

A Novel Sorbent for Solid-Phase Extraction Coupling to High Performance Liquid Chromatography for the Determination of Olaquinox in Fish Feed

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Abstract

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A new and hydrophilic molecularly imprinted polymer (MIP) selective for olaquinox was prepared by bulk polymerisation using methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the crosslinker. The synthesised polymer was characterised by Fourier-transform infrared and static adsorption experiments. Results showed that the MIP had good recognition and selective ability for olaquinox. A novel method based on molecularly imprinted solid-phase extraction coupled with high-performance liquid chromatography was developed for the separation and determination of trace olaquinox in feed samples. Under selected experimental conditions, the detection limit (signal-to-noise ratio = 3) was 42.2 ng/g, and the relative standard deviation (RSD) for five replicate extractions of 50 µg/l olaquinox was 4.9%. The method provided high recoveries ranging from 89.8% to 93.1% at three spiked levels with < 5% RSDs. This method was successfully applied for the analysis of olaquinox in feed.

Keywords: HPCL; molecular imprinting; SPE; olaquinox

Quinoxaline 1,4-di-*N*-oxide derivatives are synthetic antimicrobial agents known for growth-promoting and anti-infectious activities in the livestock feed industry (CARTA *et al.* 2005). Olaquinox (OLA), one of the best known derivatives, has been developed by the Bayer Company in Germany since the 1970s. OLA can improve feed efficiency for farm animals and prevent dysentery and bacterial enteritis (DING *et al.* 2006). However, incorrect use of OLA has side effects and toxicity. A study has indicated that OLA abuse adversely affects the environment (WANG & GENG 2002). Another study has shown that OLA can destroy the antioxidant defence system of animals (WANG & GENG 2003). Many other studies (NEUMANN *et al.* 2003; HAO *et al.* 2006) have suggested that OLA is mutagenic, carcinogenic, and phototoxic. To prevent these uncontrolled effects on human health, an accurate and reliable analytical method of monitoring OLA must be established.

Several analytical methods of determining OLA based on chromatography have been reported, including column liquid chromatography with ultraviolet (UV) detection (LIN & JENG 2001; WU *et al.* 2006) and diode-array detection (GIZZI *et al.* 2007), thin-layer chromatography (ROETS *et al.* 2001), liquid chromatography (LC)-tandem mass spectrometry (MS/MS) (POUCKE *et al.* 2003), micellar liquid chromatography (MLC) with UV detection (NASR *et al.* 2013), and LC-electrospray-MS/MS (HUTCHINSON *et al.* 2005). Chromatographic methods can be reliable and sensitive, but they also have the drawbacks of tedious procedure and time consumption. Furthermore, the analysis of trace OLA contaminant in complex matrix requires sample pretreatment to reduce the interference of sample matrix and enhance sensitivity at the same time. Conventional sample preparation techniques such as solid-phase

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extraction (SPE) restrict their uses because of low selectivity. Therefore, a simple, rapid, and selective sample preparation method of analysing trace OLA in complicated feed matrices must be developed.

Molecularly imprinted polymers (MIPs) are artificial polymer materials possessing high selectivity and specificity for template molecules. MIPs are relatively easy and inexpensive to prepare, intrinsically stable to use many times without performances loss, and suitable in harsh chemical media. MIPs as a SPE sorbent material were first reported by SELLENGREN (1994) for the selective determination of the drug pentamidine in urine samples. Since then, molecularly imprinted SPE (MISPE) has been widely applied as a simple and effective pretreatment method for the selective extraction of template molecules from highly complex environmental and biological samples (ANDERSSON 2000; FRANCESCO *et al.* 2005).

MIPs synthesised in organic solvents greatly hinder the further development of MISPE. The analysis of biological and environmental samples requires full compatibility with water, so high recognition of MIPs in aqueous media is challenging. Some researchers have demonstrated the synthesis of MIPs in polar porogen and the use of isolating template from analogues in aqueous extracts (HAUPT *et al.* 1998), but the specific recognition of MIPs needs further improvement. Some researchers have prepared MIPs using OLA as template in organic solvents (SONG *et al.* 2011; XU *et al.* 2012; ZHANG *et al.* 2013), but the preparation of MIPs using OLA as template in aqueous solvents have not reported. In this study, MIPs were prepared by bulk polymerisation in the mixed solvent of doubly deionised water (DDW) and acetonitrile. An effective and convenient technique for the detection of trace OLA in fish feed by MISPE combined with high-performance liquid chromatography (HPLC) was developed. The factors affecting preconcentration and separation of analytes were discussed, and the applicability of this method was evaluated.

