Molecularly Imprinted Polymers

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CSO, Biotage

2010年11月30日（東京）と12月2日（大阪）に開催した
ExploraSepセミナーのプレゼンテーション資料です。

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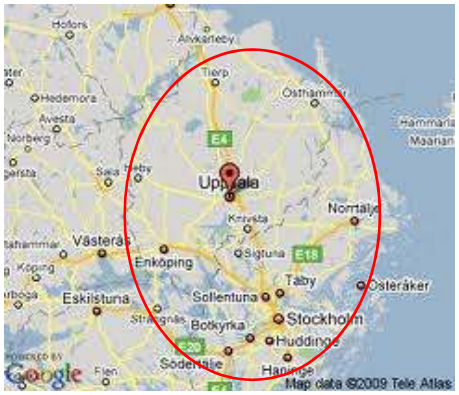
Uppsala：Summer and Winter



バイオタージ本社があるスウェーデンの風景

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Agenda

Part I

- What are MIPs – a short history?
- How do they behave?
- Examples of selectivity
- ExploraSep™ : a new screening concept

Part II

- ExploraSep and Genotoxins: Case Studies

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The concept of 'Imprinting'

In 1931 the group of Polyakov (Kiev) reported some unusual adsorption properties in **silica particles** prepared using a novel synthesis procedure. Sodium silicate had been polymerized in water using $(NH_4)_2CO_3$ as the gelating agent. After two weeks, **additives (benzene, toluene or xylene)** had been added. The silica was subsequently allowed to dry for 20–30 days, after which the **additive was removed** by extensive washing in hot water. Subsequent **adsorption studies** revealed a higher capacity for uptake of the additive by the silica than for structurally related ligands, i.e. some kind of **memory for the additive was apparent**, at least in the cases of benzene and toluene.

In 1934-5 more detailed investigations of the phenomenon were reported and the observed selectivity was explained as resulting from structural changes in the silica reflecting the nature of the additive.

1950,s Polyakov repeated and reiterated his founding experiments though they were not widely accepted or referenced

1970's Gunther Wolff (Germany) took the concept to another level….

MIPは1930年頃から研究されていた技術です。共有結合で化合物を結合するポリマーの研究を経て、より汎用性の高い現在のMIP（非共有結合により化合物を捕捉）が開発されました。

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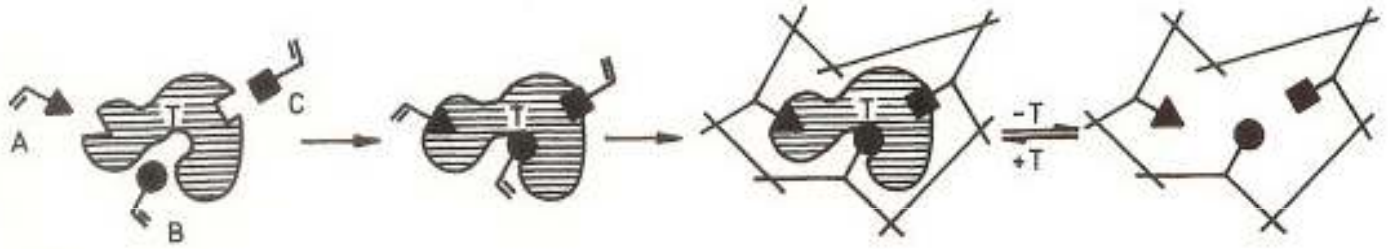
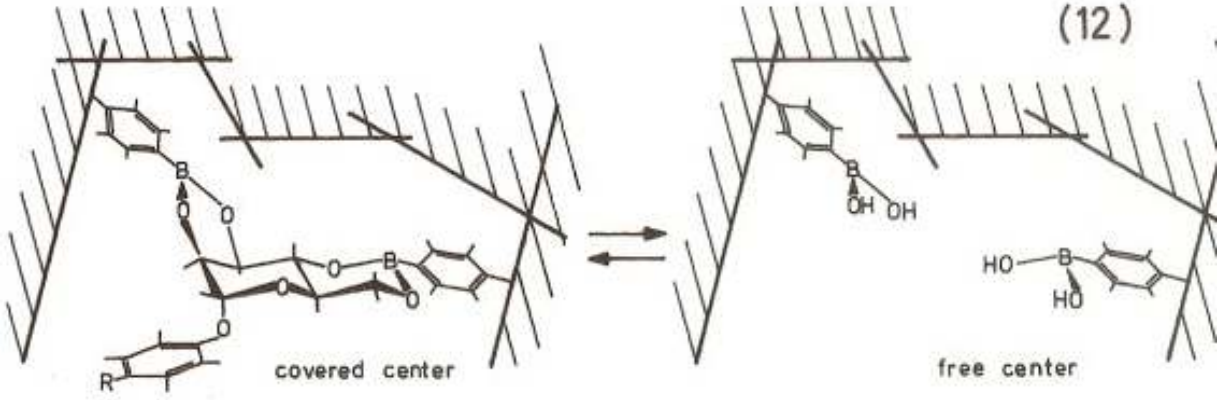
The Birth of Covalent Imprinting
Wolff (1972-78)

"Polymers with binding groups located in a definite spatial proximity and cooperativity in cavities of specific shape should show high selectivity in binding. This arrangement is similar to those of natural receptor sites."



Covalent imprinting – boronate esters with diol templates



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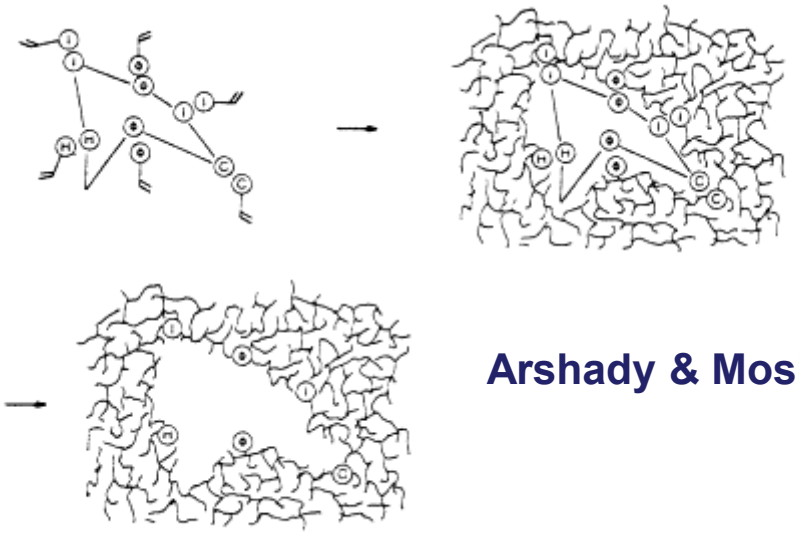
Non- covalent Imprinting – the universal method

”According to this strategy .... a monomer mixture containing ...cross-linking units is polymerized in the presence of a free substrate ..act as a template.... This is simply a mixing process and no chemical attachment to the monomeric units is required. The monomers are, however, chosen in such a way as to have non-covalent binding abilities (ie ionic, hydrogen bond, hydrophobic, charge transfer etc) complementary to those of the guest template.”



Arshady & Mosbach, 1981

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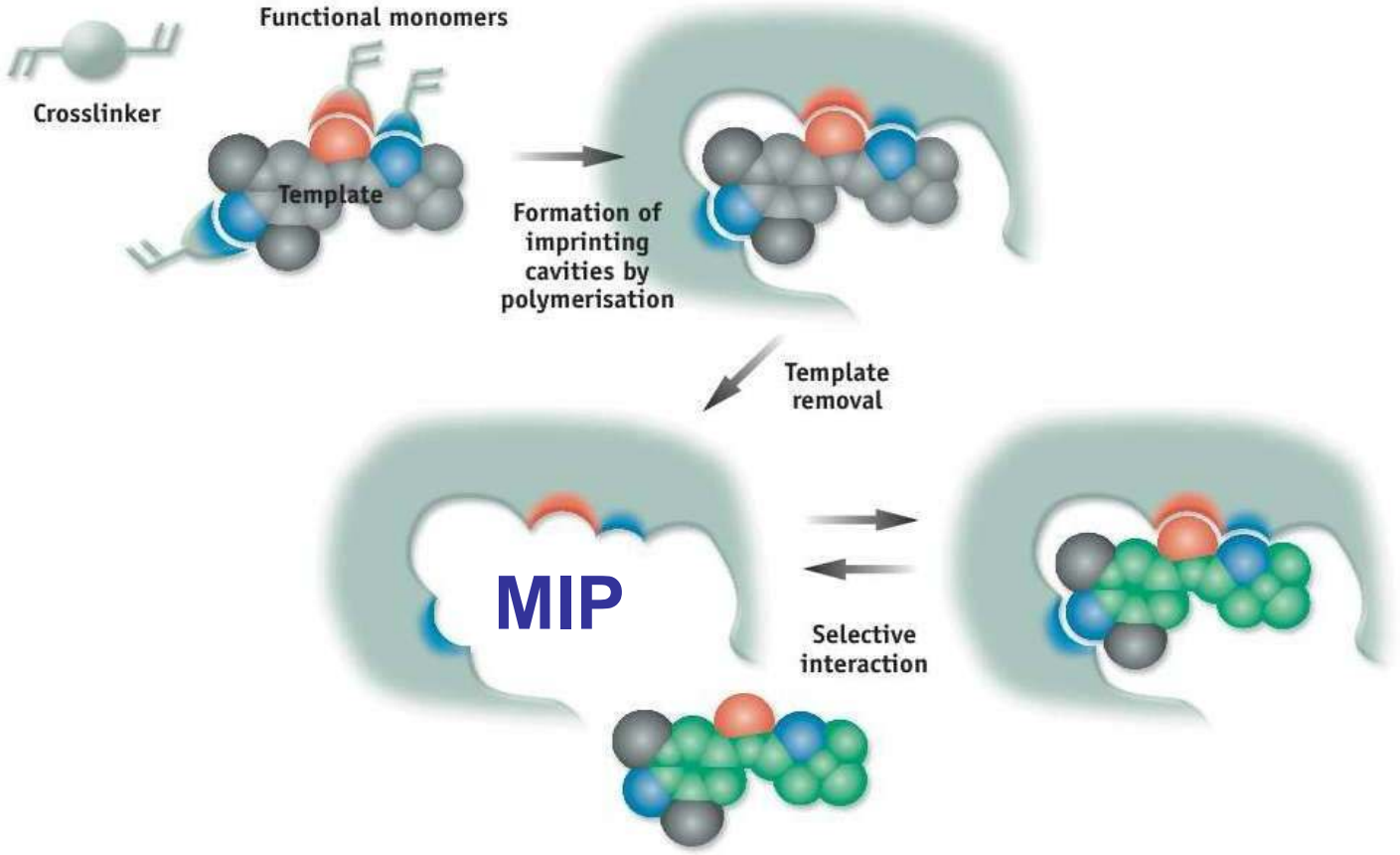
The Non-covalent Process simplified



MIPは、特定の化合物を“鋳型”にして作られます。

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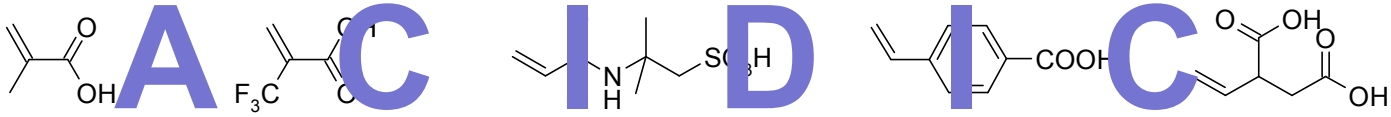
The Functional Monomer Landscape

ACIDIC



Methacrylic acid

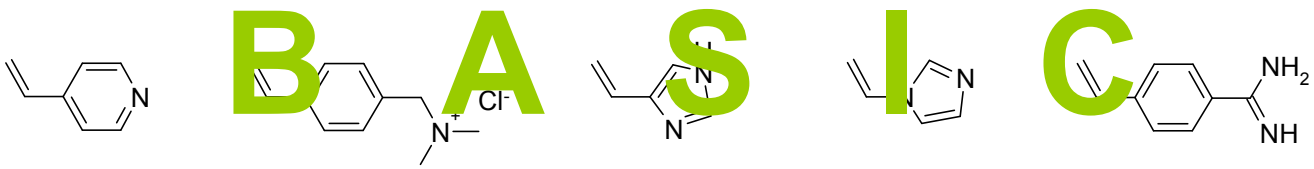
Trifluoro-methacrylic acid

Acrylamido-(2-methyl)-propane sulfonic acid

4-Vinylbenzoic acid

Itaconic acid

BASIC



4-Vinylpyridine

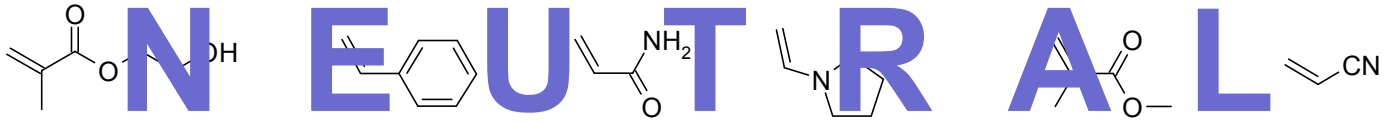
4-Vinylbenzyl-trimethyl-ammonium chloride

4(5)-Vinylimidazole

1-Vinylimidazole

4-Vinylbenzamidine

NEUTRAL



Hydroxyethylmethacrylate

Styrene

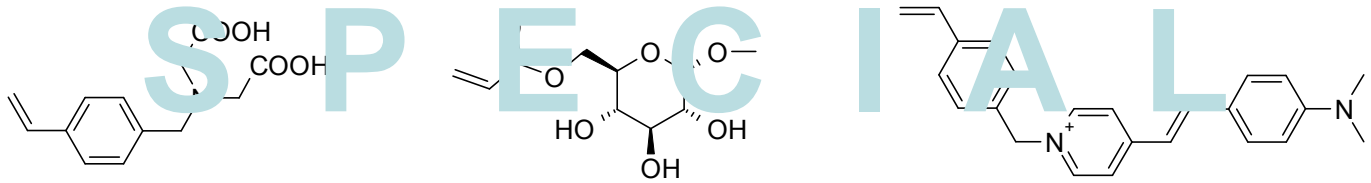
Acrylamide

Vinylpyrrolidone

Methylmethacrylate

Acrylonitrile

SPECIAL



4-Vinylbenzyl-iminodiacetic acid

Methyl-a-D-glucopyranoside-6-acrylate

Fluorescent reporter monomer

MIPは様々なモノマーで構成される特殊なポリマーです。

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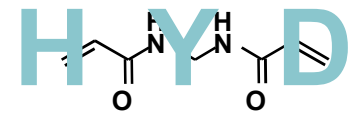
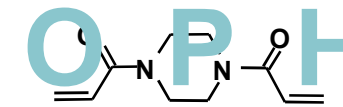
The Polymer backbone

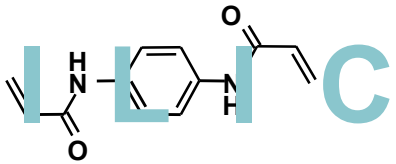
HYDROPHILIC



N,N'-Methylene-bisacrylamide

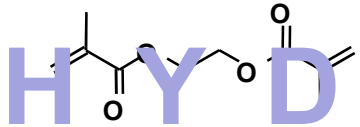


Bisacryloyl piperazine

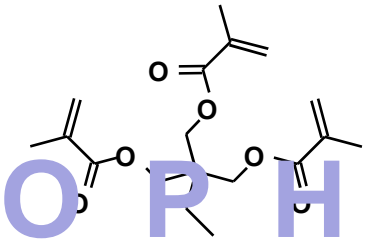


Phenylene-diacrylamide

HYDROPHOBIC

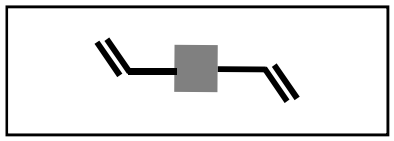


Ethyleneglycol dimethacrylate



Trimethylolpropane trimethacrylate

Divinylbenzene



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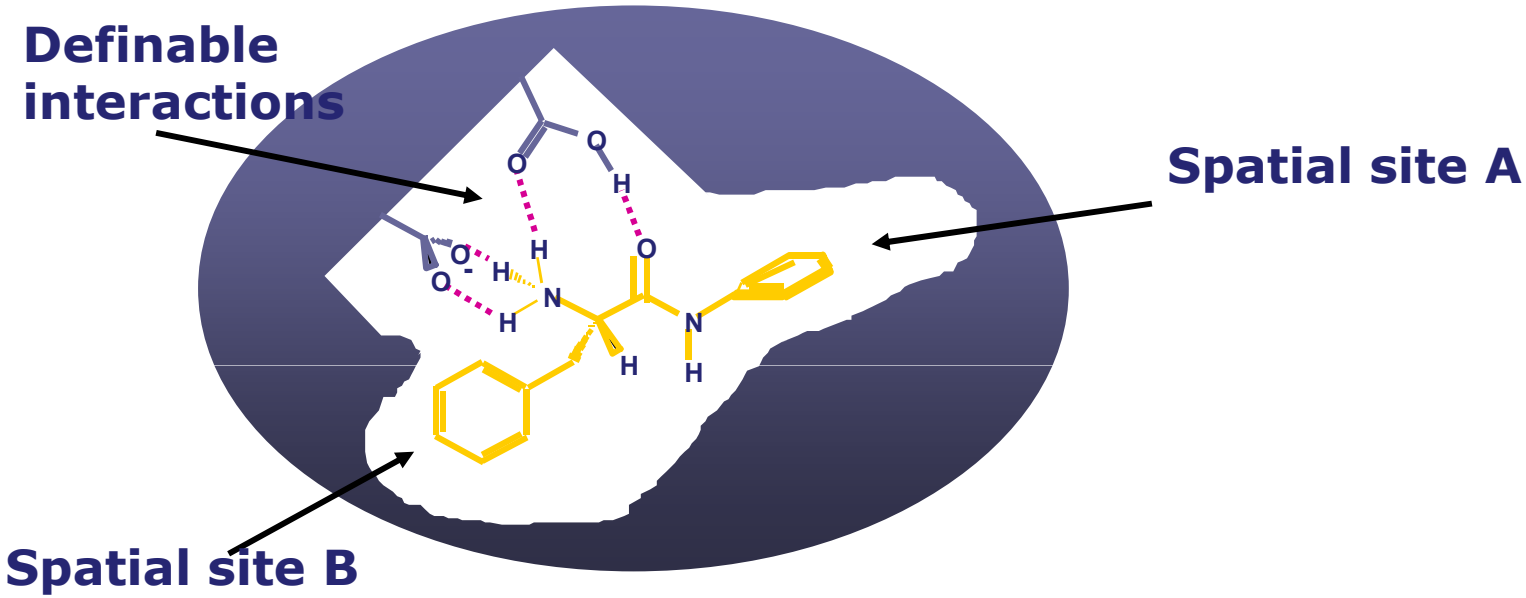
The MIP Binding Site (small molecule)



