Sigma-Aldrich Integrated Chiral Services

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Chirality 2012
Fort Worth, Texas, 10th-13th June

SIGMA-ALDRICH®

Agenda

- Introduction
- Sigma-Aldrich Integrated Chiral Services Capability
- SAFC Pharmorphix®
- Chiral Services Overview
- Chiral Method Development
- Preparative Chiral HPLC
- Resolution of Racemic Mandelic Acid by Diastereomeric Salt Formation
- Summary

Sigma-Aldrich Integrated Chiral Services Capability

- Sigma-Aldrich is a leading global partner, providing products and services to support the broad range of customer needs from research through to commercialisation
- Technologies offered by Sigma-Aldrich include Supelco® (e.g. chiral chromatography) and Pharmorphix® (e.g. solid-state services) which enable and support the research and development needs for chiral separation
- Chiral services have recently been consolidated in Cambridge, UK and include
  - Chiral screening technologies
  - Production of enantiomerically pure compounds by either preparative separations or crystallisation techniques
  - Determination of absolute stereochemistry by single crystal X-ray diffraction
- The Cambridge facility will become the worldwide hub for chiral separation services for Sigma-Aldrich and act as a dedicated point of contact for customers requiring support during research, development and commercial activities

Chiral Services has Moved to Europe!

SAFC Pharmorphix

- Physchem Profiling
- Routine Analysis
- Solid State Enhancement
- Crystallisation Development
- Structure Determination
- Lifecycle Extension
- Chiral Separation

Founded in 2003

Dedicated state of the art facility

Track record 1 in 3 compounds enter the clinic

Investigated a range of >500 compounds

- Key insights in to **stability**, **scalability**, **formulation**, **bioavailability**, and **purity** of the API
- The ability to **modify** and / or **optimize** the physical & chemical properties of the API

Pharmorphix® Overview: Equipment

X-Ray Diffraction

Single Crystal X-Ray diffraction
Variable humidity X-Ray powder diffraction

Thermal Analysis

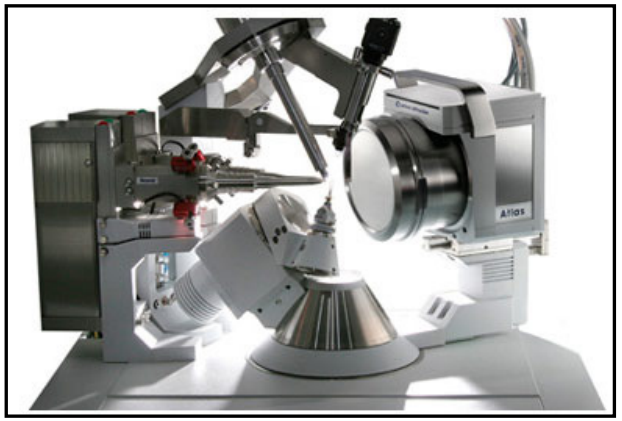
DSC and TGA instruments (Mettler and TA)
Hot-stage microscopy
Variable temperature X-ray powder diffraction

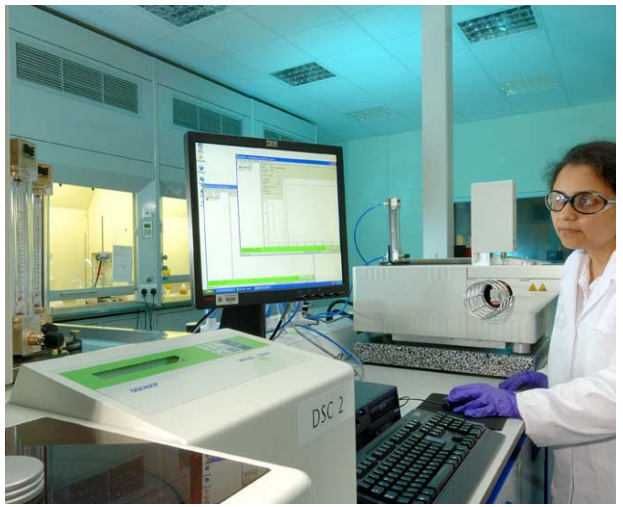
Crystallisation

Parallel reaction blocks/turbidity probes
Jacketed reactors from 250 ml to 7.5 L

Physicochemical Profiling

pKa and log P/D determination
Intrinsic dissolution





Dedicated State of the Art Facility

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Physical & Chemical Analysis

- Pharmorphix Overview
- Physchem Profiling
- Routine Analysis
- Solid State Enhancement
- Crystallisation Development
- Structure Determination
- Lifecycle Extension
- Chiral Resolution

Approximately 40 % of drug failure can be attributed to poor pharmacokinetics*

Physicochemical Performance

Adsorption/Desorption properties

Measured pKa, LogP & LogD

Physical Performance

Solubility (kinetic, Thermodynamic)

Limits drug concentration in intended media

Solid State Stability under a range of conditions

Chemical Performance

Chemical stability under a range of environments

Selecting compounds with the right balance of physicochemical properties reduces the probability of failure.

** Kennedy, DDT 2 436-444, 1997*

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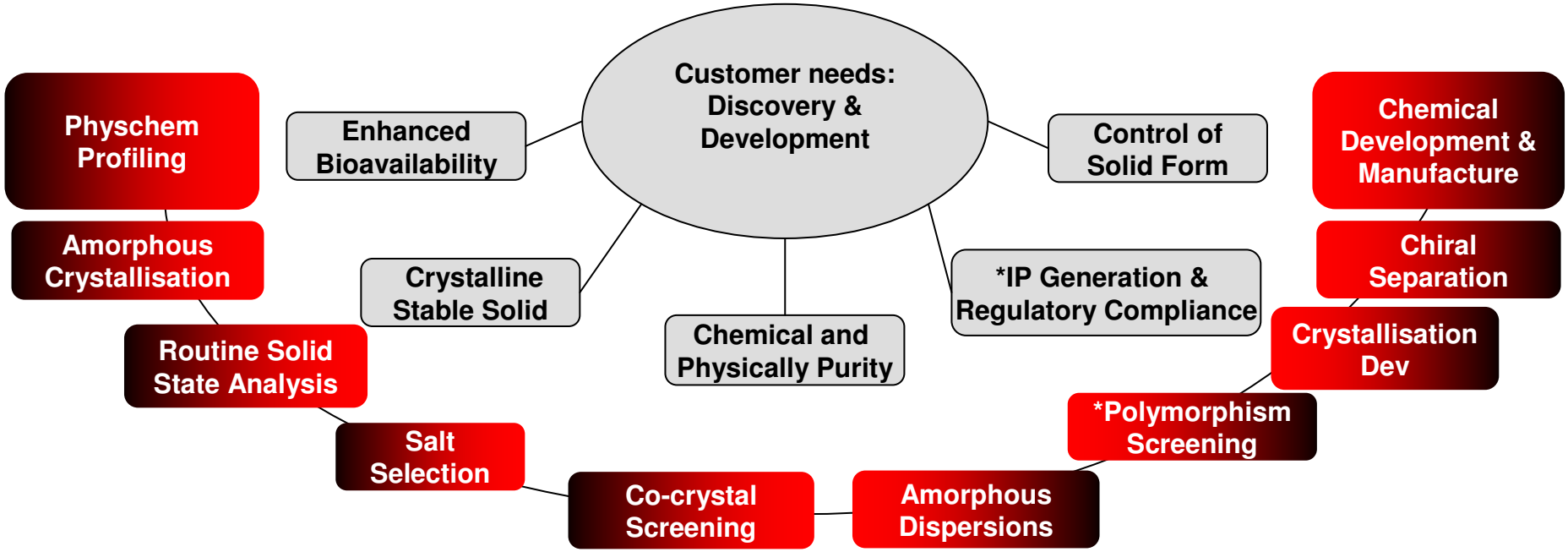
Customers and Pharmorphix®

