

Characterization of four saturated fatty acids using gradient HPLC-CAD highlighting optimized evaporation temperature control features

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Abstract

Purpose: Investigating the effects of improved evaporation temperature control features of the Thermo Scientific™ Charged Aerosol Detector P series, such as the Temperature Coupling Mode, on the analysis of four common saturated fatty acids (FAs). This highlights the HPLC-CAD application robustness including improved response for semi-volatile analytes.

Methods: In this work, a gradient LC method [1] for the determination of four FAs was used to evaluate the improved temperature control design and Temperature Coupling Mode feature of the Thermo Scientific™ Vanquish™ Detector CAD HP. The signal-to-noise (S/N) stability over a 24-hour period was studied in addition to the uniformity of FA signal response over the gradient. Finally, a recommended EvapT to maximize S/N is presented.

Results: It was demonstrated that improved evaporation temperature control alongside the Temperature Coupling Mode provided enhancements to the analysis of the four saturated FAs. An evaporation tube temperature of 25.0° C resulted in the best S/N ratio for the four FAs. Setting the Temperature Coupling Mode to 'On' further improved the overall S/N ratio at the optimum 25° C evaporation temperature. The temperature control of the CDM via the Temperature Coupling Mode had a positive effect on the response, especially for semi-volatile species when activated. Additionally, experiments showed robust application performance over a 24-hour period.

Introduction

Polysorbates (PS) are non-ionic surfactants that are used in pharmaceutical and biopharmaceutical formulations as emulsifiers and solubilizers for poorly soluble drug substances. They are a crucial component of protein formulations because they prevent protein unfolding and aggregation and help maintain the protein in its active form.[2]

Commercially available PS are complex heterogeneous mixtures, varying in composition across suppliers and even between lots from the same manufacturer. During the manufacturing process PS can be contaminated with impurities such as FAs. [3] Moreover, during storage or transit, PS in formulations can degrade also forming FAs. This is a major problem as FAs can form insoluble particles that can seriously compromise product quality, efficacy, and patient safety.[4] Thus, the ability to measure FA in both raw materials and formulations is of the upmost importance.

FAs do not contain a suitable chromophore so cannot be detected without derivatization by traditional liquid chromatographic methods such as HPLC-UV. Rather, researchers now use HPLC with charged aerosol detection for routine measurement of FAs (Figure 1). [1] The charged aerosol detector (CAD) is sensitive, has a wide linear and dynamic range, and shows uniform response for all non-volatile and many semi-volatile compounds present in a sample, including FAs.

Charged aerosol detection involves pneumatic nebulization of the mobile phase from the analytical column to form an aerosol. Aerosol droplets enter a heated evaporation tube and are de-solvated to form particles. In the charging detection module (CDM) particles are charged, and the charge is then measured using an electrometer. The amount of charge is directly proportional to the quantity of the analyte present. The temperature of the evaporation tube (EvapT) significantly influences detector performance.

