

LGC Experience in Production and Characterisation of Pure Substances & the Role of NMIs in this context



Dr Steve Wood
LGC Limited

- **Outline**
 - **Overview of LGC Limited**
 - **LGC Reference Materials Production**
 - **Accreditation Status**
 - **Pure Substance Reference Materials**
 - **Purification**
 - **Characterisation**
 - **Inter comparisons**
 - **Value assignment**

LGC Limited - Locations

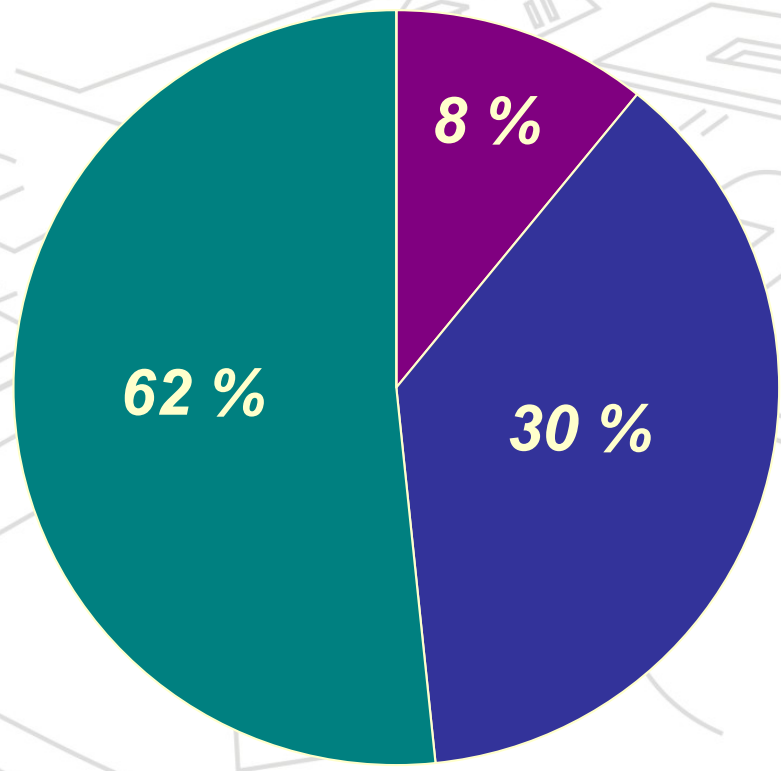


Activities at LGC Teddington

Analytical Services

- Forensic Science
- Food Safety
- Pharmaceutical
- Lifescience

Contract R&D



Quality Services

- Reference Materials
- Proficiency Testing
- Training

400 staff on Teddington site

LGC's UK National Roles

Government Chemist

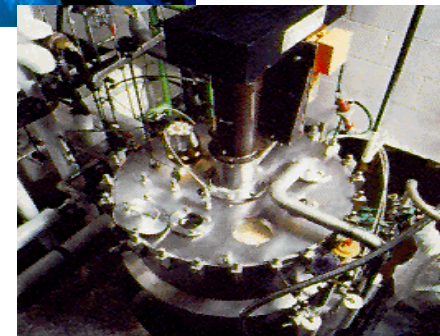
- ***Referee Analyst (20 Acts of Parliament)***
- ***Adviser to UK government & industry on regulations and scientific input***

National Measurement Institute

- ***Delivers world-class measurement science & technology and provides traceable and increasingly accurate standards of measurement for use in trade, industry, academia and government***

R&T Division

- **Programmes Supporting NMI Work**
- **NMS ChemBio (Chemical Measurements)**
 - *Development of methods & standards for improved chemical analysis*
- **NMS ChemBio (Biochemical Measurements)**
 - *Reliable measurements for the biotechnology industry*
- **MET (Measurements for Emerging Technologies)**
 - *Tissue Engineering, Bioprocessing, Point of Care Testing*



RM Production Resources

- ***5 staff experienced in RM production***
- ***3 staff in calibration analysis***
 - *Pure materials & alcohols*
- ***Technical support***
 - *20+ staff in mass spectrometry and separation science*
- ***Statistics support***
- ***Quality support***

LGC Certified Reference Materials

- ***Matrix materials***

- ***Food***
- ***Environment***
- ***Clinical***
- ***Industry***
- ***Forensic***

- ***Single substance organic materials***

- ***Purity***
- ***Melting point***
- ***Enthalpy of fusion***
- ***Elemental content***

~150 materials

Pure RM production for > 20 years

RM Production Planning

Produce a written project plan for production of each reference material

- **Production Steps**
- **Material specification**
- **Sourcing, preparation and sub-division**
- **Homogeneity assessment**
- **Stability assessment**
- **Characterisation of the assigned value(s)**
- **Calculation of the assigned value and its uncertainty**
- **Documentation and storage of the material**
- **Sales and storage and distribution**
 - **LGC Standards**

Project Planning Form	
WORKING TITLE	
(Reference:)	
PPF-Type Certification	Status: DRAFT under discussion
Project information	
Integrated scientific area:	
Action number:	
(C)RM-project responsible:	
Resources needed:	Internal Delegate: External Collaborator: 0 FTE days; €k
Action Leader:	
Service providers:	
Processing:	
Dispatch:	
Stability Monitoring:	
Quality Management:	
(C)RM identifier	(C)RM-Matrix:
CRM: <input type="checkbox"/>	Target parameters:
Proficiency testing: <input type="checkbox"/>	
Feasibility study (FWP V&V): <input type="checkbox"/>	
Others: <input type="checkbox"/>	
EUR-report already available: <input type="checkbox"/> EUR	

Relevant Guidelines

- **ISO/IEC 17025**
 - **General Requirements for the Competence of Calibration and Testing Laboratories**
- **ISO Guide 34**
 - **General Requirements for the Competence of Reference Material Producers**
- **ISO Guide to the Expression of Uncertainty in Measurement (GUM)**



Material Certification

- ***Certification Panel***
 - ***In house group***
 - ***Project team members***
 - ***Independent experts (e.g. statistics)***
 - ***Quality Team representative***
- ***European Reference Materials Co-operation***
 - ***Technical review by experts from German (BAM) and European (IRMM) metrology and reference material organisations***
 - ***ERM Panel approval***

Activities of Certification Panel

- ***Review the intended use/original specification of the material***
- ***Review the expertise of the RM producer / subcontractors***
- ***Review the approaches used to characterise the property values***
- ***Examine records on the sourcing and preparation of the RM***
- ***Review the approaches used to assess homogeneity and stability***
- ***Review supplementary information from the characterisation studies***
- ***Review statistical processing of raw analytical data and results***
- ***Record the essential details of the review and examination of data***
- ***Approve documentation for the reference material***
- ***Officially authorise the release of the RM for use***

Certificate (ISO Guide 31)



CERTIFICATE OF ANALYSIS

ERM[®] - AC020a

<i>trans</i> -5,6-Dihydro-4-methoxy-6-(2-phenylethenyl)-2H-pyran-2-one (Kavain)		
Parameter	Certified value ¹ (mass %)	Uncertainty ² (mass %)
Purity	99.8	0.2

1) The certified value is traceable to the analytical methods and standards used in the characterisation study and described in the certificate.

2) The quoted uncertainty is the half-width of the expanded uncertainty calculated using a coverage factor (k) of 2.45, which gives a level of confidence of approximately 95 %.

This certificate is valid for 12 months from the date of shipment provided the sample is stored under the recommended conditions.

The minimum amount of sample to be used is 2 mg.

NOTE

European Reference Material ERM[®] - AC020a was produced and certified under the responsibility of LGC according to the principles laid down in the Technical Guidelines of the European Reference Materials[®] co-operation agreement between BNM-LGC-IRM. Information on these guidelines is available on the Internet (<http://www.erm.com.org>).

Accepted as an ERM[®], Teddington, August 2006.

Signed: _____

Dr John Marriott, UK Government Chemist
LGC Limited
Queens Road
Teddington
Middlesex
TW11 6LY, UK



4026

All following pages are an integral part of the certificate.

ERM[®] - AC020a
Page 1 of 4

DESCRIPTION OF THE SAMPLE

A batch of D,L-kavain obtained from a commercial supplier of chemical reagents was mixed and dispensed as 10 mg units into screw-capped amber glass vials.

The material was considered to be homogeneous on the basis of High Performance Liquid Chromatography with UV detection (HPLC-UV) measurements on 10 randomly selected 2 mg portions, which showed no significant variation in purity value.

The identity of the material was confirmed by 250 MHz ¹H-NMR spectroscopy and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS/MS) utilising accurate mass measurement for determination of elemental formulae in combination with product ion MS.

The material contains a mixture of D and L kavain; the proportion of these isomers in the material is not necessarily the same as that found in nature.

INTENDED USE

The primary use of this reference material is for the calibration of methods for the determination of kavain in herbal products, foodstuffs and other relevant matrices.

ANALYTICAL METHOD USED FOR CERTIFICATION

High Performance Liquid Chromatography with UV Detection (HPLC-UV)

The purity of the material was determined by HPLC-UV using a reversed phase column (150 × 2 mm, Phenomenex Luna C18 (2), 5 µm), with an isocratic mobile phase consisting of 55 % water and 45 % acetonitrile and a flow rate of 0.2 mL/min. Solutions of kavain in acetonitrile (0.01 mass %) were prepared from 9 units of the material. The purity of the material was quantified by peak area normalisation using UV detection at λ=246 nm.

Differential Scanning Calorimetry

A Polymer Labs STA 625 differential scanning calorimeter was used, with a sample size of approximately 2 mg in aluminium pans and a heating rate of 1.3 °C/min. The instrument was calibrated using a high purity indium certified reference material (LGC6901). A total of 6 determinations were carried out.

Gas Chromatography Flame Ionisation Detection (GC-FID)

The purity of the material was determined by GC-FID using a DB-1 column (60 m × 0.25 mm id, d = 0.25 µm) with a constant flow of helium as the carrier gas (1 mL/min) and column temperature programme of 30 °C for 1 minute, ramp @ 10 °C to 40 °C, ramp @ 5 °C to 200 °C for 1 minute, ramp @ 1 °C to 270 °C for 20 minutes with the Flame Ionisation Detector (FID) at a temperature of 280 °C. Solutions of kavain in dichloromethane (0.1 mass %) were prepared from 10 portions of the material. Each solution was analysed by cold on-column injection of 1 µL. The purity of the material was quantified by peak area normalisation.

The data from these three techniques is shown in Table 1.

In addition to the above, moisture and inorganic material was also determined:

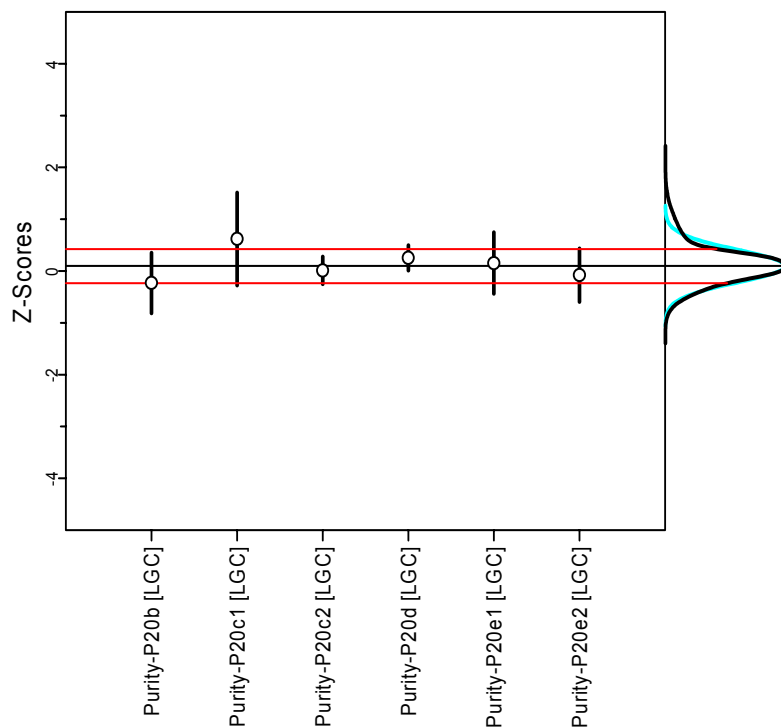
Moisture

Moisture was determined by coulometric Karl Fischer titration and the value subtracted from the purity determined by HPLC-UV and GC-FID.