Small Molecule Clinical Development



An Integrated Flexible Service to Meet Customer Needs

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Chiral Services Overview

- Chiral HPLC & GC method development screening provides
  - Methods for general use
  - LC-MS compatible methods for clinical, stability or dissolution studies
  - Method for later scale-up to prep
- Examples of possible optimisation projects
  - Methods that provide high sensitivity for trace analysis
  - Methods for API plus all impurities
  - Methods that will not have interferences from excipients in formulations
  - Resolution of parent drug and metabolites
- Loading studies
- Small scale purification (Batch LC)
- Large scale purification (SMB)

Finding the Right Chiral Column

Prior knowledge (in-house/external literature), specific functionality

and/or

Column choice and separation mode may depend on application and be influenced by

- Known solubility of compound
- Need for MS detection
- Need for preparative separation

and/or

Screen a range of column types and separation modes

- Analytical column kits available for CHIROBIOTIC, CYCLOBOND

and/or

Screening process can be contracted out

- Ideal if short of resources
- Compliment existing chiral stationary phases

Chiral Method Development Services

- This service aims to provide a result within 5 days of sample receipt for analytical method development
- A comprehensive report that includes the screening results, optimisation studies performed and final method is provided on completion of project
- This also includes elution order determination by measurement of optical rotation, along with any suggestions for further optimisation if required

HPLC chiral method development screening

- The first stage is an automated primary column screen using an established protocol that includes a comprehensive range of RP, polar organic/ionic and normal phase methods
- This primary screen generally provides 80% of our screening success
- Methods generated by the screen are verified on a second system
- Other chiral stationary phases may be investigated as required (e.g. CLC, protein based....)

GC method development screening

- Chiraldex (polar) and SupelcoDex (non-polar) phases based on cyclodextrin derivatives

Astec Cyclobond and Chirobiotic Phases

Polar Organic Mode (POM):

- Astec CYCLOBOND (1992) (e.g. 95/5/0.3/0.2, CH_3CN/MeOH/HOAc/TEA)
  - Acetonitrile is a dominant solvent
  - Acid/base additives are to suppress ionization
  - Samples have at least 2 H-bonds capability
- Astec CHIROBIOTIC® (neutral molecules)
- Chiral synthetic polymers e.g. Astec P-CAP, P-CAP-DP
- Cyclofructans
- Polysaccharides (e.g. ASTEC Cellulose DMP)

Polar Ionic Mode (PIM):

- Astec CHIROBIOTIC (2003) (e.g. 100/0.1/0.1, MeOH/HOAc/TEA)
  - Methanol is a dominant solvent
  - CSPs have ionic character
  - Acid/base additives promote ionic interactions for ionizable samples
  - ASTEC CHIROBIOTIC V2

Submitting a Sample for Chiral Screening

SUPELCO

Sample Submission Form

Customer Information:

Name: _____

E-mail: _____

Company: _____

Shipping Address: _____

_____

City/State/Zip: _____

Phone: _____

Fax: _____

Signature: _____

(Please use one form per sample.)

For Internal Use Only:
SAAS No.: _____
Sample Received: _____
Authorization: _____
Work Start: _____
Work Complete: _____
Results Shipped: _____

Sample Structure: Indicate main functional groups if structure not disclosed. (Place drawing in box or attach separately)

Sample Information:
Chemical/Name/Code: _____
Submitted Sample Amount: _____ mg *Please submit at least 25 mg*
Isomer Type: (please check one) ☐ RACEMIC ☐ DIASTEREOMERIC ☐ MIXTURE
pka: _____ UV (max): _____ *(Please Send Spectrum, if available)*
Appearance: (please check one) ☐ Powder ☐ Crystal ☐ Oil ☐ Other ☐ Color

Solubility: (please check one)				**Stability:** (please check one)			**Details**
EtOH:	☐ Soluble	☐ Partly Soluble	☐ Insoluble	Light:	☐ Stable	☐ Unstable	
MeOH:	☐ Soluble	☐ Partly Soluble	☐ Insoluble	Temp (≤ 50 °C):	☐ Stable	☐ Unstable	
IPA:	☐ Soluble	☐ Partly Soluble	☐ Insoluble	Acid (e.g. TFA):	☐ Stable	☐ Unstable	
ACN:	☐ Soluble	☐ Partly Soluble	☐ Insoluble	Base (e.g. DEA):	☐ Stable	☐ Unstable	
Hexane:	☐ Soluble	☐ Partly Soluble	☐ Insoluble	Other (moisture, air, etc.):	☐ Stable	☐ Unstable	

Application Request
Method for: ☐ Screening ☐ Method Development/Optimization ☐ Preparative ☐ LC ☐ GC
If preparative, please indicate the ultimate quantity of enantiomer required: _____
Column/condition already tried with/without success: _____

Separation Information:	**Column**	**Mobile Phase**
Column/Conditions already tried with success:		
Column/Conditions already tried without success:		

Recommendations or other useful information *(If more space required, use separate page)*: _____

May we add the results to an application presentation/publication? ☐ Yes ☐ No Conditions, if any:

Safety Information:
MSDS/Toxicity Data: (please check one) ☐ Toxic/Harmful ☐ Minimal Hazard ☐ Not Available
Bioactive: _____ If Bioactive, what type: _____
Potency/Human Exposure Issues: _____

Please contact us before submitting sample.
telephone: 800-359-3041 or 814-359-3041
fax: 800-359-3044 or 814-359-5468
e-mail: techservice@sial.com

Return Form with Sample and MSDS (if available) To:
SUPELCO
Attention: Applications Lab
595 N. Harrison Rd.
Bellefonte, PA 16823

TS07005A

- Non-disclosure agreement
- Customer asked to provide as possible
  - Safety
  - Stability
  - Solubility

Contact: chiral@sial.com

Further information:
www.sigmaaldrich.com/chiral

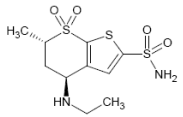
Contract Chiral Services Report

595 North Harrison Rd.
Bellefonte, Pennsylvania 16823
(814) 359-3041

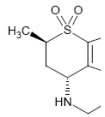
HPLC Chiral Screening Report

Analyte Description:	Dorzolamide Hydrochloride (CAS: 130693-82-2, USP 1225281), Dorzolamide Hydrochloride Related Compound A (CAS: N/A, USP 1225292, [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide, monohydrochloride)])
Supelco Sample No.:	R&D Application Request #739; Notebook 1688-18
Quote No.:	
Report to:	Internal

The sample has been tested through our method development protocol employing 3 CHIROBIOTIC (V2, T, TAG) columns and 3 CYCLOBOND (B-CD, DNP, and HP-RSP) columns with a combination of mobile phases encompassing polar ionic (PI), reversed-phase (RP), polar organic (PO) and normal-phase (NP) chromatographic modes of operation.