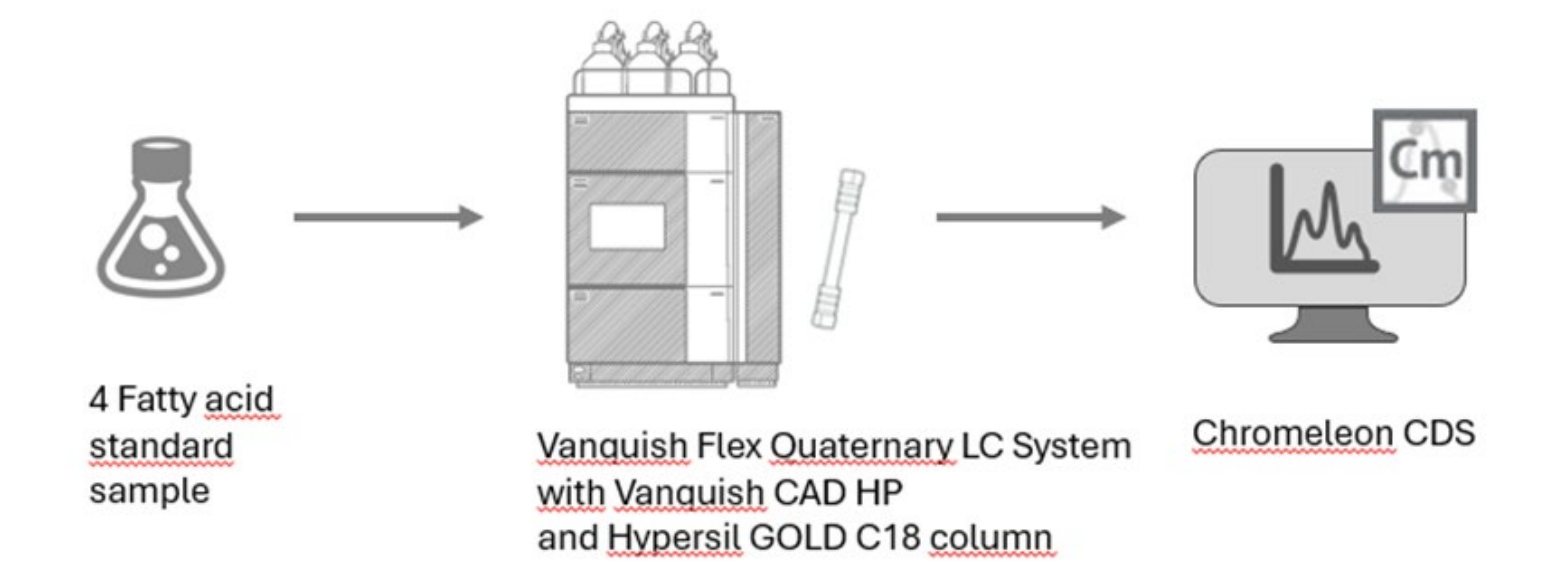


Figure 1. Schematic workflow for the analysis of four common saturated FAs.

Materials and methods

Sample Preparation

Stock solutions of the four saturated FAs were prepared as follows:

1. Prepare the diluent. The diluent consists of 15 mL acetonitrile and 5 mL water.
2. Create an individual stock of each FA (lauric acid, myristic acid, palmitic acid, stearic acid). 0.25 mg/mL of each individual FA was added in separate vials by weighing 5.0 mg FA and then adding 20 mL methanol. Sonicate for 2 minutes to dissolve.
3. Create one working solution with all four FAs at a concentration of 50 µg/mL. Pipette 200 µL of each 0.25 mg/mL FA stock solution and add 800 µL diluent.

Chromatographic Conditions

Table 1 shows the chromatographic conditions of the RP-HPLC-CAD method for the FA analysis.

Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.3.2 was used for data acquisition and processing.

Table 1. Chromatographic conditions

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™ C18 50 x 2.1 mm; 1.9 µm (P/N 25002-052130)
Solvent A	0.05% formic acid in water (v/v)
Solvent B	0.05% formic acid in acetonitrile (v/v)
Analytical	
Time (min)	%B
0	75
1	75
3	85
3.5	85
4	75
5	75
Flow rate	0.75 mL/min
Column temperature	25° C with active preheater off, forced air mode, fan speed 5
Autosampler temperature	6° C
Needle wash solution	10/90 water/ isopropanol (v/v)
Needle wash mode	Both (before and after)
Injection volume	10 µL
CAD settings	
Data collection rate: 10 Hz	
Filter setting: 5.0 s	
Power values: 1.5, 1.8	
Evaporation temperature: variable (25 – 40° C)	
Temperature Coupling Mode: On & Off	
Temperature Coupling Offset: +5.0° C	
Diverter valve position: to nebulizer	

Results

Noise vs. Evaporation Temperature

The background noise was compared at various EvapTs. It was observed that the EvapT had little effect on the noise (Figure 2).

