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Automating the Accurate Transfer of Viscous Samples for the Completely Automated Extraction of Mycotoxins from Edible Oils

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Abstract

The manual transfer of liquid samples is part of daily activities throughout the analytical laboratory. The accurate and precise transfer of liquid samples can be critical to the analytical results. Liquid samples with high viscosities pose several challenges to achieving accurate and precise delivery of desired volumes. Automating the accurate transfer of such viscous liquids would lend support to the quality of the analytical procedure, help ensure the high quality of the resulting data, and free the analyst from performing a tedious manual task.

A robotic autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques. The sampler can be configured as part of a GC or LC system or as a stand-alone bench-top workstation. An analytical balance can be included to provide weight verification of liquid transfers.

In this report, a new heated liquid syringe tool that allows viscous liquid samples to be accurately transferred is described and its performance examined. Resulting weight verification data from the performance assessment for example edible oil samples are provided. Good accuracy and precision for transferring viscous samples is demonstrated. Data is provided to demonstrate that the new heated liquid syringe tool enables completely automated extraction of mycotoxins from edible oils and LC/MS/MS analysis of the extract using a single automated analysis setup under integrated control software.

Introduction

Due to their potential therapeutic or health-promoting properties, edible oils extracted from plant seeds have gained popularity compared with animal-based fats [1]. However, adverse growth or storage conditions can lead to fungal growth resulting in contamination. As a result, a major food safety challenge for edible oils is the presence of mycotoxins, including but not limited to those produced by *Aspergillus Flavus* and *Aspergillus Parasiticus* molds, which are among the fungi that produce the toxic secondary metabolites collectively known as aflatoxins. Several mycotoxins are known to be human carcinogens. Contamination of oil seeds by toxigenic molds can lead to the seeds and the oil extracted from the infected seeds becoming unfit for consumption.

Mycotoxin levels in food and animal feed are regulated in most countries so there is great interest in a fast, sensitive, and selective analytical method. However, determining mycotoxin concentrations at trace levels in the presence of large amounts of viscous oil matrix is a challenging task. The accuracy and precision of the analytical results depend on the

extraction and cleanup methods used to isolate the mycotoxins from the complex food matrices, but also on accurate transfer of amounts of viscous liquids.

As a result of this study, we were able to show that a liquid-liquid extraction of mycotoxins from edible oil samples, including accurate transfer of the initial oil sample, was successfully automated using the GERSTEL MPS robotic sampler. Based on the presented method, analytes can be rapidly and reproducibly isolated from edible oil samples based on an automated procedure that includes subsequent LC/MS/MS analysis using the Agilent Ultivo triple quadrupole mass spectrometer.

Figure 1. MPS robotic/robotic^{PRO} sampler configured with GERSTEL automated sample preparation options.

EXPERIMENTAL

Materials. A stock solution containing aflatoxins B1, B2, G1, and G2 was purchased from Romer Labs (Biopure, Mycotoxin Mix 1, 002021). A high calibration standard was prepared by making appropriate dilutions of the mycotoxin mix stock solution using (1:1) water: methanol. Calibration standards were then prepared using a dilution ratio strategy from the high concentration sample of 1:2:2:2:2.5:2:2.

Extra virgin olive oil (cold pressed), sesame oil (pure), flax oil (organic, pure, unrefined, cold pressed), and sunflower oil (virgin, cold pressed), were purchase from local markets. A range of aflatoxin spiked edible oil samples were prepared by making appropriate dilutions of the mycotoxin mix stock solution.
A (95:5) acetonitrile: formic acid (v:v) extraction solution was prepared by combining 190 mL of acetonitrile (Sigma Aldrich, 34998) with 10 mL of formic acid (Sigma Aldrich, 695076). All other reagents and solvents used were reagent grade.