With the monomers employed interactions can involve H-bonding, charge-charge, van der Waals, hydrophobic and charge-transfer

特定の化合物をかたどったキャビティ内で、水素結合やファンデルワールス力などが作用します。

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Engineering binding and Capacity

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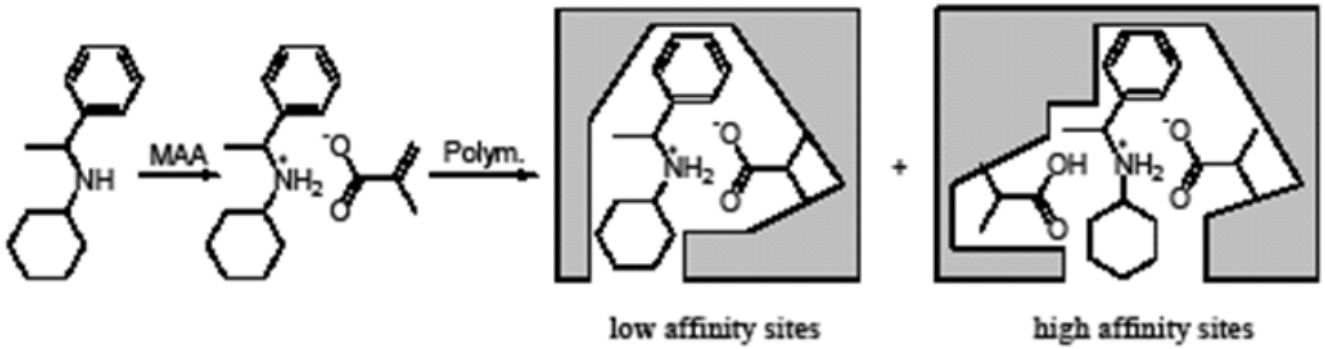
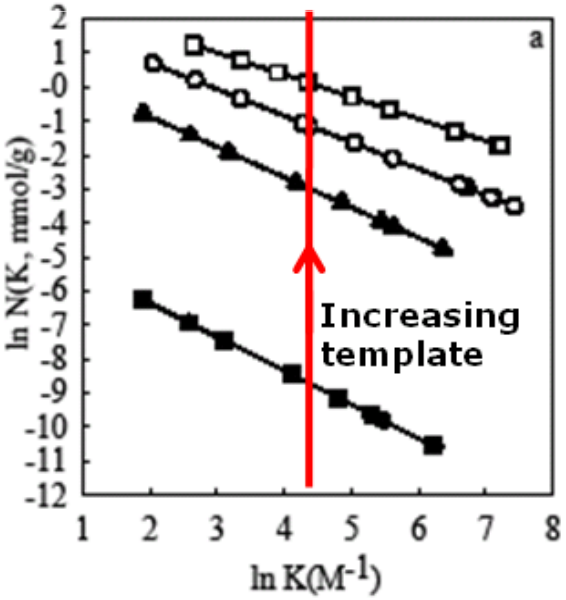
Template ratios: K and Capacity





1) Template-monomer stoichiometry controls capacity

2) The random polymerization process generates polyclonal sites

Data from Kim, H., PhD Thesis, ULouisiana, 2004

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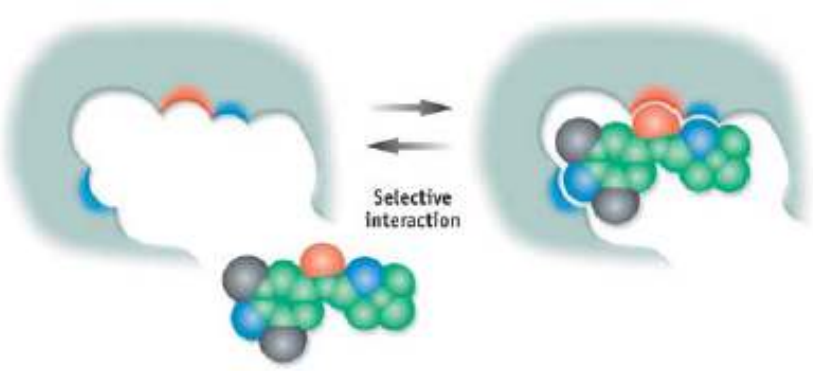
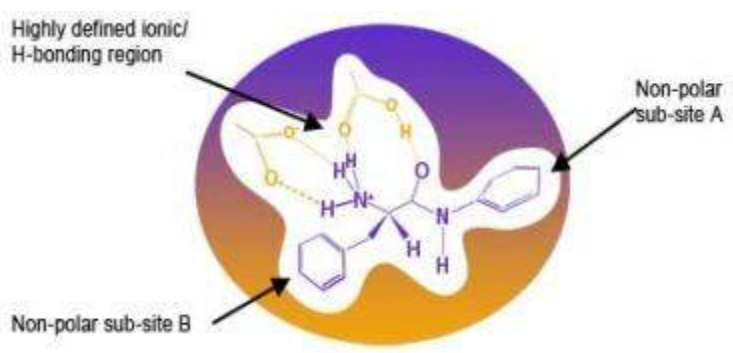
Molecularly Imprinted Polymers – a Summary

- Highly cross-linked polymer based phases
- Pre-determined selectivity for a particular analyte or group of structurally related compounds
  - Size exclusion
  - Chemical interactions in highly defined positions (H-bonding/ionic, Van der Waals interactions, π–π interactions)
- Selective target recognition
  - Mimics of polyclonal antibodies or receptors





MIPは高度に架橋された特殊なポリマーで、特定の化合物をかたどったキャビティ内においてポジション限定的に化合物と相互作用します。選択的に化合物を"認識"する仕組みは抗原抗体反応（ポリクローナル）に似ています。

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- What are MIPs?
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Part II

- ExploraSep and Genotoxins: Case Studies

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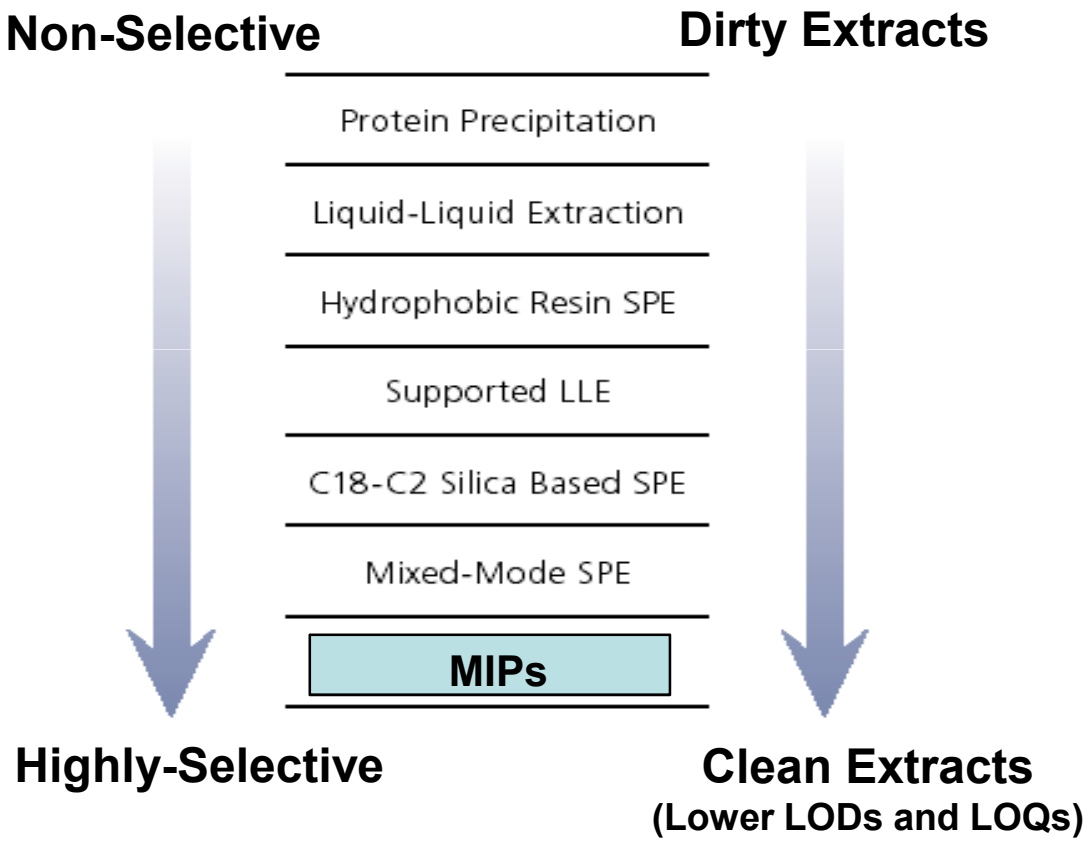
Relative Selectivity of Sample Preparation Techniques



MIPを充填した固相抽出カラム（AFFINILUTE MIP）を例に挙げ、MIPの優れたターゲット選択性を説明します。

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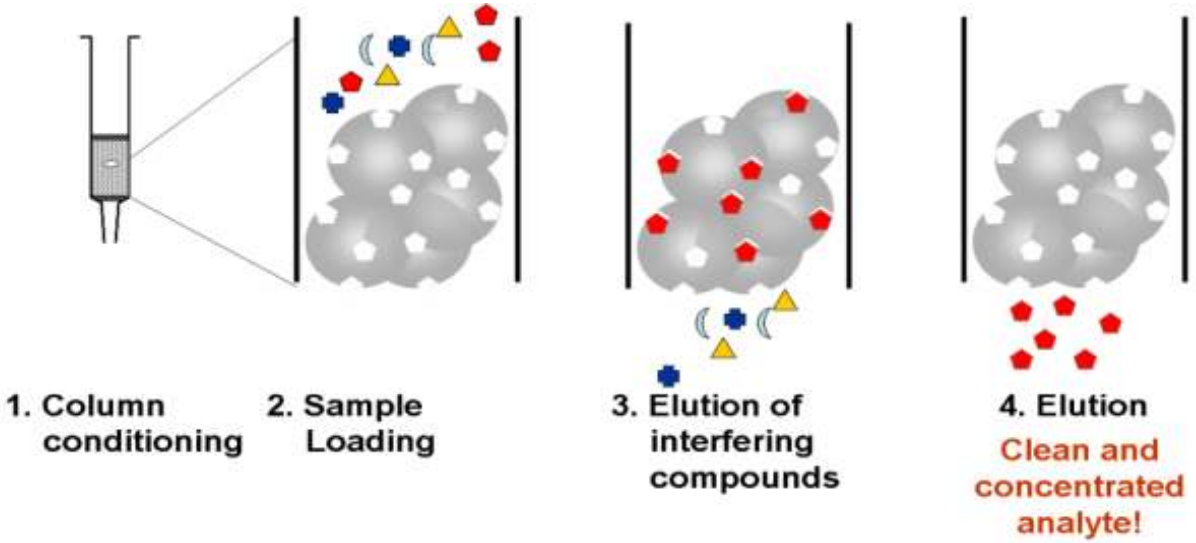
AFFINILUTE MIP SPE procedure



- MIP methodology differs from conventional SPE methodology
- Protocols for reversed phase, ionic-exchange resins etc CANNOT be used without careful comparison with the recommended MIP method
- Selectivity is typically introduced during the interference wash step with organic solvents

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AFFINILUTE MIP: Selective extraction in complex matrices

- Technical features
  - MIPs generate stronger interaction between the sorbent and the analyte
  - Interfering substances can be washed away using harsher washing conditions
  - The MIP material is stable at high temperatures, in organic solvents and at extreme pH (normally)

- Advantages
  - Cleaner extracts
  - Simplified washing protocols with less extraction steps
  - Minimized matrix effects and ion suppression
  - Higher precision

MIPを充填した固相抽出カラム（AFFINILUTE MIP）では、超微量のターゲット化合物を極めてクリーンな分析用サンプルとして抽出できます。

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AFFINILUTE MIP Phases and Applications Commercially Available For…

Drug-like

- Clenbuterol
- Beta agonists (class-selective)
- Beta blockers (class-selective)
- Beta receptors (class-selective, β-agonists & β-blockers)
- NSAIDs

Forensic

- Amphetamines
- NNAL
- TSNAs (Tobacco Specific Nitrosamines)

Food/Agricultural

- Chloramphenicol
- Fluoroquinolones
- Nitroimidazoles
- Triazine herbicides (class-selective)
- Polyaromatic hydrocarbons (PAH)

MIPを充填した固相抽出カラム（AFFINILUTE MIP）で抽出できる化合物の一覧です

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Example 1: Amphetamine drug Family

- A class of compounds including amphetamine (alpha-methylphenethylamine) and substituted amphetamines
- CNS stimulants
  - Clinically used to treat ADHD, narcolepsy and other sleeping disorders.
  - Stimulants and hallucinogens illegally used as recreational club drugs and as performance enhancers.
- Heavily regulated worldwide
- Schedule I & II drugs as reported by the DEA, USA

AFFINILUTE MIPによるアンフェタミン抽出を例に、MIPのターゲット選択性について説明します。

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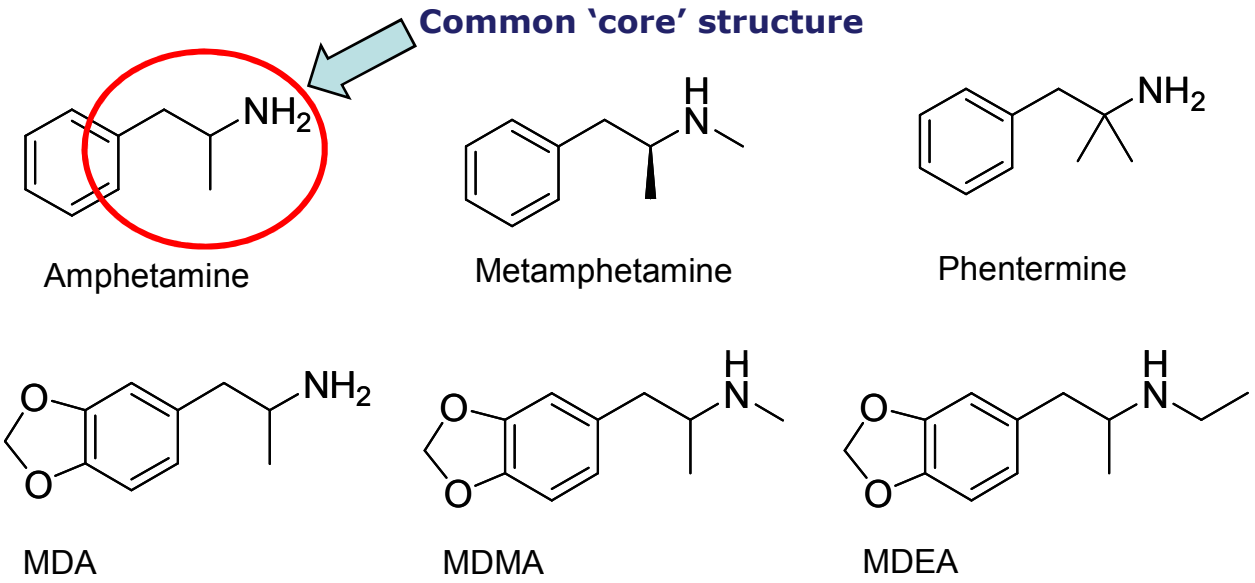
Amphetamine Structures



