (at.%)	5.6	5.4	5.2	5.0
Si/Al	3.2	3.2	3.1	3.2

dimension 2.0 cm × 0.5 cm. The edges were heat sealed. Before use, the device was cleaned with methanol under sonication for 3 min and then stored in methanol until use.

2.7. μ-SPE procedure

A 10 mL aliquot of the sample solution was added to a sample vial (12 mL). A magnetic stirring bar (15 mm × 5 mm) was placed in the solution. The μ-SPE device was then placed in a 10 mL sample (pH was adjusted to 1.5) that was stirred at 1250 rpm. The extraction was allowed to take place for 30 min before the device was removed, rinsed in water, dried with lint-free tissue and placed in a 750 μL desorption vial. Methanol (400 μL) was added and the analytes were desorbed by ultrasonication for 5 min. After desorption, the μ-SPE device was removed from the desorption vial and the extract was injected directly into the HPLC unit for analysis. The μ-SPE device could be reused after cleaning with methanol.

2.8. HPLC conditions

A Waters Alliance (model 2695) HPLC system (Milford, MA, USA) equipped with fluorescence detector was used. The chromatographic conditions were based on our previous studies [22]. The separation was performed on Poroshell 120 EC-C18 analytical column (100 mm × 4.6 mm × 2.7 μm) (Agilent Technologies, Wilmington, DE, USA) operated at 27 °C. The mobile phase consisted of acetonitrile, water, and acetic acid (49.5:49.5:1, v/v) with the flow rate of 1.0 mL min^{-1}. OTA exhibits natural fluorescence and the detector wavelengths were set at λ_{ex} 333 nm and λ_{em} 460 nm. A 20 μL aliquot of the sample was injected into the HPLC. The data were processed using licensed PowerChrom v2 software (EDAQ, Denistone East, Australia).

3. Results and discussion

3.1. LTL zeolites and its channel properties

The powder XRD patterns of LTL zeolite samples synthesized using different gel compositions are shown (Fig. 2). The results showed that the XRD patterns of synthesized LTL samples are identical as that of the simulated pattern of LTL-type material, suggesting pure crystalline LTL solids without phase impurities has been obtained.

The morphologies of LTL samples were studied under SEM analysis (Fig. 3). The nanocrystalline zeolite L are present as discrete nanocrystals sized 200 nm × 200 nm (Fig. 3a and b) while rod, cylinder and needle LTL crystals adopt a cylindrical morphology with different length and width (Fig. 3c–h). From the EDX results, the Si/Al ratio for all samples is nearly identical with the average of 3.2 (Table 1). This suggests all samples have identical framework (Si, Al) composition.

The performance of the LTL sorbent materials (nanosized, rod, cylinder and needle) as μ-SPE sorbent for the OTA extraction was evaluated. Based on the HPLC peak area, cylinder LTL was found to be the best sorbent, followed by needle LTL, rod LTL and nanosized LTL (Fig. 4). The corresponding FTIR spectra for the zeolite LTL before and after OTA absorption are shown in Fig. 5. The band at 1732 cm^{-1} in Fig. 5b was attributed to the C=O stretching vibration from the carbonyl and carboxylic acid group was not initially present in the zeolite LTL (Fig. 5a). Furthermore, the presence of the C=O stretching vibration arising from the ester group of OTA at 1718 cm^{-1} can be easily recognized.

LTL zeolite is a crystalline aluminosilicate with hexagonal symmetry and consists of a bunch of strictly parallel channels (Fig. 6a and b) [31,32]. In order to explain the observation obtained, the number of channels (n_{ch}) for all the LTL samples is calculated by using $n_{ch} = 0.267(d_Z)^2$, where d_Z is the average diameter of the crystal in nm [32] (Fig. 4). Interestingly, high OTA extraction efficiency of cylinder LTL can be related to its very large amount of parallel channels (1,068,000) whereas the nanosized LTL with 10,680 parallel channels has the lowest OTA extraction performance (Fig. 4). Thus, it can be easily observed that the extraction efficiency of OTA in LTL zeolites is highly dependent on the number of accessible channels (crystal width) for diffusion and absorption. Moreover, cylinder (3.4 μm) and needle (5.7 μm) LTL crystals with long length is also found to have significant effect in OTA extraction whereby the OTA molecules can enter the LTL pore channel from both ends, diffuse deeper into the channel and trap inside the channel more effectively when time increased.