Instrumentation. All automated PrepSequences were performed using a GERSTEL MPS robotic/robotic^{PRO} dual head sampler with the GERSTEL CF-200 centrifuge, balance (weighing option) , mVAP, quick MIX, 5-position dilutor option, a heated agitator, and the GERSTEL Heated Liquid Syringe Module (HLM) as shown in Figure 1. All subsequent analyses were performed using an Agilent 1260 HPLC, outfitted with an Agilent Poroshell 120 EC-C18 column (3.0 x 50 mm, 2.7 µm), coupled to an Agilent Ultivo triple quadrupole mass spectrometer with jet stream electrospray source. Sample injections were made using a GERSTEL robotic^{PRO} sampler with the LCMS tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 2 µL stainless steel sample loop.

Automated Prep Sequence. A manual method for liquid-liquid extraction of mycotoxins from edible oils [2] was automated using the MPS robotic/robotic^{PRO} dual head sampler. The automated steps performed are listed below (steps 2-13).

1. User places edible oil sample into a 10 mL vial and places the vial onto the MPS.
2. MPS moves vial into incubator at 60°C for 10 minutes.
3. MPS transfers 1.5 mL of the edible oil sample using HLM (at 65°C) into an empty 10 mL vial.
4. MPS adds 7.5 mL (95:5) acetonitrile: formic acid (v/v).
5. MPS mixes the vial content by agitation for 10 minutes at 2000 rpm.
6. MPS centrifuges vial for 5 minutes at 2000 g.
7. MPS transfers 4 mL of supernatant into a clean, empty, 10 mL vial.
8. MPS adds 4 mL hexane.
9. MPS mixes the vial content by agitation for 10 minutes at 2000 rpm.
10. MPS centrifuges vial for 5 minutes at 2000 g.
11. MPS transfers 2.5 mL of the lower layer into an empty, round bottom, 4 mL vial.
12. MPS evaporates the extract to dryness at 45°C.
13. MPS reconstitutes using 500 µL (1:1) methanol: water.
14. MPS injects (or, optionally, filters through a 0.2 µm filter then injects) the reconstituted extract into the LC-QQQ.

Analysis conditions LC

Pump:	gradient (800 bar), flowrate = 0.2 mL/min	
Mobile Phase:	A - 0.1 % formic acid in water	
	B - 0.1 % formic acid in methanol	
Gradient:	Initial	10 % B
	0.5 min	10 % B
	1.0 min	50 % B
	3.0 min	50 % B
	3.5 min	60 % B
	7.5 min	65 % B
	8.0 min	90 % B
	11.0 min	90 % B
	11.5 min	10 % B
	14.0 min	10 % B
Run time:	14 minutes	
Injection volume:	2.0 µL (loop over-fill technique)	
Column temperature:	30°C	

Analysis conditions MS

Operation:	electrospray positive mode
Gas temperature:	250°C
Gas flow (N_2):	8 L/min
Nebulizer pressure:	30 psi
Sheath gas heater:	350°C
Sheath gas flow (N_2):	11 L/min
Capillary voltage:	4000 V
Nozzle voltage:	500 V
Delta EMV:	0 V

Mass spectrometer acquisition parameters are shown in Table 1 with qualifier ions.

Table 1. Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion [m/z]	Product Ion [m/z]		Frag. Voltage [V]		Collision Energy [V]	
Aflatoxin G2	331.1	313.1	115	190	190	24	80
Aflatoxin G1	329.1	311.1	243.1	180	180	20	28
Aflatoxin B2	315.1	287.1	259.1	190	190	24	28
Aflatoxin B1	313.3	285.1	241.1	190	190	20	40

Results and Discussion

Raising the temperature of a viscous sample decreases its viscosity. The ability to control the temperature of both the sample and the syringe being used to transfer the sample is important in order to achieve reliable and accurate transfer of viscous samples using syringe based autosamplers. Figure 2 shows how increasing the temperature of propylene glycol, (cP=42 at 27°C) leads to an improvement in transfer volume accuracy for the viscous liquid standard.