Dorzolamide Hydrochloride



Dorzolamide Related (

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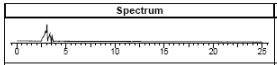
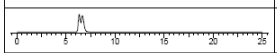
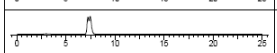
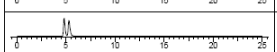
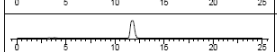
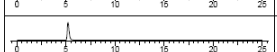
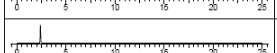
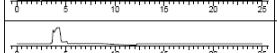
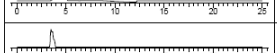
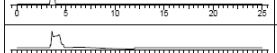
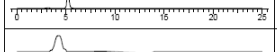
Summary of Primary Screen:

Spectrum	Column	mode	elution	File
	CHIROBIOTIC TAG	RP	No Retention	C:\acd\AC D_Chrom1 373.cdf
	CHIROBIOTIC TAG	PIM	Separation	C:\acd\AC D_Chrom1 384.cdf
	CHIROBIOTIC V2	RP	Separation	C:\acd\AC D_Chrom1 397.cdf
	CHIROBIOTIC V2	PIM	Separation	C:\acd\AC D_Chrom1 404.cdf
	CHIROBIOTIC T	RP	No Separation	C:\acd\AC D_Chrom1 416.cdf
	CHIROBIOTIC T	PIM	No Separation	C:\acd\AC D_Chrom1 423.cdf
	Cyclobond I 2000	RP	No Retention	C:\acd\AC D_Chrom1 435.cdf
	Cyclobond I 2000	POM	Unknown	C:\acd\AC D_Chrom1 442.cdf
	Cyclobond 2000 HP-RSP	RP	No Retention	C:\acd\AC D_Chrom1 461.cdf
	Cyclobond 2000 HP-RSP	POM	Unknown	C:\acd\AC D_Chrom1 485.cdf
	Cyclobond 2000 DNP	RP	No Separation	C:\acd\AC D_Chrom1 497.cdf
	Cyclobond 2000 DNP	POM	Unknown	C:\acd\AC D_Chrom1 504.cdf

Positive Results of Primary Screen:

The following combinations of stationary phas provided evidence of enantiomeric selectivity

- CHIROBIOTIC V2: PI mode (best)
- CHIROBIOTIC V2: RP mode
- CHIROBIOTIC TAG: PI mode

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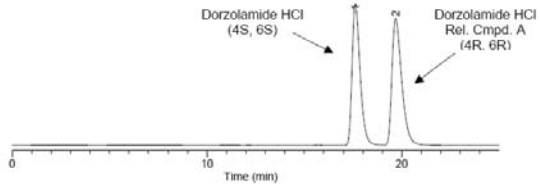
Chromatographic Results:

1:1 Dorzolamide HCl:Dorzolamide Related Compound A:

UV



Conditions:

Column: CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
Mobile Phase: 100:5 MeOH:H_2O, 3.81 mM NH_4TFA (or 0.05% NH_4TFA)
Temperature: 22 °C
Flow Rate: 0.3 mL/min
Detection: UV at 254 nm
Injection Volume: 10 µL
Sample: 1.0 mg/mL in MeOH

Peak 1 retention time (R_{t1}): 17.61 min.
Peak 2 retention time (R_{t2}): 19.68 min.

Chromatographic Results:

2:1 Dorzolamide HCl:Dorzolamide Related Compound A:

SIGMA-ALDRICH®

Chiral HPLC Purification

Small Scale HPLC Purification

- Preparative HPLC method development
- Conversion of methods for solvent optimisation
- Loading studies
- mg to multi-gram scale

Process HPLC

- Lab scale to automated production scale
- Normal phase columns, 1-50cm diameter
- Development and scale-up capabilities from g to tonne scale
- ISO 9000 certified

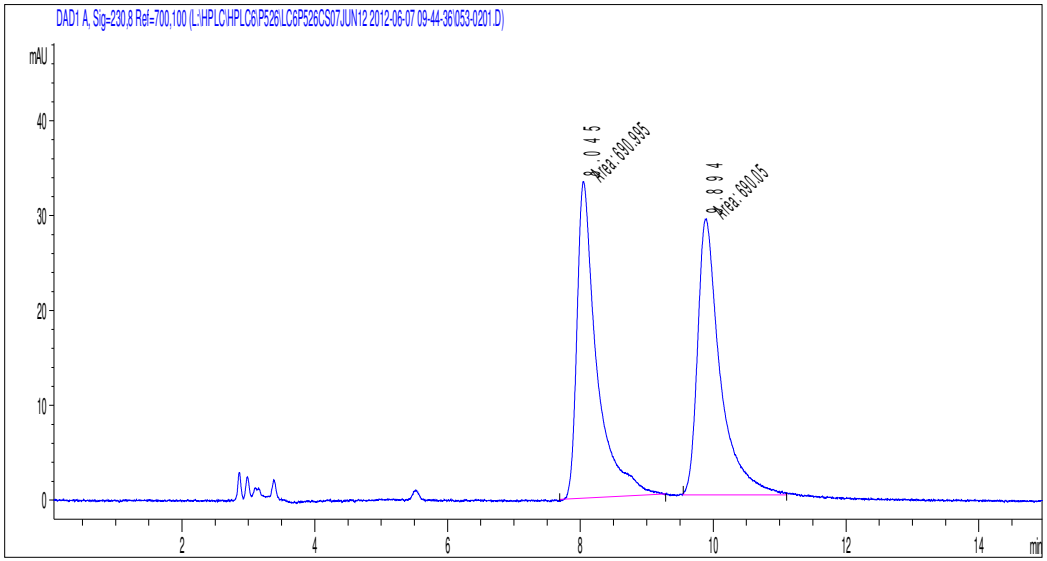
Small to Medium Scale SMB

- Highly flexible facility
- SMB feasibility/method development service
- Multi-gram to kg scale
- Lab scale SMB with throughput of 5-70 g/day
- Medium scale SMB for multi-kg scale purification (40mm column diameter)

Production Scale SMB

- FDA approved
- Multi kg to tonne scale
- Operates under cGMP
- Six 450 mm diameter columns
- Extensive experience in
  - Process optimisation
  - Solvent recovery
  - Process transfer to SMB

Resolution of Mandelic Acid Enantiomers



Experimental conditions:

Column:	Astec Cellulose DMP, 250 x 4.6mm, 5µm
Mobile Phase:	Hexane: IPA: TFA (v/v) 875:125:2.5
Temperature:	ambient
Flow Rate:	1.0 mL/min
Detection:	UV at 230 (8) nm
Injection Volume:	5 µL
Sample:	500 µg/mL in IPA

Peak 1 retention time: 8.05 min. (S)-Mandelic acid

Peak 2 retention time: 9.90 min. (R)-Mandelic acid

Elution order confirmed by separate injection of (R)- and (*S*)- Mandelic acid

Chiral separation via diastereomeric salt formation Principle

RS + R* → RR* + SR*

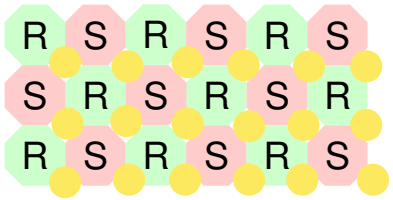
Racemic Base Chiral acid * Salts with difference in solubility

Crystalline salts with different solid state properties:

- X-ray powder diffraction patterns
- Thermal profile (different melting points)
- IR spectra
- Solubility

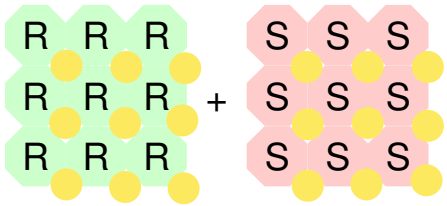
The diversity of chiral discrimination

Chiral resolving agent



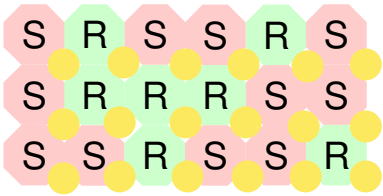
Racemic compound

Most of the cases



conglomerate

~ 5% of the cases



Mixed crystal (solid solution)

Crystallisation of a conglomerate gives efficient purification to the single enantiomer.

Pharmorphix can screen for conglomerates (specific solid phase properties)

Important points

Amorphous salts won't show any chiral discrimination except for particular cases (e.g. amorphisation of crystalline solvate during isolation)

You can not predict if a salt will crystallise in a conglomerate system !!