Methamphetamine "crystal meth", "ice" – potent stimulant
MDA, MDEA & MDMA (Ecstasy) – psychedelic stimulant

MDA (3,4-Methylenedioxyamphetamine), MDMA (3,4-methylenedioxy-N-methamphetamine) MDEA (3,4-methylenedioxy-N-ethylamphetamine)

AFFINILUTE MIPアンフェタミンは"クラス選択性"があり、アンフェタミンに似た構造の他の化合物も捕捉することができます。

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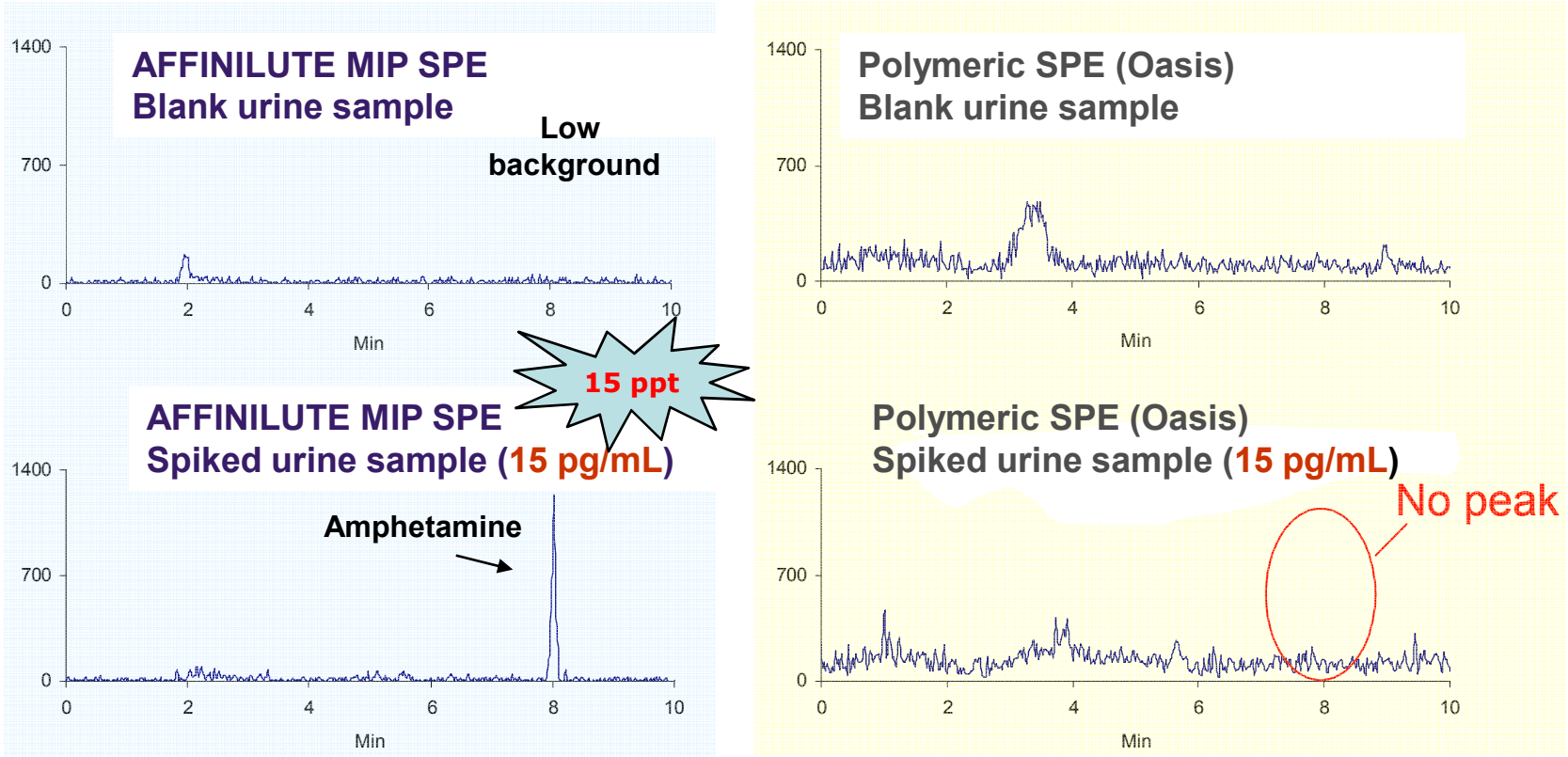
AFFINILUTE MIP : High Sensitivity

AFFINILUTE MIP vs Conventional Hydrophilic Polymer†

Urine extracts (MRM 136/118)



❁ ” AFFINILUTE MIP Amphetamines gives an Astronomically Low Background” –
❁ *Texas Veterinary Diagnostic Lab*

MIPは、夾雑物が多量に存在する環境下でもpptレベルでターゲット化合物を捕捉することができます。

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Higher recovery and reproducibility

AFFINILUTE MIP vs Conventional Hydrophilic Polymer†

	% Recovery		% RSD	
	Affinilute	Hydrophilic polymer SPE	**Affinilute**	Hydrophilic polymer SPE
Methamphetamine	101	100	1,41	5,16
Amphetamine	104	90	3,90	14,30
Phentermine	104	64	6,11	26,36
MDA	113	*	9,84	*
MDMA	97	86	2,52	8,10
MDEA	106	7	6,60	37,80

High Recoveries for all compounds using AFFINILUTE

High Reproducibility using AFFINILUTE

† *M.-R. Fuh, T.-Y. Wu and T.-Y. Lin, Talanta, 2006, 68:987-991*

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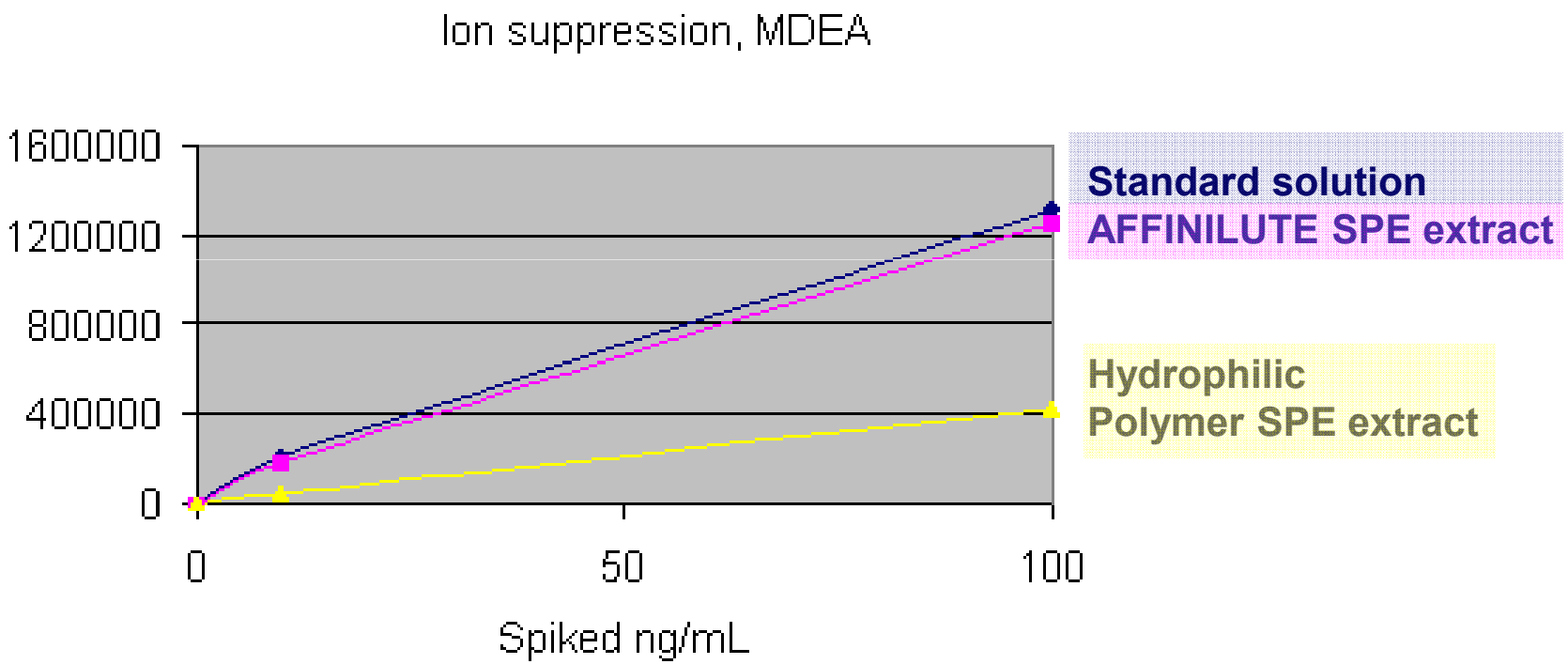
Lowered Ion-Suppression

AFFINILUTE MIP vs Conventional Hydrophilic Polymer



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Example 2 : NNAL

- Carcinogenic nicotine metabolite measured in urine of smokers and passive smokers
- A nitrosamine so within the genotoxic 'class'
- Current methods - elaborate and time-consuming
- Trace level determination required

AFFINILUTE MIPによるタバコ特異ニトロソアミン代謝物（NNAL）抽出を例に、MIPの選択性の高さを説明します。

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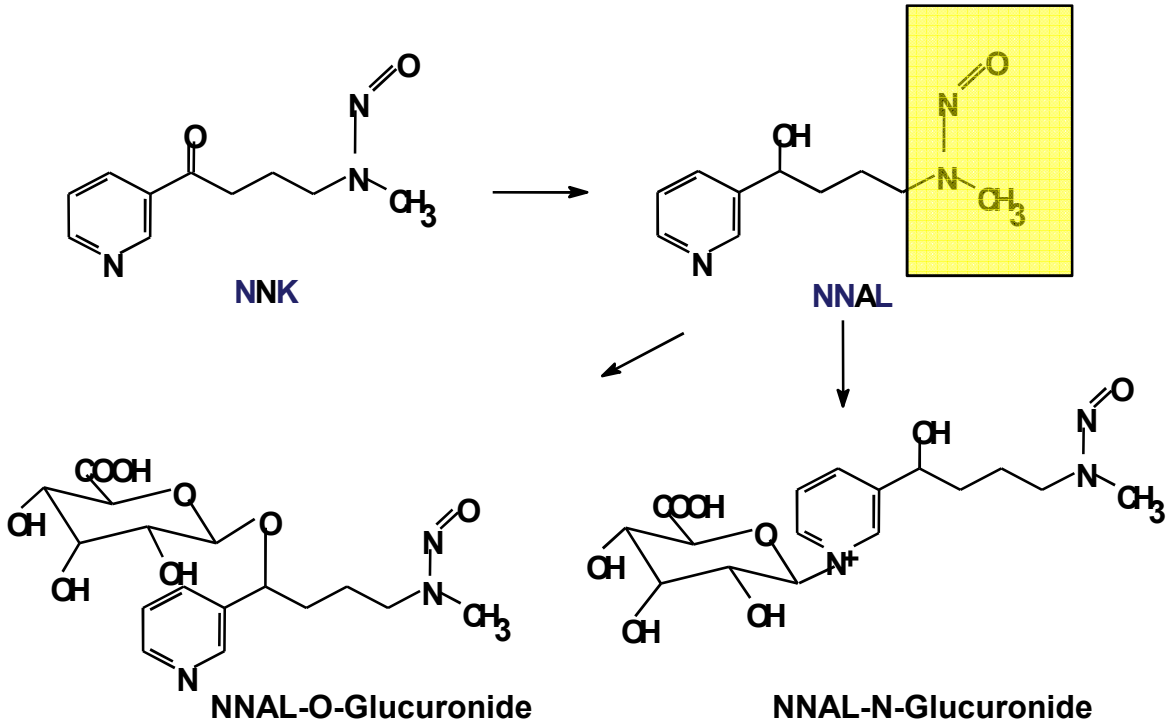
NNAL Structure



Note: -N-N=O is a PGI (genotoxin) functional group

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Affinilute™ NNAL

Conventional method

Analysis of NNAL. To 20–100 ml (smokers) or 100–500 ml (non-smokers) of urine, 4 ng *iso*-NNAL (internal standard, generous gift from Dr Dietrich Hoffmann, Valhalla, NY, USA) were added. Samples from non-smoker were concentrated on a rotary evaporator at 40 °C and 3 kPa to 100 ml. The sample was adjusted to pH 5.0 with hydrochloric acid. The aqueous solution was transfered to a glass column (450 mm × 40 mm, G3 frit) filled with Extrelut® (Merck, Darmstadt, Germany) and allowed to soak for 30 min. The column was eluted with 250 ml ethyl acetate (Code 3427, Promochem, Wesel, Germany), and the eluate was evaporated to 5 ml at 40 °C and 20 kPa. The concentrate was added to 5 ml water, adjusted to pH 2.0 with hydrochloric acid, and transfered to a separation funnel. After washing the aqueous layer three times with ethyl acetate, the pH was adjusted to 5.0 with aqueous sodium hydroxide and absorbed on a column containing Extrelut®. The column was eluted with 80 ml ethyl acetate, the eluate dried over anhydrous sodium sulphate, and concentrated to 2 ml *in vacuo*. The final extract was applied to 8 g aluminium oxide (activity II-III, ICN Biomedicals, Eschwege, Germany), equilibrated with 20 ml ethyl acetate (glass column, 150 mm × 15 mm, G2 frit). The column was washed with 10 ml ethyl acetate. Unconjugated NNAL was eluted with 20 ml ethyl acetate/methanol (10:1 v/v) (methanol, Code 9835, Promochem, Wesel, Germany) and evaporated to 1 ml. The purified fraction was transfered to a reaction vial, the solvent was removed under nitrogen, and the residue was derivatized by adding 48 µl bis-(trimethylsilyl)acetamide (BSA) and 2 µl trimethylchloro silane (TMCS) (Aldrich, Steinheim, Germany) at 50 °C for 30 min.

Over 20 laborious steps
Results in 3 days
Insufficient detection limits

Affinilute method

Extraction Procedure:
Recommended flow rate is 0.5 mL/min except for analyte elution 0.2 mL/min.
Gentle vacuum should be applied between each interference elution.

Sample pre-treatment: Urine diluted 1:1 with dest. water

Column conditioning: Condition the column with:
- 1 mL of DCM
- 1 mL of MeOH
- 1 mL of dest. water

Sample application: Apply the sample to the column (1-10 mL)

Interference elution: Elute the interferences with:
- 1 mL of water (elution of salt and matrix components)
- 10 minutes of vacuum (~ -0.7 bar) to dry the column
- 1 mL of toluene
- 1 mL of toluene/DCM (9:1)
- 1 mL of toluene/DCM (4:1) (selective wash, elution of hydrophobic bonded interferences)
- 2 minutes of vacuum to remove the toluene

Results after 0.5 h! 