Inorganic Content

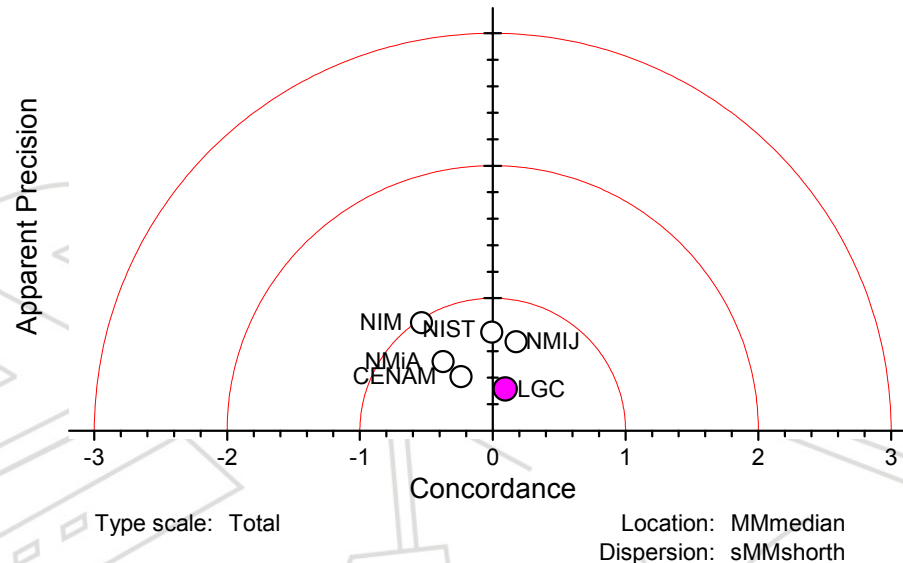
The percentage of inorganic material was assessed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), and confirmed by ash determination at 550 °C. The value was subtracted from the purity value determined by HPLC-UV and GC-FID. The percentage inorganic content was not subtracted from the DSC purity value as this technique allows for the presence of inorganics in the sample.

International Inter-comparison Studies



- **LGC has demonstrated its competence in the measurement of the purity of organic substances through participation in the BIPM CCQM P20 series of inter-comparison studies on the following materials:**
 - **CCQM-P20.b: Ortho-xylene**
 - **CCQM-P20.c: Atrazine (2 samples, high and low purity)**
 - **CCQM-P20.d: Chlorpyrifos**
 - **CCQM-P20.e: Theophylline (2 samples, high and low purity)**

Comparison with other NMIs

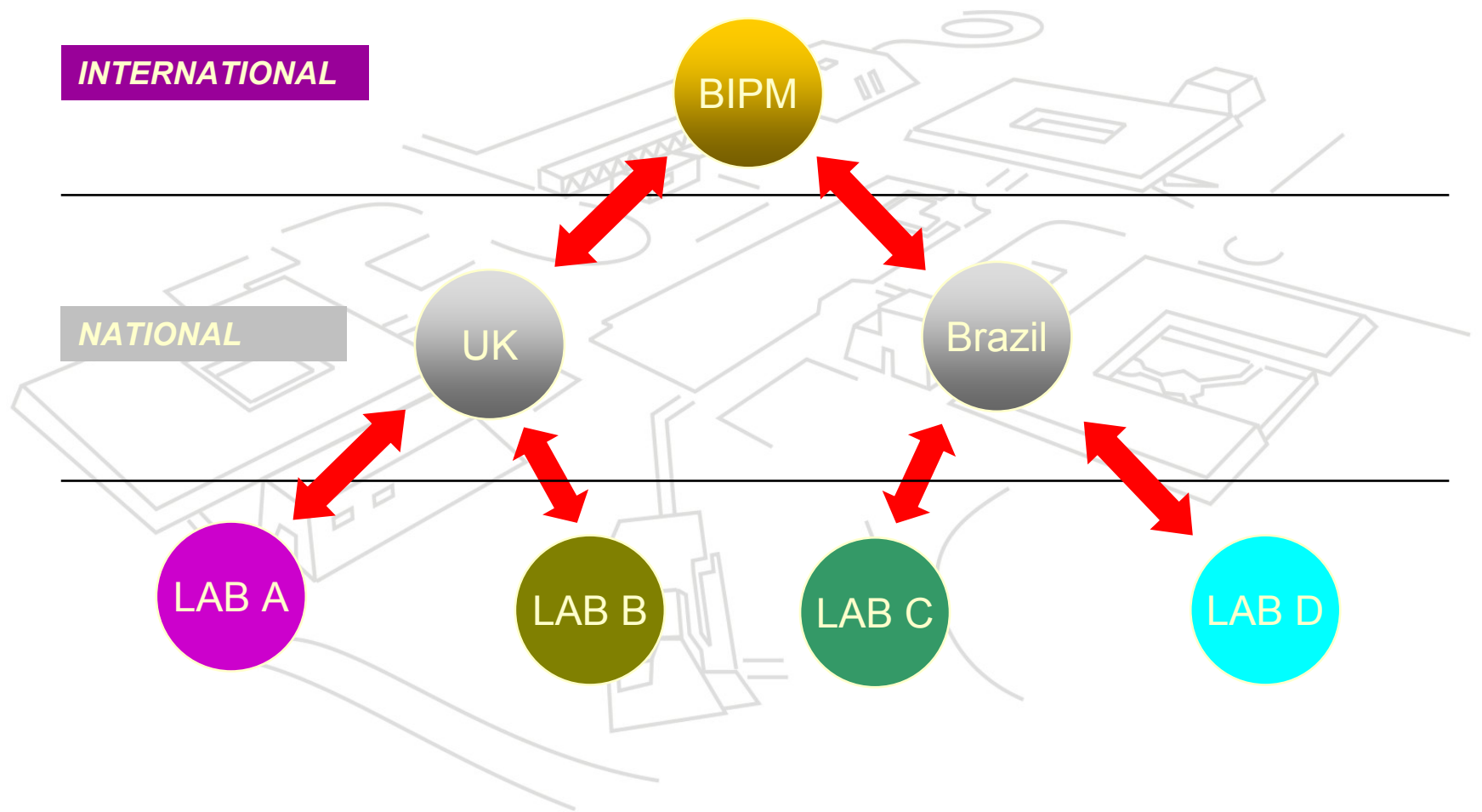


- LGC's performance in these studies is compared with the national measurement institutes from Australia (NMIA), China (NIM), Japan (NMIJ), Mexico (CENAM) and the USA (NIST)**

INTERNATIONAL



NATIONAL



Production of pure substance RMs at LGC

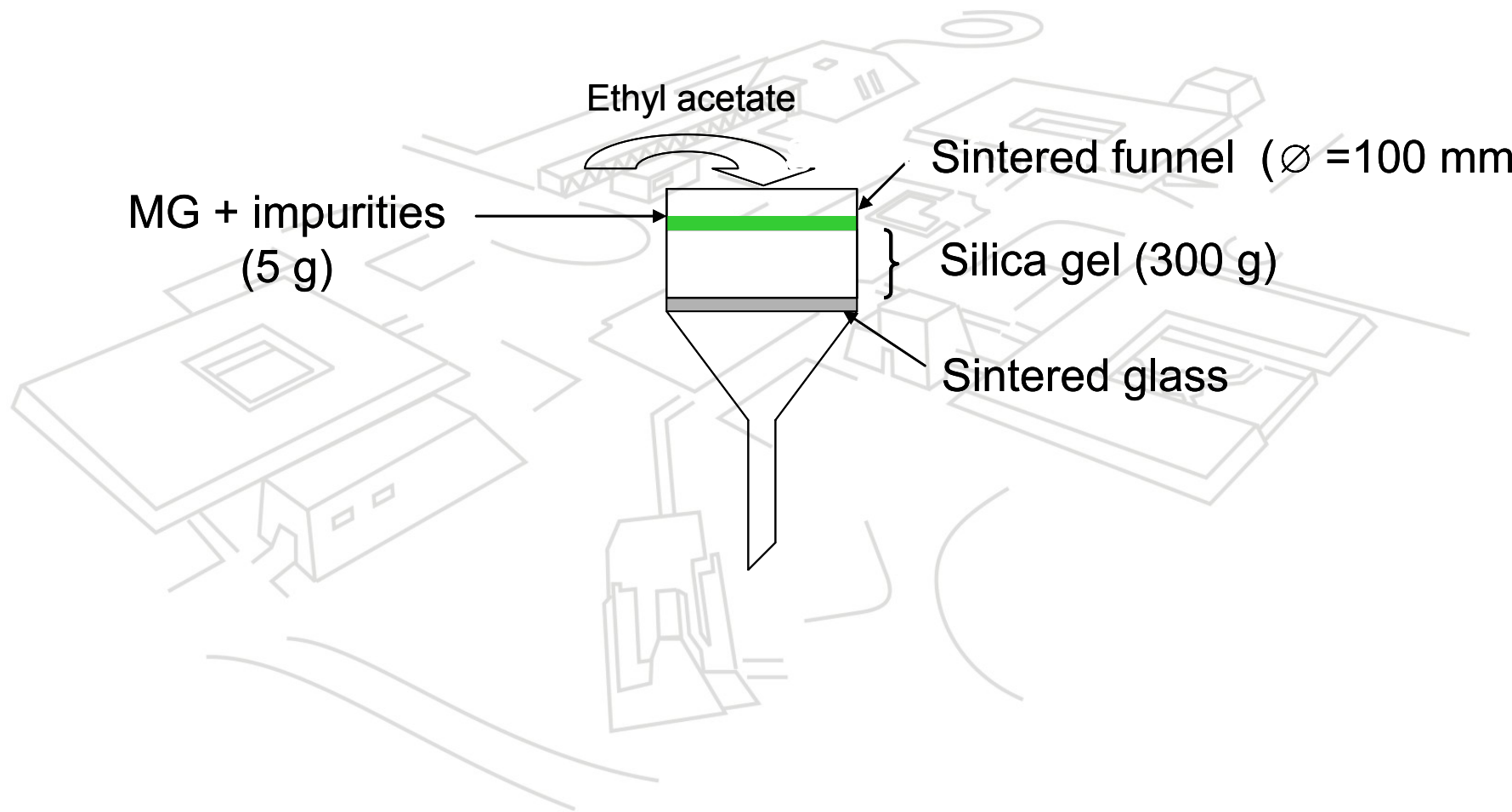
- > 20 years
- Accredited in 2006 by UKAS for RM production to ISO Guide 34 & ISO/IEC 17025
 - wide accreditation scope
 - including pure organic compounds.
- Pure organic certified RMs at LGC:
 - Melting point materials
 - Malachite Green / LMG
 - Solvent Yellow 124
 - Kavain
 - Theophylline
 - Digoxin



Multiple Independent Methods

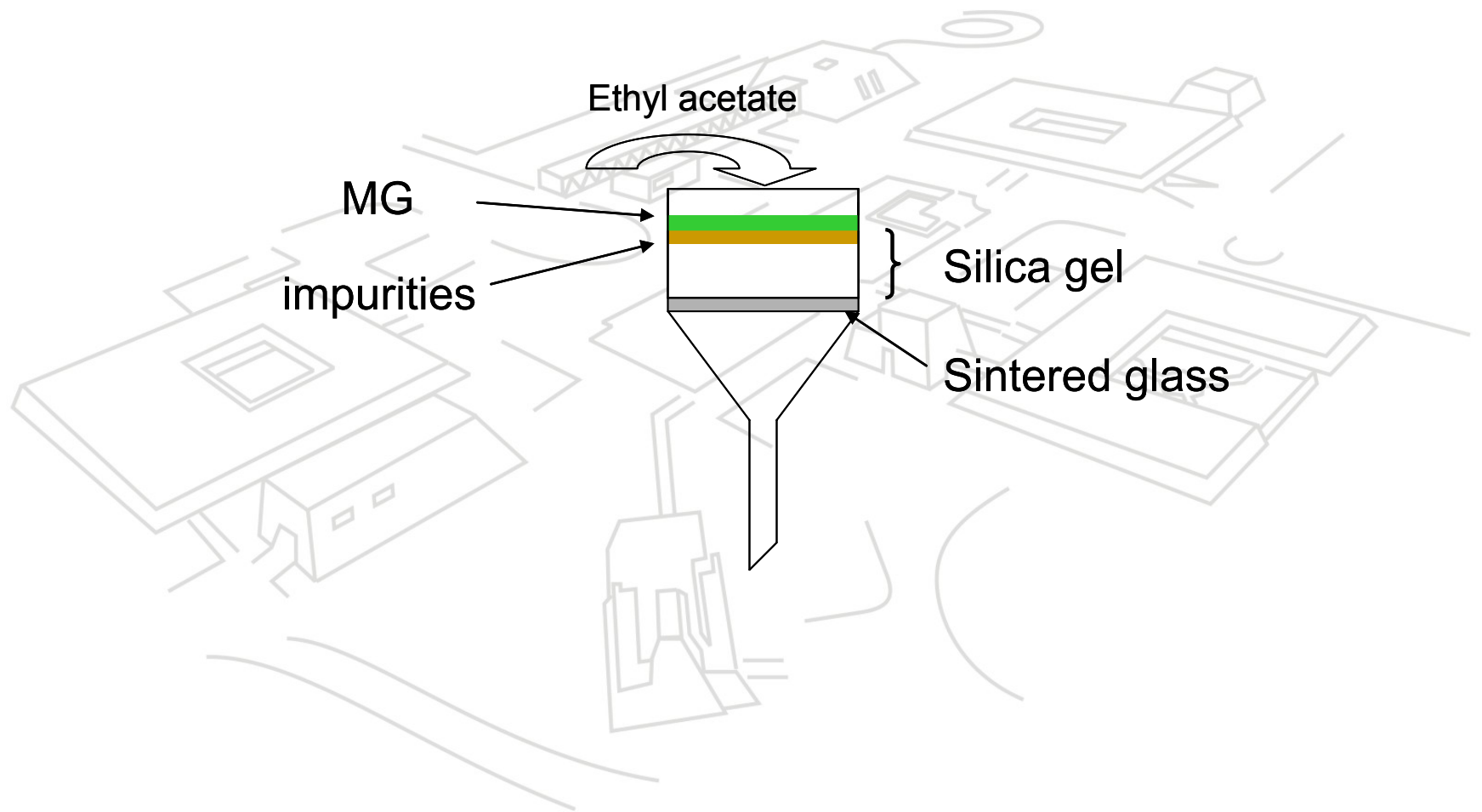
- **Used for pure materials**
 - e.g. Theophylline, kavain, saccharin, BHA, BHT
 - multiple methods
 - HPLC
 - GC-FID / GC-MS
 - DSC
 - calculate a mean value
 - correct for moisture and ash content
 - determine uncertainty
- **Moisture**
 - Karl Fischer titration
 - loss on heating
- **Ash content**
 - ICP-OES/MS
 - Ashing
 - Thermal Gravimetric Analysis

