ANALYTICAL CHARACTERIZATION OF POLYSORBATE 80

Case Study

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November 2020

In it for life

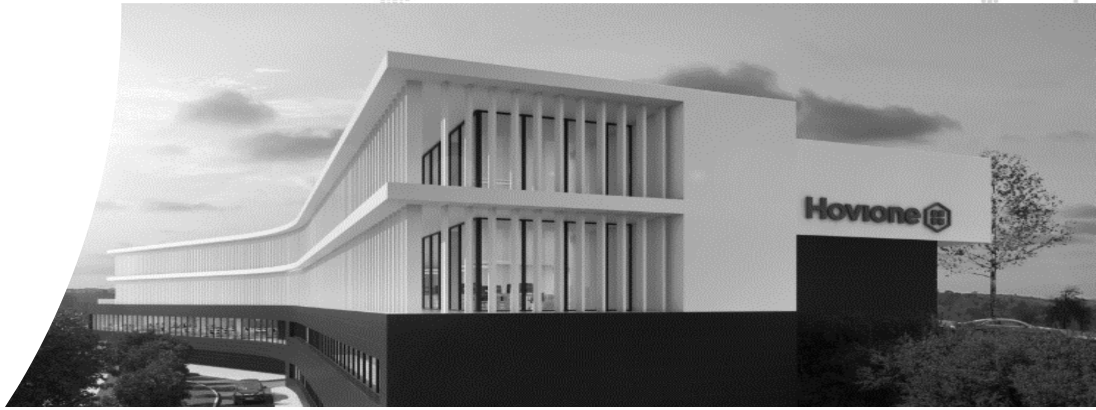
POLYSORBATES AT HOVIONE

With 4 US FDA inspected facilities, Hovione is committed to deliver biopharma excipients with outstanding quality

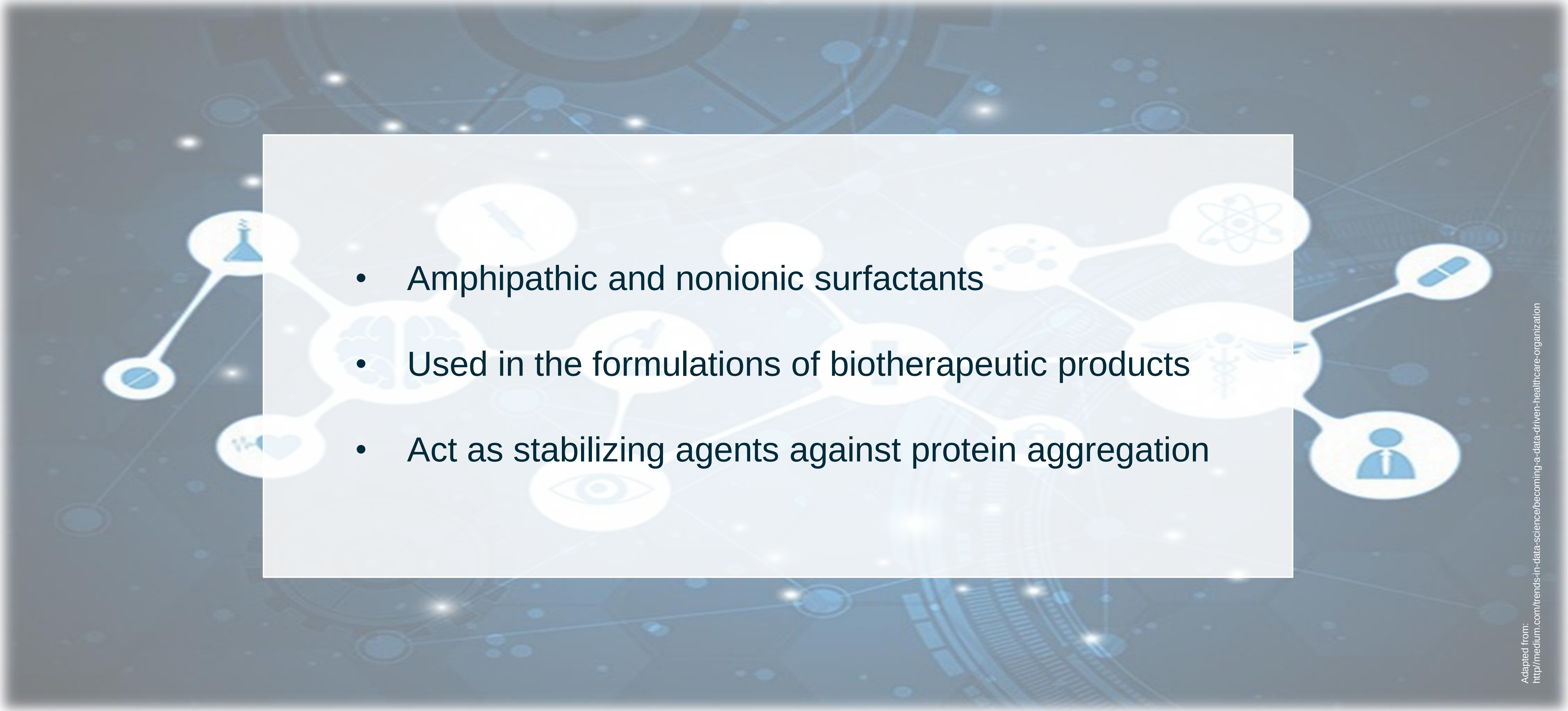
As a solution seeking company, we embraced the challenging domain of polysorbates addressing the issue it presents in the formulation of biopharmaceuticals

Case study:

Hovione has conducted a mapping of commercial Polysorbate 80 and compared those with Hovione´s Biopharma grade



POLYSORBATES



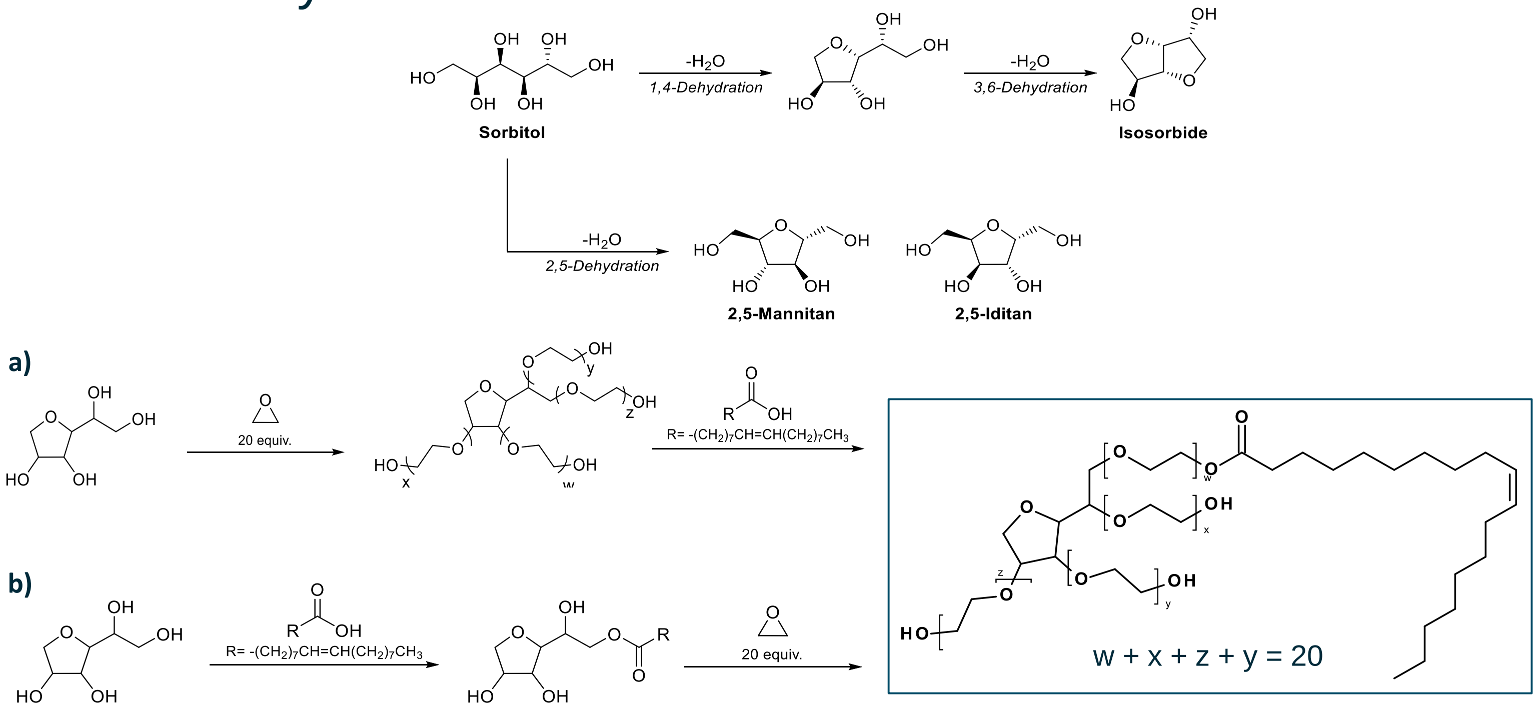
- Amphipathic and nonionic surfactants
- Used in the formulations of biotherapeutic products
- Act as stabilizing agents against protein aggregation

