
Overcoming Obstacles in Establishing Pharmacopoeial Reference Standards: Insights from Case Studies

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Outline

- ✓ Role of Reference Standards in the Quality of Medicines
- ✓ Case Studies Ph.Eur. / EDQM
- ✓ Case Studies USP
- ✓ Conclusions

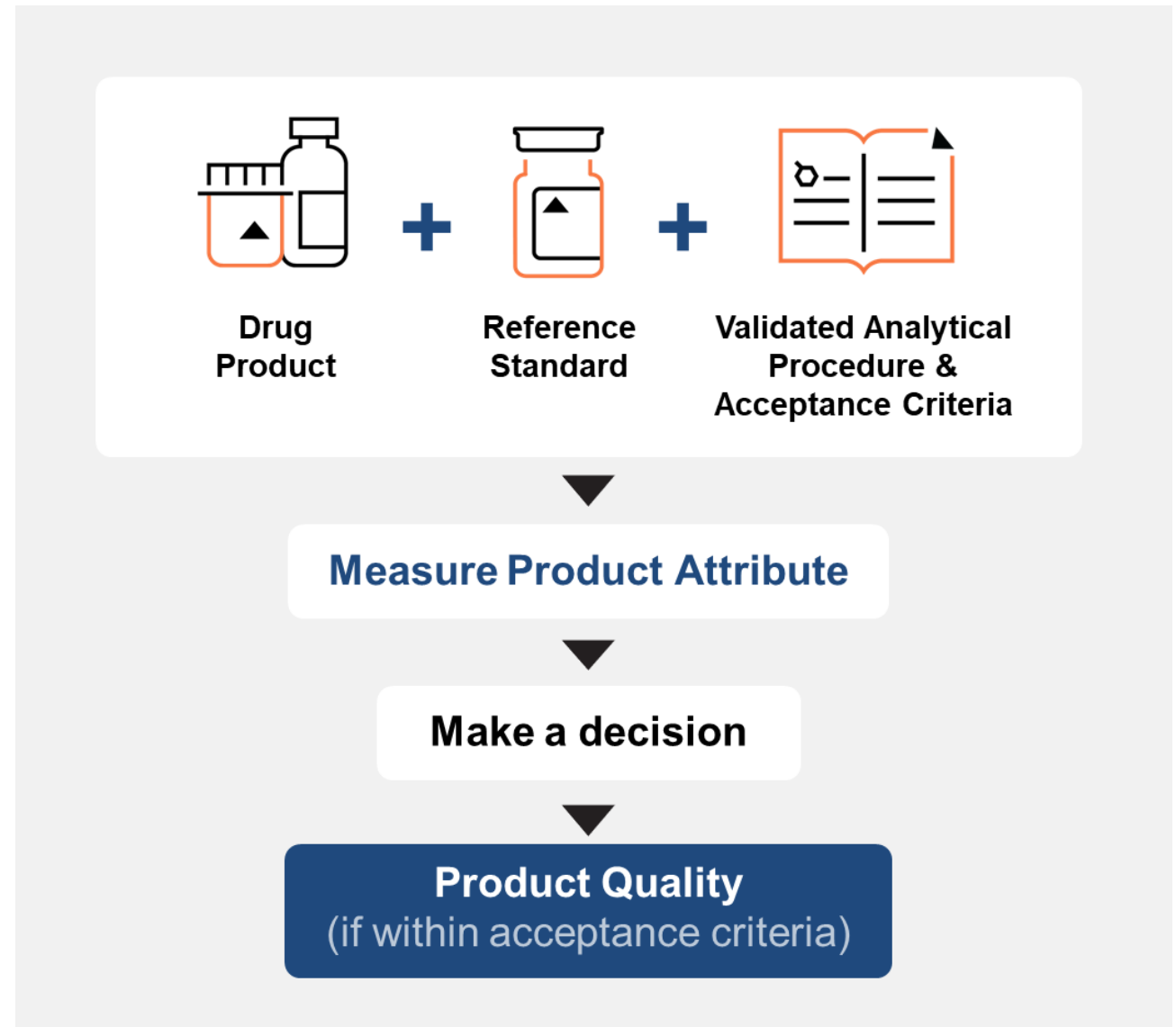
Ensuring Quality of Medicines

What is Quality?

The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity. (ICH Q6A)

How to determine Quality?

- by their design
- development
- in-process & GMP controls
- process validation
- by specifications



Risk to Quality: Issues with Reference Standards

Ensuring accuracy of the Reference Standard assigned value is paramount during the whole RS lifecycle (development AND valid use period)

A high quality Reference Standard provides accurate assigned value

Several factors involved in the RS development and lifecycle can cause inaccurate assigned values, including identity issues

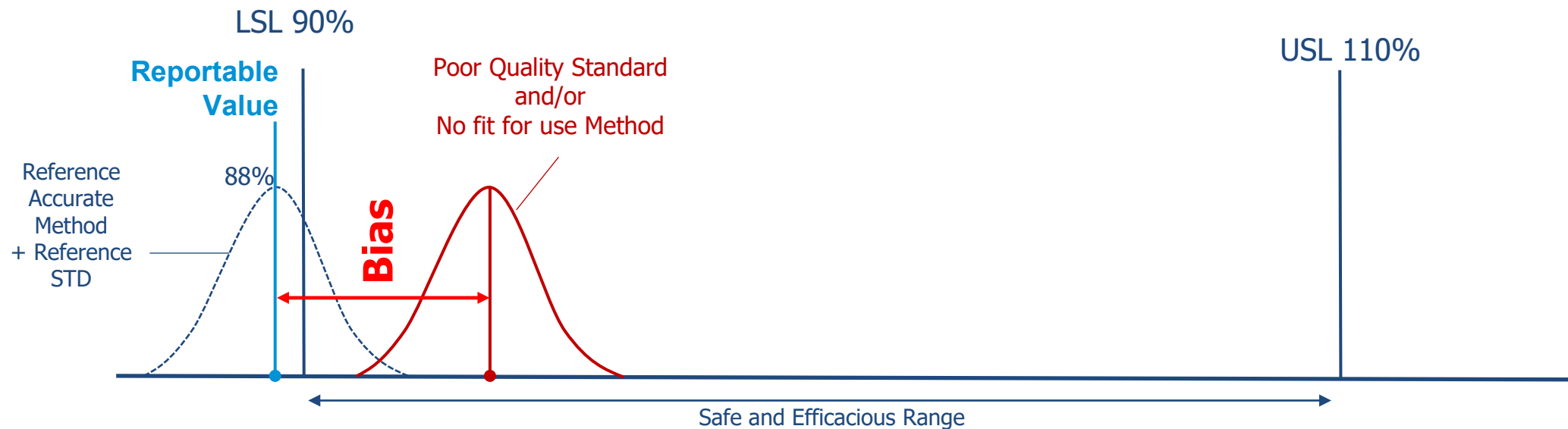


Risk to Quality – Reference Standards and Analytical Procedures

What Significant Bias can Cause

Incorrect Result

High probability of accepting OOS product



Impact of an Incorrect Result

On the patient? On your business?
Product Recall? Market withdrawal?

USP Reference Standards

USP General Notices 5.80. USP Reference Standards

- USP Reference Standards are authentic specimens that have been approved as suitable for use in USP or NF tests and assays (see USP Reference Standards <11>).

USP General Chapter <11> USP REFERENCE STANDARDS

- When approved as suitable for use in USP or NF tests and assays, USP RS also assume official status and legal recognition in the United States and other jurisdictions that recognize the USP or NF (see GN, 2.30 Legal Recognition).
- USP RS, when they are physical materials, are Reference Materials as defined in the *International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM)*.