Purification by chromatography

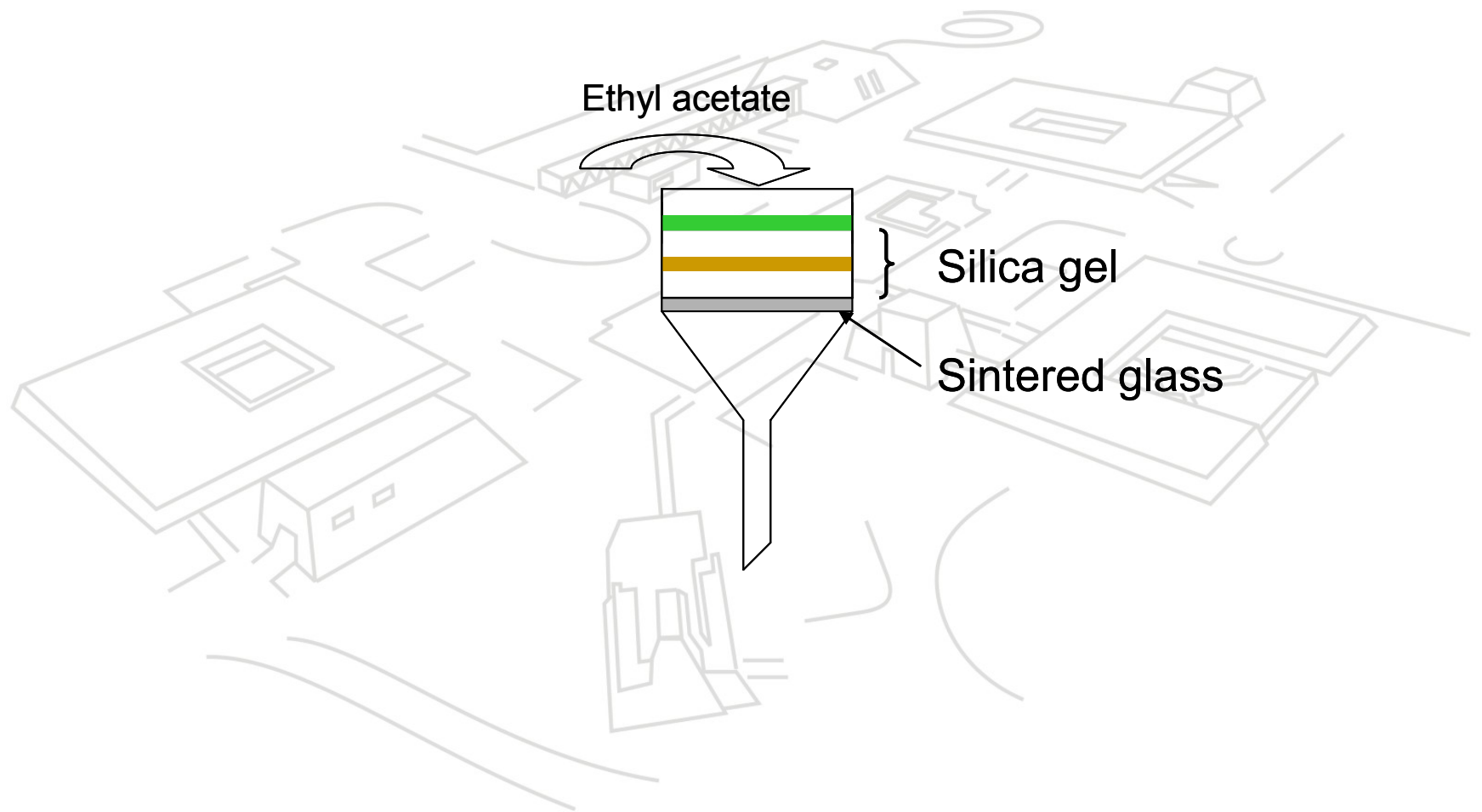


* 60Å, Particle size 40-63 micron, Fisher, DAVISIL

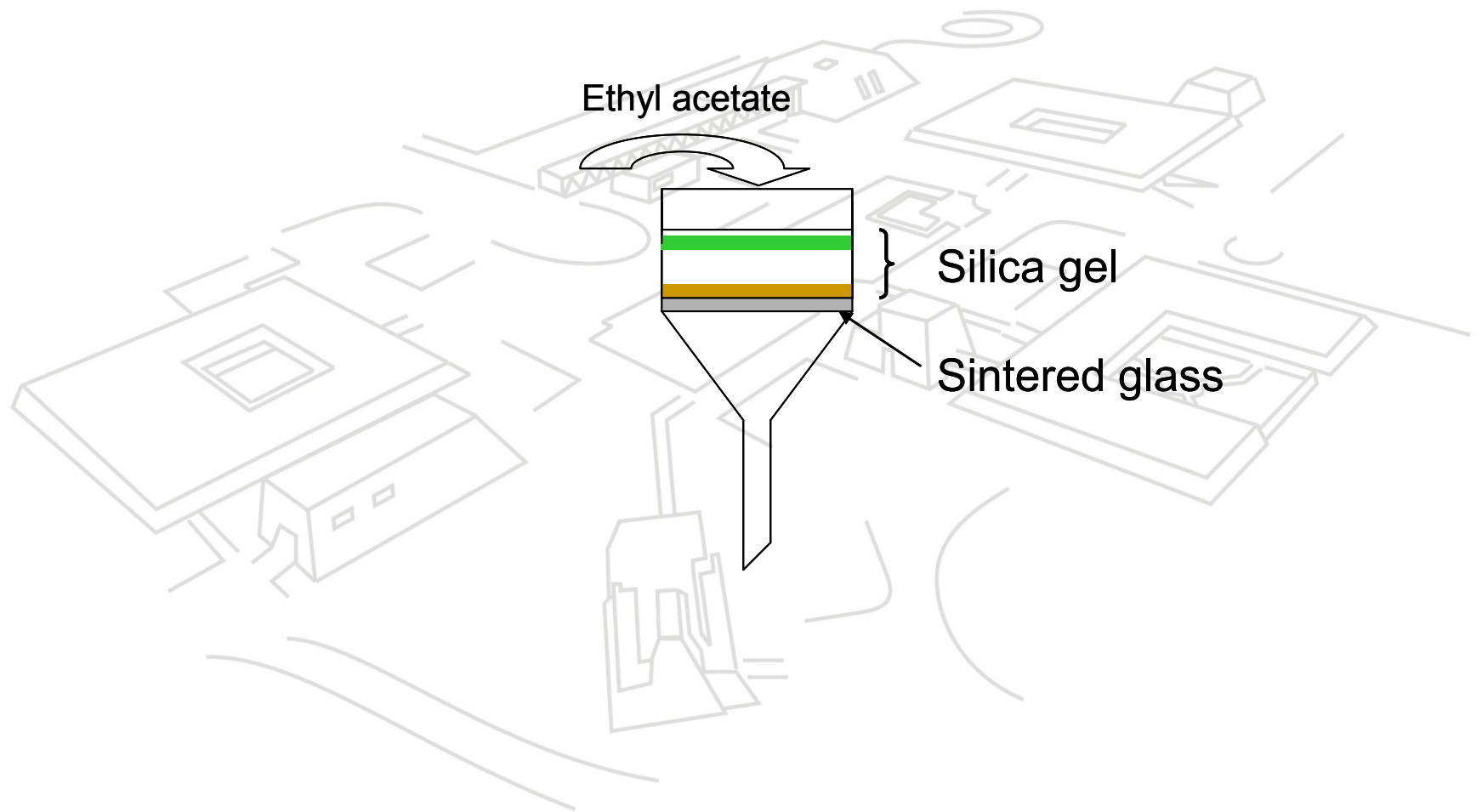
Purification by chromatography



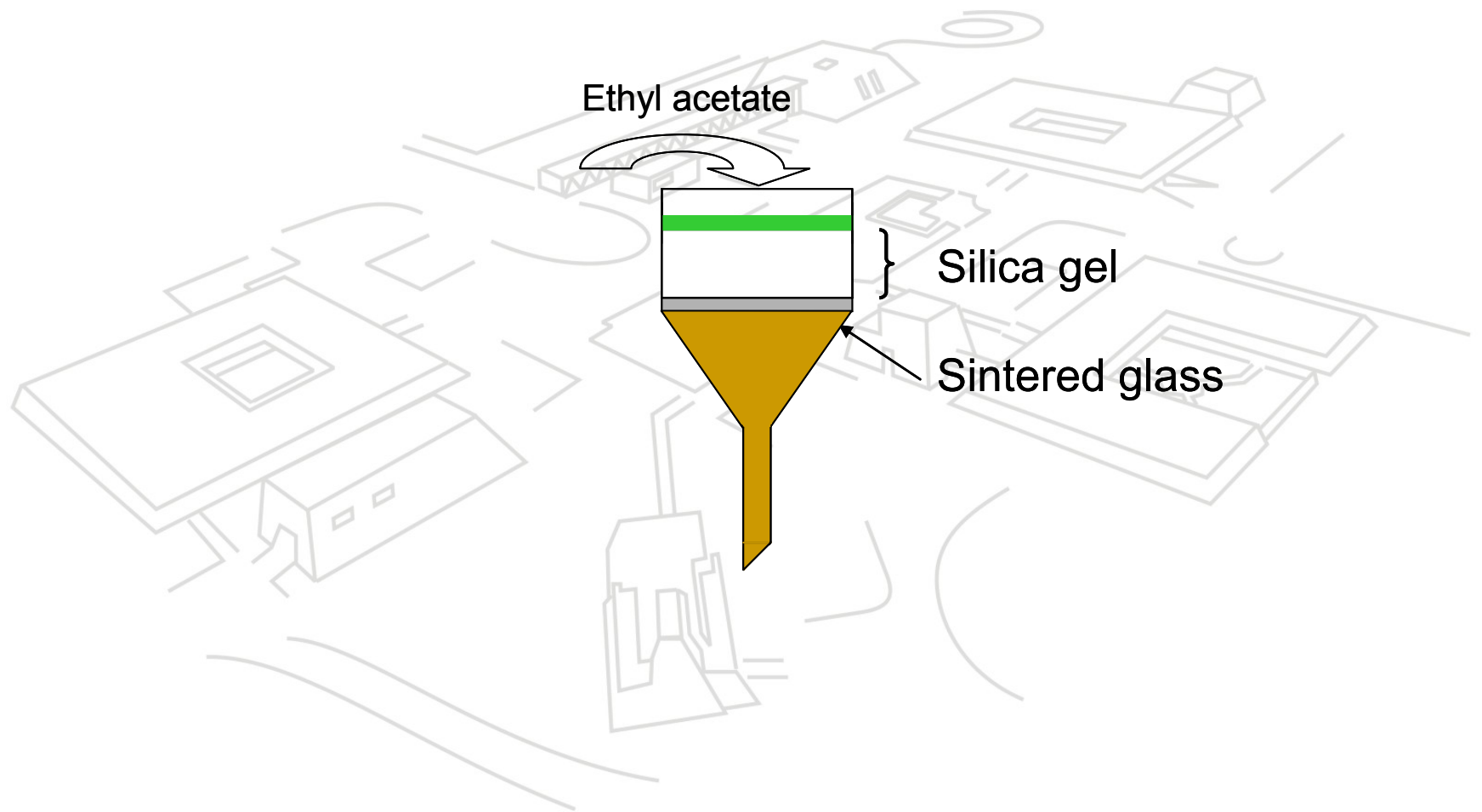
