Simulating E-Studies using HRAM-GCMS and HRAM-LC/MS/MS Screening at 1 ng/mL level

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Objective

Our aim is to propose a systematic and effective extractables study design for evaluating potential extractables and leachables from Prefilled Syringes (PFS). This design is specifically oriented to identify and semi quantify a set of potential leachables covering both a realistic and exaggerated profile.

Extraction of PFS from drug reservoir

PTFE surface of coated stopper

DP reservoir filled with extraction solvent

Modern prefilled syringes typically incorporate a Polytetrafluoroethylene (PTFE) coating on the rubber stopper surface that directly contacts the drug product. Extractables studies that indiscriminately extract such stoppers may overlook the effect of this protective coating, leading to an overestimation of the number and concentrations of extractables.

Rubber stopper as used in PFS.
PTFE coated area is marked red

Study Layout: Simulation studies or classical layout?

The design of extractables studies is heavily influenced by their specific goals, e.g.: whether the aim is material characterization or at assessing realistic and worst-case potential leachables.

Classical layout for extractables studies:

- Extraction of primary packaging materials in organic and aqueous solvents disregarding the drug product (DP) formulation evaluated.
- Often, parts were simply placed in extraction vessels, thus e.g. from a PTFE coated stopper of a prefilled syringe, coated and uncoated surface areas were extracted – although the non-coated surface has no DP contact.
- As extraction is done with solvents not related to the formulation, the amounts detected do not reflect the amounts expected to be present in the DP at the end of storage.

"State-of-art" approach for extractables studies :

- Extraction of the assembled syringe directly targeting the drug reservoir → all components of the PFS that are in contact with the drug product during storage are extracted simultaneously in one step.
- Solvents simulating as well as exaggerating the extraction efficiency of the drug product.
- The extraction time and temperature are adapted to the actual storage conditions.
- Accelerated aging simulation by temperature elevation over longer periods of time to simulate the product's shelf life based on ASTM F1980-16 [3].

Simulating extractables studies allow estimation of the amounts expected to migrate into the drug product at the end of shelf time.

AET of simulating extractables screenings

The AET used in simulating E-studies must cover the safety concern threshold (SCT) and the % of intended storage time covered in the simulating extractables study.

$$AET_{Device} = SCT * \frac{V_{Device}}{V_{Dose}} \qquad AET_{Extract} = \frac{SCT * T_{Simulated} * V_{Device}}{F * V_{Dose} * V_{Extract}}$$

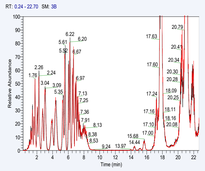
AET_{Device}	Analytical evaluation threshold for the extracted substance per device [µg/device]
$AET_{Extract}$	Analytical evaluation threshold for the extracted substance in the extract generated [ng/mL]
SCT	Safety concern threshold (e.g. 1.5 µg/day)
V_{Device}	Volume of drug product intended to be stored in the device [mL]
V_{Dose}	Volume of dose per day [mL/day]
$V_{Extract}$	Solvent volume for extraction of one device [mL] – if extraction is done from PFS inside
F	Safety factor for uncertainty of semi-quantification, PQRI def. 2 (≥ 5 would be scient. appropriate)
$T_{Simulated}$	Adjustment for partial coverage of shelf time in Extractables Study (example: 33% covered => 0.333)

Example: 1.0 mL prefilled syringe used for storage and a daily dosage of 1.0 mL DP/day.

$Dose\ 1.0\frac{mL}{day}$: $AET_{Extract} = \frac{1.5\ \mu g/day * 0.333 * 1.0\ mL}{5 * 1.0\ mL/day * 1.0\ mL}$ = 100 ng/mL

$Dose\ 10\frac{mL}{day}$: $AET_{Extract}$ = 0.01 µg/mL = 10 ng/mL

- With higher dosages from PFS, very low AET's need to be covered, requiring highly sensitive screening methods, to achieve the 100% ID rate now requested by FDA and EMA.
- Polymeric excipients (e.g. PS20/PS80) may require additional dilution steps, further lowering the AET.



Simulation studies of DP's thus often require AET's as low as 1.0 - 10 ng/mL in complex matrix such as polysorbates.

Experimental Setup and Methods

Low AET require consequent use of HRAM technology to ensure high identification rates and sensitivity

HRAM-LC/MS/MS Screening (Q-Exactive or Exploris 120 & Ultimate UHPLC)

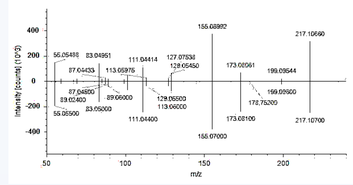
- APCI, HESI and ESI available
- R = 120'000
- Always MS1 and MS2 data generated
- Mass accuracy of typically ± 3 mmu
- High quality, accurate MS1 and MS2 down to **1.0 ng/mL i**n both polarities

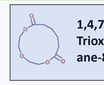
HRAM-GC/MS Screening (Exploris GC/MS)

- EI, PCI and NCI available
- R = 30'000
- MS1 with mass accuracy ± 0.3 mmu in calibrated range – allows calculation of formula from all fragments
- EI spectra at 1 ng/mL
- PAL Autosampler for liquid injection & Headspace
- NIST fits usually > 800

