Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Analysis of active substances and co-formulants in ppp

The use of standardised Inhouse and multi methods

Outline

1. **Background**
   - Definition of a multi method / standardised method
   - Benefits of multi and standardised methods
   - Laboratory for formulation analysis

2. **Procedures used in the laboratory for formulation analysis**
   - Overview of general procedure
   - Sample preparation
   - Conditions used in HPLC/UV analysis
   - Conditions used in GC/FID analysis
   - Inhouse multi method

3. **Conclusion**

Standardised methods / multi methods

- **Analysis of a sample is time consuming if a validated method is not available**
  - research for existing methods
  - testing for appropriate conditions (sample preparation, solvents, instrumental setup etc.)

- **Assumption:**
  Almost every laboratory in market control has a specific inhouse method or at least certain conditions as a starting point for analysis
  - Optimisation depending on analyte and formulation type

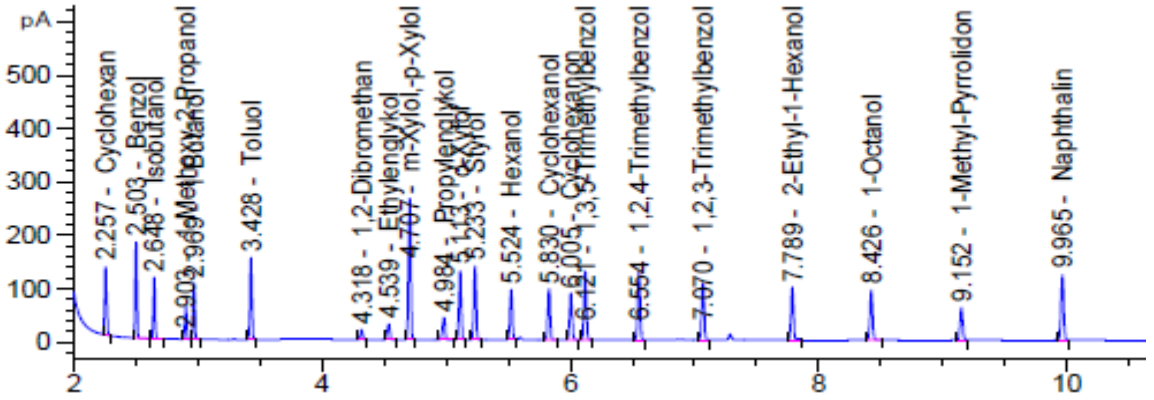
What is a multi method?

Two possible definitions

Classic definition:

- analysis of several analytes in just one chromatographic run
- optimised for these specific analytes

→ **One specific method which can be used to analyse defined substances**



What is a multi method?

Two possible definitions

New definition (in context of ppp quality control):

- Standardised conditions, e.g.
  - defined sample preparation procedures,
  - defined solvents,
  - specific set of columns (GC and HPLC)
- Can be used universally for several analytes and formulation types
- Can also be used as a starting point for further method optimisation for single-analyte methods

→ **Standardised method**

Standardised methods / multi methods

What are the benefits?

- **Time-saving**
  - Less time needed for method development / optimisation
  - Less research for existing methods
  - One method for multiple analytes and matrixes

- **Cost-effective**
  - Less equipment required (instruments, columns, solvents, …)

The use of a standardised method shortens the analytical process of a sample and makes the laboratory work more efficient!

Laboratory for formulation analysis

What do we do at the BVL?