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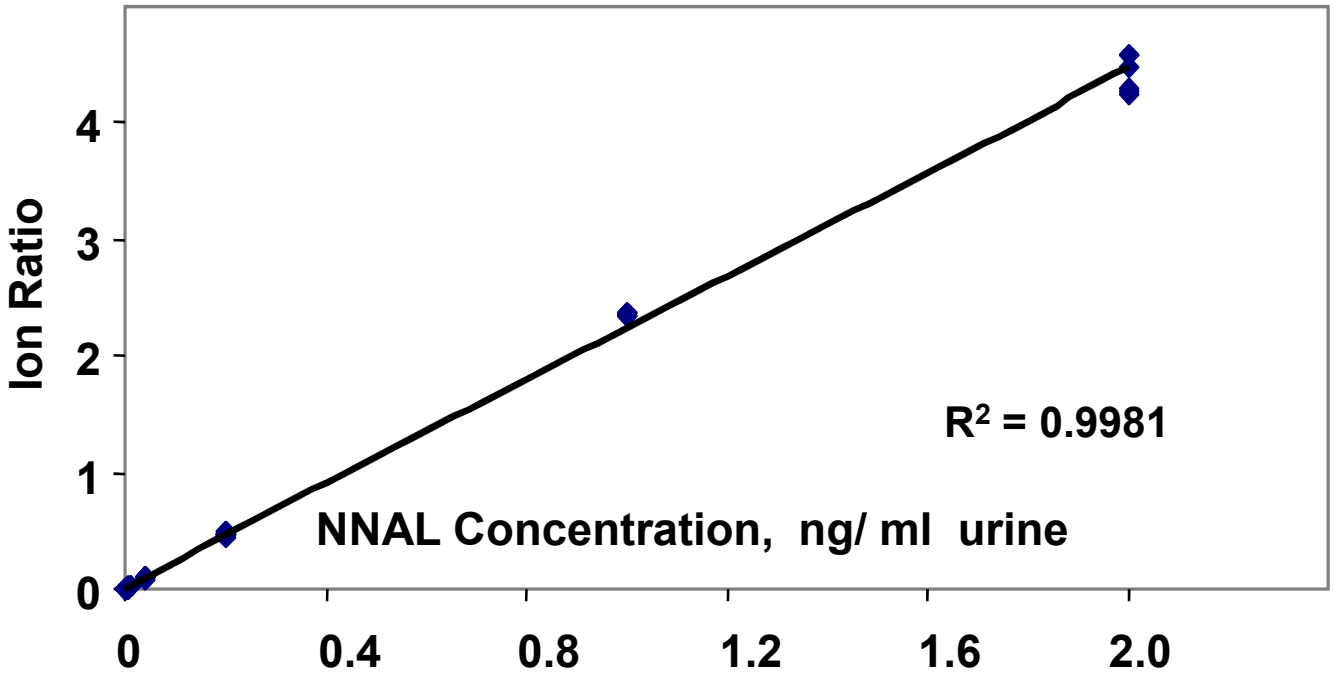
Affinilute™ NNAL - sensitivity



Sensitivity down to 0.1-0.2 ppb

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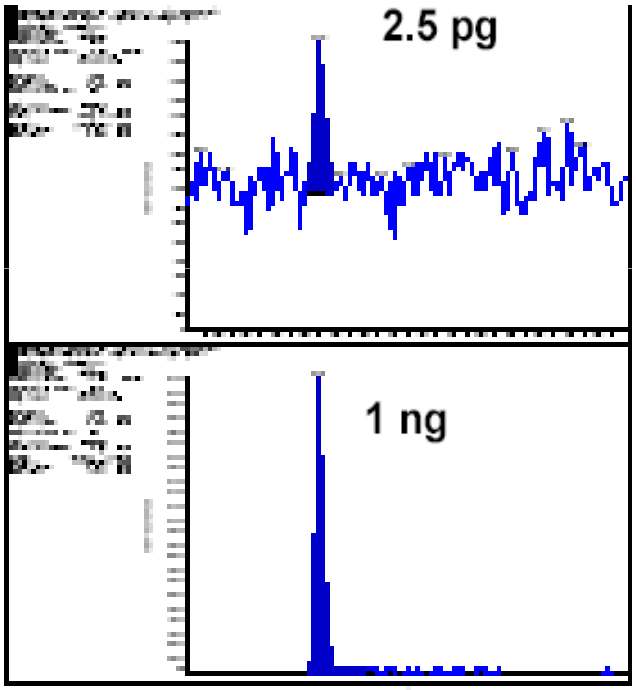
Affinilute™ NNAL – the sensitivity LIMIT



★ ”Using MIP in SPE was a success for NNAL measurement” ★

John Bernert, CDC Atlanta

Faster and more efficient SPE by using MIP allows higher sample throughput

Allows high sensitivity – detection of 2 ppt possible!

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Agenda

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- What are MIPs?
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- ExploraSep™ : a new screening concept

Part II

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目的のターゲット化合物を捕捉できるMIPを探す方法として、ExploraSepプレートによるスクリーニングを説明します。

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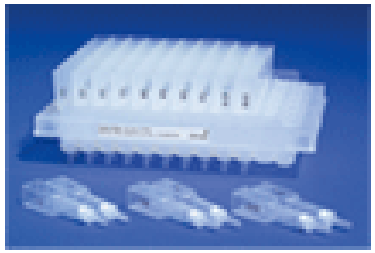
ExploraSep™ : Exploiting Similarity

ExploraSep ▶ more...

Screening Plates

Highly specific scavengers for removal of impurities including genotoxins



Target compounds are screened on multiwell plates containing a large number of different MIPs generated against many different templates and containing different monomer chemistries

How and why does ExploraSep work?

ExploraSepは96ウェルプレートフォーマットの製品で、
様々な化合物をテンプレート（鋳型）にして作成した様々なMIPが搭載されています。

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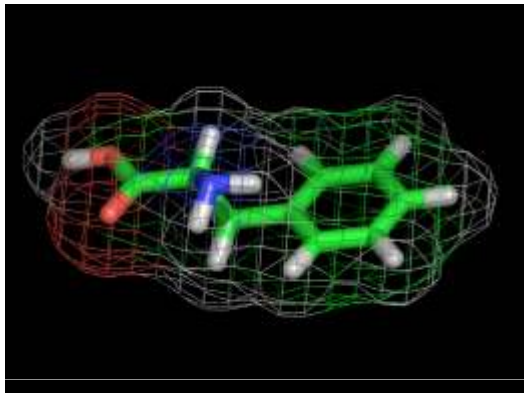
Similarity – the Concept

- Virtually all ways to measure molecular similarity are based on either (or both!) of these things:
  - The structure of the molecule (2D, 3D)
  - The properties of the molecule
- In general, an object A (molecule) is described by a set of *n* features:

$$\mathbf{X}_A = \{X_{1A}, X_{2A}, X_{3A}, \ldots X_{nA}\}$$

This is generally known as a fingerprint

- In addition, a scoring scheme is needed
  - A similarity measure or
  - A dissimilarity (distance) measure



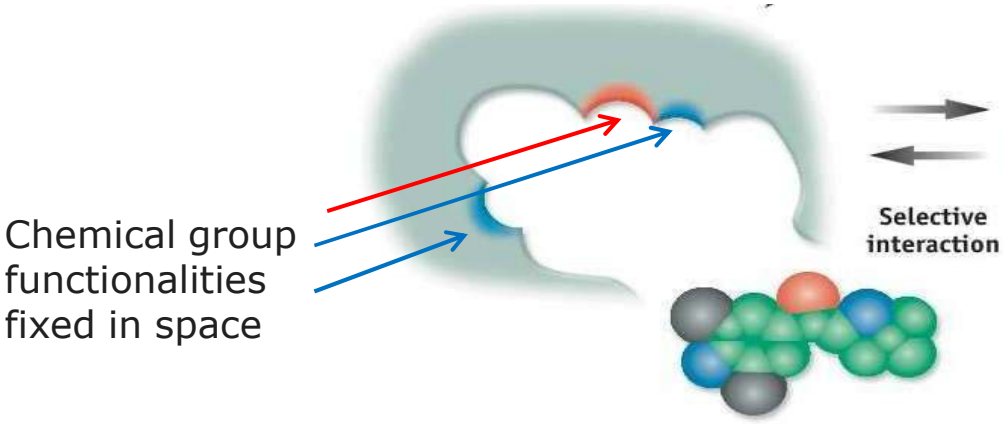
Most of the time we use the concept of similarity because of the similar property principle:

Structurally similar molecules are expected to exhibit similar physical properties or biological activities

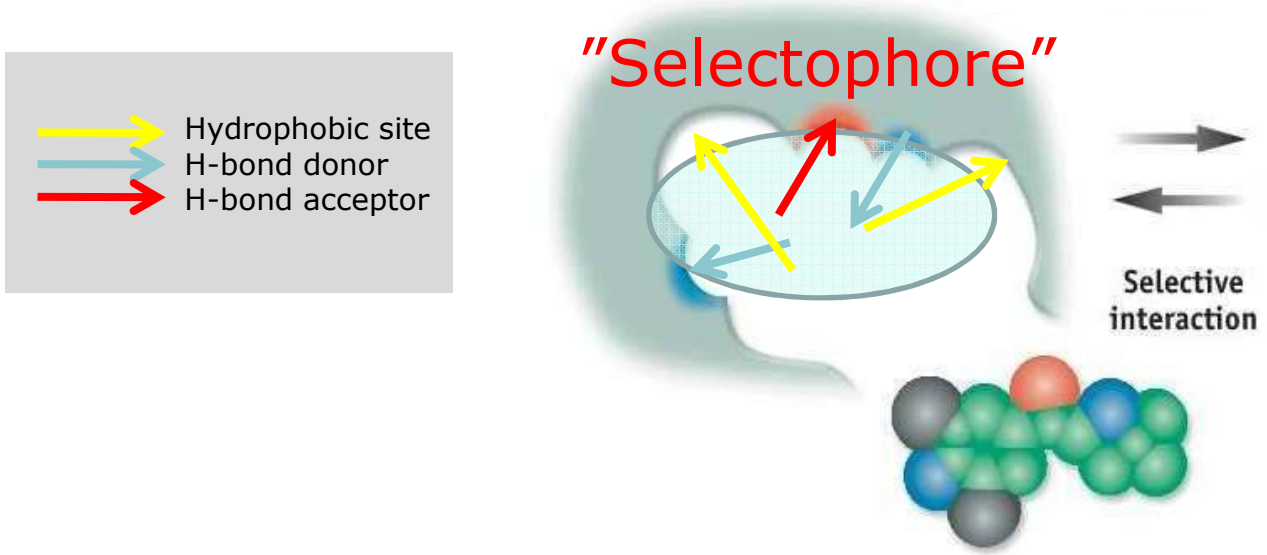
メディシナルケミストリーにおける”ファーマコフォア”に良く似た概念で、MIPの交差反応性（クラス選択性）を“セレクトフォア”と定義して説明します。

Biotage

Similarity leads to cross-reactivity



Other molecules that share common features may also fit into the MIP binding site



Biotage

Selectophore concept Summary

- Comparable to pharmacophore concept in medicinal chemistry*
- Pharmacophore =“a set of structural/chemical features in a molecule that is recognized at a receptor site and is responsible for the biological activity”
- Similar molecules can bind to the same site – otherwise Pharma would have no business!
- It is to be expected that the selectivity ‘profile’ of a MIP binding site will be at least as flexible as a biological receptor site

*Gund, P., *Prog. Mol. Subcell. Biol.* **1977**, 5: pp 117–143

MIPには交差反応性（クラス選択性）があるので、鋳型にした化合物そのもの以外でも、構造と化学的特性が似ていれば保持（結合）することができます。

Biotage

ExploraSep Chemistries

ExploraSep ▶ more...

Screening Plates

Highly specific scavengers for removal of impurities including genotoxins

128 resins

Plate A (acidic functionalities)
- Hydrophobic and carboxylic acidic moieties
- Successful target analytes - amines, amides, nitrosamines, esters, carboxylic acids

Plate U (proprietary 'urea' functionalities)
- Hydrophobic moieties and urea groups
- Successful target analytes - phosphates, phosphonates, sulphates, sulphonates, anions of carboxylic acids

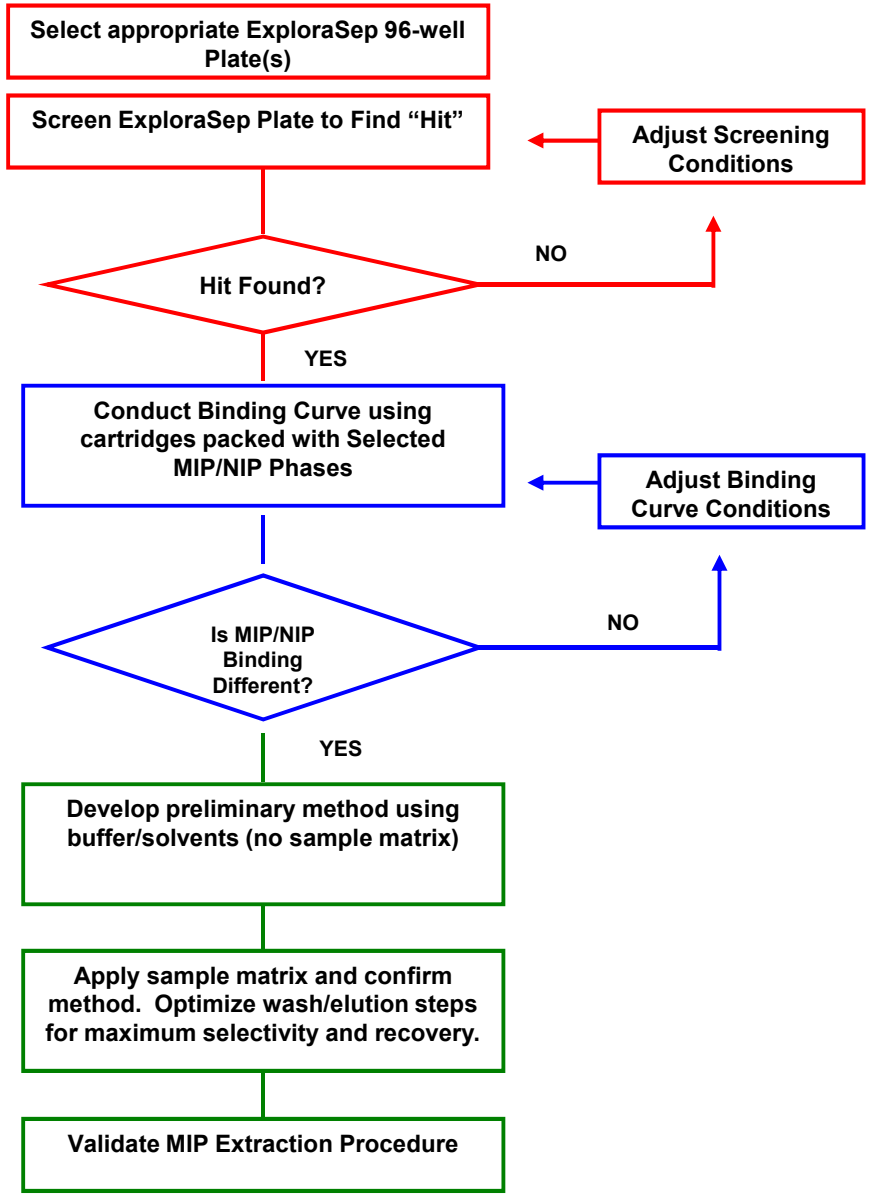
Plate H (aromatic functionality)
- Hydrophobic aromatic moieties
- Successful target analytes - non-polar and aromatic compounds

Plate C (CHO or multi-OH binding functionality)
- Hydrophobic moieties and hydroxy functionalities
- Successful target analytes - 1,2- and 1,3-diols, and α-hydroxy carboxylic acids