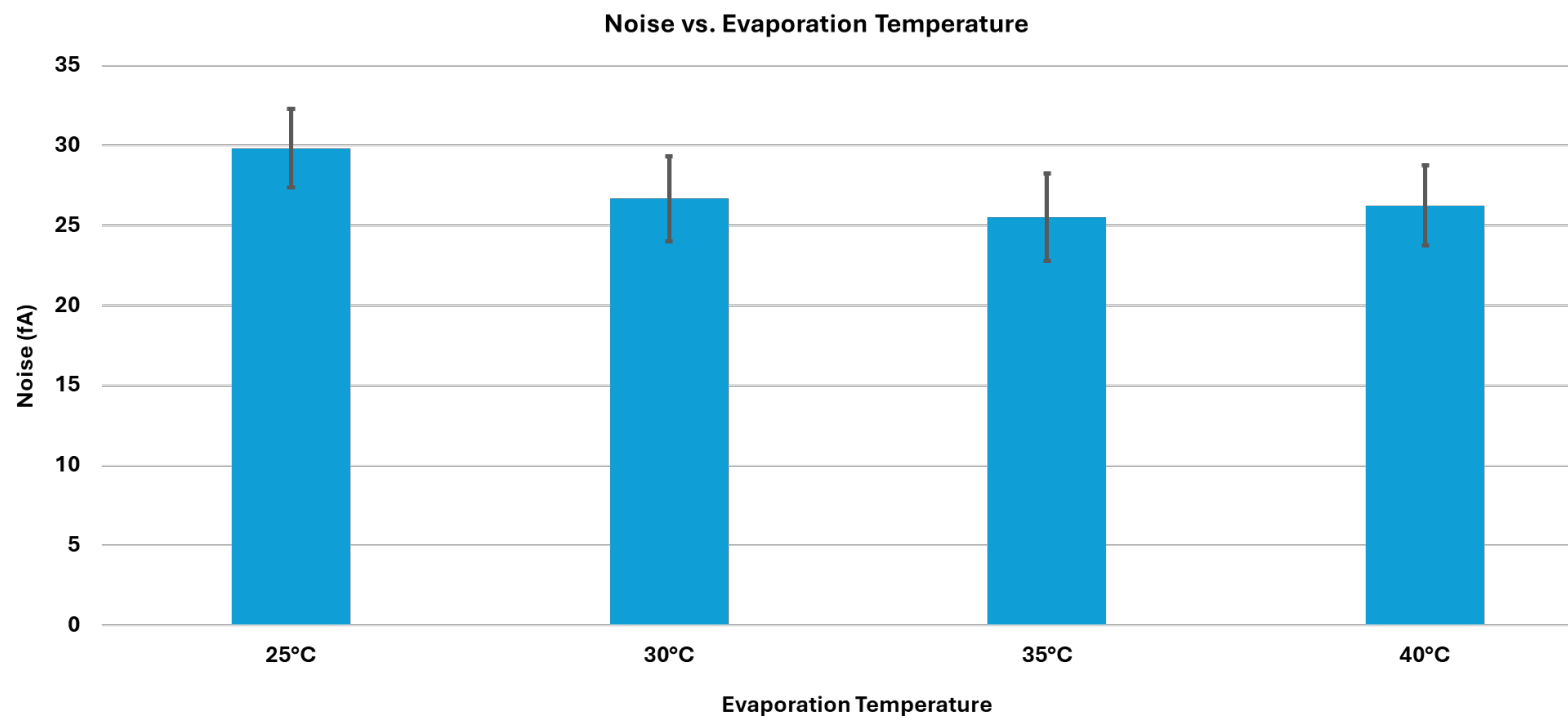


Figure 2: Changes in the evaporation temperature showed insignificant influence on the noise. The noise was calculated by averaging the noise over the time of the gradient (0.5 – 3.0 minutes) in a blank run where the SD, shown as the error bars, is derived from n=3.

S/N vs. Evaporation Temperature

As shown in Figure 3, the S/N for each of the FAs decreased as EvapT increased as analytes became more volatile. Represented FAs are lauric acid > myristic acid > palmitic acid > stearic acid in order of their volatility, where lauric acid is the most volatile. From the S/N comparison at the respective temperature for the FAs, it was determined that an evaporation temperature of 25.0° C was optimal.

This study was repeated over a 24-hour period to ensure method robustness. The highest relative standard deviation was observed as being less than 10%. Except for lauric acid, its S/N was 10x less than the other FAs and at 40° C was even close to the limit of quantitation (S/N = 10).