Screening of diverse chiral resolving agent is necessary to maximise the chance of finding the right system.

Screening of solvents is necessary to take advantage of solvate formation (e.g. a methanolate salt could be a conglomerate as the non solvated salt could form a racemic crystal).

Most of the time, the results are not interpreted properly. (Solid phase knowledge is necessary to understand the system.)

Screening

Aims: identify potential system (chiral agent, solvent)

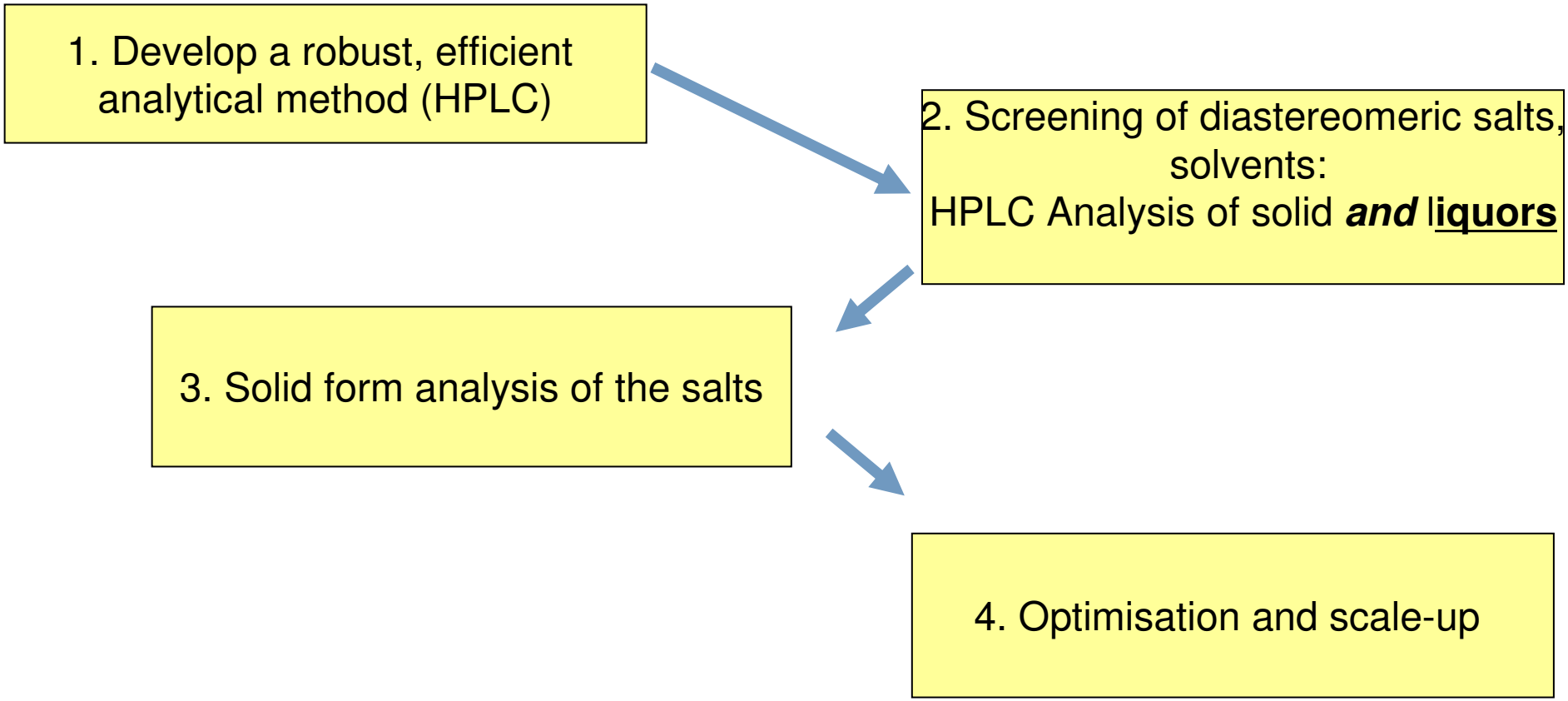
- 3 to 5 solvents systems x 20 chiral resolving agents are typically screened (~ 5-15mg per reaction of racemate is required).
- Selection of acid based on pKa and diversity.
- Slow cooling to promote crystallisation – slow evaporation, maturation to encourage crystallisation.
- Samples are inspected (microscope or XRPD) to check if they are crystalline

Scale-up / Optimisation of conditions

Concentration, solvents, temperature influence the recovery and e.e.

Pharmorphix can rationalise the system to find the optimum conditions to get the maximum recovery with the best e.e.

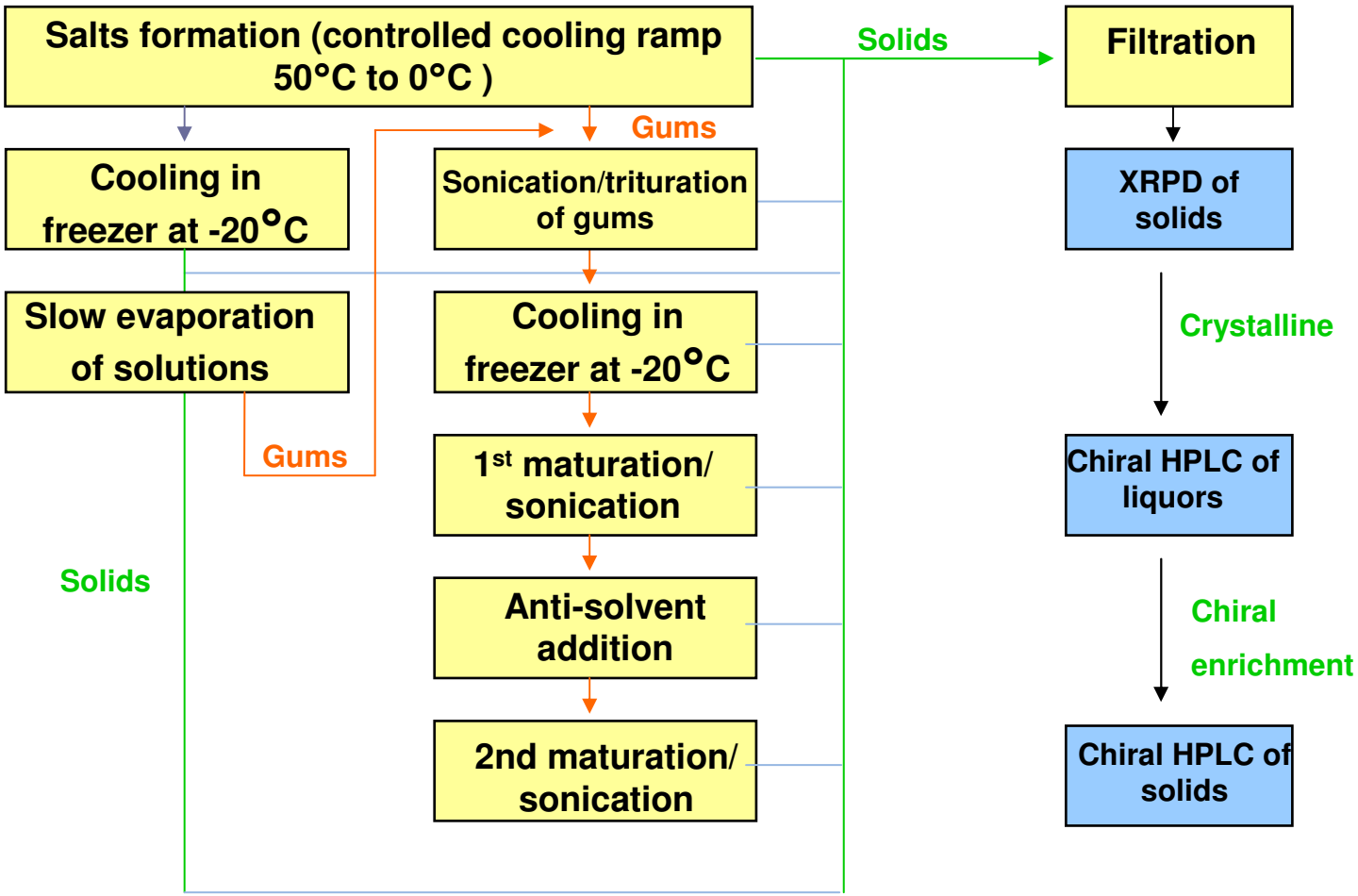
Diastereomeric Crystallisation Flowchart



