Analytical strategies to monitor polysorbate in biotherapeutics

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PS20 stability and degradation pathways

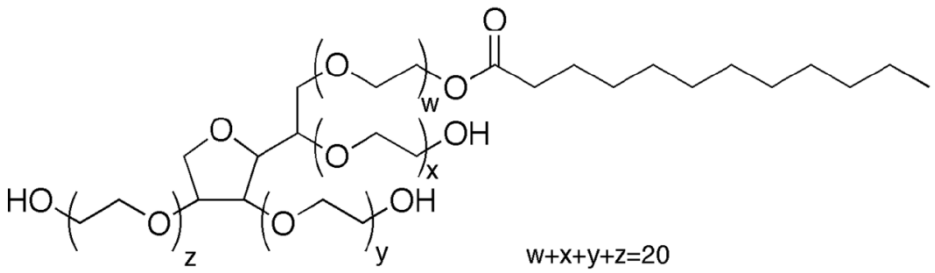
PS20 quantification and characterization

PS20 degradation products

Considerations for control strategy

Polysorbate in biotherapeutics
Structure and use

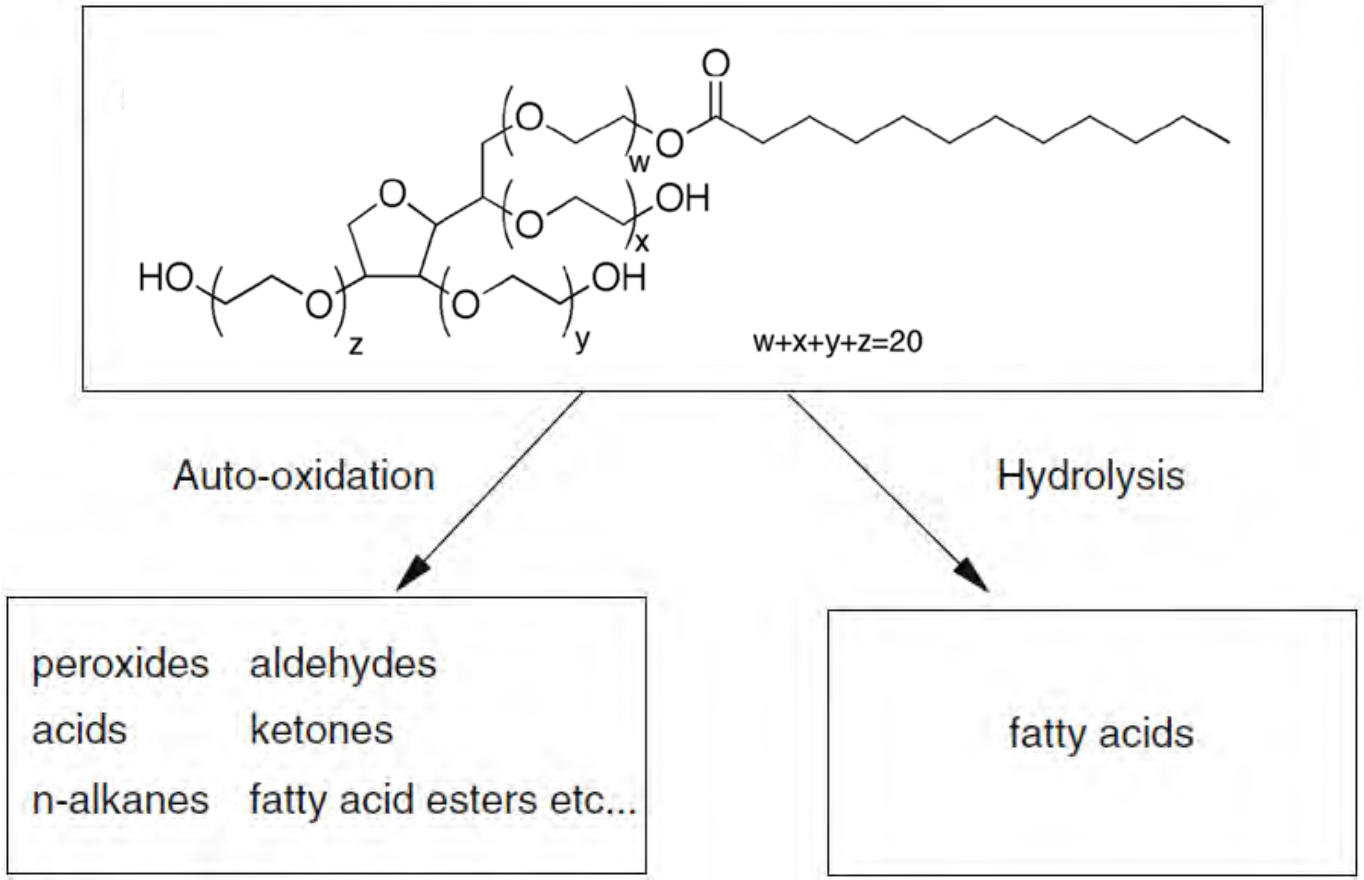
- Polysorbates (PS) are widely used as surface active agents in biopharma
  - ~ 3 out of 4 biologics include polysorbate as part of their formulation
- Ability to form micelles and thus particularly preventing protein aggregation as a result of adsorption at interfaces (air/water)
- Consist of hydrophilic polyoxyethylene (POE) sorbitan linked to fatty acids by an ester bond



