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High-Performance Thin-Layer Chromatography analysis of E 471 and E 472 emulsifiers: analytical approaches and challenges

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Analysis of E 471 and E 472 emulsifiers – agenda

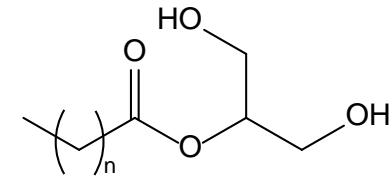
- Introduction
 - What are E 471 and E 472 emulsifiers and challenges with their use?
 - Why high-performance thin-layer chromatography (HPTLC)?
- Analysis of E 471 emulsifiers by HPTLC
 - fingerprint analysis and quantification in whipping cream
 - changes during storage
- Analysis of E 472 emulsifiers by HPTLC
 - fingerprint analysis and quantification in foamed foods
 - automated data evaluation

Introduction

Introduction: What are E 471 and E 472 emulsifiers?

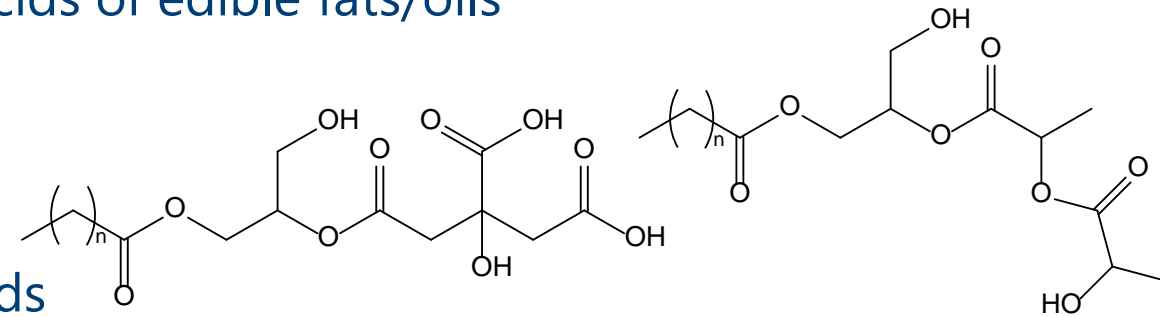
E 471 emulsifiers

- mono- and diacylglycerols (MG/DG) of fatty acids of edible fats/oils
- "pure" MG or mixtures of MG/DG



E 472 emulsifiers

- organic/fruit acid esters of MG/DG of fatty acids



Group	Name	Abbreviation
E 472a	Acetic acid esters of MG/DG of fatty acids	ACETEM
E 472b	Lactic acid esters of MG/DG of fatty acids	LACTEM
E 472c	Citric acid esters of MG/DG of fatty acids	CITREM
E 472d	Tartaric acid esters of MG/DG of fatty acids	TARTREM
E 472e	Mono and diacetyl tartaric acid esters of MG/DG of fatty acids	DATEM
E 472f	Mixed acetic and tartaric acid esters of MG/DG of fatty acids	MATEM

→ Used in various foods (e.g., dairy products)



Introduction: Challenge with E 471 and E 472 emulsifiers

(Highly) complex mixtures

- no characterized purified substances
- composition varies depending on raw material/production
- different by-products (glycerol, free fatty acids (FFA))