Case study

- Resolution of (R,S) Mandelic acid
- Chiral HPLC method has been previously optimised

General procedure



Screen - Summary

(R)-Phenylglycinol from IPA (liquor:35/65 – solid:55/45)

(R)-Phenylglycinol from IPA/water (90/10) (liquor:39/61 – solid:52/48)

The salts were scaled up (~ 200 mg) and characterised to check if they were forming conglomerates

Experimental

About 200 mg of Mandelic acid was mixed with 180 mg of (R)-(-)-2-Phenylglycinol in 7 mL of 2-propanol.

The mixture was homogenised by heating (clear solution).

And cooled down to room temperature to obtain the precipitation of the salts.

→ After filtration, the solid was analysed by chiral HPLC (50/50)

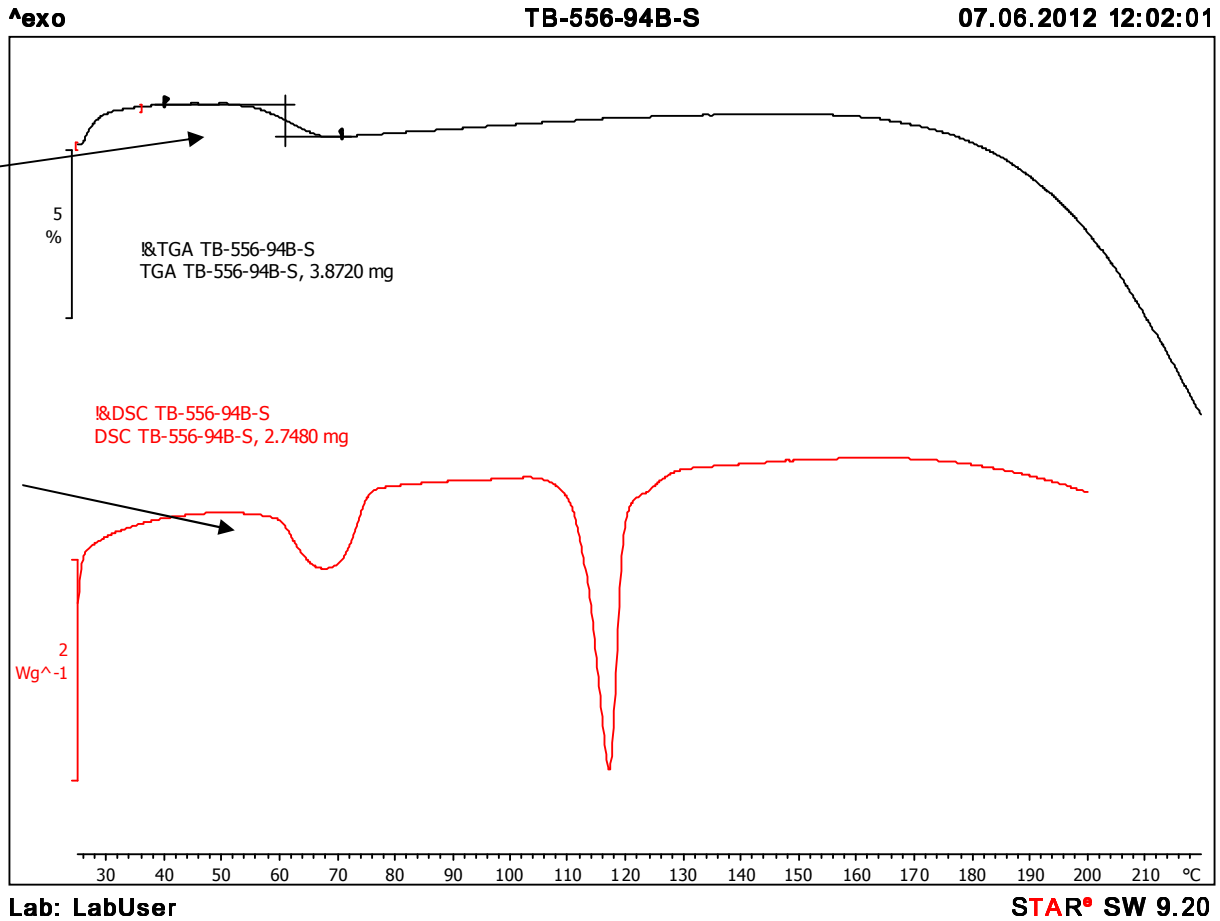
The experiment was repeated with 2-propanol + 1% water.

→ After filtration, the solid was analysed by chiral HPLC (56/44)

Thermal analysis of the filtered salt isolated from IPA/water (99/1)

Weight loss

Endotherm associated to dehydration



Does the resolution involve the formation of an hydrate ?

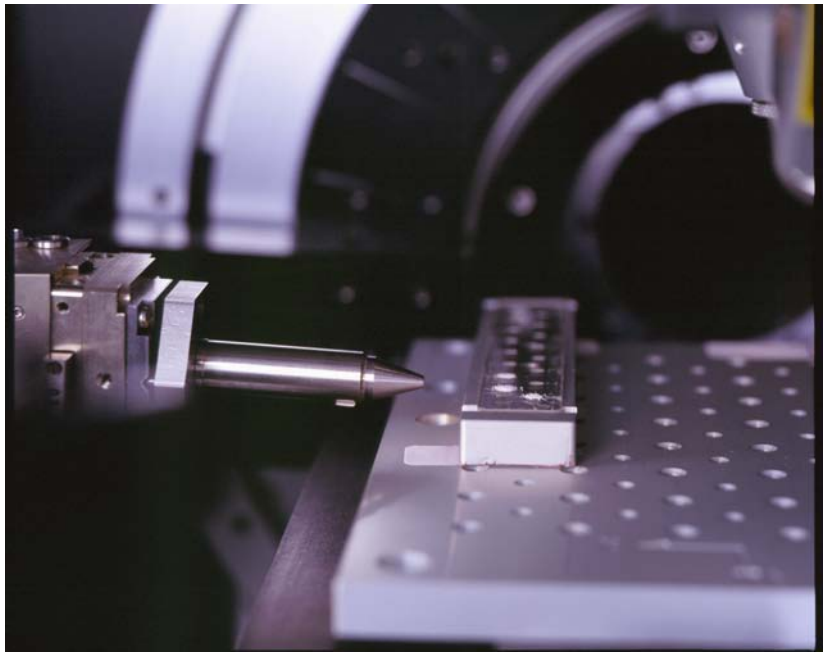
Understanding the system

(R)-Phenylglycinol (R)-mandelate salt in IPA
(R)-Phenylglycinol (R)-mandelate salt in water
(R)-Phenylglycinol (S)-mandelate salt in IPA
(R)-Phenylglycinol (S)-mandelate salt in water

