

Excipient Analytics & Reverse Engineering

Novel methods allow a better understanding & more precise analysis of even complex excipients. This help to de-risk and speed up product development

RaDes GmbH
Hamburg, Germany
May 2026



RADES

RATIONAL DESIGN
OF FORMULATIONS

Opportunities & added value of advanced excipients analytics

Many important surfactants, solubilizers & emulsifiers are complex excipients and consist of dozens or hundreds of subspecies and are insufficiently described by pharmacopeial specifications.

For many such excipients, our novel methods allow for a detailed qualitative & quantitative analysis.

Important benefits include:

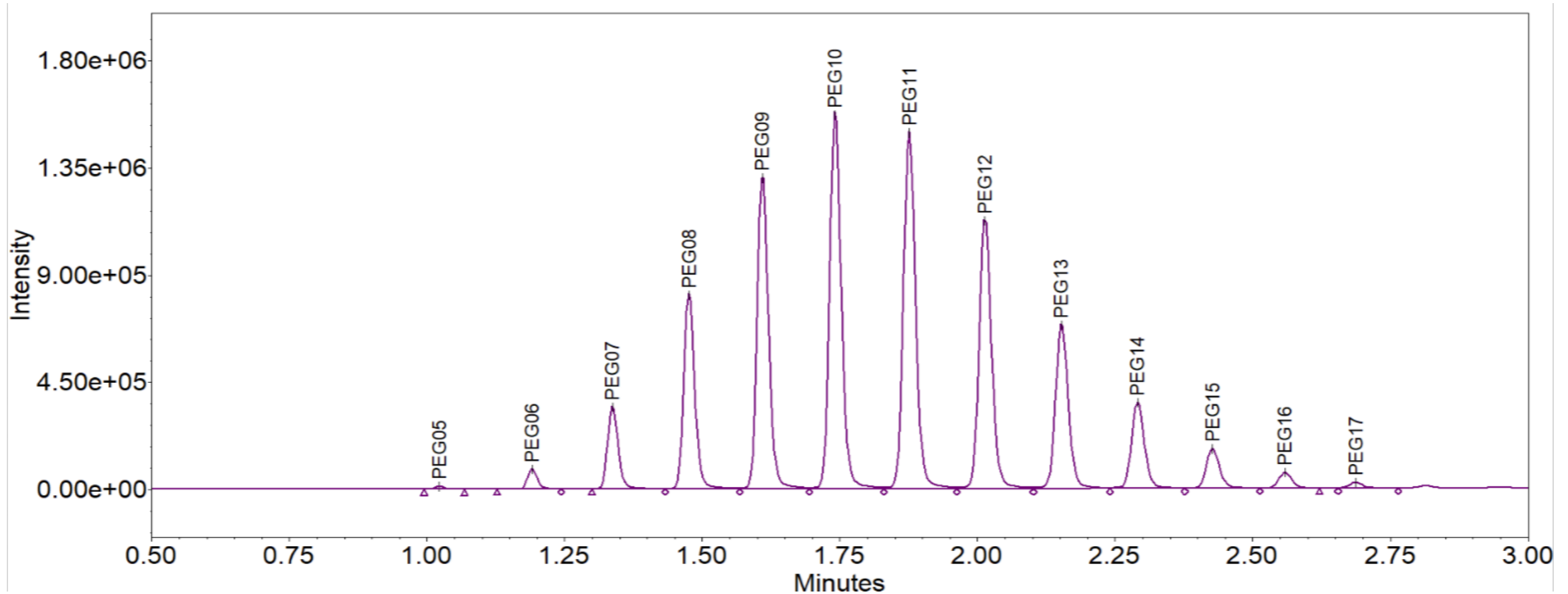
1. **Reverse engineering:** exact quantification and qualitative fingerprinting, which often allows identification of the supplier. This **de-risks & speeds up generic development** projects
2. **Batch-to-batch and supplier-to-supplier variability** can be identified and quantified to support **supplier selection, troubleshooting, and process robustness**. In-house specifications can be developed if needed
3. **Optimization of product development:** Identification of critical material and critical quality attributes (**CQAs & CMAs**) for robust product design & to meet the expectations of authorities (new EMA guideline on quality & equivalence of topicals)

Polyethoxylated Excipients

with a special focus on polysorbates

Polyethylene Glycols

This is **PEG400** (= polyethylene glycol 400, macrogol 400). **It's a mixture.**



Chromatogram of PEG400. The numbers indicate the number of polyoxyethylene units. One unit is around 44 Da. PEG10 has an approximate molecular weight of 440 Da.

- It contains relevant amounts from PEG06 (approx. **260 Da**) to PEG16 (approx. **700 Da**)
- The **Gaussian-like distribution of subspecies** around a mean is a major reason for the complexity of polyethoxylated excipients