Variations in composition

- variations in techno-functionality
- affecting food quality

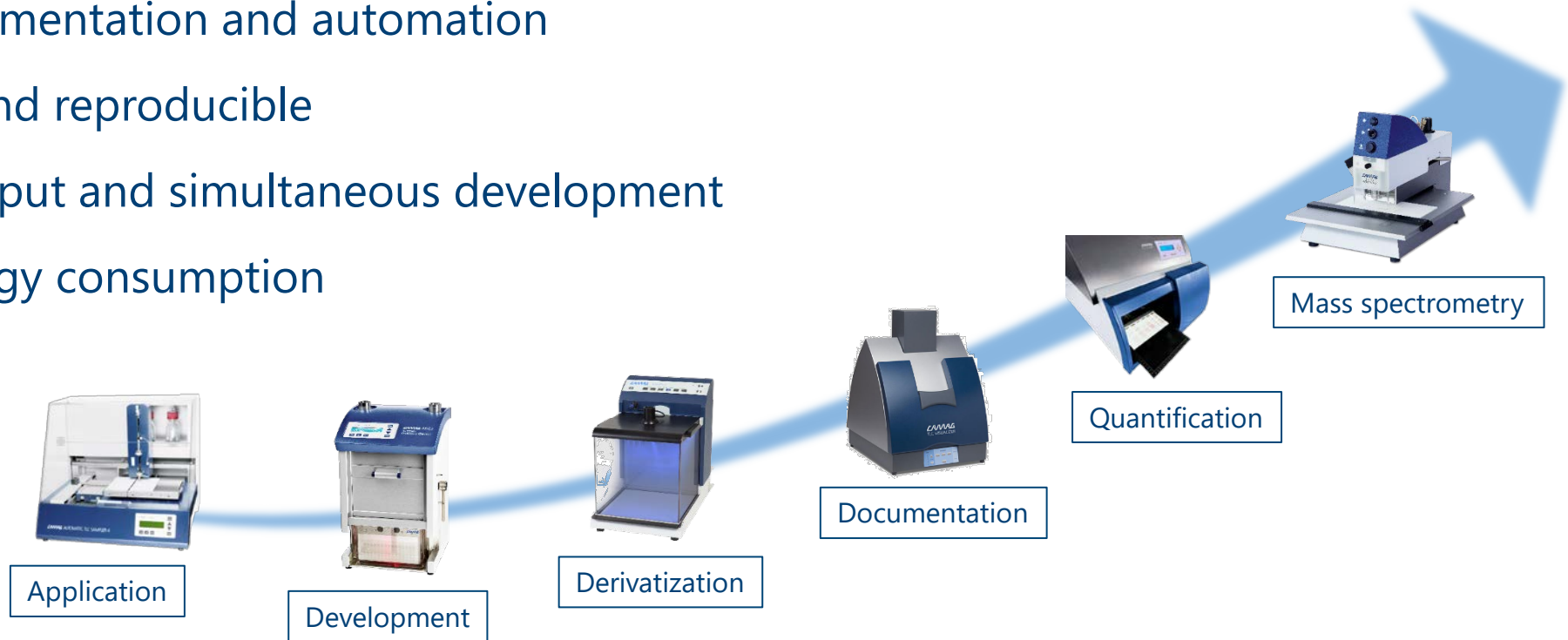


→ **fast** and **easy-to-perform** methods for **E 471** and **E 472** analysis needed

- characterization of the composition
- quantification in food (dairy products, foamed food formulations)

High-performance thin-layer chromatography (HPTLC)

- separation of analytes on planar thin-layers
- many stationary/mobile phases, selective detection options
- high degree of instrumentation and automation
- selective, sensitive, and reproducible
- high sample throughput and simultaneous development
- low solvent and energy consumption
- fingerprint analysis

