Pharma and biopharma

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Enhancing semi-volatile analyte detection in HPLC-CAD using temperature coupling mode for optimized evaporation control

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Abstract

Purpose: Accurate quantification of fatty acids (FAs) in pharmaceutical and biopharmaceutical formulations, such as polysorbates, is critical, as their presence can impact product quality, stability, and patient safety.

In this study, an UHPLC-Charged Aerosol Detector (CAD) method was used to evaluate the impact of advanced temperature control in Charged Aerosol Detection technology, with a focus on the Temperature Coupling Mode.

Methods: A reversed-phase UHPLC-CAD method in gradient mode was run with a mobile phase containing water, acetonitrile and formic acid. Different evaporation tube temperatures (EvapT) were applied, with Temperature Coupling Mode either "ON or "OFF.

Results: Results demonstrate that an EvapT of 25 ˚C provides optimal signal-to-noise (S/N) ratios for saturated FAs. Importantly, activation of the Temperature Coupling Mode significantly improves analyte response, particularly for semi-volatile compounds. Signal enhancements up to ~47% were observed for the most volatile FA (lauric acid), with a decreasing impact on less volatile species.

Introduction

Polysorbates are widely used in biopharmaceutical formulations but can degrade or contain FAs impurities that must be accurately measured to ensure product quality and safety. Charged Aerosol Detection is commonly used for FA analysis, though its performance depends strongly on EvapT. Higher EvapT improves S/N ratios but can reduce sensitivity for semi-volatile compounds like FAs, while lower EvapT has the opposite effect. The Thermo Scientific™ Vanquish™ Charged Aerosol Detector P series introduces a Temperature Coupling Mode that links EvapT with the charging detection module (CDM) temperature, overcoming limitations of earlier systems with fixed CDM temperature of 40 ºC. This coupling improves response and measurement reliability for semi-volatile species, enabling better optimization of FA analysis[1].

Materials and methods

Sample preparation

Individual stock solutions of lauric acid, myristic acid, palmitic acid, and stearic acid were prepared at a concentration of 0.25 mg/mL in methanol. A combined working solution containing all four FAs was then prepared at 50 µg/mL in 75% acetonitrile.

Test method

Table 1. Chromatographic conditions on a Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system with the CAD HP

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)	
Gradient	Time [min]	B [%]
	0	75
	1	75
	3	85
	3.5	85
	4	75
	5	75
Column Temp.	25 ˚C	
Inj. volume	10 µL	
CAD settings	Data collection rate: 10 Hz	
	Filter setting: 5.0 s	
	PV settings: PV 1.8	
	Evaporation temp: Variable (25–40 ˚C)	
	Temperature Coupling Mode: On & Off	

Figure 1. Screenshot of the part in the instrument method to activate the Temperature Coupling Mode and set the offset between EvapT and CDM temperature in a range of -5˚ C to +5˚ C

