Determination of Patulin in Apple Juice and Apple-Derived Products Using a Robotic Sample Preparation System and LC-APCI-MS/MS

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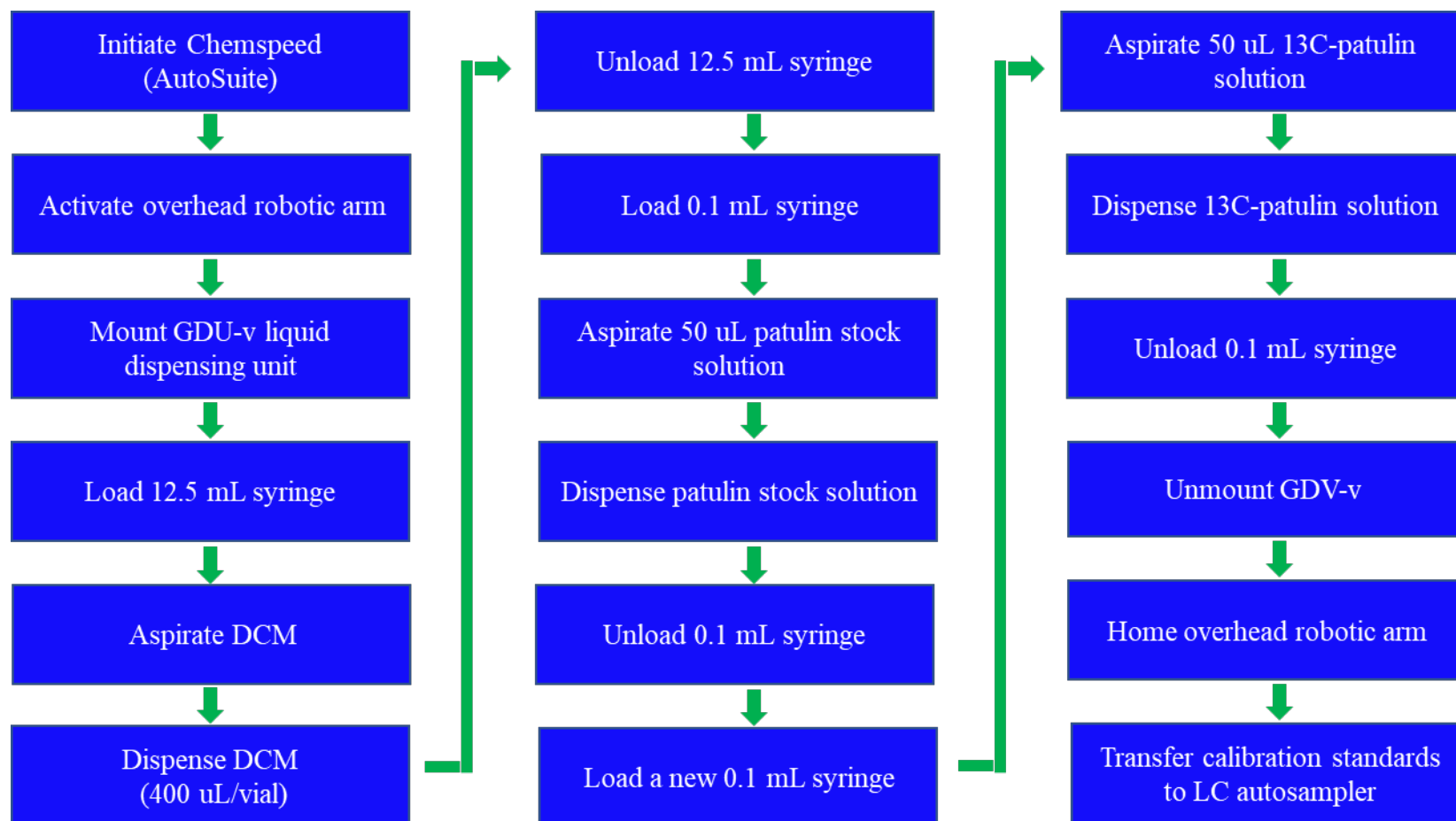
Figure S1. Chemspeed workflow for calibration standard preparation.