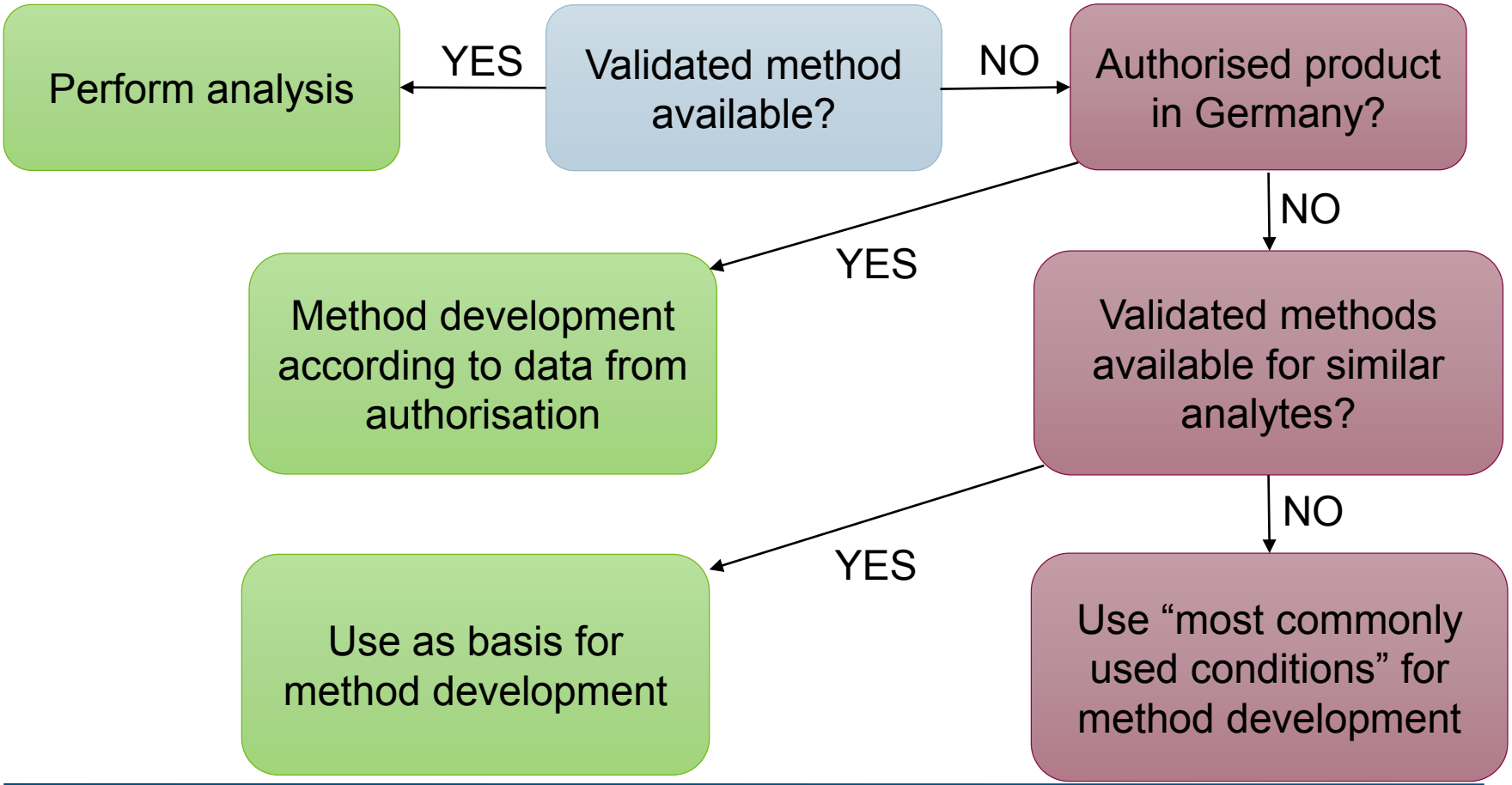
- **Kinds of samples**
  - Planned samples according to control plan
  - Suspicious samples

  } market control
  - Samples from authorisation process
  - Parallel trade samples
  - Other samples

- **Parameters**
  - Physical, chemical and technical characteristics
  - Active substances, co-formulants, impurities and foreign substances

General procedure

Determining the content of active substance



Laboratory for formulation chemistry

Validated methods

HPLC/UV

- 143 single-analyte methods
- 3 multi methods

GC/FID

- 46 single-analyte methods
- 1 multi method

LC/MS

- 7 single-analyte methods

GC/MS

- 7 single-analyte methods
- 1 multi method
- 1 screening method

→ Need for a multi method to reduce number of used methods

Laboratory for formulation chemistry

Preparation of samples

Homogenisation e.g. shaking (liquid ppp) or stirring (solid ppp)