MATERIAL AND METHODS

Chemicals and material. OLA, mequindox, and quinocetone were obtained from the Institute for the Control of Agrochemicals of Ministry of Agriculture (Beijing, China). Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich (Milwaukee, USA). 2,2-Azobisisobutyronitrile (AIBN) was obtained from Tianjin Chemical Reagent Factory (Tianjin, China). Fish feed was purchased from the animal market of Taian

(Shandong, China). HPLC-grade methanol and acetonitrile were purchased from Tianjin Yongda Chemical Co. (Tianjin, China). All other reagents were analytical grade. DDW (18 M Ω /cm) obtained from a Water Pro. Water system (Labconco Corp., Kansas City, USA) was used throughout the experiments.

Apparatus. To determine the adsorption capacity of the imprinted and non-imprinted polymer, a Cary 50-Bio UV spectrometer (Varian Australia Pty Ltd., Victoria, Australia) and a US-501 horizontal shaker (Beijing, China) were used. Fourier-transform infrared (FTIR) spectra (4000–400 cm⁻¹) in KBr were recorded using a Vector 22 spectrometer (Bruker, Rheinstetten, Germany). A Strata Scx C₁₈ SPE column (200 mg/3 ml) obtained from Phenomenex Inc. (Torrance, USA) was used. The HPLC system consisted of two LC-10ATVP pumps and a Shimadzu SPD-10AVP UV detector (Kyoto, Japan). All separations were conducted on an analytical reversed-phase Thermo C₁₈ column (4.6 mm × 250 mm, Agela Technology, Newark, USA) at a mobile phase flow rate of 1.0 ml/minute. CLASS-VP software was used to acquire and process spectral and chromatographic data. The mobile phase was methanol/water (15 : 85, v/v), the injected sample volume was 15 μ l, and detection was performed at 372 nm.

Preparation of MIPs. About 0.263 g of OLA (1 mmol) was dissolved in 4.0 ml of DDW and 6.0 ml of acetonitrile solution at 60°C and then mixed with 0.173 g (2 mmol) of MAA. The mixture was magnetically stirred for 30 min, and then 0.793 g (4 mmol) of EGDMA was added. After 10 min of stirring, 60 mg of AIBN was added and the mixture was stirred for 30 minutes. Then, the mixture was sealed and thermally initiated in a water bath at 60°C for 24 hours. After polymerisation ended, the rigid polymer was ground and sieved. The resulting particles were flushed with methanol and DDW to remove the unreacted reagents and template molecules and dried in a vacuum oven at 100°C for 10 hours. Following Soxhlet extraction with 300 ml of methanol/acetic acid (8 : 2, v/v) for 24 h, the polymer was washed with 300 ml of methanol for 4 h to be free of OLA (Figure 1), which was verified by the analysis of the methanol eluent using HPLC at 372 nm. Finally, the product was dried at 80°C for 10 h to obtain the MIP material.

For comparison, a non-imprinted polymer (NIP) was also prepared following the same procedure but without the template.

Characterisation of MIPs. To investigate adsorption and recognition ability in aqueous medium, 20 mg of imprinted polymer and non-imprinted