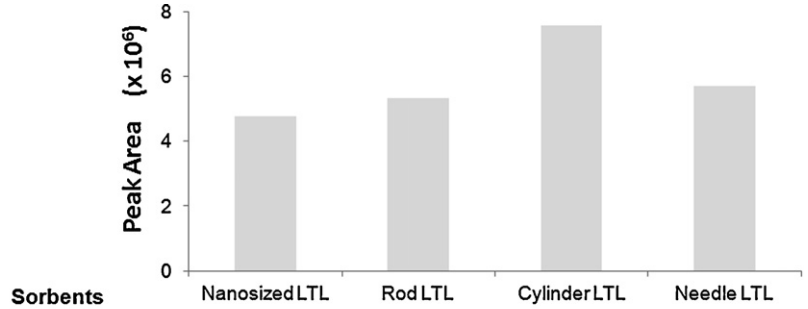

Fig. 4. Effect of LTL zeolites (with different diameter, length and number of parallel channels) on μ-SPE. Extractions were carried out using 5 ng mL^{-1} OTA. μ-SPE conditions: samples were extracted for 20 min with 5 min desorption by ultrasonication using 400 μL of methanol; 10 mg of sorbent was used.

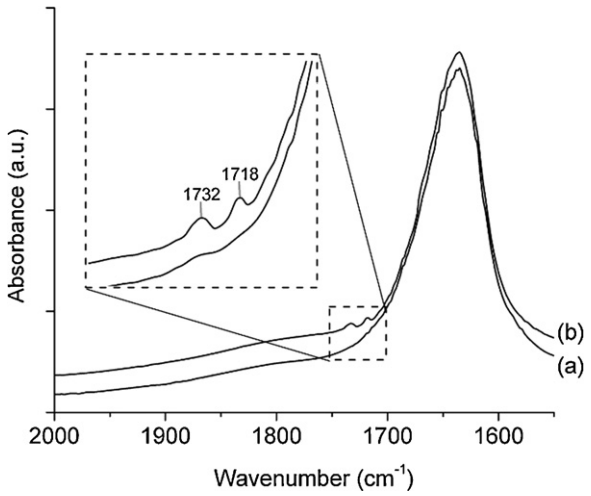

Fig. 5. FTIR spectra of (a) zeolite LTL and (b) zeolite LTL incorporated with OTA.

It is well known that the one-dimensional channel system of LTL zeolite determines the possibility of a molecule (shape and polarity) to be inserted into its channels [33,34]. As the main channel of LTL have the smallest free diameter of about 0.71 nm, while the largest diameter inside is 1.26 nm (Fig. 6c) only molecules with diameter less than 0.71 nm is allowed to enter the channels [35]. The three possible orientations of a molecule inside LTL channel are shown in Fig. 6d where a molecule larger than two unit cells is forced to align parallel to the channel (case 1). Shorter molecules tend to arrange as shown for case 2 or 3 [33]. In the present study, OTA has a diameter of 0.50 nm which can be conveniently inserted into the channel of LTL. The length of OTA is 1.60 nm. Thus, OTA can only align along the *c*-axis (case 1) since the length of a unit cell is 0.75 nm (Fig. 6e and f).

3.2. Optimization of μ-SPE procedure

Several extraction conditions were investigated to evaluate the different factors that affect the extraction efficiency. Since μ-SPE is an equilibrium-driven process, the efficiency is dependent on the partitioning of the analyte between the aqueous phase and the sorbent. Optimization was carried out by triplicate analysis with 5 ng mL^{-1} OTA. The parameters studied were the type of zeolite LTL and mass, pH, salt addition, extraction time, extraction speed and desorption conditions (solvent type and time).