Results

Effects of evaporation tube temperature on sensitivity

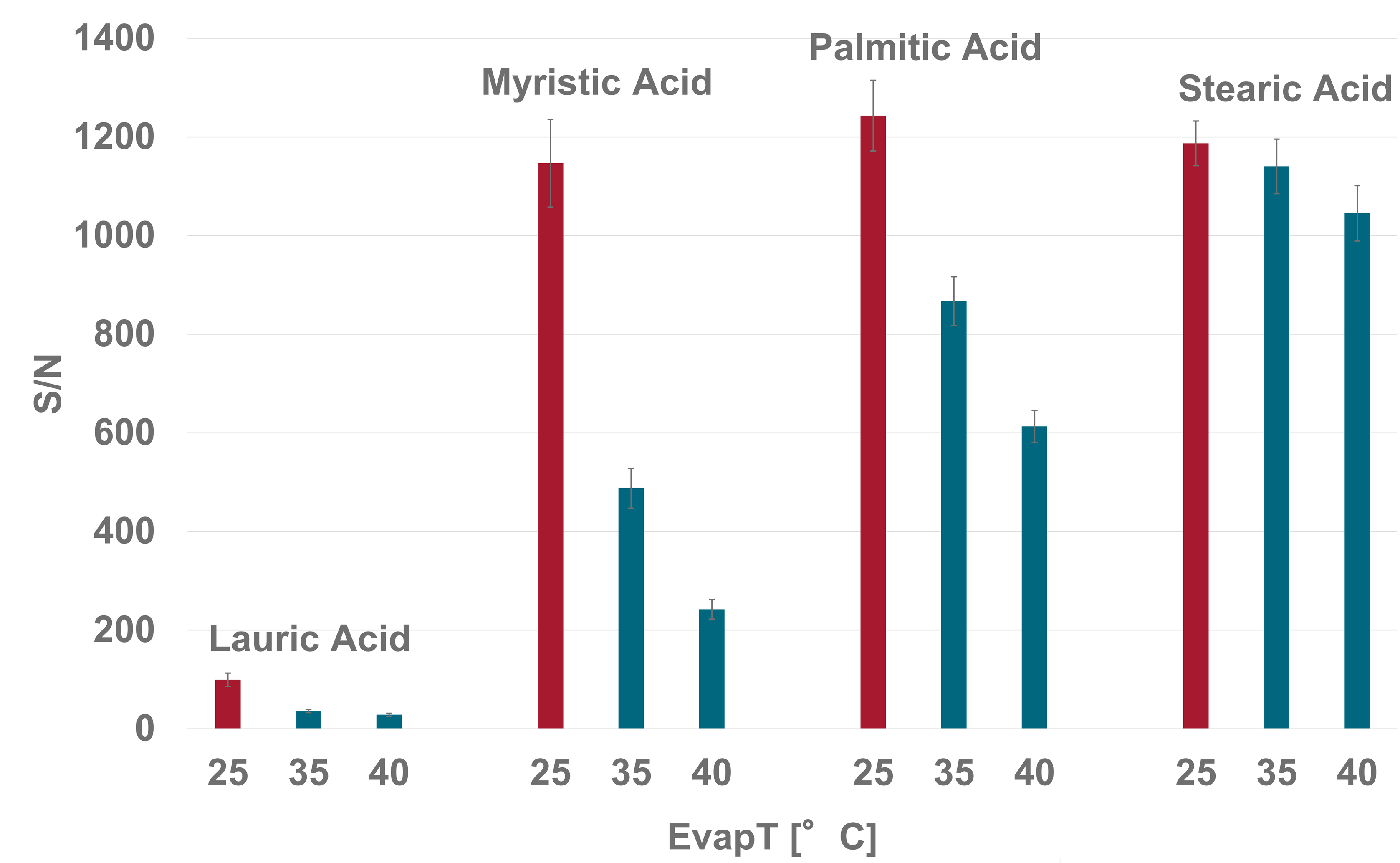

Figure 2. The S/N for each of the FAs decreased as EvapT increased, especially when analytes became more volatile. Lauric acid is the most volatile, stearic acid is the least. The optimal EvapT of 25 ˚C is highlighted in red. The standard deviation is displayed via the error bars for n = 18

Effects of temperature coupling mode on sensitivity

Increase in S/N Coupling ON	+46.8%	+35.7%	+20.3%	+9. 4%
Volatility	****	***	**	*

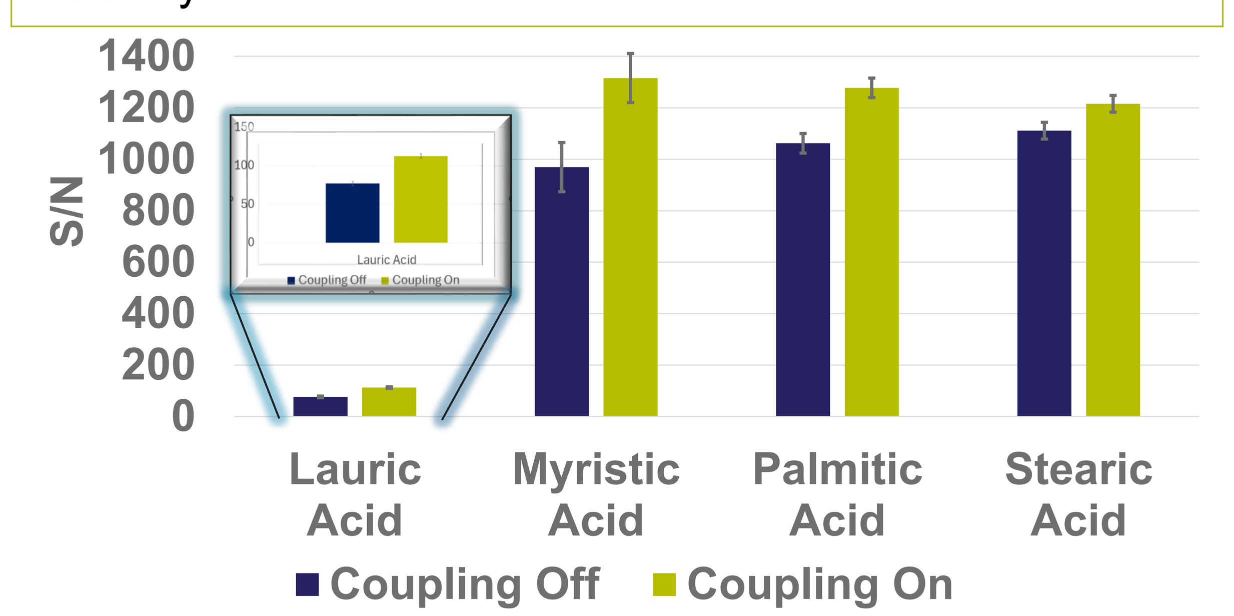

Figure 3. Temperature Coupling Mode "ON" vs "OFF" at EvapT 25 ˚C. The evaluation is based on the S/N ratio obtained for the four FAs. Lauric acid, the most semi-volatile in the mixture, shows the greatest improvement in S/N with + 46.8%. The error bars show the standard deviation for n = 6

Conclusions

- Advanced temperature control in CAD improved method sensitivity up to ~47% for semi-volatile fatty acids.
- The best overall S/N was achieved at 25.0 ˚ C EvapT with Temperature Coupling Mode enabled.
- The optimized CAD approach supports more sensitive FA impurities and degradants quantification in pharmaceutical and biopharmaceutical formulations.

References

1.Thermo Fisher Scientific Application Note 003867: Characterization of four saturated fatty acids using gradient HPLC-CAD highlighting optimized evaporation temperature control features, **2025**

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