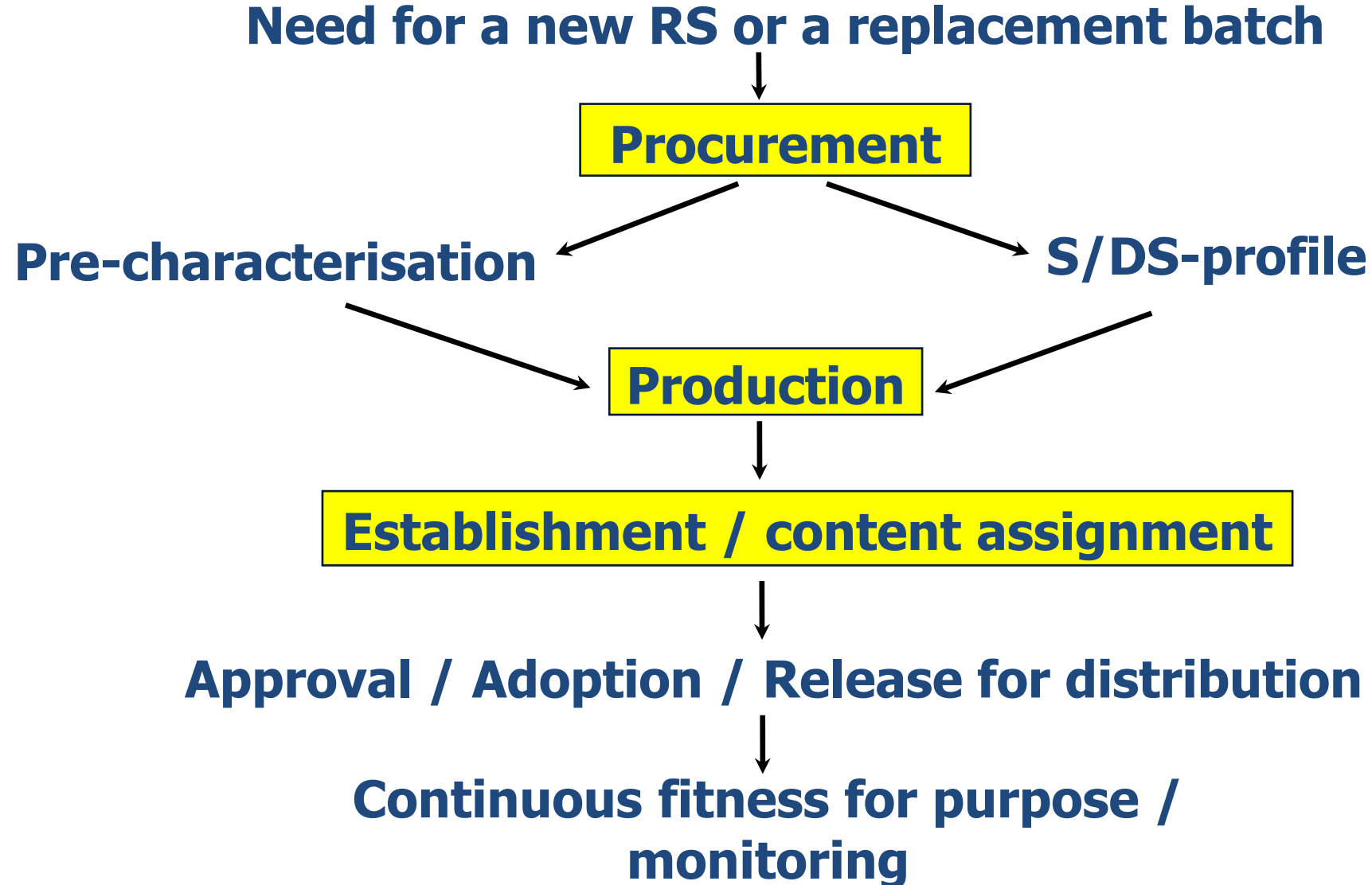
Ph.Eur. Reference Standards

Ph.Eur. 5.12. Reference Standards

- **European Pharmacopoeia chemical reference substance (CRS)**
A substance or mixture of substances **intended for use as stated in a monograph or general chapter** of the European Pharmacopoeia.
- A substance or preparation to be established as a primary standard is **characterised by a variety of analytical techniques** chosen to demonstrate its suitability for use.
- The **extent of testing** and the number of laboratories involved in the establishment of a CRS depend on the use of the CRS and **are tailored to ensure fitness for purpose.**

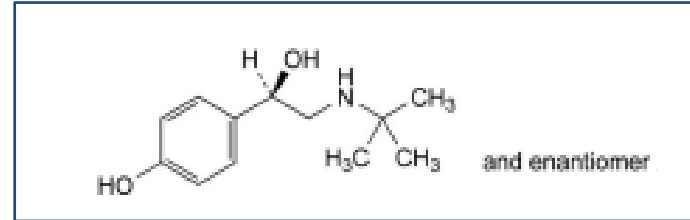
Case Studies Ph.Eur.

Ph.Eur. Reference Standard Establishment Process



Procurement – Quality of candidate material

Salbutamol impurity B



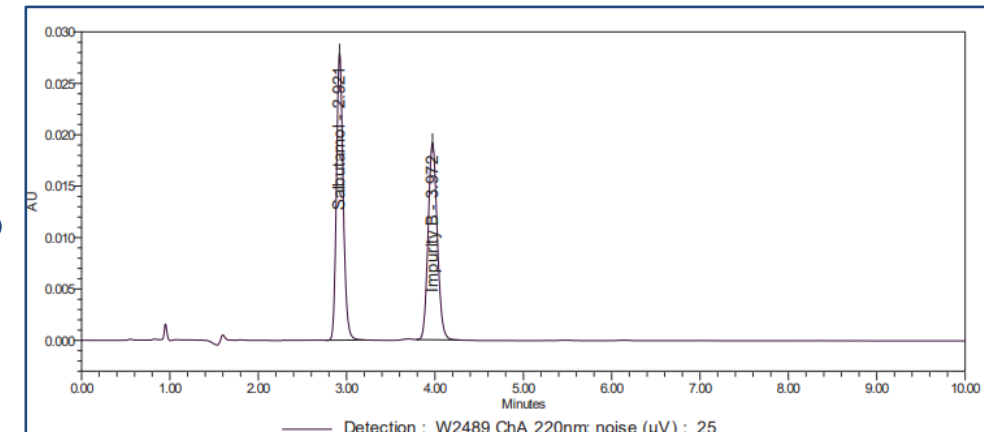
Use: Peak identification in LC test for related substances in a previous version of the Ph. Eur. monograph for salbutamol.

Current CRS: HCl salt – new candidate: Sulfate salt

CoA: Identity confirmed by MS and NMR

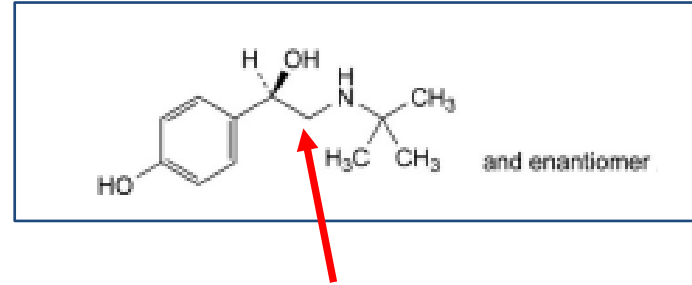
Testing results:

- LC: relative retention matched with current CRS
- MS: in accordance with sum formula
- IR spectrum: slight differences
- NMR: ¹H-NMR minor difference



Procurement – Quality of candidate material

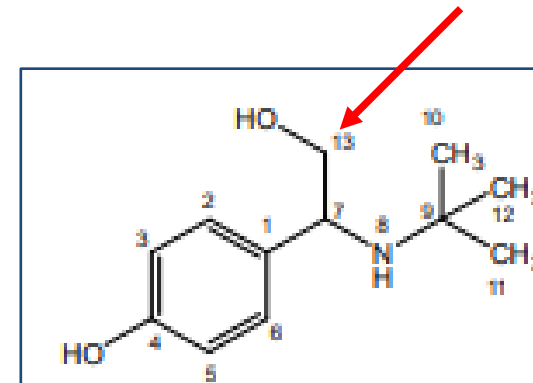
Salbutamol impurity B



Further NMR investigation:

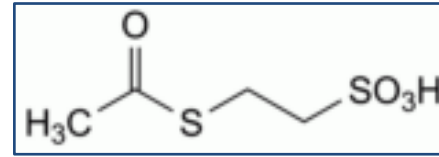
The NMR spectra of salbutamol impurity B new candidate material were found not in accordance with the structure of impurity B.

In particular, the correlations observed in 2D-NMR experiments (1H-1H COSY and 1H-13C HMBC) do not tally with the structure of impurity B, but with that of an isomer of impurity B



Procurement – Quality of candidate material

Mesna impurity C CRS



Use: External standard in LC test for related substances in Ph. Eur. monograph for mesna.

