

# Automating the Preparation of Matrix Matched Calibration Standards for the Analysis of Food Contaminants by LC/MS/MS

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## KEYWORDS

Sample Preparation, High Throughput Lab Automation, Food Safety, Pesticides, LC/MS/MS

## ABSTRACT

The manual preparation of calibration standards and QC samples is part of the daily activities of laboratories that analyze food samples for contaminants. The accurate and precise transfer of liquid standards and solutions is critical for ensuring the validity of the analytical results. Automating the accurate transfer of liquid standards and solutions helps to improve the quality of the analytical procedure while freeing the analyst from a manually tedious task.

In the work presented here, matrix matched calibration standards were prepared automatically by a robotic autosampler commonly used for sample introduction in GC/MS or LC/MS. The resulting precision and accuracy data from calibration curves and QC sample analysis in example food commodities are provided. Accuracy and precision for QC samples were found to be: 97.4 % and 4.25 % (Apples), 100 % and 8.4 % (Pears), 97.7 % and 3.96 % (Sweet Potatoes), 95.7 % and 3.59 % (Green Beans), 98.2 % and 2.79 % (Carrots), (96.8 % and 3.34 % (Beef) and 97.6 % and 4.31 % (Turkey). Data is provided showing the use of an automated filtration option that enables completely automated preparation of QuEChERS derived food extracts with subsequent analysis by LC/MS/MS

## INTRODUCTION

In recent articles, Lehotay [1] and Sapozhnikova [2] provide validation data for the determination of contaminants in various food commodities using the GERSTEL MultiPurpose Sampler (MPS) robotic to automate the cleanup and injection to GC of QuEChERS derived sample extracts with high precision and accuracy. In this study, we show that the same equipment can be used to prepare the required matrix matched standards required for analyte quantitation.

As a result of this study, we were able to demonstrate successful automation of the preparation of matrix matched calibration standards for a range of LC amenable pesticides using the GERSTEL MPS robotic sampler. Using this method, matrix matched calibration curves and QC samples were rapidly, accurately and reproducibly prepared for a variety of QuEChERS derived food commodities. In addition, the GERSTEL MPS robotic was successfully used to perform automated filtration combined with injection into an LC-MS/MS system configured using the Agilent Ultivo Triple Quadrupole Mass Spectrometer.

**EXPERIMENTAL**

**Materials.** Pesticide stock solutions were obtained from Restek. An intermediate stock solution containing all pesticides monitored was prepared by combining the appropriate volumes of each pesticide stock solution to give a final concentration of 2200 ng/mL. Spiking solutions used for the preparation of the calibration standards were prepared using the intermediate stock solution. Separate spiking solutions were prepared for the preparation of QC samples. An internal standard stock solution was prepared in acetonitrile at a concentration of 2200 ng/mL for both atrazine-d<sub>3</sub> and diazinon-d<sub>10</sub>.

Comminuted samples of apples, pears, sweet potatoes, green beans, carrots, beef, and turkey were obtained from a local market. Sample preparation for these blank matrices followed the previously reported QuEChERS approach described [1,2]. All other reagents and solvents used were reagent grade.

**Instrumentation.** All automated PrepSequences were performed using a GERSTEL MPS robotic<sup>PRO</sup> sampler equipped with a 100 µL and a 1000 µL syringe. All subsequent analyses were performed using an Agilent 1260 HPLC with an Agilent Eclipse Plus C18 RRHD column, (2.1 x 50 mm, 1.8 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. Sample injections were made using a GERSTEL robotic<sup>PRO</sup> 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. Prior to injection, each sample was filtered using the GERSTEL Fast Filtration Option as shown in Figure 1.



Figure 1. MPS robotic<sup>PRO</sup> sampler with the GERSTEL Fast Filtration Option.

**Automated Prep Sequence.** Preparation of the matrix matched calibration curves and QC samples was automated using an MPS robotic<sup>PRO</sup> sampler. Final concentrations of the calibration standards were prepared using a dilution ratio strategy from the high concentration sample of 1:2:5:2:5. The QC samples were prepared at final concentrations of 2.5 ng/mL and 25 ng/mL, respectively.