- Chain length and relative content of specific fatty acids is defined in pharmacopoeia
  - PS20: 40-60% Laureate, i.e. saturated C12

Polysorbate in biotherapeutics
Known degradation pathways

Ravuri et al. 2011



Potential impact on product quality

Arising free radicals can cause protein modifications

Fatty acids can form insoluble particles

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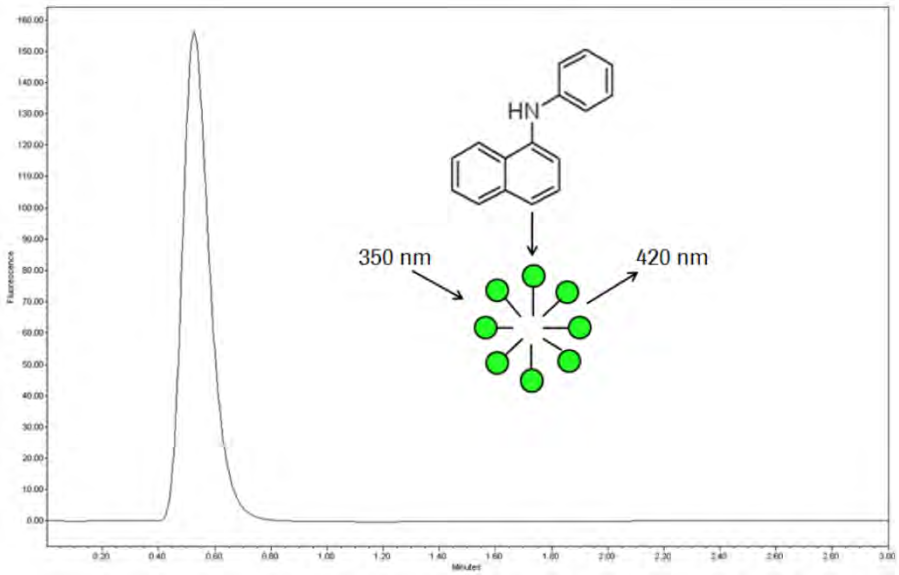
Analytical strategies for polysorbates
Quantification