Adapted from:
http//medium.com/trends-in-data-science/becoming-a-data-driven-healthcare-organization

a) *"Effect of Polysorbate 80 Concentration on Thermal and Photostability of a Monoclonal Antibody",* AAPS PharmSciTech, Vol. 14, No. 1, March 2013
b) *"Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways"* 2007 American Pharmacists Association

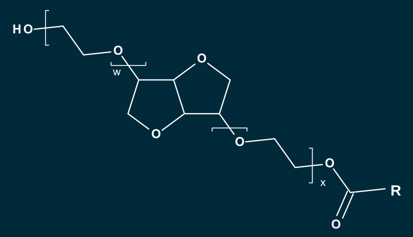
POLYSORBATE 80

Common synthesis root:

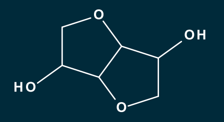


Polysorbate 80

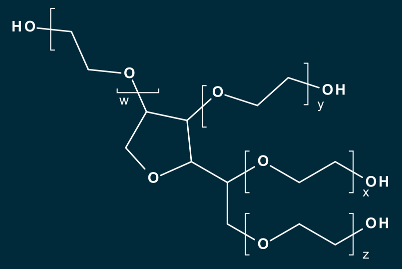
Polysorbates present a variety of components such as partial esters of fatty acids, sorbitol and its anhydrides along with approximately 20 polymerized ethylene oxide units



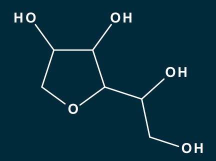
POE Isosorbide ester



Isosorbide

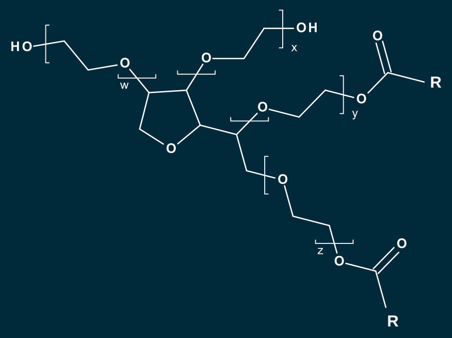


POE Sorbitan

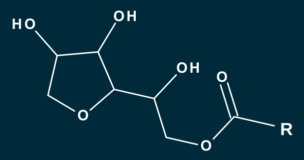


Sorbitan

\+



POE Sorbitan diester



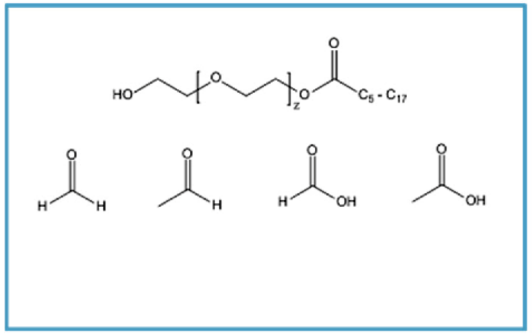
Sorbitan ester

Chapter 5 :Acid-Catalyzed Dehydration of Sorbitol to 1,4-Sorbitan, 2016

CHALLENGES

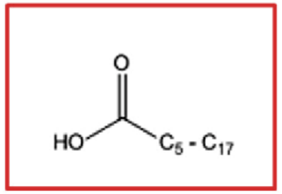
Polysorbates Degradation

- Oxidation resulting in hydroperoxide formation and side chain cleavage
- Hydrolysis and release of free fatty acids



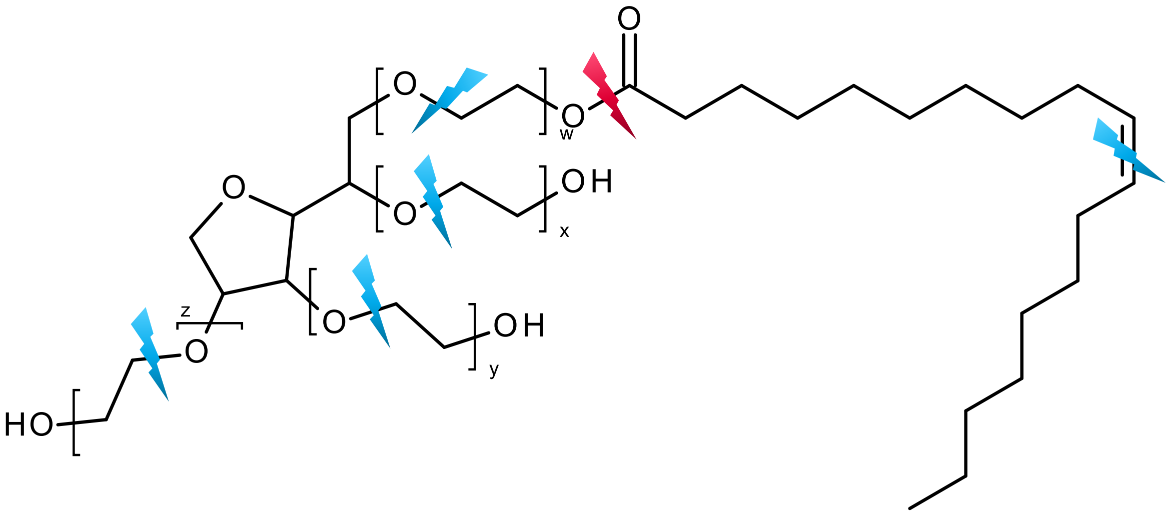
Oxidation

Fatty Acid Esters
Aldehydes
Ketones
Acids
Peroxides
Alkanes



Hydrolysis

Fatty Acids



a) *Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways"* 200t7 American Pharmacists Association
b) Adapted from: *"Novel markers to track oxidative polysorbate degradation in pharmaceutical formulations"*, Journal of Pharmaceutical and Biomedical Analysis, Volume 157, 5 August 2018, Pages 201-207

Analytical Selection

- **Compendial methods**

Further characterization:

- **Volatiles** finger printing by GC-FID
- Polysorbate **profile** by HPLC-PDA
- **Fatty acids** screening by GC-FID
- **Peroxide** quantification by HPLC and UV/VIS using catalase
- **Elemental impurities** by ICP-MS

Case Study

Adapted from:
https://www.overtec.it/articoli/analisi-acque/analisi-acque
https://www.watertestingservicesllc.com/

VOLATILE IMPURITIES

VOLATILES FINGER PRINTING BY GC

Degradation products:

Alkyl aldehydes and Alkanes

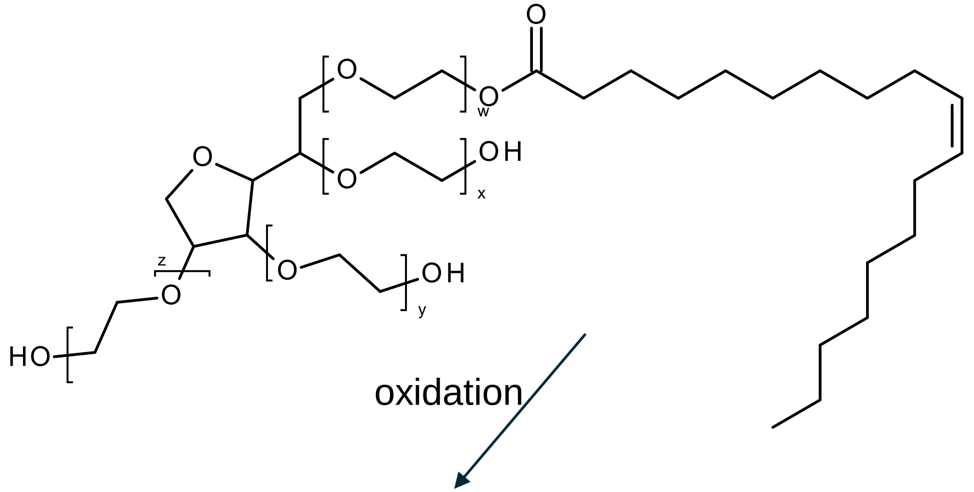
- Oxidative scission in the vicinity of the olefin

Formates and esters

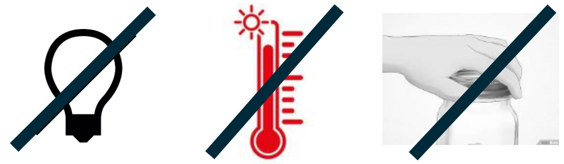
- Peroxide scission in the propagation step of POE autoxidation

