

Optimization of a SPE for a LC-MS/MS general unknown screening procedure by means of computational techniques

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Outline

● Introduction and goal

- IDA fundamentals

● Study set-up

- SPE
- DoE

● Results

- Screening design
- Optimization design

● Conclusions

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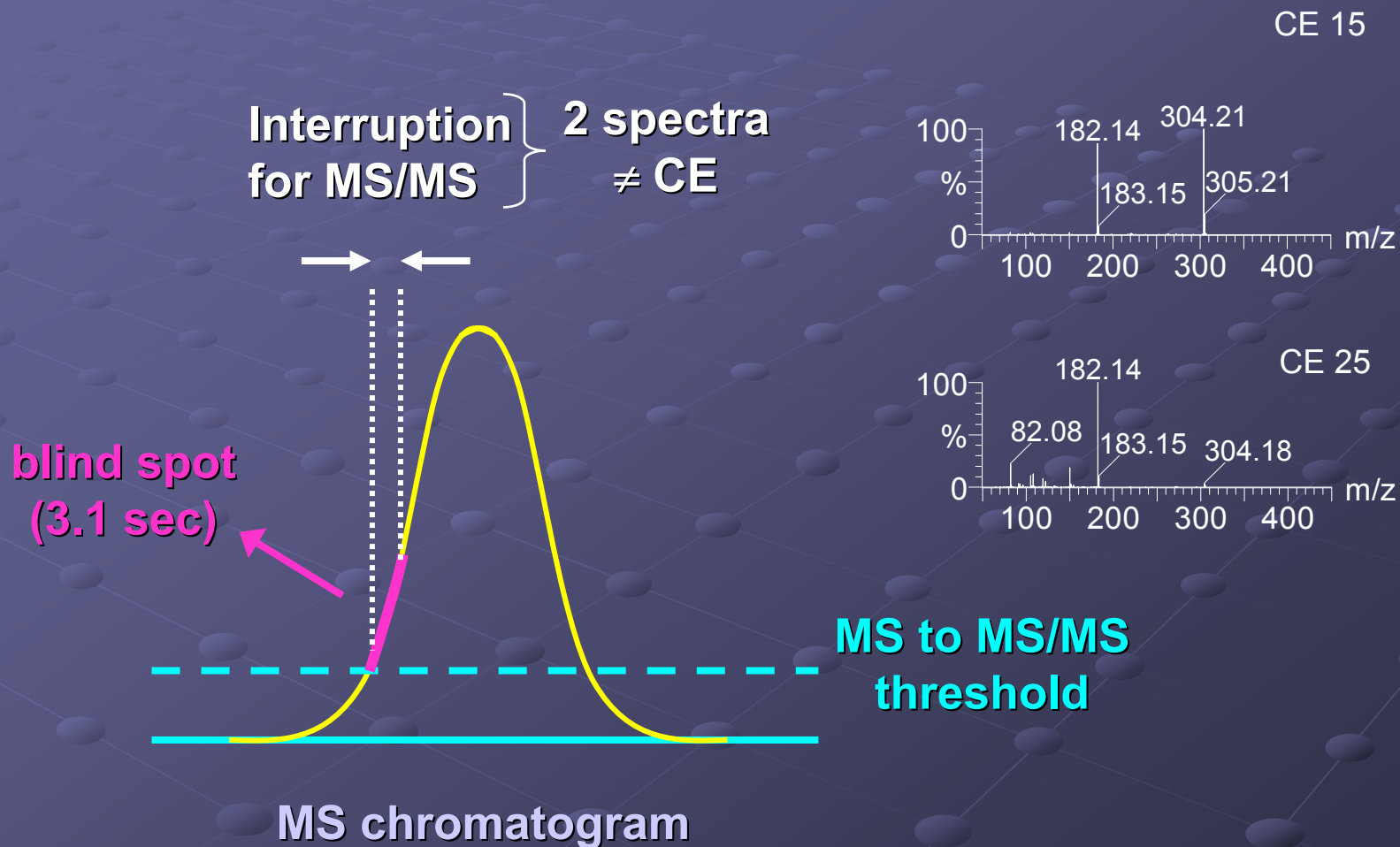
● Conclusions

