Novel applications of multi-detector HPLC systems in pharmaceutical analysis

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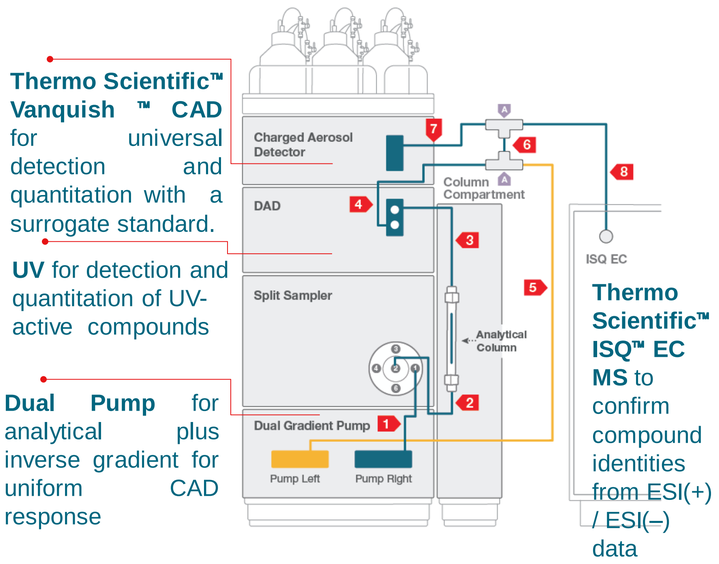
Abstract

Purpose: Provide a UHPLC method with simultaneous CAD and MS detection to monitor extractables and leachables from polymeric sources (polyurethane, nylon, PVC). Differentiate CAD semi-volatiles and volatiles before quantitation for exceptional quantitation accuracy of unknowns. Also provide UHPLC methods for multidetector analysis of polysorbate 80 and for multidetector / high resolution MS analysis of E/L.

Methods: a multi-step gradient was run on a reversed phase column. A make-up flow was applied post-column to maintain a constant solvent composition (inverse gradient). The flow was split with an approximate ratio of 1:1 to a CAD and a single quadrupole MS detector or 10:1 to a CAD and a Thermo Scientific™ Orbitrap Exploris™ 120 MS.

Results: Unknowns in E/L samples can be quantitated by CAD with a single surrogate standard, identified with high resolution MS and confirmed with single quad MS. Polysorbate composition can be monitored at the sub-class and single-component level.

System



No.	Connection between	Description
1	Pump right outlet – Injection valve port 1 (= "From pump")	Viper capillary, ID x L 0.10 × 350 mm, MP35N, P/N 6042.2340
2	Injection valve left port 2 (= "To column") – Column inlet	Active pre-heater, 0.10 × 380 mm, MP35N, P/N 6732.0110
3	Column outlet – DAD inlet	Post-column cooler, ID × L 0.10 × 240 mm, MP35N, P/N 6732.0510
4	DAD outlet – T-piece	Viper capillary, ID x L 0.10 × 550 mm, MP35N, P/N 6040.2360
5	Pump left outlet – T-piece	Viper capillary, ID x L 0.10 × 950 mm, MP35N, P/N 6042.2395
6	T-piece – T-piece	Viper capillary mixer, 25 µL, MP35N, P/N 6042.3020
7	T-piece – Charged Aerosol Detector inlet	Viper capillary, ID x L 0.10 × 650 mm, MP35N, P/N 6042.2370
8	T-piece – MS inlet	Viper capillary, ID x L 0.10 × 650 mm, MP35N, P/N 6042.2370

System setup decisions

Figure 1. Solvent density measured after split point B (MS detector branch) for the polysorbate assay. Constant density is reached with the capillary mixer, but not with the standard capillary. Efficient mixing of the analytical and make-up flow are essential to achieve stable detector response.