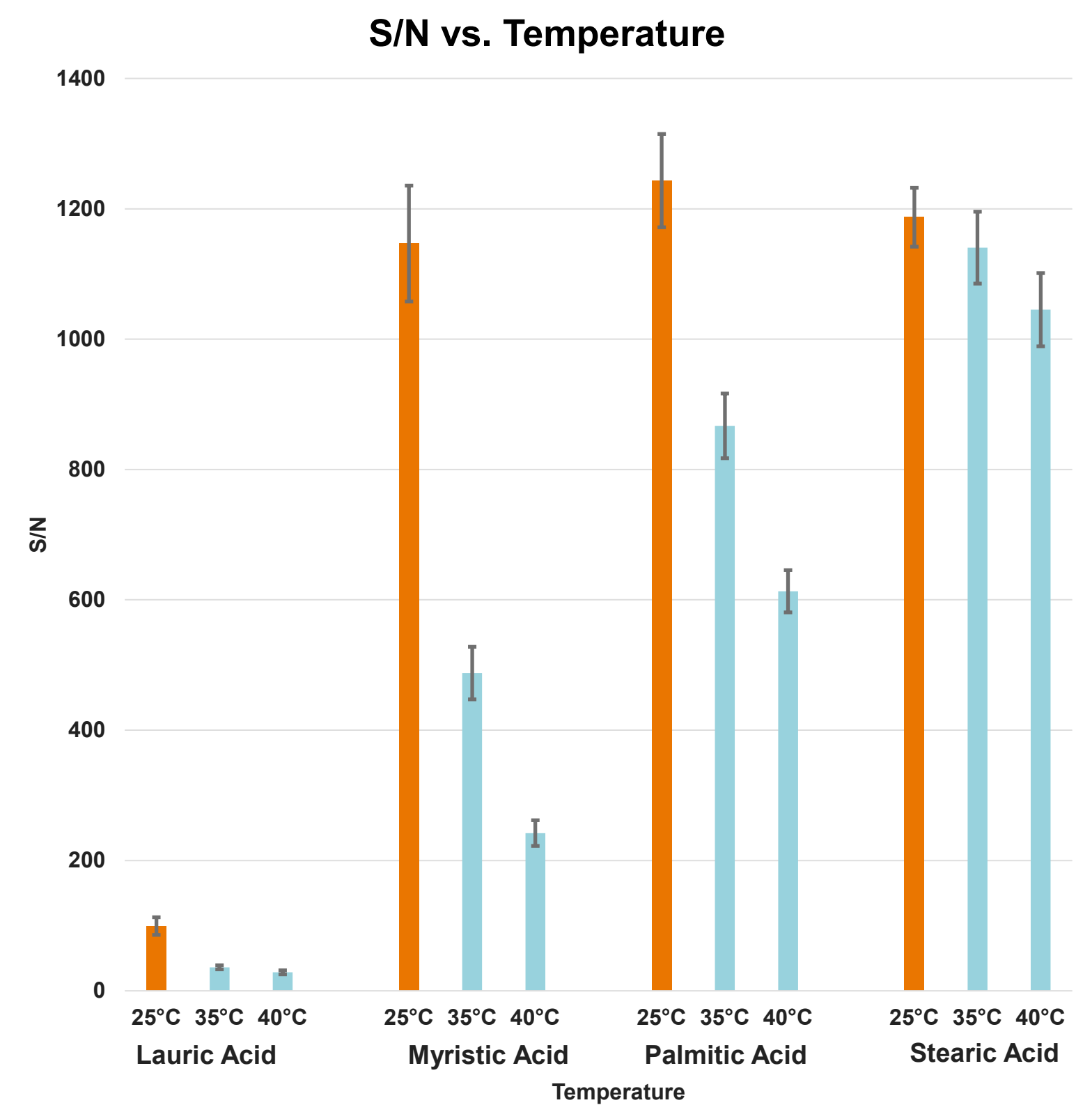
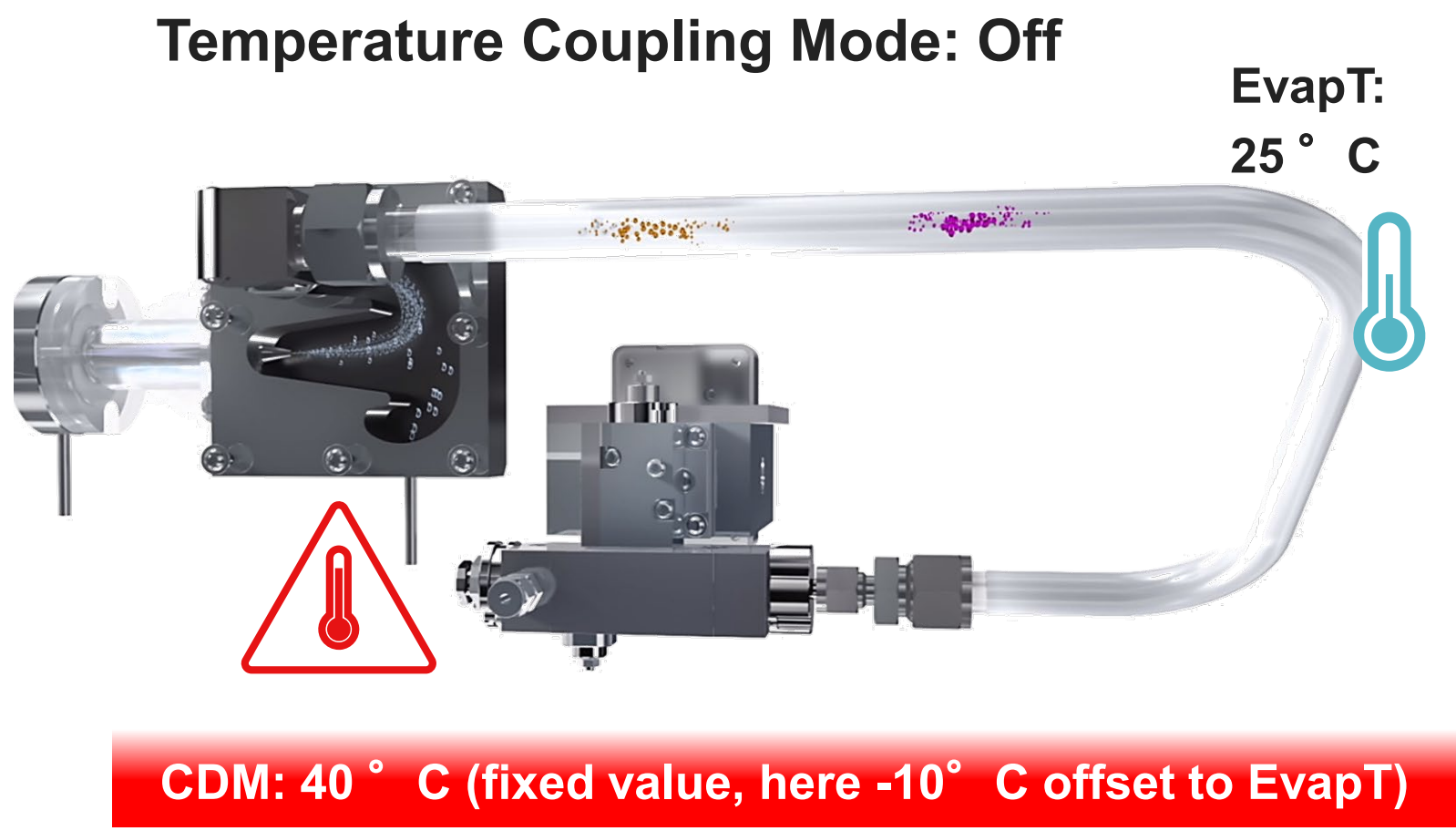