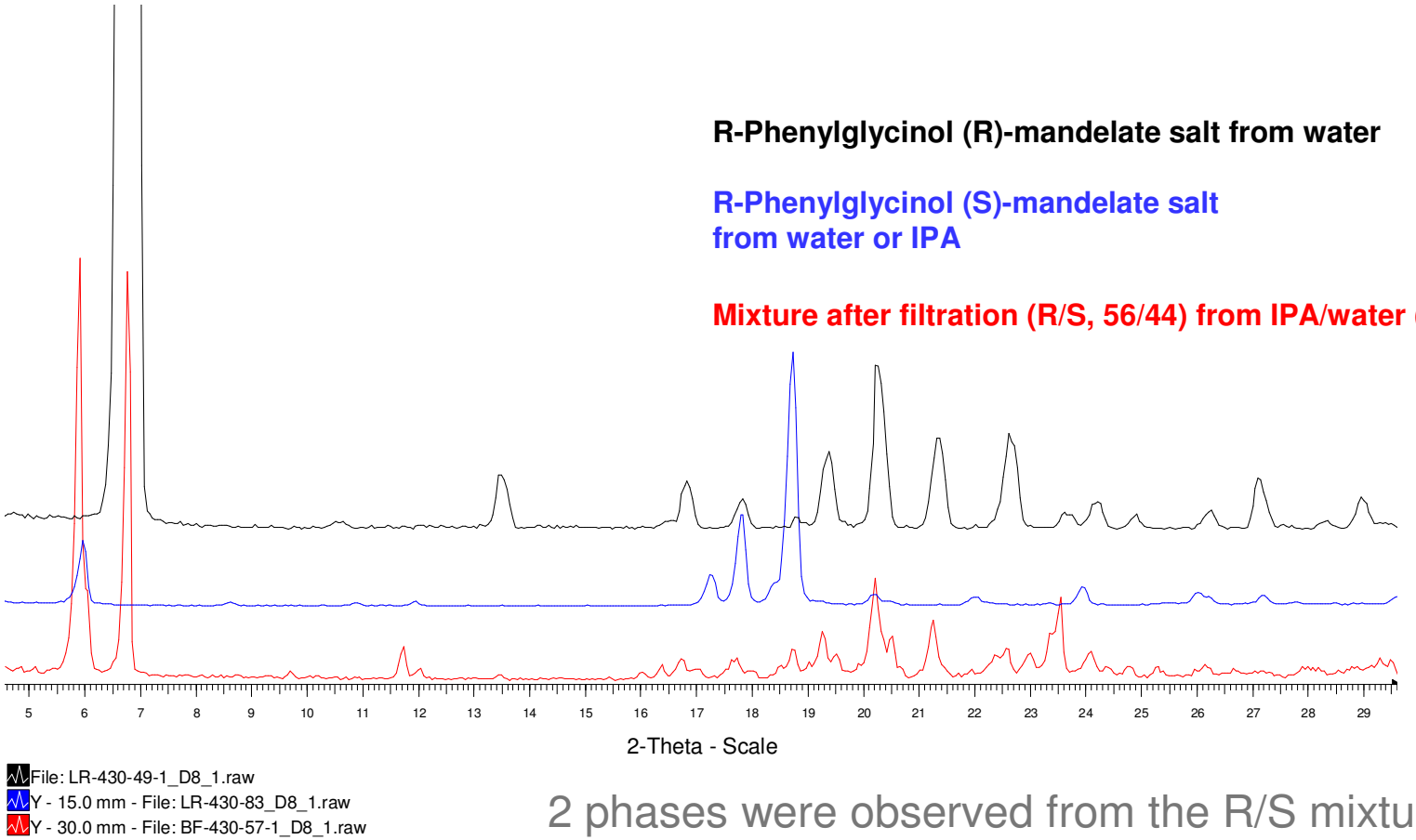
were prepared

The solid phases were characterised by XRPD, TGA, DSC.