*Analysis conditions LC*

Pump:	gradient (800 bar), flowrate = 0.45 mL/min
Mobile Phase:	A - 5mM ammonium formate in water w/0.1 % formic acid B - 5mM ammonium formate in methanol w/0.1 % formic acid
Gradient:	Initial 2 % B 0.5 min 2 % B 1.0 min 50 % B 4.0 min 65 % B 16.0 min 100 % B 18.0 min 100 % B 18.1 min 2 % B
Run time:	21.5 minutes
Injection volume:	2.0 µL (loop over-fill technique)
Column temperature:	45°C

*Analysis conditions MS*

Operation:	electrospray positive mode
Gas temperature:	250°C
Gas flow (N <sub>2</sub> ):	11 L/min
Nebulizer pressure:	40 psi
Sheath gas heater:	350°C
Sheath gas flow (N <sub>2</sub> ):	11 L/min
Capillary voltage:	3500 V
Nozzle voltage:	500 V
Delta EMV:	0 V

The mass spectrometer acquisition parameters are shown in Table 1 with qualifier ions. A retention time window value of 1 minute was used for each positive ion transition being monitored during the dynamic MRM experiment.

Table 1. Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion [m/z]	Fragmentor Voltage [V]	Product Ion [m/z]		Collision Energy [V]		Retention Time [min.]
Bifenthrin	440.1	90	181	165.7	5	35	12.21
Azoxystrobin	404.3	100	372	344.1	5	15	4.24
Ethion	385	80	199	171	0	5	8.34
Fluquinconazole	376	80	349	307.1	23	23	4.87
Propargite	373.3	150	81	57.1	25	25	9.16
Etoxazole	360.1	140	141	113	28	50	9.22
Tolyfluanid	347	60	237.9	137	3	20	5.78
Triflumizole	346	80	278	73	5	10	6.91
Fenarimol	331	130	268	81	16	20	4.94
Pyriproxyfen	322.1	100	185	96.1	16	8	8.18
Flusilazole	316	150	247	165	12	24	5.41
Diazinon-d <sub>10</sub>	315.3	90	170	154	15	15	5.87
Kresoxim methyl	314.1	80	267.1	222.2	0	5	5.66
Tebuconazole	308.1	120	124.9	70	47	40	5.76
Flutriafol	302.1	100	123	70.1	28	12	3.5
Imazalil	297.1	100	201	159	10	15	3.33
Triadimenol	296.1	75	99	70.1	10	4	4.84
Triadimefon	294.1	120	197	69	15	20	4.73
Paclobutrazol	294.1	110	125	70.1	40	20	4.67
Penconazole	284.1	120	159	70	20	15	5.6
Imidacloprid	256.4	100	209	175	10	15	2.21
Linuron	248.9	110	181.9	160.1	10	10	4.08
Cyprodinil	226	160	133	93	28	40	5.17
Atrazine-d <sub>5</sub>	221.1	80	179.1	137	10	15	3.52
Dichlorvos	221	110	109	79	12	24	2.88
Atrazine	216.1	90	174	132	10	15	3.52
Thiabendazole	202	140	175	131	20	30	2.27
Carbaryl	202	70	145	127	5	25	3.11
Pyrimethanil	200	150	106.9	82	24	24	3.93

## RESULTS AND DISCUSSION

Figure 2 shows a representative mass chromatogram overlay from the analysis of a low QC sample prepared by the automated prep sequence using QuEChERS derived apple matrix.

