PERFORMANCE EVALUATION OF AN AUTOMATED QuEChERS WORKFLOW PLATFORM FOR ANALYSIS OF PESTICIDES IN FRESH PRODUCE

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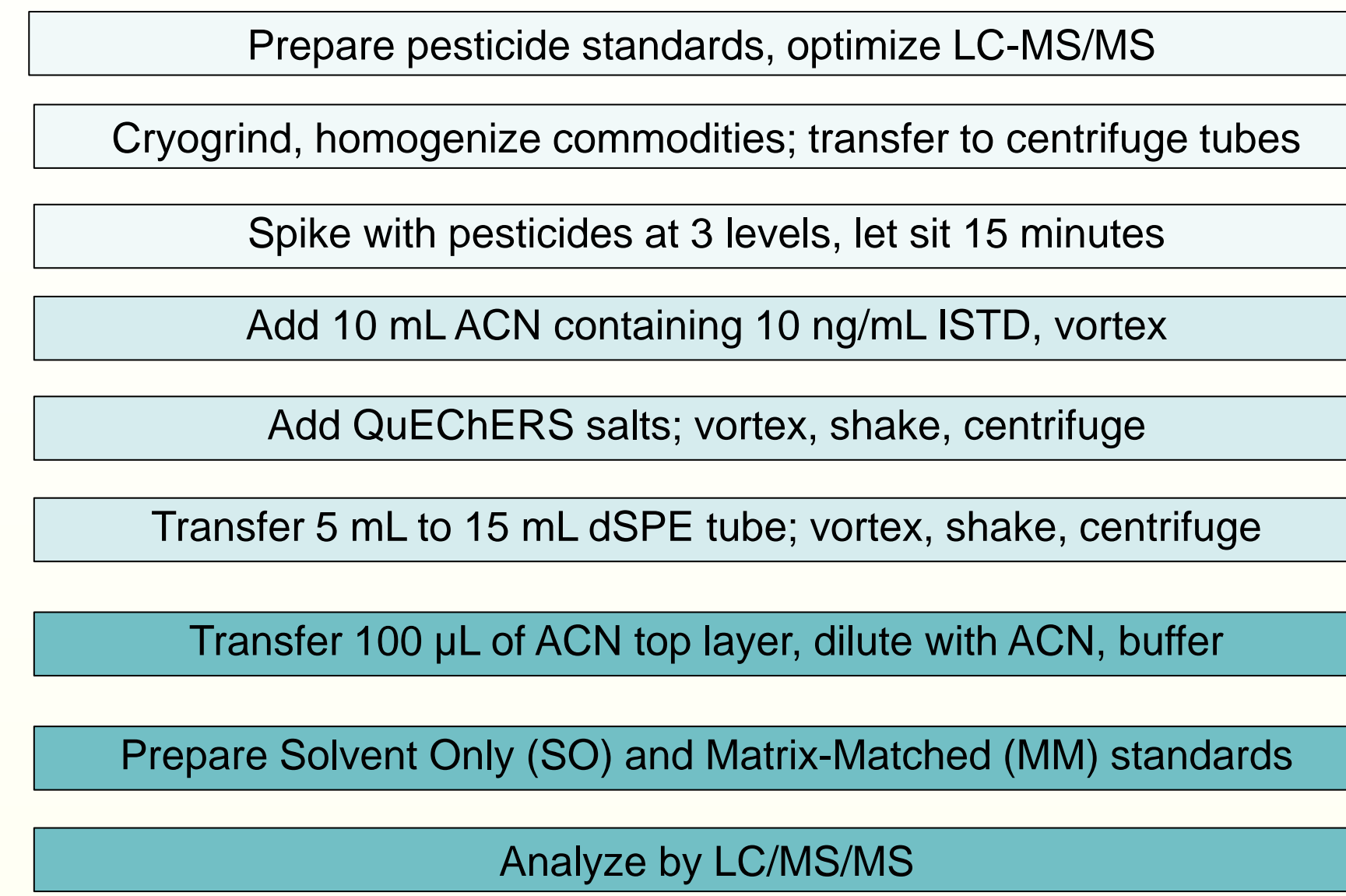
ABSTRACT