Purification by chromatography



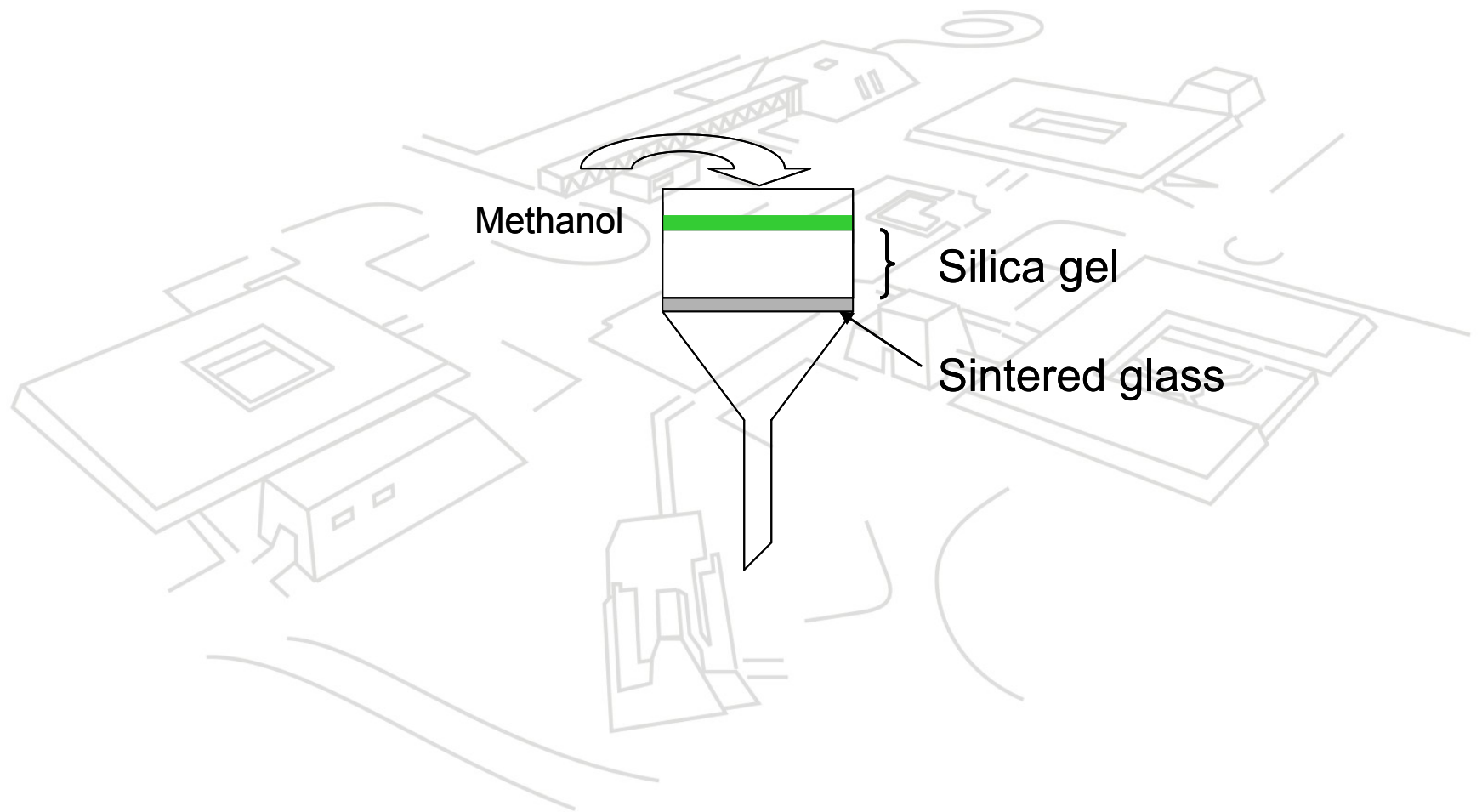
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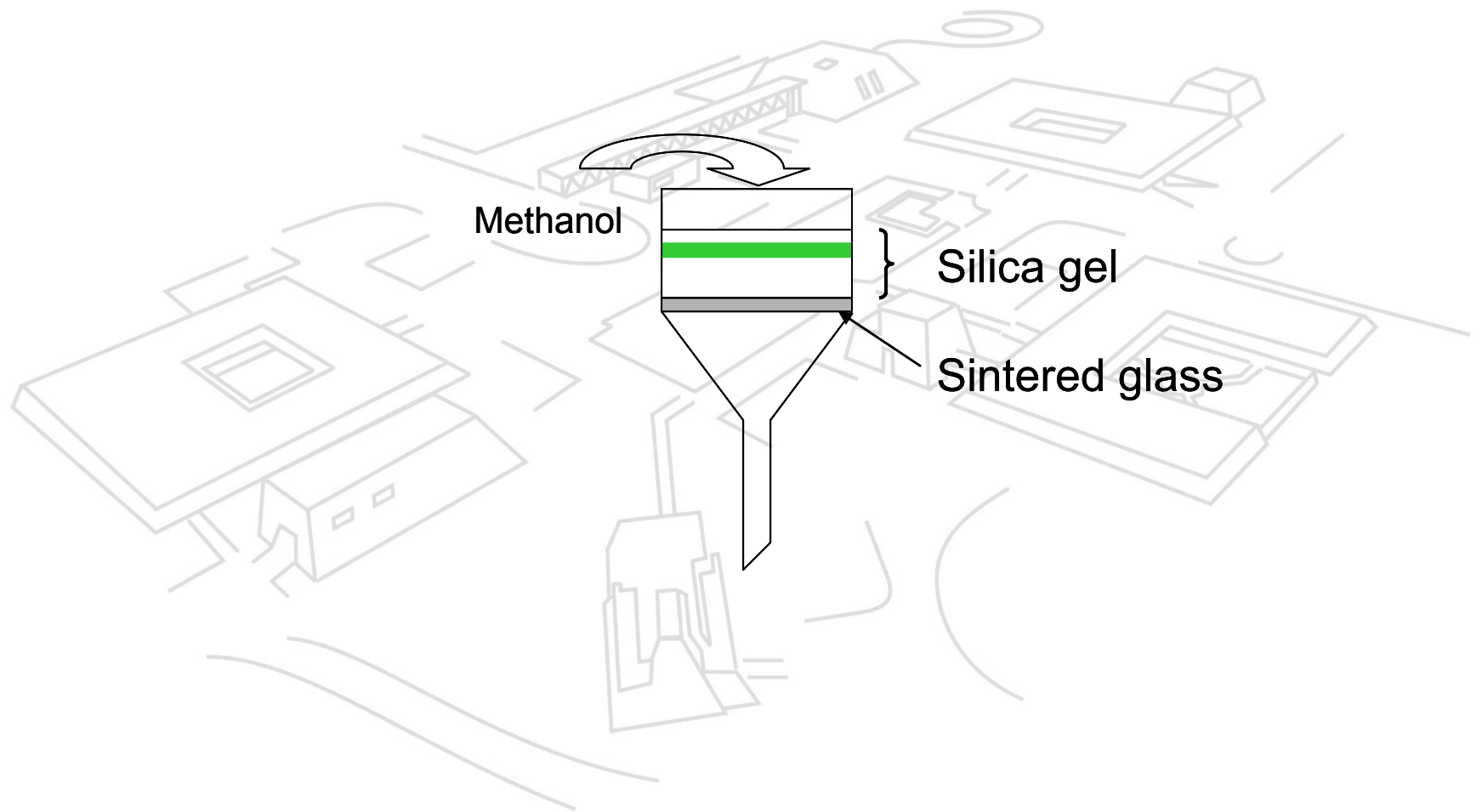
Purification by chromatography



