AN EFFICIENT METHOD FOR THE DETERMINATION OF TRACE EXCIPIENT IMPURITIES IN BIOTHERAPEUTIC DRUG PRODUCTS CONTAINING POLYSORBATE

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INTRODUCTION

Polyoxyethylene sorbitan-based nonionic surfactants such as PS-80 (Figure 1) are commonly used in formulated biopharamceuticals to reduce protein denaturing, aggregation, and adsorption to surfaces. Degradation of surfactants in formulated drugs can decrease overall product efficacy and safety, thus requiring methods to demonstrate drug products are safe and efficacious. Assessment has become increasingly critical with the recent discovery linking trace residual esterases with polysorbate degradation in formulated drug products.

As manufacturing processes are modernized as part of a pharmaceutical quality system (ICH Q10), legacy methods such as HPLC-based separations using wide-bore columns often lack the sensitivity and efficiency needed by today's standards when considering factors such as post-column dispersion and long run-times and their impact on assay performance. Efficient methods that are robust and can incorporate technology that offer improved sensitivity and throughput would be beneficial in ensuring drug product and safety over the products lifecycle.

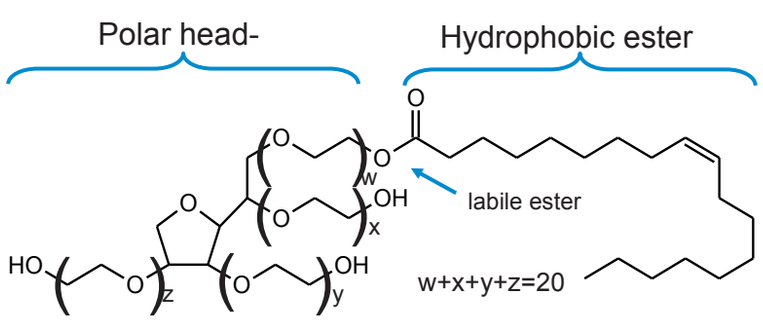

Figure 1. *Polyoxyethylene sorbitan monooleate. PS-80 contains a polar head-group and a hydrophobic tail where the head group is comprised of approximately 20 polyoxyethylene (POE) groups.*

METHODS

LC Conditions:
LC System: ACQUITY UPLC® H-Class Bio System
Detectors: ACQUITY UPLC® TUV w/5-mm Titanium flow cell
Absorption Wavelength: 200 nm
Column: ACQUITY UPLC Protein BEH C4 Column, 300Å, 1.7 µm, 2.1 mm X 100 mm p/n 186004496)
Column Temperature: 30 °C
Vials: TruView LCMS certified 12x32mm (p/n 186005669CV)
Sample Temperature: 10 °C
Injection Volume: 1 µL

Mobile phase:
A: H_2O, 0.1% FA
B: MeCN, 0.1% FA

Isocratic conditions:
Flow: 0.200 mL/min

		Recovery		Formulated Sample		
		control	experimental	control	Esterfied FA experimental	Free FA experimental
Reagent	Conc.	Volume (µL)	Volume (µL)	Volume (µL)	Volume (µL)	Volume (µL)
IS	0.3% in MeCN	10	10	10	10	10
sample	~	15	15	0	20	20
MeCN	neat	275	0	290	0	0
H_2O	neat	0	15	0	5	35
NaOH	0.5M	0	30	0	30	0
Incubate 65 °C	~	1 hr	1 hr	1 hr	1 hr	0 hr
HCl	1.0M	0	15	0	15	0
NaCl	saturated	0	50	0	50	50
H_2O	neat	0	0	0	0	15
LLE (top layer)	~	no	yes	no	yes	yes
MeCN	neat	0	75	0	90	90
MeCN	neat	0	100	0	100	100
MeCN	neat	0	100	0	100	100
Final volume after LLE		300	300	300	300	300