Dicarboxylic Acids

- Degradants of oleic acid



Acids	Aldehydes
n-alkanes	Ketones
Formates	Esters



a) "*Degradation of polysorbates 20 and 80: studies on thermal autoxidation and hydrolysis.*", J Pharm Sci. 2011 Feb
b) "*Polysorbate Degradation and Quality*", Challenges in Protein Product Development pp 25-62,21 June 2018

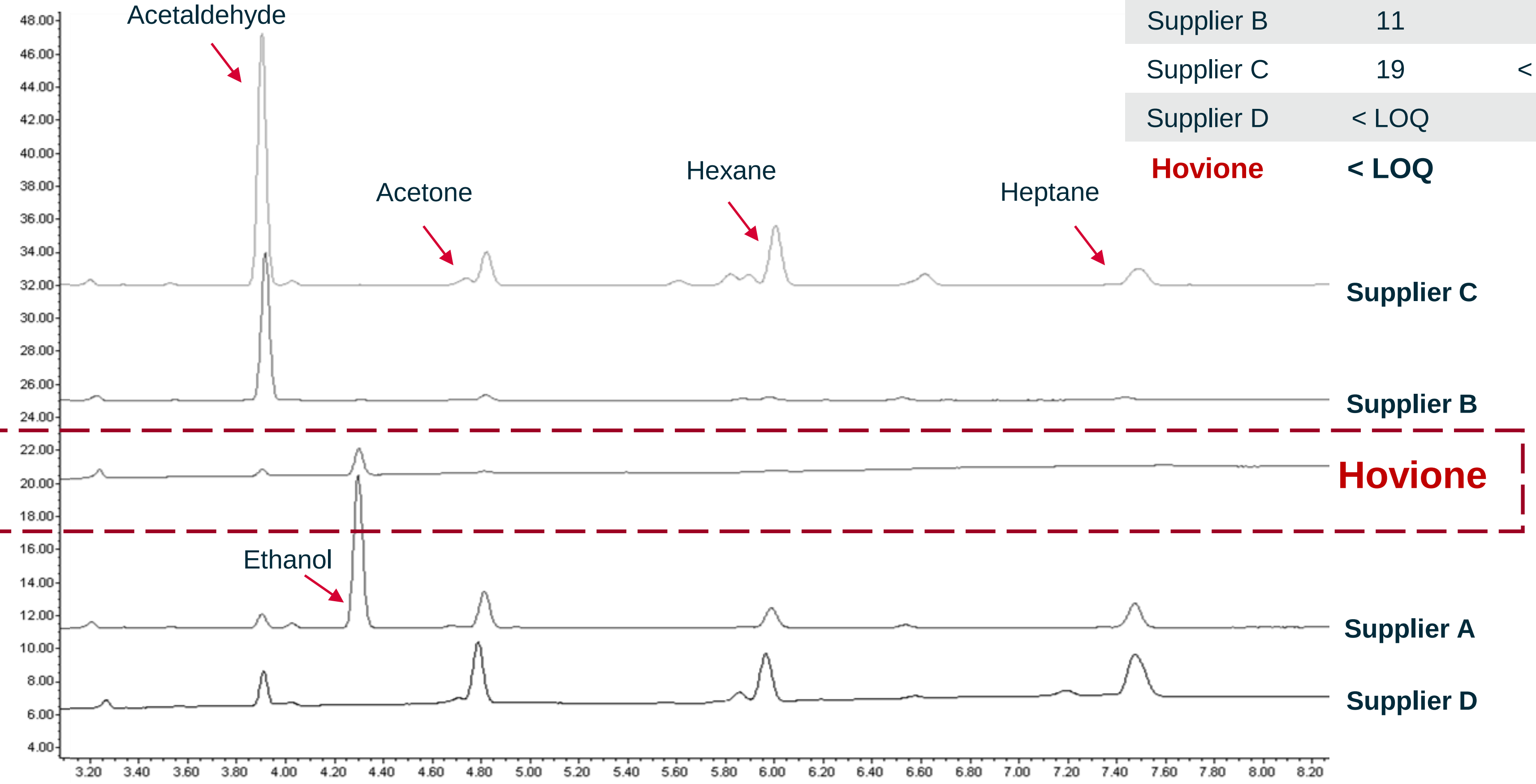
VOLATILES FINGER PRINTING BY GC

Supplier to Supplier variability

Method 1: GC-Headspace

	Volatiles (ppm)				
	Acetaldehyde	Ethanol	Acetone	Hexane	Heptane
Supplier A	< LOQ	58	< LOQ	< LOQ	< LOQ
Supplier B	11	ND	< LOQ	< LOQ	< LOQ
Supplier C	19	< LOQ	< LOQ	< LOQ	< LOQ
Supplier D	< LOQ	ND	< LOQ	< LOQ	< LOQ
Hovione	**< LOQ**	**11**	**ND**	**ND**	**ND**

ND: Not detected



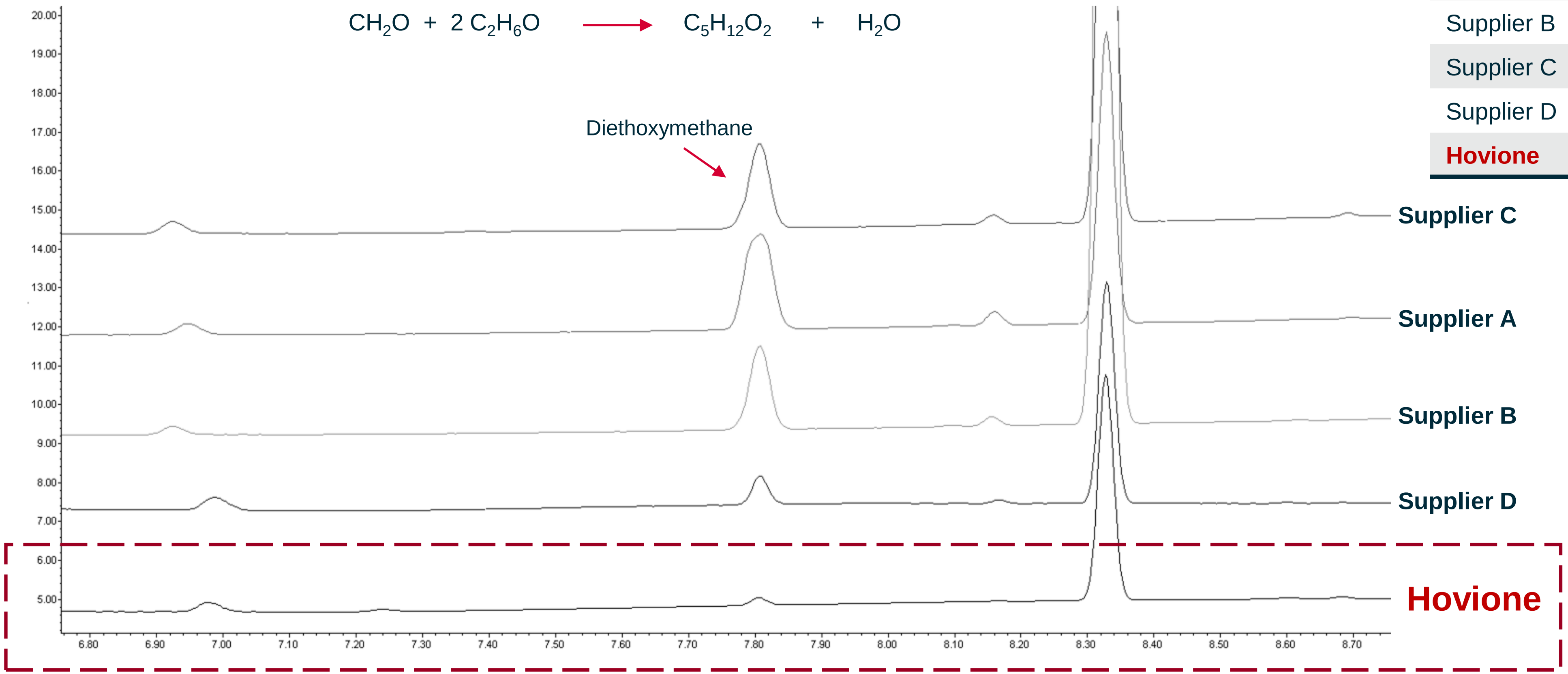
Adapted from EP Polysorbate monograph: Ethylene oxide and dioxane impurities

VOLATILES FINGER PRINTING BY GC

Method 2: GC-Headspace for Formaldehyde

Formaldehyde Ethanol Diethoxymethane

$CH_2O + 2\,C_2H_6O \rightarrow C_5H_{12}O_2 + H_2O$



Supplier to Supplier variability

	Formaldehyde (ppm)
Supplier A	8
Supplier B	6
Supplier C	2
Supplier D	**At LOQ level**
Hovione	**< LOQ**