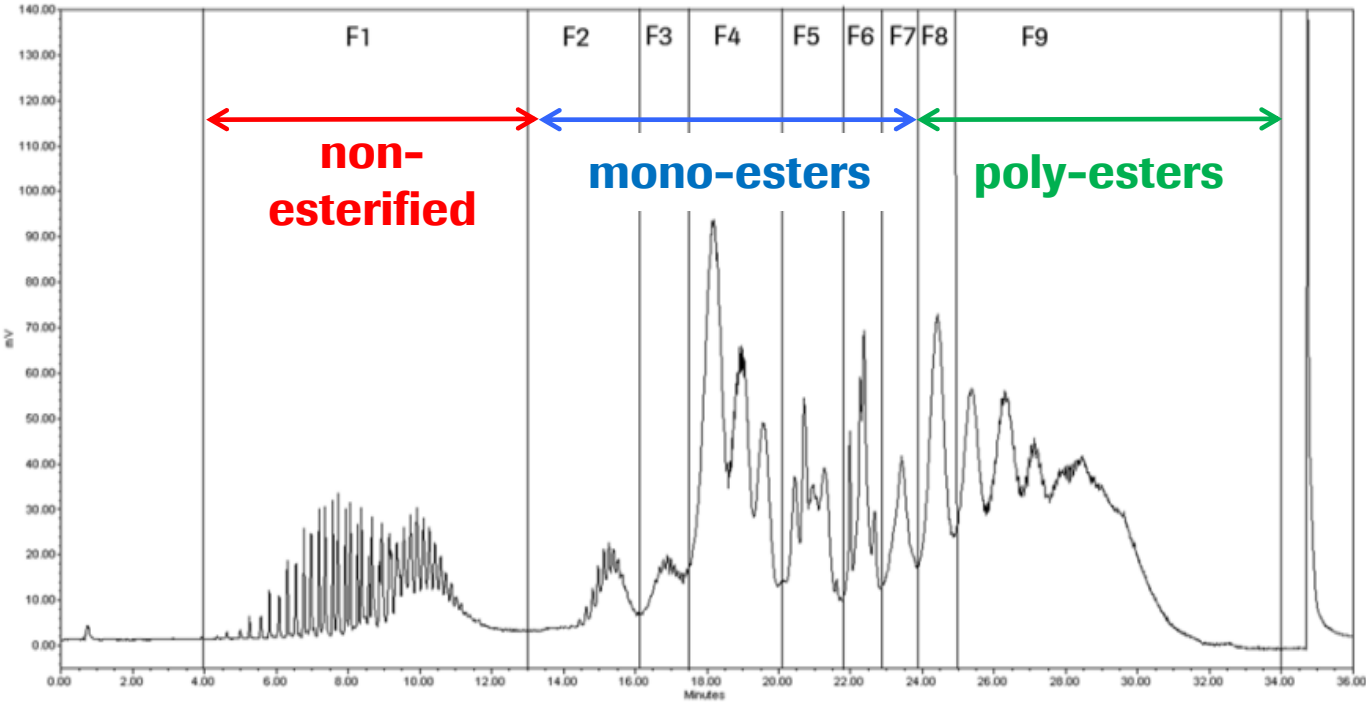
- **Fluorescence Micelle Assay (FMA)**
  - Relies on micelle forming ability of the surfactant
  - Increase in fluorescence of NPN upon micelle formation



- **Mixed Mode HPLC with ELSD/CAD detection (MM-ELSD/CAD)**
  - Mixed mode anion exchange or cation exchange column (eg Oasis MCX)
  - PS20 detection using ELSD or CAD detector
  - Also suitable for surfactants that do not form micelles, eg Poloxamer 188

Analytical strategies for polysorbates
Characterization

- Heterogeneity of PS20 can be further characterized using a shallow gradient RP-ELSD/CAD method (Hewitt et al. 2011)
- Method is capable of resolving mono- from poly-ester species