Figure 3. Effects of EvapT on the S/N for the four FAs in the working solution. The optimal EvapT of 25° C is highlighted in orange. It is representative of the average S/N over the entire 24-hour sequence (n=18). The SD is displayed via the error bars.

Temperature Coupling Mode

Case 1: semi-volatiles analyzed at EvapT = 25° C.



Case 2: nonvolatile lipids run at analyzed at EvapT = 50° C.

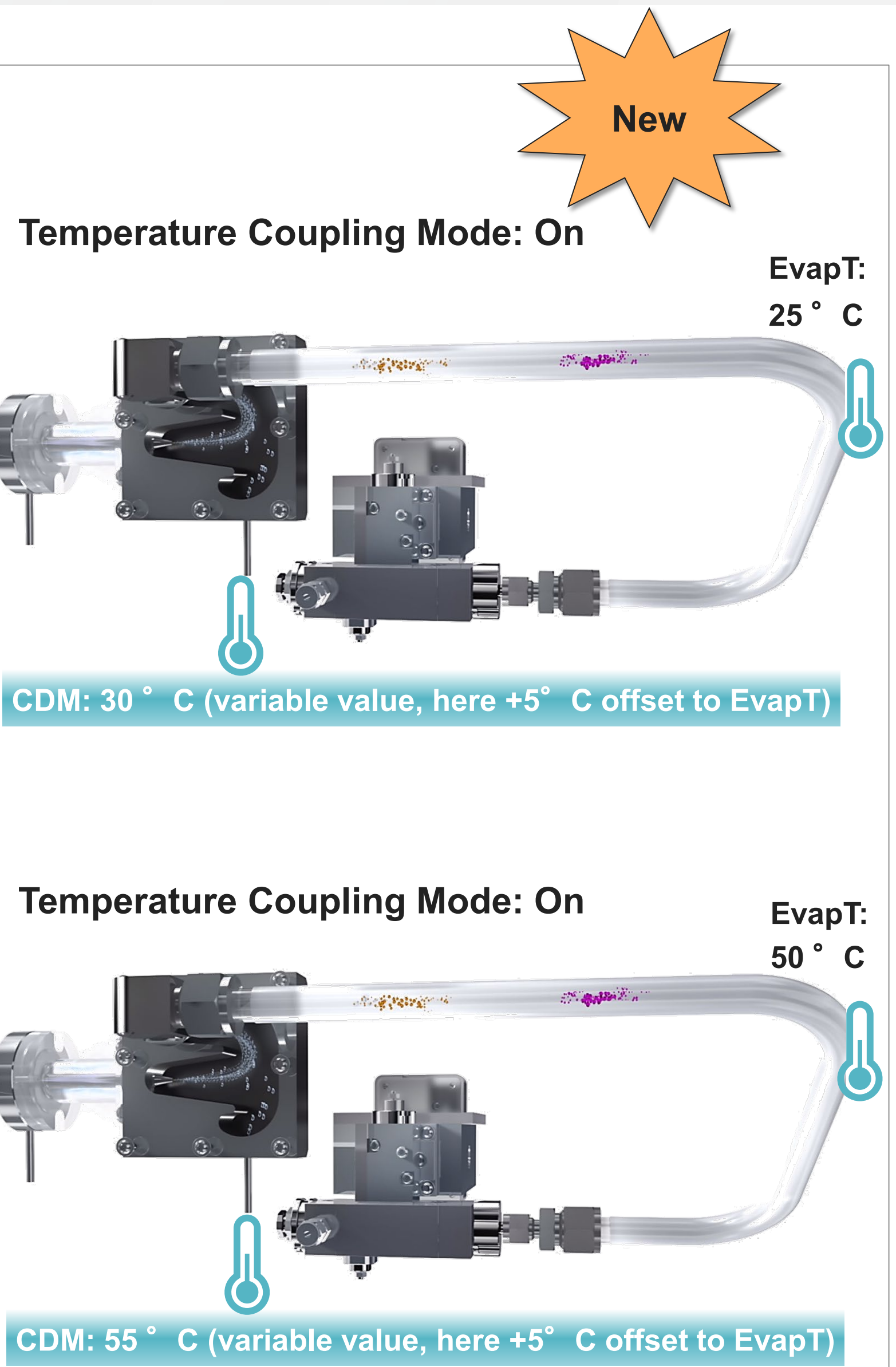
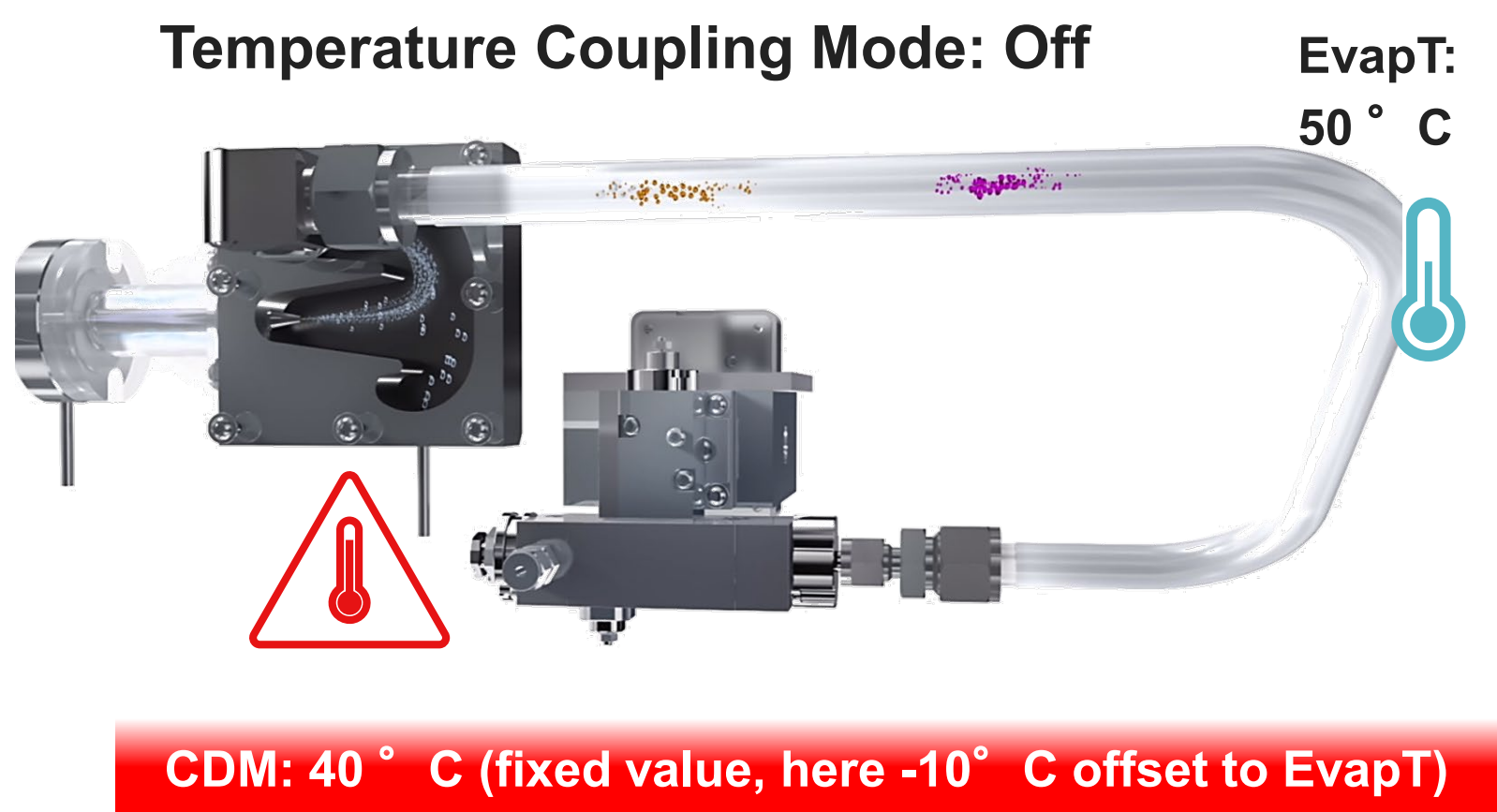