Initiate Chemspeed (AutoSuite) → Activate overhead robotic arm → Mount ScrewCapper → Open 15 mL centrifuge vials → Unmount ScrewCapper → Mount GDU-v → Load 12.5 mL syringe → Aspirate and dispense apple juice (1.0 g/vial) → Unload 12.5 mL syringe →

Load 0.1 mL syringe → Aspirate and dispense 13C-patulin solution (50 uL/vial) → Unload 0.1 mL syringe → Unmount GDU-v → Mount 4-channel liquid handling unit → Aspirate and dispense DCM (2 mL/vial) → Unmount 4-channel liquid handling unit → Mount ScrewCapper → Cap 15 mL centrifuge vials →

Unmount ScrewCapper → Initiate shaking plate (5min, 800 rpm) → Mount MultiGripper → Transfer 15 mL centrifuge vials to centrifuge buckets → Initiate DENSO robotic arm → Transfer centrifuge buckets to centrifuge (5 min, 4500 rpm) → Transfer centrifuge buckets out of centrifuge → Transfer 15 mL centrifuge vials back to the original rack → Open centrifuge vials using ScrewCapper →

Unmount ScrewCapper → Mount 4-channel liquid handling unit → Transfer DCM layer to 2 mL LC autosampler vial (1.0 mL) → Unmount 4-channel liquid dispensing unit → Mount ScrewCapper → Cap 15 mL centrifuge vials → Unount ScrewCapper → Home overhead robotic arm → Transfer LC autosampler vials to LC autosampler
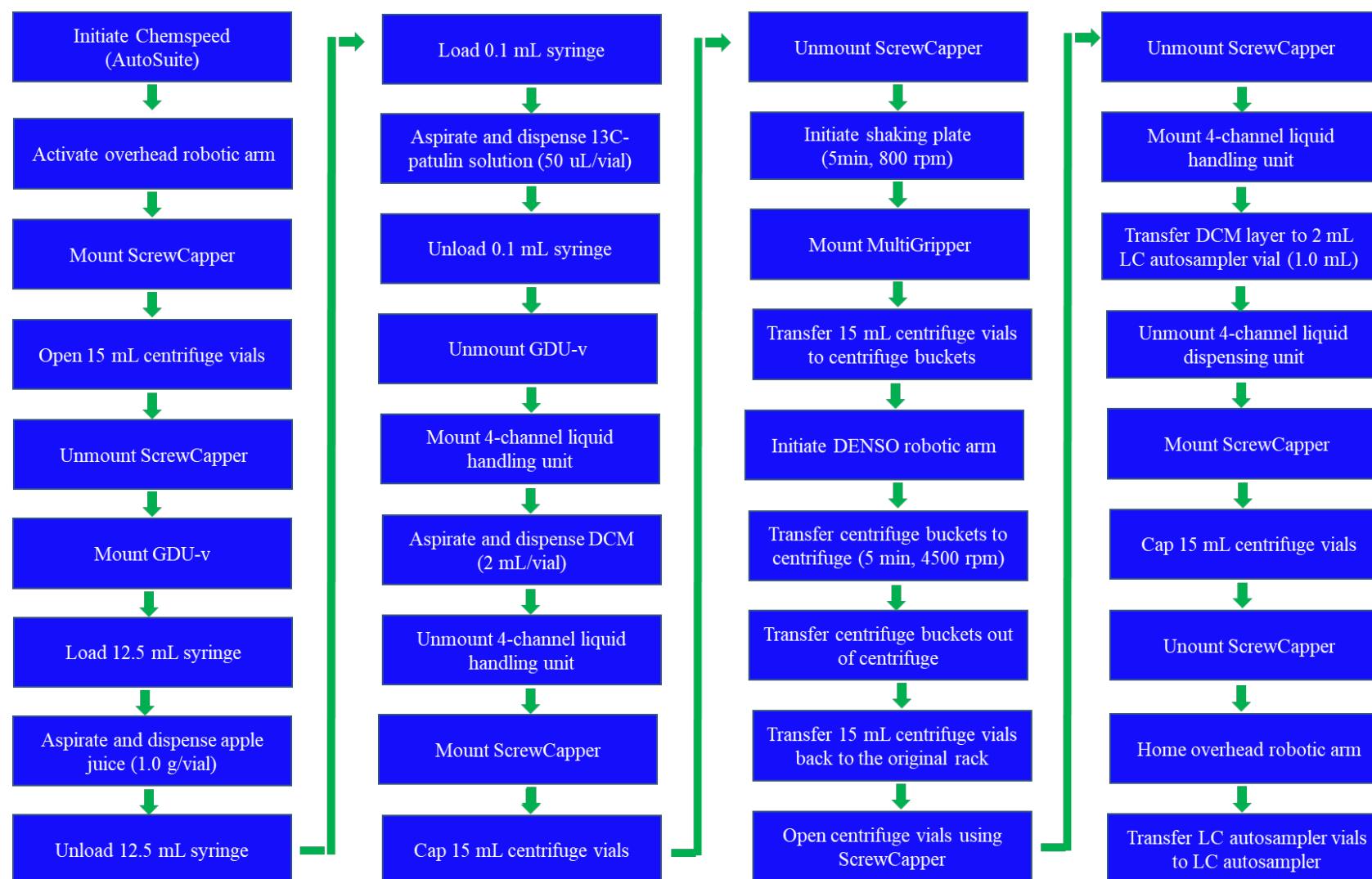
Figure S2. Chemspeed workflow for automated sample preparation.
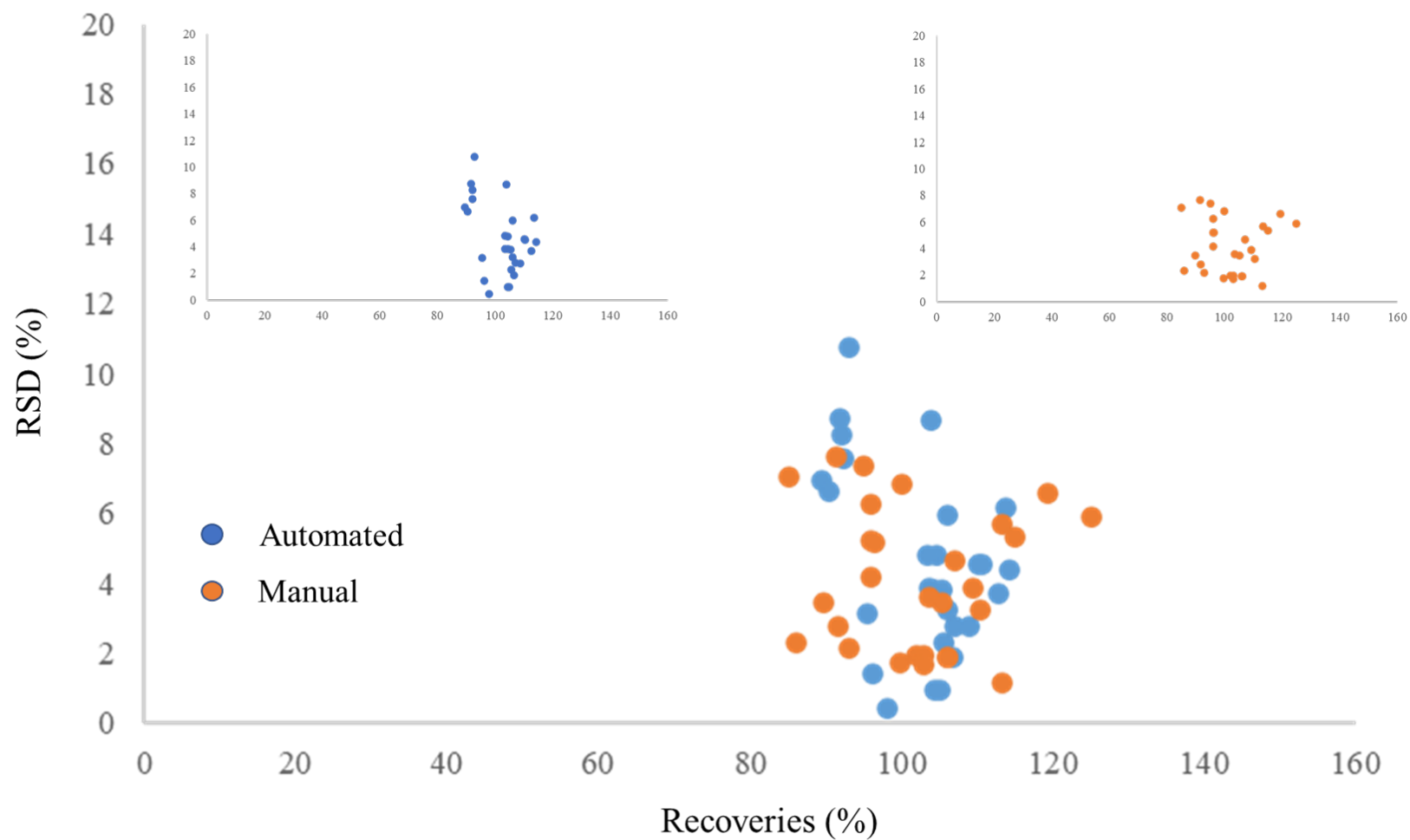
Figure S3. Overlap of recoveries and RSDs of automated and manual sample preparation.
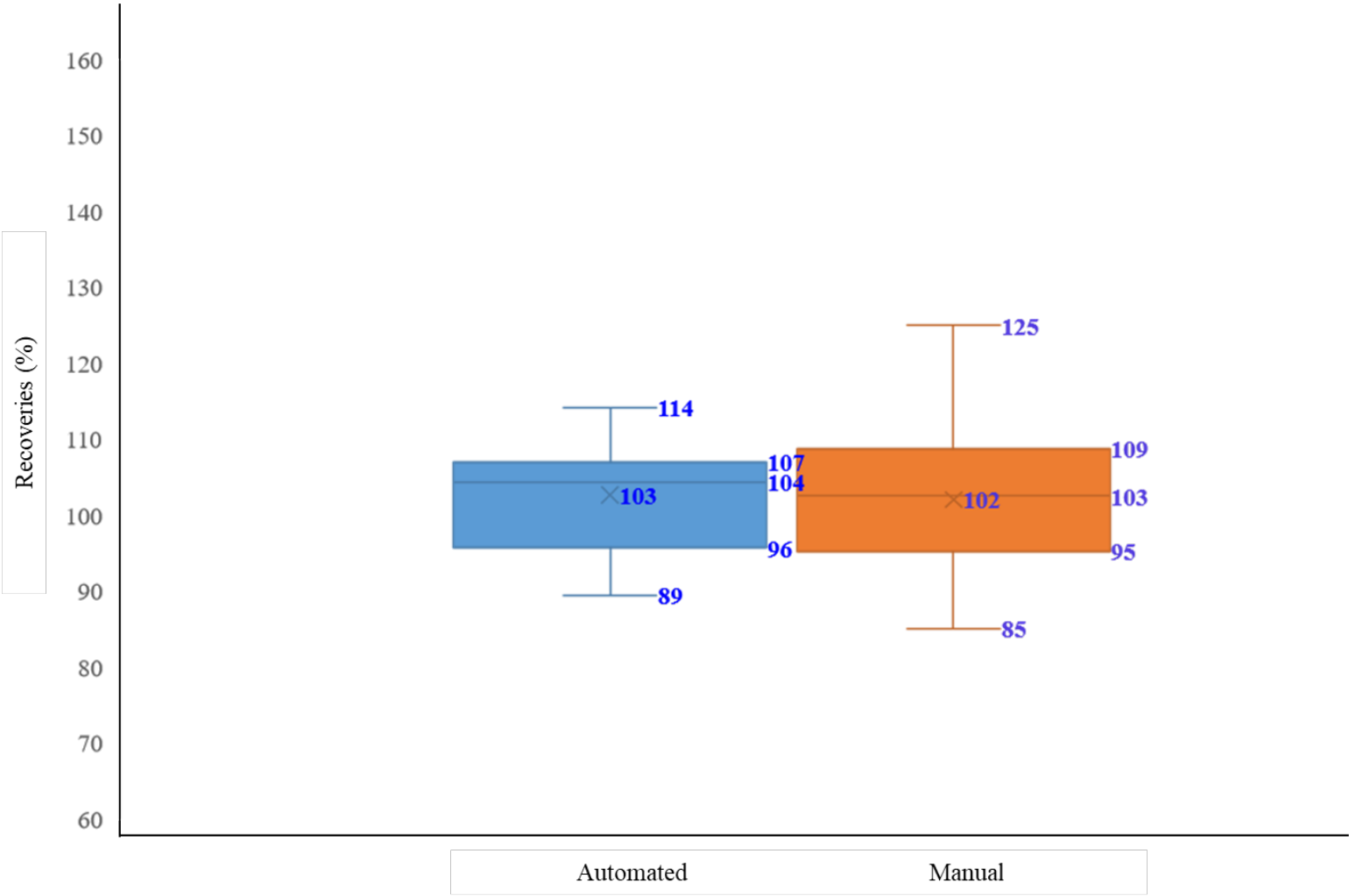
Figure S4. Boxplot of recoveries (range, mean, and lower and upper quartiles) of automated and manual sample preparation. No outliers were detected.