The Teledyne Tekmar AutoMate Q-40 is a robotic system designed to optimize and automate the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) sample preparation workflow. The performance of this system was evaluated in comparison to a manual sample preparation workflow using the AOAC 2007.01 unbuffered QuEChERS methodology where all samples were analyzed using liquid chromatography – tandem mass spectrometry (LC-MS/MS). Matrices used in this study were cabbage, valencia orange, raisin and purple corn flour. A Restek LC Multiresidue Standards Kit for Pesticide Analysis, Teledyne Tekmar QuEChERS extractions salts and C18/PSA dSPE cleanup and an ABSciex Qtrap 5500 mass spectrometer were employed. LC parameters included the use of a Restek Ultra-Aqueous C_{18} Column (100 mm x 2.1 mm, 3 μm), 10 mM NH_4HCO_2 buffer (A: water, B: methanol) with 0.1% formic acid, 0.5 mL/min flow rate with 5 μL sample injection and MS/MS parameters were optimized individually. Results were determined for the instrument (limit of detection, limit of quantitation and linearity) and method (method detection limit, recoveries, relative standard error) and will be compared to determine the statistical differences (if any).

INTRODUCTION

The ability to automate manual steps in the laboratory setting is a growing area of interest, particularly in workplaces which receive time-sensitive or large volumes of fresh produce for pesticide analysis, or in method development and validation laboratories. The Teledyne Tekmar AutoMate Q40 automated QuEChERS platform is outfitted with three reservoirs for spiking of standards or preservative, which can occur simultaneously during delivery of the extraction solvent or can be added to the final extract. Samples are vortexed prior to addition of the QuEChERS salts and then vortexed and centrifuged. A pre-determined volume of the supernatant is transferred to a dispersive solid phase cleanup vial and the sample is once again vortexed and centrifuged. Lastly, a specified volume is transferred to a final extract vial where the analyst performs the remainder of the steps. This process takes approximately four hours to complete twenty samples. The analyst then dilutes and filters the sample and prepares any calibration standards. before analysis by LC-MS/MS.

OVERALL WORKFLOW

- Prepare pesticide standards, optimize LC-MS/MS
- Cryogrind, homogenize commodities; transfer to centrifuge tubes
- Spike with pesticides at 3 levels, let sit 15 minutes
- Add 10 mL ACN containing 10 ng/mL ISTD, vortex
- Add QuEChERS salts; vortex, shake, centrifuge
- Transfer 5 mL to 15 mL dSPE tube; vortex, shake, centrifuge
- Transfer 100 μL of ACN top layer, dilute with ACN, buffer
- Prepare Solvent Only (SO) and Matrix-Matched (MM) standards
- Analyze by LC/MS/MS

