Automation of AOAC 970.16 Bitterness of Malt Beverages and AOAC 976.08 Color of Beer through Unique Software Control of Common Laboratory Instruments with Real-Time Decision Making and Analysis

Application Note FB0212

Keywords

Gilson GX-271 ASPEC™ with 406 Single Syringe Pump, TRILUTION® LH v3.0 Liquid Handling Software, Agilent 8453 UV-Visible Spectrophotometer with Multicell Transport, Torrey Pines Scientific EchoTherm™ SC20XT.

Introduction

A typical laboratory is frequently looking for ways to increase consistency and throughput. A common way to achieve this is through finding an automated solution for manual techniques. One of the largest hurdles for automation of manual methodology, no matter how simple, is the necessity of decision making based on real-time sample analysis, which typically requires the coordination of input from multiple components with varying manufacturers. In this study, a unique, single software control of a liquid handler, spectrophotometer equipped with multiple flow cells and shaker block with temperature control capabilities brings each component together into a single automated solution from multiple manual AOAC methods.

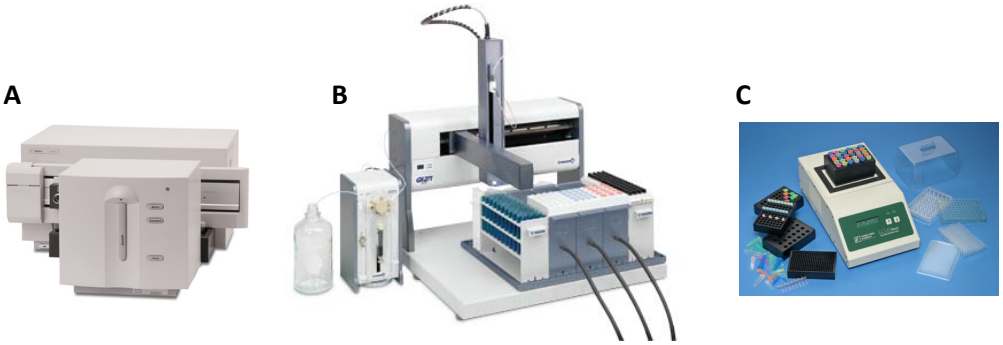

Figure 1. A) Agilent 8453 UV-Visible Spectrophotometer with Multicell Transport, **B)** Gilson GX-271 ASPEC™ with 406 Single Syringe Pump, **C)** Torrey Pines Scientific EchoTherm™ SC20XT.

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Materials & Methods

The focus of this experiment was to utilize unique software control of common laboratory equipment to allow for real-time protocol alteration by automated equipment. Through the use of conditional logic based upon inputs from a spectrophotometer and shaker block, TRILUTION® LH v3.0 Software was able to alter the course of the protocol to accommodate necessary changes. An example of the conditional logic used to determine if filtering of the beer sample was necessary during AOAC method 976.08 is displayed below in Figure 2. Figure 3 demonstrates the software protocol used to ensure proper sample temperature for AOAC method 970.16. In each of the protocols, the IF steps (orange tasks) are used to direct future operation.