Figure 4: Relationship between EvapT, CDM temperature, and Temperature Coupling Mode in the Vanquish CAD HP showing how the measurement of semi-volatiles can be improved. In Case 1, the EvapT is set to 25° C. By Temperature Coupling Mode set to ON, additional evaporation of the semi-volatile analytes is minimized due to the lower CDM temperature at 30° C, which is beneficial for the resulting S/N ratio. In Case 2, the EvapT is set to 50° C. The ability to raise the CDM temperature to 55° C can avoid potentially producing residue from condensation of volatile substances after the CDM.

Coupling Mode On vs. Coupling Mode Off

The Vanquish CAD P series contains enhanced temperature control, for the evaporation tube and the CDM. In the Vanquish CAD HP, as in previous versions of CADs, the CDM temperature is always accurately stabilized to 40° C by default, regardless of the EvapT used. The resulting temperature difference between the EvapT and CDM can adversely affect analyte response – especially for semi volatile species requiring a low EvapT. This is now addressed in the Vanquish CAD HP through a feature called Temperature Coupling Mode (see Figure 4).

For this application, the recommended EvapT is 25.0° C (see Figure 3). FA response was then measured with the Temperature Coupling Mode on and off at the EvapT of 25.0° C (Figure 5). As presented in Table 2, responses for all FAs could be improved when the Temperature Coupling Mode is switched on. As expected and explained in Figure 4, the improvement in S/N matched the analyte's semi- volatility with lauric acid (lowest MW and the most semi-volatile) showing the greatest improvement and stearic acid (highest MW and less semi-volatile) showing the least.