ExploraSepは4種類あり、それぞれに32種類のポリマーが載っています（n=3）。

Biotage

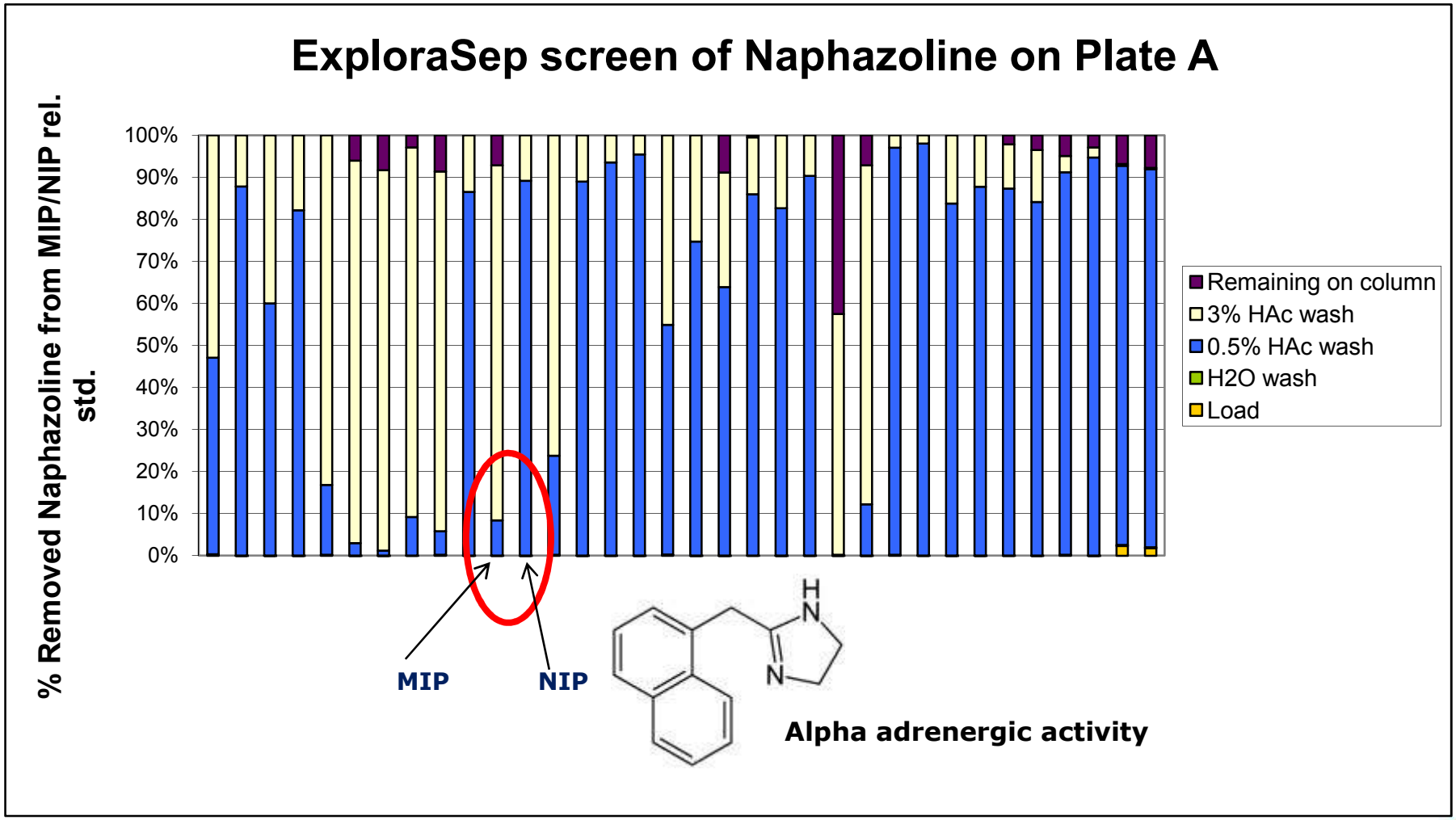
Overview of ExploraSep Process



ExploraSepによるMIPスクリーニングから、最終的に実用のためのプロトコルを決定するまでのプロセスの概要です。

Biotage

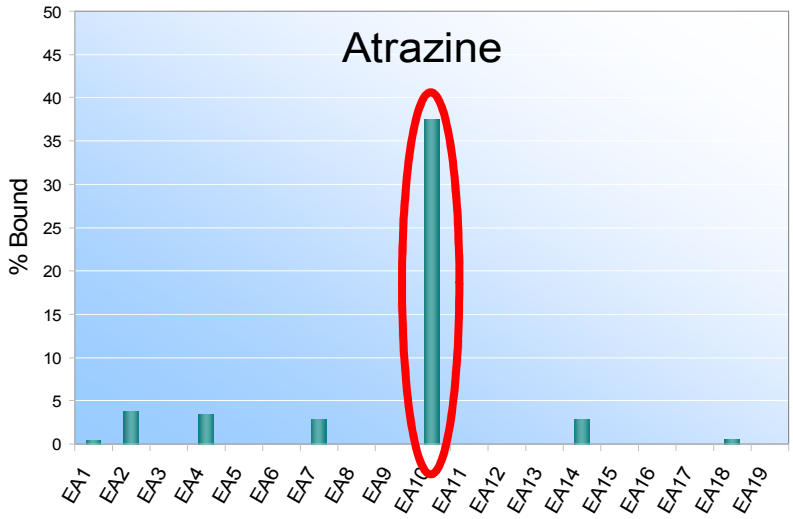
ExploraSep Screening: The Process

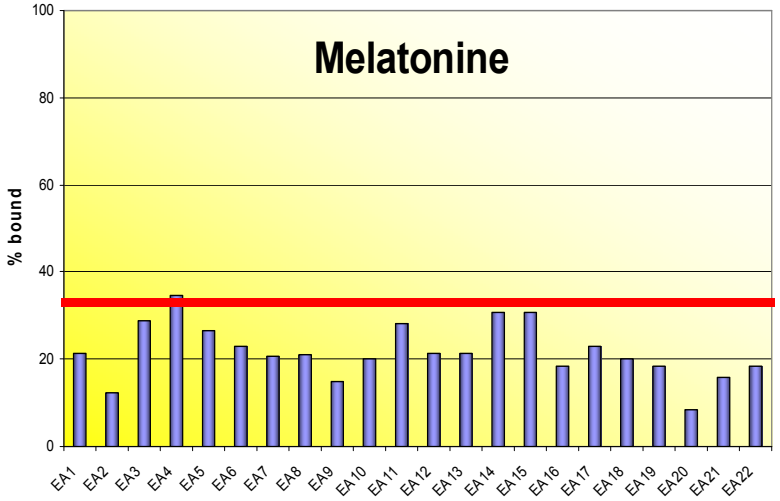


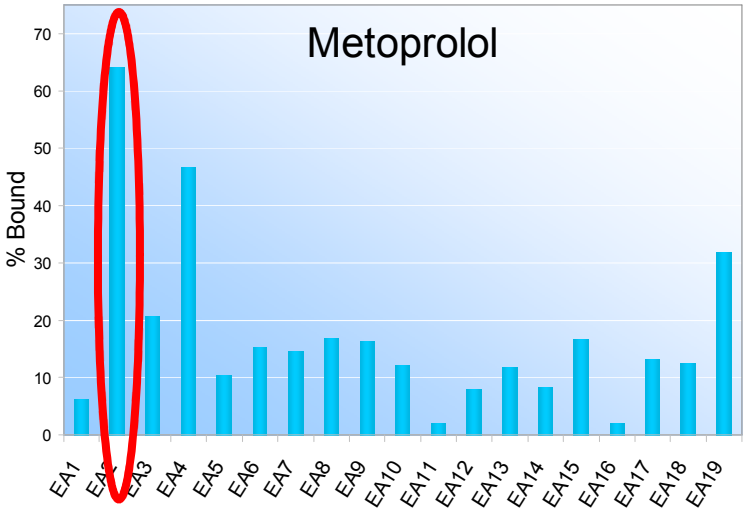
ExploraSepには、各種MIPと、それぞれのMIPに対応するNIP(non-imprinted polymer)が載っています。NIPは、MIPと全く同じモノマー組成で、テンプレート(鋳型)化合物を使わずに作成したポリマーです。

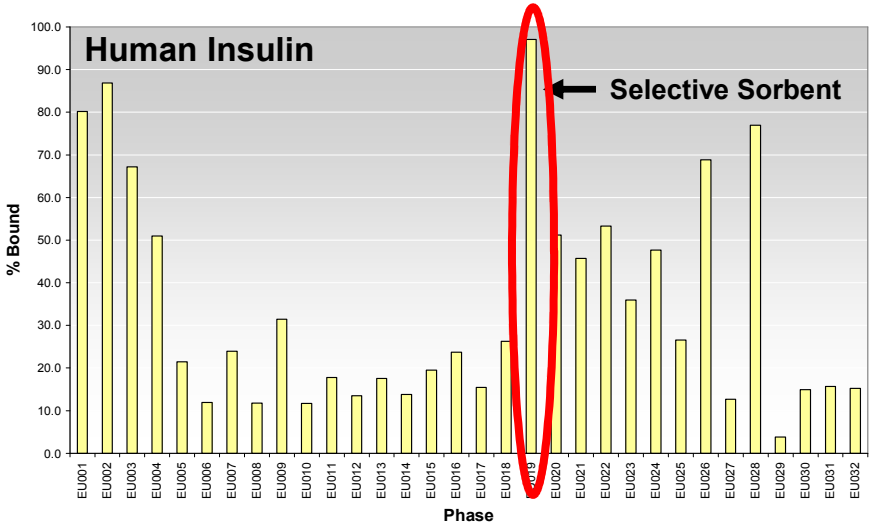
Biotage

ExploraSep Screening Examples





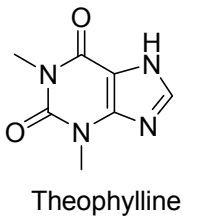




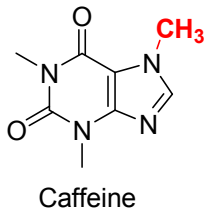
棒グラフの縦軸はポリマーに捕捉(保持)された量(%)です。

Biotage

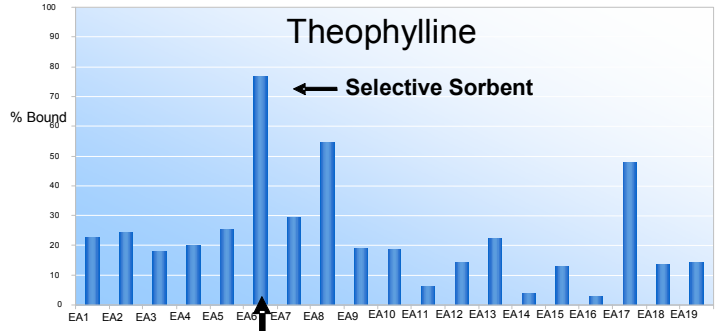
ExploraSep: Sensitive to small differences

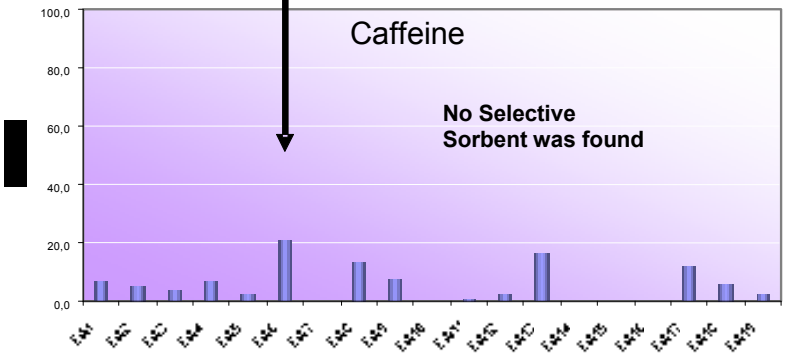


Theophylline



Caffeine





A MIP that binds theophylline but not caffeine
Differential binding with only one methyl group difference!

Biotage

Summary

- MIPs are artificial binding sites in polymers and can be made to operate in **SPE** or **chromatography** mode
- MIPs are **stable** to all organic solvents and normally extremes of pH (depends on monomer chemistry)
- MIPs can be made selective for a restricted group of **highly related** molecules OR a 'CLASS' of chemically **similar** compounds
- MIPs can be manufactured for use at **process scale**
- New separation materials for most compounds of interest to pharma can be discovered using **ExploraSep**

Biotage

End of Part I

Biotage

Agenda

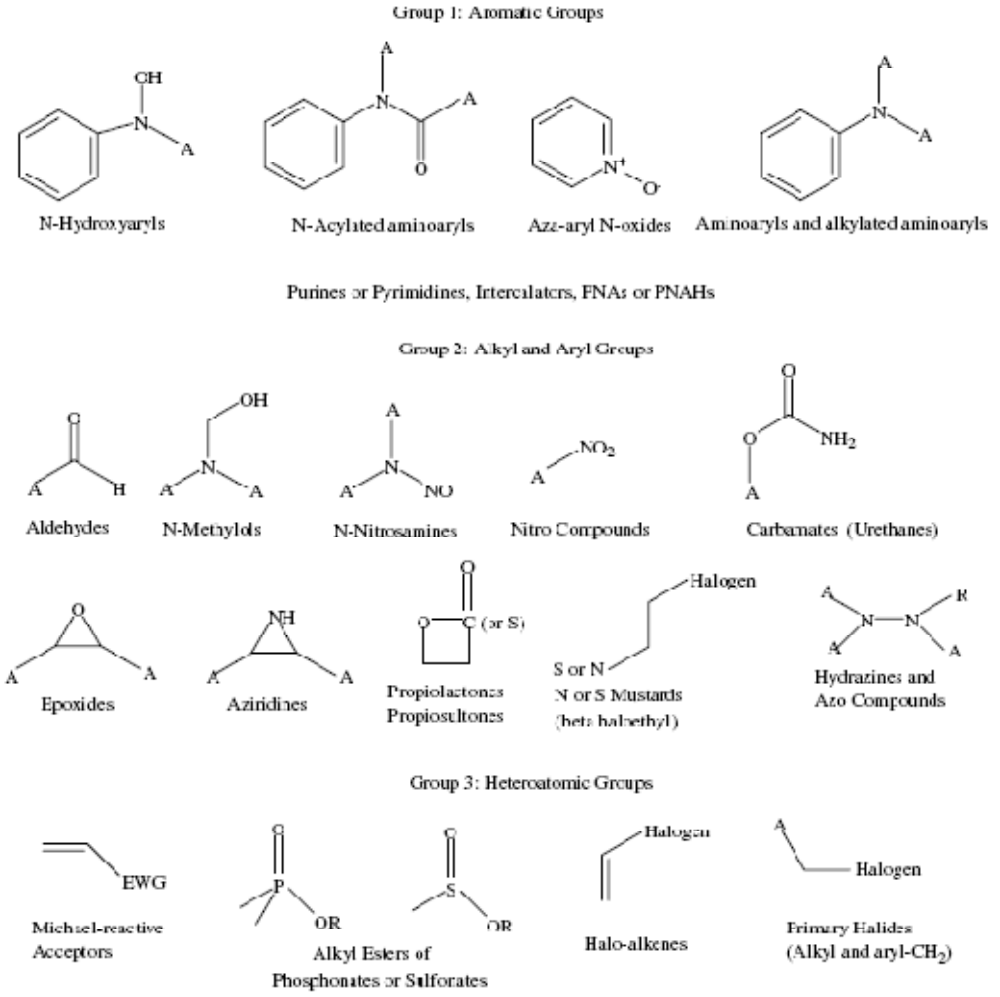
Part I

- What are MIPs?
- How do they behave?
- Examples of selectivity
- ExploraSep™ : a new screening concept

- Part II
- ExploraSep and Genotoxins: Case Studies

Biotage

Potential Genotoxic Impurities (PGI): Structural Alerts



(From Müller et al, Regulatory Toxicology and Pharmacology 44 (2006) 198–211)

Biotage

Genotoxic impurities and ExploraSep™

The Analytical Challenge

- Validated methods for multiple API's and PGI are required
- The genotoxic compounds may be closely related to the API

The Process Chemistry challenge

- Investigate process conditions that may create PGI
- Map the effect of all reaction conditions that effect PGI formation
- Investigate process changes required to control PGI concentrations
- Develop back-up strategy to scavenge PGI with high yield of API

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PGI Screening Process

- ExploraSep
  - Screen each of 4 plates in clean solvent mixture containing API and PGI
  - Take 'hits' and develop separation method (SPE or chromatography
- Comprehensive data
  - Raw data supplied with analysis showing polymer of choice for further development
- Rapid Results
  - Projects completed on agreed schedule at fixed price
- Proven Scale-up
  - MIP production already at 500 Kg/year. Option of bulk resin, SPE cartridges, pre-packed Flash cartridges or HPLC preparative columns

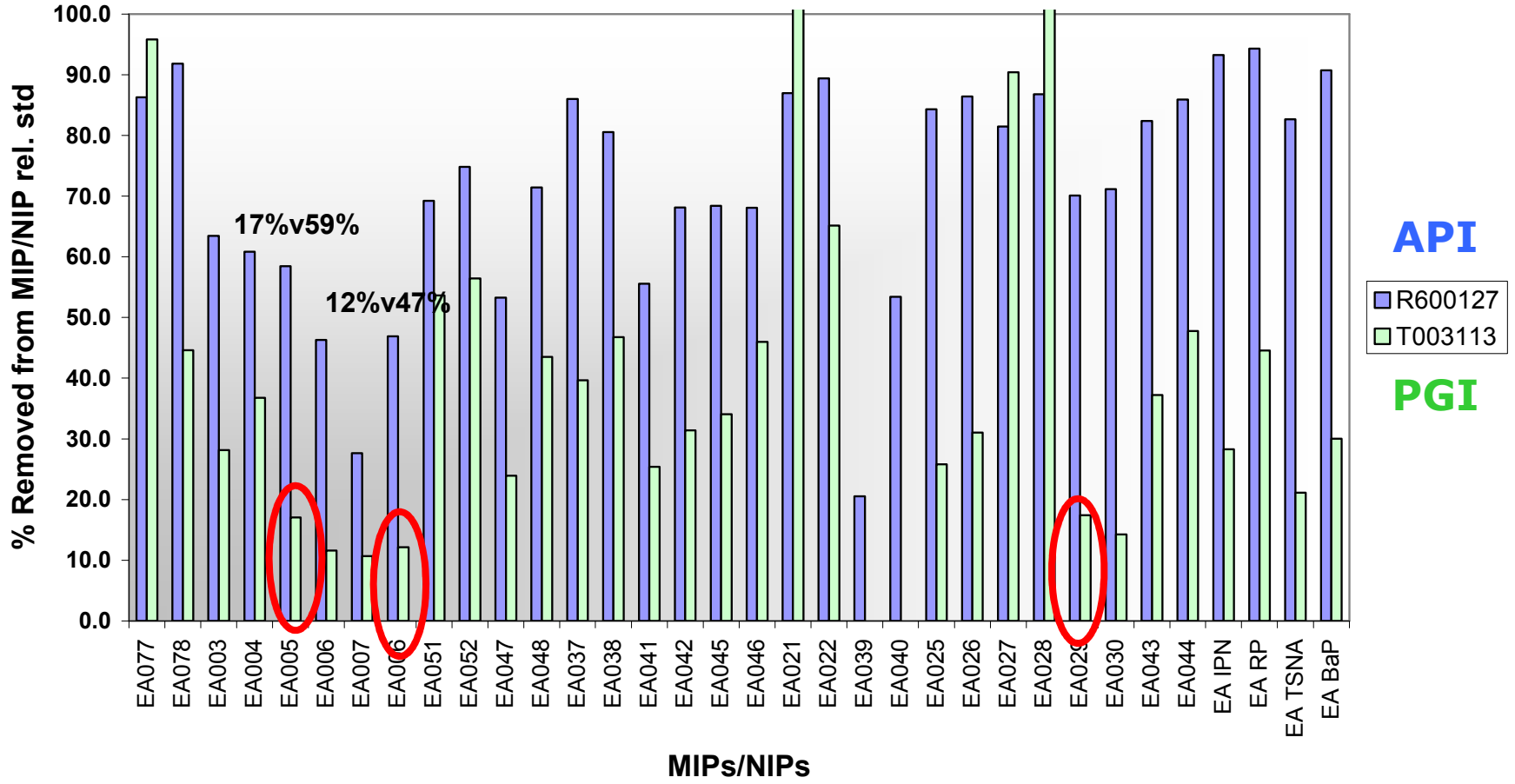
Biotage

Case Study 1
A large US Pharma

Biotage

CS 1: Screening for Genotoxin over API

ExploraSep Plate A: loaded in toluene



Weak'hits'