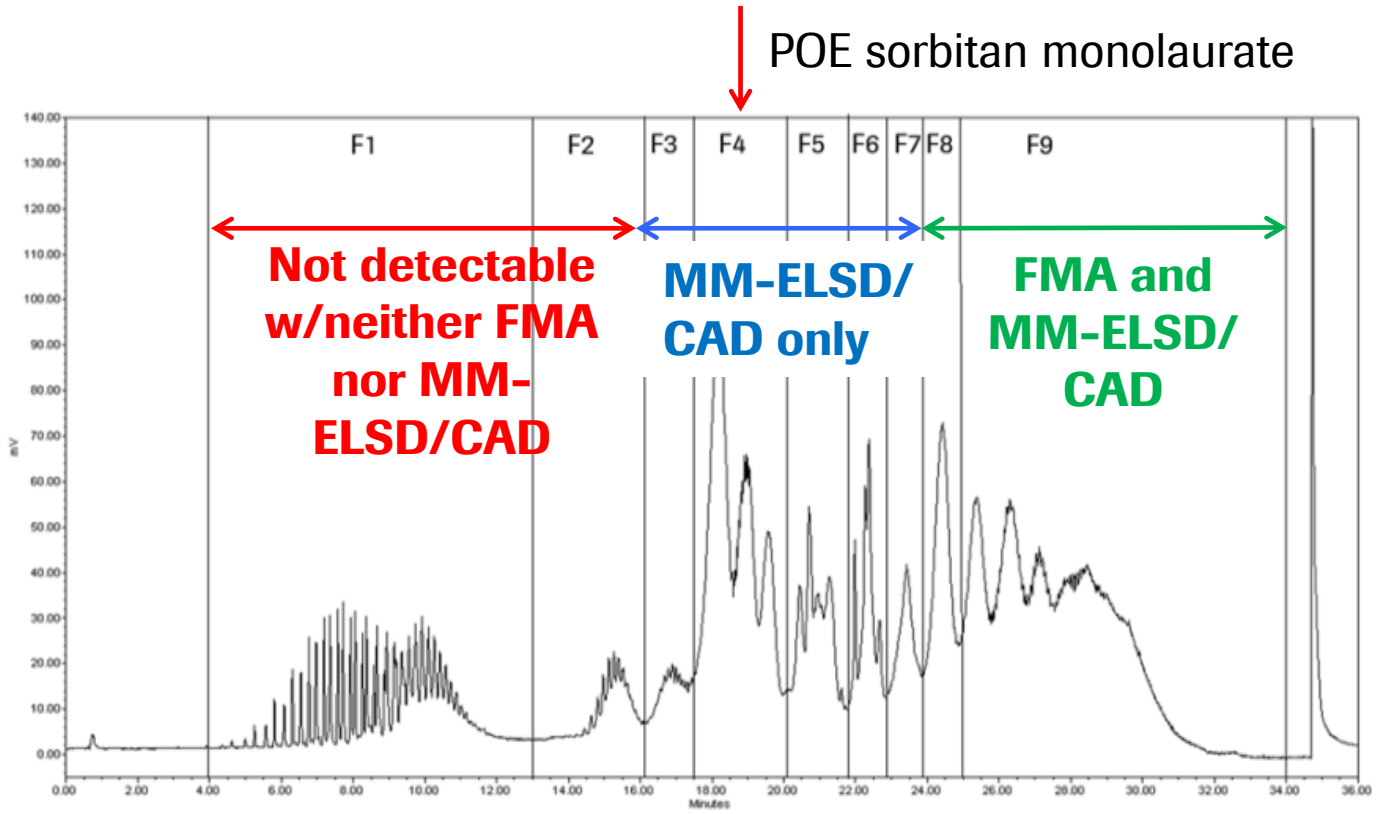


ID	Subspecies
F1	POE, POE isosorbide, POE sorbitan
F2	POE isosorbide monooctylate
F3	POE isosorbide monodecanylate
F4	POE sorbitan monolaurate, POE isosorbide monolaurate, POE monolaurate, POE sorbitan monomyristate
F5	POE isosorbide monomyristate, POE monomyristate, POE sorbitan monopalmitate
F6	POE isosorbide monopalmitate, POE monopalmitate
F7	POE isosorbide monodecanoade
F8	POE sorbitan dilaurate
F9	POE sorbitan laurate/myristate, POE sorbitan dimyristate, POE sorbitan myristate/palmitate