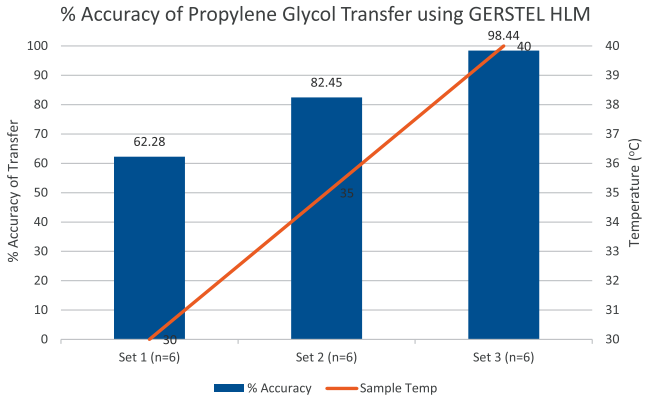

Figure 2. % Accuracy of propylene glycol transfer using the GERSTEL Heated Liquid Syringe Module.

Identical volumes of olive oil (cP=40 at 38°C), sesame oil (cP=41 at 35°C), flax oil (cP=29 at 38°C) and sunflower oil (cP=49 at 25°C) were placed into the heated agitator at 60°C for 10 minutes and replicate aliquots of each were then transferred to individual vials using the Heated Liquid Syringe Module (65°C). Table 2 shows the resulting precision and accuracy data for replicate transfers of each edible oil.

Table 2. Precision and accuracy of edible oil transfer using the Heated Liquid Syringe Module.

Replicate	Olive Oil [g]	Sesame Oil [g]	Flax Oil [g]	Sunflower Oil [g]
1	1.3199	1.3305	1.343	1.3281
2	1.3192	1.3298	1.3431	1.3298
3	1.3189	1.3312	1.3438	1.3301
4	1.3180	1.3276	1.3439	1.3284
mean	1.3190	1.3298	1.3435	1.3291
SD	0.000787	0.00156	0.000465	0.000997
% CV	0.0597	0.1172	0.0346	0.0750
% Diff from Theo.	-5.45	-4.68	-3.70	-4.72

Figures 3a and b show representative stacked mass chromatograms resulting from extracted samples of flax oil (a) and sunflower oil (b) spiked with mycotoxin at concentrations of 10 ng/mL (Aflatoxins B1, G1) and 2.5 ng/mL (Aflatoxins B2, G2).

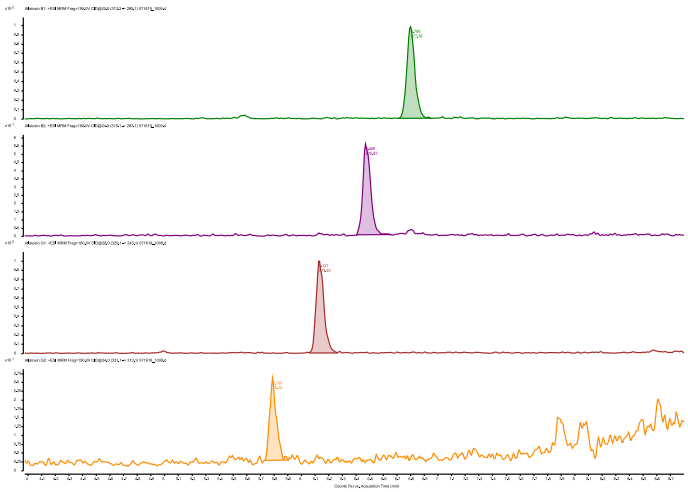

Figure 3a. Representative stacked mass chromatograms resulting from extraction of aflatoxin spiked flax oil.