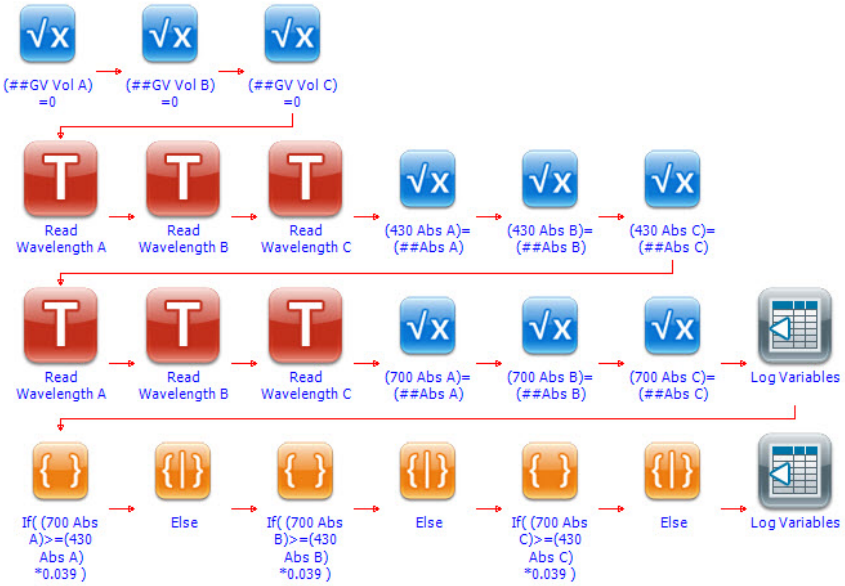

Figure 2. TRILUTION LH v3.0 Protocol for Conditional Logic in Beer Filtration.

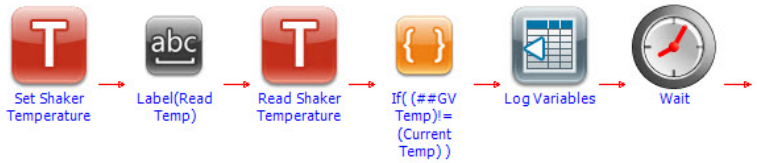

Figure 3. TRILUTION® LH v3.0 Protocol for Conditional Logic in Temperature Equilibration.

AOAC method 976.08 for Color of Beer results are derived from a simple spectrophotometer reading at two wavelengths after degassing. Based on the absorbance readings, the sample may require filtration and the readings repeated. The study showcases online absorbance readings for the software to react in real-time and use conditional logic to perform the automated rework of the sample when necessary.

AOAC method 970.16 for Bitterness of Malt Beverages requires temperature controlled samples to undergo liquid liquid extraction (LLE) prior to absorbance readings. Control of the chilled shaker block allows for a record of sample temperature at run time and shaking of the sample when required. Online transfer to the spectrophotometer records absorbance values, taking sample readings immediately and without the need for physical intervention.

Samples and solvents

- Beers supplied by microbrewery (Figure 4)
- Isopropanol (B&J, P/N 10071758)
- Hydrochloric Acid (Sigma, P/N 258148)
- 2,2,4-Trimethylpentane (isooctane)(Sigma, P/N 155012)
- 1-Octanol (Sigma, P/N 293245)
- NanoPure Water

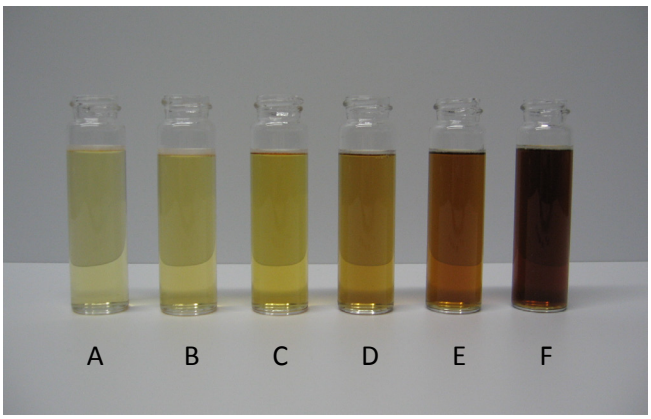

Figure 4. Beer Samples Used for the Study; A: American Pale Lager, B: Cream Ale, C: American Pale Ale, D: German Pilsner, E: English Brown Ale, F: Bock.