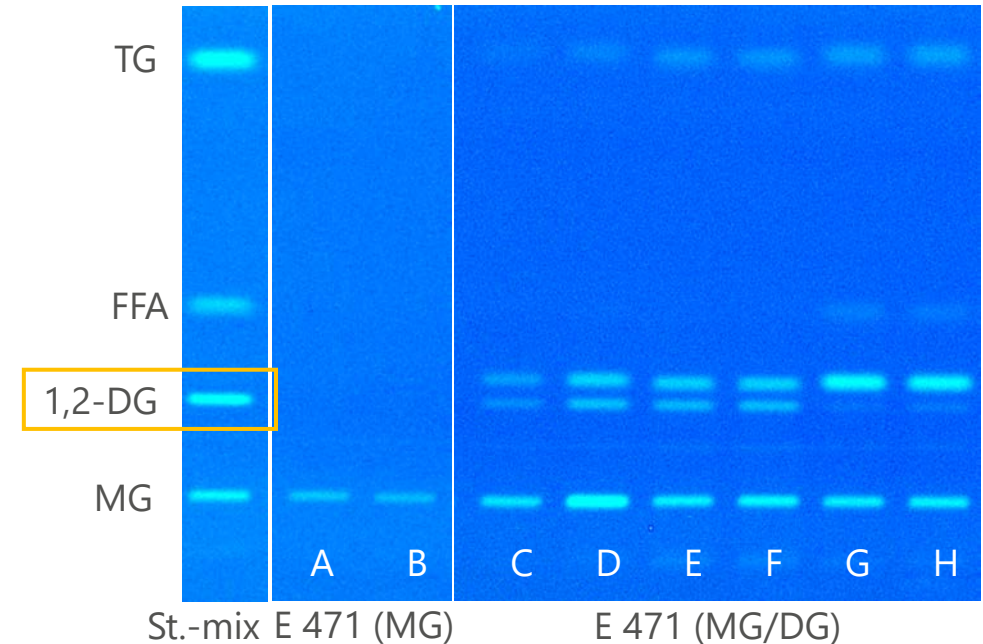


Analysis of E 471 emulsifiers by HPTLC

Analysis of E 471 emulsifiers – fingerprint and quantification

HPTLC–FLD^[1]

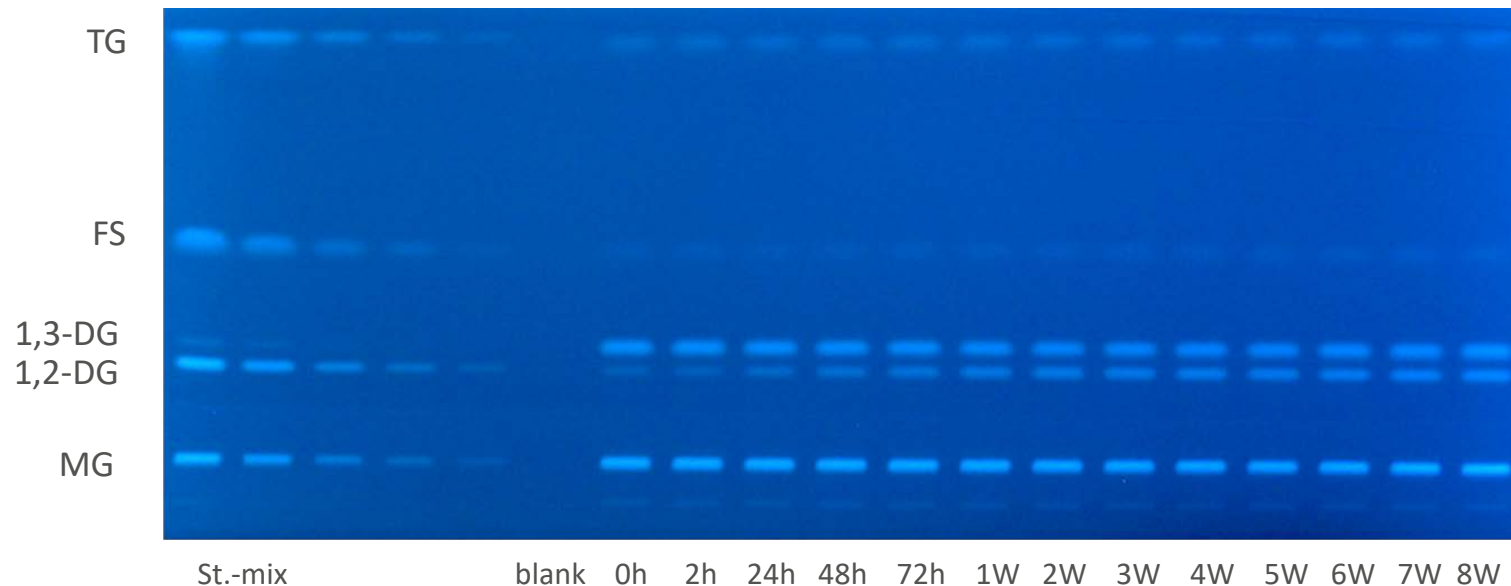
- layer: Lichrospher HPTLC silica gel
- impregnation: fluorescent dye (primuline)
- development: 1st: diethyl ether
2nd: *n*-pentane/*n*-heptane/diethyl ether
- detection: fluorescence scan at UV 366/>400 nm