Identification of non-volatile compounds by HRAM-LC/MS

Confirmation via MS2 Data in Solvias EXLEA database



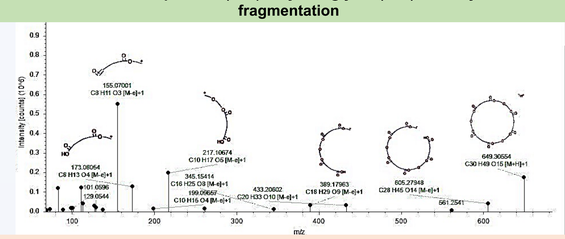


1,4,7-Trioxacyclotridecane-8,13-dione

Top: Spectrum from actual study

Bottom: Reference spectrum from Solvias EXLEA data base

Confirmation of adipic acid (n=3) ethylene glycol (n=6) ester by simulated fragmentation



HRAM-GC/MS Identification Workflow

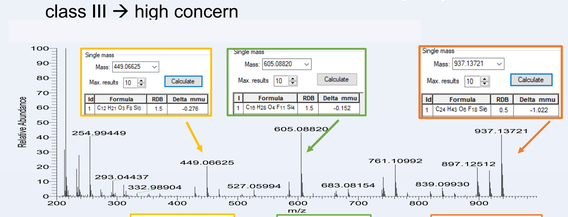
EI Spectrum (HRAM)	PCI/NCI Spectra (HRAM)
Search NIST, Wiley… ("as with quad spectra")	ChemSpider, PubChem .. with proprietary software
Fits > 850 = ID	Molecular Ion + HRAM fragment Fit = ID
Fits > 750 + HRAM fit = ID	No molecular ion but good fragment fit ⇒ Group ID: Branched alkane e.g.

Total ID or Group assignment: 98 - 100%

Increased identification ratio with our workflow compared to classical NIST search (at least an ID of the compound class will be achieved)

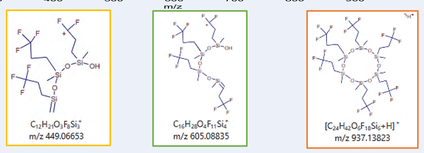
Identification of unknown compounds by HRAM-GC/MS

- Extracted compounds in E&L studies are often not available in NIST type libraries
- Unknown compounds are treated toxicologically as Cramer class III → high concern



Calculated masses and structure examples

M+H (937.137) is 300 Da above largest CAL-MIX ion of GC/MS system

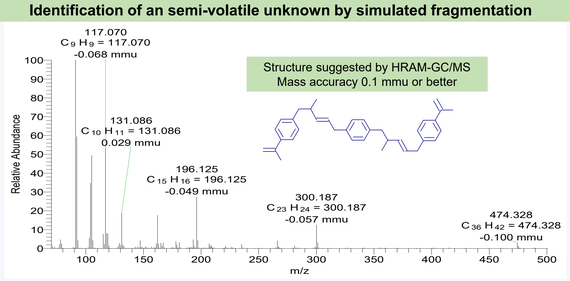


$C_{13}H_{21}O_3F_9Si_2^+$ m/z 449.06653

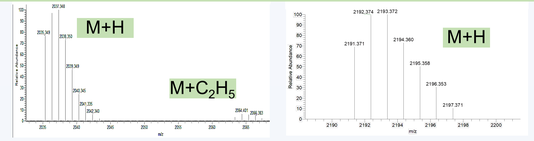
$C_{16}H_{20}O_4F_{11}Si_2^+$ m/z 605.08835

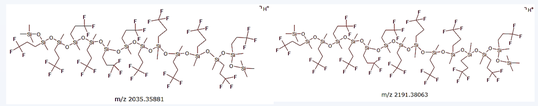
$[C_{24}H_{41}O_6F_{13}Si_4+H]^+$ m/z 937.13823

Identification of an semi-volatile unknown by simulated fragmentation



PCI-HRAM-GC/MS measurements





Measured: 2035.349 : Δm ~ -10 mmu

Measured: 2191.371: Δm ~ - 10 mmu

- Unknown compounds can be identified due to high mass accuracy and resolution of the systems
- Identification of compounds up top 2500 Da is possible by PCI-HRAM-GC/MS

Results and conclusions

1. HRAM-MS allows performing extractables studies in extracts simulating the DP formulation with AET's adapted to the SCT of the drug product – using AET down to 1 ng/mL.
2. Simulated extractables studies based on HRAM-MS technology give pharmaceutical manufacturers a complete picture of all impurities expected to migrate from the packaging material into their drug products.
3. Combining commercial libraries (NIST, Wiley etc.), proprietary data bases (e.g. Solvias EXLEA with HRAM-MS1 and MS2 data of more than 7000 E&L specific compounds) will minimize percentage of unidentified compounds to less than 5%.
4. For NVOC analysis, HRAM-LC/MS/MS typically achieves ID rates between 98-100% of all organic compounds detected with MW 150-2000 Da.
5. For VOC and SVOC analysis use of HRAM-GC/MS, allows adding a 2nd identification approach to the "classical" NIST search – which also significantly increases identification ratios, compared to simply library searching.

References

1. USP <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems
2. USP <1664> Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems
3. Standard Guide for accelerated aging of sterile barrier systems for medical devices (ASTM F1980-07)

For any question, please mail to

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