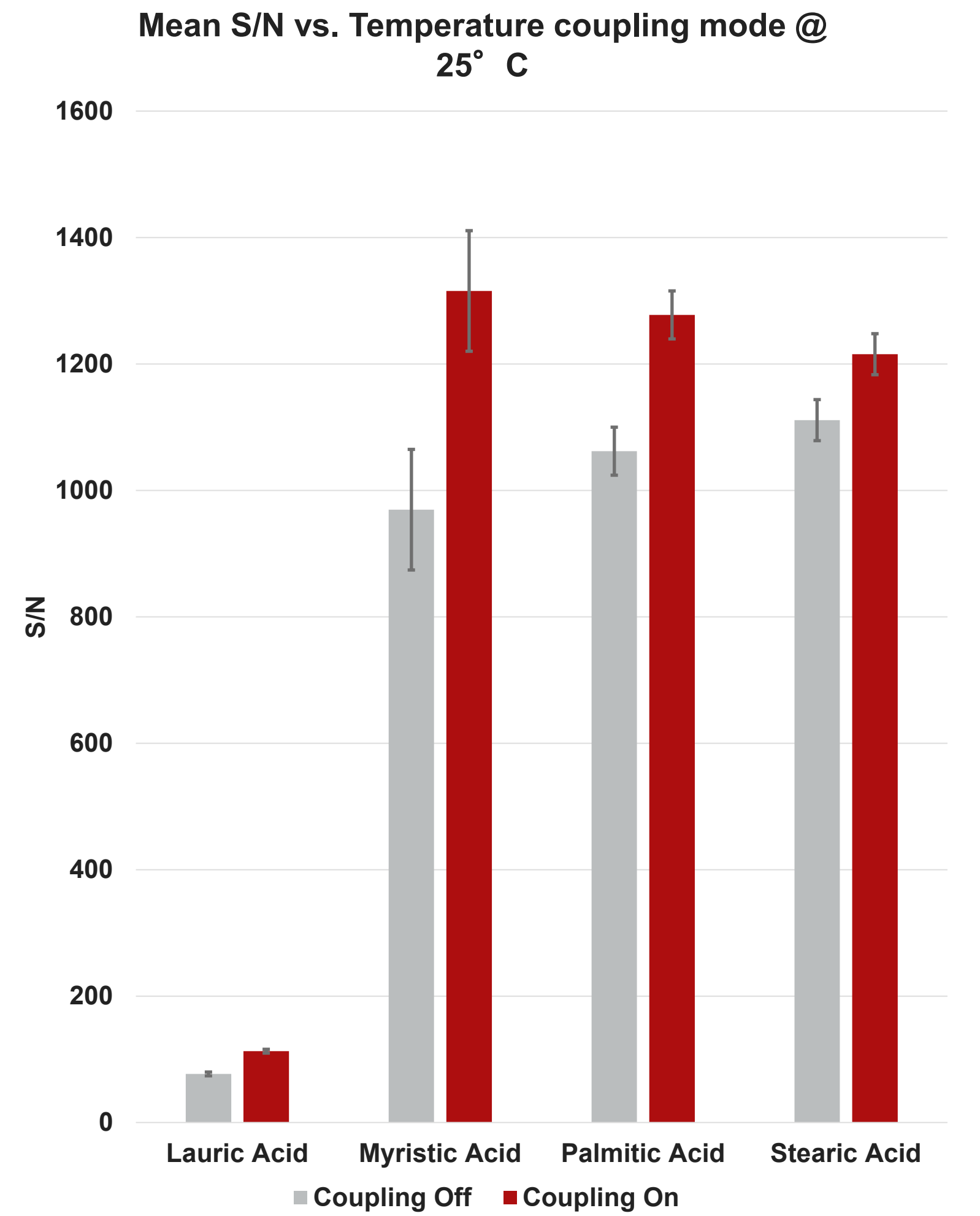


Figure 5: The mean S/Ns of the FAs were improved when the Temperature Coupling Mode is ON (n=6). The SD is displayed via the error bars.

Table 2: The % increase in S/N when the Temperature Coupling Mode is turned on.

Lauric Acid	Myristic Acid	Palmitic Acid	Stearic Acid
46.8%	35.7%	20.3%	9.4%

Conclusions

In this work, it was demonstrated that the improved evaporation temperature control alongside the Temperature Coupling Mode provided improvements to the analysis of the four saturated FAs.

- An evaporation tube temperature of 25.0° C resulted in the best S/N for the four FAs.
- The setting of the Temperature Coupling Mode On, improved the overall S/N for the four FAs at the optimum 25° C EvapT.
- The temperature control of the CDM via the Coupling Mode has a positive effect on the response especially for semi-volatile species when turned on.
- Experiments showed a robust application performance over a 24-hour period.

References

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