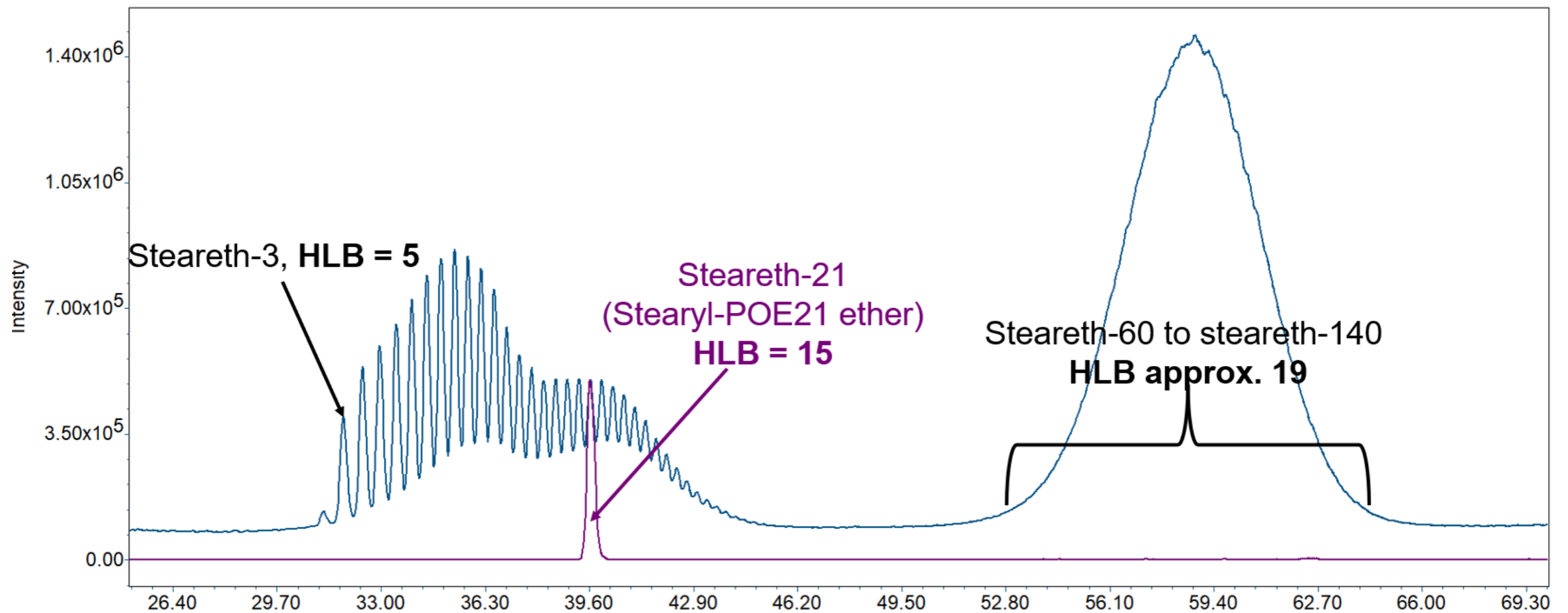
Polyethoxylated excipients

Polyethoxylated excipients are the most important class of excipients in liquid and semi-solid pharmaceutical formulations. Almost every formulation contains one or more of these excipients.

Examples:

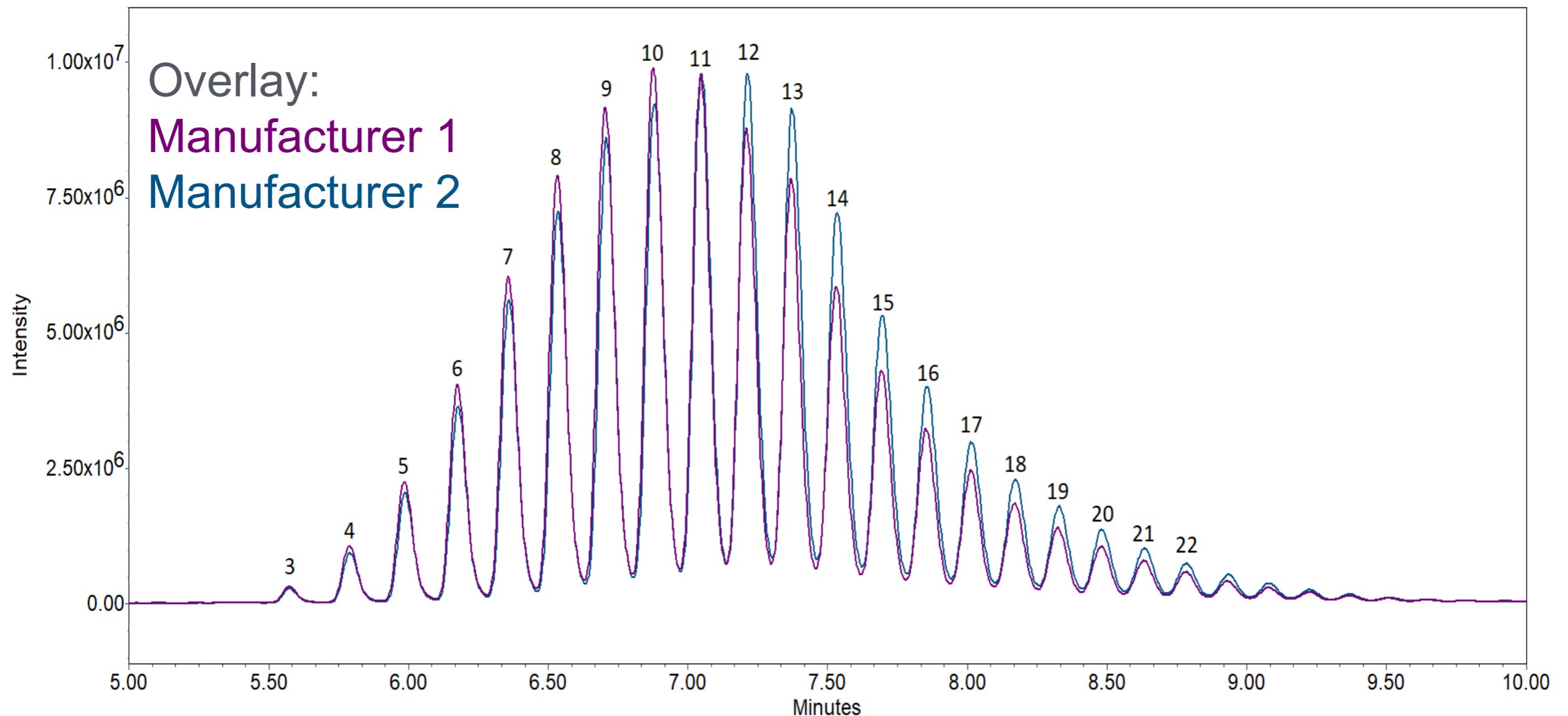
1. Pure **polyethylene glycols** (PEG, macrogols) => solvent, ointment base
2. **Poloxamers** (polyoxyethylene–polyoxypropylene block copolymers) => solubilizer, thickener, stabilizer for proteins
3. **Polysorbates** (PEG-sorbitan fatty acid esters) => solubilizer, emulsifier, stabilizer for proteins
4. **PEG fatty acid esters** => emulsifier, solubilizer, surfactant
5. **PEG alkyl ethers** => emulsifier, solubilizer, surfactant
6. **PEG-glyceryl and PEG hydrogenated castor oil** derivatives => solubilizer, surfactant
7. **PEGylated phospholipids** => stabilizers of LNP, e.g. for mRNA delivery

PEG alkyl ethers: Steareth-21: Wide variety of species



- “Steareth-21” (Brij S721) contains less than 5% of steareth-21
- HLB values of components range from 5 to 19
- It itself is a complex emulsifier and could vary greatly according to specifications

Lauromacrogol 400: Variability Between Manufacturers

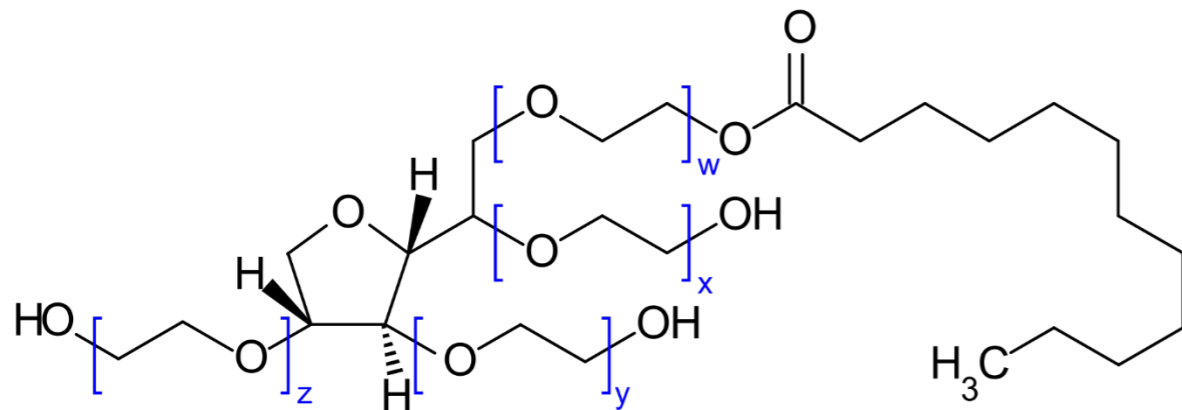


- Nominal number of PEG units per Pharm. Eur. monograph of lauromacrogol (also known as “Polidocanol”) as active ingredient is 9.
- Broad distribution of PEG units with maximum at 10 (manufacturer 1) and 12 (manufacturer 2)