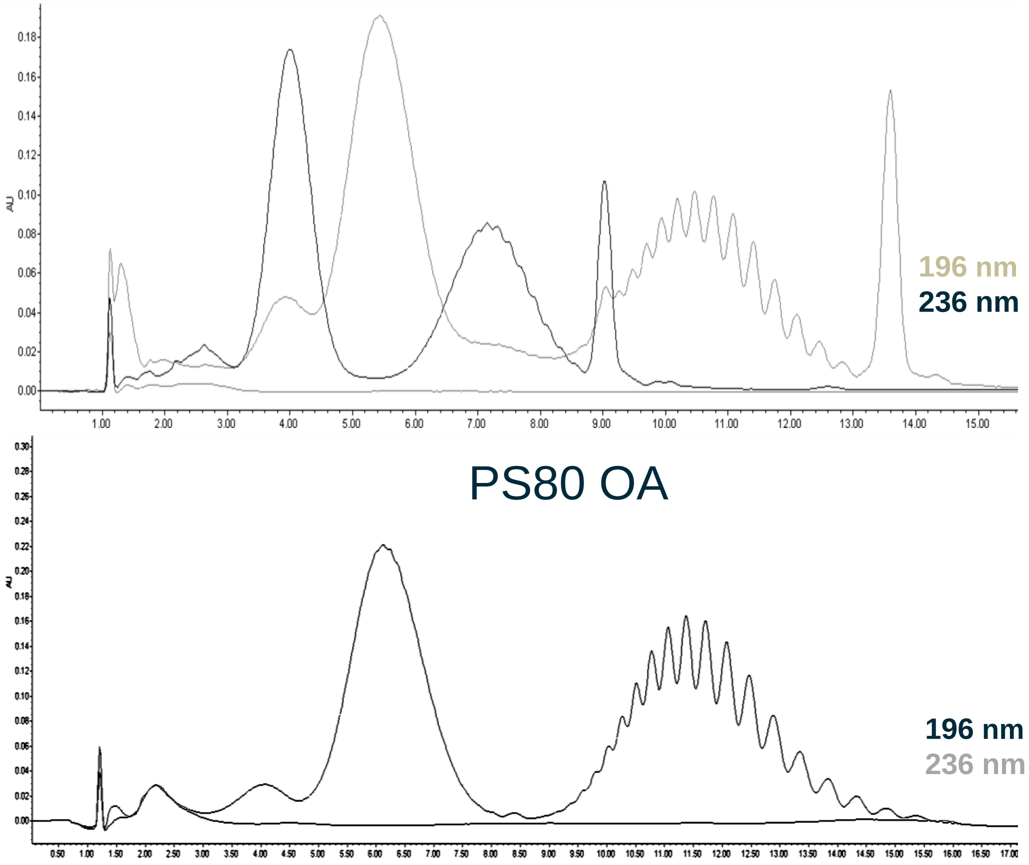
Adapted from: *"A Robust Static Headspace GC-FID Method to Detect and Quantify Formaldehyde Impurity in Pharmaceutical Excipients"* 2018

PROFILE

PROFILE ASSESSMENT

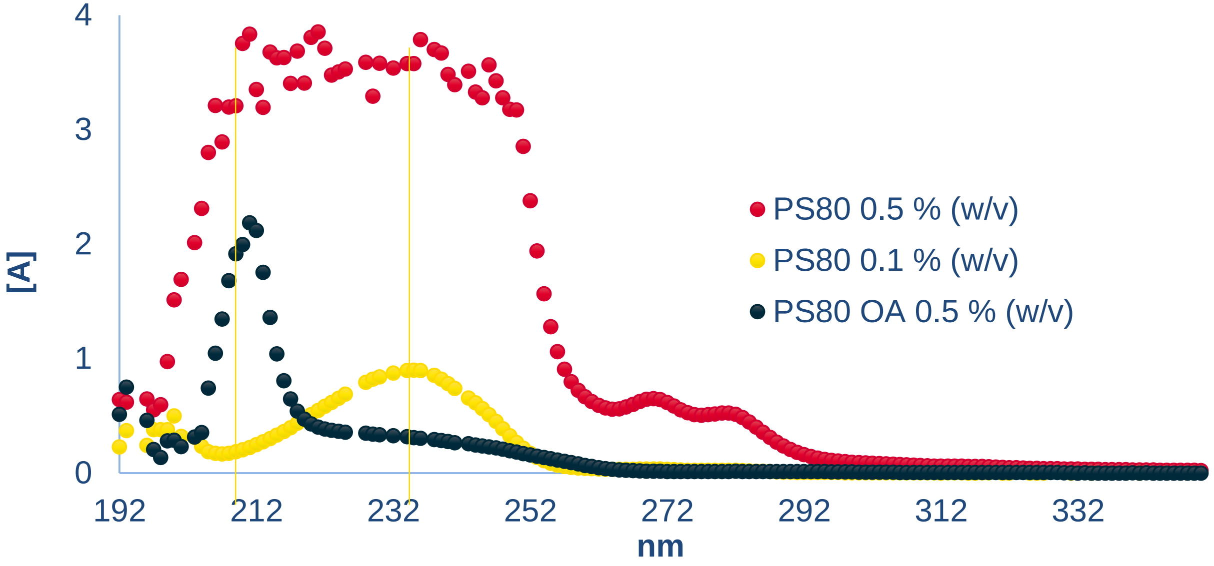
Method: HPLC-PDA and UV

PS80



Eluting at **196 nm**: PEG chains, Sorbitan, Sorbitol dehydration impurities esterified with one or more oleic acid chain

Eluting at **236 nm**: PEG chains, Sorbitan, Sorbitol dehydration impurities esterified with other esters (such as C18:2) depending on the purity of the oleic acid used

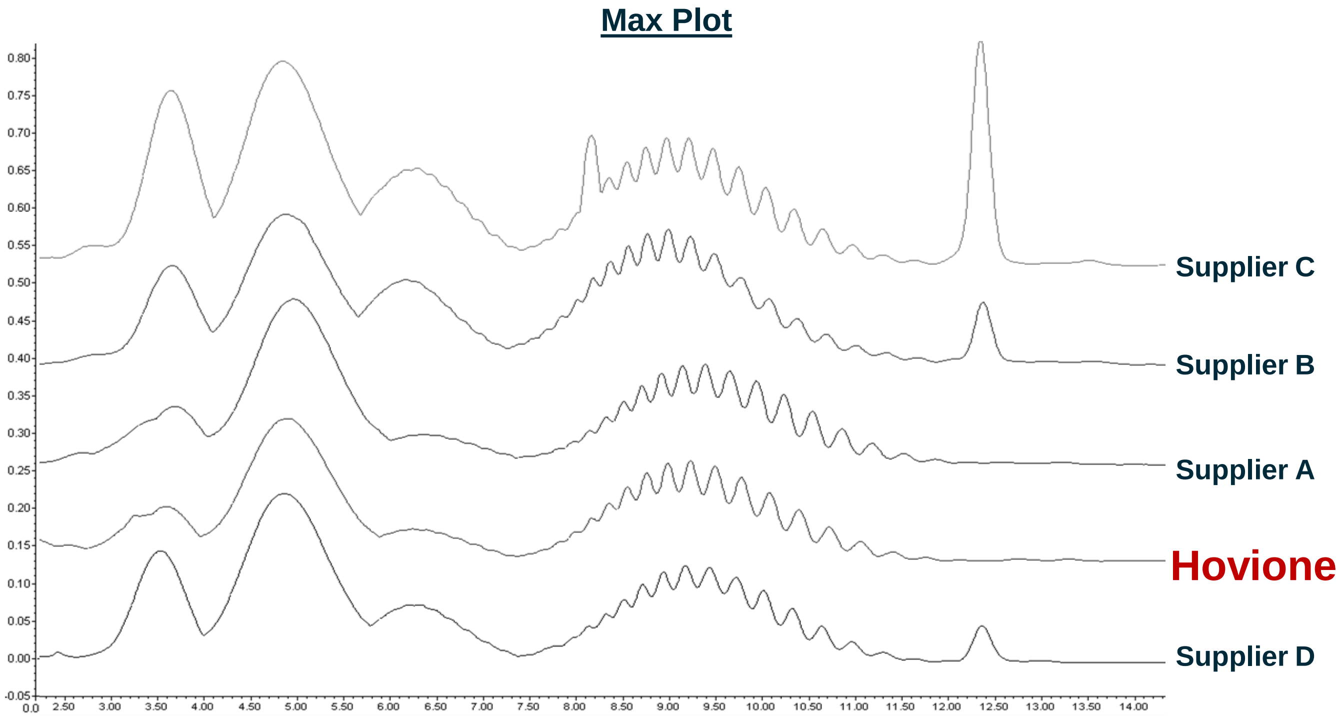


Adapted from: White paper (Agilent): Analysis of Tween 80 by high performance liquid chromatography with diode array detection (**2018**), 1-6