Introduction & goal

- **G**eneral **U**ncertain **S**creening procedures
 - ~ essential in forensic toxicology
 - ~ no foreknowledge
 - ~ **I**nformation-**D**ependent **A**cquisition (IDA)*
- SPE optimization ~ IDA

* Rapid Commun. Mass Spectrom. 14, 1787-1792 (2000)

IDA fundamentals



Introduction

SPE optimization

```
graph TD; A([SPE optimization]) --> B[OFAT  
(One-Factor-At-a-Time)]; A --> C[DoE  
(Design of Experiments)];
```

OFAT

(One-Factor-At-a-Time)

- ☞ No 2- and 3-way interactions
- ☞ 2048 (2^{11}) experiments
(11 factors)

DoE

(Design of Experiments)

- Screening design
- Multi-level optimization design

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Study set-up - SPE

Conditioning



Sample appl.



Washing



Drying

Elution



● MeOH

● Ammonium acetate buffer

▲ Compound of interest

■ Impurity

● Matrix

○ Ammonium acetate buffer + %MeOH

● Hexane

● Water

● Elution solvent

Study set-up - SPE

Conditioning



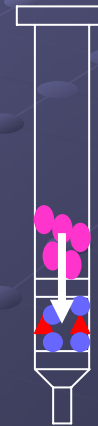
Sample appl.



Washing



Elution



Drying

Volume & flow (factor 1 to 8)

- MeOH
- Ammonium acetate buffer
- ▲ Compound of interest
- Impurity
- Matrix

- Ammonium acetate buffer + %MeOH
- Hexane
- Water
- Elution solvent

Study set-up - SPE

Conditioning



Sample appl.

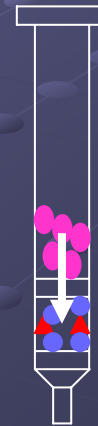


Washing



Drying

Elution



Factor 11

Factor 9 & 10

- MeOH
- Ammonium acetate buffer
- ▲ Compound of interest
- Impurity
- Matrix

- Ammonium acetate buffer + %MeOH
- Hexane
- Water
- Elution solvent

Molarity
Percentage

Study set-up - SPE

Conditioning



3mL MeOH
3mL buffer (pH 9)

Sample appl.



2mL blood

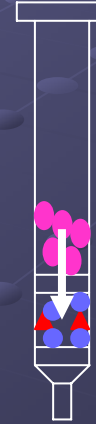
Washing



?

Drying
?

Elution



MeOH + 1%AA

- MeOH
- Ammonium acetate buffer
- ▲ Compound of interest
- Impurity
- Matrix

- Ammonium acetate buffer + %MeOH
- Hexane
- Water
- Elution solvent

Study set-up - DoE

● Definition of:

- Factors & experimental domain
- Responses
- Screening design
 - ↳ Plackett - Burman
- Optimization design
 - ↳ Central composite design

Study set-up - DoE

	Factors	Symbol	-1	+1	Unit
1	Flow 1st wash	A	0.5	5	mL/min
2	Volume 1st wash	B	1	6	mL
3	Buffer molarity (1st wash)	C	10	200	mM
4	% MeOH (1st wash)	F	10	60	%
5	Flow 2nd wash	E	0.5	5	mL/min
6	Volume 2nd wash	H	0	3	mL
7	Flow water	D	0.5	5	mL/min
8	Volume water	G	1	6	mL
9	Drying time	K	0	6	min
10	Flow elution	J	0.5	5	mL/min
11	Volume elution	L	1	6	mL