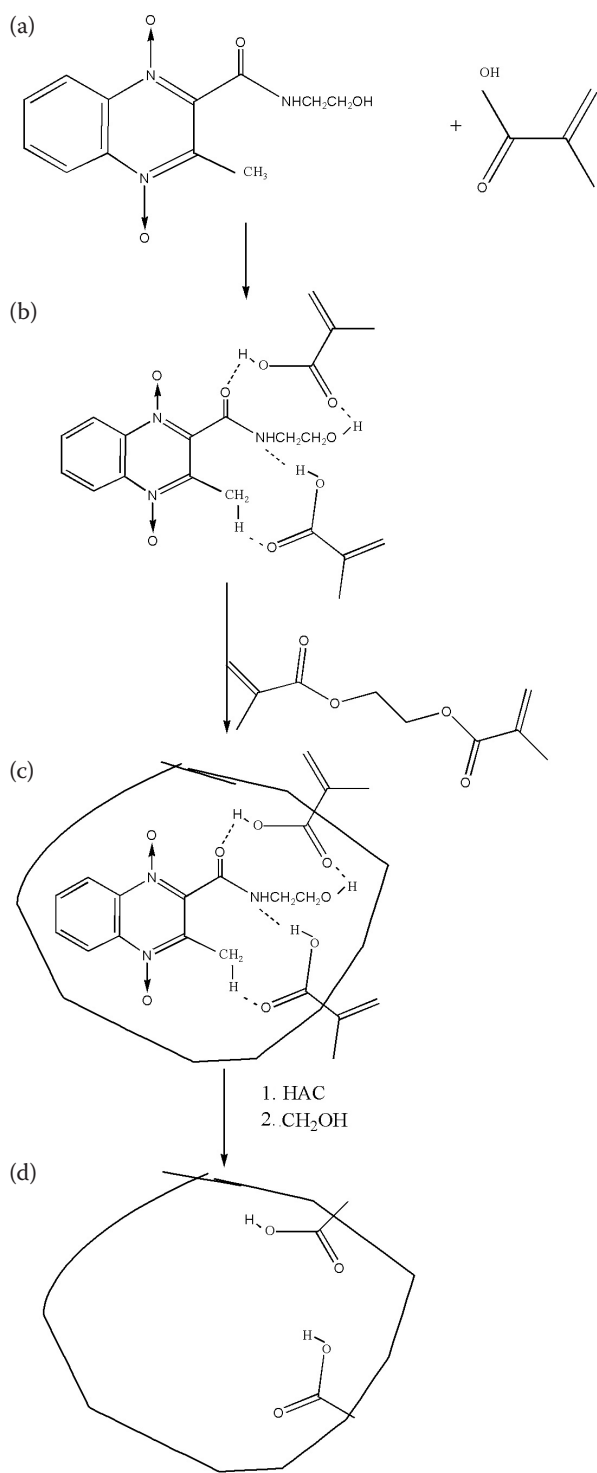


Figure 1. Schematic representation of the molecularly imprinted polymer used in this study

polymers were equilibrated with 10 ml of DDW solution containing OLA at different concentrations (20–100 mg/l) in a 50 ml volumetric flask. The mixtures were mechanically shaken for 4 h at room temperature with a horizontal shaker and then separated by centrifugation (4000 rpm) for 20 minutes. The

supernatants were measured for unextracted OLA by UV spectrometry at 372 nm, and the adsorption capacity (Q) was calculated.

The uptake kinetics of the imprinted polymer for 100 mg/l OLA was evaluated. After shaking for 5, 30, 60, 90, 120, 180, and 240 min at room temperature and separating by centrifugation (4000 rpm) for 20 min, the adsorption capacity of unextracted OLA in the supernatant was determined by UV spectrometry.

Selective recognition studies were performed by adsorbing OLA and structurally related compounds of mequindox and quinocetone (100 mg/l) with imprinted polymer and non-imprinted polymers. The supernatants were measured for the unextracted mequindox and quinocetone at 372 and 314 nm, respectively.

Procedure of MISPE combined with HPLC (MISPE-HPLC). To evaluate the applicability of MIPs as a functionalised sorbent for the extraction and determination of trace OLA in samples coupled with HPLC, 200 mg of MIP or NIP was packed into an empty SPE column. The MISPE cartridge was initially rinsed with 5 ml of methanol and DDW, followed by loading 50 ml of standard OLA solution at a flow rate of 1.2 ml/minute. OLA was selectively preconcentrated onto the sorbent-packed cartridge. After sample loading, the cartridges were thoroughly conditioned with 0.5 ml of methanol/DDW (40 : 60, v/v) at 6.0 ml/min to remove impurities and then eluted with 0.5 ml portions of methanol/DDW (85 : 15, v/v) at 1.2 ml/min to completely desorb the analytes adsorbed onto the SPE microcolumn. The elution fraction volume was accurately measured. The effluents were filtered with a 0.45 μ m filter membrane, and 15 μ l of the filtrate was injected into HPLC for analysis. The peak areas at 372 nm were used for data evaluation. Finally, the cartridges were sequentially flushed with 5 ml of methanol and DDW for the next sample preconcentration. Under these conditions, a complete cycle of the MIP and HPLC separation and determination of OLA lasted for 42 minutes. For comparison, the same procedure was adopted with C₁₈ SPE cartridges for the extraction of OLA standard solution. The detection limit was determined by serial dilution of a standard solution to obtain S/N ratios of 3 : 1 (SNYDER *et al.* 1997).