CoA: sodium salt – content by quantitative NMR: 98.7 %.

Results from initial testing:

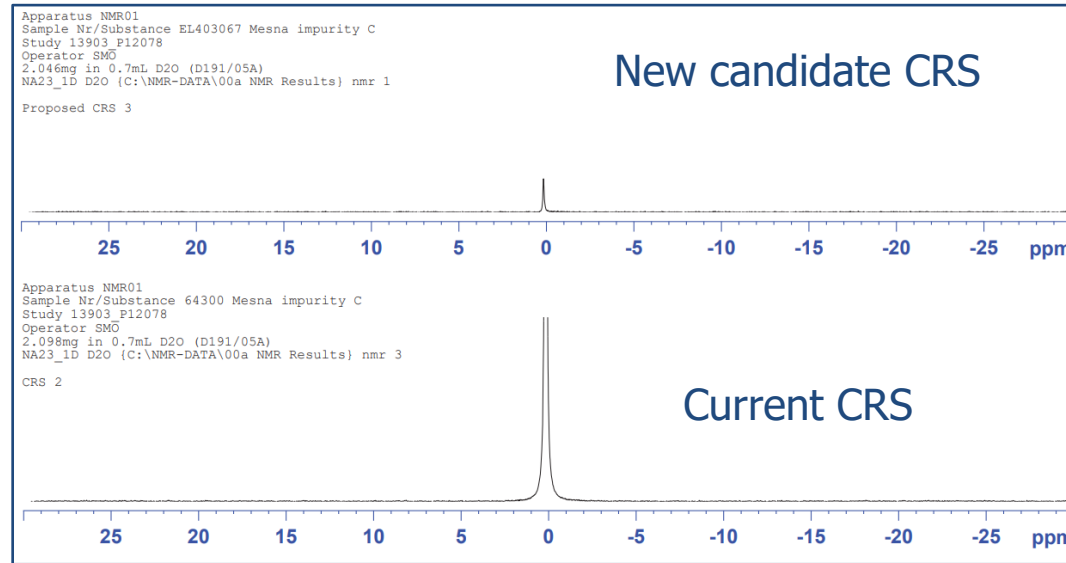
- IR: different from previous CRS batch (sodium salt)
- MS: in accordance with sum formula
- ¹H-NMR: concordant with current CRS
- Water by micro determination: < 0.1 %
- Residual solvents by HS-GC: < 0.1 %
- LC related substances (versus dilution of test solution): n.d.

Content by mass balance:
100 %

- Content by quantitative NMR (expressed as sodium salt): **91.8 %**
- Content by LC assay versus previous CRS batch (expressed as sodium salt): **91.5 %**

Procurement – Quality of candidate material

Mesna impurity C CRS



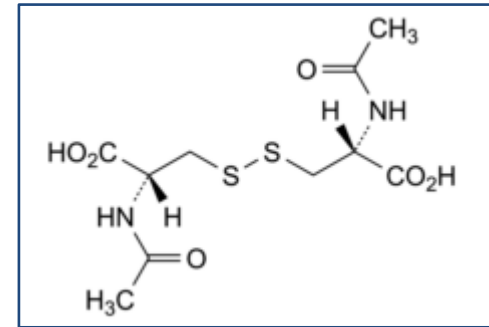
²³Na NMR spectrum

Results from additional testing:

- NMR: no prominent impurity signals; ²³Na spectrum different from previous CRS batch (almost no ²³Na seen).
- MS: abundant potassium adducts present in positive ionisation mode.
- In-house LC-CAD method: confirmation of presence of large amounts of potassium; only traces of sodium detected.

Procurement – Quality of candidate material

Acetylcysteine impurity C CRS



Use: External standard in the LC test for related substances for the quantification of impurity C in a previous version of the Ph.Eur. monograph for acetylcysteine

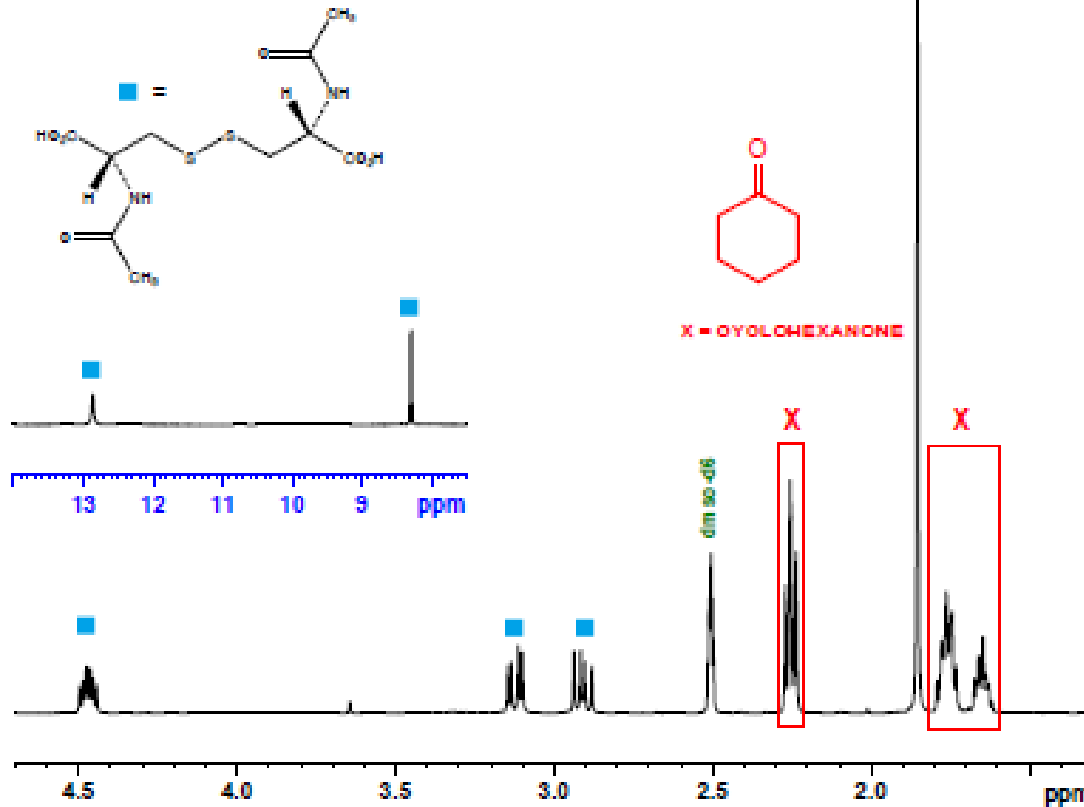
Analytical results:

- Loss on drying: **2.2%**
- LC-purity using the LC method for related substances given in the monograph acetylcysteine: **95.6%**
- Inorganic impurities: **0.8%**
- **Content by qNMR: 63%**

Procurement – Quality of candidate material

Acetylcysteine impurity CRS

Apparatus NMR01
ERS Nr/Substance 53214 acetylcysteine impurity C
Study 09032
Operator BMO
29.623mg in dmsd-d6 (D194/03E)
PROTON128_EDQM DMSO (C:\NMR-DATA\00a NMR Results) run 9