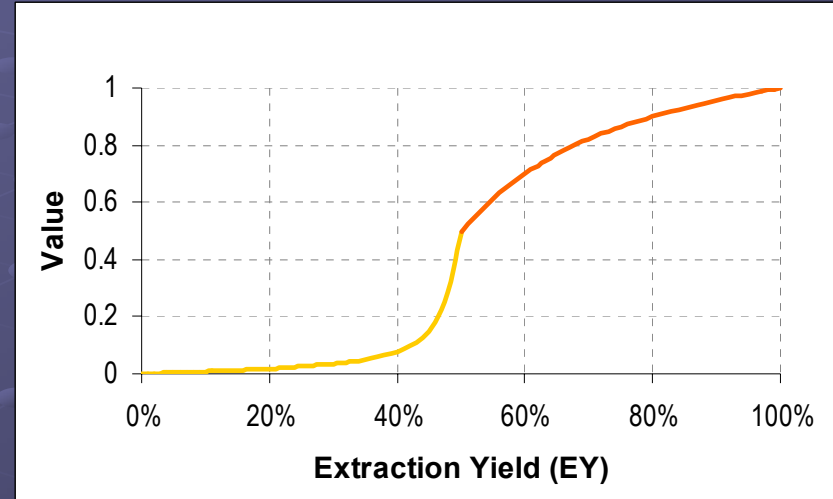
Study set-up - DoE

Responses

R 1 Overall extraction yield (EY)

R 2 Number of compounds effectively retrieved by IDA

R 3 Total number of ions detected by IDA



Characteristics:

if $EY < 50\%$:

less than linear **penalty**

if $EY > 50\%$:

more than linear **reward**

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Results – Screening design

● Plackett-Burman with fold-over

⇒ 24 experiments

⇒ ANOVA analysis: $p < 0.05$ ~ significant

● model

● model terms

⇒ Validation of the models

✓ normal probability plot residuals

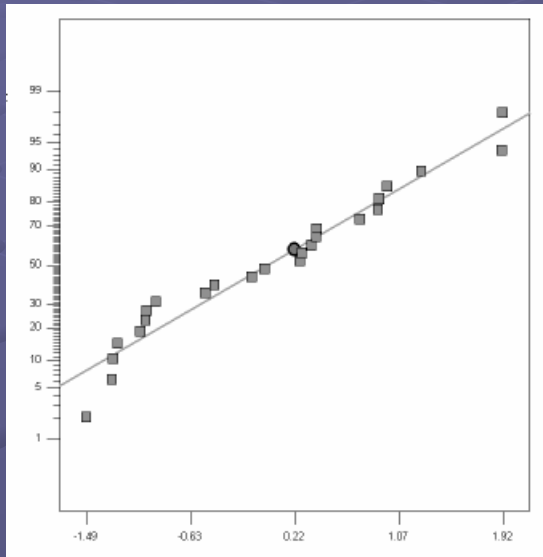
✓ distribution plot residuals ↔ predicted