Analytical strategies for polysorbates

Choice of method is critical to monitor PS degradation

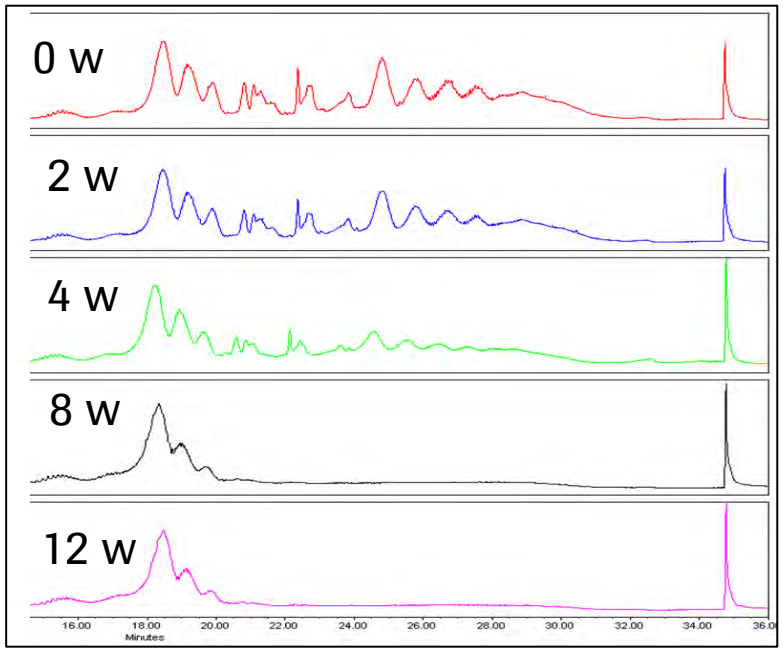
- Since FMA relies on micelle formation, its response is not uniform across the different PS20 ester species (Lippold et al 2017)
- Main component, POE sorbitan monolaureate, only detectable w/ MM-ELSD/CAD



Analytical strategies for polysorbates

Case studies

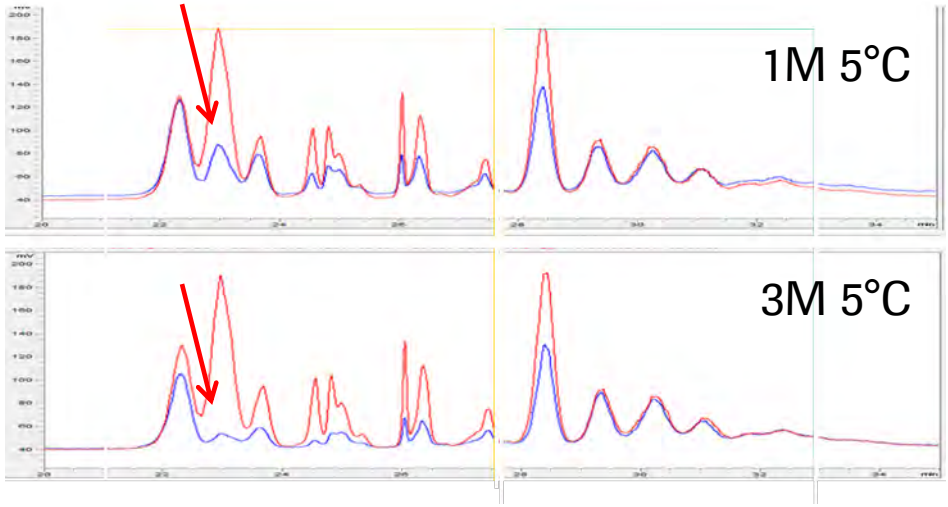
Thermal 40°C (oxidative) Degradation



mono-esters ⟷ poly-esters

- Polyesters are degraded more readily w/ temperature stress (Auto-Oxidation)
- FMA *underestimates* PS20 content since it cannot quantify monoester species