Weigh sample (≥ 200 mg) into volumetric flask and add appropriate solvent

Sonication 15 min at 20 °C

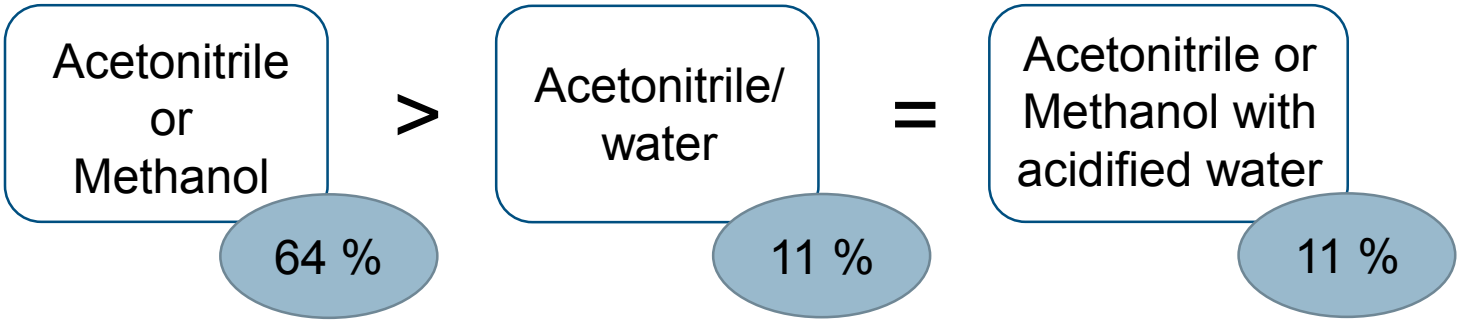
Dilution and filtration (0.45 µm), if necessary

Analysis by HPLC/UV or GC/FID with external calibration

Analysis of active substances

Most commonly used conditions

- **Solvent:**

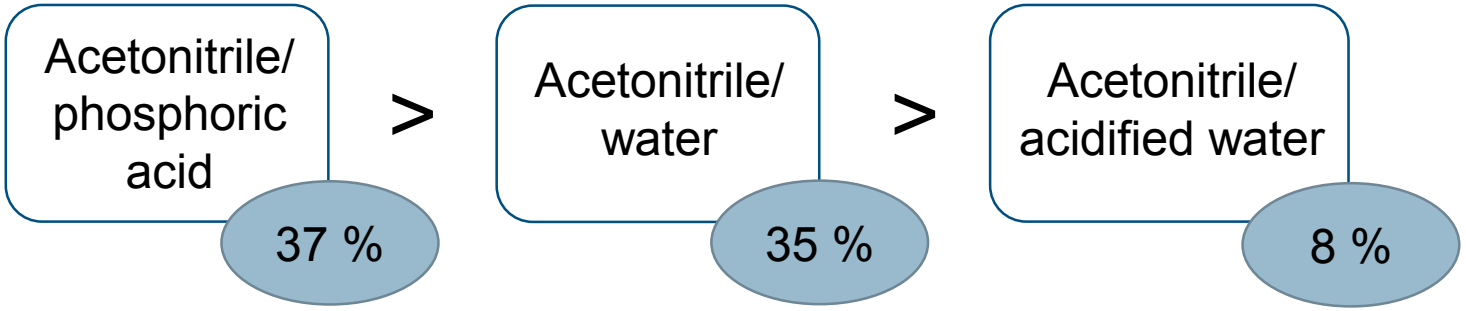


Remaining methods using e.g. buffer or other organic solvents

Analysis of active substances

Most commonly used conditions

- **Mobile Phase:**



  - Mainly used: isocratic elution
  - Composition of the mobile phase is analyte-dependent

Analysis of active substances

Most commonly used conditions

- **Column:** LiChrospher 100 RP-18, 250 x 4 mm (5 µm)
- **Column temperature:** 30 °C
- **Flow rate:** 1.5 mL/min
- **Injection volume:** 5 µL
- **Wavelength:** depending on analyte

Summary

HPLC/UV - Analysis of active substances

Starting conditions for standardised inhouse methods

- **Solvent:** Acetonitrile
- **Column:** LiChrospher 100 RP-18, 250 x 4 mm (5 µm)
- **Column temp.:** 30 °C
- **Mobile Phase:** Acetonitrile/ water (0.1 % phosphoric acid) isocratic elution
- **Flow rate:** 1.5 mL/min
- **Injection vol.:** 5 µL