✓ Outlier T plot

✓ Box Cox plot

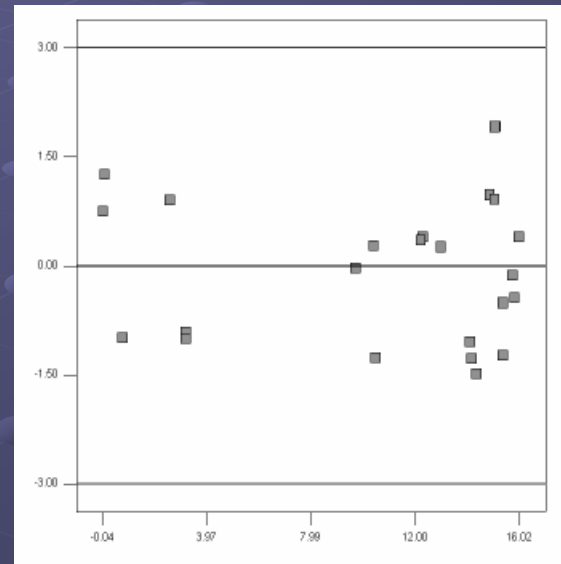
Results – Screening design

Normal % probability



Studentized residuals

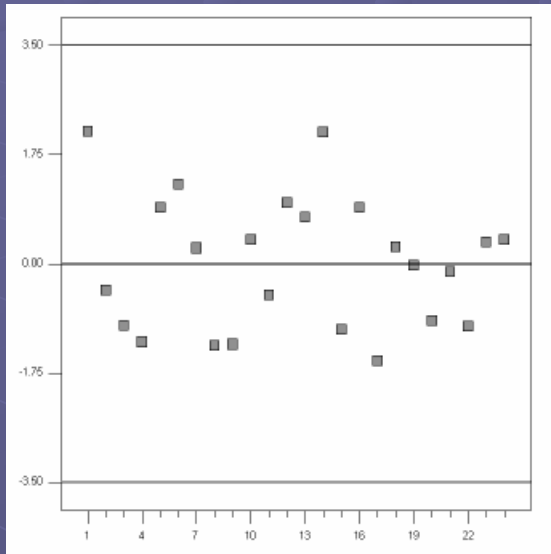
Studentized residuals



Predicted

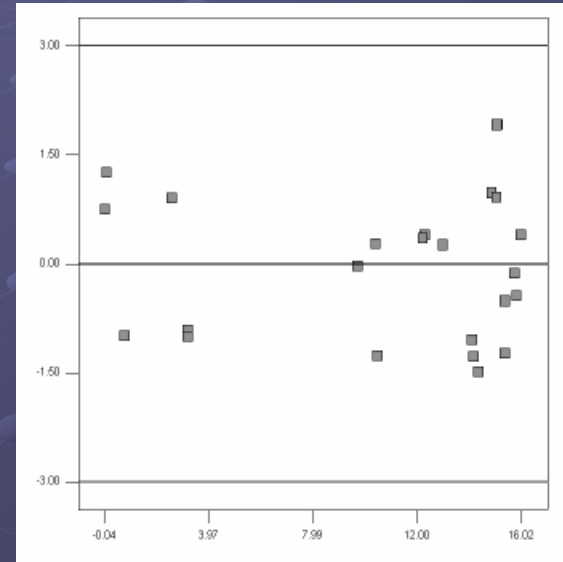
Results – Screening design

Outlier T



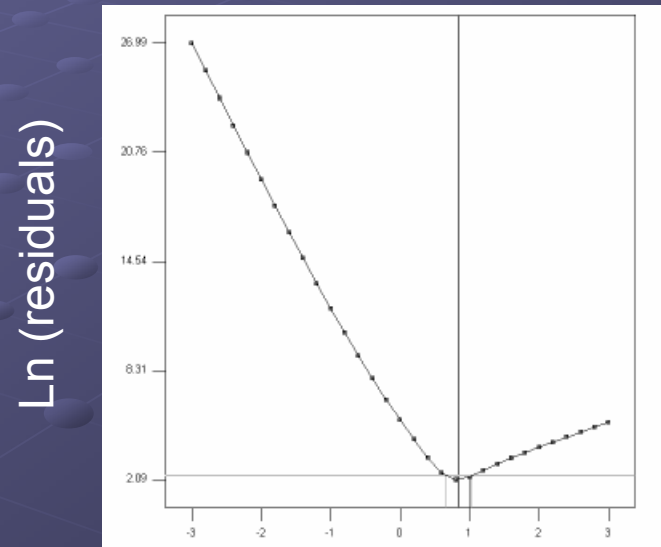
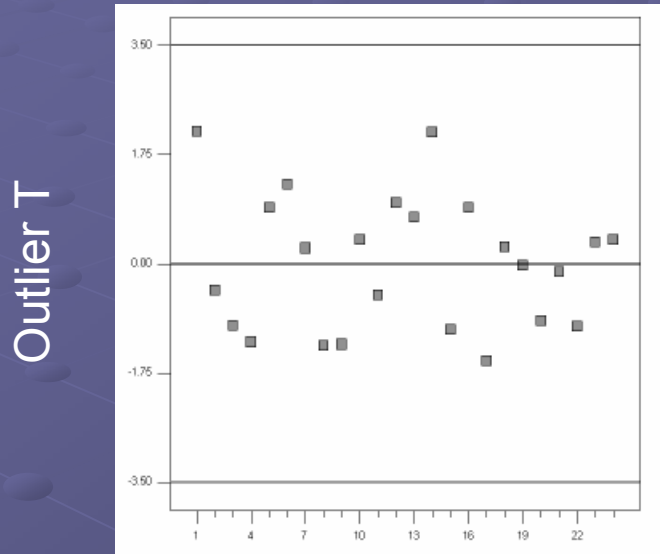
Run number