3.2.1. Type zeolite LTL and mass

Initially, 10 mg of LTL zeolites (nanosized LTL, rod LTL, cylinder LTL and needle LTL) were used for the extraction of OTA. Cylinder LTL was found to have better interaction with OTA (Fig. 4). The influence of cylinder LTL mass for the extraction (5–30 mg) was also investigated. When more than 25 mg zeolite was used, no additional enhancement of peak area was observed. Thus, 25 mg of cylinder LTL was used in all experiments.

3.2.2. Effect of pH and salt addition

The pH of the sample solution was adjusted to the acidic range in order to deionize the molecule and to promote its extraction. In this study, the pH of the sample solutions was varied from 1 to 3 by the addition of hydrochloric acid (1.0 M). It was found that the optimum pH for OTA extraction was pH 1.5.

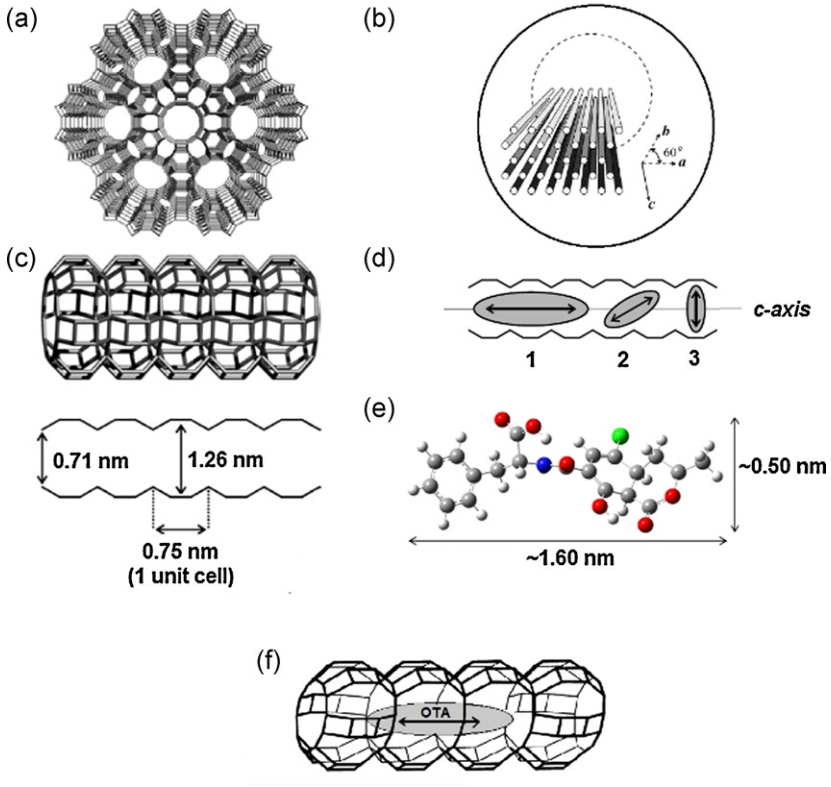

Fig. 6. (a) Top view of the structure of zeolite LTL illustrating its hexagonal framework. It shows a channel surrounded by six neighbouring channels. (b) Schematic view of some channels in a hexagonal zeolite LTL crystal with cylinder morphology [32]. (c) Side view of a channel that consists of 0.75 nm long unit cells with a van der Waals opening of 0.71 nm at the smallest and 1.26 nm at the widest place. (d) Schematic illustration of different orientation of molecules in the main channel of LTL [33]. (e) The width and length of OTA. (f) Schematic illustration of OTA in the main channel of LTL.