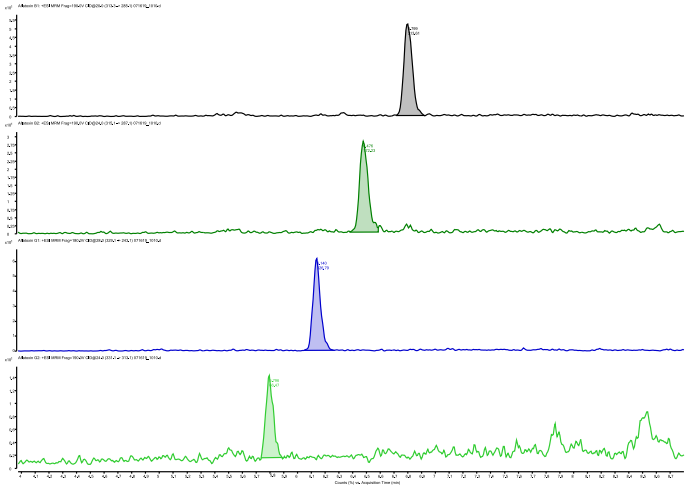

Figure 3b. Representative stacked mass chromatograms resulting from extraction of aflatoxin spiked sunflower oil.

As shown in Table 3, the ability to accurately and reproducibly transfer edible oil samples during the automated extraction procedure leads to reproducible data for the replicate extracts of olive oil spiked with mycotoxins.

Table 3. Precision of mycotoxin extractions from olive oil using automated extraction procedure.

Response:	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
Olive Oil 1	260	120	288	46
Olive Oil 2	251	108	287	45
Olive Oil 3	239	124	282	43
Olive Oil 4	240	116	259	43
mean	243	116	276	44
SD	6.51	8.00	14.6	1.04
%CV	2.68	6.90	5.30	2.40

A representative calibration curve for Aflatoxin B1 is shown in Figure 4. Regression analysis for all mycotoxin compounds analyzed within this method resulted in R^2 values of 0.999 or greater.

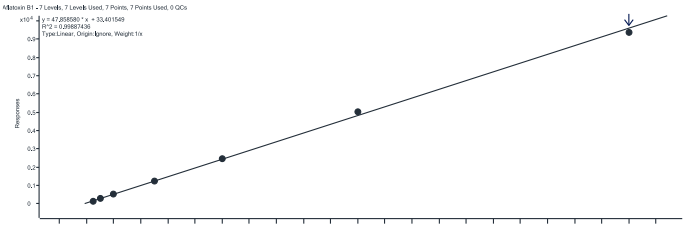

Figure 4. Representative calibration curve for Aflatoxin B1.

The recovery of mycotoxins using the automated extraction was assessed by comparing the results obtained from mycotoxin spiked edible oil samples with those resulting from spiking the extracts of blank edible oil samples after the extraction. Table 4 shows the resulting % recovery of each mycotoxin from each edible oil examined.

Table 4. % Recovery of mycotoxins from edible oils using the automated extraction procedure.

% Recovery	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
Olive Oil	95.9	103	95.7	129
Sesame Oil	83.8	93.7	92.1	131
Flax Oil	87.7	104	88.0	126
Sunflower Oil	84.3	101	90.2	101
Ave. % Recovery	87.9	101	91.5	122

Conclusions

As a result of this study, we were able to show:

- An extraction procedure for mycotoxins in edible oils was readily automated using the GERSTEL MPS robotic^{PRO} sampler, including introduction of the extract to LC/MS/MS and analysis based on Agilent Ultivo triple quadrupole mass spectrometer.
- Viscous edible oil samples can be transferred accurately and precisely using the GERSTEL Heated Liquid Syringe Module.
- Mycotoxins can be reproducibly extracted from edible oil samples using an automated extraction procedure with an average precision of 4.32 % (range: 2.40 % – 6.90 %RSD).
- The recovery of mycotoxins from edible oil samples using the automated extraction procedure and LC/MS/MS analysis averaged 101 % (range: 87.9 % - 122 %).

References

[1] Bhat, R and Reddy, K.R.N, "Challenges and issues concerning mycotoxins contamination in oil seeds and their edible oils: Updates from last decade.", *Food Chemistry*, 215 (**2017**), 425-437.

[2] Eom, T., et al., "Multiclass mycotoxin analysis in edible oils using a simple solvent extraction method and liquid chromatography with tandem mass spectrometry.", *Food Additives & Contaminants: Part A*, **2017**, Vol. 34, No. 11, 2011-2022.

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