- **Wavelength:** analyte-dependent

HPLC/UV - Analysis of active substances

Validation data

- 8 methods (ca. 6 %) using the standardised conditions
- Mainly SC formulations

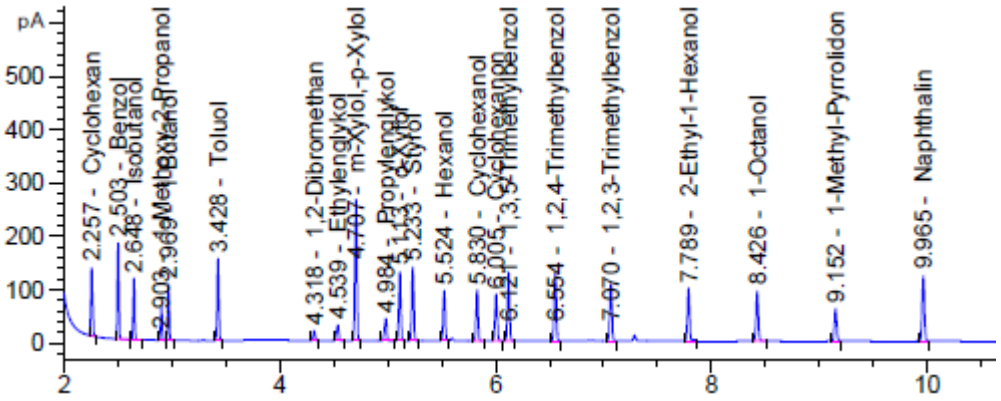
Recovery:	97.6 - 100.8 %	comply with EU SANCO/3030
Repeatability:	RSD 0.12 – 0.83 %	
Linearity:	≥ 0.99	

→ Conditions suitable for analysis of ppp

Analysis of active substances and co-formulants

Most commonly used conditions

- GC conditions are already standardised
- Conditions are chosen according to inhouse multi method for co-formulants



Analysis of active substances and co-formulants

Most commonly used conditions

- **Solvent:** Acetone
- **Column:** Zebron ZB-1701
  30 m x 0.32 mm x 0.25 µm
- **Injector:** split injection
  injection volume 1 µL
  injection port: 250 °C
- **Carrier gas:** Helium, constant flow 2 mL/min
- **Detector gas:** Hydrogen 40 mL/min
  Synthetic air 250 mL/min

Analysis of active substances and co-formulants

Most commonly used conditions

Analyte	Split	Temperature programme [°C]	FID temp. [°C]	Run time [min]
Active substances	50:1	35 (0.5 min), ramp 35 °C/min, 280 (5 min)	250	12.5
Co-formulants	20:1	45 (1 min), ramp 10 °C/min, 200 (1 min), ramp 10 °C/min, 280 (5 min)	300	30.5

- Impurities and foreign substances are analysed according to co-formulants
- if necessary: starting point for further method development

Analysis of active substances and co-formulants

Validation data

- Methods using the standardised conditions:
  - 6 for active substances (ca. 38 %) (EC and WG formulation)
  - 1 multi method for co-formulants containing 24 analytes (SC formulation)

	a.s.	co-formulants
Recovery [%]	97.7 – 101.9	97.0 – 100.6
Repeatability [RSD %]	0.15 – 2.18	0.14 – 2.57
Linearity	≥ 0.99	≥ 0.98

comply with EU SANCO/ 3030

→ Conditions suitable for analysis of ppp

Co-formulants, foreign substances and screening purposes

Inhouse multi methods

GC/FID

- Validated multi method with 24 substances (extending scope)
- Quantitative analysis

GC/MS

- Confirmation of GC/FID results
- Screening (qualitative analysis)

- General conditions are identical (solvent, injector conditions, temp. programming, …)
- Some conditions differ to fit for MS analysis (e.g. gas flows)

Co-formulants, foreign substances and screening purposes

Inhouse multi methods

Classical multi method
to quantify 24 substances simultaneously

According to “new” definition
because conditions are used as starting point for method development / optimisation

Conclusion

standardised or multi methods…

- Helpful tools to make the work in formulation laboratories more efficient
- Reduce the number of single-analyte methods by using standardised conditions
- A standardised / multi method would be helpful for harmonisation of market control

Nevertheless:

Access to methods supplied during authorisation is recommended for market control because of important information / advice for determining certain analytes in specific formulations types.

Thank you very much for your attention!

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