Polysorbates: Composition (1)

Polysorbate 20 (PS20) is a complex mixture containing components of different chemistry, polarity, and molecular weight

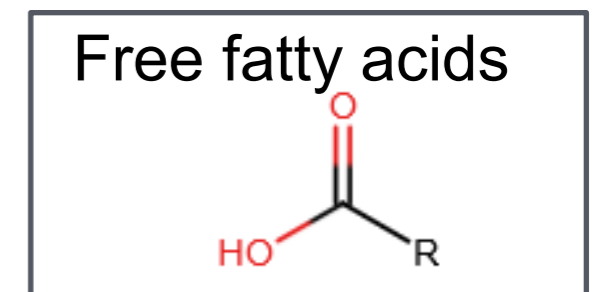
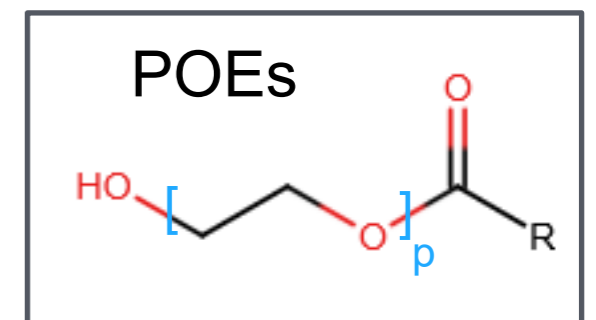
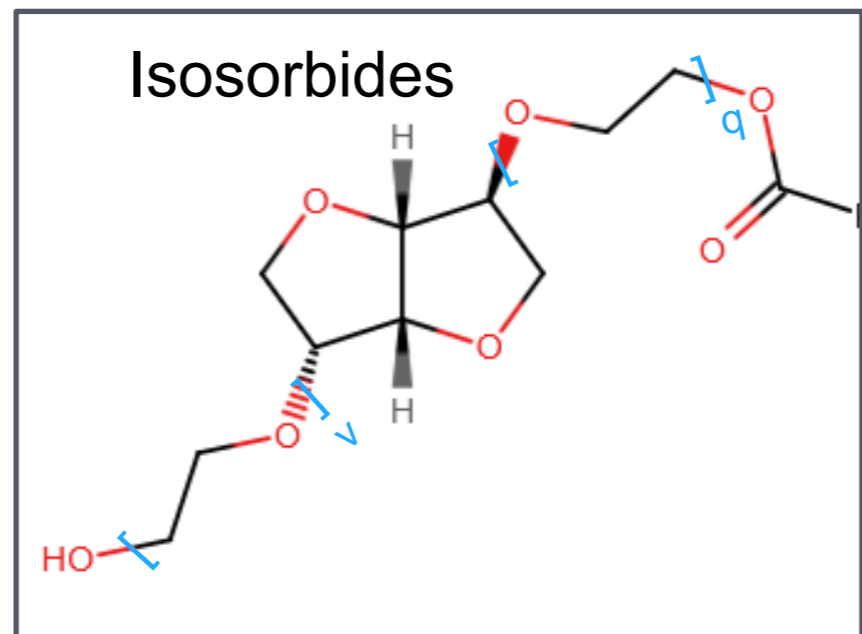
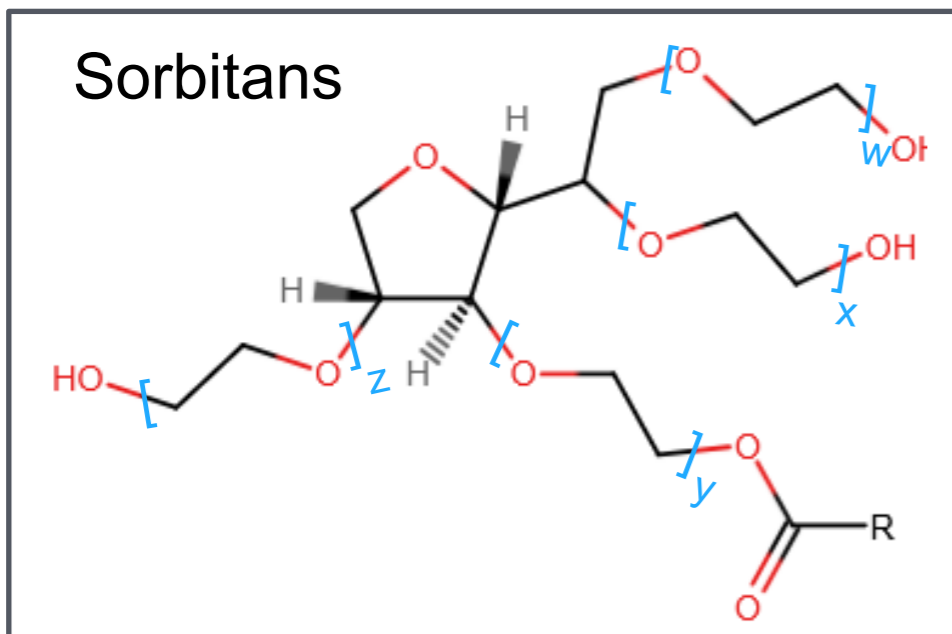
- Formal structure of PS20 according to Ph.Eur. and USP-NF:



$$w + x + y + z = 20$$

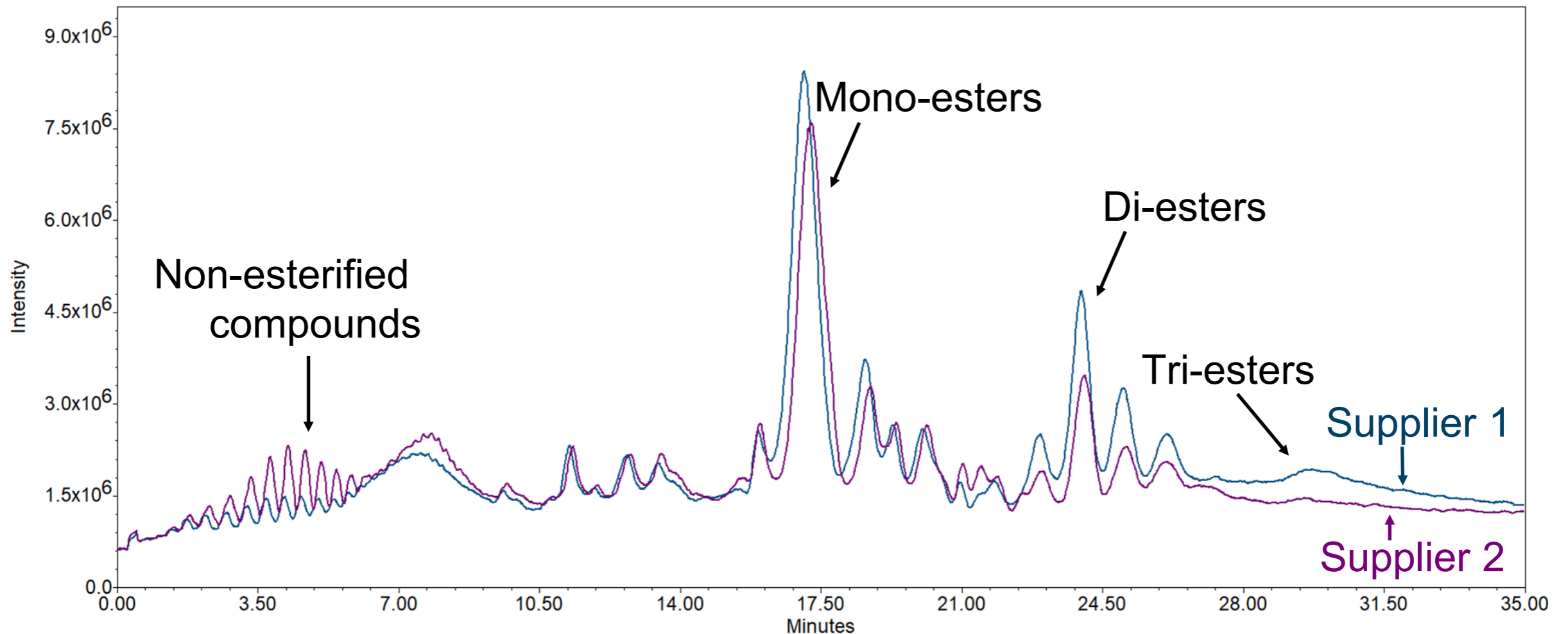
= POE (20) sorbitan monolaurate

- True, complex composition of polysorbates:



Polysorbates: Composition (2)

Due to its complexity, polysorbates from different suppliers show differences in composition:



- Polysorbate 20 from suppliers 1 and 2 showed difference in non-esterified compounds as well as in mono- versus di- and tri-esters
- Material from supplier 2 appears to be more “lipophilic”
- The differences can result in different protein stabilization properties and solubilization

Polysorbates: Composition (3)

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Research paper


Unravelling the polysorbate 20 composition: A fusion of UPLC-MS analysis and stochastic modelling[☆]

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Analysis 1

Determination of percent of unesterified sorbitans, isosorbides and POEs

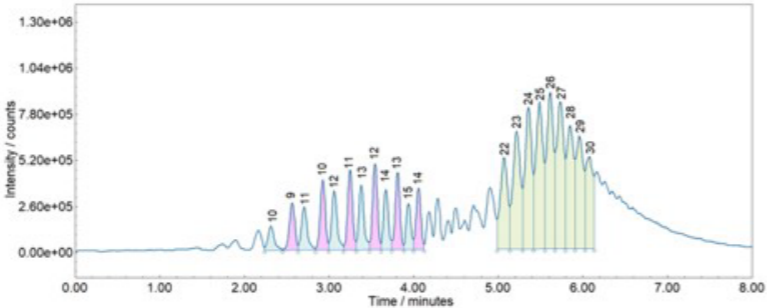


Calculation of polysorbate 20 composition (27'775 individual compounds are calculated)

Conversion of the molar percentages into weight percentages


Analysis 2

Determination of homologous distribution and content of sorbitans, isosorbides and POEs




Certificate of analysis

Composition of fatty acids



Calculation of polysorbate 20 saponification and hydroxyl value and verification as control values against the certificate of analysis



Ground-breaking new insights to polysorbate 20 composition by RaDes:

- 27775 (!) PS20 components were identified
- Fully characterized by:
 - ✓ substance class
 - ✓ number of esters
 - ✓ fatty acid combination
 - ✓ number of oxyethylene (OE) units

Polysorbates: Application of RaDes analytical method

Importance in generic development projects:

Batch-to-batch comparisons and compositional differences between suppliers

Comparison between substance classes

Manufacturer	Type / Quality	Batch	Mol %			Weight %		
			sorbitan	isosorbide	POE	sorbitan	isosorbide	POE
Croda	HP	1938157	51.9	30.3	17.8	69.0	20.3	10.6
Croda	HP	1244268	51.8	30.3	17.9	68.4	20.8	10.8
KLK Kolb	Kotilen-L/1	1-2508621	53.3	35.9	10.8	69.8	23.6	6.5
Nanjing Well Pharma Co LTD.	PS 20 for injection	20171030-1k	58.2	37.2	4.6	71.9	25.3	2.8
Nanjing Well Pharma Co LTD.	PS 20 for injection	20190501-1k	65.2	33.0	1.8	77.2	21.8	1.0
Croda	Super Refined	0001204756	59.4	15.4	25.1	75.1	10.4	14.5
Croda	Super Refined	0001308151	57.7	16.5	25.9	73.6	11.0	15.4
Mean:			56.8	28.4	14.8	72.1	19.0	8.8
%RSD:			8.57	31.28	63.76	4.58	31.24	63.12

Variability within pharmacopeial specifications can influence:

- Droplet size distribution
- Viscosity & rheological profile
- Release behaviour