Scheme 1: Overall workflow for pesticide standard preparation and preparation of fresh produce samples using the QuEChERS method
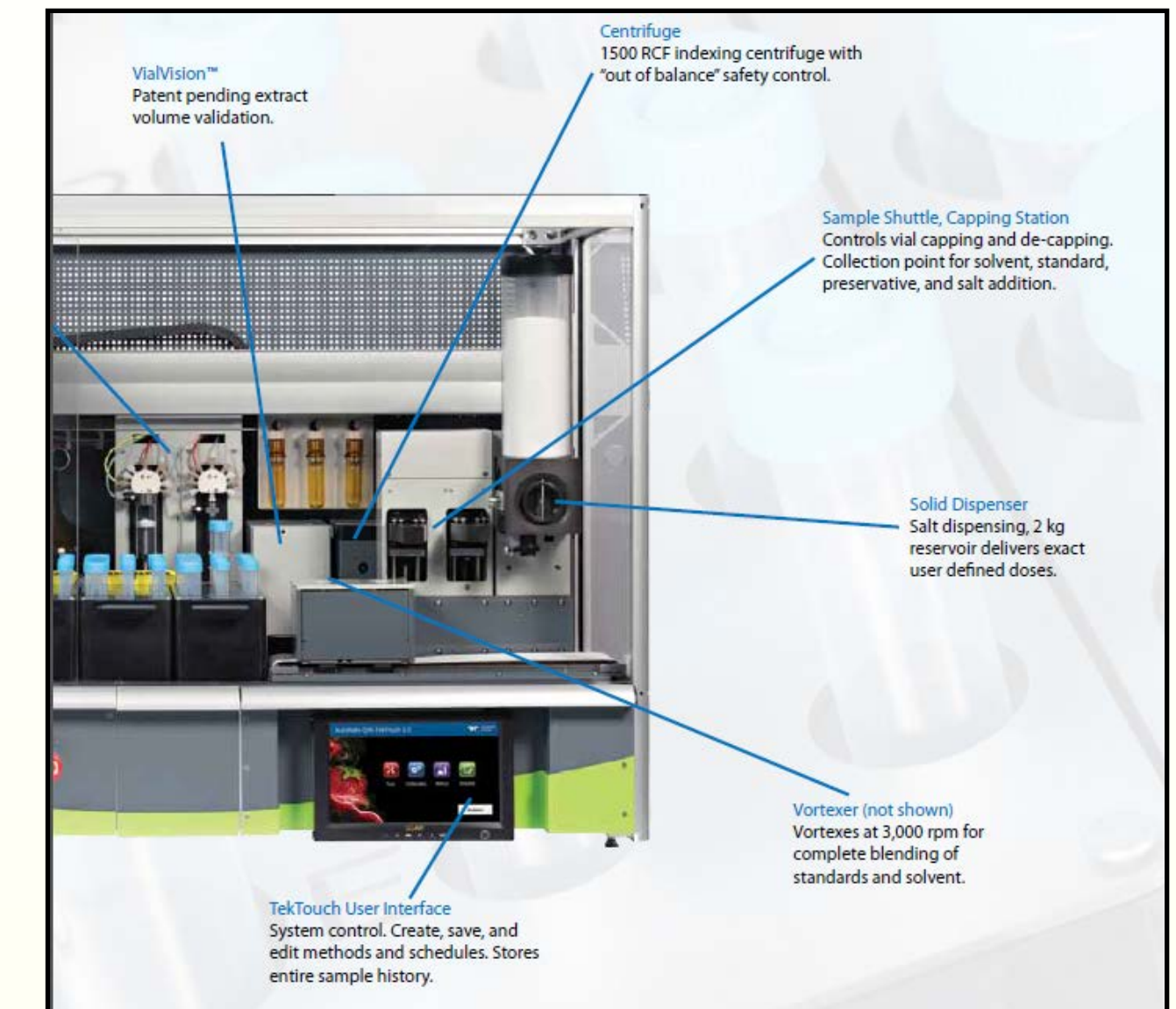

Figure 1: Teledyne Tekmar AutoMate Q40 Automated QuEChERS Platform (www.teledynetekmar.com)

RESEARCH OBJECTIVES

1. Evaluate the Automate Q40 for performance in the areas of limit of detection (LOD), limit of quantitation (LOQ), linearity (R2), method detection limit (MDL), percent recovery (REC) and percent relative standard deviation (RSD) for 204 pesticides in four matrices.