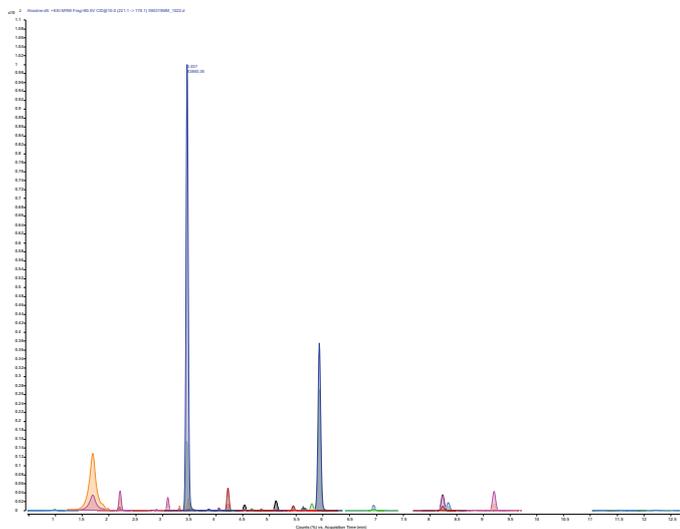


Figure 2. Representative overlay mass chromatogram from matrix matched apple low QC sample.

The representative calibration curves for (A) bifenthrin, (B) kresoxim methyl, and (C) tebuconazole from the analysis of matrix matched, green bean calibration standards are shown in Figure 3. Average regression analysis for all pesticide compounds from all food commodities analyzed within this method resulted in  $R^2$  values of 0.99 or greater.

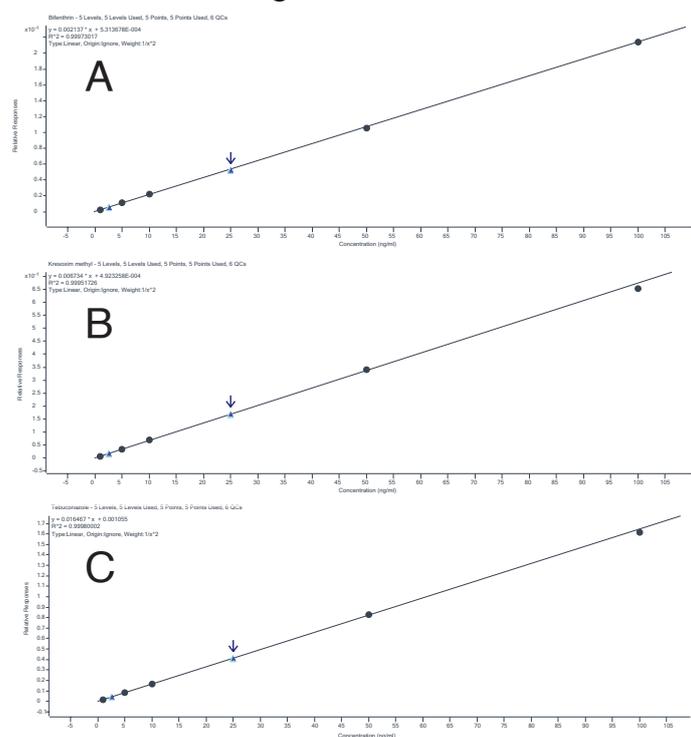


Figure 3. Representative calibration curves for matrix matched green beans. (A): Bifenthrin, (B): Kresoxim methyl, (C): Tebuconazole.

Table 2 shows the average accuracy of QC samples prepared by the automated prep sequence using QuEChERS derived food commodities. Table 3 shows the average precision data of QC samples prepared by the automated prep sequence using QuEChERS derived food commodities. Examples of individual accuracy and precision data for all 27 pesticides monitored in (A) pears, (B) sweet potatoes, and (C) turkey are shown in Figure 4. These data show that matrix matched calibration standards and QC samples can be prepared by the GERSTEL MPS sampler with high precision and accuracy.

Table 2. Average accuracy of matrix matched QC samples.

	Ave. %Accuracy	Min. %Accuracy	Max. %Accuracy
SO	97.2	88.7	105
Apples	97.4	85.7	104
Pears	100	89.5	113
Sweet Potatoes	97.7	85.9	109
Green Beans	95.7	88.6	101
Carrots	98.2	92.1	104
Beef	96.8	91.2	100
Turkey	97.6	91.9	99.1

Table 3. Average precision of matrix matched QC samples.