RESULTS AND DISCUSSION

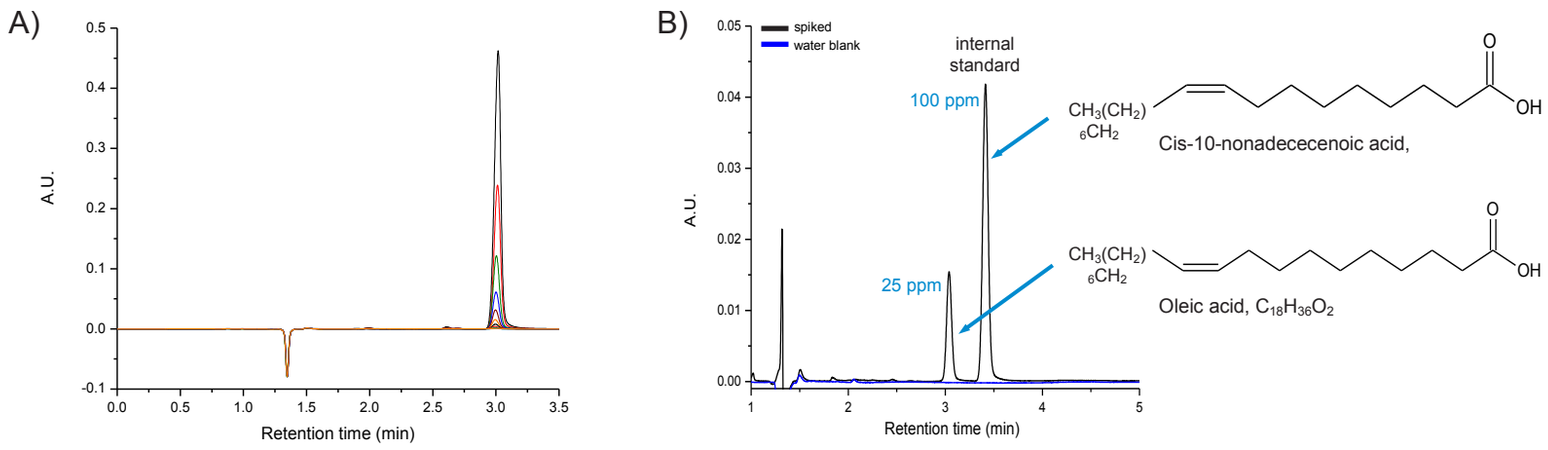

Figure 2. *Dynamic range and internal standard evaluation. A) A serial dilution series of an oleic acid standard using concentrations ranging from 1000 ppm (0.1%) to 0.24 ppm (0.000024%) was performed using a 5-min isocratic method (65%B) on a UPLC platform. B) Cis-10 nonadecenoic acid was identified as a suitable internal standard that is baseline resolved from oleic acid with no observable carry-over. The high throughput separation showed minimal dispersion and a high degree of linearity demonstrating UPLC platforms are well suited for UV-based assays that are efficient and sensitive in the detection of oleic acid degradation species.*

A)

		Control		Experimental				
		Oleic acid	Int. Stnd.	Oleic acid	Oleic acid	Int. Stnd.	Int. Stnd. correction factor	Oleic acid corrected area
sample	Expected OA (ppm)	Area	Area	Area	Recovery (%)	Area		
1	400	10053	2608	8253	82.09	2085	1.29	10322
2	200	5438	2716	4202	77.28	2002	1.34	5701
3	100	2754	2571	2430	88.26	2325	1.16	2688
4	50	1376	2654	1304	94.80	2472	1.09	1400
5	25	708	2619	653	92.23	2440	1.10	701
6	12.5	375	2968	344	91.59	2514	1.07	406
7	6.25	184	2677	180	98.18	2493	1.08	194

B)

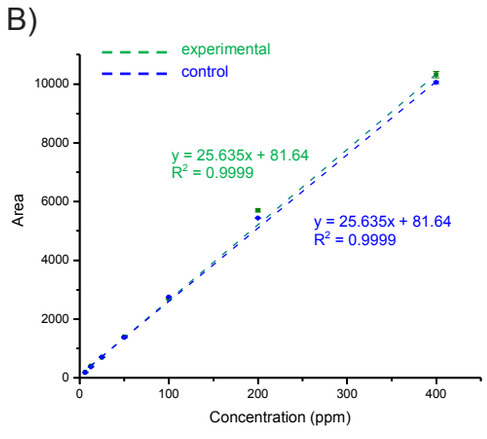

Figure 3. *Liquid-liquid extraction evaluation. A) Two sets of oleic acid samples representing experimental and control were prepared to assess recovery efficiency of a low-volume liquid-liquid extraction technique. Using the isocratic method in Figure 2, corrected peak area for the oleic acid was determined using the average response of the IS in the control sample. B) The high degree of linearity and agreement between both data sets over the dynamic range of the assay (slope ratio = 1.03) demonstrate oleic acid and a corresponding IS can be extracted over a broad working range with conditions that are compatible with direct injection of samples for efficient and accurate analysis of fatty acids.*