2. Compare performance of AutoMate Q40 to manual workflow performed by novice laboratory analyst

MATERIALS AND METHODS

- Comminuted, homogenized commodities
  - cabbage, valencia orange (10.0 g ; raisin, purple corn flour (5.0 g)
- Restek Multiresidue Pesticide Standards Kit (204 pesticides in 10 ampoules)
- QuEChERS Extraction Solvent: acetonitrile containing 10 ng/mL ISTD
- Teledyne Tekmar Products (required)
  - 50 mL centrifuge tubes
  - Extraction Salts: 4 mg $MgSO_4$, 1 mg NaCl
  - dSPE Cleanup: 15 mL centrifuge tube; (1,200 mg $MgSO_4$, 400 mg PSA)
- Waters Acquity UHPLC with ABSciex Qtrap 5500 mass spectrometer
  - Restek Ultra-Aqueous C_{18} Column (100 mm x 2.1 mm, 3 μm)
  - 10 mM NH_4HCO_2 buffer (A: water, B: methanol) with 0.1% formic acid
  - 12 minute LC analysis; initial 90% A, final 0% A ; 3 minute re-equillibration
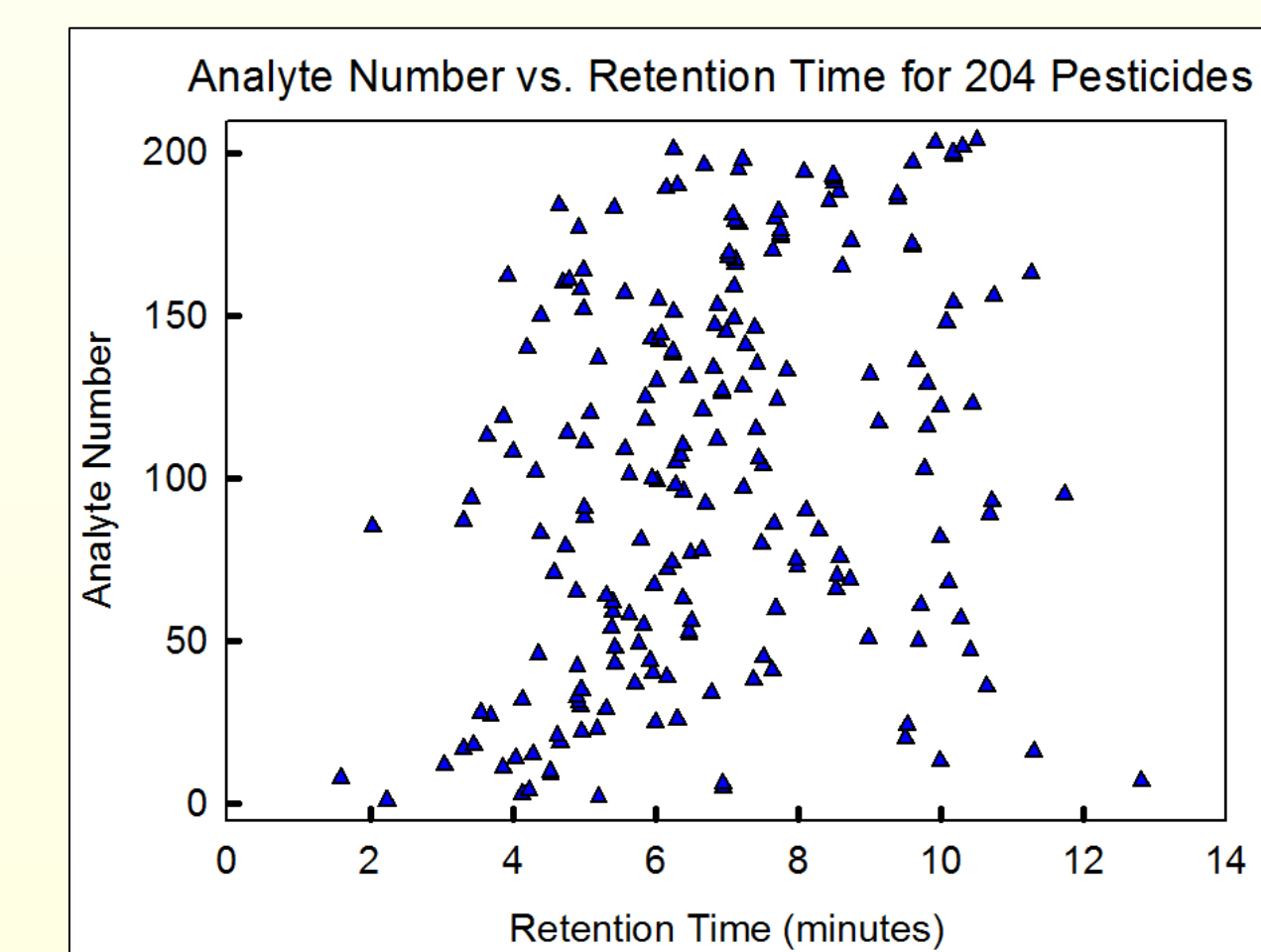 - 0.5 mL/min flow rate, 5 μL sample injection