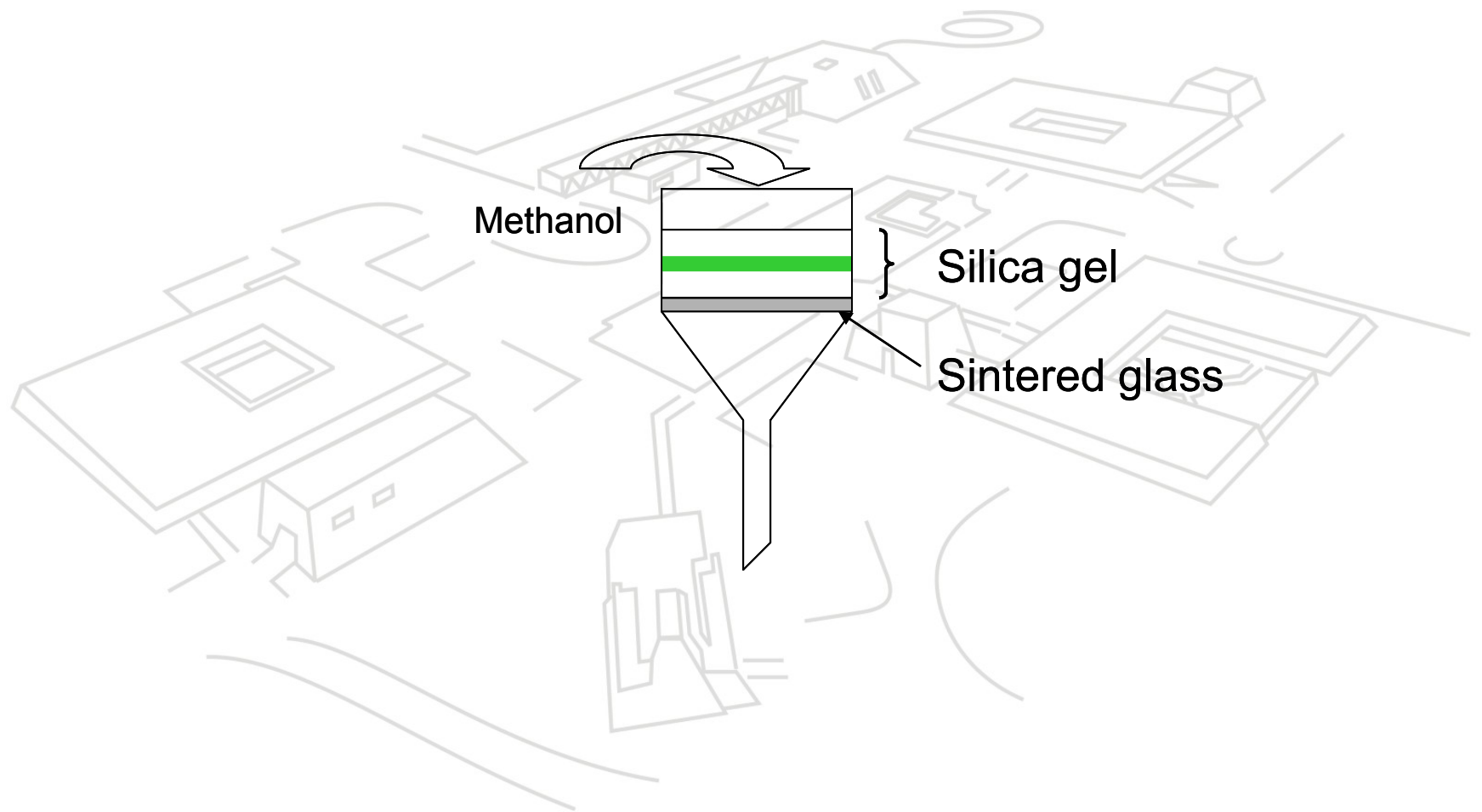
Purification by chromatography



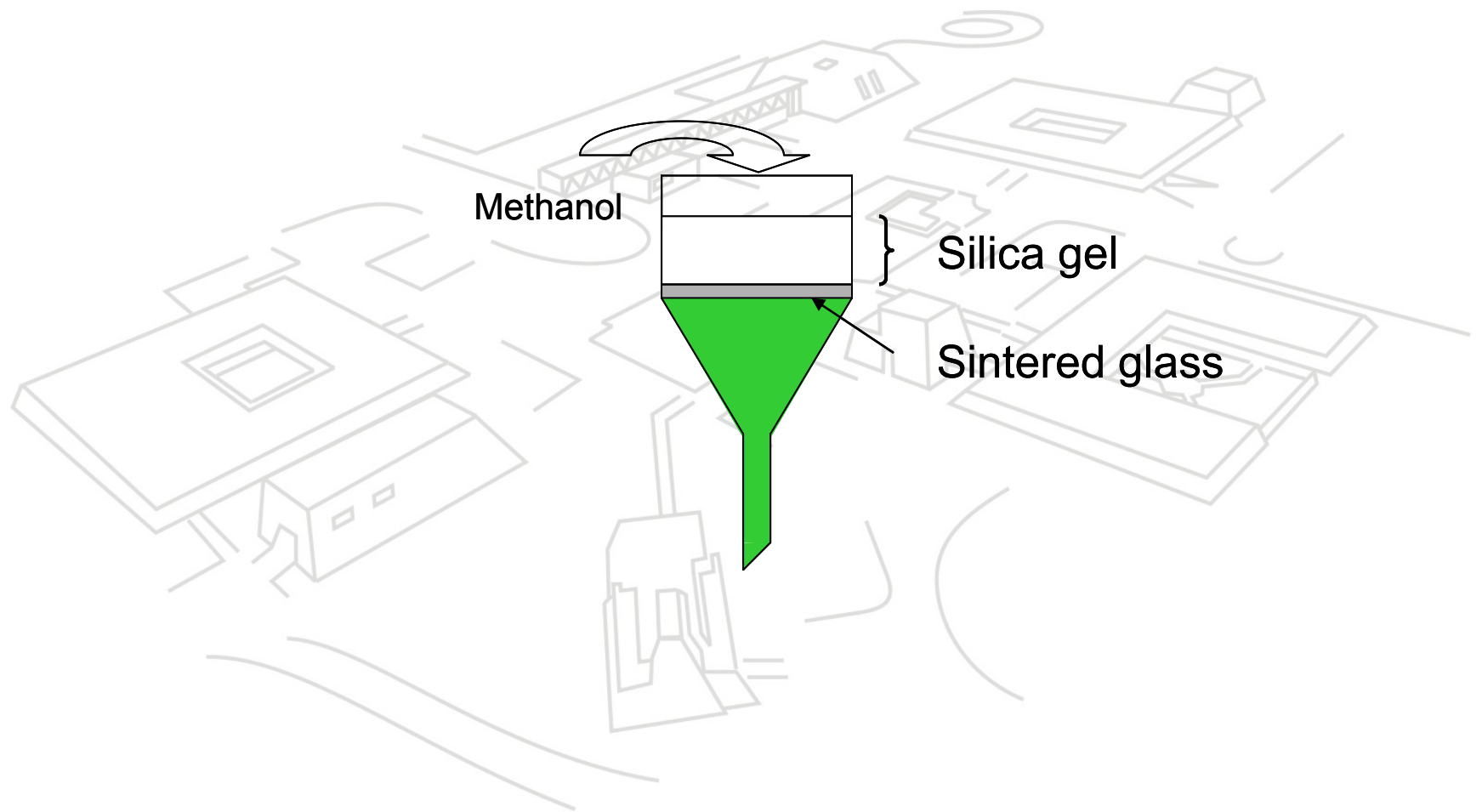
Purification by chromatography



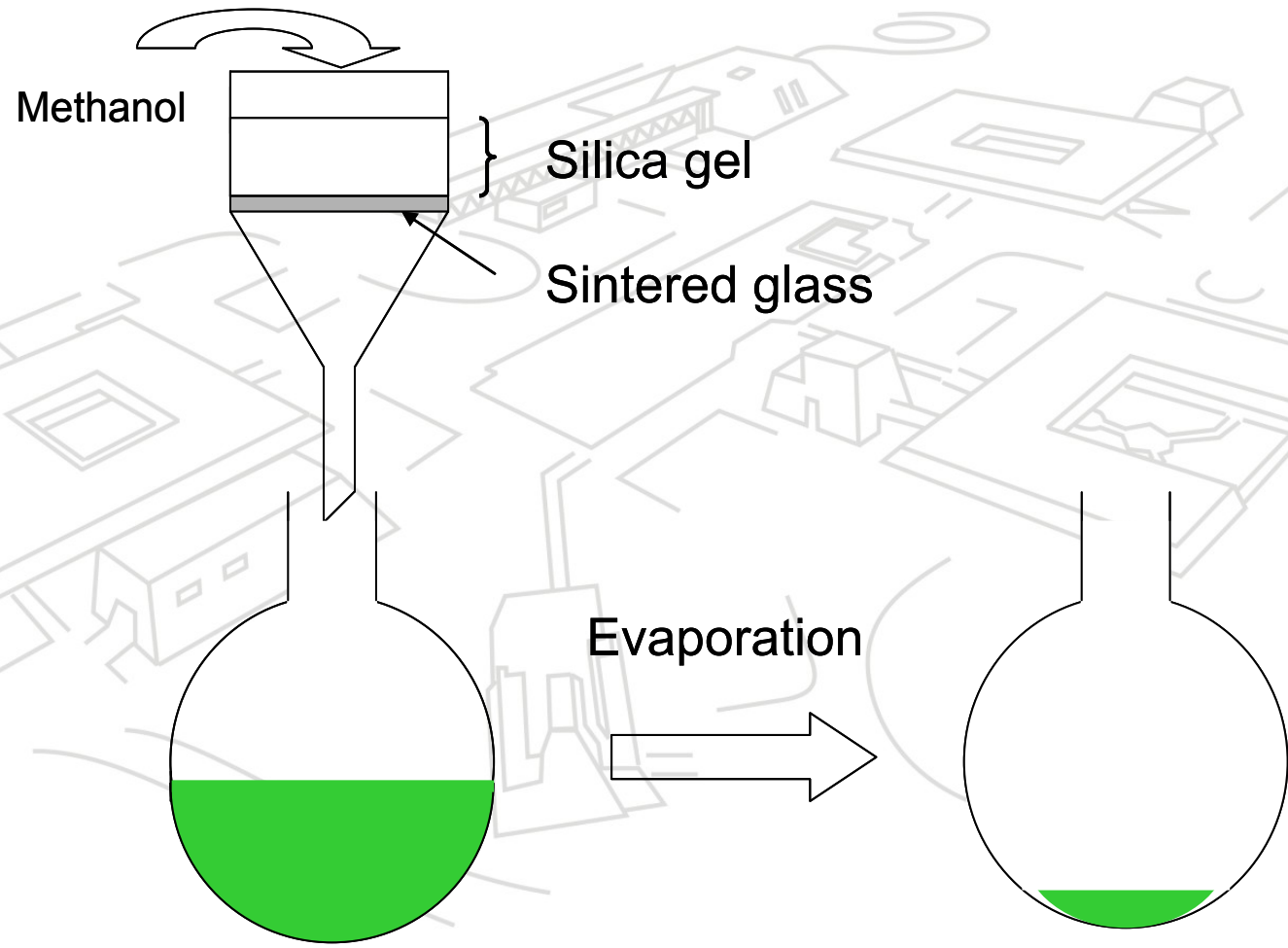
Purification by chromatography



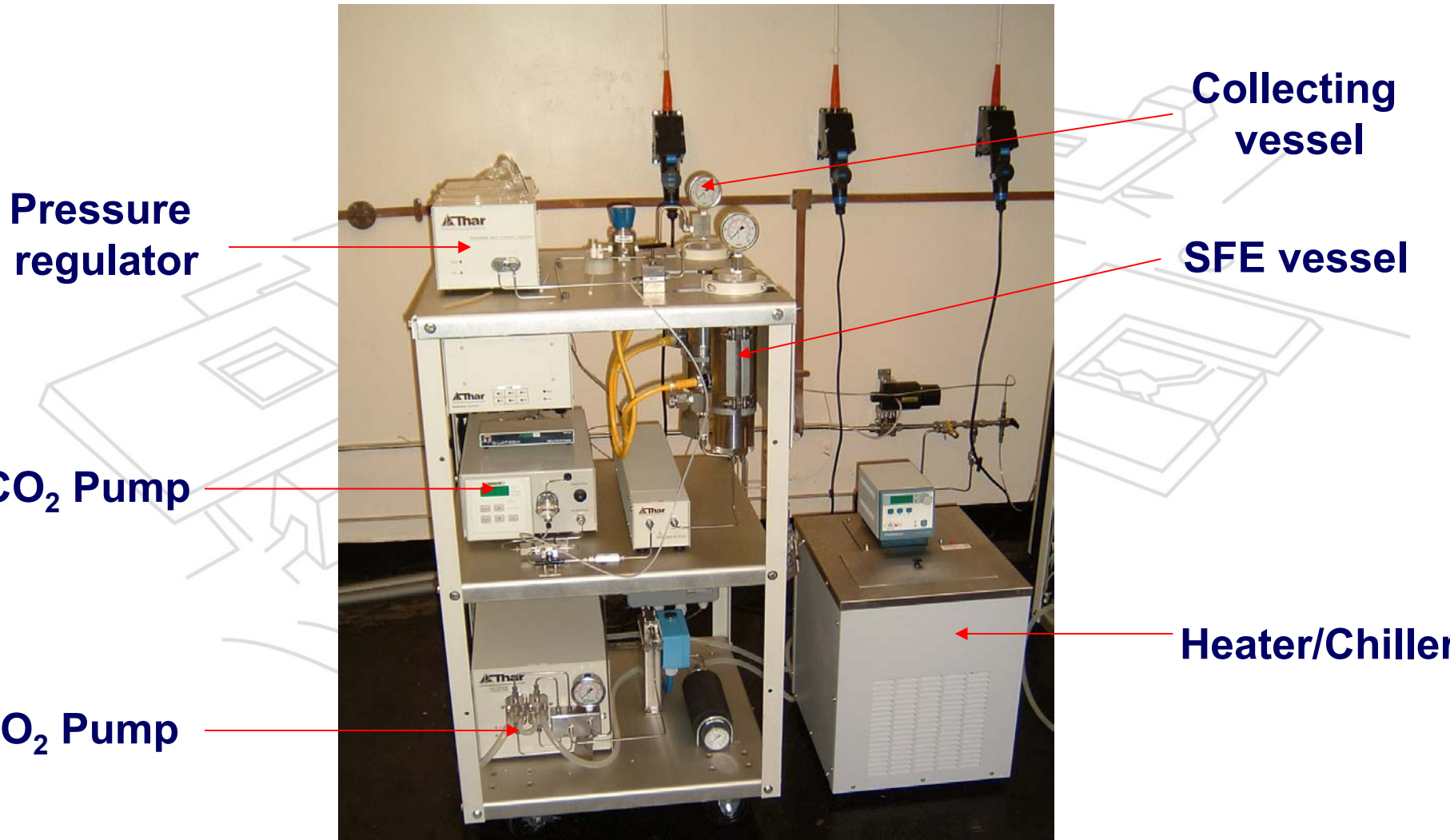
Purification by chromatography