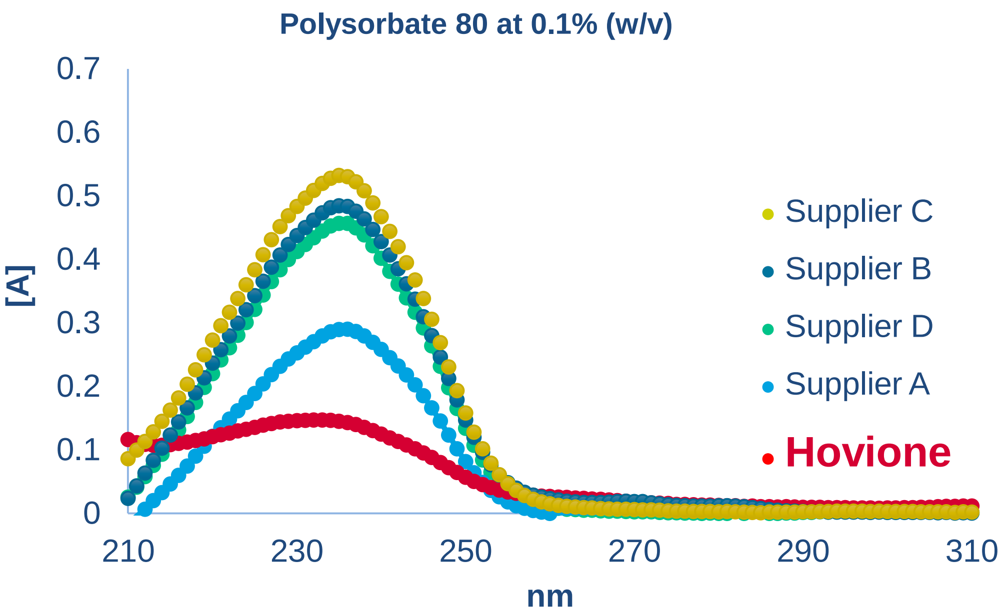
Adapted from: Characterization of polysorbate 80 with liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopy: Specific determination of oxidation products of thermally oxidized polysorbate 80 (**2011**)

Supplier to Supplier Variability

Method: HPLC-PDA for Profile



Supplier to Supplier variability



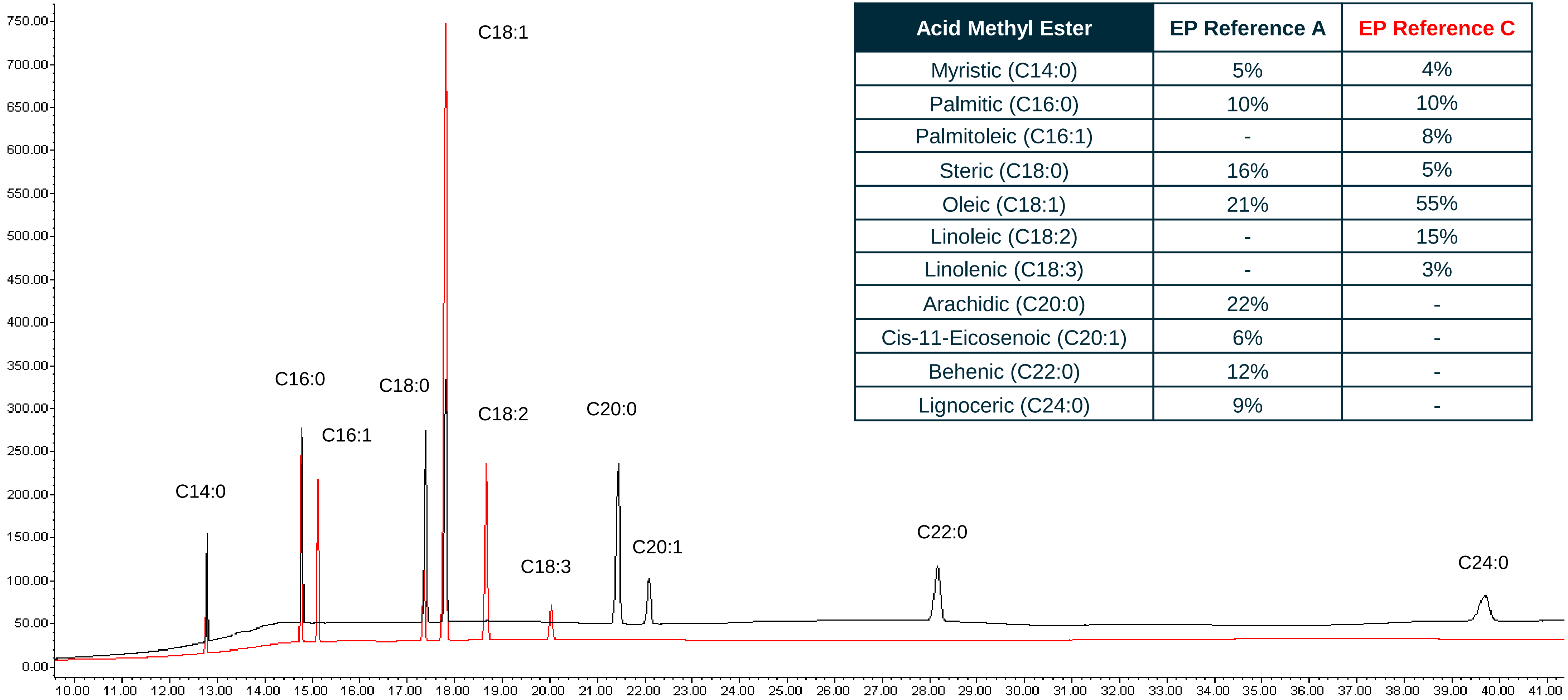
Adapted from: White paper (Agilent): Analysis of Tween 80 by high performance liquid chromatography with diode array detection (2018), 1-6

FATTY ACIDS

Adapted from: https://gmdexports.com//

FATTY ACIDS SCREENING BY GC

Method: GC-FID



Acid Methyl Ester	EP Reference A	EP Reference C
Myristic (C14:0)	5%	4%
Palmitic (C16:0)	10%	10%
Palmitoleic (C16:1)	-	8%
Steric (C18:0)	16%	5%
Oleic (C18:1)	21%	55%
Linoleic (C18:2)	-	15%
Linolenic (C18:3)	-	3%
Arachidic (C20:0)	22%	-
Cis-11-Eicosenoic (C20:1)	6%	-
Behenic (C22:0)	12%	-
Lignoceric (C24:0)	9%	-

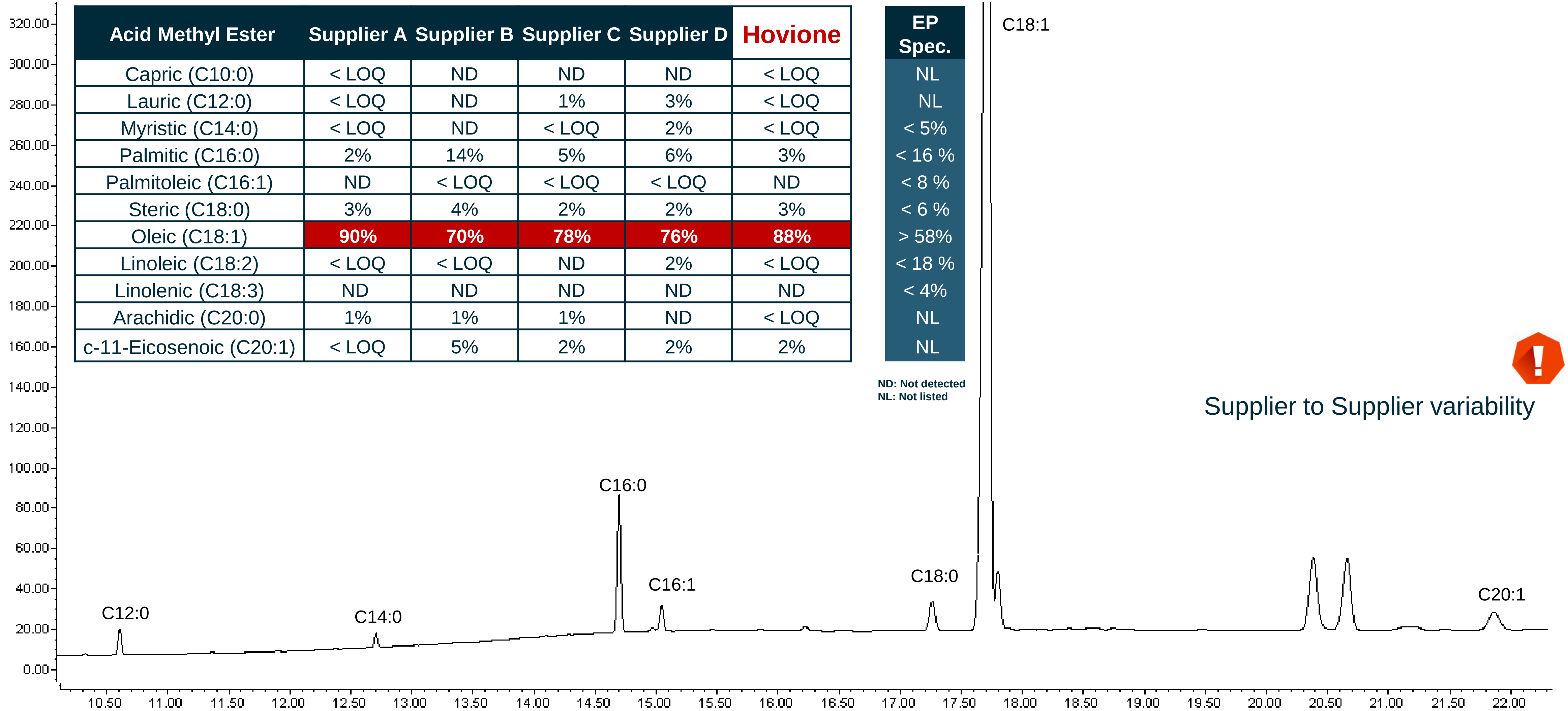
Adapted from EP Polysorbate monograph: Composition of fatty acids