Studentized residuals



Predicted

Results – Screening design



Results – Screening design

	Desired outcome	Symbol	R 1 ↑	R 2 ↑	R 3 ↓
1	Flow 1st wash	A		+1	
2	Volume 1st wash	B	-1	-1	+1
3	Buffer molarity (1st wash)	C	-1	-1	
4	% MeOH (1st wash)	F	-1	-1	+1
5	Flow 2nd wash	E			
6	Volume 2nd wash	H	-1	-1	
7	Flow water	D			
8	Volume water	G			
9	Drying time	K			
10	Flow elution	J			
11	Volume elution	L			

Results – Screening design

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Results – Screening design

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4	% MeOH (1st wash)	F	-1	-1	+1
5	Flow 2nd wash	E			
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4	% MeOH (1st wash)	F	-1	-1	+1
5	Flow 2nd wash	E			
6	Volume 2nd wash	H	-1	-1	
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Results – Screening design

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4	% MeOH (1st wash)	F	-1	-1	+1
5	Flow 2nd wash	E			
6	Volume 2nd wash	H	-1	-1	
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8	Volume water	G			
9	Drying time	K			
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Results – optimization design

● CCD

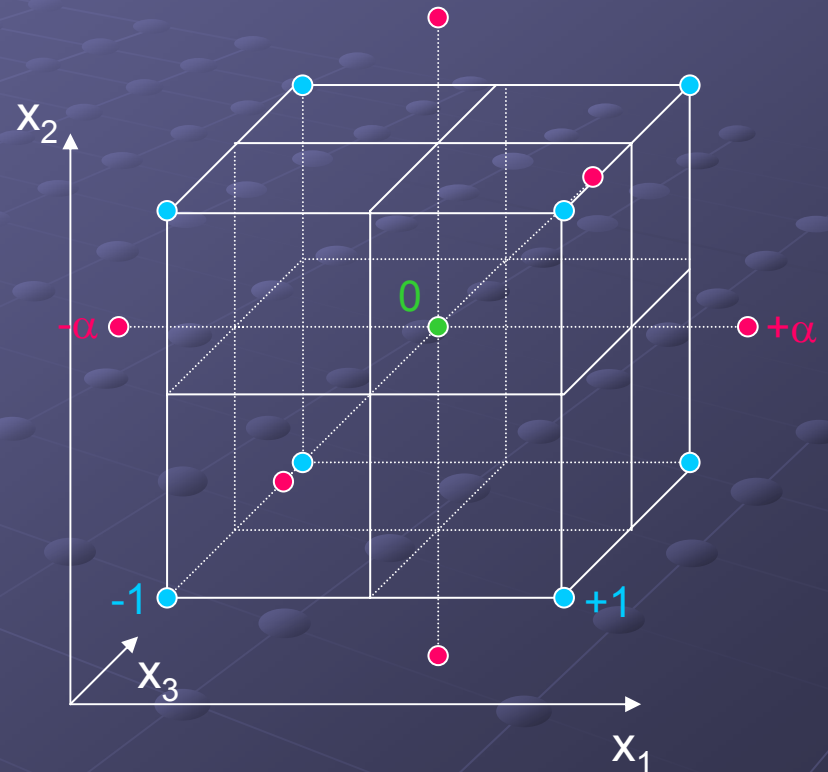
⇒ 37 experiments

⇒ ANOVA analysis

● model

● model terms

⇒ Validation



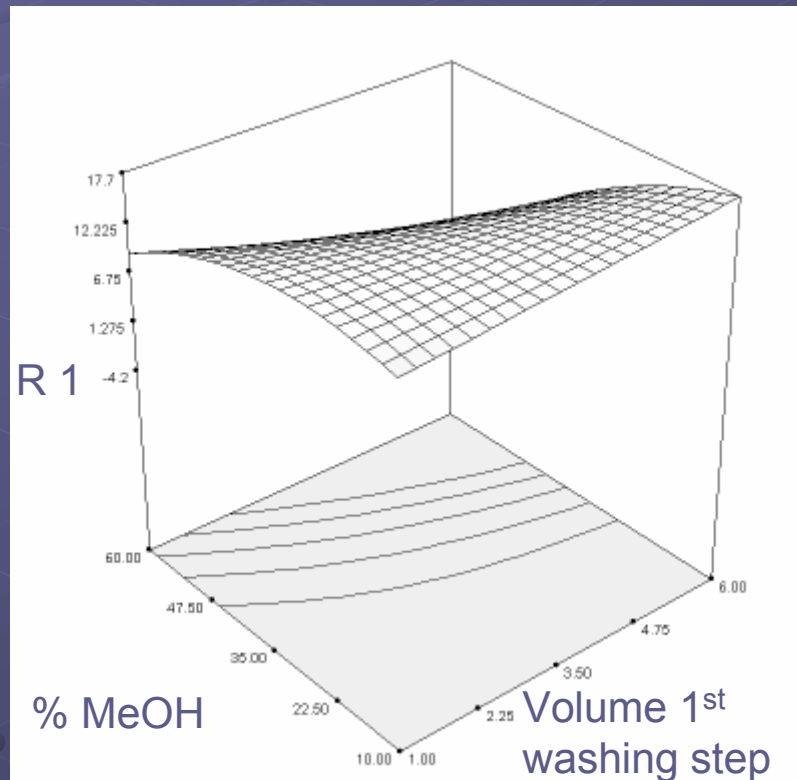
● Axial or star points

● Cube points

● Center point

Results – optimization design

● Graphical optimization



X = A: volume 1st
washing step

Y = B: % MeOH

C: Molarity (60mM)

Results – optimization design

● Numerical optimization

SOLUTIONS

	Volume 1st wash	% MeOH	Molarity buffer	R 1	R 2	R 3	Desirability
1	4.83	16.07	60	16.2	17	26.86	1
2	3.99	10.95	60	16.2	17	26.19	1
3	4.67	11.72	60	16.6	17	26.6	1

Results - SPE

Conditioning



3mL MeOH
3mL buffer (pH 9)

1mL/min

Sample appl.



2mL blood

0.5mL/min

Washing

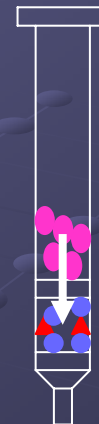


5mL buffer+15%MeOH
1mL water

5mL/min

~~Driving~~

Elution



1mL MeOH + 1%AA

0.5mL/min

- MeOH
- Ammonium acetate (AmmAc) buffer
- ▲ Compound of interest
- Impurity
- Matrix
- Ammonium acetate buffer + %MeOH
- Water
- Elution solvent

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Conclusions

● **DoE** : useful **tool** for SPE optimization