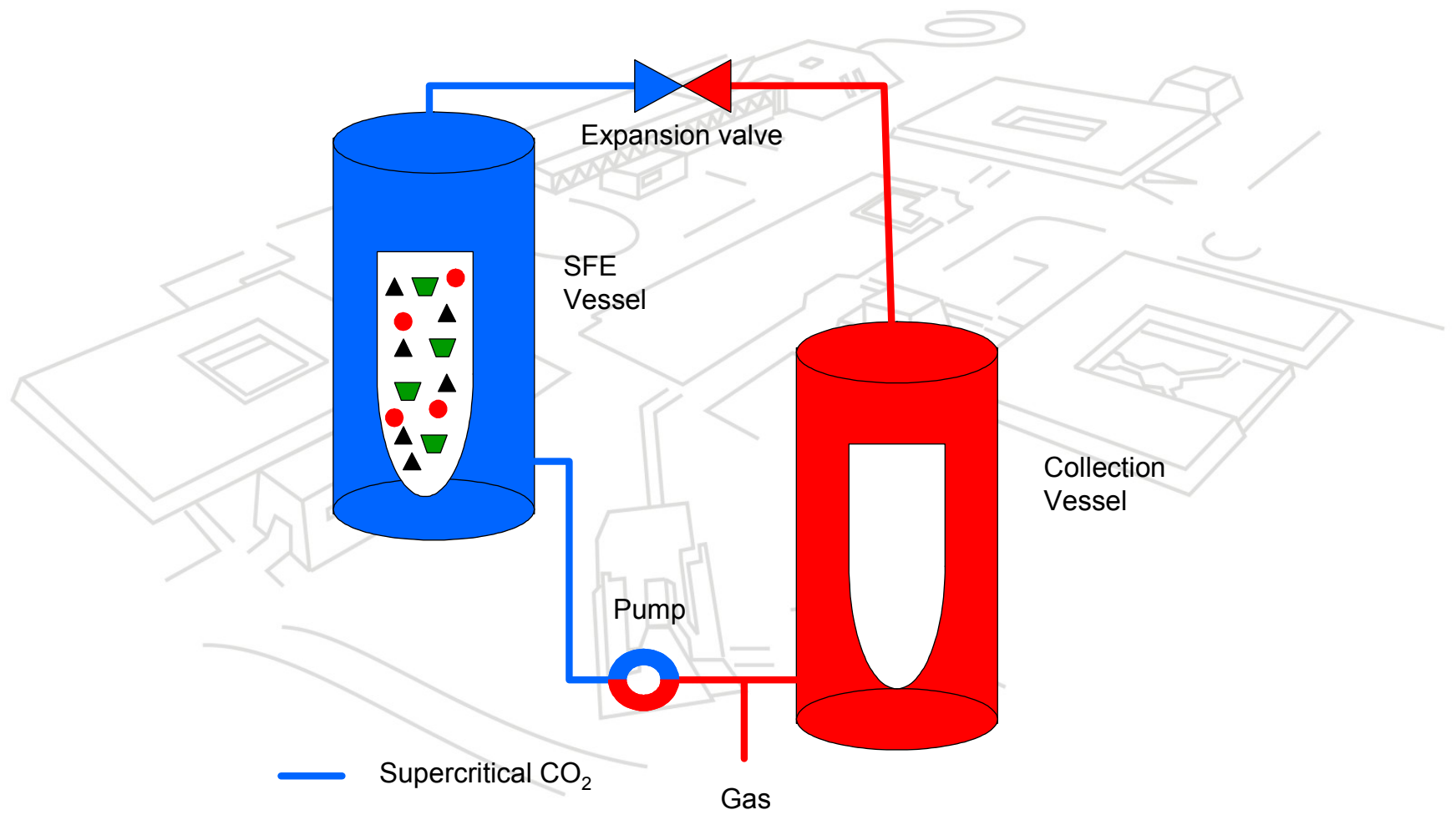
Purification by chromatography



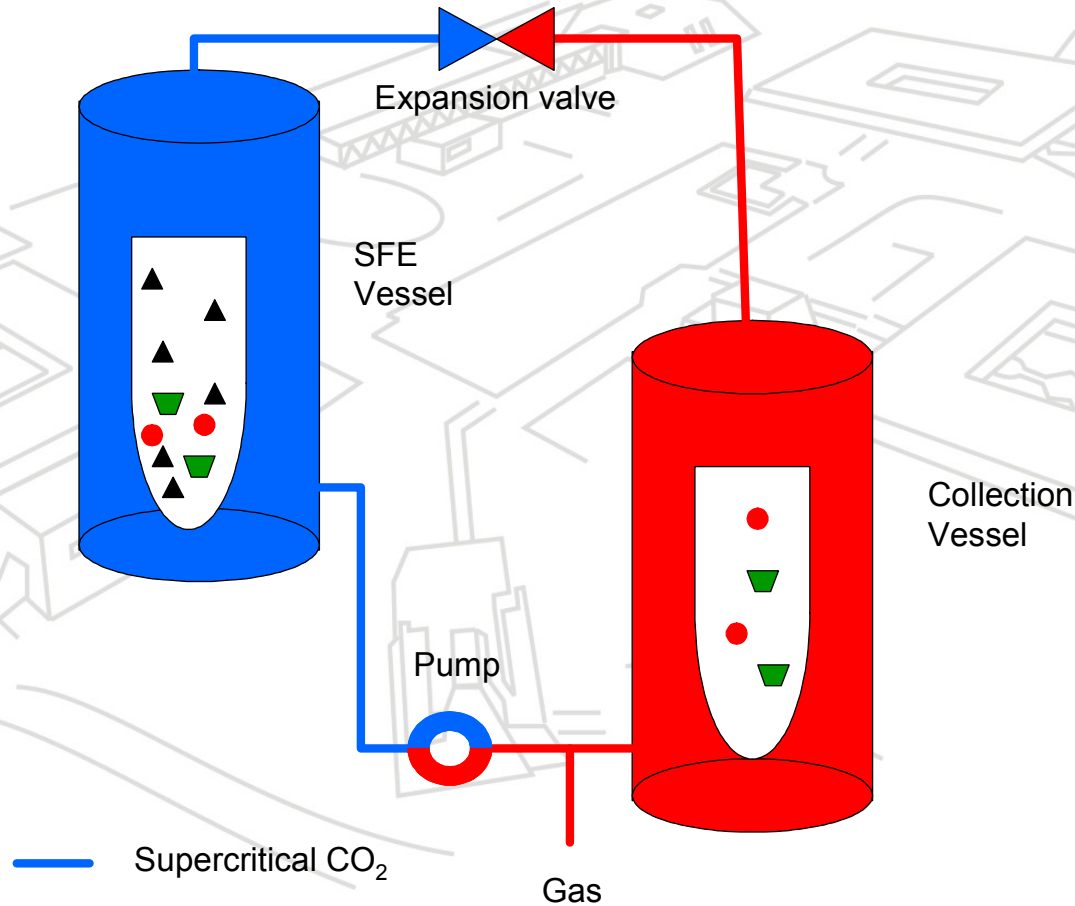
Purification by Supercritical Fluid Extraction



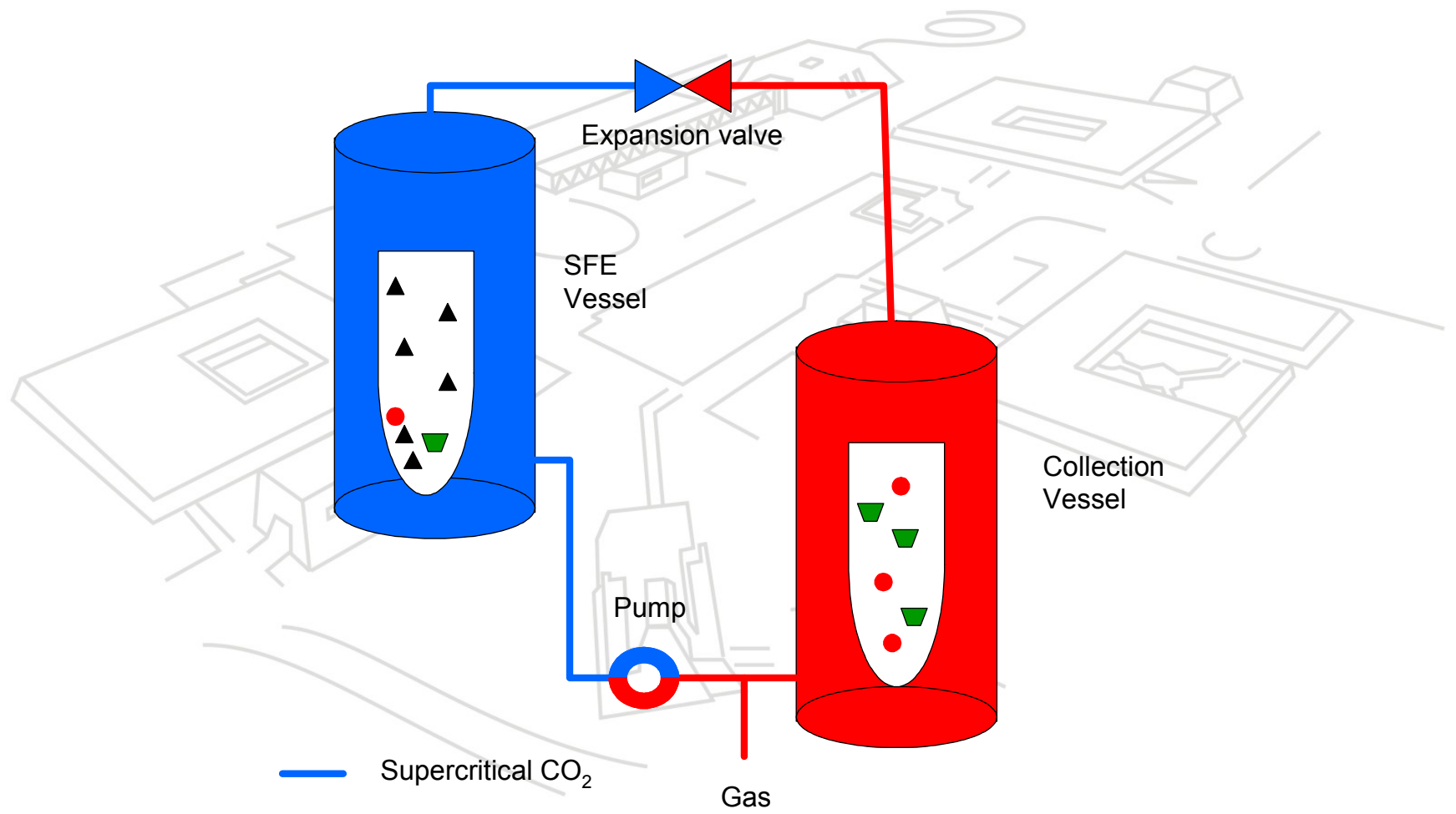
Supercritical Fluid Extraction - Principle



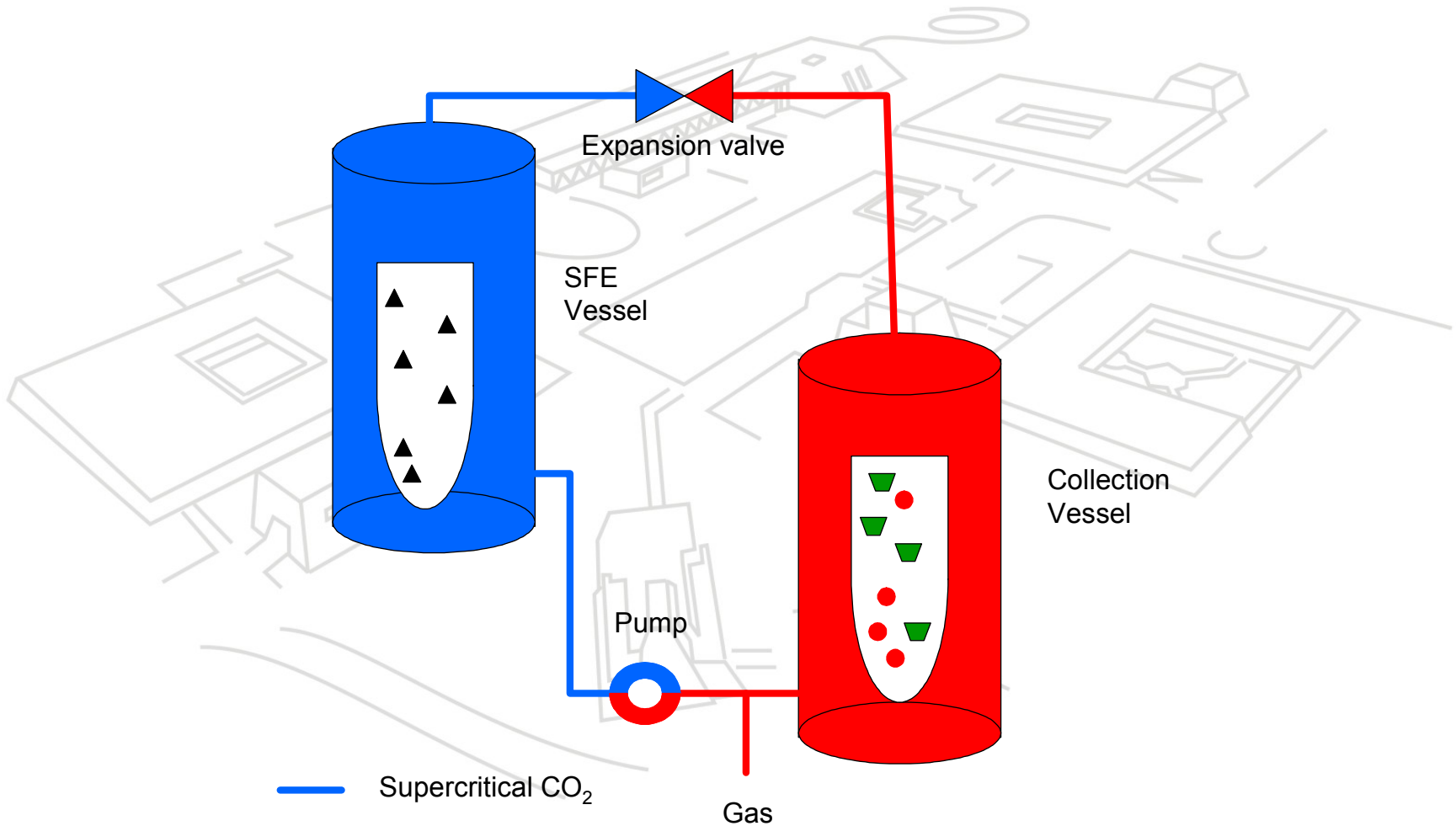
Supercritical Fluid Extraction - Principle