	Ave. %Precision	Min. %Precision	Max. %Precision
SO	4.72	1.27	13.1
Apples	4.25	1.40	19.0
Pears	8.40	1.66	23.6
Sweet Potatoes	3.96	1.08	16.4
Green Beans	3.59	1.90	10.4
Carrots	2.79	1.27	10.0
Beef	3.34	1.17	8.8
Turkey	4.31	1.52	10.5

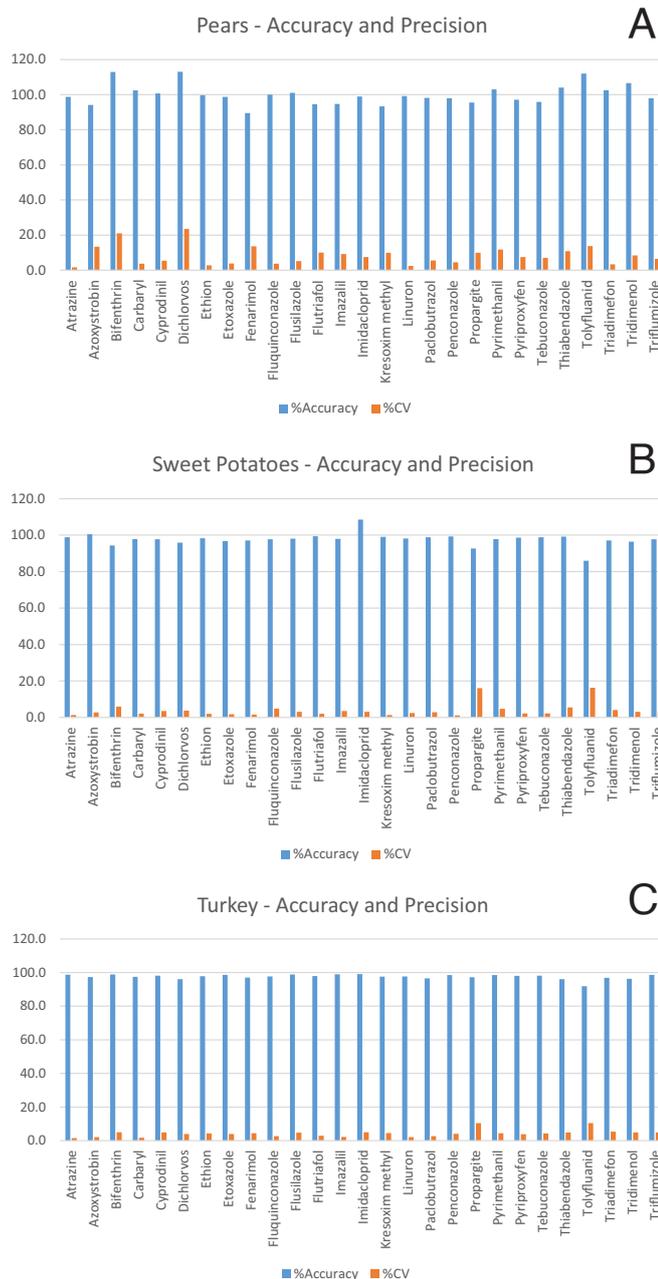


Figure 4. Resulting accuracy and precision for (A): Pears, (B): Sweet Potatoes, (C): Turkey.

Matrix effects were calculated for each analyte as the difference between the slope of the matrix matched calibration curve and solvent-only calibration curve divided by the slope of solvent only calibration curve [2]: %ME = [(slope of MM calibration curve–slope of SO calibration curve)/slope of SO calibration curve] × 100 %. Results of the average % MEs are found in Table 4 and shown graphically for all 27 pesticides from all food commodities in Figure 5. The ruggedness of the method is supported by that fact that these matrix matched samples did not undergo any additional cleanup, only filtering, and the calculated % MEs were still only determined to be within ±40 % with the average being within ±11.4 %.