Figure 2: Analyte number vs. retention time for 204 pesticides

Sample Type	Sample Name	Desired Conc.	Volume Stock to Add	Fortification Solution
Water Blanks 1-2	WB 1-2	0 ng/mL	500 μL	ACN
Matrix Blanks 1-4	XB 1-4	0 ng/mL	500 μL	ACN
Matrix Spike 10 ng/mL	XS-5 1-4	10 ng/mL	200 μL	0.5 μg/mL
Water Spike 50 ng/mL	WS-50 1-4	50 ng/mL	250 μL	2.0 μg/mL
Matrix Spike 50 ng/mL	XS-50 1-4	50 ng/mL	250 μL	2.0 μg/mL
Matrix Spike 250 ng/mL	XS-250 1-4	250 ng/mL	500 μL	5.0 μg/mL

Table 2: Sample spiking procedure using 0.5, 2.0 and 5.0 μg/mL Fortification Solutions

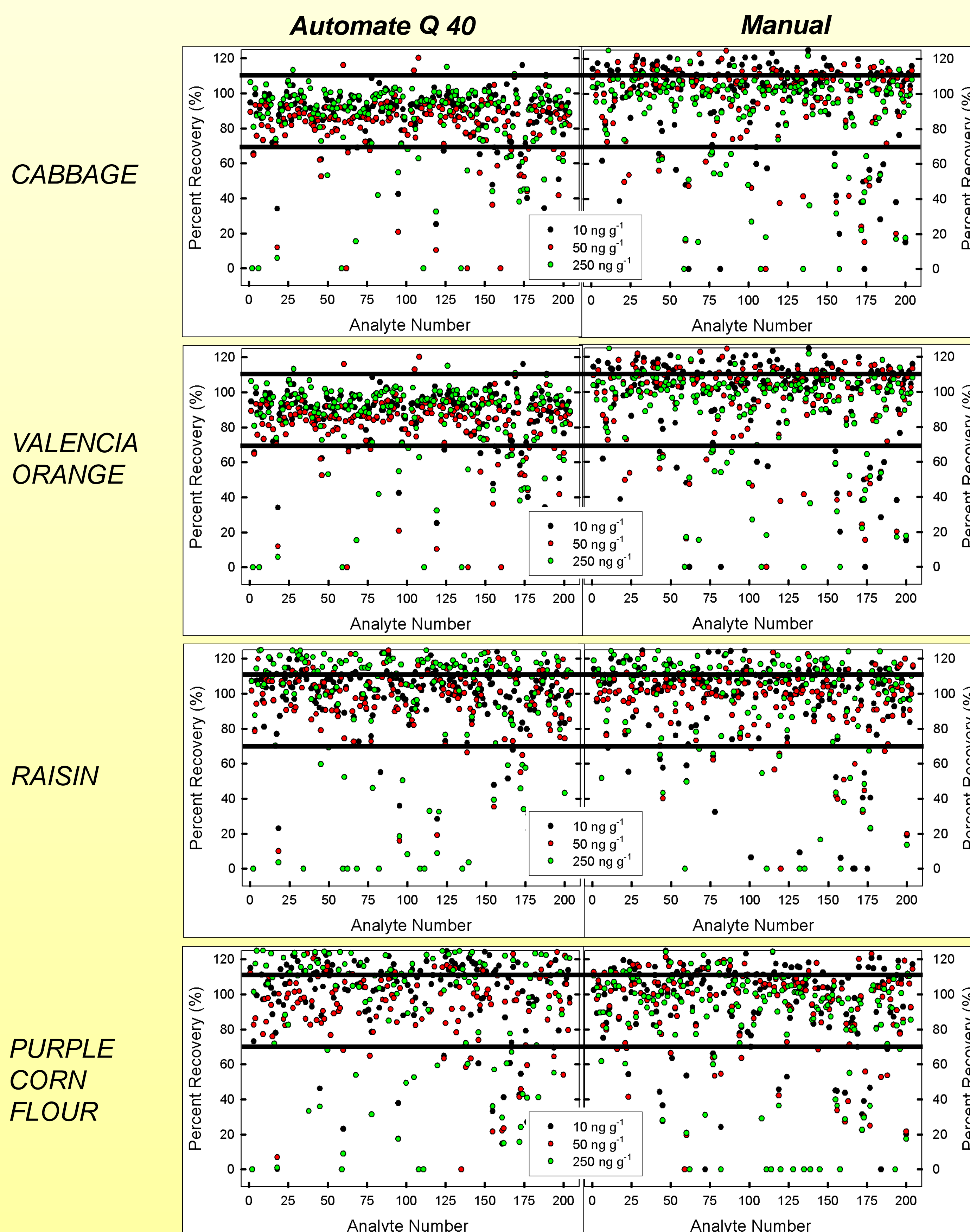## RESULTS A: PERCENT RECOVERY OF 204 PESTICIDES

Figure 3: Percent recovery data for cabbage, valencia orange, purple corn flour and raisin spiked with 204 pesticides at 10, 50 and 250 parts per billion (ppb). Data acquired using the AutoMate Q40 is shown on the left and data acquired manually is shown on the right. Black bars represent the recovery range 70% to 110% for reference.