Sample preparation. Fish feed was purchased from the animal market of Taian in July, 2012 (Shandong, China). To check the accuracy of the developed MISPE-HPLC method, the feed sample for spiking was determined to be free of OLA. In a typical procedure, 2.0 g of blank feed was separately weighed into a 100 ml conical flask and then spiked with

1.0 ml of standard solution (0.2, 0.4, and 0.75 mg/l) in a 100 ml conical flask. After incubation for 1 h, the spiked samples were ultrasonicated with 3×10 ml methanol/DDW (5:95, v/v) solution for 30 minutes. The resulting extractions were collected and centrifuged at 3000 rpm for 30 min, and the supernatants were filtered for the MISPE procedure.

RESULTS AND DISCUSSION

FTIR analysis of MIPs and non-imprinted polymers. Figure 2 compares the FTIR spectra of OLA, imprinted polymer, and non-imprinted polymer. In the FTIR spectra of OLA (Figure 2a), the observed features at around 1079 and 3421 cm^{-1} represent C-O and O-H stretching vibrations, respectively, indicating that OLA had the -OH group. In the FTIR spectra of imprinted polymers (Figure 2c), the observed features at around 1726 cm^{-1} indicate C=O stretching. The observed feature at around 3541 cm^{-1} indicates O-H stretching vibration. These results demonstrated that MAA existed and may have reacted with OLA, thus, MIP was synthesised. The imprinted and non-imprinted polymers (Figure 2b) showed similar locations and appearances of the major bands, indicating that the template was removed from the imprinted polymer after extraction.

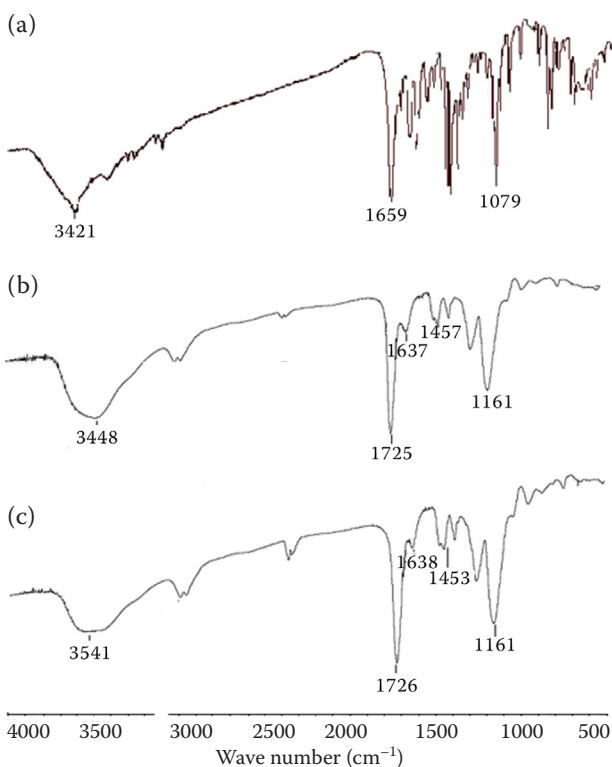


Figure 2. FT-IR spectra of olaquinox (a), non-imprinted polymers (b), and imprinted polymer (c)

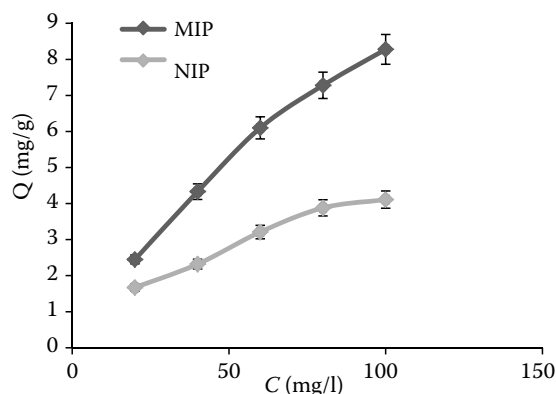


Figure 3. Adsorption isotherms of the imprinted (MIP) and non-imprinted polymer (NIP). The results in the graphs represent mean values calculated from three replicates ($n = 3$)

Characterisation of adsorption ability. The binding affinity of the resulting MIP was evaluated by equilibrium adsorption experiments, and 20 mg of imprinted or non-imprinted polymer was incubated with 10 ml of OLA at different concentrations in DDW standard solution. The adsorption data (Figure 3) showed that the binding capacities of imprinted or non-imprinted polymer increased with increased initial OLA concentration. The adsorption capacity of the imprinted polymers ($8.28 \pm 0.23 \text{ mg/g}$) was twofold higher than that of non-imprinted polymers ($4.11 \pm 0.21 \text{ mg/g}$) at 100 mg/l concentration. These results demonstrated that the imprinted polymer may have several imprinted cavities and specific binding sites caused by the imprinting effect.