Fatty Acids screening by GC

Acid Methyl Ester	Supplier A	Supplier B	Supplier C	Supplier D	Hovione	EP Spec.
Capric (C10:0)	< LOQ	ND	ND	ND	< LOQ	NL
Lauric (C12:0)	< LOQ	ND	1%	3%	< LOQ	NL
Myristic (C14:0)	< LOQ	ND	< LOQ	2%	< LOQ	< 5%
Palmitic (C16:0)	2%	14%	5%	6%	3%	< 16 %
Palmitoleic (C16:1)	ND	< LOQ	< LOQ	< LOQ	ND	< 8 %
Steric (C18:0)	3%	4%	2%	2%	3%	< 6 %
Oleic (C18:1)	**90%**	**70%**	**78%**	**76%**	**88%**	> 58%
Linoleic (C18:2)	< LOQ	< LOQ	ND	2%	< LOQ	< 18 %
Linolenic (C18:3)	ND	ND	ND	ND	ND	< 4%
Arachidic (C20:0)	1%	1%	1%	ND	< LOQ	NL
c-11-Eicosenoic (C20:1)	< LOQ	5%	2%	2%	2%	NL

ND: Not detected
NL: Not listed

Supplier to Supplier variability



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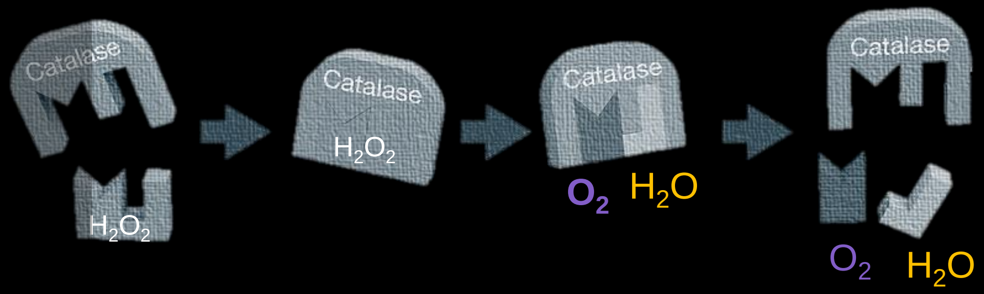
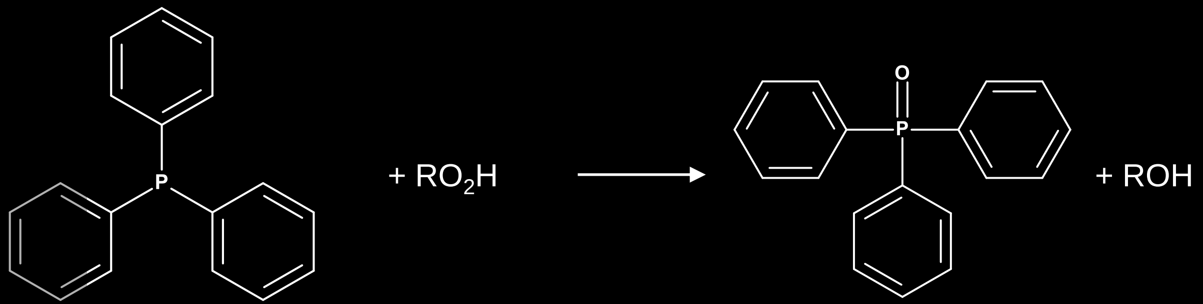


Adapted from : https://fineartamerica.com/featured/action-of-catalase-on-hydrogen-peroxide-mikkel-juul-jensen.html

Peroxides



HYDROPEROXIDES CONTENT BY UV/VIS

Method 1: UV/VIS

Fox Assay II Methodology

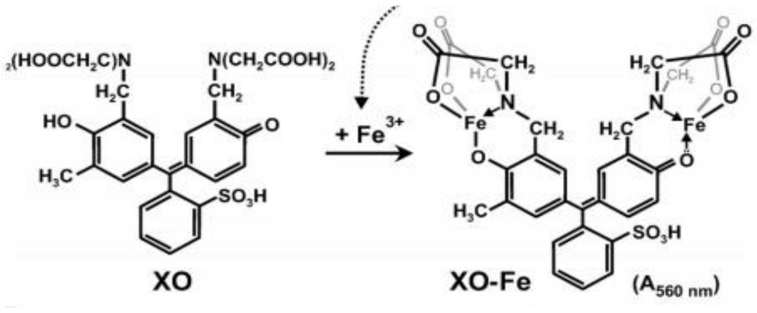
Total hydroperoxides content

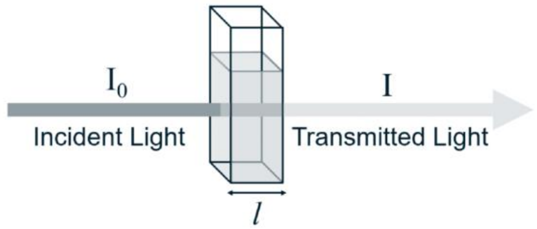
- Reduction of Hydroperoxides by Fe (II) under acid conditions

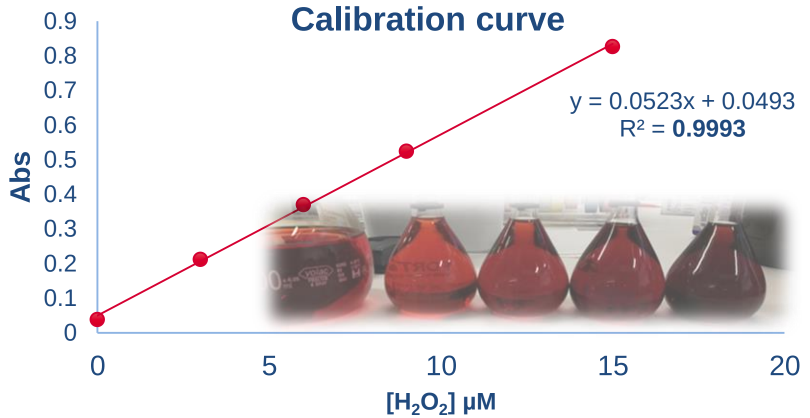
$$Fe^{+2} + H_2O_2 \dashrightarrow Fe^{+3} + OH^- + HO^\cdot$$

$$Fe^{+2} + ROOH \dashrightarrow Fe^{+3} + OH^- + RO^\cdot$$

- The resulting ferric ion forms a strong complex with xylenol orange







- The complex has maximum absorbance at 560 nm

Adapted from : "*Method for the simultaneous determination of free/protein malondialdehyde and lipid/protein hydroperoxides*", *Free Radical Biology and Medicine 2013*
Adapted from: "*Hydroperoxide Assay with the Ferric–Xylenol Orange Complex*", School of Biological Sciences, Macquarie University, Sydney 2109, Australia, 1998
Adapted from: https://www.edinst.com/blog/the-beer-lambert-law/

HYDROPEROXIDES CONTENT BY UV/VIS

Method 1: UV/VIS

Catalase Methodology

Alkoxy peroxides content

- Catalase reacts with the free peroxide but not with the alkoxy peroxide

$$ROOH + 2\,H_2O_2 \xrightarrow[\text{Quenching}]{\text{Catalase}} ROOH + 2H_2O + O_2$$

- The quenched sample is then analyzed by the Fox Assay II

Free peroxides content

- The difference between the total hydroperoxides and the alkoxy peroxides gives the amount of free peroxide

Adapted from: "*Evaluation of Hydroperoxides in Common Pharmaceutical Excipients*", Pharmaceutical Analysis Chemistry, Merck Research Laboratories,2006

HYDROPEROXIDES CONTENT BY UV/VIS

	Total Hydroperoxides (ppm)	ROOH (ppm)	ROOH (%)	H_2O_2 (%)
Supplier A	68	33	49	51
Supplier B	120	46	38	62
Supplier C	148	65	44	56
Supplier D	412	172	42	58
Hovione	64	36	44	56