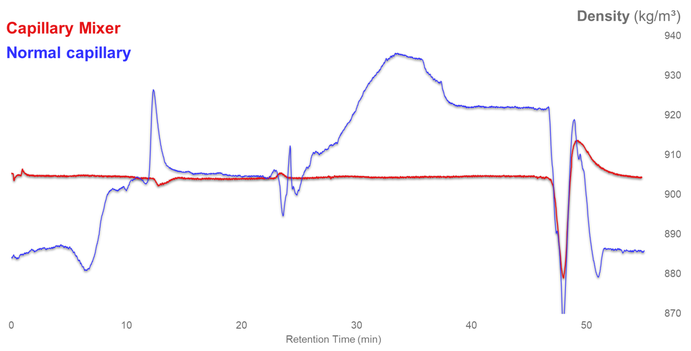


Figure 2. Inverse gradient compensation merges calibration curves to roughly one line.

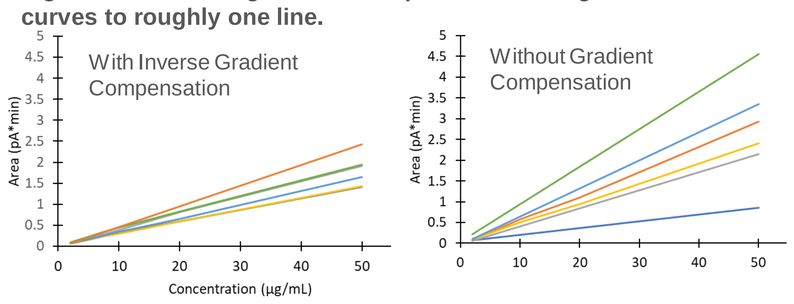
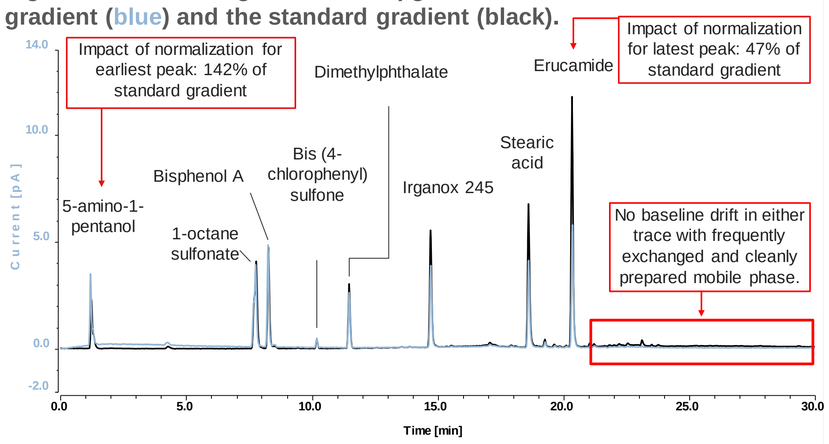


Figure 3. Chromatograms of 10 µg/mL standard with the inverse gradient (blue) and the standard gradient (black).



Extractables and leachables method

Column	Thermo Scientific™ Hypersil GOLD™ C18 3.0 × 100 mm, 1.9 µm, P/N 25002-103030
Column temp.:	60 ° C (forced air), active pre-heater
Injection volume:	10 µL
Flow rate:	0.45 mL/min
Mobile phase:	A: 2.5 mM ammonium acetate in water
	B: 2.5 mM ammonium acetate in methanol
CAD	Data acquisition rate: 10Hz, Filter 5.0
	Evaporation Temperature 35 ° C
	Power Function: 1.1
ISQ EM	Ion polarity : +3000 V and - 2000 V
	Full scans per injection: **1** (+) 90-400 *m/z* **2** (+) 400-1250 *m/z* **3** (-) 90-1250 *m/z*
	Vaporizer : 255 ° C; Ion transfer tube: 350 ° C
	Sheath gas: 46.4 psig; Aux gas: 5.3 psig