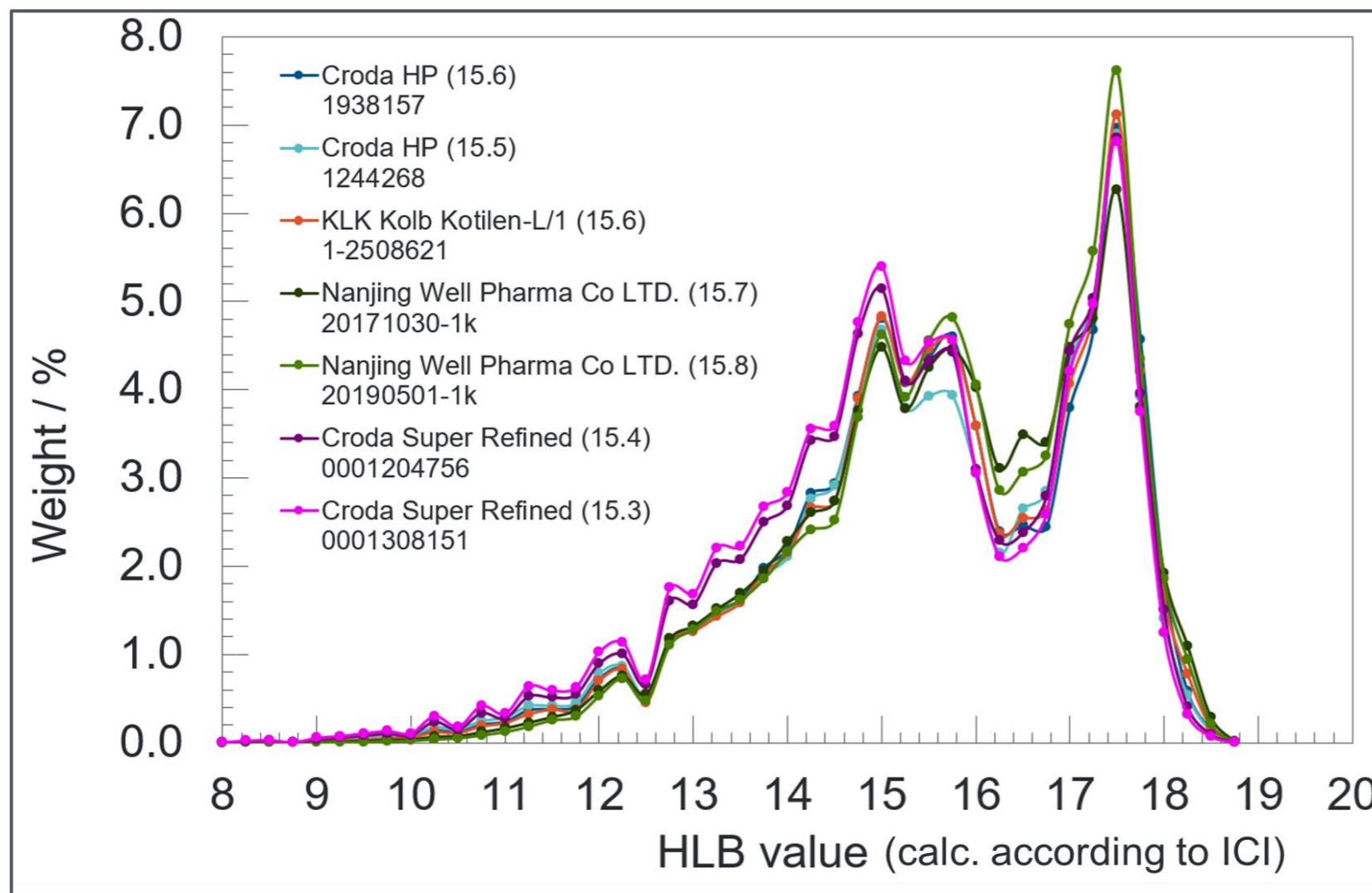
=> To achieve equivalence with an originator, it may be necessary to identify both the correct quantity and the right quality of the polysorbate used

Polysorbates: Application of RaDes analytical method

Benefits in formulation design:

Polysorbates from different suppliers vary in HLB (or Cc in HLD concept), which affects solubilization potential and emulsion stability. Solubility in various solvents can also differ

- Comparing them allows you to select the best suited type for formulation performance & robustness



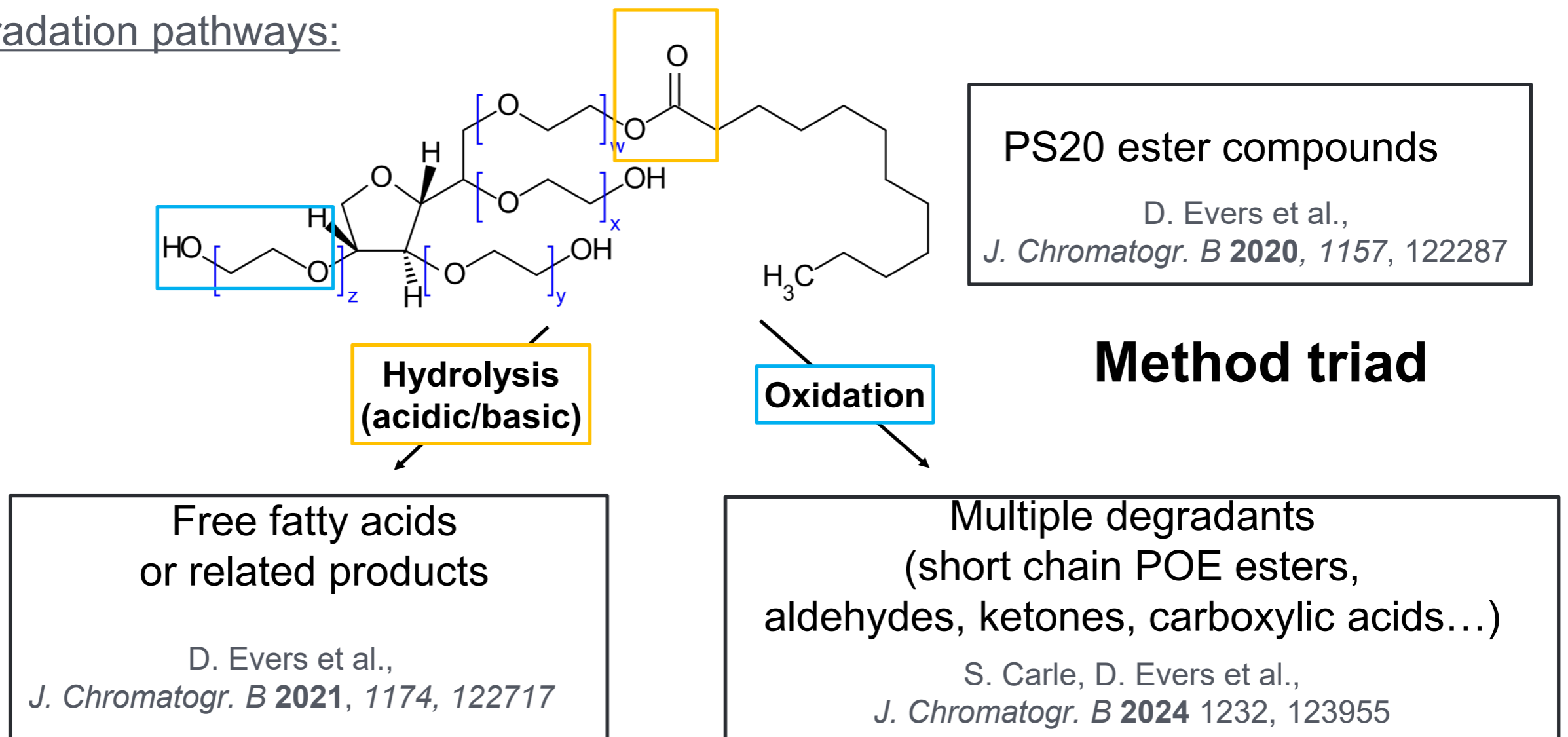
$$HLB = \frac{(M_{\text{sorbitan or isosorbide}} + M_{\text{POE}})}{M_{\text{molecule}}}$$

HLB range	Use
4 – 6	W/O emulsions
7 – 9	Wetting agents
8 – 18	O/W emulsifiers
13 – 15	Detergents
15 – 18	Solubilizing

Polysorbate analytics in biopharmaceutical products

- Polysorbates are the most important stabilizers for biopharmaceutical formulations, such as injectable antibodies.
- However, polysorbates can degrade, which can lead to formulation instability and the formation of particles or precipitates in injectables.