The effect of salt on the extraction efficiency was determined by adding 5, 10, 15, 20 and 30% (w/v) of sodium chloride, respectively, to 10 mL sample solutions. The extraction efficiency decreased as the salt concentration is increased because the viscosity of the aqueous sample is significantly higher when large amounts of sodium chloride was added, causing a reduction in the mass transfer process. Thus, the absence of salt was more suitable to the extraction process.

3.2.3. Effect of extraction time and stirring speed

Mass-transfer is a time-dependent process, therefore the extraction times between 10 and 60 min were studied. As expected, longer extraction time gave better extraction efficiency, the maximum efficiency was achieved at 40 min.

Stirring of the solution enhances mass transfer in the sample solution and induces convection in the membrane phase. The effect of stirring speeds (250–1500 rpm) was investigated and was found that higher extraction efficiency was obtained when the solution was stirred at 1250 rpm.

3.2.4. Effect of desorption solvent and time

After the extraction, analytes were desorbed in organic solvents *via* ultrasonication. Various organic solvents (namely methanol, acetonitrile, acetone and toluene) with different polarity were tested. According to solvent polarity/polarizability scale the empirical polarity of methanol, acetonitrile, acetone and toluene were 0.9, 0.9, 0.88 and 0.66, respectively. Methanol was found to be the best desorption solvent as the highest peak areas were obtained followed by acetonitrile. No peak was detected when toluene was used as desorption solvent.

The desorption (ultrasonication) time was investigated between 3 and 15 min with 400 μL methanol. Optimum desorption was achieved at 5 min. As desorption time increased beyond 5 min, a slight decrease in the peak area was observed. Since it is an equilibrium-driven process, re-adsorption of the analytes to the sorbent may be one of the reasons for the decrease in peak area [20].

3.2.5. Adopted extraction conditions

The adopted conditions were: 25 mg of long cylinder LTL as the extraction sorbent; pH 1.5; without addition of salt; extraction

Table 2
Method validation parameters obtained from the matrix matched calibration.

Sample	Linear range (ng g^{-1})	Regression equation	r^2	LOD (ng g^{-1})	LOQ (ng g^{-1})
Coffee	0.5–25	$y = 1.0 \times 10^5 x - 8.6 \times 10^5$	0.9987	0.09	0.28
Cereal	0.1–25	$y = 4.8 \times 10^5 x - 1.3 \times 10^5$	0.9952	0.03	0.08

Table 3
Recoveries, intra-day and inter-day data of spiked coffee and cereal samples.

Spiked sample (ng g^{-1})	Mean recovery (%) ± %RSD ($n = 9$)	Intra-day (%RSD, $n = 5$)	Inter-day (%RSD, $n = 25$)
Coffee			
0.5	92.7 ± 5.0	2.0	4.9
10	92.2 ± 2.2	1.2	5.5
25	99.5 ± 1.0	1.1	6.8
Cereal			
0.5	91.7 ± 1.0	1.2	5.1
10	98.8 ± 1.7	1.7	3.1
25	101.0 ± 3.3	1.4	1.6

RSD, relative standard deviation.

Table 4
Comparison of the analytical methods for the determination of OTA.

Instrument	Sample preparation	Type of sample	Linear range (ng mL^{-1}/ng g^{-1})	LOD	LOQ	Repeatability (% RSD)	Recovery (%)	Reference
HPLC-FL[a]	μ-SPE[c]	Coffee	0.5–25	0.09	0.28	<2.0 ($n = 5$)	92–100	Current method
	μ-SPE	Cereal	0.1–25	0.03	0.08	<1.7 ($n = 5$)	91–101	Current method
	MI-μSPE[d]	Coffee	0.5–50	0.06	0.19	<2.3 ($n = 5$)	91–99	[22]
	MI-μSPE	Grape juice	0.5–50	0.02	0.06	<1.0 ($n = 5$)	99–101	[22]
	MI-μSPE	Urine	0.5–50	0.02	0.08	<0.9 ($n = 5$)	96–99	[22]
	SPME[e]	Coffee	3–32	0.30	2.00	<3.3 ($n = 5$)	–	[14]
	HF-LPME[f]	Wine	0.25–10	0.20	0.25	<7.0 ($n = 3$)	74–79	[15]
	MSPD[g]	Cereal	0.25–20	0.05	0.19	<7.0 ($n = 5$)	77–89	[37]
LC–MS/MS[b]	PLE[h]	Breakfast cereal	0.25–10	0.1	0.25	<10.0 ($n = 3$)	80–88	[1]
	DLLME[i]	Wine	0.0025–400	0.005	0.015	<5.8 ($n = 6$)	97–102	[18]
	In-tube SPME	Nuts and grains	0.5–20	0.09	–	<5.1 ($n = 6$)	89–90	[19]