Polysorbate method

Column	Thermo Scientific™ Accucore™ C18 2.1x100 mm, 2.6 µm (p/n 17126-152130)
Column temp.:	50 ° C (forced air), active pre-heater
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Mobile phase:	A: 5 mM ammonium formate (formic acid to pH 4.8)
	B: 50/50 acetonitrile / isopropanol (v/v)
CAD	Data acquisition rate: 20Hz, Filter 3.6
	Evaporator Temperature 50 ° C
	Power Function: 1.5
ISQ EM	Ion polarity : + @ 3kV (- 3kV for oleic acid)
	Full scans: (+) 350-2000 m/z or (-) 200-600
	Vaporizer : 227 ° C; Ion transfer tube: 150 ° C
	Sheath gas: 42.9 psig; Aux gas: 4.8 psig

Results: quantitation decisions

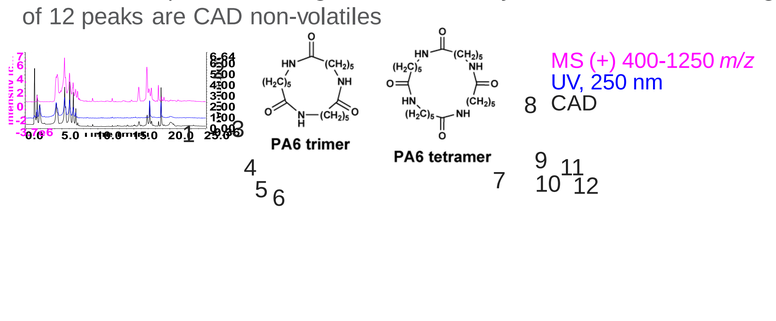
CAD accurately quantifies analytes using surrogate standards. A **key requirement** is that analytes **behave as non-volatiles** under the method conditions. A **screening procedure** for this non-volatile behavior was defined by Eckardt *et al.* in 2018:

- Compare RF at 35 deg C and 50 deg C evaporation temperatures:
- Quantitate if $Q_{50/35} \geq 0.85$

Using this screening, CAD relative response for the nonvolatiles in the standard has an RSD of 15%, compared to UV_{230} RSD of 105% and MS RSD of 109%.

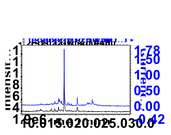
Results: E/L sample quantitation

Figure 4. Nylon-based wound dressings (shown below), as well as polyurethane and PVC-based substances can leach a number of oligomers that are well quantified using CAD. For this nylon burn wound dressing, 10 of 12 peaks are CAD non-volatiles



1 PA-6 trimer
2 PA-6 tetramer
3 PA-6 pentamer
4 PA-6 hexamer (oligos of caprolactam)
5 PA-6 heptamer
6 PA-6 octamer
7 non-volatile, m/z = 614.0, 658.0 (+)
8 non-volatile, m/z = 556.0, 600.0 (+)
9 *semi-volatile, m/z* = 615.0, 659.0 (+)
10 non-volatile, *m/z* = 642.0, 658.0 (+)
11 non-volatile, *m/z* = 540.0, 584.0 (+)
12 *semi-volatile, m/z* = 671.0, 714.0 (+)

Figure 5. Polyurethane wound dressing, 100 mg sample shaken in 2 mL 50/50 isopropanol/water, 50 ° C, 72 h. Three of six peaks are CAD non-volatiles and can be accurately quantified with the surrogate standard. Mass list from: Groh, KJ et al., DOI: 10.1016/j.scitotenv.2018.10.015



2 CAD
TIC (+), 90 – 400 *m/z*

1 3 4 5 6

Identity	CAD $Q_{50/35}$	UV 230	MS
1) Unknown	0.42	x	280.3 +
2) Oleamide	0.58	x	282.4 +
3) Unknown	0.75	x	148.3 +
4) Unknown	0.92	x	338.4 +
5) Unknown	1.2	✓	473.4 -
6) Unknown	0.88	✓	608.4 -

E/L further reading

E/L with CAD, Single Quad MS, UV: Application Note 1630

Nonvolatility, Eckardt et al. 2018 https://doi.org/10.1016/j.chroma.2018.08.051

E/L with CAD, HRAM MS, UV: Application Note 1401 and AN 1950

Results: polysorbate samples

Figure 6. CAD chromatograms showing the effects of lipase-induced degradation of PS80. Comparison between the control sample (black) and degraded sample (gold). Sorbitan and isosorbide mono-esters are hydrolyzed to polyols. The peak of oleic acid released by the hydrolysis is visible in the gold trace.