The uptake kinetics of OLA by the imprinted polymer was also examined at 100 mg/l concentration, as shown in Figure 4. After shaking for 5 min, the adsorption capacity of $5.34 \pm 0.09 \text{ mg/g}$ imprinted polymer was

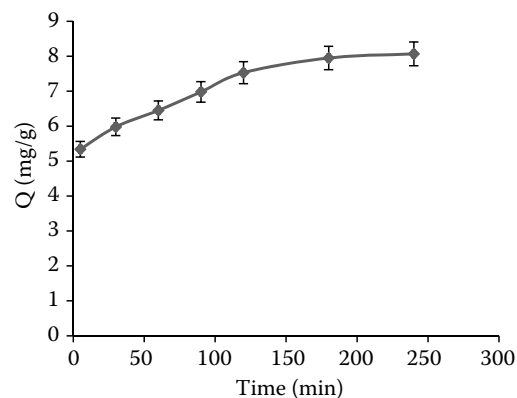


Figure 4. Kinetic uptake plot of the imprinted polymer. The results in the graphs represent mean values calculated from three replicates ($n = 3$)

obtained, i.e. 66.19% of the saturated adsorption capacity; moreover, adsorption almost reached equilibrium within 3 hours. The rapid adsorption kinetics of the imprinted polymer was an obvious advantage for its application in SPE for the determination of OLA residues in animal feedstuff or food products.

Results of selective recognition studies showed that the adsorption capacity of imprinted polymer toward OLA, quinocetone, and mequindox at 100 mg/l was 8.28 ± 0.23 , 1.14 ± 0.08 , and 2.47 ± 0.11 mg/g, respectively; the adsorption capacity of non-imprinted polymer toward OLA, quinocetone, and mequindox was 3.21 ± 0.19 , 3.16 ± 0.18 , and 3.47 ± 0.21 mg/g, respectively. This finding demonstrated that the MIP was able to selectively recognise OLA from its structural analogues. This finding may be due to the imprinting effect, i.e. the difference in molecular interactions and structures. During the preparation of the imprinted polymer, the template of OLA was incorporated into organic polymer networks. Subsequently the imprinted polymers had complementary cavities to OLA in shape, size, and spatial arrangement after removal of the template molecule.

Optimisation of MISPE. To evaluate the applicability of the imprinted sorbent for the separation and determination of trace OLA by HPLC, chemical and flow variables such as composition and volumes of elution solution, sample acidity, and loading flow rate were optimised to achieve good sensitivity and precision of this method.

Organic solvents have strong elution ability and interrupt the enrichment of target molecules on MIPs. Therefore, different eluents were investigated to identify the influence on desorption of OLA from the imprinted cartridges (Figure 5a). The imprinted sorbent cartridge was initially washed with 0.5 ml of methanol/water (40:60, v/v) and then eluted with 0.5 ml of different proportions of methanol/water to desorb the template.

Results showed that the absorbed OLA was slightly eluted by methanol/water at a ratio of 80:20 (v/v), and a small peak was observed in the chromatograms. The largest chromatographic peak of OLA was observed after elution with methanol/water (85:15, v/v) and then decreased with increased methanol content. Thus, methanol/water (85:15, v/v) was selected as the eluting solvent, and the loaded OLA can be fully desorbed.

To determine the optimum eluent volume applied in the eluting step, different volumes (0.1–1.5 ml) of methanol/water (85:15, v/v) were tested (Figure 5b). With increased eluting solvent volume from 0.1 ml to 0.5 ml, the chromatographic peak of OLA rapidly increased and then decreased within the range of 0.5–1.5 ml. Therefore, 0.5 ml of methanol/water (85:15, v/v) was used as the eluting solution.