- Our findings suggest that nearly **60%** of the peroxides present in Polysorbate 80 are $\mathbf{H_2O_2}$

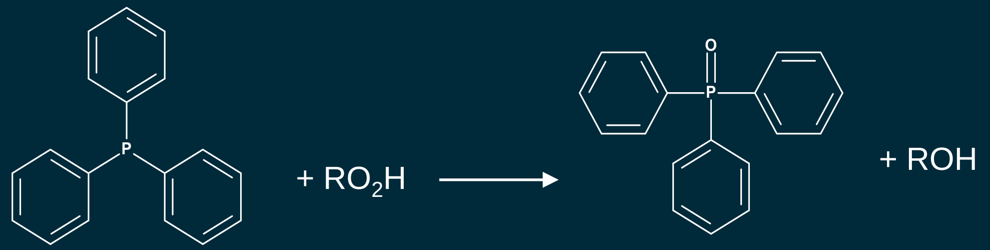
Supplier to Supplier variability

HYDROPEROXIDES CONTENT BY LC

Method 2: HPLC-PDA

- **Triphenylphosphine** (TPP) reacts rapidly and quantitatively with H_2O_2 and ROOH to yield 1 mole of Triphenylphosphine oxide (TPPO) for each mole of peroxides reacted

- Polysorbates are added to a standard solution of 0.1 mg/mL TPP in methanol. The sample is then allowed to react for about **15 min** prior to HPLC analysis



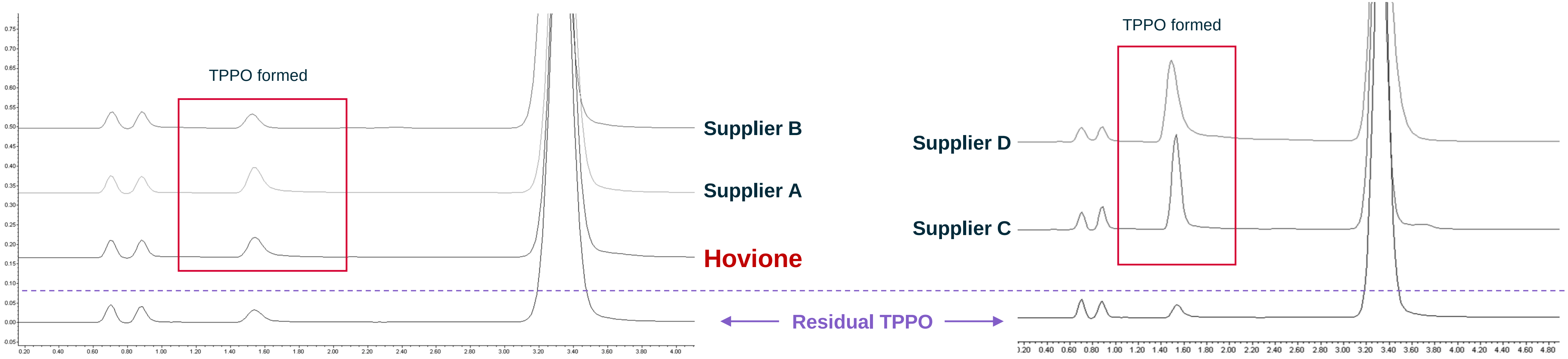
Challenge

- TPP undergoes **slow air oxidation,** gradually oxidizing to TPPO even in the absence of peroxides

Adapted from: *"Evaluation of Hydroperoxides in Common Pharmaceutical Excipients"*, Pharmaceutical Analysis Chemistry, Merck Research Laboratories,16 June 2006

HYDROPEROXIDES CONTENT BY LC

Challenge

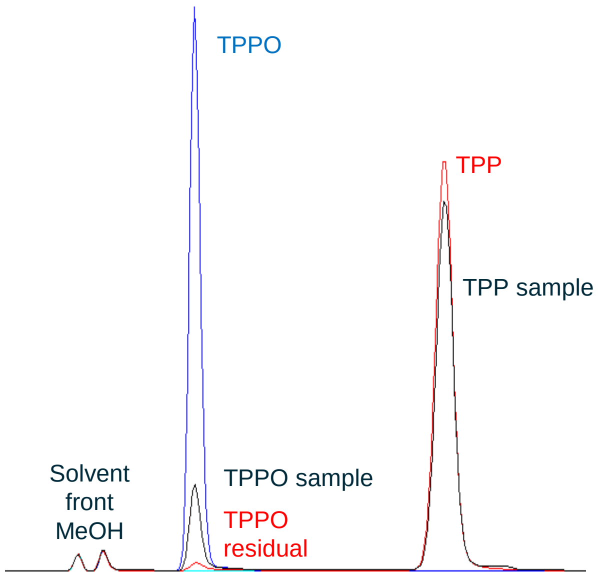


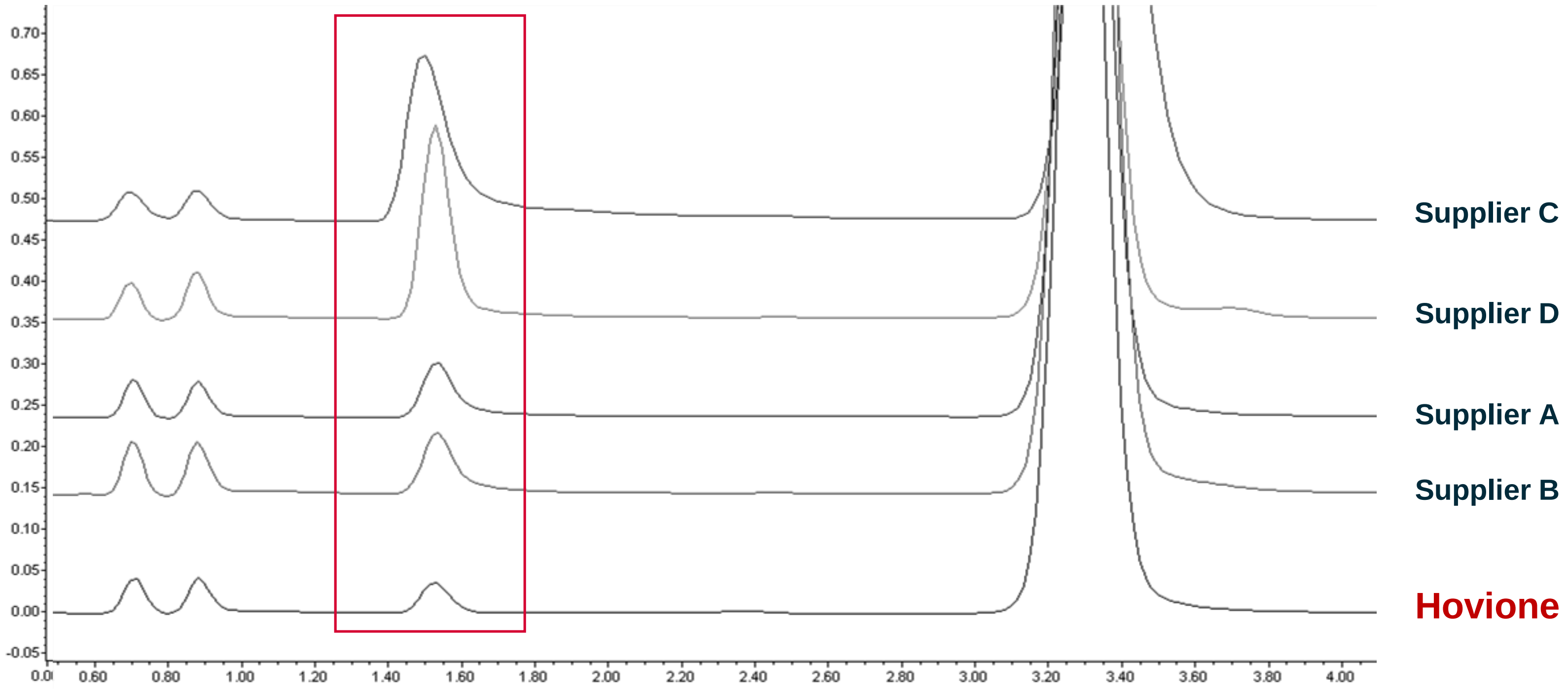
- Low peroxide content is difficult to quantify accurately due to the residual TPPO
- Method suitability for low peroxide samples should be assessed