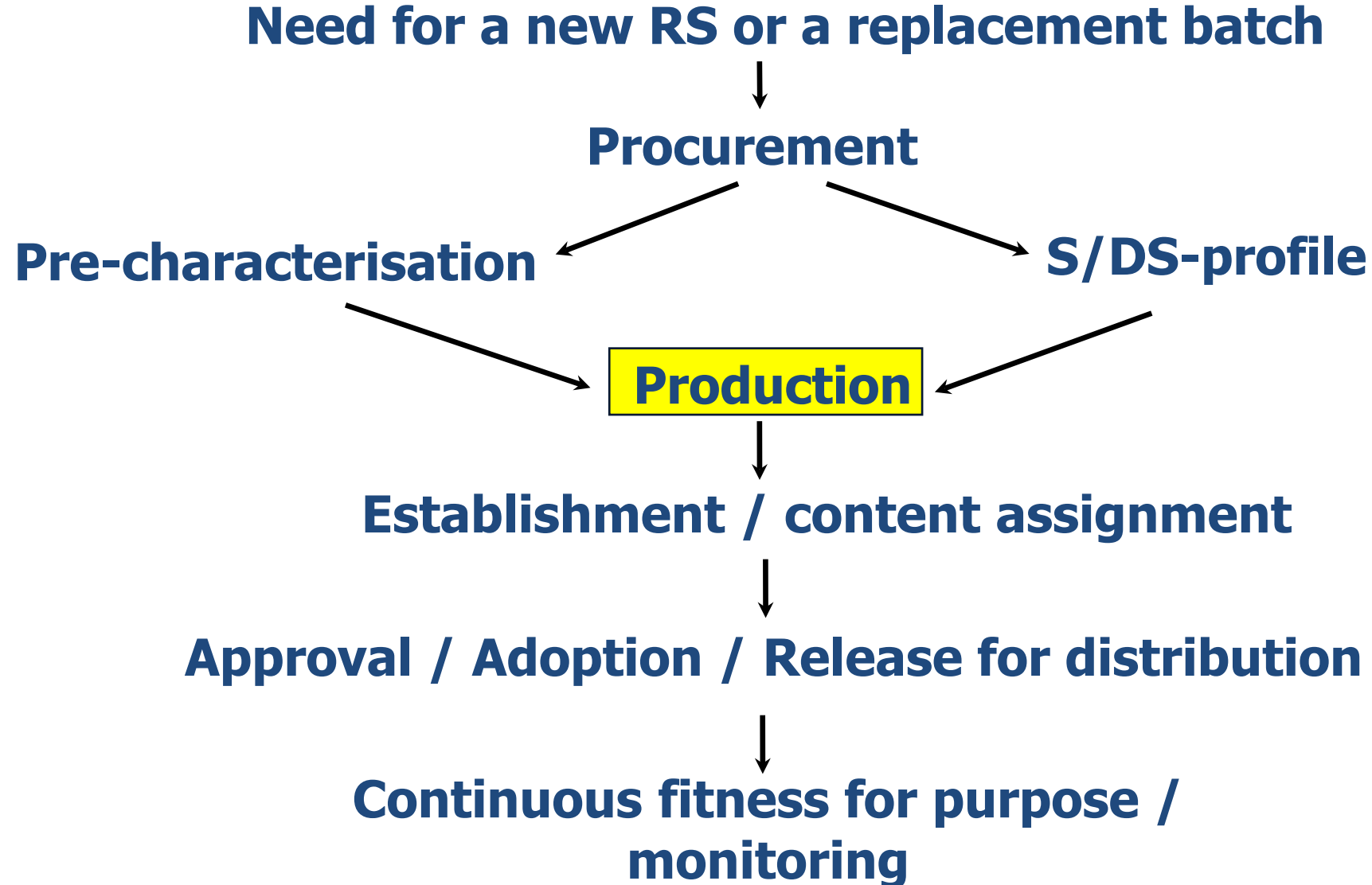
In the NMR spectrum additional signals were detected.

According to further investigation identified as *cyclohexanone*.

Quantification by qNMR and GC: 32.2%

This results in an assigned content of 61.9%.

Ph.Eur. Reference Standard Establishment Process

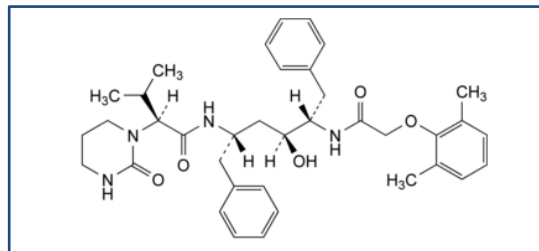


Manufacturing – Equipment Cleaning

Cleaning validation is a procedure of establishing evidence that cleaning processes for manufacturing equipment prevents product contamination.

Manufacturing – cleaning validation

Lopinavir CRS



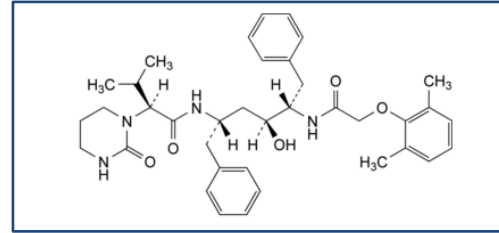
Use: Reference standard for LC assay in the Ph.Eur. monograph for lopinavir

Bulk material: sourced from GMP compliant pharmaceutical manufacturer

CoA: No residual solvents

Manufacturing – cleaning validation

Lopinavir CRS



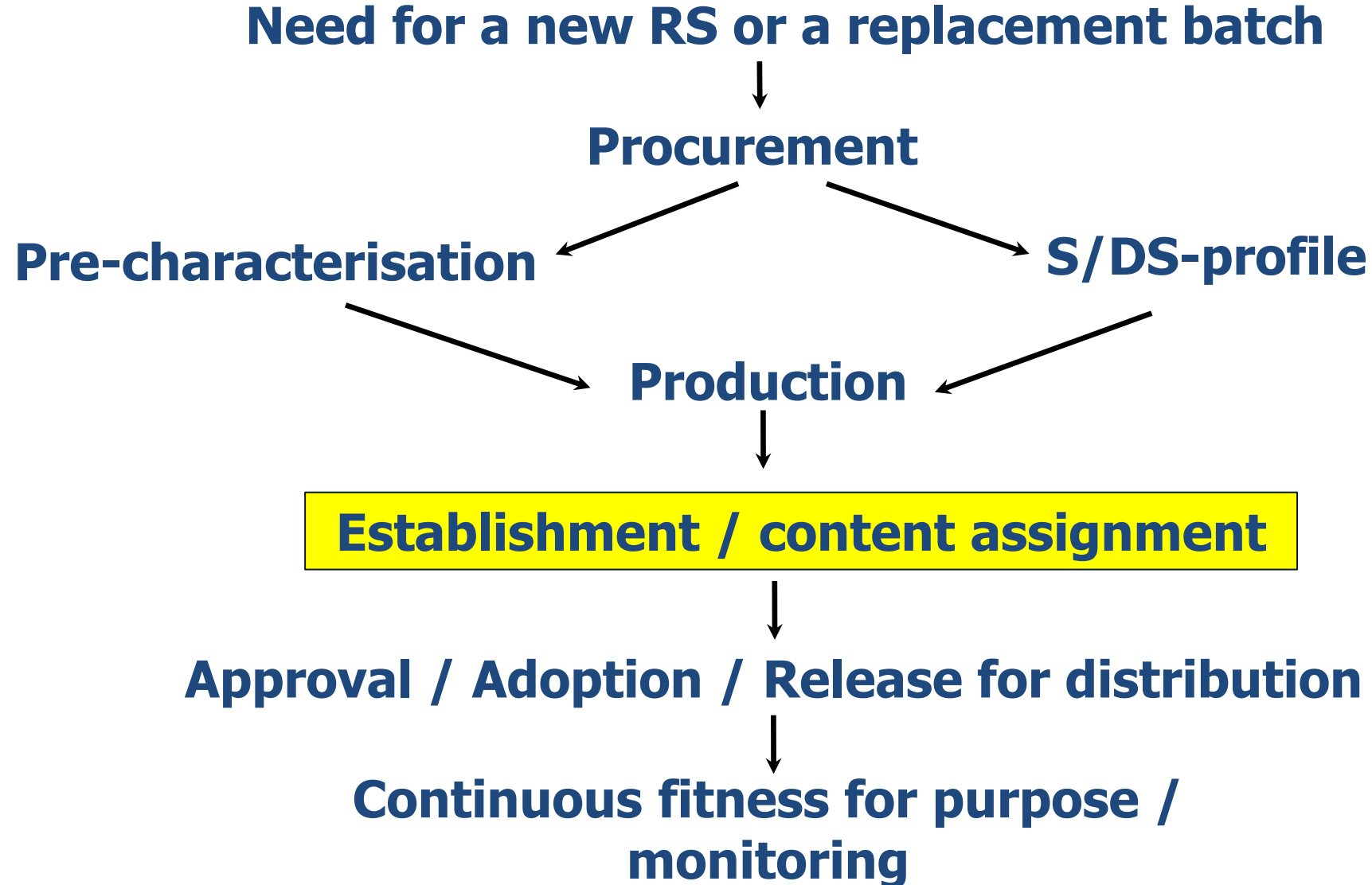
Testing of manufactured CRS:

- Semi-Micro Determination of Water: 3.4 %
- Thermogravimetry: 3.6 %
- Residual solvents analysis by HSGC: 0.9 % EtOH

Investigation:

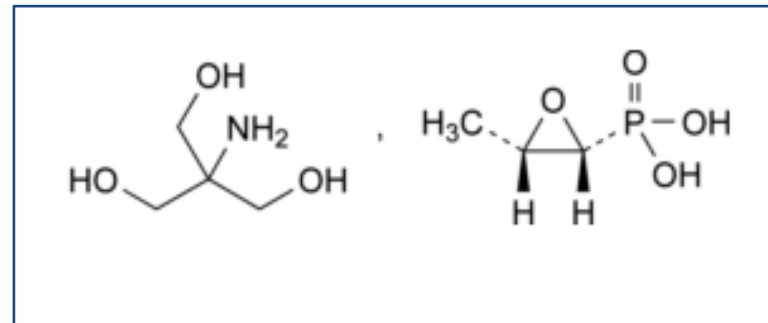
- Ethanol was used in the cleaning of the production glove box
- Lopinavir shows a strong tendency to absorb EtOH

Ph.Eur. Reference Standard Establishment Process



Mass Balance

Fosfomicin trometamol CRS



Use: Reference Standard for LC assay in the Ph.Eur. monograph for fosfomicin trometamol

Content assignment by mass balance:

Assigned content = (100 % - water % - residual solvents % - inorganic impurities %) x [(100 % - sum of impurities by LC %) / 100 %]

Mass Balance

Fosfomycin trometamol CRS

Content assignment using mass balance approach based on monograph methods.

Methods	
Related substances	0.14 %
Water	0.08 %
Residual solvents	n.d.
Sulfated ash	< 0.1%

Content = 99.8 % (m/m)

Mass Balance

Fosfomicin trometamol CRS

Content verification by orthogonal techniques

qNMR results (% m/m):

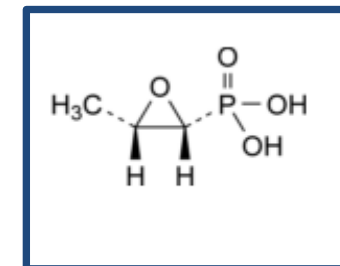
- Fosfomicin: 52.4 %
- Trometamol: 47.3 %
- Fosfomicin + Trometamol: 99.7 %

Molar fractions:

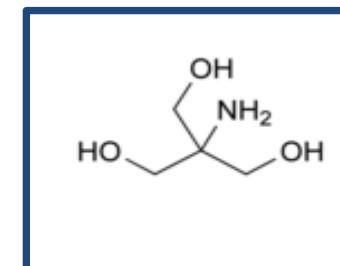
- Fosfomicin: 3.80 mmol/g
- Trometamol: 3.90 mmol/g

Excess trometamol: 1.3 % m/m

Fosfomicin $C_3H_7O_4P$
M = 138.06 g/mol



Trometamol $C_4H_{11}NO_3$
M = 151.12 g/mol

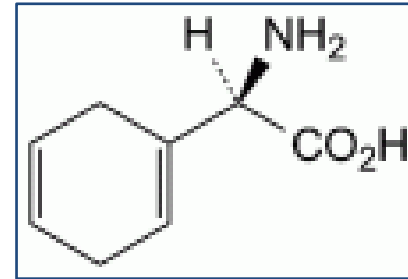


Content based on modified mass balance approach:

$(100\% - \text{water}\% - \text{excess trometamol}\%) \times (100\% - \text{related substances}\%) / 100\% = \mathbf{98.5\%}$

Determination of impurities - UV-Response Factors

Cyclohexa-1,4-dienylglycine CRS



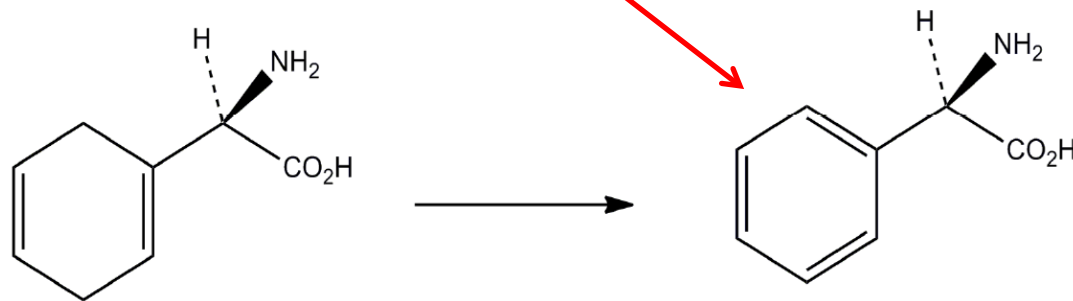
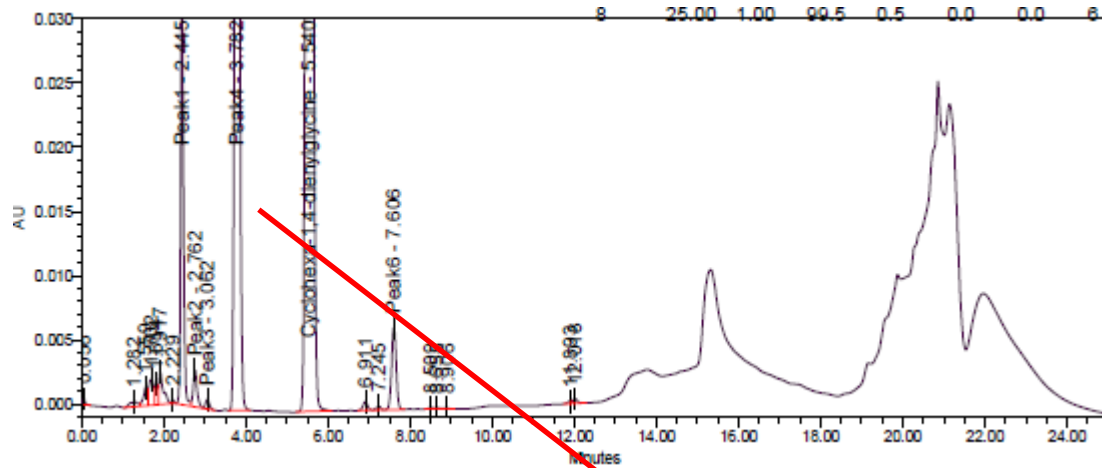
Use: In a previous version of the Ph.Eur. monograph for cefradine as external standard in the LC test for related substances for the quantification of impurity B

Analytical testing results CRS establishment:

- Loss on drying: **0.1% m/m**
- LC-purity (LC method for related substances given in the monograph of cefradine using a 0.1% dilution of the test solution as a reference for quantification at 220 nm): **70.7% m/m**
- Content by qNMR: **above 85% m/m**

Determination of impurities - UV-Response Factors

Cyclohexa-1,4-dienylglycine CRS



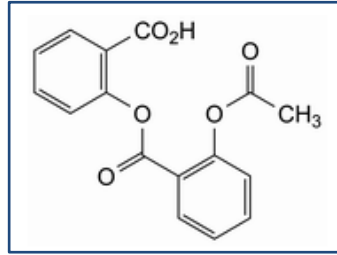
At the given wavelength of 220 nm the impurity eluting as peak 3 was identified as phenylalanine having a significantly higher specific absorbance.

Quantification of this peak was therefore carried out using phenylalanine as ext. std.

This results in an assigned content of 86.7%.