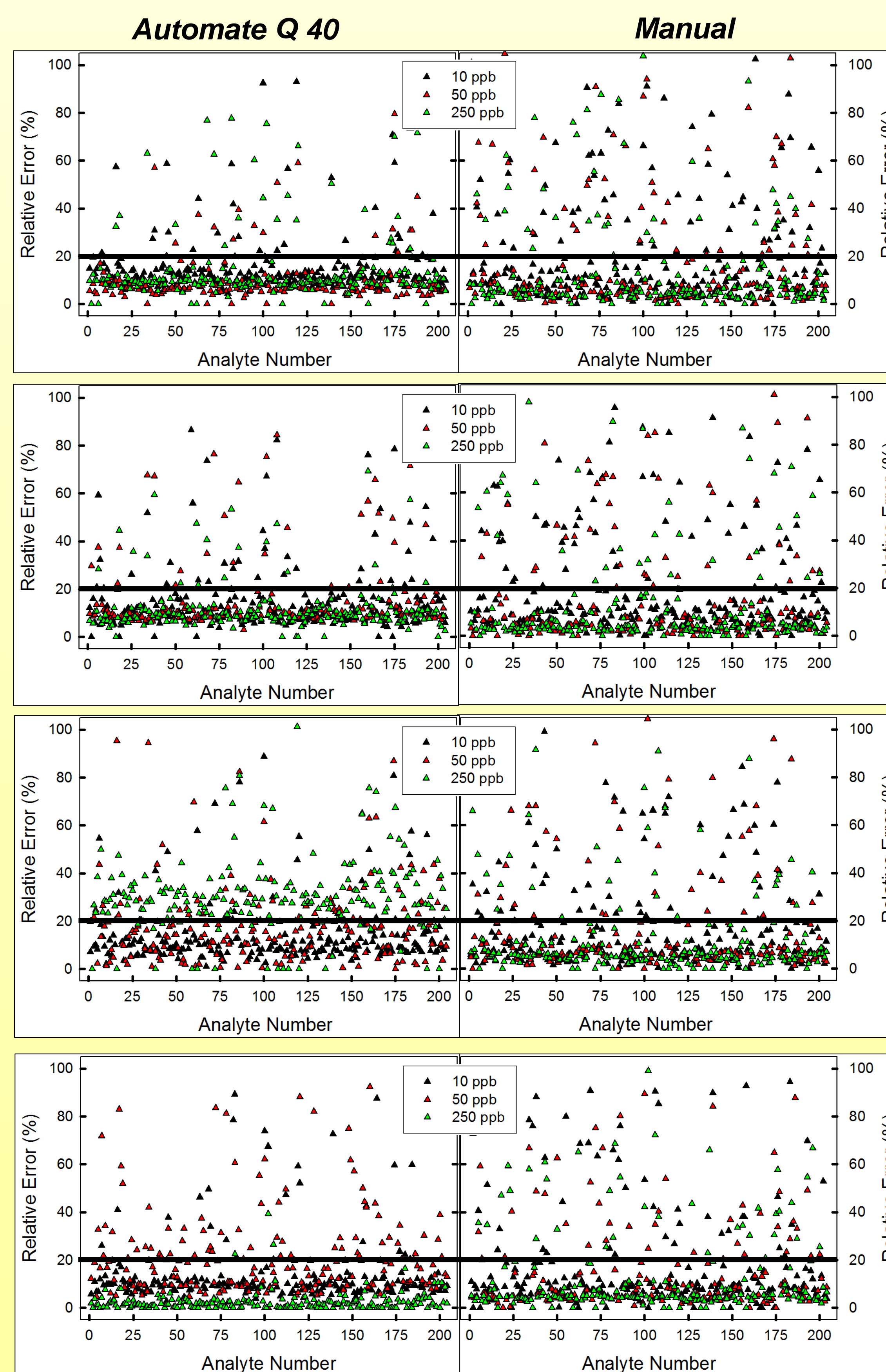## RESULTS B: PERCENT RELATIVE STANDARD DEVIATION

Figure 4: Percent relative standard deviation data for cabbage, valencia orange, purple corn flour and raisin spiked with 204 pesticides at 10, 50 and 200 parts per billion (ppb). Data acquired using the AutoMate Q40 is shown on the left and data acquired manually is shown on the right. Black bar represents RSD = 20% for reference.

RESULTS C: COMPARISON

Overall Performance

A t-value was calculated for 204 pesticides at three spike levels in four matrices using the equation below:

$$t(observed) = \frac{x_1 - x_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where x is the average recovery, σ is the standard deviation and n is the sample size. The degrees of freedom, and the critical value of the t-distribution given the degrees of freedom and a 0.025 two-sided significance level were determined. Values of t(observed) larger than t(critical) were deemed significantly different and were summed for each spike level and are presented in the right panel below. Values of t(observed) less than or equal to t(critical) were deemed "similar", meaning that there is insufficient information to believe that results are different

CABBAGE

	Similar	Different
10 ppb	163 (80%)	41 (20%)
50 ppb	146 (72%)	58 (28%)
250 ppb	174 (85%)	30 (15%)
Avg. ± St. Dev	79% ± 7%	21% ± 7%

VALENCIA ORANGE

	Similar	Different
10 ppb	175 (86%)	29 (14%)
50 ppb	165 (81%)	39 (19%)
250 ppb	144 (71%)	60 (29%)
Avg. ± St. Dev	79% ± 8%	21% ± 8%

RAISIN

	Similar	Different
10 ppb	149 (73%)	55 (27%)
50 ppb	198 (97%)	6 (3%)
250 ppb	196 (96%)	8 (4%)
Avg. ± St. Dev	89% ± 14%	11% ± 14%

PURPLE CORN FLOUR

	Similar	Different
10 ppb	181 (89%)	23 (11%)
50 ppb	184 (90%)	20 (10%)
250 ppb	166 (81%)	38 (19%)
Avg. ± St. Dev	87% ± 5%	13% ± 5%