Single crystals were also obtained, they were analysed by single crystal XRD



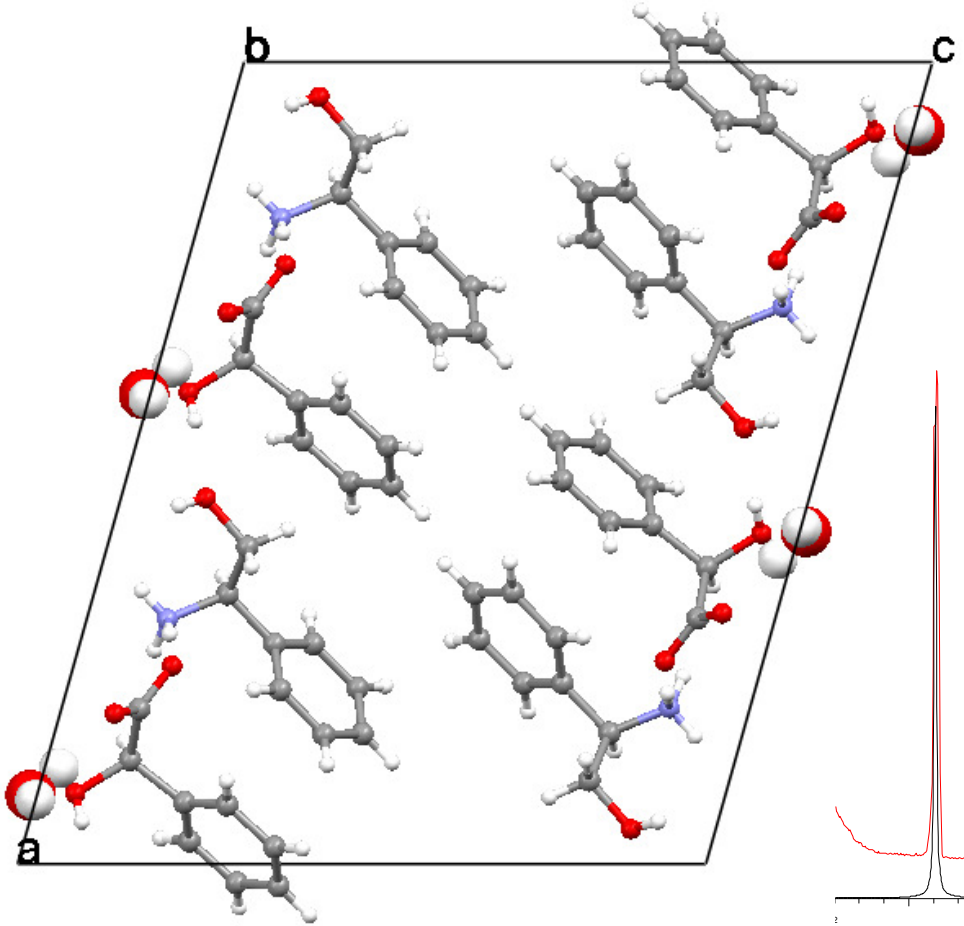
XRPD analysis



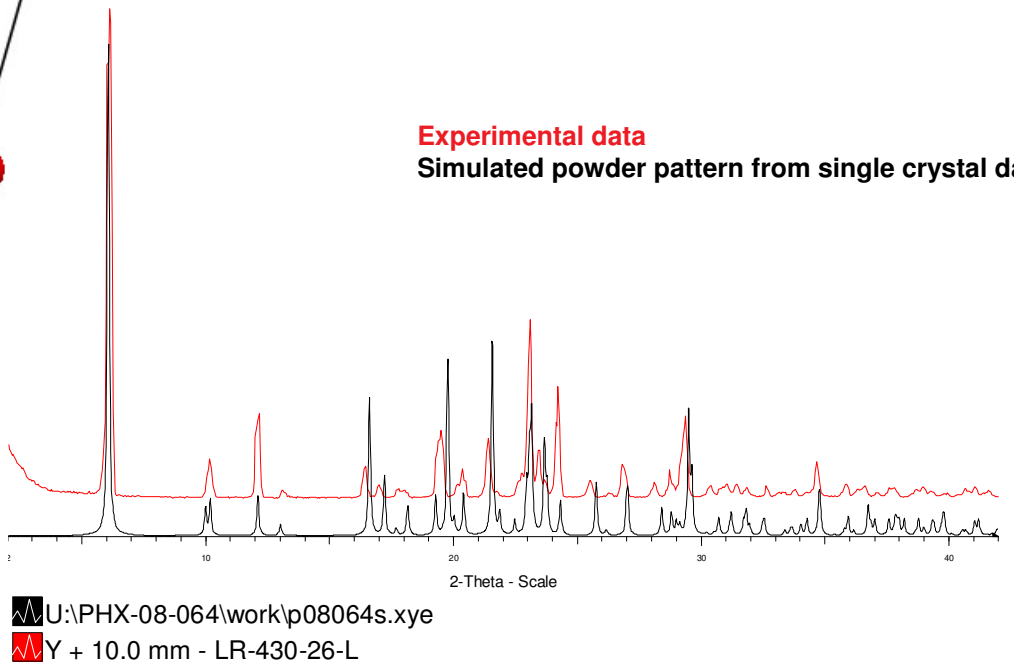
2 phases were observed from the R/S mixture

→ Conglomerate

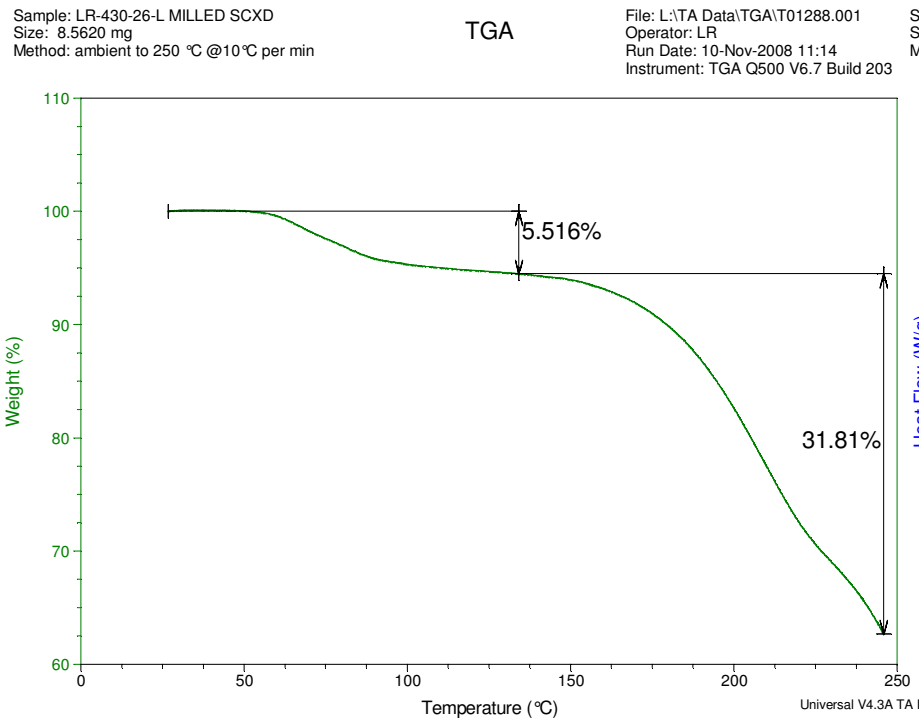
Single Crystal data of (R)-Phenylglycinol (R)-mandelate salt