[a] High performance liquid chromatography coupled with fluorescence detector.
[b] Liquid chromatography–mass spectrometry.
[c] Micro-solid phase extraction.
[d] Molecular imprinted polymer micro-solid phase extraction.
[e] Solid phase microextraction.
[f] Hollow fiber liquid-phase microextraction.
[g] Matrix solid phase dispersion.
[h] Pressurized liquid extraction.
[i] Dispersive liquid–liquid microextraction.

Table 5
OTA concentrations in samples analyzed.

Sample analyzed	No. of positive sample	OTA found[b] ($ng\,g^{-1}$)
Coffee		
Roasted coffee (11)[a]	4	nd–1.47
Instant coffee (9)	7	nd–9.33
Cereal		
Breakfast cereal (15)	10	nd–0.46
Infant cereal (10)	4	nd–0.77

[a] Number of sample.
[b] Range of OTA detected; nd, not detected.

time, 40 min at room temperature; stirring speed, 1250 rpm; desorption solvent, methanol; desorption time, 5 min. Under these conditions, the extraction efficiency of 68% was obtained. Extraction efficiency (E_e) was evaluated by the following equation:

$$E_e = \frac{C_f V_f}{C_i V_i} \times 100\%$$

where C_f and C_i are the concentrations of the analyte found in the final extract (desorbed analyte) and present in the original sample solution, respectively. V_f is the volume of the concentrated extract, and V_i is the volume of the original sample solution. Enrichment factor (E_f) was calculated based on the following equation:

$$E_f = \frac{C_f}{C_i}$$

where C_f is the concentration of analyte in the final extract and C_i is the initial concentration of analyte in the sample solution before the extraction. Under the above optimum conditions, an enrichment factor of 17 was achieved.

3.3. Carry-over effect

After the first desorption, a used μ-SPE device was cleaned with 2 mL of methanol for 10 min (ultrasonication) to remove the residual analyte. The same device was again desorbed for 3 min to test for carry-over effects. No analytes were detected. In fact, the μSPE devices were found to be reusable for about 20 extractions. Device-to device variation was also tested for six samples and acceptable relative standard deviation (<10%) was obtained.

3.4. Method validation

3.4.1. Linearity, LOD and LOQ

Under the optimized μ-SPE conditions, calibration curve of OTA standards were constructed by diluting appropriate volumes of the working standard solution with water into seven different concentrations ($0.1–25\,ng\,mL^{-1}$). For each level, three replicate extractions were performed. The regression equations and correlation of determination were $y = 1{,}036{,}698x + 438{,}894$ and $r^2 = 0.9999$, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were determined as reported by Aurora-Prado et al. [36].

$$\mathrm{LOD} = 3.3\frac{s_a}{b}, \qquad \mathrm{LOQ} = 10\frac{s_a}{b}$$

where s_a is the standard deviation of the intercept and b is the slope of the regression line obtained from the calibration graph. The LOD and LOQ for OTA standards were 0.01 and $0.04\,ng\,mL^{-1}$, respectively.