Table S1. Calibration standards, sample preparation, and associated statistics used for uncertainty estimation of patulin measurements in incurred samples.

Statistical components	Automated	Manual
Slope coefficient, a	0.006257	0.005724
Standard error of slope, S_a	0.000066	0.000107
Coefficient of determination, R^2	0.999211	0.997570
Intercept, b	-0.036147	-0.009426
Standard error in intercept, S_b	0.023154	0.037208
Average of calibration Conc., C_{cal}	196	196
Variance of calibration Conc., V_{cal}	747377	747377
Average of patulin/^{13}C-patulin ratio in calibration standards, Y_{cal}	1.190024	1.112320
Standard error in patulin/^{13}C-patulin ratio, S_r	0.057439	0.092301
Average of patulin/^{13}C-patulin ratio in sample, Y_{sample}	0.230336	0.172715
$Y = (Y_{sample} - Y_{cal})^2$	0.921002	0.882856
$X = a^2 V_{cal}$	29.3	24.5
Average measured patulin in the sample (μg/g, n =4), C_{sample}	21.1	14.3
Standard error of C_{sample} based on a nine-point calibration curve (μg/g), S_{sample}	2.9	5.1
Relative standard uncertainty of patulin in the sample, u_{sample}	0.14	0.36
Relative standard uncertainty of patulin CRM, u_{CRM}	0.0015	0.0015
Relative standard uncertainty of LC-APCI-MS/MS, $u_{LC-MS} = (u_{sample}^2 + u_{CRM}^2)^{0.5}$	0.14	0.36
Relative standard uncertainty of sample preparation ($u_{sample\ preparation}$)	0.13	0.18
Relative standard uncertainty of balance, $u_{gravimetaric\ calibration}$	0.001	0.000025
Relative standard uncertainty of pipetting, $u_{volumetric\ calibration}$	0.01	0.006
Relative standard uncertainty, $u = (u_{LC-MS}^2 + u_{sample\ preparation}^2 + u_{gravimetaric\ calibration}^2 + u_{volumetric\ calibration}^2)^{0.5}$	0.19	0.40
Relative expanded uncertainty (k=2), $U = u \times k$	0.38	0.80
Patulin concentration with expanded uncertainty in the sample (ng/g, k=2)	21.1 ± 8.0	14.3 ± 11.4

Supplemental Information

Metrological traceability of patulin measurements were established using a CRM and calibration data.

A bottom-up approach was used to identify key uncertainty sources. Standard (u) and expanded (U) uncertainty (k=2, 95% level of confidence) of the two patulin measurements were calculated using the relative standard uncertainty from major contributing factors, including an LC-MS instrument, sample preparation, the CRM, and gravimetric and volumetric apparatuses used for the analysis (JCGM 100:2008).

$$u = \left(\sum_{i=1}^{n} u_i^2\right)^{1/2} = \left(u_{\text{instrument}}^2 + u_{\text{sample preparation}}^2 + u_{\text{gravimetric}}^2 + u_{\text{volumetric}}^2\right)^{1/2}$$

$$u_{\text{instrument}} = \left(u^2{}_{\text{samp}} + u^2{}_{\text{CRM}}\right)^{1/2} = 0.14$$

$$u_{\text{sample preparation}} = 0.13$$

The relative standard uncertainty of the method was calculated using pooled uncertainty data from the between-matrix variabilities at 10, 50, 200, and 1000 ppb (Table 3).

$$u_{\text{gravimetric}} = 0.001$$

The relative standard uncertainty of the analytical balance of GDU-v was estimated using the readability, 1 mg. The relative uncertainty for a 1,000 mg test portion is 0.001.

$$u_{\text{volumetric}} = 0.01$$

The relative standard uncertainty (1 μL) of the 100 μL pipettes was obtained from the calibration report. This yields a relative standard uncertainty of 0.01.

$$u = \left(u_{\text{instrument}}^2 + u_{\text{sample preparation}}^2 + u_{\text{gravimetric}}^2 + u_{\text{volumetric}}^2\right)^{1/2} = \left(0.13^2 + 0.14^2 + 0.001^2 + 0.01^2\right)^{1/2} = 0.19$$

$$U = k \times u \ (k = 2)$$

$$U = 2 \times 0.19 = 0.38 \ (k=2)$$