The effect of sample loading flow rate and time on MISPE was studied. The peak areas of OLA increased with decreased sample loading flow rate from 2.0 ml/min to 1.2 ml/min for 50 ml loading, and no variation occurred with decreased sample loading flow rate from 1.2 ml/min to 0.5 ml/minute. The influence of sample loading time on OLA adsorption was investigated with 50 µg/l OLA at a sample flow rate of 1.2 ml/minute. Results indicated that the chromatographic peak areas almost linearly increased with increased sample loading time up to at least 42 minutes. Therefore, 1.2 ml/min flow rate and 42 min of sample loading time were chosen as the experimental conditions for further studies.

Different sample acidities were investigated within the pH ranges of 4.0–12.0 at a sample flow rate of 1.2 ml/min for 42 minutes. Results showed that the maximum chromatographic peak area of OLA was reached within the pH range of 7.0–8.0. Outside this optimum pH range, OLA had low retention on the MIP column, and the chromatographic peak area decreased. Thus, pH 7.5 was chosen for the subsequent step.

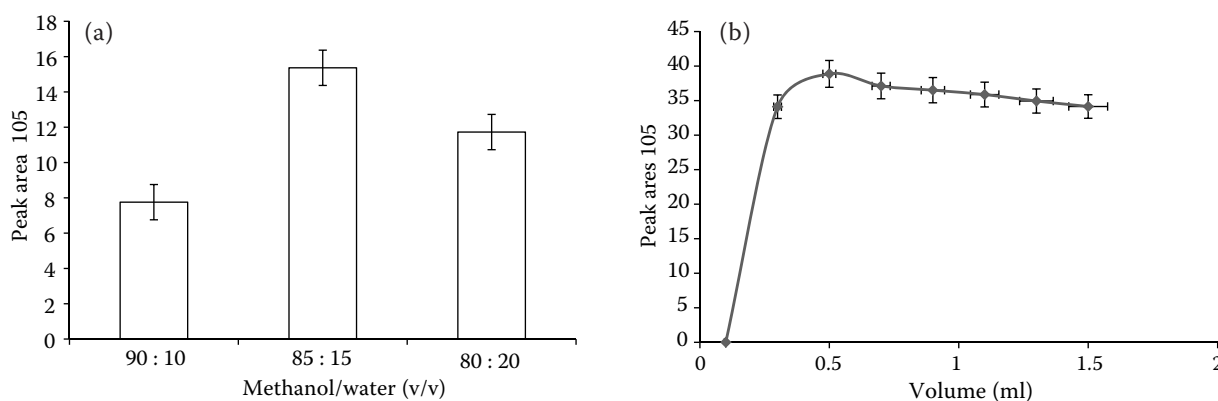


Figure 5. The effect of eluent (a) composition and (b) volume on the elution. The results in the graphs represent mean values calculated from three replicates ($n = 3$)

Applicability and merits of MISPE-HPLC. The selective adsorption of OLA by imprinted, non-imprinted, and C_{18} sorbent were observed by passing 50 ml of standard aqueous solution containing 50 $\mu\text{g/l}$ OLA at a flow rate of 1.2 ml/min (Figure 6). Results showed that the non-imprinted cartridge had a low concentration effect on OLA (Figure 6c), but OLA obviously appeared in the chromatogram after eluting from the imprinted cartridge (Figure 6b), indicating that OLA was selectively extracted onto the imprinted sorbent and the selectivity of the imprinted sorbent for OLA was very high. The selectivity and enrichment of MISPE and C_{18} SPE (Figure 6a) were compared under the same conditions. Results showed that the C_{18} cartridge had a concentration effect but no selectivity for OLA, so the chromatographic peak area was low. The imprinted cartridge had better selectivity and higher concentration on OLA than the C_{18} extraction column (Figure 6c). These results clearly indicated that this imprinted polymer was more suitable than an SPE sorbent for the selective separation and extraction of OLA before feed analysis.

The merits of the present MISPE-HPLC method for the determination of OLA were evaluated under optimal experimental conditions. The data indicated that at a loading flow rate of 1.2 ml/min for 50 ml loading, the enrichment factor of MISPE was 80. The detection limit ($S/N = 3$) based on three times the S/N of the baseline near the analyte peak was 42.2 ng/g, and the relative standard deviation for five replicate extractions of 50 $\mu\text{g/l}$ OLA was 4.9%.