Matrix matched calibrations were done by spiking known amount of OTA into coffee and cereal that were originally free from OTA. This approach enables the assessment of possible matrix effects to be evaluated. Linear range for coffee was $0.5–25\,ng\,g^{-1}$ while cereal was $0.1–25\,ng\,g^{-1}$. The LOD for spiked coffee and cereal

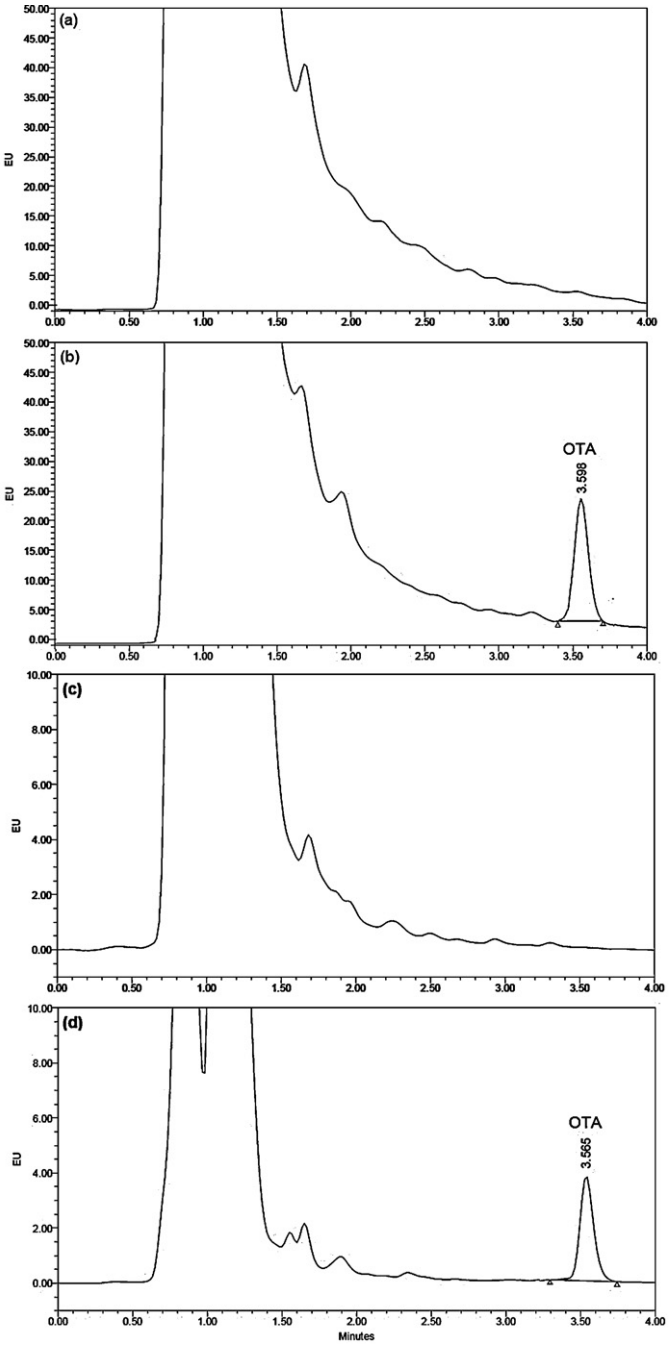

Fig. 7. Typical chromatograms of (a) blank coffee, (b) instant coffee containing OTA ($9.33\,ng\,g^{-1}$), (c) blank cereal and (d) infant cereal containing OTA ($0.77\,ng\,g^{-1}$).

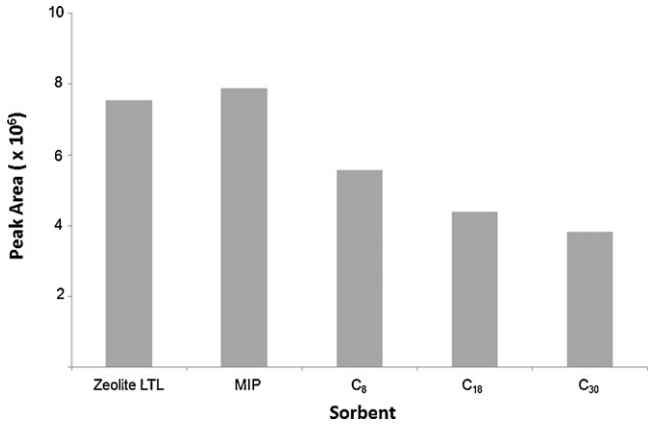

Fig. 8. Effect of different sorbents on μ-SPE. Extractions were carried out using 5 ng mL^{-1} OTA. μ-SPE conditions: samples were extracted for 20 min with 5 min desorption by ultrasonication using 400 μL of methanol; 10 mg of sorbent was used.