Determination of impurities - Late eluting impurities

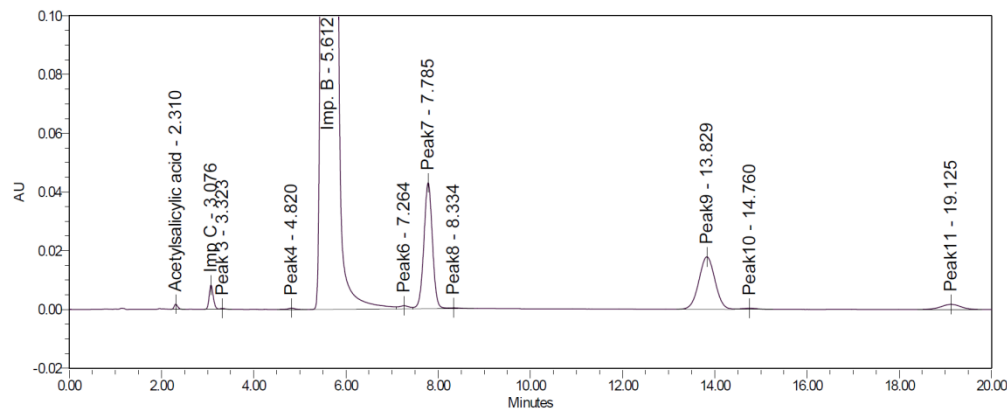
Carbasalate impurity B CRS



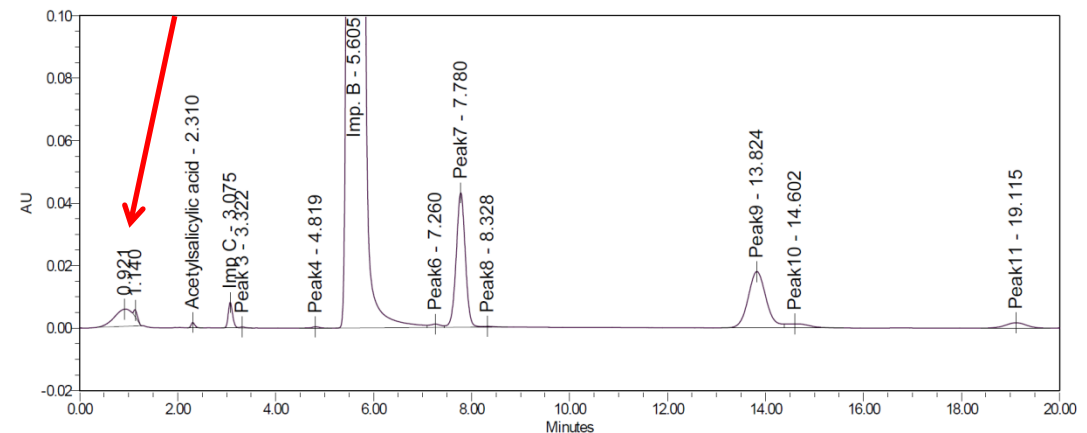
Use: External standard in LC test for related substances in a previous version of the Ph. Eur. monograph for carbasalate calcium.

LC runtime according to monograph: 8 times the retention time of acetylsalicylic acid (18.4 min)

1st Injection



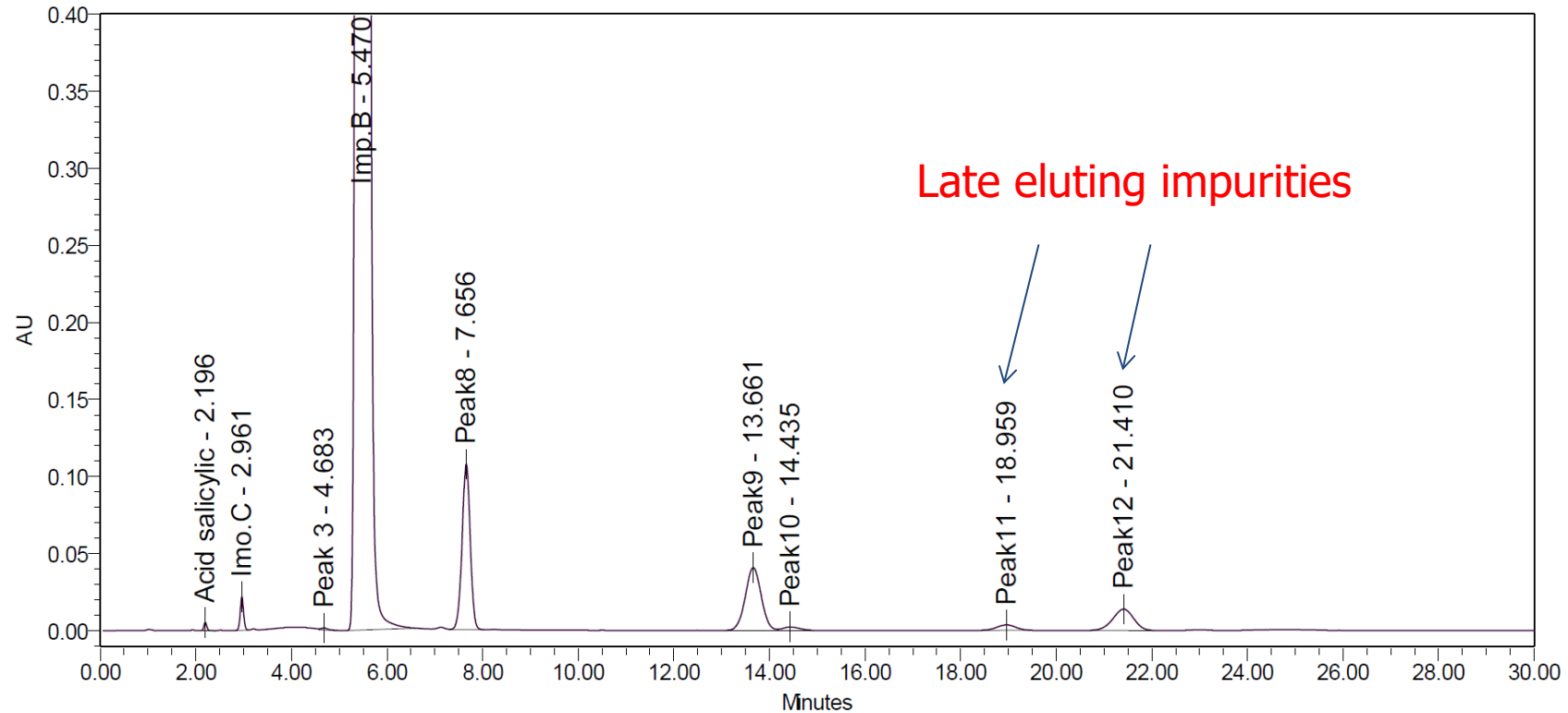
2nd Injection



Determination of impurities - Late eluting impurities

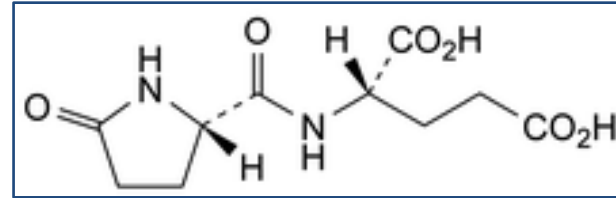
Carbasalate impurity B CRS

LC runtime prolonged to 30 min



Determination of impurities – undetected impurities

Pidolate impurity B CRS



Use: External standard in LC test for related substances in Ph. Eur. monograph for magnesium pidolate.

CoA: No content information

Results from initial testing:

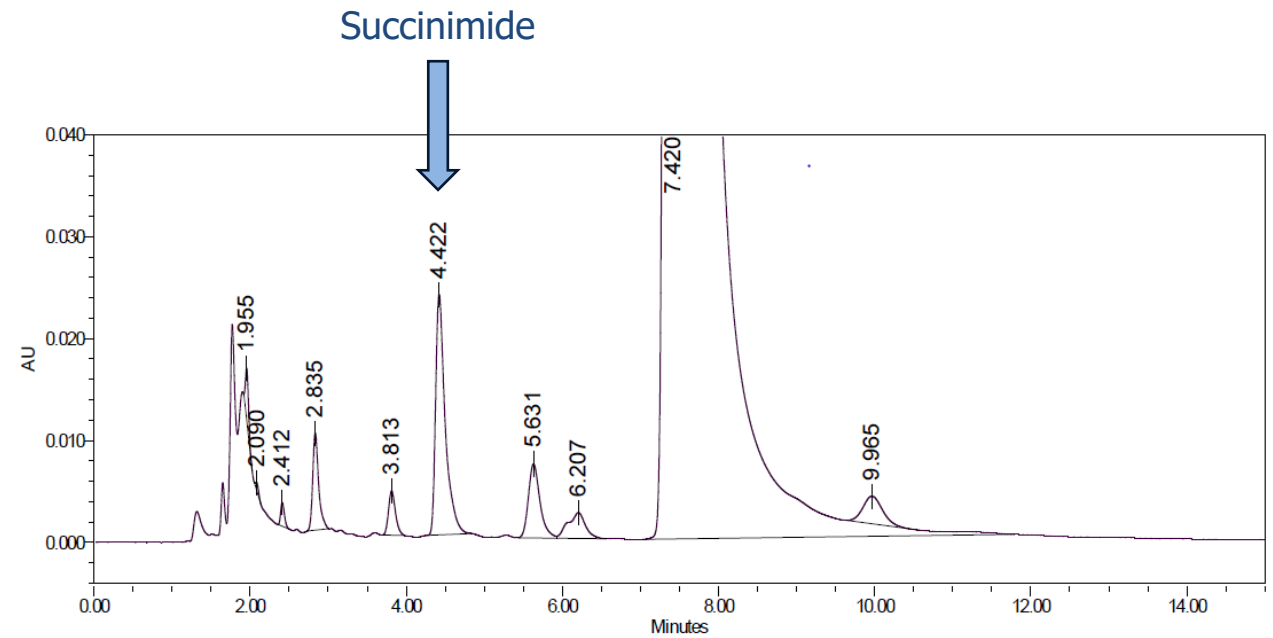
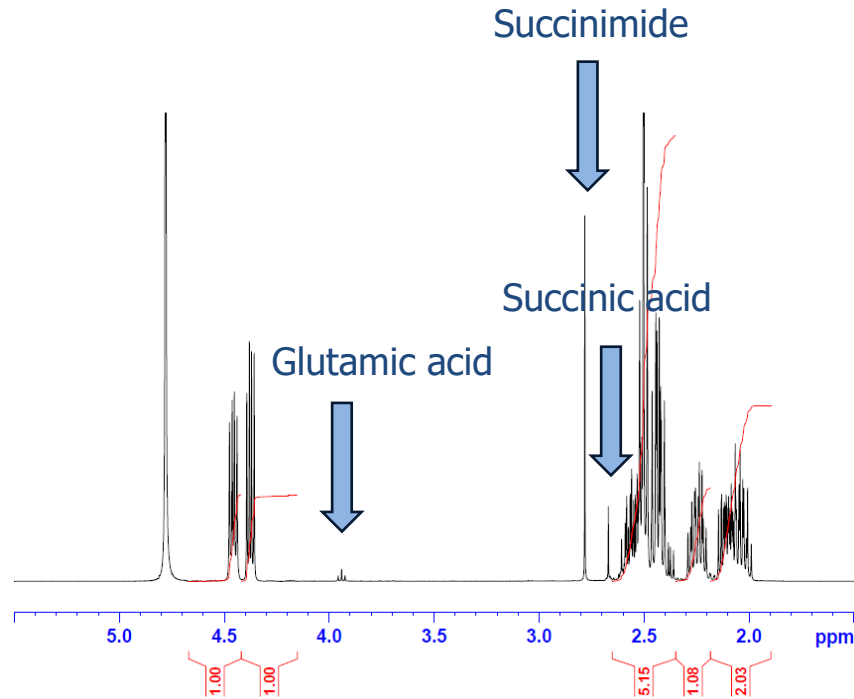
- Water by micro determination: 0.6 %
- Residual solvents by HS-GC: < 0.10 %
- Micro ash by thermogravimetry: < 0.10 %
- LC related substances (versus dilution of test solution): total 2.4 %
- Content by quantitative NMR: **estimation 92 %** (baseline correction, narrow integral range)

Preliminary content by mass balance:
97 %

Determination of impurities – undetected impurities

Pidolate impurity B CRS

Investigation by NMR



Determination of impurities – undetected impurities

Pidolate impurity B CRS

Additional testing:

- In-house LC-CAD method: confirmation of presence of glutamic acid and succinic acid. Determination of content: 1.7 % and 0.7 %, respectively.
- LC test for related substances:
 - glutamic acid: elution in the solvent front
 - succinic acid: not detected (poor response)
 - succinimide: corresponds to major impurity peak – different response compared to impurity B – quantification vs succinimide: 2.9% instead of 0.8% → total via LC related substances: 4.5% instead of 2.4%.

Total amount of organic impurities: **6.9 %**

Final content by mass balance:
92.7 %

Case Studies USP

Insufficient Assessment of Material

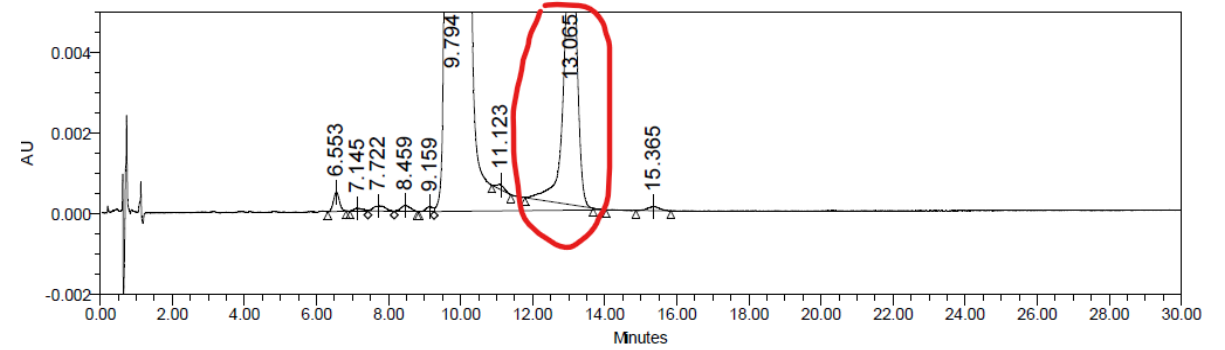
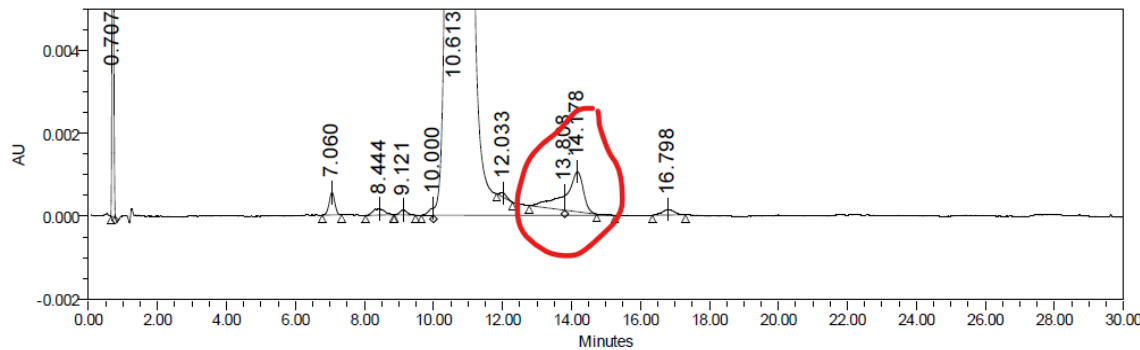
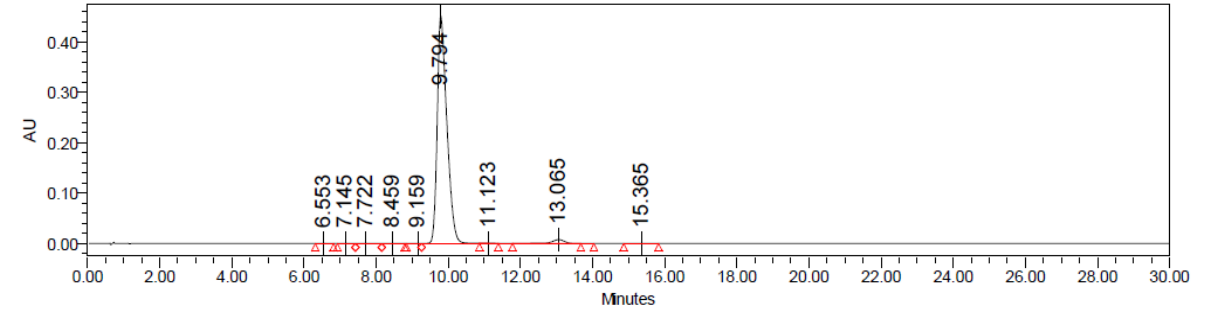
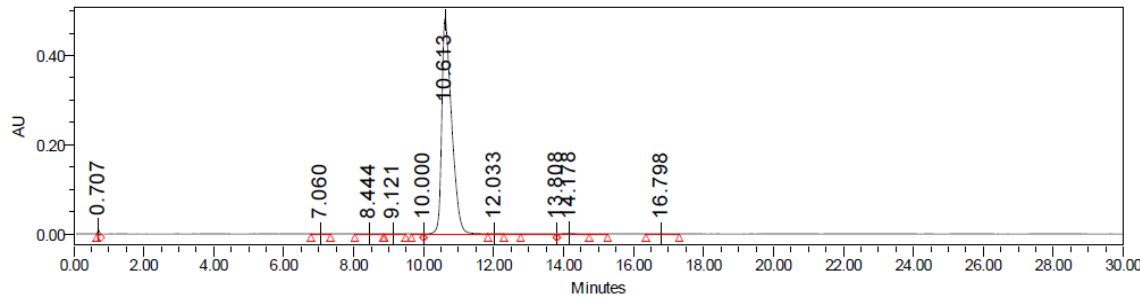
Failure in Establishing Purity