The application and accuracy of MISPE-HPLC for the preconcentration and determination of OLA in animal food samples was investigated. Fish feed determined to be free of OLA was spiked with 0.1, 0.2, and 0.375 $\mu\text{g/g}$ OLA and analysed, and the results are shown in Table 1. For each sample and concentration, three measurements were performed. The results were satisfactory with recoveries ranging from 89.8% to 93.1%. The MISPE column was stable and can be used for more than 20 cycles of the procedure without loss of sensitivity. Thus, MISPE-HPLC was a simple and sensitive method for the determination of trace OLA residues in feed samples.

Table 1. Recoveries of olaquinox in actual samples – fish feed (mean \pm RSD, $n = 3$)

Spiked level ($\mu\text{g/g}$)	Recovery of olaquinox (%)
0.375	93.1 \pm 4.3
0.200	91.4 \pm 4.6
0.100	89.8 \pm 4.8

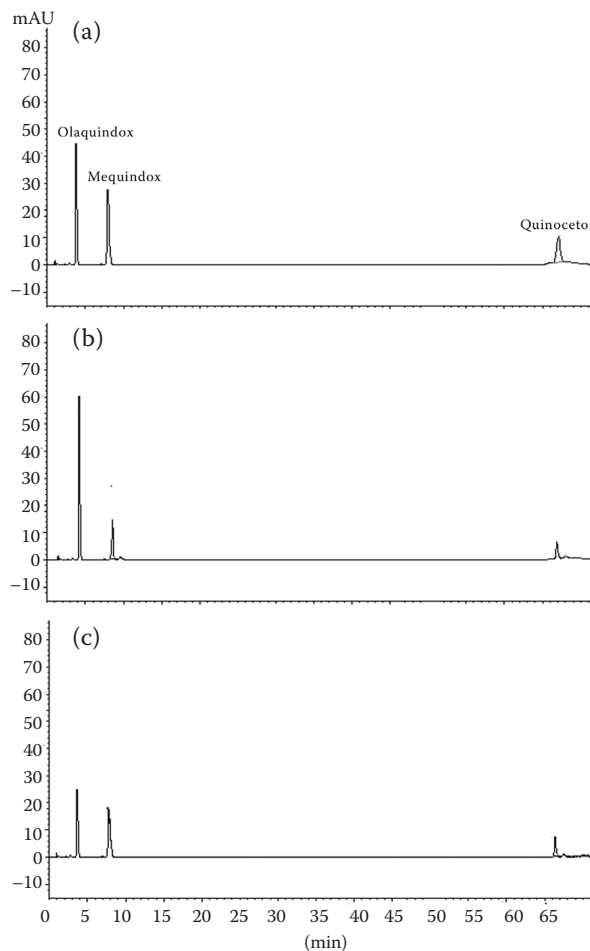


Figure 6. Chromatograms of 0.05 mg/l of olaquinox standard solution after solid phase extraction preconcentration by (a) C_{18} , (b) imprinted sorbent, and (c) non-imprinted sorbent

CONCLUSIONS

In this study, a new and hydrophilic OLA-imprinted polymer was prepared by bulk polymerisation technique. The prepared material showed good selectivity, improved stability and high adsorption capacity for OLA and was suitable to be used as a sorbent for SPE. Thus, a sensitive and simple method of MISPE-HPLC for determination of trace OLA was successfully developed. This method was evaluated for the analysis of fish feed sample spiked with low levels of OLA and good recoveries were achieved. This study will provide a sensitive and rapid method for the detection of OLA residues in feed.

References

ANDERSSON L.I. (2000): Molecular imprinting for drug bioanalysis: A review on the application of imprinted polymers