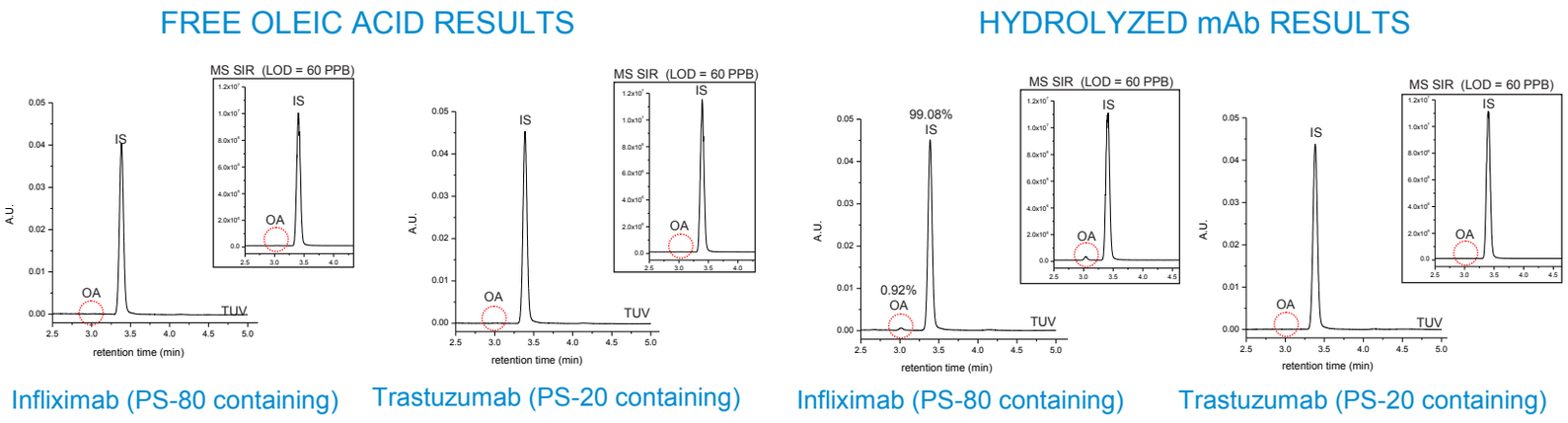

Figure 4. *Assay applicability and specificity. Free oleic acid was not detected in UV and MS chromatograms of representative samples using the proposed LLE technique. Esterfied oleic acid was observed to be present in the infliximab sample in the form of PS-80 at less than 1 % relative to the IS (99.08%) peak when the samples underwent base hydrolysis. The absence of an oleic acid peak in the hydrolyzed trastuzumab sample highlights the specificity of the assay and confirms that the infliximab sample showed no detectable signs of degradation in the free oleic acid test demonstrating the assays applicability in the detection of PS-80 degradants in formulated samples.*

Method	oleic acid conc. (ppm)	PS-80 (mg)	PS-80 conc. (vol %)	PS-80 conc. (mg/mL)	RSD (%)
multi-point calibration (using Figure 2A data)	12.91	0.00119	0.0051	0.059	4.40
single-point calibration (using Figure 2B data)	13.95	0.00129	0.0055	0.065	4.07

Table 1. *Quantitation Comparison. Using the oleic acid concentration, a single-point calibration method offered an efficient means to extrapolate PS-80 concentration in a high throughput manner when compared to a more resource dependent multi-point method.*

CONCLUSION

- **A robust method in the analysis of PS-80 degradants**
- **A deployable UV-based workflow in regulated environments**
- **Minimal sample preparation for improved productivity**
- **Ability to perform high throughput analysis**

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