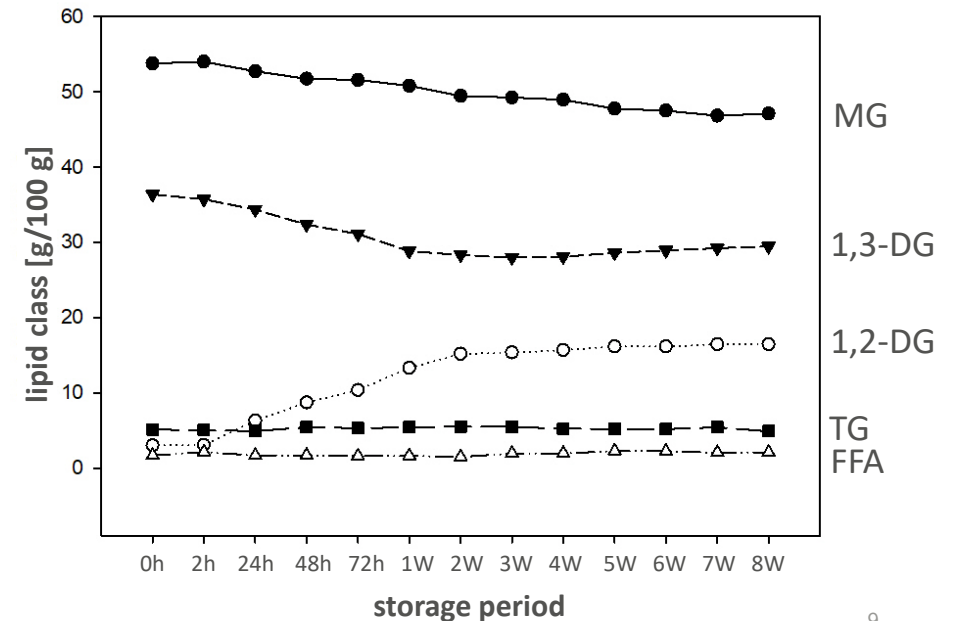
- separation of substance classes of E 471
- **simple-to-perform visual analysis (fingerprint)**, fast comparison between products/lots
- **quantification** based on one calibration substance (1,2-DG), sum value for each class^[2]

Analysis of E 471 emulsifiers – changes during storage

- Influence of high temperature (70-80°C) during storage
 - chemical composition/techno-functional properties (whipping cream)
 - example: MG/DG emulsifier, mainly saturated free fatty acids (FFA)^[3]



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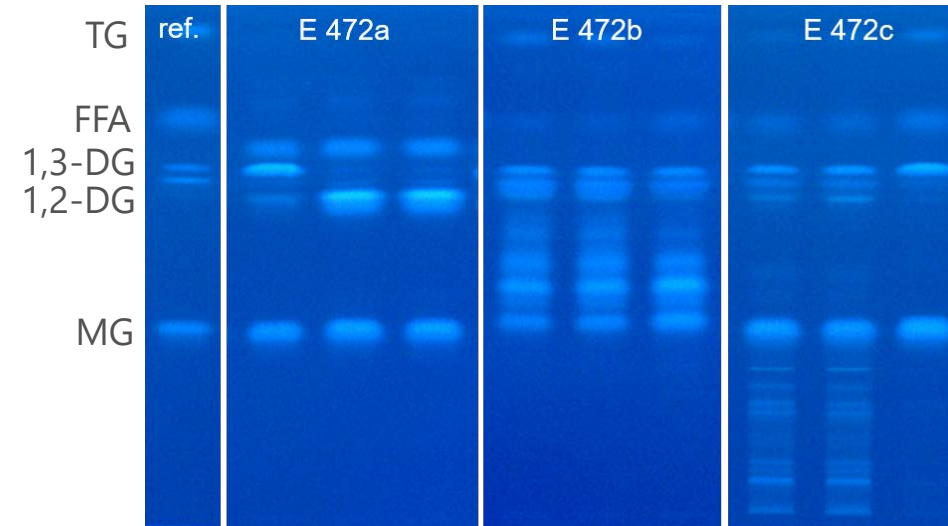
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Analysis of E 472 emulsifiers by HPTLC