HYDROPEROXIDES CONTENT BY LC

Supplier to Supplier variability

Example





- Qualitative method: fast and easy to set up
- Fast peroxide screening

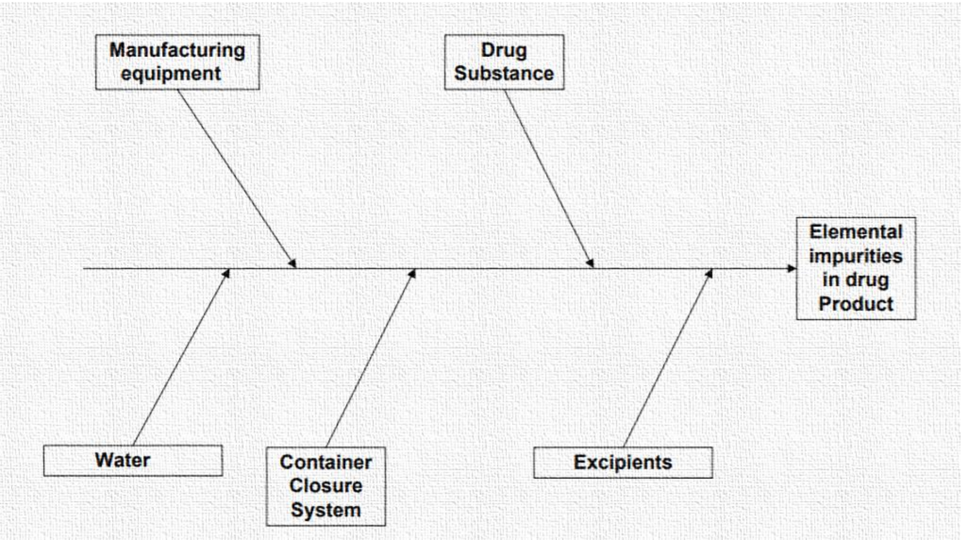
Time between sample preparation and injection should be constant

ELEMENTAL IMPURITIES

Adapted from:
https://br.freepik.com
https://pt.dreamstime.com

Elemental impurities by ICP-MS

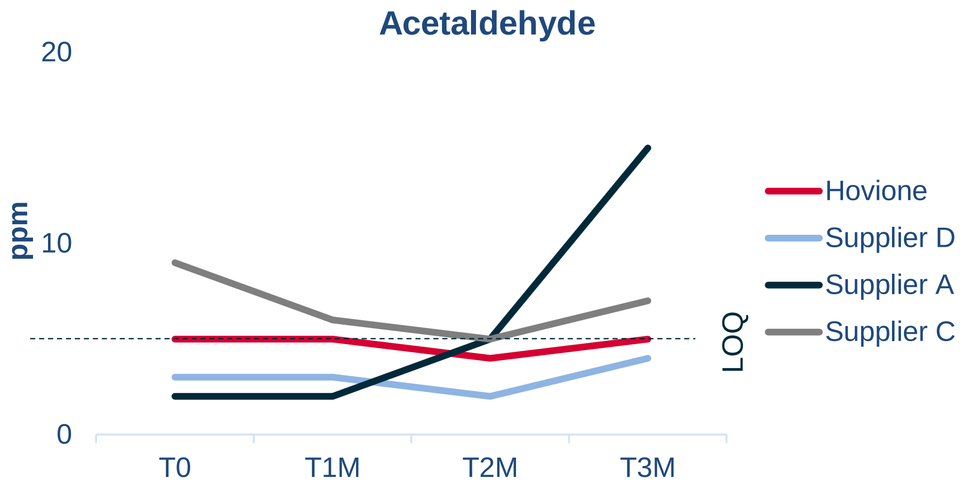
Method: ICP-MS

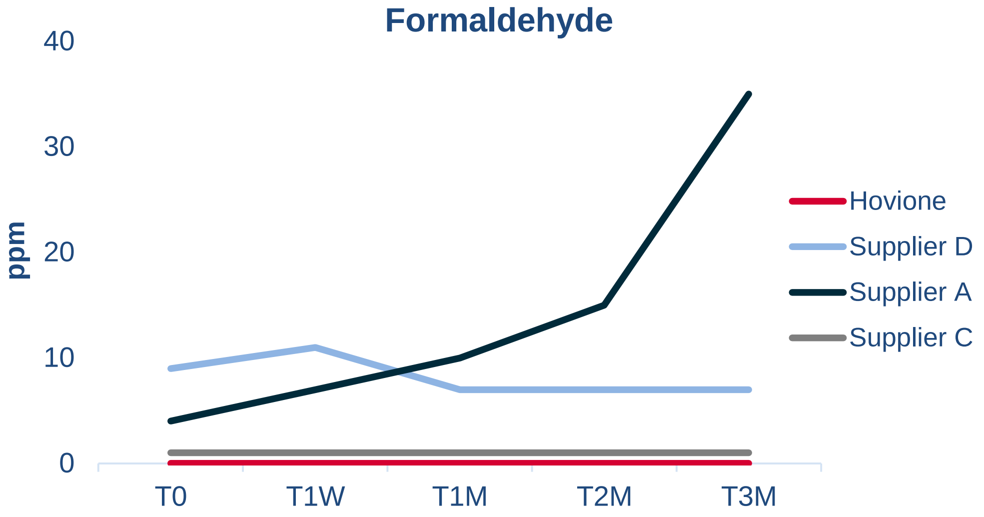


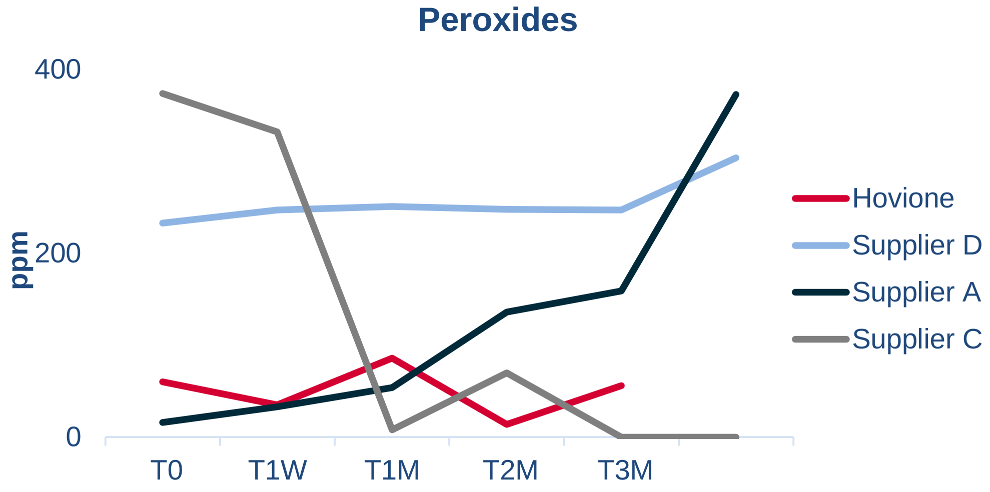
Elemental Impurities	Specifications (parenteral) ppm	Supplier A ppm	Supplier B ppm	Supplier C ppm	Hovione ppm
Cd	≤ 0.2	< LOQ (0.148)	< LOQ (0.148)	< LOQ (0.148)	< LOQ (0.148)
Pb	≤ 0.5	< LOQ (0.071)	< LOQ (0.071)	< LOQ (0.071)	< LOQ (0.071)
As	≤ 1.5	< LOQ (0.137)	< LOQ (0.137)	< LOQ (0.137)	< LOQ (0.137)
Hg	≤ 0.3	0.093	0.096	0.094	0.094
Co	≤ 0.5	< LOQ (0.006)	< LOQ (0.006)	< LOQ (0.006)	< LOQ (0.006)
V	≤ 1	< LOQ (0.060)	< LOQ (0.060)	< LOQ (0.060)	< LOQ (0.060)
Ni	≤ 2	< LOQ (0.174)	< LOQ (0.174)	< LOQ (0.174)	< LOQ (0.174)
Li	≤ 25	< LOQ (2.359)	< LOQ (2.359)	< LOQ (2.359)	< LOQ (2.359)
Sb	≤ 9	< LOQ (0.456)	< LOQ (0.456)	< LOQ (0.456)	< LOQ (0.456)
Cu	≤ 30	< LOQ (2.701)	< LOQ (2.701)	< LOQ (2.701)	< LOQ (2.701)
Mo	≤ 150	< LOQ (8.618)	< LOQ (8.618)	< LOQ (8.618)	< LOQ (8.618)

Adapted from: http://www.shimadzu.com.br/analitica/cursos/icp-ms/curso_icp-ms-1.shtml
ICH guideline Q3D (R1) : https://www.ich.org/page/quality-guidelines

Stability Data







Conclusions

Volatiles by GC-FID: Degradation Products

- Understanding product stability, purification and identify degradation agents

Profile by HPLC-PDA: Variability indicator

- Stability studies throughout samples and supply chain fingerprinting

Peroxides by HPLC and UV/VIS: Peroxide determination

- Critical attribute in product quality and stability, since it is the major responsible for oxidative degradation of Polysorbate containing formulations

Fatty acids by GC-FID: Fatty acids screening

- Supply chain fingerprinting

Elemental impurities by ICP-MS: Trace metals

- Compliance with ICH guidelines

Thank you for your attention

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