Hydrolytic degradation
(sample, control)



mono-esters ⟷ poly-esters

- Hydrolytic degradation is usually specific, i.e. either mono- or polyester are more readily degraded
- When monoesters are degraded, FMA *overestimates* true PS20 content

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PS20 degradation products

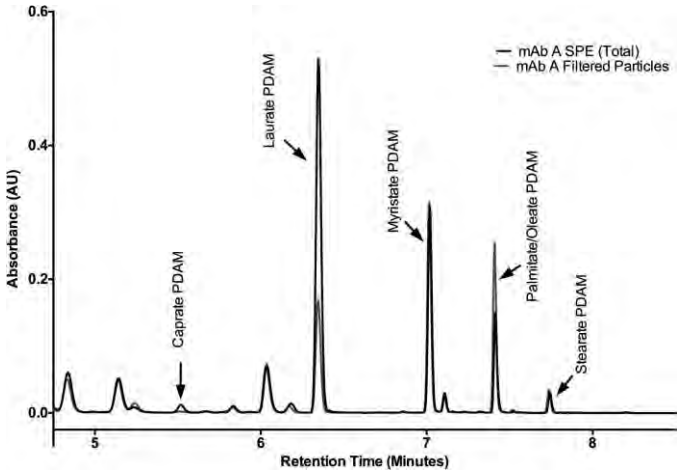
Considerations for control strategy

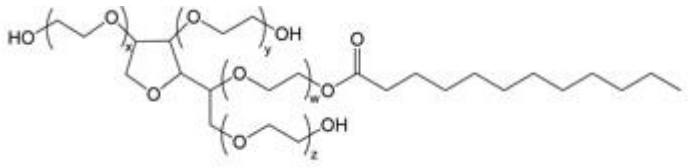
Analytical strategies for polysorbate
Quantifying degradation products

- Degradation products from oxidative PS degradation can be detected using stir-bar-sorptive absorption coupled to GC-MS (Ravuri et al. 2011)

- Hydrolytic degradation results in formation of free fatty acids which can be de-tected by UPLC (following fluorescent labeling, Tomlinson et al. 2015) or by LC-MS

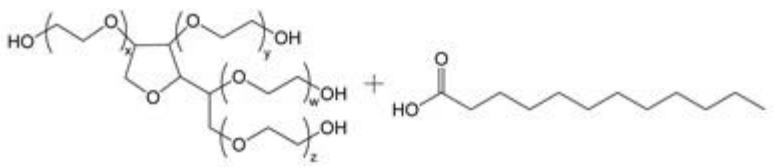
- Molar ratio of degraded PS20 and free fatty acids can give insight into major degradation pathway and confirm hydrolytic degradation





x mole of polysorbate

Hydrolytic degradation



x mole of FA formed

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Considerations for control strategy

Considerations for Drug Product control strategy
Driven by process and product knowledge

- PS20 should be monitored throughout the development lifecycle of a molecule, eg during formulation studies as well as during development stability studies

No change in PS content over product shelf life	Significant change in PS content but no impact on Product CQAs	Significant change in PS content and impact on product CQAs
▪ Leverage available data from development studies ▪ PS20 content not part of DP release or DP stability control strategy ▪ Monitoring only (eg as IPC)	▪ Provide justification that PS is not a critical excipient (incl. assessment on impact of potential degradation products) ▪ PS20 content not specified for DP release and stability	▪ Include PS content for DP release and stability ▪ Include appropriate justification for EoSL limit incl. potential impact of PS degradation products

Doing now what patients need next