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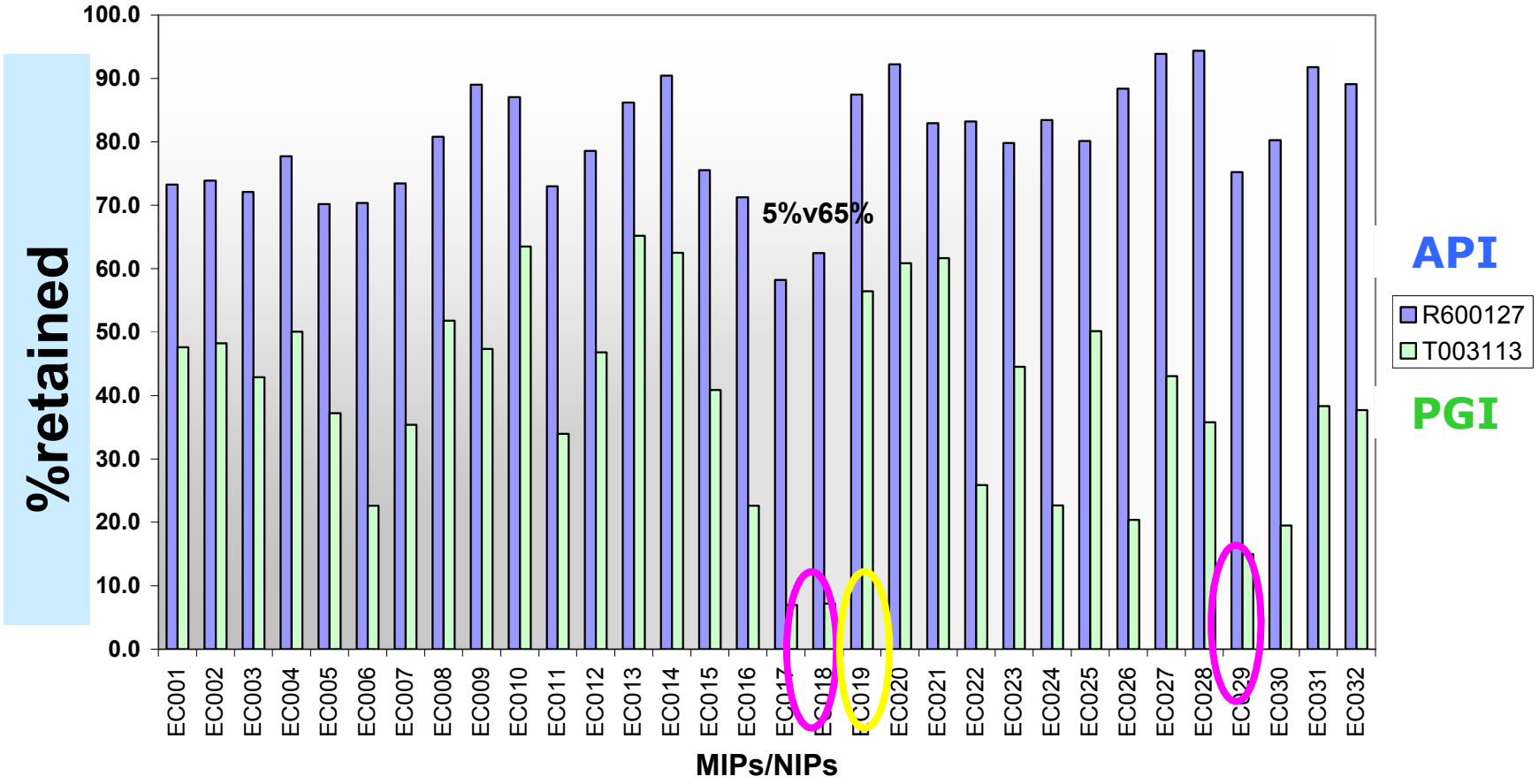
CS 1: Selectivity for Genotoxin over API

ExploraSep Plate C: loaded in toluene



Several hits that differentiates PGI (T003113) and API (R600127)

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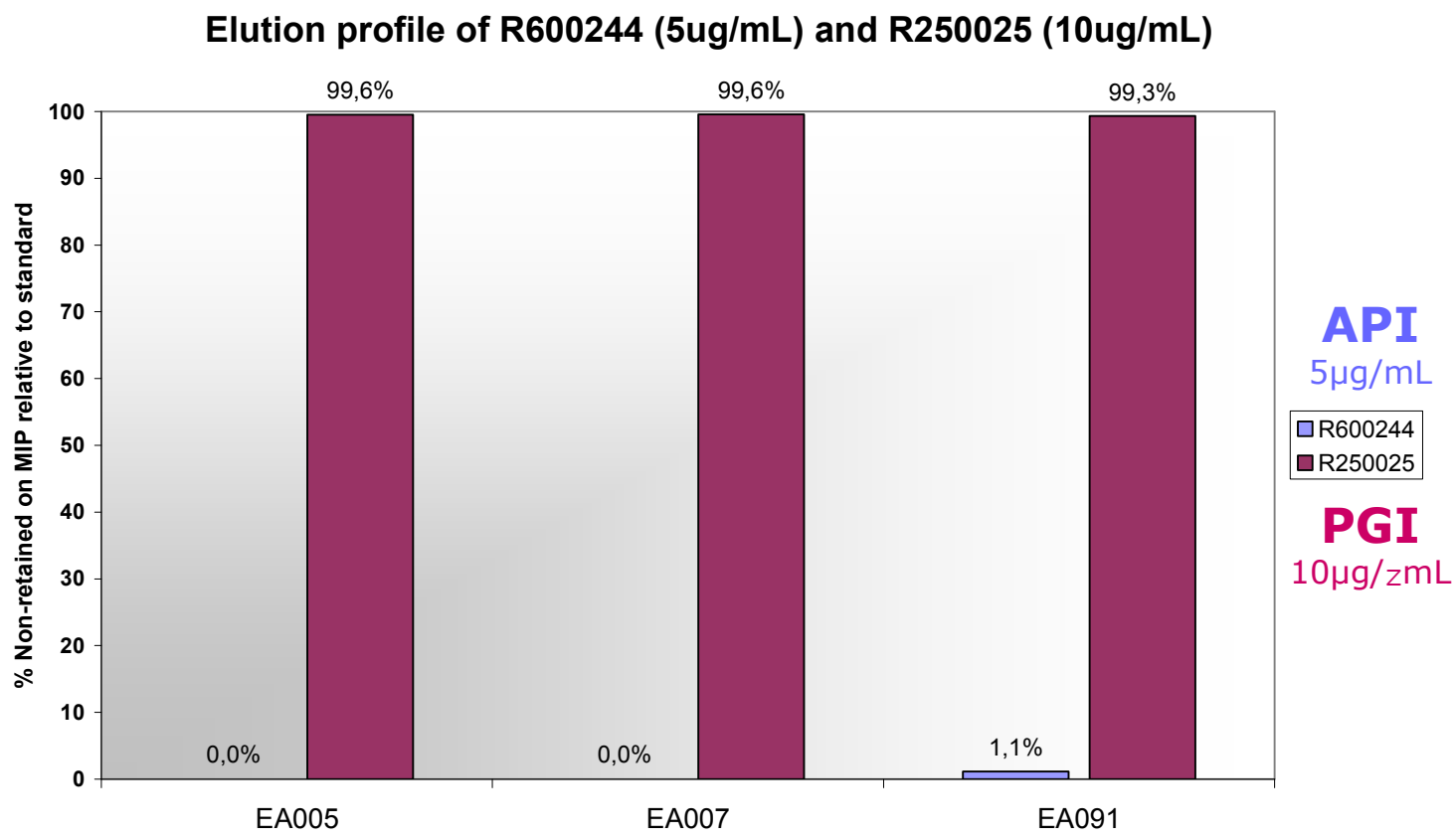
CS 1: API binds and PGI elutes



High Selectivity forR600244 (API) over R250025 (PGI)

(0% = non-detectable by lc/ms/ms)

Biotage

CS 1: Conclusion

- First API-PGI set screened on Plate C identifies several candidates with selectivity for PGI for further evaluation. EC 029 selected for further evaluation

- Second API-PGI set screened on plate A shows selectivity for API over PGI. EA 005 and EA 007 selected for further evaluation.

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Case Study 2
A large European Pharma

Biotage

Case Study 2

- Structures confidential but:
  - PGI contains amide and piperazine moieties.
  - API contains piperazine, indole and dione moieties.
- Log P
  - PGI ~ 1.3
  - API ~ 3.3.

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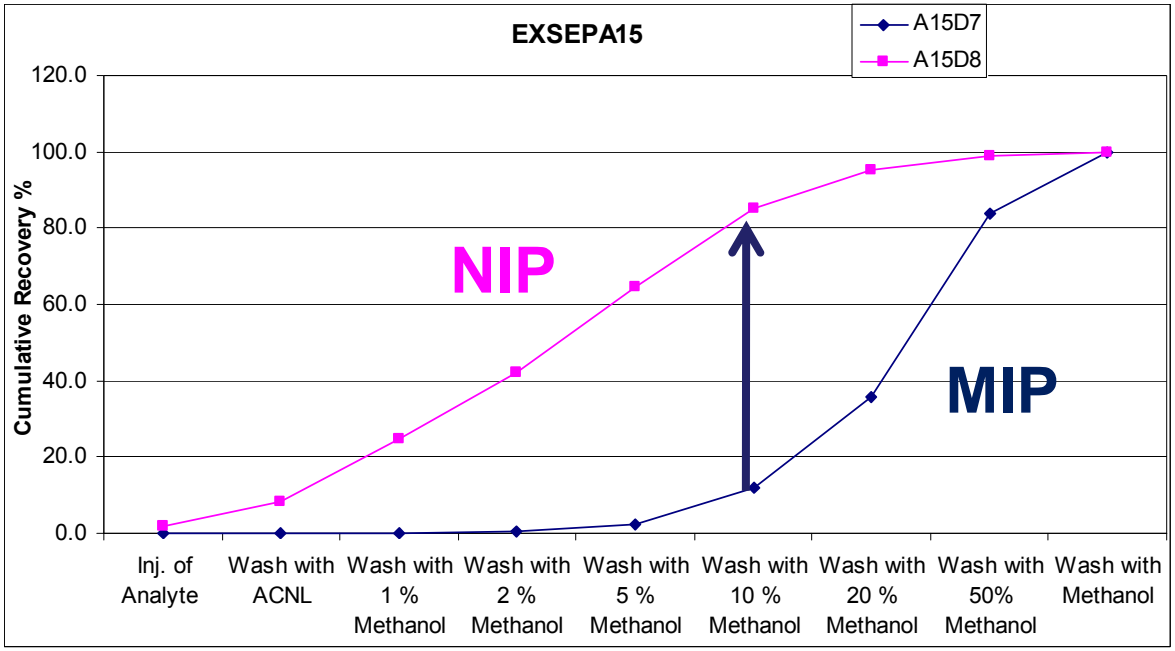
Case Study 2



Binding curve for genotoxin (d7) and API (D8) on MIP EA15 derived from ExploraSep A Plate.

Arrow shows difference between imprinted (MIP) and non-imprinted (NIP) polymer

Biotage

Case Study 2 (cont)

Conclusion

- Quite good selectivity on several of the polymers on the A plate : EA07, EA15 and EA23
- Polymers were packed in HPLC columns and supplied to customer

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Case Study 3
A European CMO

Biotage

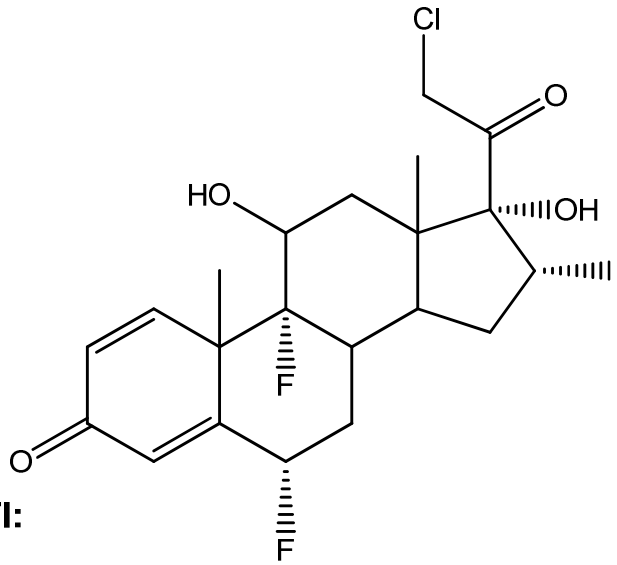
Case Study 3

- All genotoxin structures known but only a few of the APIs.
- Log P of Methyl p-Toluenesulfonate (PGI) ~ 2.0, log P of 21-chlorodiflorasone (API) ~ 2.7.
- Log P of 1,3-diisopropylurea (PGI) ~ 0.6, log P of API ~ 2.7.
- All screened with ExploraSep

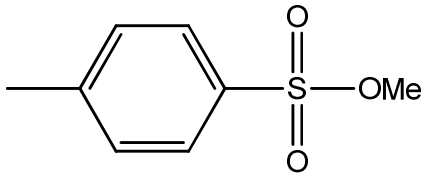
Biotage

Case Study 3 (cont)

API or Intermediate:

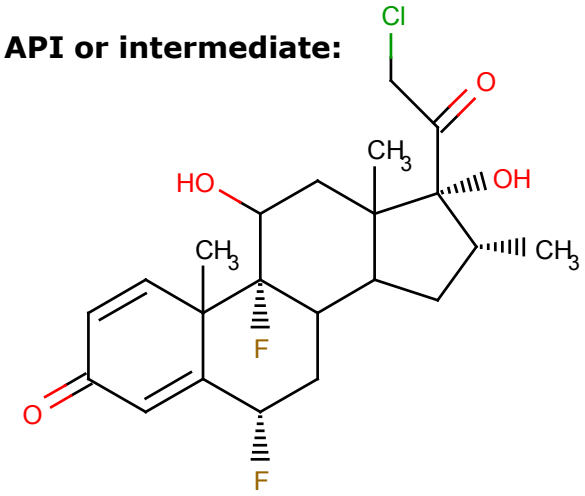


GTI:

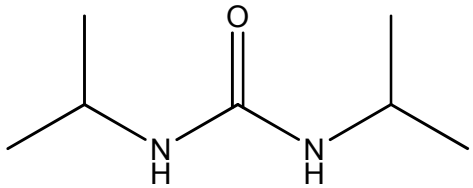


Methyl p-Toluenesulfonate

API or intermediate:



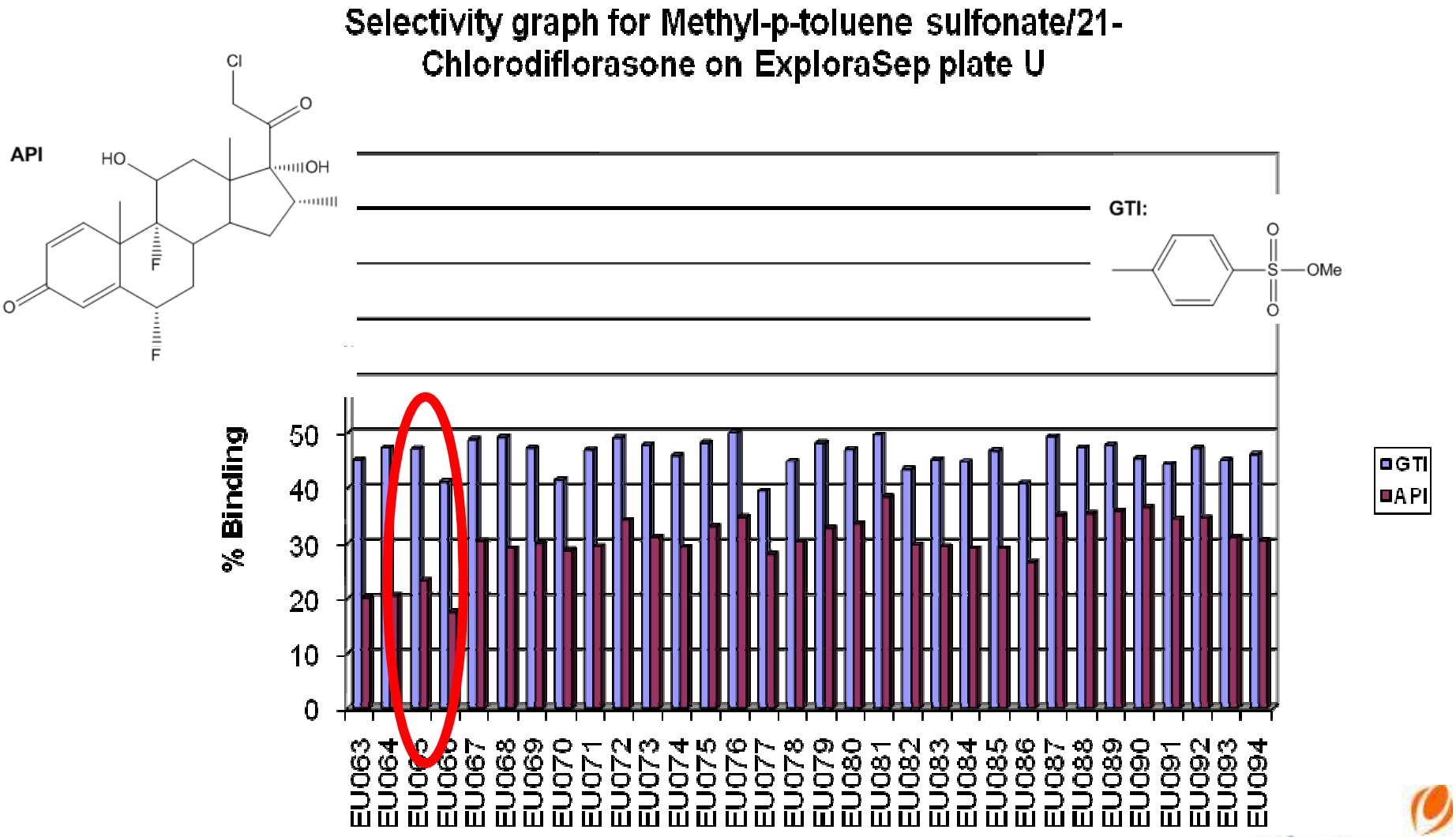
GTI:



1,3-diisopropylurea

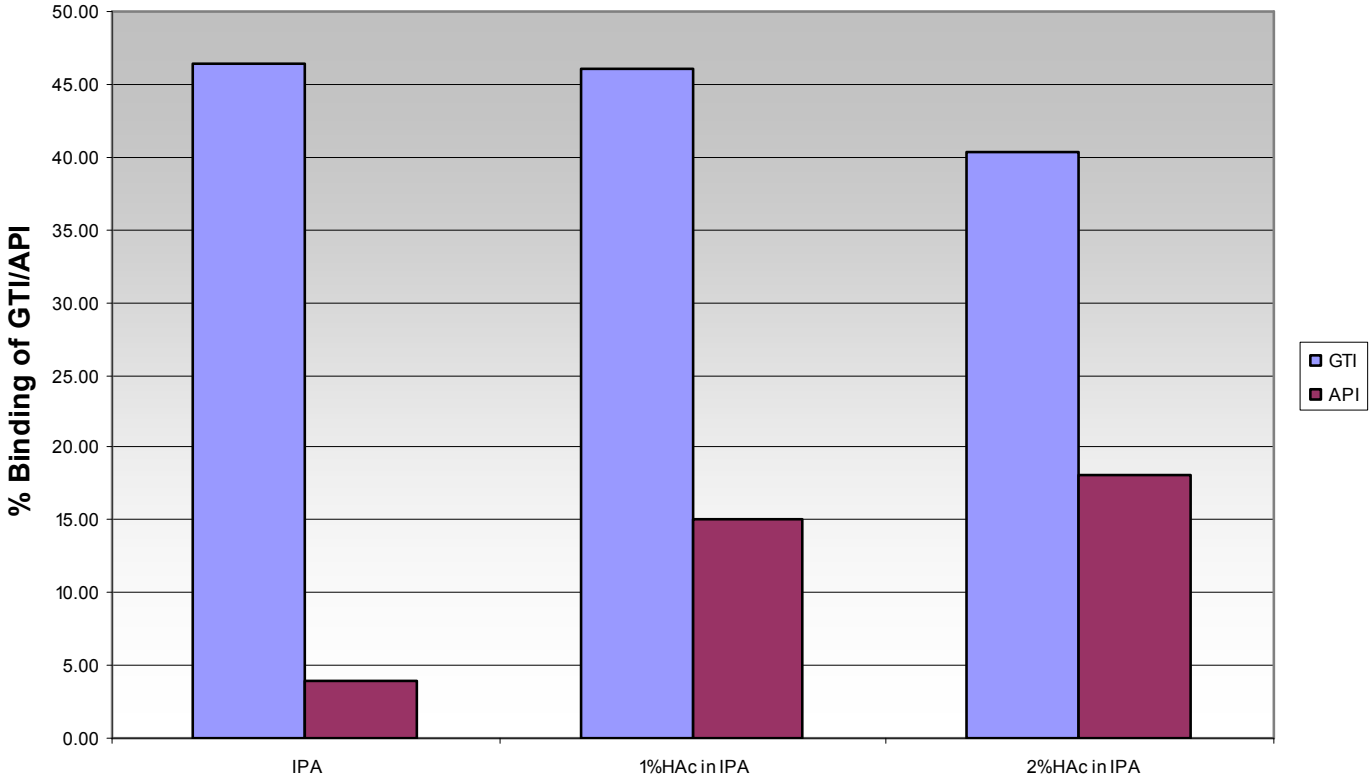
Biotage

Case Study 3 (cont)



Biotage

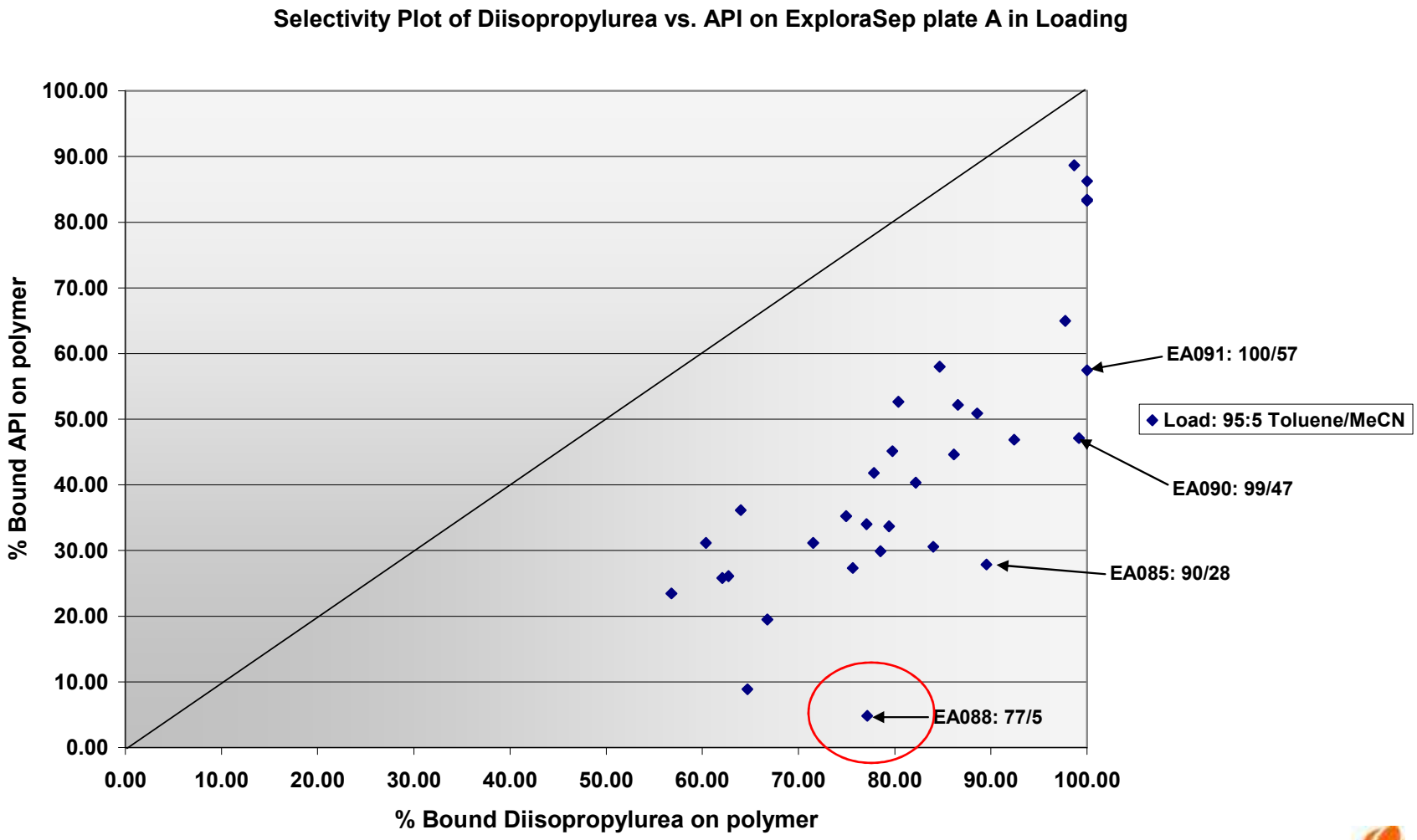
Case Study 3 (cont)



Selectivity graph of methyl p-toluenesulfonate (PGI)/API in loading on EU064

Biotage

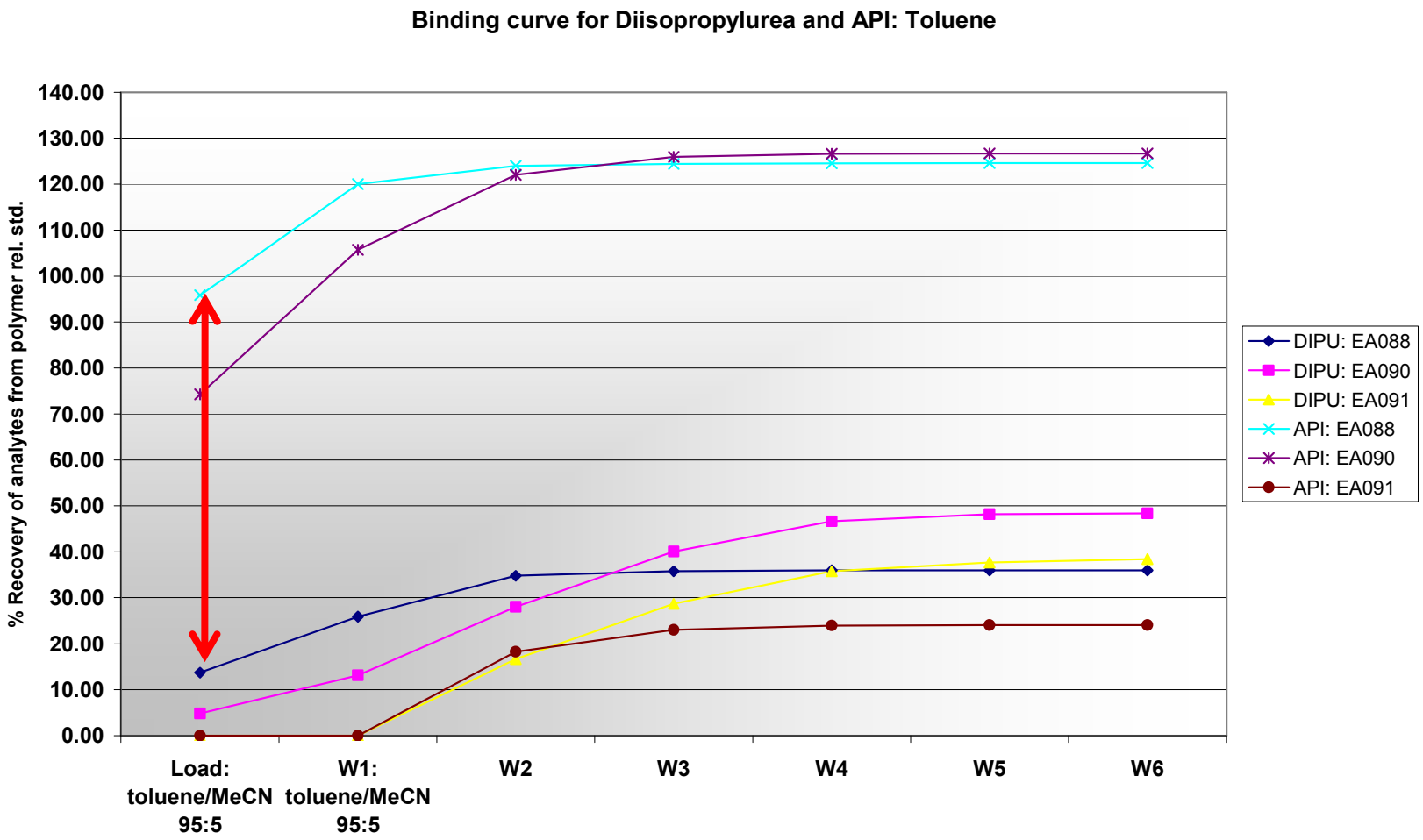
Case Study 3 (cont)



Further evaluation of additional A plate candidates

Biotage

Case Study 3 (cont)



Arrow shows API versus PGI extraction on EAO88
= Starting point for optimized separation

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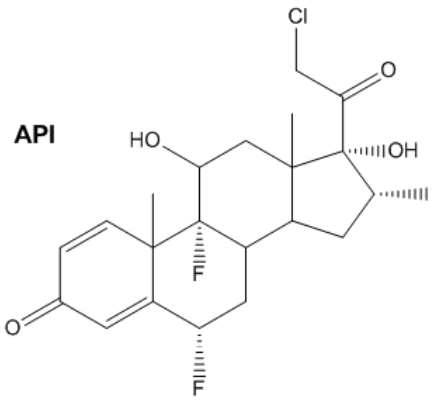
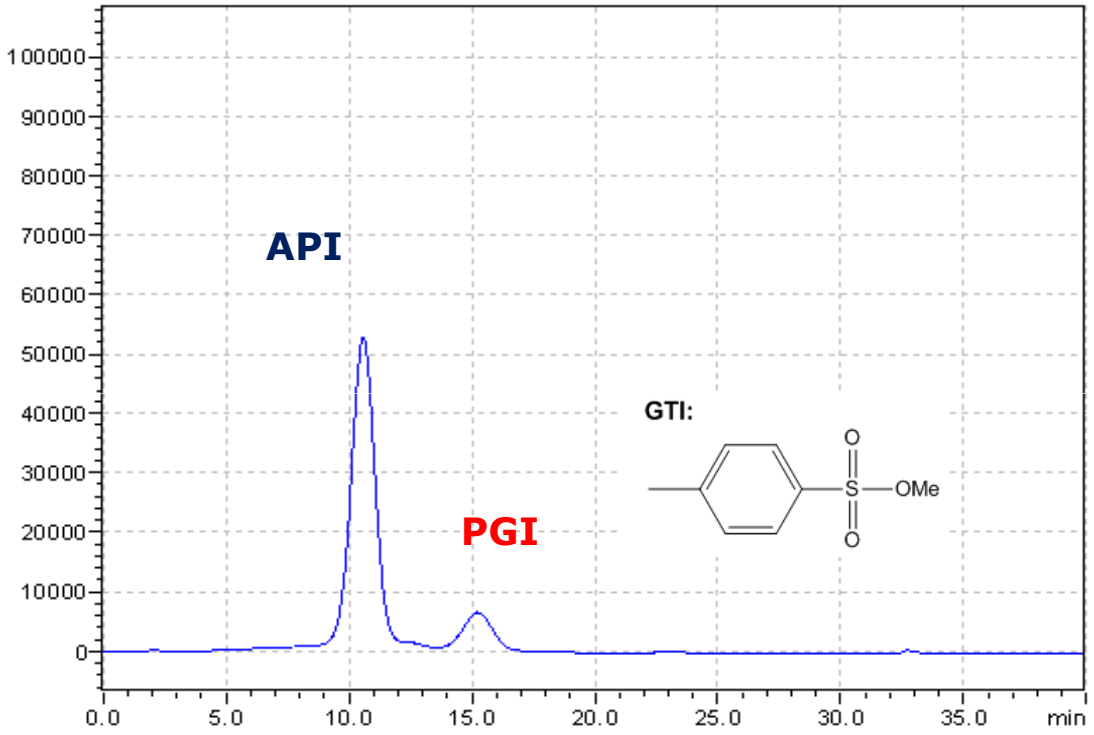
Case Study 3 (cont)