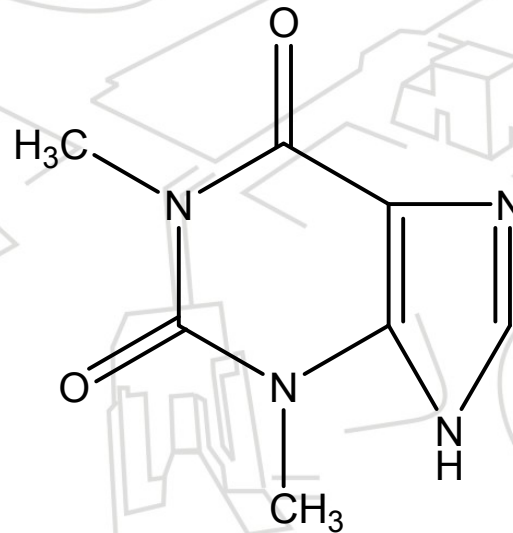
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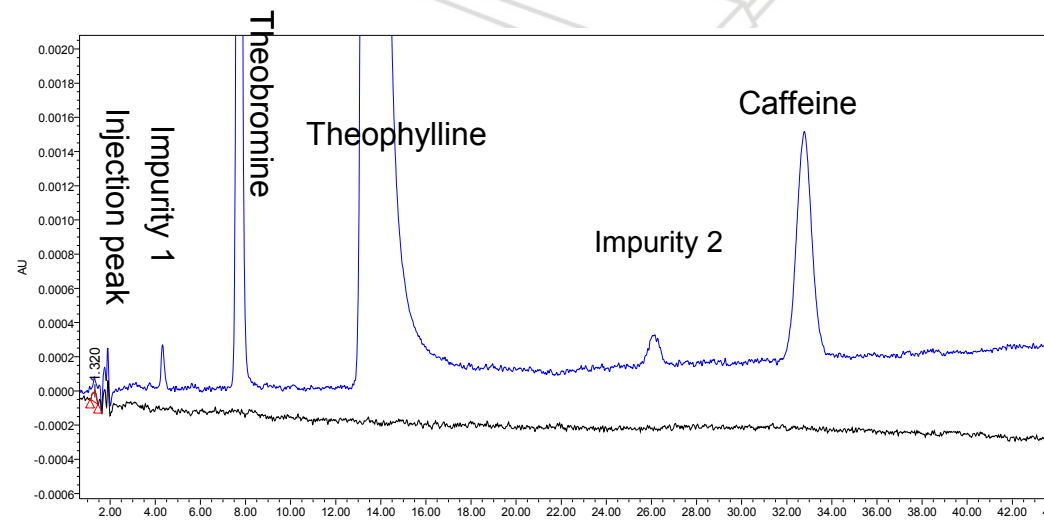
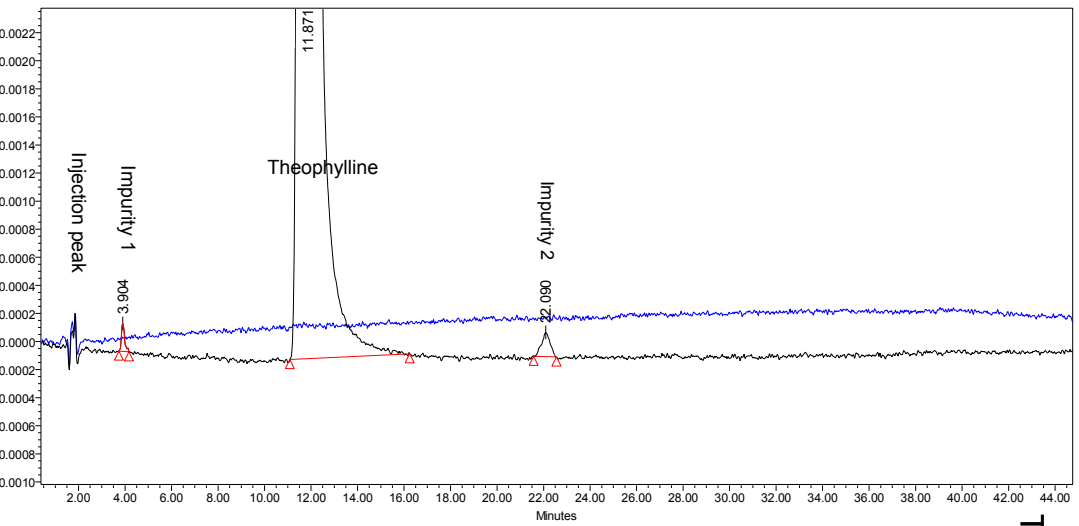
Case Study 1 – Theophylline

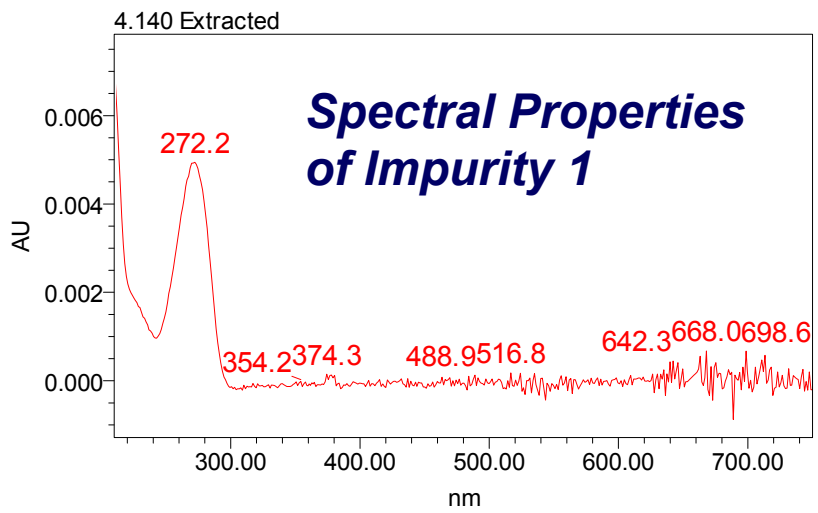
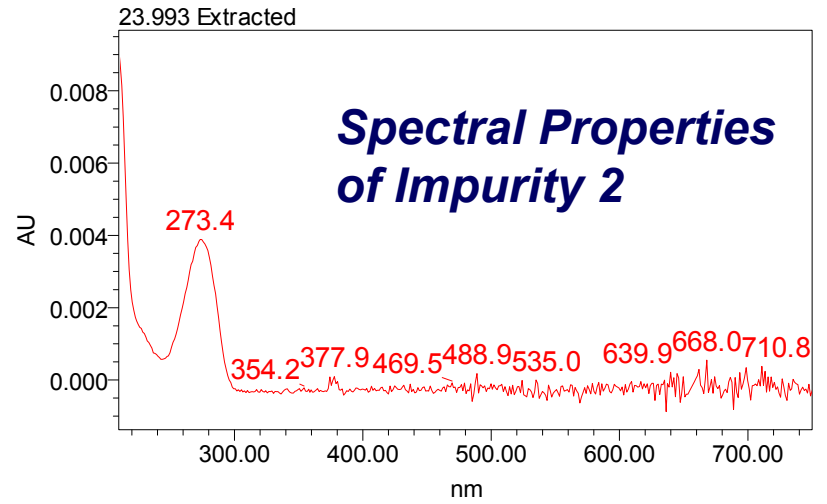
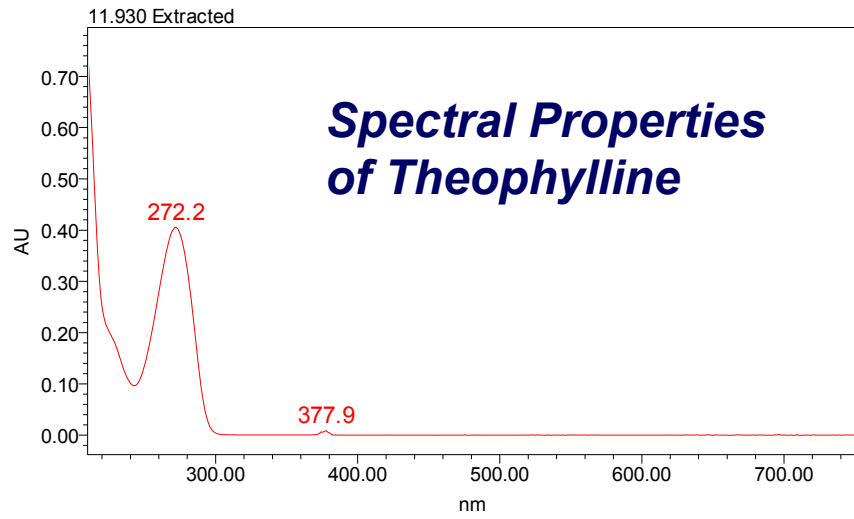


Theophylline

- **Commercial supply:**
 - **Confirmation of identity by mass spectrometry and ^1H NMR.**
 - **Packaged into appropriate numbered glass vials (septum and crimp capped).**
 - **Tested for homogeneity.**
- **Characterization using as many quantitative techniques as possible, in this case:**
 - **DSC,**
 - **HPLC UV,**
 - **GC-FID.**
- **Moisture determined using oven Karl Fischer.**
- **Inorganic screen by ICP-OES & ICP-MS**
- **Impurity identities confirmed by LC-MS**

HPLC-DAD





Certified Value

- **HPLC (n=9, s.d = 0.004)** **99.938 %**
- **DSC (n=6, s.d. = 0.01)** **99.98 %**

Unweighted mean used to assign the purity value for this material

- **Impurity 1:** **0.02 %**
- **Impurity 2:** **0.05 %**
- **KF moisture (n=2, s.d. = 0.0071)** **0.015 %**
- **Inorganics (ICP-OES / ICP-MS)** **< 0.1 %**
- **Overall Purity:** **99.95 %**
- **Standard uncertainty** (u_{char}) **0.022 %**

Stability

- **Accelerated testing**
 - *e.g. for temperature*

Storage Time (months)	Storage Temperature			
	40°C	20°C	4°C	-18°C
1	✓	✓	✓	✓
2	✓	✓	✓	✓
3	✓	✓	✓	✓

- **Carried out under isochronous conditions**
 - *Removes long term analytical variations due to*
 - *instrument drift*
 - *use of different calibration materials*
 - *changing environmental conditions*
 - *changes in analyst or laboratory reagents etc*
- **Long term testing**
 - *Compare results with samples stored under 'reference' conditions*

Expiry Date & Uncertainty

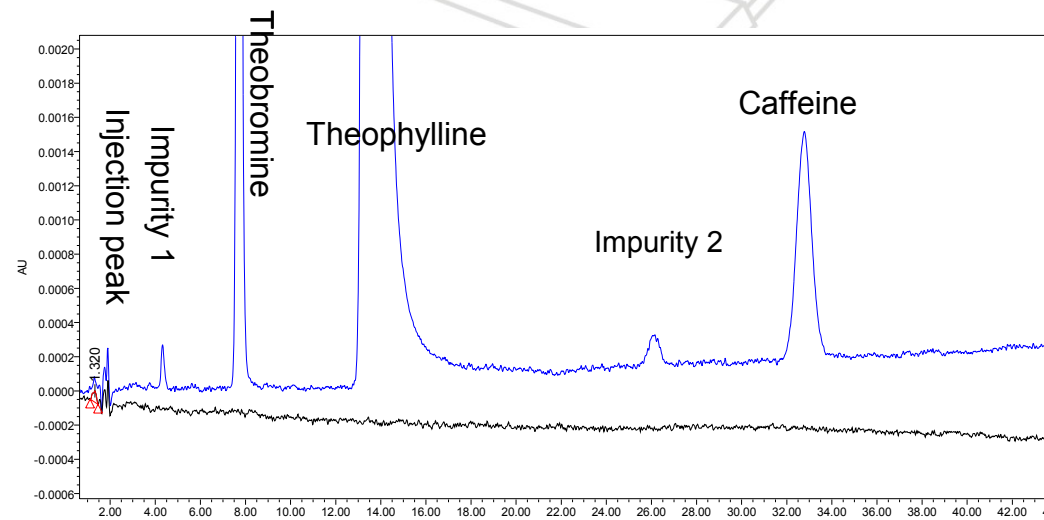
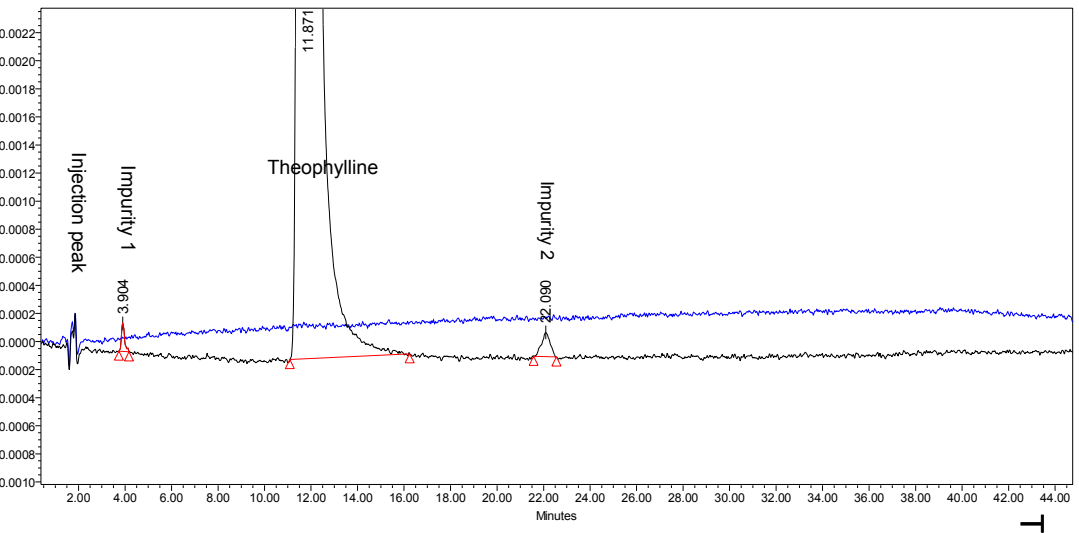
- **Stability testing**
 - **Expiry date - when a material is considered no longer suitable for sale**
 - **Stability uncertainty (u_{stab})**
- **Uncertainty**