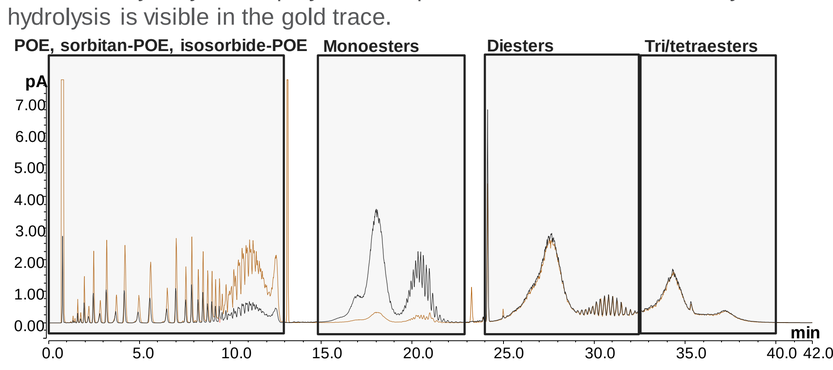
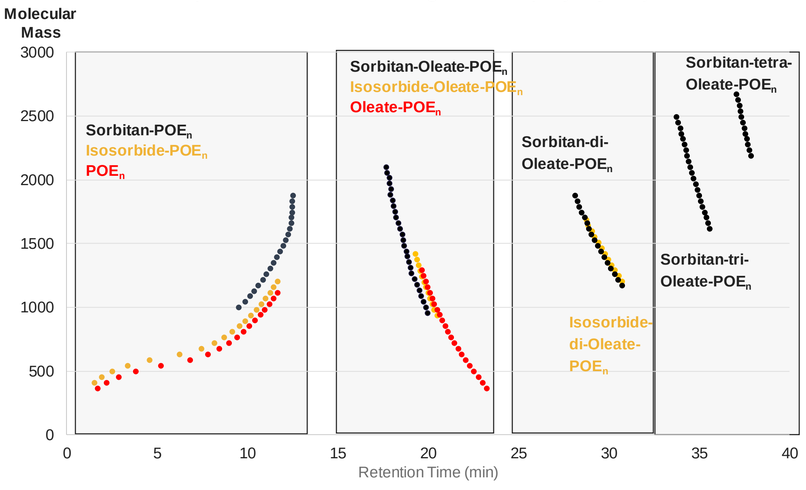


Figure 7. Retention time dependency on the mass of PS80 components. The retention time depends on the degree of esterification, and the compound class (sorbitan or isosorbide). Within a specific class, retention time depends on size, *i.e.* length of the polyoxyethylene branch.



Polysorbate further reading

Polysorbate with CAD, SQ MS, UV: Application Note 73979

Conclusions for both multidetector applications

- A well-established method for extractables and leachables quantification and for producing data for a single-quadrupole MS library based on RRT and mass was implemented along with laboratory best practices and streamlined data analysis to save time and money and improve detector signal and quantification accuracy.
- The UHPLC multidetector system enables complete analysis of PS80. The system can be used to elucidate degradation mechanism or comparison of PS80 from different batches
- The uncertainty factor was minimized and quantification was improved by: 1) classifying unknowns as non-volatile or semi-volatile, 2) using an inverse gradient, and 3) thoroughly mixing the analytical and inverse gradients before the flow split.
- The inverse gradient did not adversely affect MS LOQs and improved the uncertainty factor.
- Baseline drift in CAD and MS was avoided by proper eluent hygiene

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