Analysis of E 472 emulsifiers – fingerprint

HPTLC–FLD^[4]

- layer: HPTLC silica gel
- development: 1st: chloroform/methanol/H₂O/formic acid
2nd: *n*-heptane/diethyl ether/formic acid
- derivatization: fluorescent dye (primuline)
- detection: fluorescence scan at UV 366/>400 nm

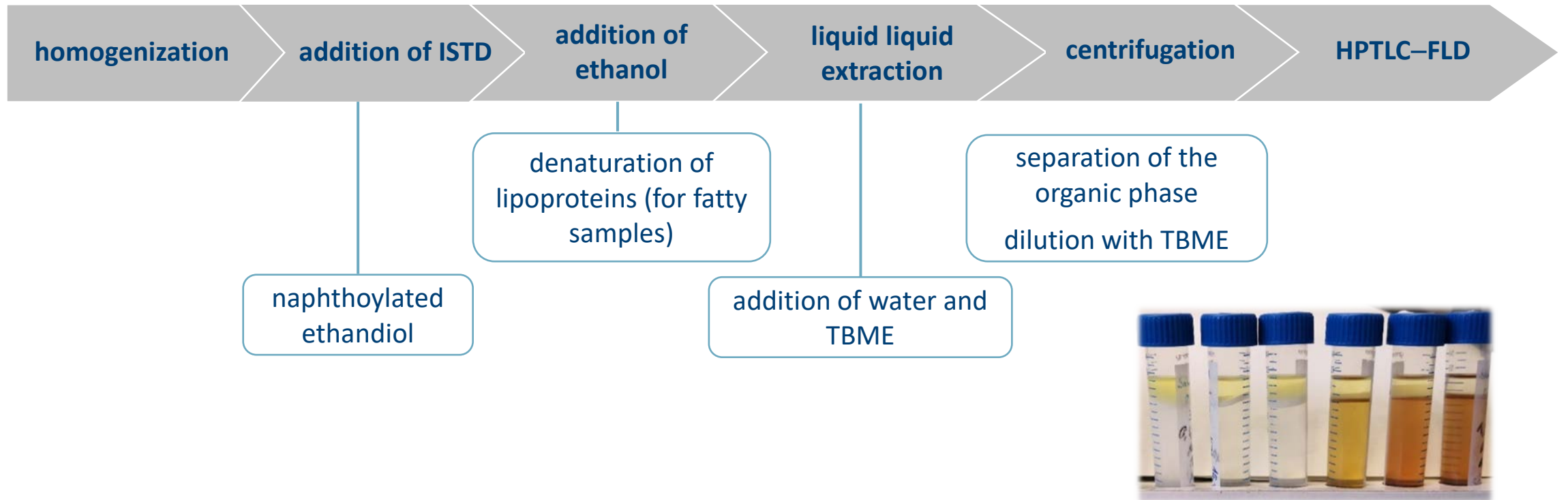


E 472 emulsifiers (samples and lots)

- separation of substance classes of E 472 subclasses a-c
- **simple-to-perform visual analysis (fingerprint)**
- **comparison** and **characterization** of E 472 emulsifiers