are 0.09 and 0.03 ng g^{-1}, respectively, while the LOQ for spiked coffee and cereal are 0.28 and 0.08 ng g^{-1}, respectively (Table 2).

3.4.2. Recovery, intra-day and inter-day precision

Recovery studies were carried out by spiking OTA to the non-contaminated coffee and cereal at different concentrations of OTA. Five replicate samples were studied at each concentration. Good recoveries were obtained for all samples, ranging from 91.7 to 101.0% (Table 3).

Intra-day precision (repeatability) was estimated at three concentration levels of OTA that were spiked to the samples. Inter-day precision (reproducibility) was performed by spiking to the matrix with three concentration levels of OTA and all samples were analyzed on five different days. Intra-day and inter-day precisions for peak areas, expressed as the percentage relative standard deviation (RSD), were 1.1–2.0% and 1.6–6.8%, respectively, indicating the good precision of the developed method.

3.5. Comparison with previously reported methods

The analytical characteristics of the present method were compared with the other reported methods (Table 4). The sensitivity of the current method (reflected in LOD and LOQ) is similar to the earlier μ-SPE work reported using MIP [22] and the other microextraction techniques that uses HPLC-FL system [14,15,37]. The sensitivity is also similar to the in-tube SPME [19] and pressurized liquid extraction technique [1] with MS detection. The μ-SPE technique that either uses zeolite or MIP as sorbent resulted in better recoveries than the HF-LPME [15] and matrix solid phase dispersion method [37].

3.6. Analysis of real samples

The developed method was applied to the determination of OTA in coffee and cereal samples. Among the twenty coffee samples analyzed, four roasted coffee samples and seven instant coffee samples were contaminated with OTA. The levels of OTA in roasted coffee ranged from 0.30 to 1.47 ng g^{-1} (Table 5) and in instant coffee ranged from 0.51 to 9.33 ng g^{-1}.

For cereal samples, ten out of fifteen breakfast cereal samples were contaminated by OTA. The found levels were lower than the legal limit (3 ng g^{-1}), ranging from 0.28 to 0.46 ng g^{-1}. Among the ten infant cereals analyzed, four were detected with OTA ranging from 0.28 to 0.77 ng g^{-1}. One of these infant cereal sample exceeds the maximum level set by EU regulations (0.5 ng g^{-1} for infant cereal). Typical chromatograms obtained (Fig. 7) indicate that the OTA peaks are not interfered by other matrix components.

3.7. Comparison with other sorbents

The extraction efficiency of the zeolite LTL was compared to other commercial sorbents. Zeolite LTL exhibited comparable extraction efficiency to the more expensive MIP sorbent, and is clearly superior to other common sorbents such as C_8, C_{18} and C_{30} (Fig. 8).

4. Conclusions

Zeolite LTL crystals with different morphologies were prepared and characterized. When used as sorbent in the μ-SPE format for the extraction of OTA, the cylinder LTL zeolite (with very large amount of parallel channels and long length) gave the best performance, followed by the needle LTL, rod LTL and the nanosized LTL. The resultant zeolite showed good selectivity and extraction efficiency, comparable to those of the MIP [22]. The high recoveries (91.7–101.0%) and satisfactory precision (1.1–6.8%) obtained suggest that the method can be a viable option for the analysis of OTA in different food matrices. The preparation of the zeolite is very simple and uses inexpensive starting materials.

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