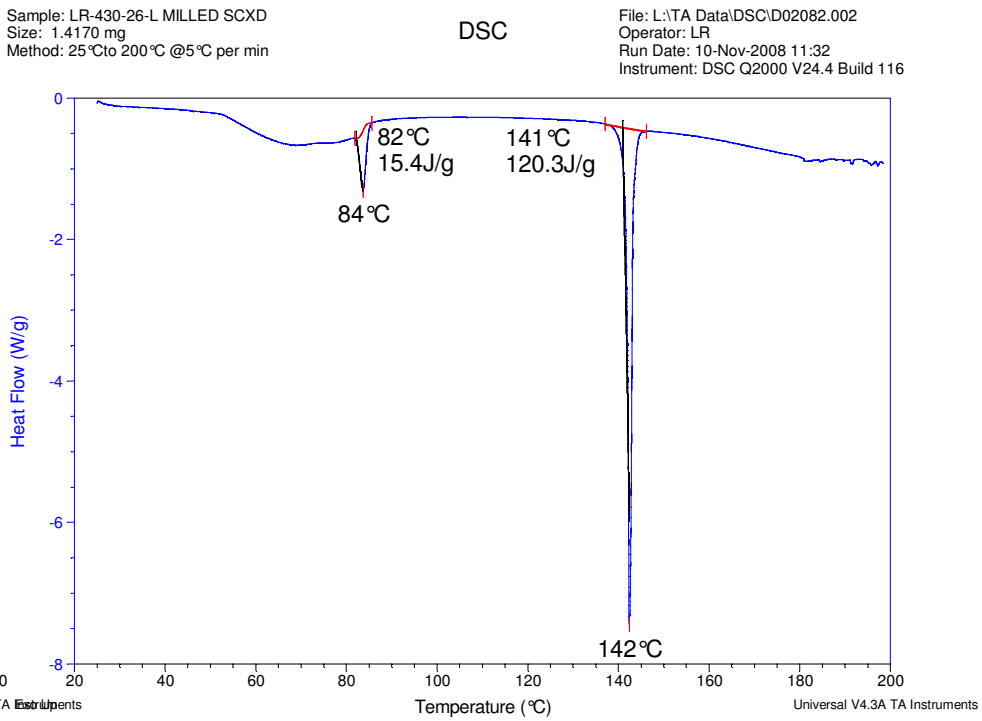


Monohydrate of
(R)-Phenylglycinol mandelate salt



TGA/DSC of (R)-Phenylglycinol (R)-mandelate hydrate salt

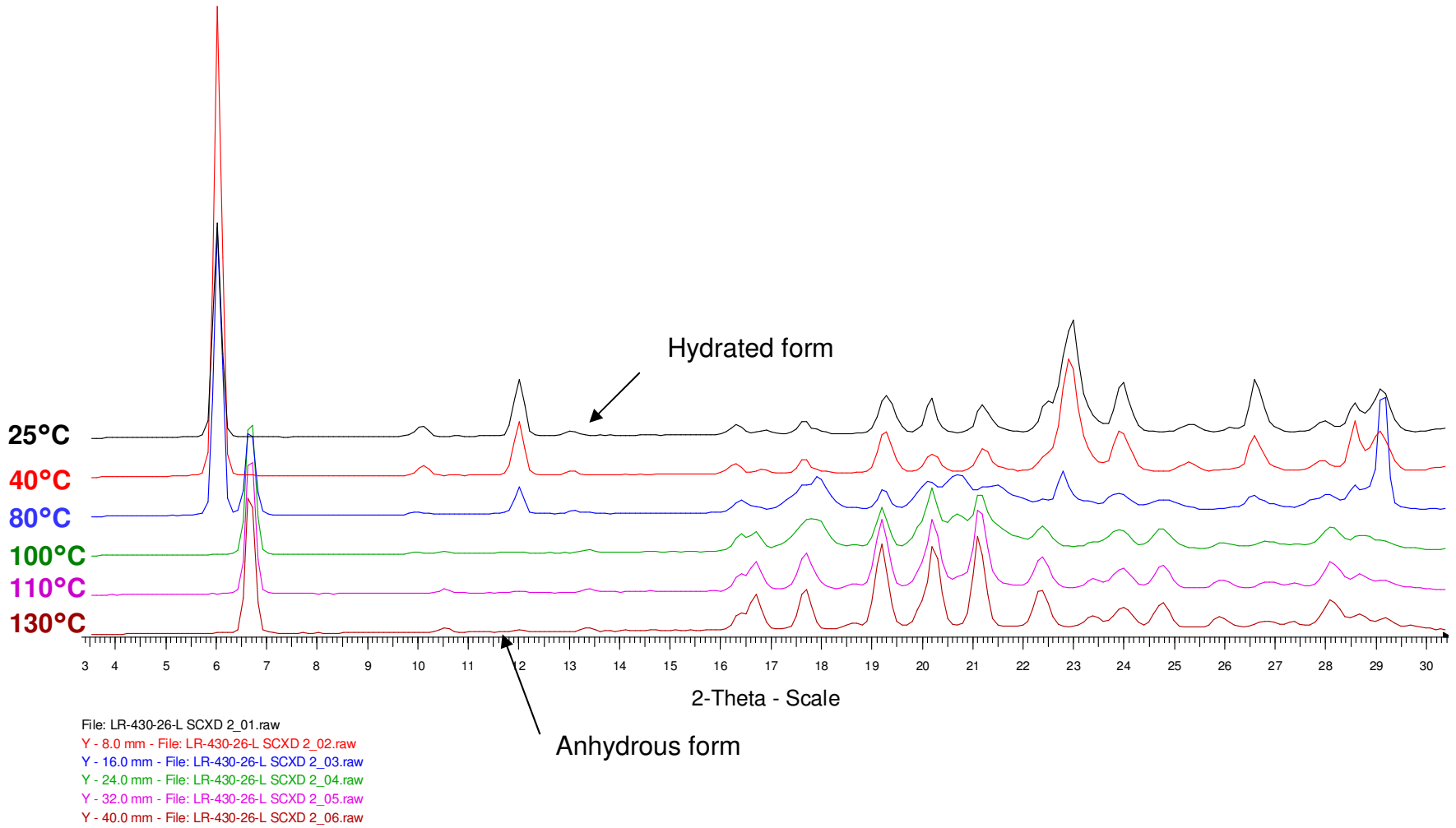




TGA 10°C/min
Mass loss of 5.5% w/w
Theoretical mass loss for a monohydrate: 5.8% w/w

DSC 5°C/min
Dehydration complete at 82°C
Melt of an anhydrous form at 141°C

VT-XRPD of (R)-Phenylglycinol (R)-mandelate hydrate salt



Summary of analysis

- In IPA/water, a physical mixture of crystals containing the (S)-Mandelic acid and crystals containing the (R)-Mandelic acid is obtained (XPRD)

- Confirmation that the resolution required the formation of a hydrated salt (single crystal, thermal data and XRPD)

Dilution/recrystallisation

20 mg of salt (56/44) was reslurried in 200, 300 or 400 µl of IPA/water (99/1) (heat/cool cycle for 24h then equilibration at RT).

The 3 samples were filtered and analysed.

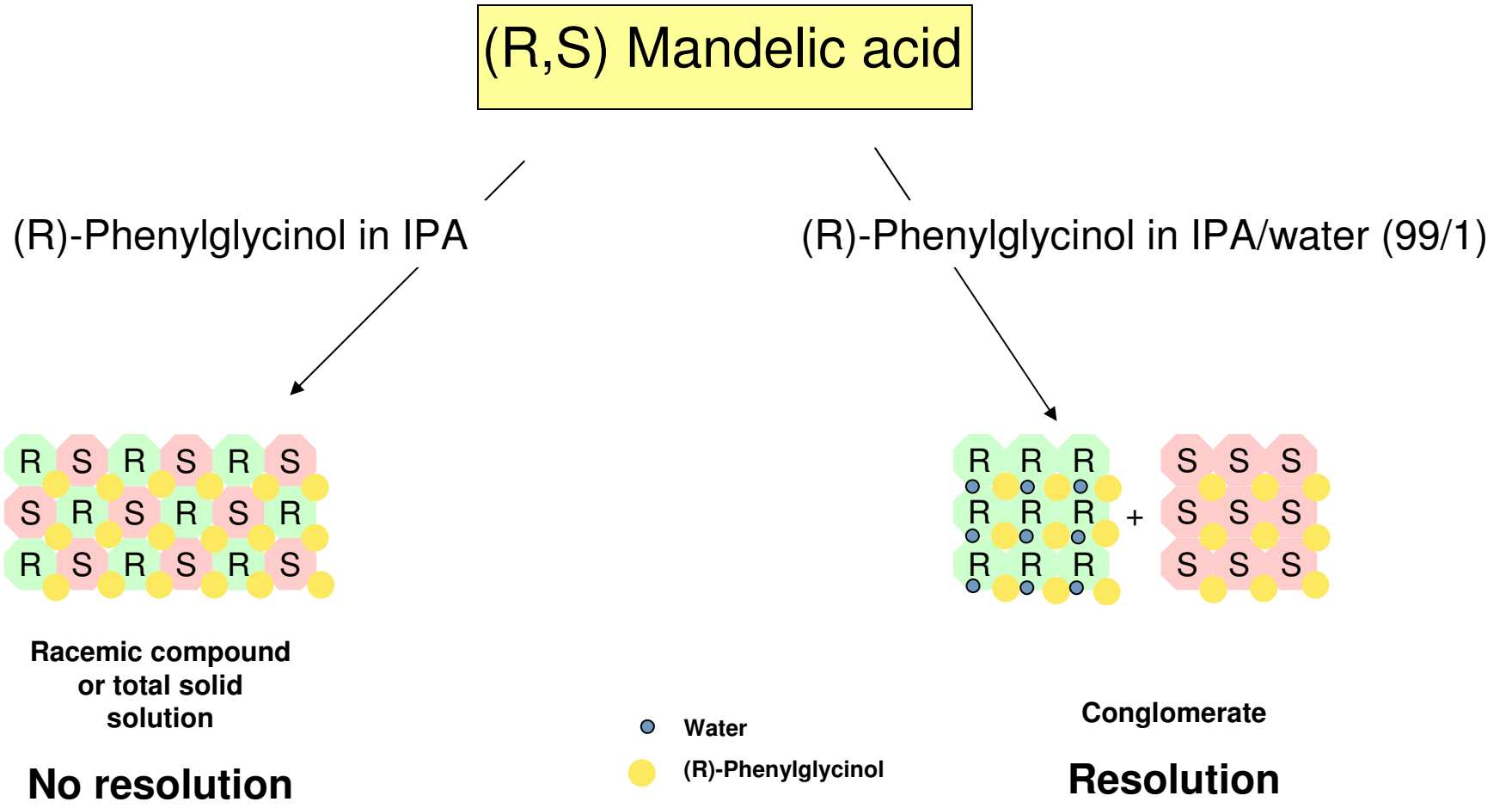
15 volumes of solvent →

Experiment Name	Solvent (µl)	Composition of solid (R/S)	Composition of liquor (R/S)
TB-556-95A	200	93/7	40/60
TB-556-95B	300	97/3	39/61
TB-556-95C	400	98/3	45/55

The resolution was repeated using 1 g of racemic Mandelic acid.

(R)-Mandelic acid was isolated with 95% ee.

Summary of the case study



Key Capabilities for Resolution Studies

Chromatography services (column screens and small scale purification)

Determination of absolute stereochemistry

Rapid screening and characterisation of crystalline diastereomeric salts

At-hand X-ray diffraction and associated specialised experience

Suite of further physicochemical techniques (e.g. DSC, TGA) for characterisation of physical properties of crystalline salts

Wide range of chiral acids and bases for diastereomeric resolution of racemic acids and bases (>150)

Process transfer to our manufacturing sites

Acknowledgement

Ludovic Renou and Baptiste Fours for the crystallisation study

Carrie Sheard for the chiral HPLC analysis