	Data in Supplier's Certificate of Analysis (CoA)	Analysis by USP
Organic Impurity by HPLC	not reported	2.3% at 235 nm
Counter Ion	6.82%	2.39%
Water Content	2.80%	1.70%
Impurities Detected by Other Techniques	not reported	estimated >40%
Purity	98% (by HPLC)	55% (by qNMR)

- ▶ This is a material with high content of carbohydrate impurities. USP detected it from TLC (not detectable from HPLC at 235 nm).
- ▶ Root Cause: Not proper assessment of Material – no orthogonal methods used.

Suitable Stability & Degradation Assessment

Degradation due to material packaging under inappropriate conditions



Root Cause: Trace amount oxygen introduced during sealing of ampule

This material is packed in inert atmosphere (no oxygen, e.g., ampule filled with nitrogen).

What if **inappropriate** conditions for packing the materials are established?

What if appropriate handling/storage conditions are **NOT** included on the label?

Other Things That Could Go Wrong

USP RS development: Bad bulk examples detected during collaborative testing
High risk of going unnoticed during in-house testing in just one lab

	Product Description	Issue Description	Conclusion	Investigation & Root Cause
1	"Herbal Marker Compound"	CoA stated 97%. Purity only 70%.	Failure to meet specification	HPLC from supplier did not show the impurities that USP found (different methods).
2	"Antibiotic"	It is a sodium salt instead of free acid	Potency/Assay issue	Wrong identity sent by supplier.

Things That Could Go Wrong

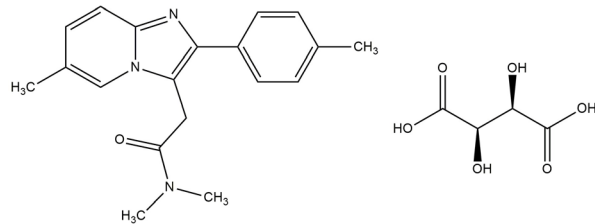
USP RS development: Bad bulk examples detected during collaborative testing High risk of going unnoticed during in-house testing in just one lab

	Product Description	Issue Description		Investigation & Root Cause
3	" <i>meta</i> -disulfamoylbenzene"	Assay value lower, organic impurities higher than monograph specification	Failure to meet specification	Supplier used different HPLC method.
4	"Impurity of a direct thrombin inhibitor"	High ethanol value	Failure to meet specification	Two independent suppliers had issues with residual solvents. The original monograph sponsor also had 4% alcohol solvent present in their material.
5	"Racemate of a dopamine agonist"	Collaborator observed single peak instead of two peaks in SST chromatogram	Failure to meet specification	This bulk is not a racemate. Supplier sent wrong material.

Suitable Stability & Degradation Assessment

Unsuitable Packaging Material and Amount per Vial of In-House RS

- ▶ Zolpidem Tartrate is known as a hygroscopic molecule
- ▶ Label instruction shows that water must be determined before use
- ▶ In-house Standard: "Well-Characterized" Zolpidem Tartrate filled in vials of 2g – **Is this suitable?**



 **REFERENCE STANDARD**
ZOLPIDEM TARTRATE CIV 200 mg
NDC # 00216-2785-01

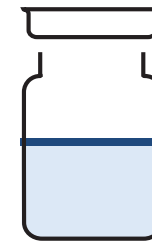
 Warning! Harmful if swallowed. May cause drowsiness or dizziness.

For quantitative applications, determine the water content titrimetrically at the time of use. Use as is material and correct weight for water content. Use a value of 1,000 mg of zolpidem tartrate per mg of material on the anhydrous basis. Keep container tightly closed. Protect from light. Material is hygroscopic. Store in a freezer.

See certificate for any additional information.
USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666
Cat. No. 1724907 Material mfd. in France

- ▶ Results of Water Determination on different days:

	Water Content
First day open	0.85%
After storage 1 week	1.92%
After storage 1 month	2.58%



Top layer presents higher concentration of moisture

The material exposed on the surface will absorb more water than the rest of the material present in the vial; The result may not be representative of the quantity removed for later weighing. (not a homogenous material)

Suitable Stability & Degradation Assessment

Unsuitable Packaging Material and Amount per Vial of In-House RS

- ▶ In-house Standard: "Well-Characterized" Zolpidem Tartrate commercialized in vials of 2g – **Is this suitable?**

Potency (anhydrous basis)	Water content measured at time of analysis (Surface)	RSD%	Water specification	Real content of water on the vial
100%	2.58%	6%	NMT 3.0%	0.85%

Potency used 97.42%

Real Potency 99.15%

- ▶ Water determination test and the use of not fit for use procedures or bad practices:
 - Not proper amount of sample used in the test
 - Not performed in triplicate
 - No acceptance criteria established



Suitable Stability & Degradation Assessment

Unsuitable Packaging Material and Amount per Vial of In-House RS

- ▶ Assay using HPLC-UV Procedure

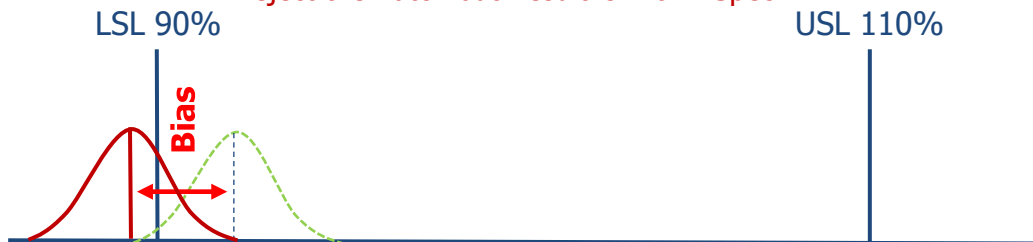
Standard Solution (Average Area Replicates)	Concentration (mg/mL)	Concentration corrected by Water Determination (mg/mL)*	Concentration corrected by Real Water Content (mg/mL)**
15423	0.5156	0.5023	0.5112

*Apparent Potency Used: 97.42% (2.58% Water measured on surface)
 **Real Potency: 99.15% (0.85% of water)

Scenario 1 – Batch close to LSL

Sample	Area	SMP concentration (mg/mL)	Assay% (Apparent Potency)	Assay% (Real Potency)
SMP 1	14428	0.5254	89.4	91.0

"True Value" of API in the Drug Product: 91%
 Reject the Batch but Result is within Spec



Scenario 2 – Batch close to USL

Sample	Area	SMP concentration (mg/mL)	Assay% (Apparent Potency)	Assay% (Real Potency)
SMP 1	17721	0.5254	109.8	111.8

"True Value" of API in the Drug Product:
 111.8%

LSL 90% Accept the Batch but Result is Out of Spec USL 110%



Conclusions

- Procure high quality candidate material from a reliable source
- Identity confirmation of candidate material is not trivial
- Use suitable controlled manufacturing conditions
- Use of orthogonal methods is of utmost importance
- It is extremely important to assign accurate values to quantitative reference standards
 - Otherwise, bias in analytical results is very likely later in QC
- It is equally important to ensure the quantitative values remain valid during the whole life-cycle of RS batch
 - Unit size can be crucial
- Collaborative testing increases probability of detecting flaws with candidate materials

Acknowledgements

USP

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EDQM Public Relations team

Thank you for your attention



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