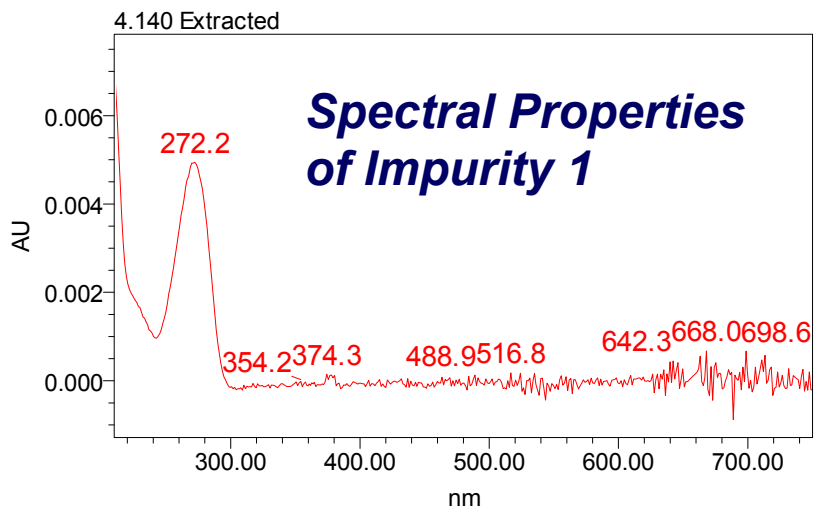
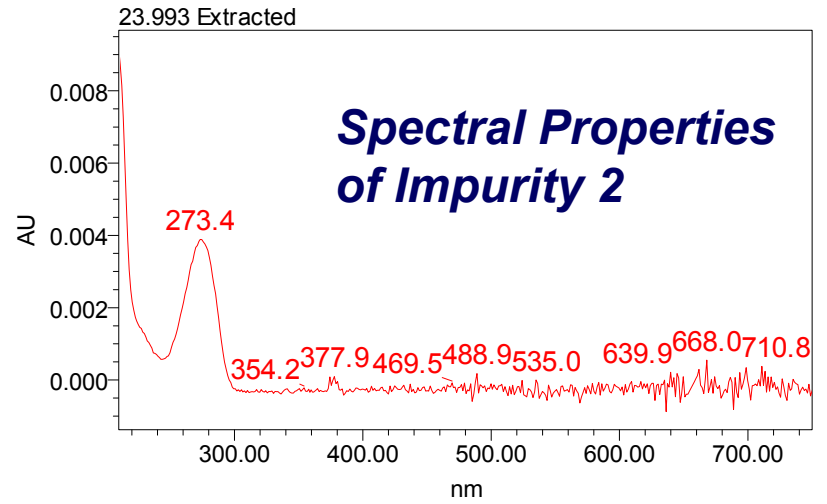
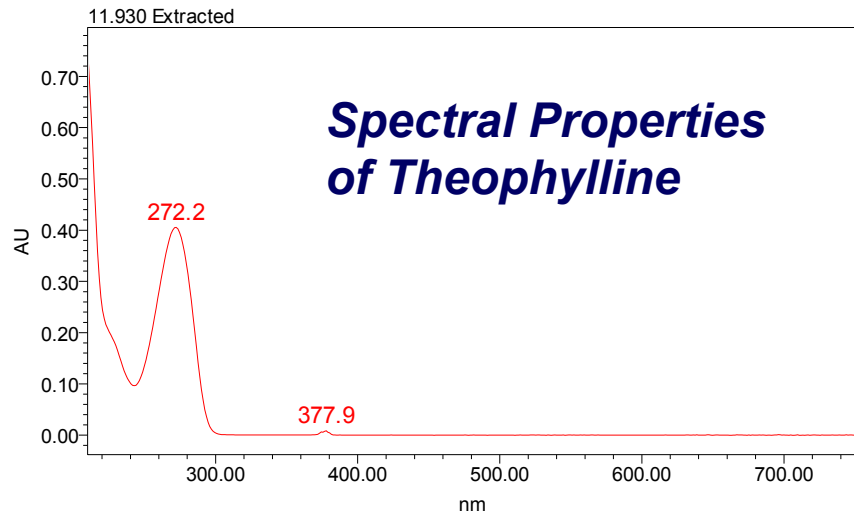
$$U_{CRM} = k \times \sqrt{u_{char}^2 + u_{hom}^2 + u_{stab}^2}$$

Theophylline

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- **Long term testing**

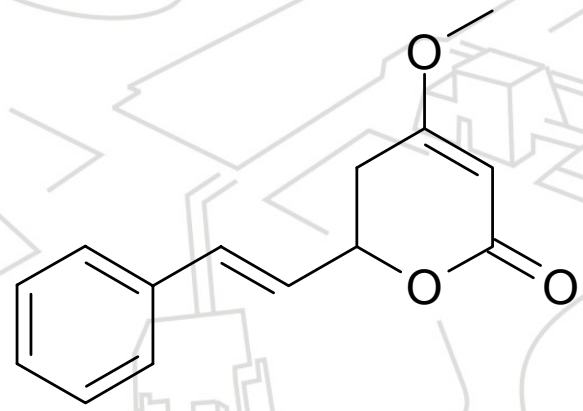
- *Compare results with samples stored under 'reference' conditions*

Expiry Date & Uncertainty

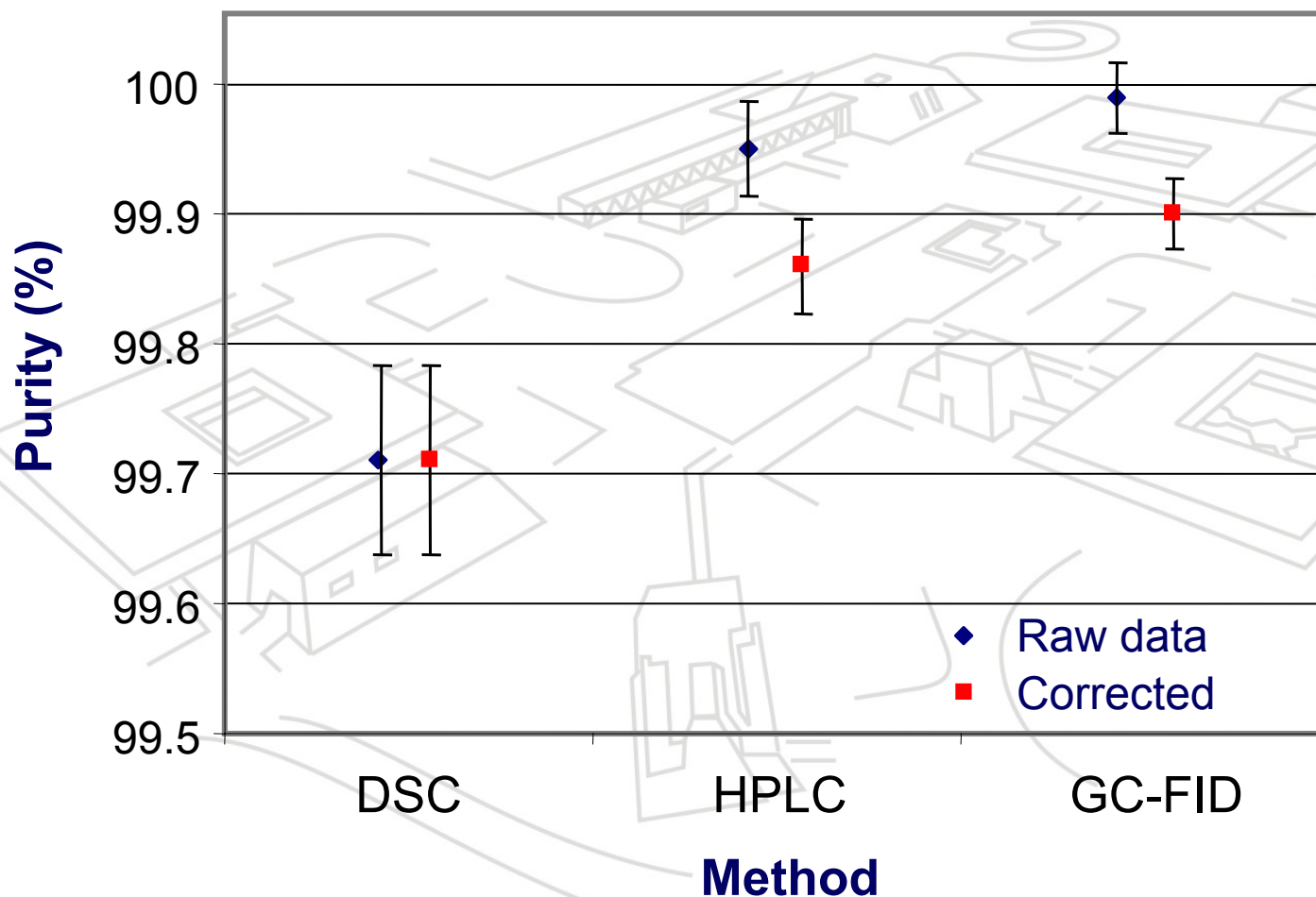
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- **Uncertainty**

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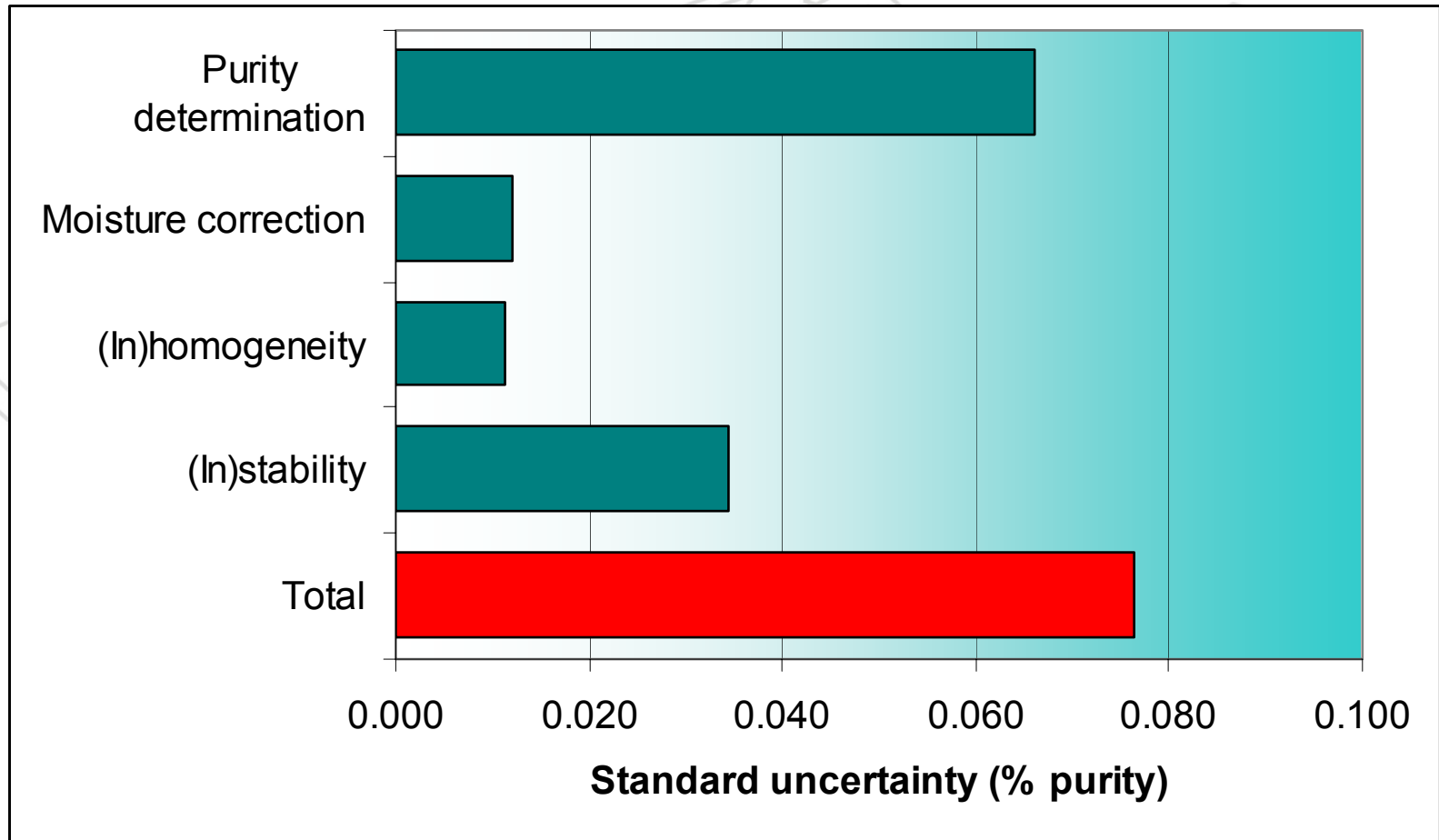
Case Study 2 – Kavain



Kavain characterisation



Kavain: Uncertainty contributions



Issues & challenges

- **Thermally unstable materials**
 - **Not amenable to thermal techniques e.g. gas chromatography or differential scanning chromatography**
- **Materials with no chromophore**
 - **E.g. some amino acids**

Further information

- ***Recent/pending publications***

- ***Production of malachite green oxalate and leucomalachite green reference materials certified for purity. Anal Bioanal Chem. Published on line 16 April 2008***
- ***Development and accreditation to ISO/IEC 17025 calibration status of a melting point measurement facility for the UK. thermochimica acta (submitted)***

Acknowledgements

- **Thierry le Goff**
- **Ben Joseph**
- **UK Department of Innovation, Universities and Skills**
 - **Chemical and Biological Metrology Knowledge Base Programme**
 - **www.nmschembio.org.uk**