AUTOMATE STANDARD PREPARATIONS FOR FOOD ANALYSES – A REAL-WORLD EVALUATION

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INTRODUCTION

- **Sample preparation is often time consuming and tedious but is a critical step in many chemical analyses.**
- **Any error in sample prep will affect the final results and is costly.**
- **Automation of sample preparation can reduce human error and improve the accuracy and precision of the analyses.**
- **Automation of the entire sample preparation workflow is challenging. So, we focus on the most common operation, which is the dilution and mixing. This operation is needed in preparation of standard or sample solutions from stock solutions.**
- **The scope of this work is to evaluate the Andrew+ robot and OneLab software for the standard and sample solution preparations in diverse food testing assays.**

Figure 1. *Andrew+ Pipetting Robot together with OneLab cloud-native software.*

METHOD

Evaluation strategy

The strategy of the evaluation was to test with a wide range of assays for different analytes, involving different techniques and solvents. The analytes included salt or ion (sodium chloride), vitamins (retinol, vitamin D, folic acids), sugar (galactose), amino acids, and another nutrient (carnitine and choline). The analytical techniques that were involved included chromatography based techniques, such as ion chromatography-conductivity detection (IC-CD), liquid chromatography-fluorescence detection (LC-FLR), liquid chromatography-ultraviolet/visible spectrometry (LC-UV/Vis), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and non-chromatography based techniques, such as electrochemical detection and microbiological-turbidity detection. The solvents included those common to food analytical labs, such as water, methanol, acetonitrile, and hexane. Hexane is a volatile solvent and is difficult to pipette.

AUTOMATION PROTOCOL EXAMPLES

Example 1: Carnitine by LC-MS/MS with Andrew+

Analysis technique: LC-MS/MS
Diluent: Acetonitrile/water mixture
Manual dilution: 1 to 10 mL manual dilution in a test tube
Robot dilution: vials and mixing: Transferring 150 µL sample solutions and 1350 µL diluent (ACN/water) into 2 mL

Dominos and pipettes:
Tip Insertion System Domino (p/n 186009612)
5mL Tip Rack Holder Domino (p/n 186009599)
2mL HPLC Vial Rack Domino (p/n 186010091)
Microtube Domino (p/n 186009601)
50mL Conical Centrifuge Tube Domino (p/n 186009614)
Andrew Alliance single-channel pipette 10-300 µL (p/n 186009606)
Andrew Alliance single-channel pipette 100-5000 µL (p/n 186009608)

Figure 2. *Andrew+ deck configuration for the "Carnitine by LC-MS/MS" protocol. Domino positions on the Andrew+ working deck: [1] Tip Insertion System Domino, [2] 5mL Tip Rack Holder Domino, [3] 2mL HPLC Vial Rack Domino, [4] Microtube Domino, [5] 50mL Conical Centrifuge Tube Domino.*

Example 2: Standard solution preparation—Vitamin A by LC-FLR with Andrew+

Analysis technique: LC-FLR
Diluent: Hexane
Manual dilution: Serial dilution of 1 to 10 mL of standard solutions with diluent (hexane) in 50 mL centrifuge tubes and mixing
Robot dilution: Transferring 1 to 10 mL standard solutions and diluent (hexane) into 50 mL centrifuge tubes and mixing

Dominos and pipettes:
Tip Insertion System Domino (p/n 186009612)
10mL Tip Rack Holder Domino (p/n 186010098)
50mL Conical Centrifuge Tube Domino (p/n 186009614)
Andrew Alliance single-channel pipette 50-1000 µL (p/n 186009766)
Andrew Alliance single-channel pipette 500-10000 µL (p/n 186009767)

RESULTS

1) Standard solution preparation

The evaluation was carried out in two main steps. The first step was to evaluate the performance of the Andrew+ robot in standard solution preparation only. The detector response ratios from the robot prepared standard solutions were compared against the standard operation procedure (SOP) dilution ratios to assess the accuracy in serial dilution and mixing. The results were summarized in Table 1.

Table 1. *Accuracy of automated serial dilution and mixing of standard solutions in different assays*

	Analyte	Technique	Diluent	Operations	Total dilution ratio	Accuracy (dilution ratio)	
						Andrew+	Manual
1	Sodium	IC-CD	Water	Serial dilution: 5 mL standard solutions mix with 5 ml water and mixing	1:64	-0.5% to 3.0%	N/A
2	Retinol	LC-FLR	Hexane	Serial dilution of 1 - 10 mL of standard solutions with hexane and mixing	1:250	-0.5% to 2.3%	-3.8% to 0%
3	Vitamin D_3	LC-UV/Vis	Methanol	Serial dilution of 0.4 - 10 mL of various standard with diluent and mixing	1:250	0.2% to 1.7%	-0.3% to 3.5%
4	Galactose	IC-ED	water/methanol	Serial dilution of 1 - 3 ml of standard solutions with diluent and mixing	1:500	-0.63% to 0.65%	N/A

- The accuracy by robot operation ranged from -2.8% to 3.0%, as compared to -5.0% to 4.2% by human.

2) Andrew+ in food analyses

The second step was to evaluate the robot in real-world food analyses. The Andrew+ robot was implemented in the sample preparation procedure, i.e., the standard solution serial dilution and the sample solution final dilution. The food analysis results using robot prepared standard and sample solutions were compared with those from manual preparation. The results were summarized in Table 2.

Figure 3. Andrew+ deck configuration for the "Standard Solution Preparation - Vitamin A by LC -FLR" protocol. Domino positions on the Andrew+ working deck: [1] Tip Insertion System Domino; [2] 10mL Tip Rack Holder Domino, [3] 50mL Conical Centrifuge Tube Domino.

RESULTS

Table 2. *Relative difference in food analysis results between robot and human operations in standard solution preparation.*

	Analyte	Technique	Diluent	Operations	Total dilution ratio	Number of samples	Difference % (compared to manual results)
5	Folic acid	Microbiological-turbidity	Water	Serial dilution of 0.4 - 10 ml of standard solutions with diluent and mixing	1:125	5	-3.8% to 5.7%
6	Cysteine and methionine	LC-FLR	Water	Single dilution from 10 µl to 500 µl and mixing	1:50	3	Cysteine: -0.5% to 1.5%; Methionine: -0.7% to 2.3%
7	Amino acids	LC-UV/Vis	Water	Single dilution from 100 µl to 500 µl and mixing	1:5	4	Within -1.2% to 2.5% for 14 amino acids in 4 samples
8	Carnitine	LC-MS/MS	ACN/Water	Single dilution from 150 µl to 1500 µl and mixing	1:10	4	Within -1.1% to 0.4% for 3 samples and 6.7% in one sample.

- The robot operation results were comparable to the human operation results. There is no bias introduced in the food analysis by implementing Andrew+ in the sample preparation procedure.

3) Precision in sample preparation with Andrew+

Table 3. *Precision of automated dilution and mixing of sample solutions in water (n=8).*

Analyte	Technique	Operations*	Precision (RSD)
Choline	IC-CD	10 µL sample mixed with 490 µL water	2.00%
		250 µL sample mixed with 250 µL water	0.30%
		100 µL sample mixed with 4900 µL water	0.70%
		2500 µL sample mixed with 2500 µL water	0.60%

CONCLUSION

After our extensive evaluation of Andrew+ and OneLab platform in our routine food analysis lab, we found its performance meets the requirements in accuracy and precision in sample preparation. It can improve the analytical productivity, lab safety, traceability, and reduce human error. It is easy to use and can be incorporated as part of the sample preparation for a wide range of analyses.

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