Degradation pathways:



Polysorbate analytics in biopharmaceutical products

RaDes developed novel and powerful UPLC-MS analytical methods a single sample preparation procedure to characterize PS20 with regards to:

- i) ester stability,
 - ii) free fatty acids,
 - iii) oxidation markers in biopharmaceutical products
- Instead of reporting only a single polysorbate content, detailed assay information of individual markers is reported.
 - There is significantly higher selectivity compared to, for example, a CAD detector due to an additional mass spectrometric separation dimension.
 - The fatty acid determination is very sensitive and robust using an isolator column.
 - The results of the fatty acid analysis are expressed as relative (% decomposed) and absolute (ng/mL) values.
 - Distinct degradation patterns are obtained for hydrolytic, oxidative, and enzymatic degradation, and even enzyme-specific patterns may be recognized.

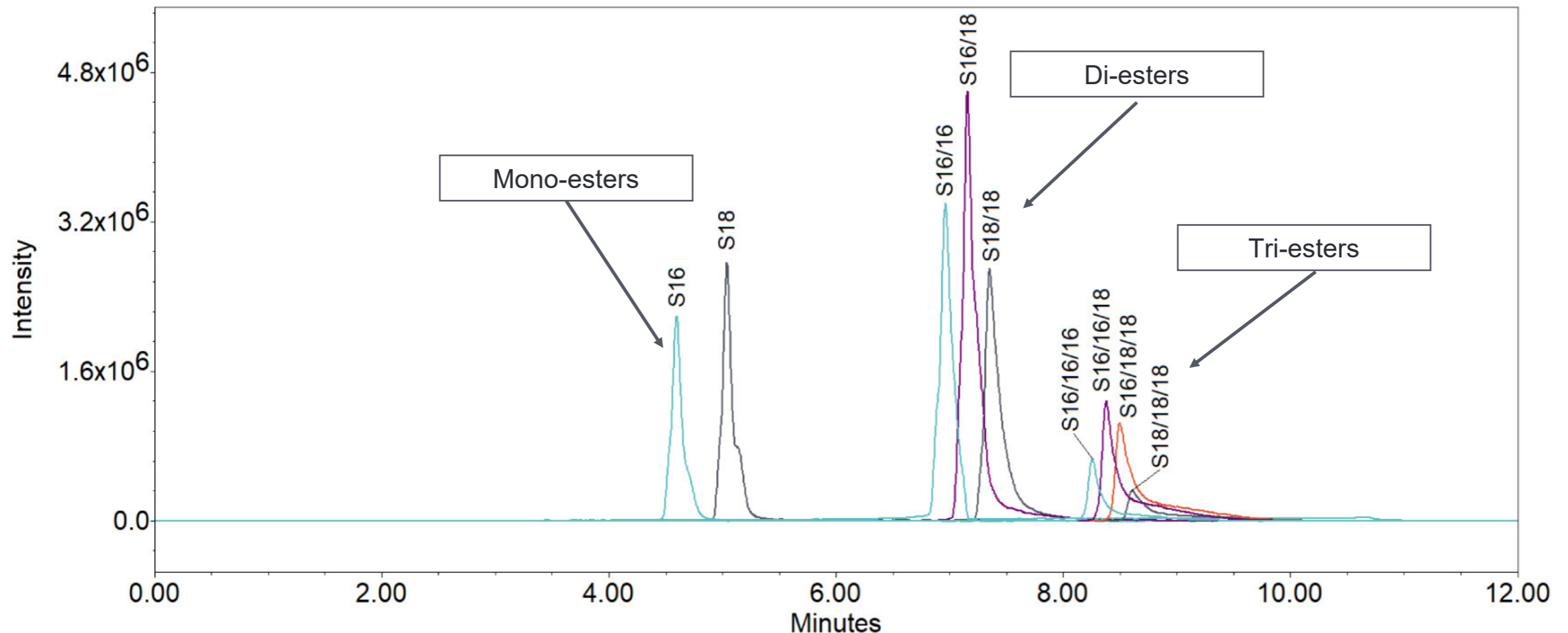
References:

- [D.-H. Evers, et al. Development and validation of a selective marker-based quantification of polysorbate 20 in biopharmaceutical formulations using UPLC QDa detection. Journal of Chromatography B 1157 \(2020\) 122287](#)
- [D.-H. Evers, et al. Hydrolytic polysorbate 20 degradation – Sensitive detection of free fatty acids in biopharmaceuticals via UPLC-QDa analytics with isolator column. Journal of Chromatography B 1174 \(2021\) 122717](#)
- [S. Carle, D-H. Evers, E. Hagelskamp, P. Garidel, J. Buske. All-in-one stability indicating polysorbate 20 degradation root-cause analytics via UPLC-QDa, Journal of Chromatography B, 1232 \(2024\) 123955](#)

Further excipients (non-polyethoxylated)



Further Excipients: Sorbitan monostearate (e.g. Span®)