Apparatus

- Gilson GX-271 ASPEC™ with 406 Single Syringe Pump
  - 10 mL syringe
  - GX Transfer Port Assembly (Special 1785)
  - Special 1891 for on bed placement of SC20XT
- Agilent 8453 UV-visible Spectrophotometer
  - Multicell Transport (8-cell)
  - (8) Flow Cell (10 mm, 430 µL)
- Torrey Pines Scientific EchoTherm™ SC20XT Heater, Chiller, and Orbital Shaker with Aluminum block for (6) 50 mL centrifuge tubes
- Phenomenex Single-fritted Empty 6 cc SPE Cartridge (P/N AH0-8942)
- Phenomenex 0.45 µm 13 mm Nylon Filter (P/N AF0-0500)

Protocols

- AOAC Method 976.08 Color of Beer:
  - Room temperature beer was opened and transferred to a 50 mL centrifuge tube
  - The beer was shaken on the SC20XT shaker unit for 90 minutes to degas
  - 3 aliquots of beer were transferred via the GX-271 ASPEC™ and transfer ports to flow cells on the 8453
  - UV-Visible spectrophotometer
  - Readings were taken at 430 nm and 700 nm
  - If $A_{700\ nm} \geq A_{430\ nm} * 0.039$, the sample was deemed turbid and the GX-271 ASPEC transferred the beer sample to the filter cartridges
  - If $A_{700\ nm} < A_{430\ nm} * 0.039$, the sample was deemed free of turbidity and the SRM was calculated
- AOAC Method 970.16 Bitterness of Beer:
  - 10 mL of beer was manually transferred to a 50 mL centrifuge tube and placed on the SC20XT shaker unit
  - The SC20XT was set to 10° C and checked every 30 seconds until the temperature was met; sample equilibrated 5 minutes
  - 25 µL 1-octanol was transferred via the GX-271 ASPEC to the beer sample, and the sample was shaken slightly to combine
  - 1 mL of 3 M hydrochloric acid and 20 mL of isooctane were transferred via the GX-271 ASPEC to the beer sample.
  - N_2 gas was bubbled through the beer: isooctane mixture via the 406 Single Syringe Pump to perform the liquid-liquid extraction.
  - The isooctane layer was manually transferred to small vials
  - The isooctane was transferred via the GX-271 ASPEC and transfer ports to flow cells on the 8453 UV-Visible spectrophotometer
  - A reading was taken at 275 nm and the BU value was calculated

Results

TRILUTION® LH v3.0 Software was successfully able to incorporate and control multiple laboratory devices and react in real-time to inputs from the devices to modify experimental protocols. Detailed explanation of the protocols and logic can be found in Figures 5 and 6.

Microbrewery beer sample test results in Table 1 show some inconsistencies with the values provided by the brewery; however, they show the same trends that were anticipated.