Table 3: Results of t-test for statistical significance between results obtained using the AutoMate Q40 platform and a manual technique for cabbage, orange, raisin and purple corn flour at three spike levels.

DISCUSSION

The performance of the AutoMate Q40 in comparison to manual sample preparation for LOD, LOQ, linearity (R²) and MDL were very comparable (data not shown) where LOD, LOQ and MDL values were less than or equal to 0.1 ppb for most analytes. R² values were greater than or equal to 0.998 for most analytes for both systems. Percent recovery ranged from 70% to 110% for cabbage and valencia orange (AutoMate) and were ~10% greater at all spike levels for manual preparation. For purple corn flour and raisin data, no obvious differences are apparent although recoveries range 80% to 120%. Percent relative standard deviation for all samples was less than or equal to 20% except raisin (AutoMate) at 250 ppb spike which extended to 40%. Results of the t-test show that 80-90% of analytes perform similarly when using the AutoMate versus a manual preparation. Evaluation of the data deemed to be significantly different did not reveal any trends.

SUMMARY AND CONCLUSION

Four matrices including cabbage, orange, raisin and purple wheat flour were evaluated using a mixture of 204 pesticides at three spike levels of 10, 50 and 250 ppb. Samples prepared using the AutoMate Q40 were diluted, filtered and analyzed using LC-MS/MS with manually prepared solvent-only and matrix-matched calibration standards whereas all other samples were prepared manually by a novice analyst. Percent recoveries and relative error were determined for all samples from the AutoMate and manual preparation. Similarities or differences in the data were determined using the t-statistic. Overall, the performance of the AutoMate Q40 is comparable to that of a manual QuEChERS preparation.

ACKNOWLEDGEMENTS