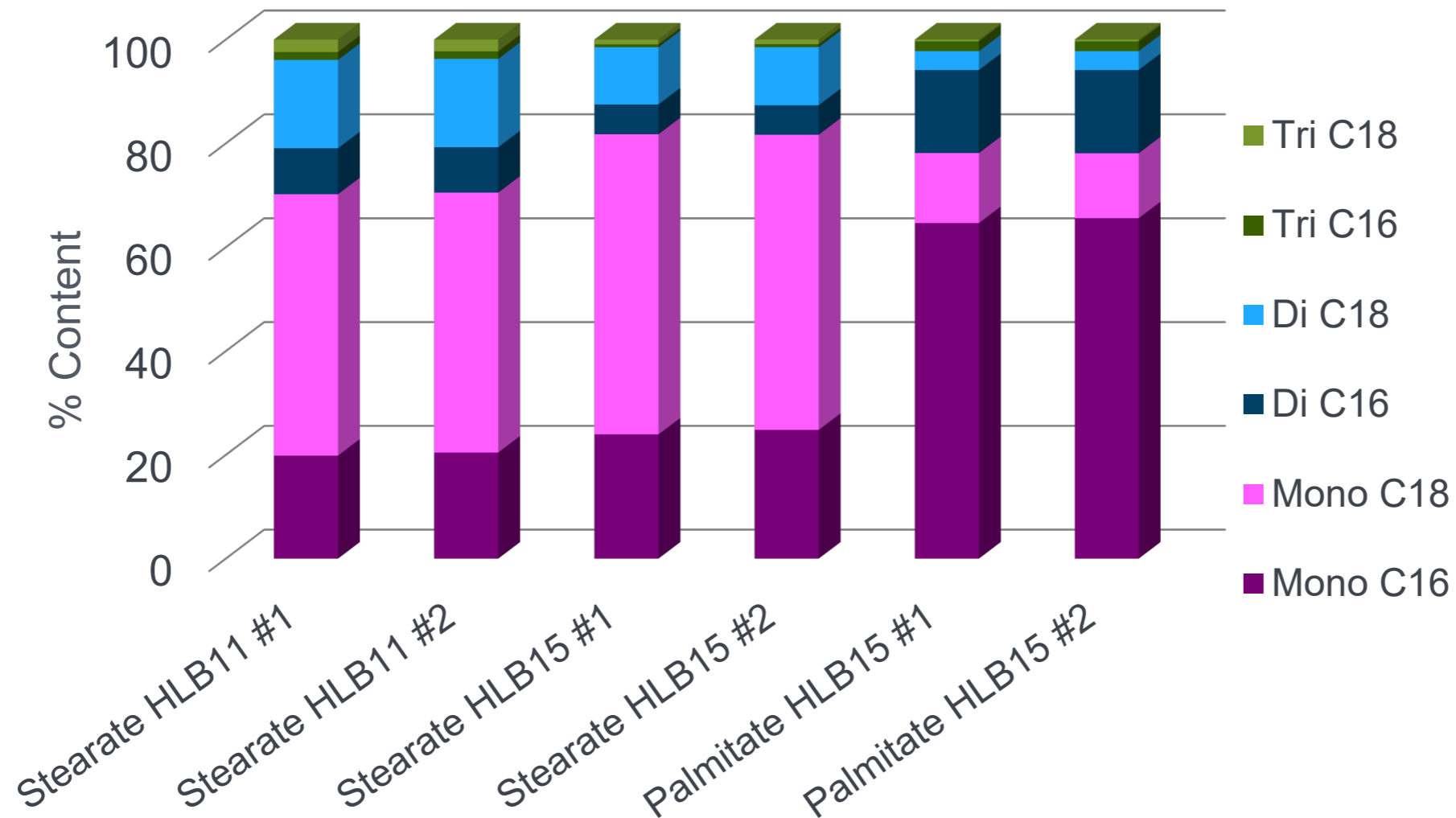


Spectrum of SE 60 with intensity over time: first S16 and S18 as monoesters, second S16/16, S16/18, and S18/18 as diesters, and third S16/16/16, S16/16/18, S16/18/18, and S18/18/18 as triesters

- “Sorbitan monostearate” (Span 60) contains the monoesters sorbitan monostearate and sorbitan monopalmitate.
- In addition, it also contains more than 50% di- and tri-esters. These have significantly lower polarity leading to lower HLB values and water solubility.

Further Excipients: Sucrose stearates (sugar esters)

- Sucrose stearates are a mixture of mono-, di-, and a small amount of tri-esters
- Higher HLB types (HLB15) show a lower amount of di-esters than HLB11
- In “stearates” (C18) a relevant amount of palmitate (C16) and in “palmitate” a relevant amount of stearate is present
- At the same HLB (15), palmitates require a lower amount of mono-esters than stearates



Further Excipients

Further complex pharmaceutical excipients that we can analyze using modern methods to gain insights that help to speed up and de-risk development projects include:

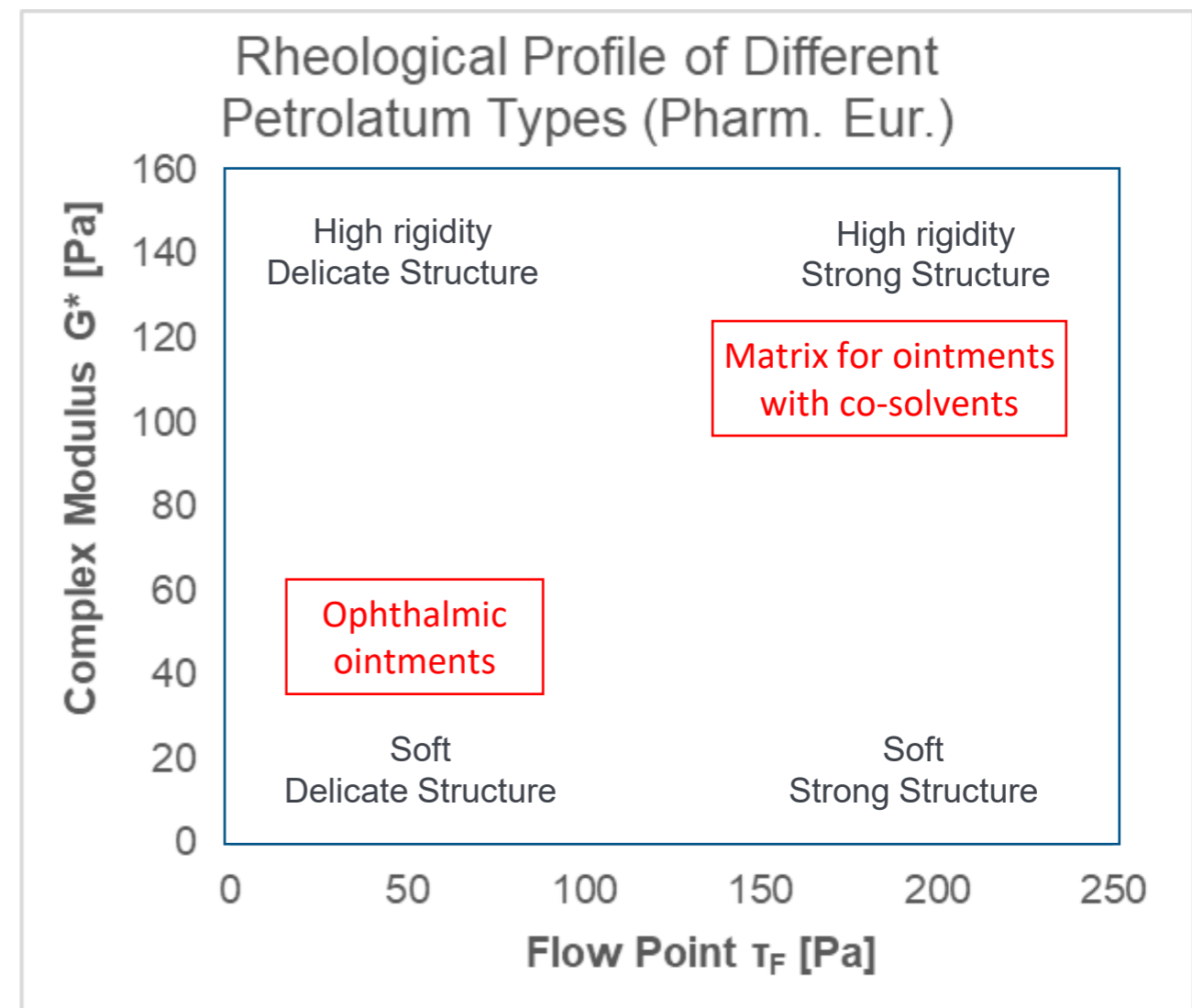
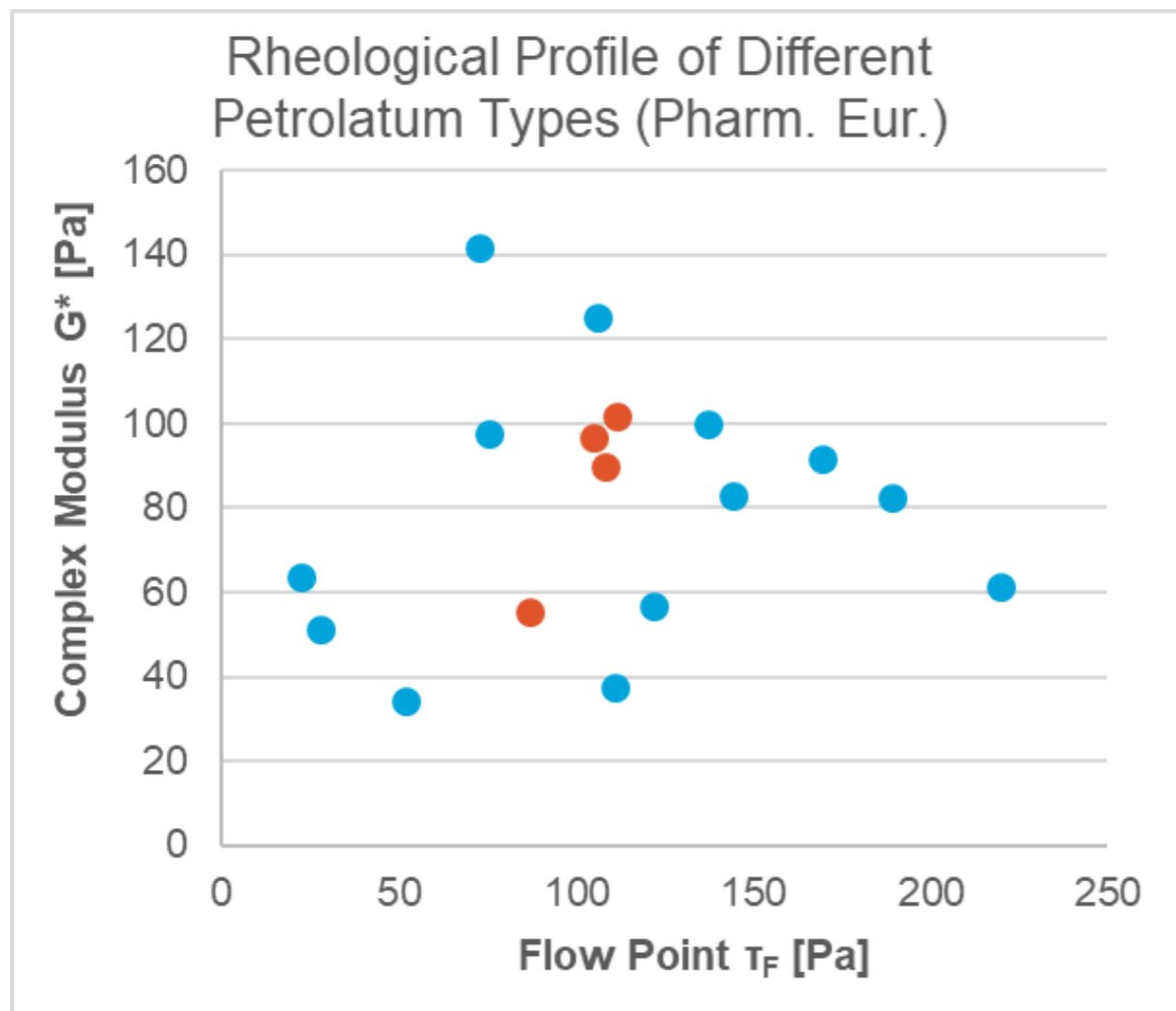
- Mono- & di-glycerides
 - Triglycerides, such as MCT
 - Petrolatum & other hydrocarbons
- **contact us if you work with a complex excipients you want to understand better!**

Our in-depth knowledge and experience in this field enables us to efficiently develop additional methods for other complex excipients.

Physical characterization of semi-solid excipients

Complex, semi-solid excipients, such as petrolatum, can be characterized by rheological analysis:

- Even with the pharmacopeial specifications, petrolatum varies significantly in rheological properties
- These differences may impact critical formulation attributes and process parameters
- To achieve equivalence in generic development, it is crucial to identify the right petrolatum variant



RaDes GmbH – Contact

If you are interested to learn more about how we can support your projects by our leading capabilities in excipient analytics, contact us!

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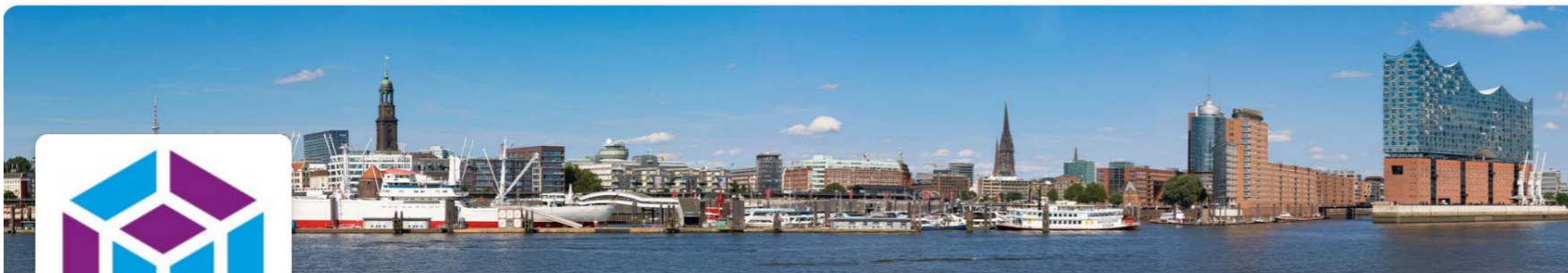
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