Table 4. Average % matrix effects for food commodities examined.

	Ave. %ME	Min. %ME	Max. %ME
Apples	2.65	-22.9	15.0
Pears	11.4	-22.9	40.3
Sweet Potatoes	3.94	-9.77	37.6
Green Beans	6.38	-5.52	36.4
Carrots	-0.513	-25.7	32.3
Beef	-2.94	-40.4	31.5
Turkey	0.189	-24.7	32.5

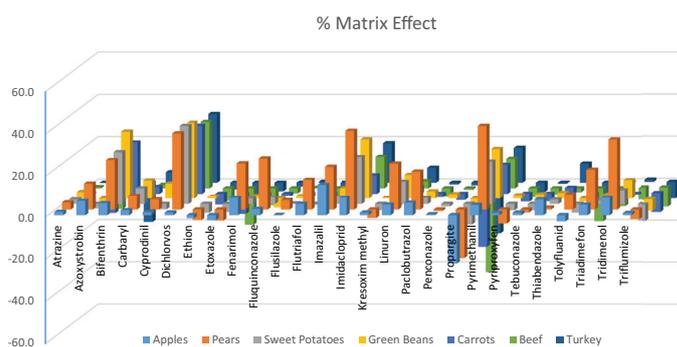


Figure 5. %Matrix effects for all pesticides in all food commodities.

The recovery of the pesticides monitored using the automated filtration option was confirmed by comparing the results of spiked pesticide samples against the same spike pesticide samples that were filtered prior to analysis. Figure 6 shows the resulting % recovery of each pesticide following automated syringe filtration.

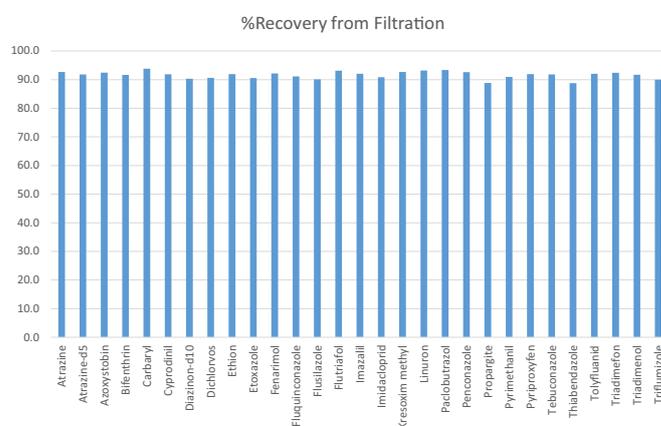


Figure 6. Recovery of pesticides following automated filtration.

## CONCLUSIONS

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

- The preparation of matrix matched calibration curves for a range of LC amenable pesticide standards were successfully automated using the GERSTEL MPS robotic<sup>PRO</sup> sampler.
- Average regression analysis for all pesticide compounds from all food commodities analyzed within this method resulted in R<sup>2</sup> values of 0.99 or greater.
- Accuracy and precision for all QC samples were found to be: 97.4 % and 4.25 % (Apples), 100 % and 8.4 % (Pears), 97.7 % and 3.96 % (Sweet Potatoes), 95.7 % and 3.59 % (Green Beans), 98.2 % and 2.79 % (Carrots), (96.8 % and 3.34 % (Beef) and 97.6 % and 4.31 % (Turkey).
- For the 27 LC amenable pesticides analyzed, the calculated %ME's were found to average 2.6 % (Apple), 11.4 % (Pear), 3.9 % (Sweet Potatoes), 6.4 % (Green beans), -0.5 % (Carrots), -2.9 % (Beef), and 0.2 % (Turkey).

## REFERENCES

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- [2] Sapozhnikova, Y., "High-throughput analytical method for 265 pesticides and environmental contaminants in meats and poultry by fast low pressure gas chromatography and ultrahigh-performance liquid chromatography tandem mass spectrometry", *J. Chrom. A*, **2018**, 1572, 203-211.



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