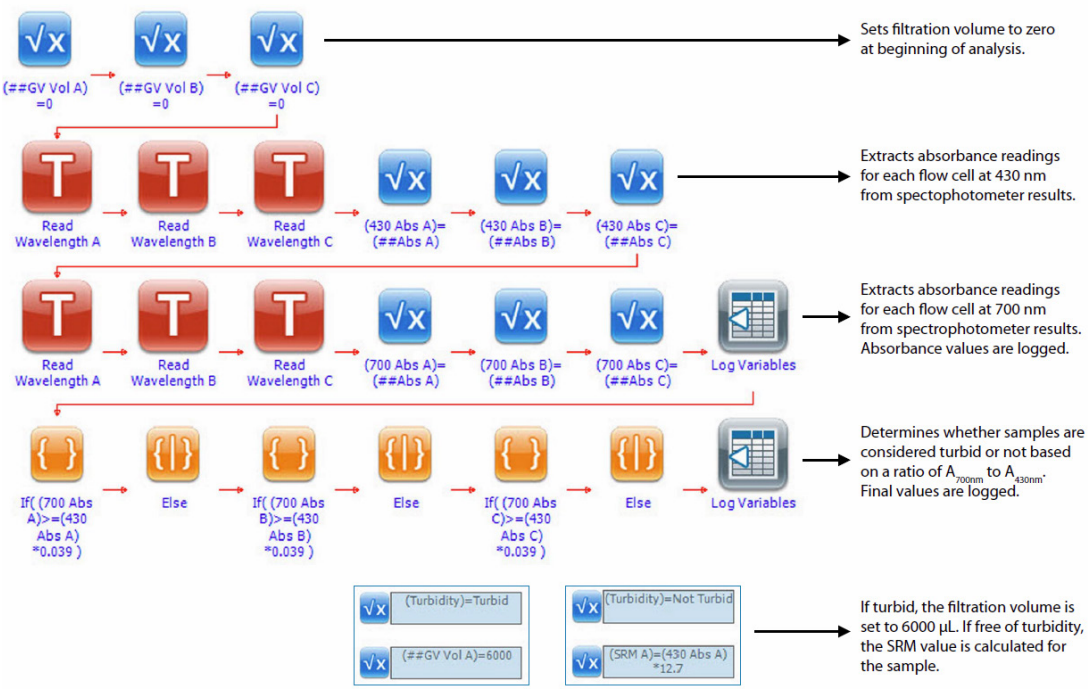

Figure 5. TRILUTION LH v3.0 Protocol Detail for Conditional Logic in Beer Filtration.

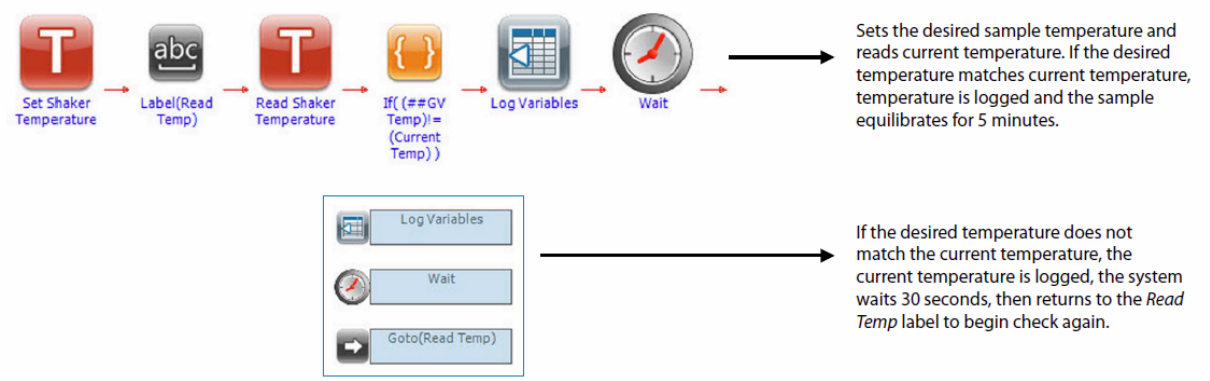

Figure 6. TRILUTION® LH v3.0 Protocol Detail for Conditional Logic in Temperature Equilibration.

Table 1. Beer Characteristics Provided and Testing Results.

Beer	Style	Provided Color (converted from EBC to SRM)	Tested Color (SRM)*	Provided BU**	Tested BU
A	American Pale Lager	2.4	3.8	7	12.5
B	Cream Ale	4.7	5.6	11	14
C	American Pale Ale	5.8	8.1	35.5	23.5
D	German Pilsner	12.1	10.4	20	18
E	English Brown Ale	24.5	21.6	20	18.5
F	Bock	59.6	48.9	19.5	13

* Note: None of the microbrew beers passed the turbidity requirement, even after filtration. A macrobrew beer was also tested for method verification and did pass the turbidity requirement. ** Note: Beer was tested 6 months after provided numbers were given; potential degradation of bitterness compounds is a possible explanation for the result inconsistency.

Summary

Automation of AOAC methods 970.16 and 976.08 using conditional logic to modify experimental protocols in real time was successful. Further method and procedural optimization can be done to improve result correspondence.