- to solid-phase extraction and binding assay. *Journal of Chromatography B*, **739**: 163–173.
- CARTA A., CORONA P., LORIGA M. (2005): Quinoxaline 1,4-dioxide: A versatile scaffold endowed with manifold activities. *Current Medicinal Chemistry*, **12**: 2259–2272.
- DING M.X., WANG Y.L., ZHU H.L., YUAN Z.H. (2006): Effects of cyadox and olaquinox on intestinal mucosal immunity and on fecal shedding of *Escherichia coli* in piglets. *Journal of Animal Science*, **84**: 2367–2373.
- FRANCESCO P., CARMELO G., FRANCESCA I., RITA M., UMILE G.S., NEVIO P. (2005): Molecularly imprinted solid phase extraction for detection of Sudan I in food matrices. *Food Chemistry*, **93**: 349–353.
- GIZZI G., VINCENT U., HOLST C.V., JONG J.D., GENOUEL C. (2007): Validation of an analytical method for the determination of carbadox and olaquinox in feedstuff by liquid chromatography coupled to UV and/or diode array detection. *Food Additives and Contaminants*, **24**: 1226–1235.
- HAO L.H., CHEN Q., XIAO X.L. (2006): Molecular mechanism of mutagenesis induced by olaquinox using a shuttle vector pSP189/mammalian cell system. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **599**: 21–25.
- HAUPT K., DZGOEV A., MOSBACH K. (1998): Assay system for the herbicide 2, 4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Analytical Chemistry*, **70**: 628–631.
- HUTCHINSON M.J., YOUNG P.B., KENNEDY D.G. (2005): Confirmatory method for the analysis of carbadox and olaquinox in porcine feeding stuffs using LC-electrospray MS-MS. *Food Additives and Contaminants*, **22**: 113–119.
- LIN S.Y., JENG S.L. (2001): High-performance liquid chromatographic determination of carbadox, olaquinox, furazolidone, nitrofurazone, and nitrovin in feed. *Journal of Food Protection*, **64**: 1231–1234.
- NASR J.J., SHALAN SH., BELAL F. (2013): Determination of carbadox and olaquinox residues in chicken muscles, chicken liver, bovine meat, liver and milk by MLC with UV detection: Application to Baby Formulae. *Chromatographia*, **76**: 523–528.
- NEUMANN N.J., BLOTZ A., WASINSKA-KEMPK G., ROSENBRUCH M., LEHMANN P., AHR H.J. (2005): Evaluation of phototoxic and photoallergic potentials of 13 compounds by different *in vitro* and *in vivo* methods. *Journal of Photochemistry and Photobiology B: Biology*, **79**: 25–34.
- POUCKE C.V., KEYSER K.D., BALTUSNIKIENE A., MCEVOY J.D.G., PETEGHEMA C.V. (2003): Liquid chromatographic-tandem mass spectrometric detection of banned antibacterial growth promoters in animal feed. *Analytica Chimica Acta*, **483**: 99–109.
- ROETS E., QUINTENS L., KIBAYA R., HOOGMARTENS J. (2001): Quantitative determination of olaquinox in animal feed. *Journal of Planar Chromatography – Modern TLC*, **14**: 347–349.
- SELLERGREN B. (1994): Direct drug determination by selective sample enrichment on an imprinted polymer. *Analytical Chemistry*, **66**: 1578–158.
- SNYDER L.R., KIRKLAND J.J., GLAJCH J.L. (1997): Quantitation (including trace analysis). In: *Practical HPLC Method Development*. John Wiley & Sons, Inc; New York: 643–684.
- SONG J., QIAO X., CHEN H., ZHAO D., ZHANG Y., XU Z. (2011): Molecularly imprinted solid-phase extraction combined with high-performance liquid chromatography for analysis of trace olaquinox residues in chick feeds. *Journal of the Science of Food and Agriculture*, **91**: 2378–2385.
- WANG K., GENG Y. (2002): Pathological study on the acute olaquinox poisoning in carp. *Acta Veterinaria et Zootechnica Sinica*, **6**: 565–569.
- WANG K., GENG Y. (2003): Studies on haematological changes in common carps by subacute toxicity test of olaquinox. *Acta Hydrobiologica Sinica*, **1**: 23–26.
- WU Y.J., WANG Y.L., HUANG L.L., TAO Y.F., YUAN Z.H., CHEN D.M. (2006): Simultaneous determination of five quinoxaline-1,4-dioxides in animal feeds using ultrasonic solvent extraction and high performance liquid chromatography. *Analytica Chimica Acta*, **569**: 97–102.
- XU Z.X., SONG J.M., LI L.Q., QIAO X.G., CHEN H.H. (2012): Development of an on-line molecularly imprinted chemiluminescence sensor for determination of trace olaquinox in chick feeds. *Journal of the Science of Food and Agriculture*, **92**: 2696–2702.
- ZHANG H.Y., WEI Y.W., ZHOU J.H., XU Z.X., TIAN S., HE J.X. (2013): Preparation and application of a molecular imprinting matrix solid phase dispersion extraction for the determination of olaquinox in chicken by high performance liquid chromatography. *Food Analytical Methods*, **6**: 915–921.

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