Analysis of E 472b emulsifiers – quantification in foamed foods

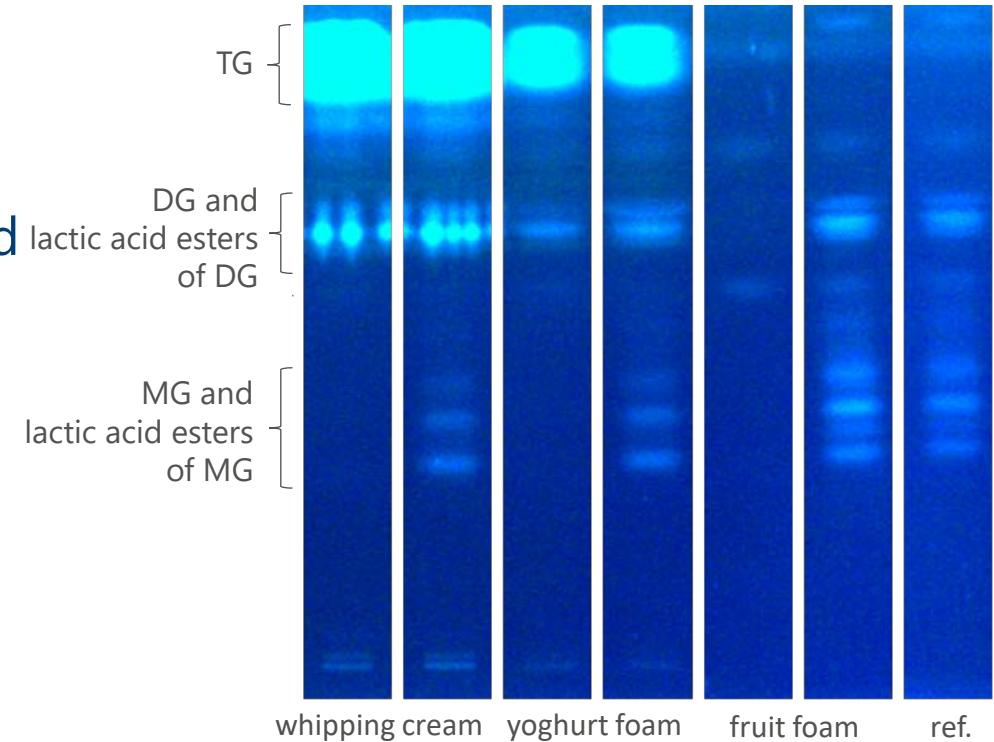
- analysis of E 472b emulsifiers in foamed food formulations^[5] – sample preparation



Analysis of E 472b emulsifiers – quantification in foamed foods

HPTLC–FLD^[5]

- layer: HPTLC silica gel 60 F₂₅₄
- development: 1st: chloroform/methanol/H₂O/formic acid
2nd: *n*-heptane/diethyl ether/formic acid
- 1st detection: absorption scan at UV 254 nm
- derivatization: fluorescent dye (primuline)
- 2nd detection: fluorescence scan at UV 366/>400 nm
- calibration: solvent standard, 0.1-2.0% E 472b