Separation in LC mode on EA088
Strong retention of toxic contaminant obtained

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CS 3: Conclusions

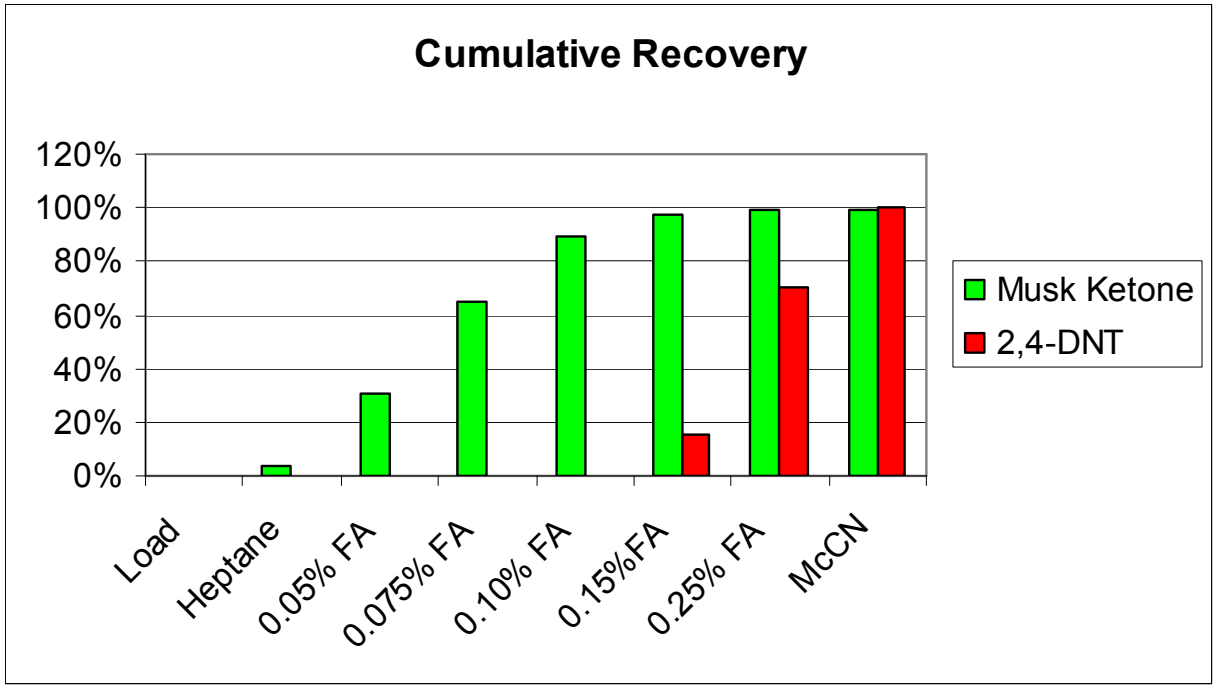
- Initial screening showed weak hits
- Additional synthesis and screen showed stronger hits
- Packing of strongest hit in HPLC mode showed satisfactory separation

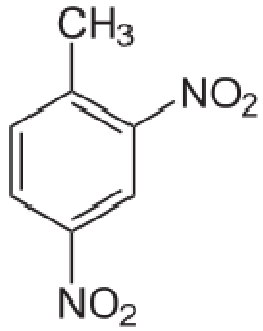
Biotage

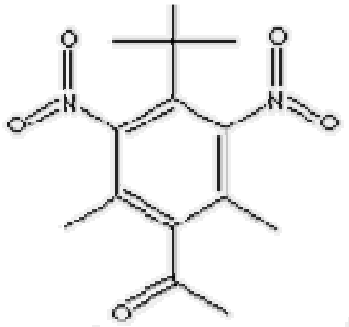
Case Study 4
European consortium study

Biotage

Case Study 4







Recognition of molecules carrying the same functional group (-NO_2)

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Summary

- The cross-reactivity of MIPs allows selective binding of other molecules
- This ’Similarity’ concept has been used to separate API’s from PGI’s
- All polymers identified in ExploraSep screening can be produced at pilot and/or Process scale
- ExploraSep is a powerful tool in separation method discovery

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Additional Information

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- How can MIPs be scaled up?

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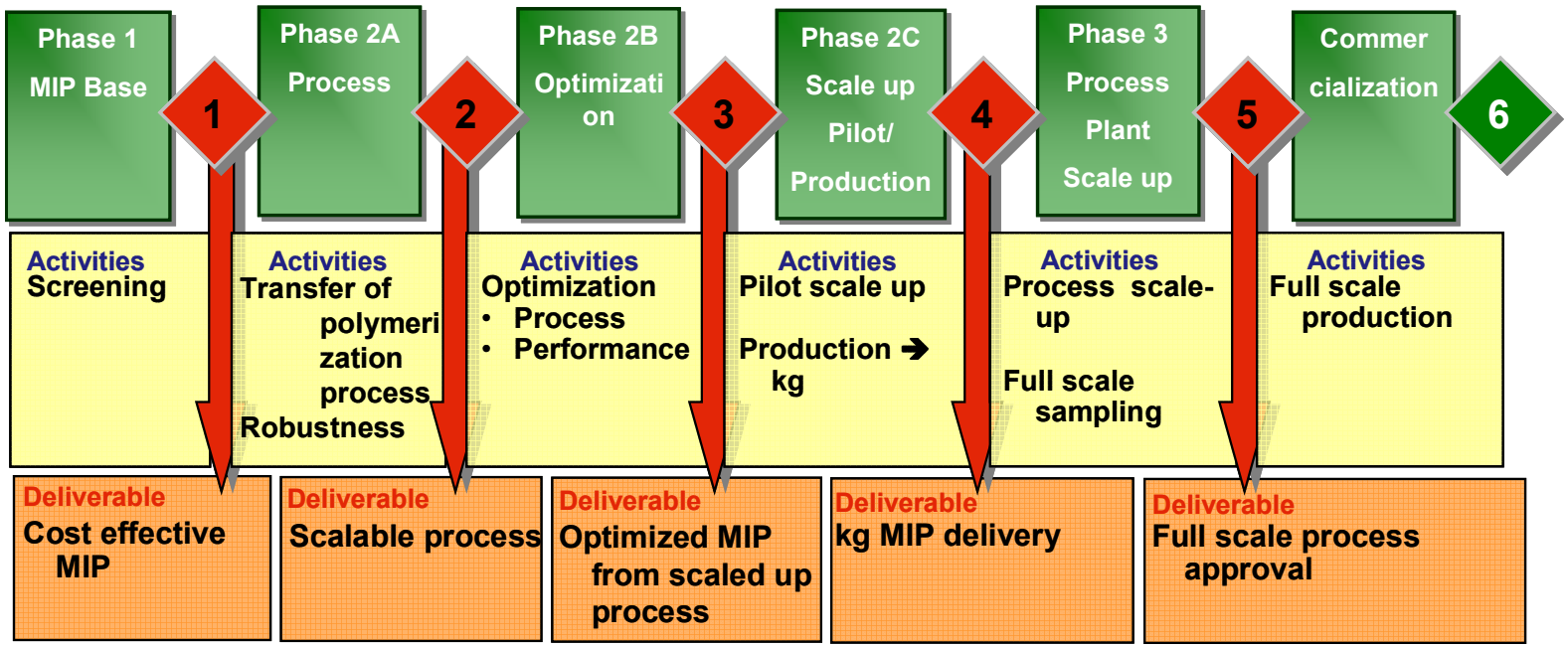
A Stage Gate approach to Production

The process path



- Applying stage-gate process for efficiency and risk reduction
- Utilising DoE (Design of Experiments) tool to attain robust product and control

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Tools in materials development

- A select group of experts
- Innovative MIP chemistry ➔ large IP portfolio
- Polymer chemistry at the research frontier
  - Novel concepts
  - New methods
- Modeling

Chemometric predictions and Monte Carlo models

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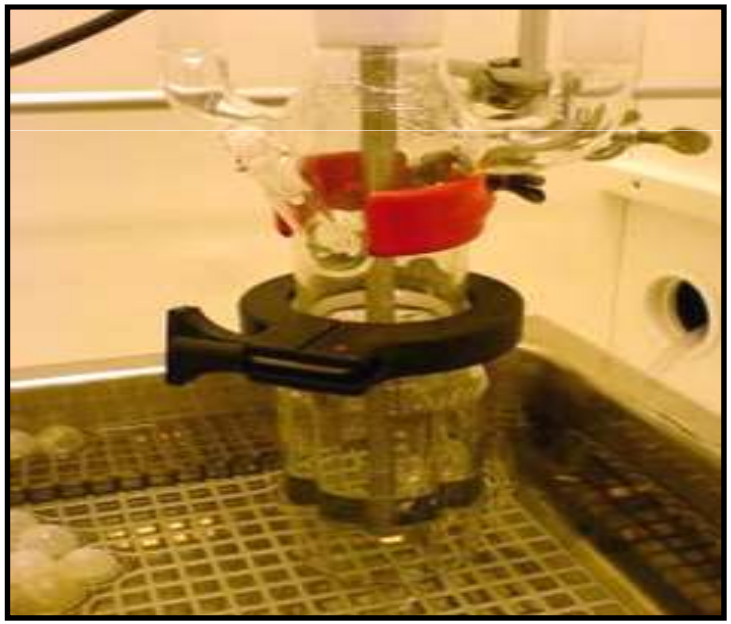
Scale up – DOE Starting point

Focus

- Polymer properties
- PSD (particle size distribution)

Parallel 50ml

0,5 L reactors



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Transfer to pilot scale …

2 L

10 L

30 L

50 L

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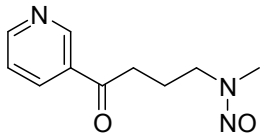
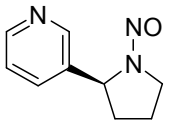
Example – Selective removal of nitrosamines in presence of nicotine

- To selectively remove a group of 4 nitrosamines at a total concentration of 70 ng/ml from a tobacco extract
- The structurally related nicotine should be retained in the mixture - at a concentration of 700 µg/ml
- Nitrosamines are structurally related and are at 10000x lower concentration

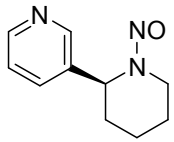
- Nitrosamines to be removed



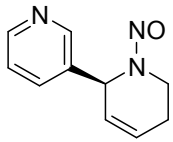
NNK



NNN

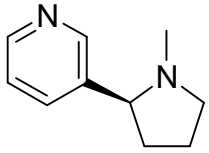


NAB



NAT

- Nicotine to be retained



(S)-(-)-Nicotine

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Optimization of the MIP recipe

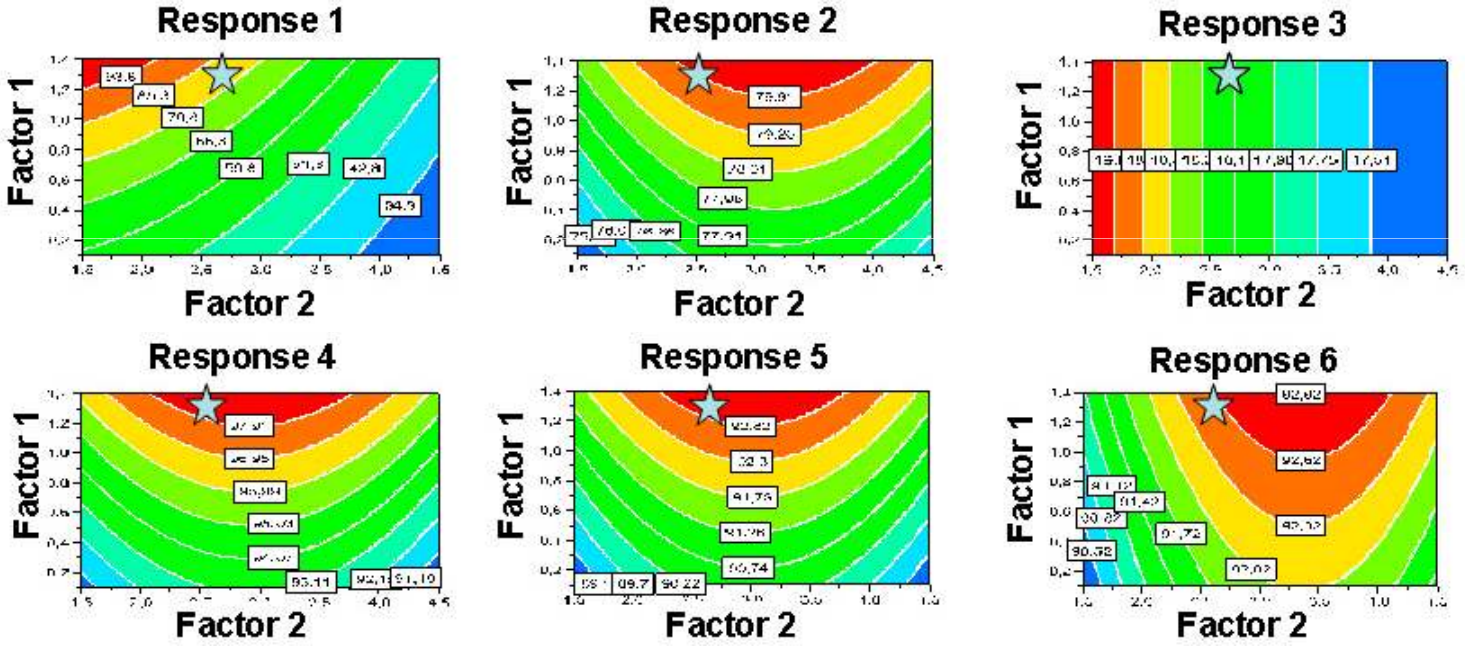
DOE computer aided optimization of the recipe

- Final DOE on “best” existing recipe
- 5 Parameters checked
- Coefficients analyzed with software MODDE
  - contour plots for the responses

Biotage

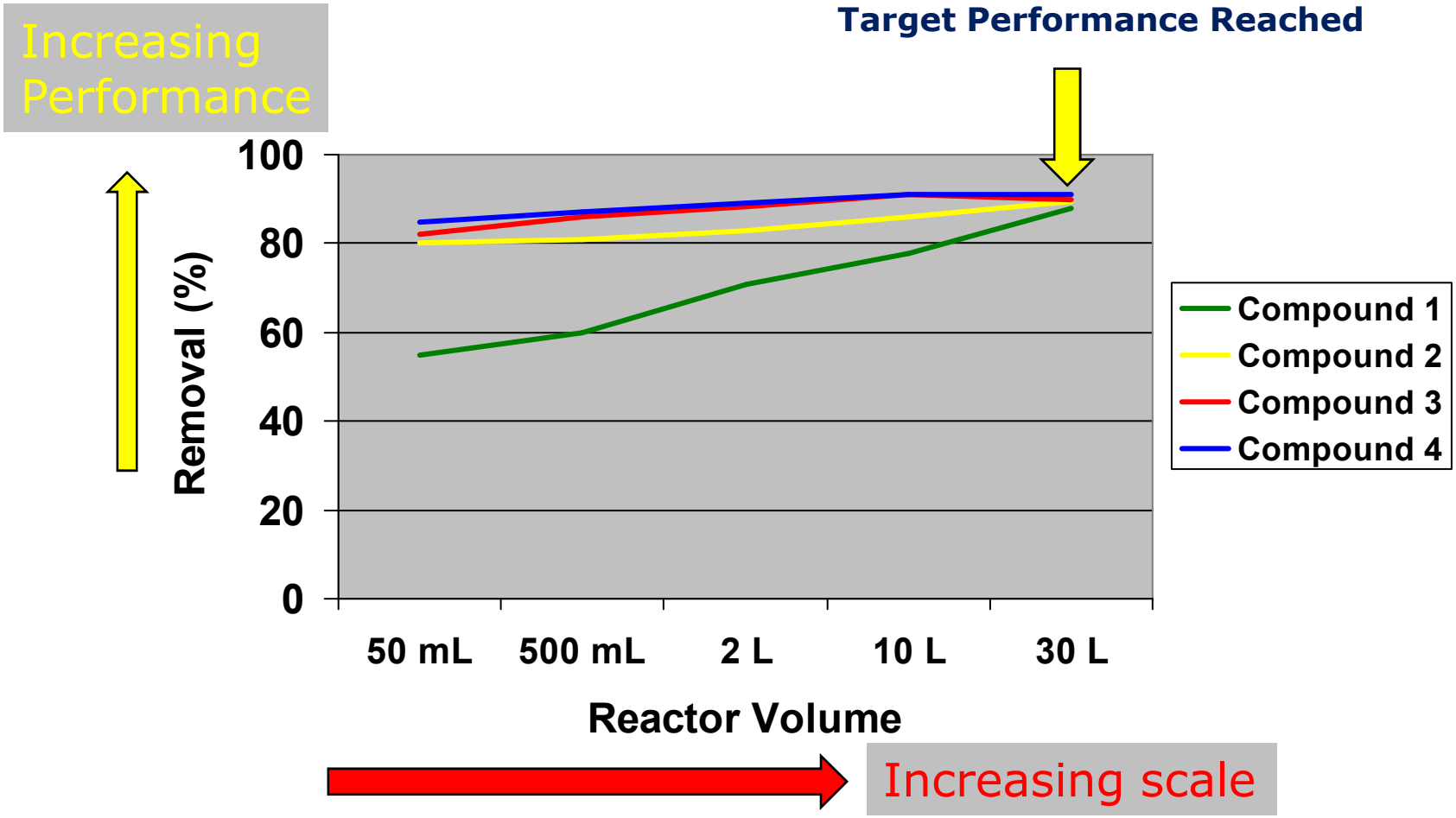
The DOE Responses

DoE driven process scale-up: Optimisation/model



Biotage

The Final Design: Pilot Scale (25kg)



Biotage