→ determination of E 472b based on MG and lactic acid esters of MG

→ amounts (sum value) in five commercial products: 110-590 mg/100 g

Analysis of E 472b emulsifiers – automated data evaluation

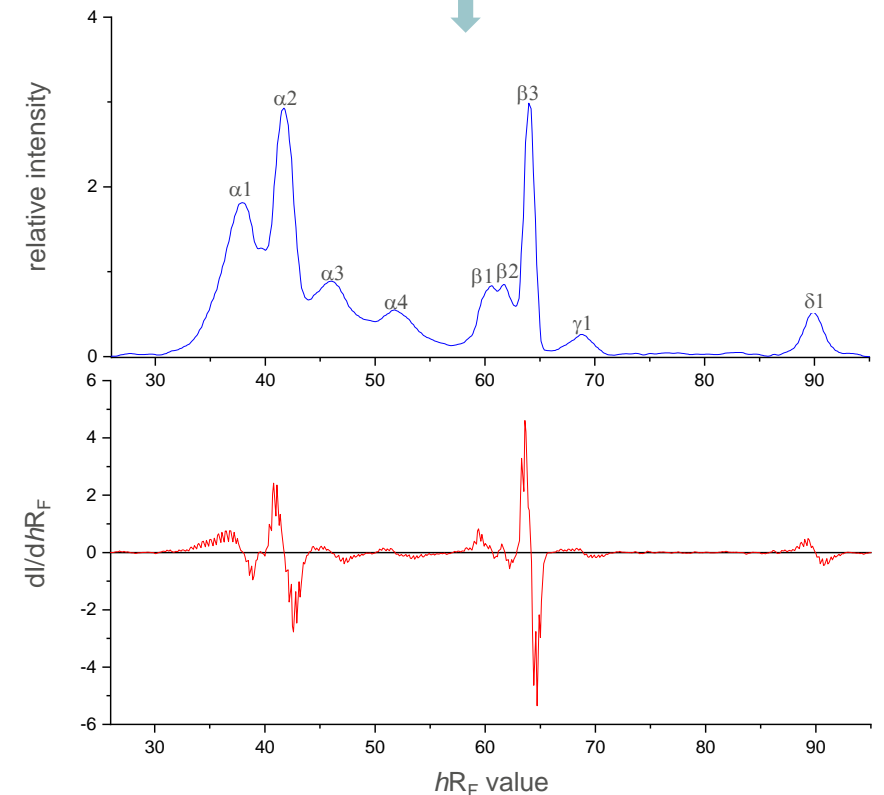
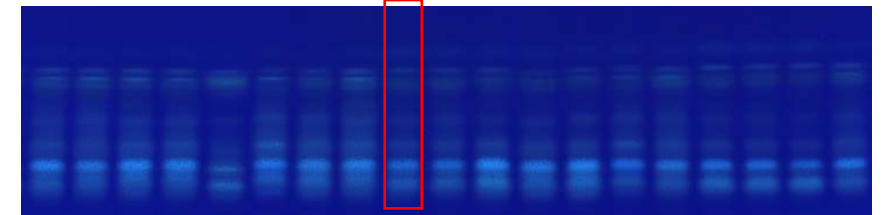
- digital fingerprint and barcode for comparison^[6]

selection of a set of emulsifiers ($n = 21$)

6 fold analysis of emulsifiers by HPTLC–FLD
reproducible hR_F values? ✓
reproducible fingerprints? ✓

signal selection (α - δ) and data preprocessing (ISTD)

determination of maximal signal intensities



Analysis of E 472b emulsifiers – automated data evaluation

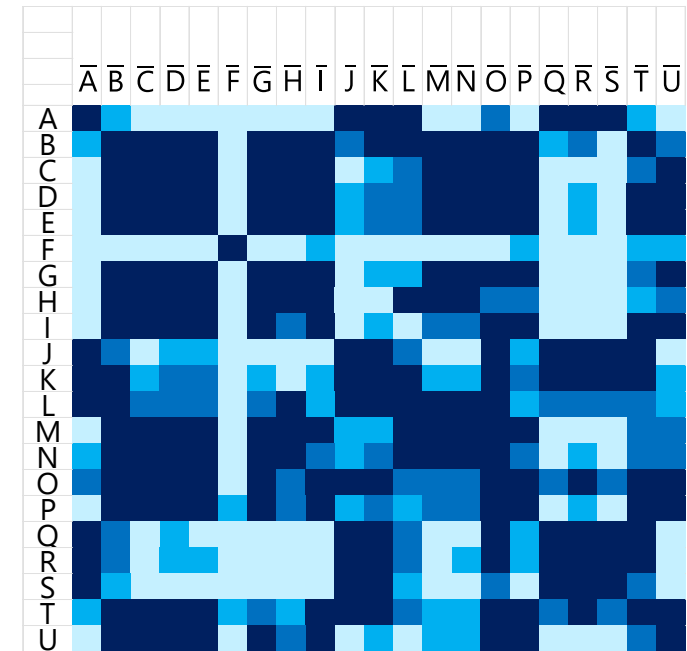
- digital fingerprint and barcode for comparison^[6]

calculation of Spectral Similarity Scores (SSS)

- intensities of one/an emulsifier are compared with the mean values of the intensities of 21 emulsifiers
 - each comparison leads to one SSS
 - 21 SSS per emulsifier (“numeric fingerprint/barcode”)

graphical visualization (→ SSS fingerprint)

- degree of correlation ↔ color code
 - visual comparison between emulsifiers possible
 - emulsifier grouping based on SSS fingerprint similarity



→ easy visualization how similar a specific emulsifier is to another

→ applications: comparison of emulsifier lots, evaluation of storage influence



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Thank you for your attention

