A Structured Approach in the Optimization of a Headspace and PTV-based injection for the Analysis of Volatile Poisons

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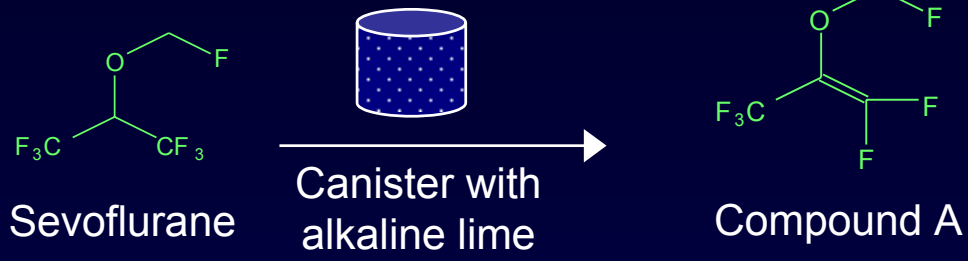
Outline

1. Introduction
2. Aim
3. Development of the injection procedure
4. Optimization of the injection procedure
5. Conclusion

Introduction

- Volatile poisons ?

Compound A



- ✓ Degradation product of sevoflurane
- ✓ Upon contact with alkaline CO_2-absorbents in low-flow and closed-circuit anaesthesia
- ✓ Known nephrotoxic in rats: LD_{50} 120 -1100 ppm
  Threshold 50 ppm
- ✓ Toxic in humans ?

Aim

1. From an anaesthesiological viewpoint:

   To assess “safe” sevoflurane administration

2. From an analytical viewpoint:

   Sound quantitation of trace levels of compound A in vapor phase samples

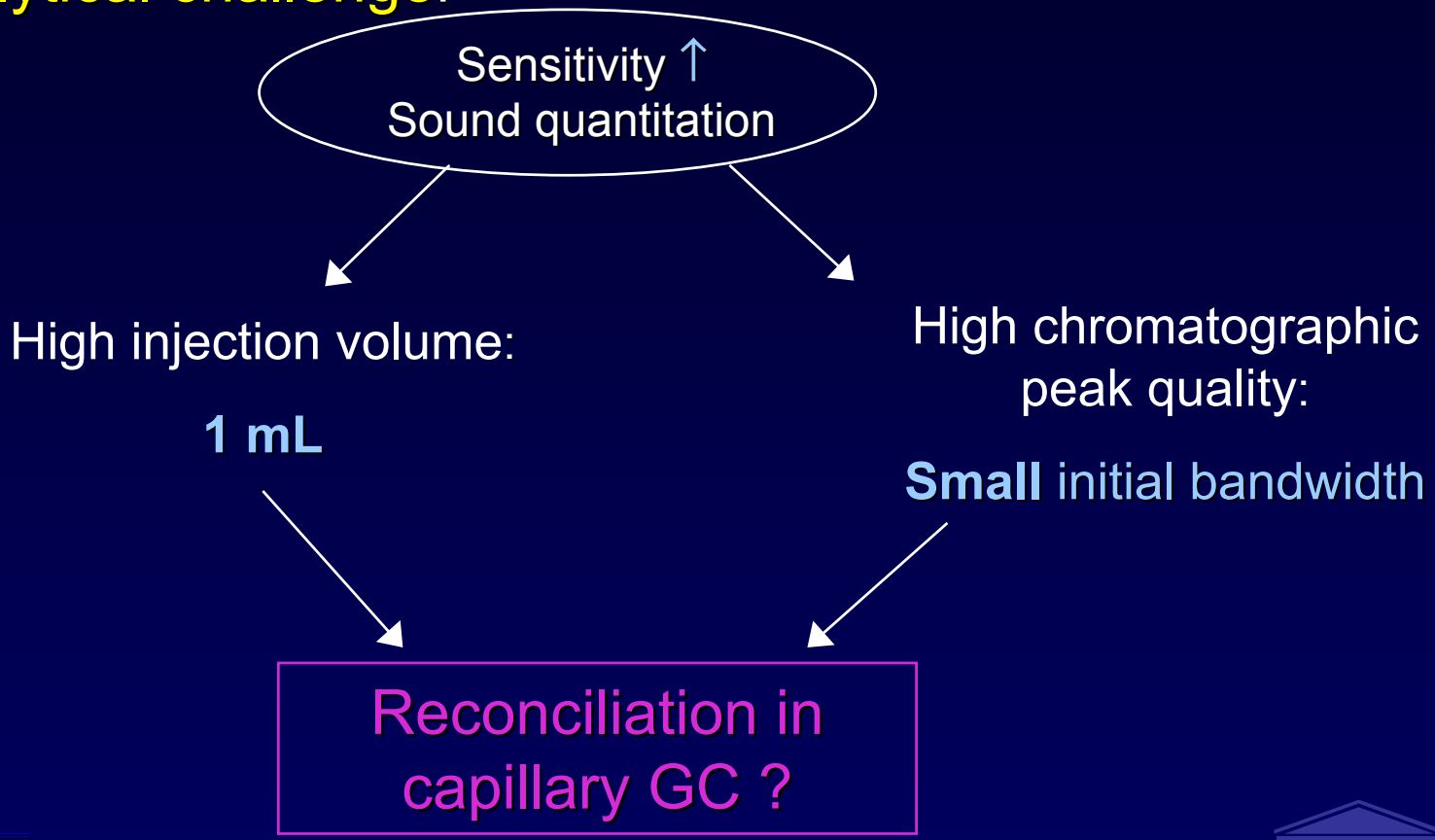
3. From a toxicological viewpoint:

   Identify and quantify (ab)use of volatiles

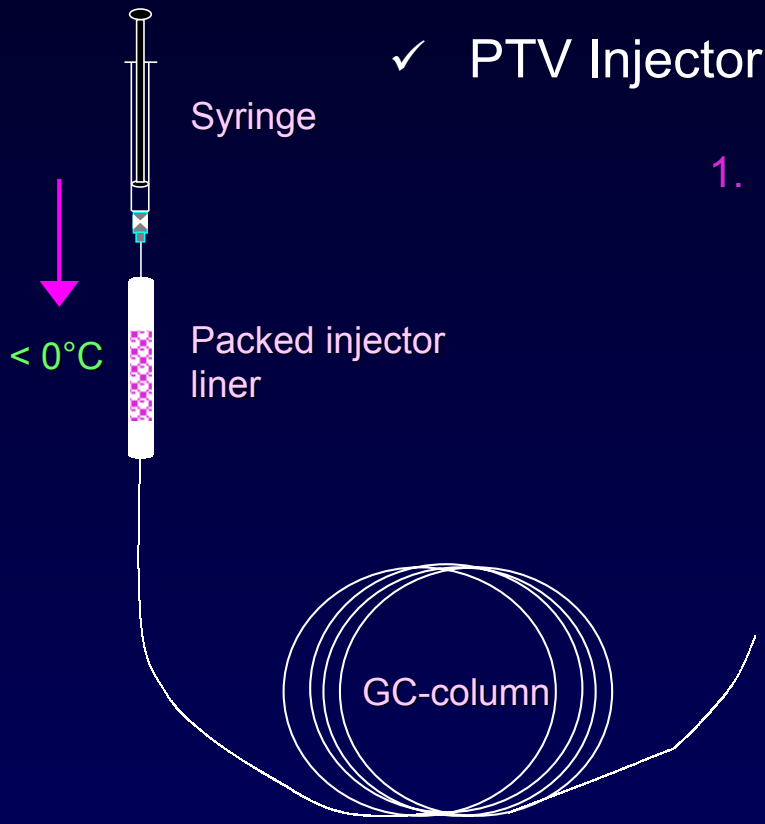
   → Headspace based injection
   → Capillary GC-MS

Development of the injection procedure

- Analytical challenge:

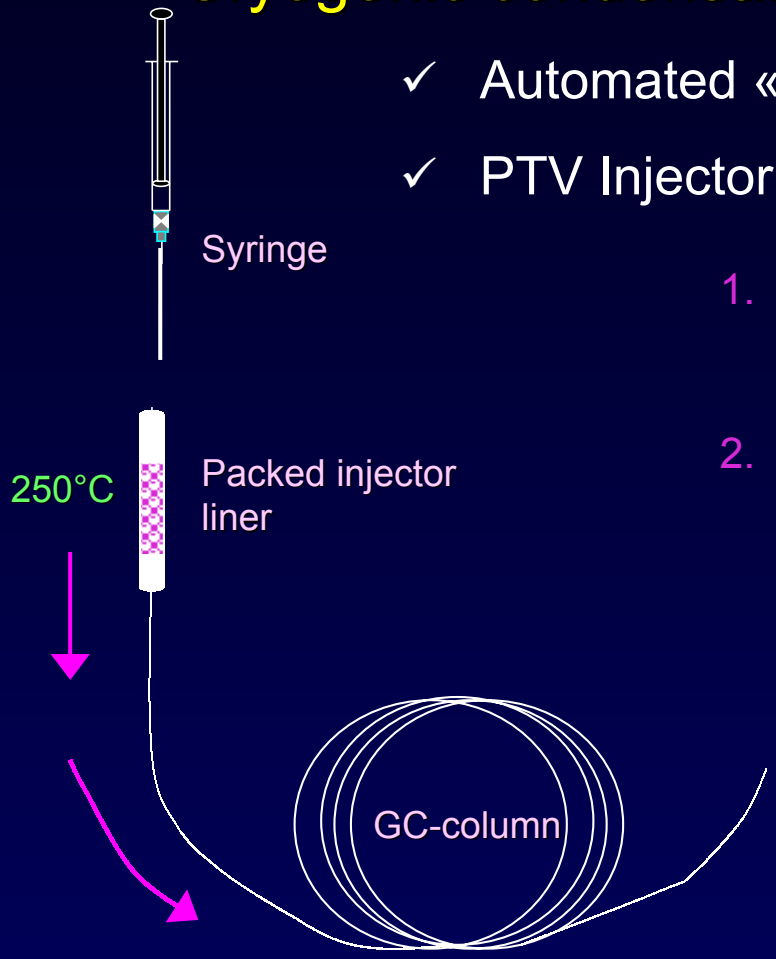


- Cryogenic condensation + flash desorption:
  - ✓ Automated « headspace » autosampler
  - ✓ PTV Injector



1. Initially: < 0°C (liquid N_2)
   → Adsorption to Tenax TA + preconcentration

- Cryogenic condensation + flash desorption:
  - ✓ Automated « headspace » autosampler
  - ✓ PTV Injector



1. Initially: – 80°C (liquid N_2)
   → Adsorption to Tenax TA + preconcentration
2. Subsequently: T° ↑
   → Desorption + transfer to the analytical column

Optimization of the injection procedure

- Fully automated procedure:
  - ✓ 20 instrumental parameters
  - ✓ Control:
    1. Headspace autosampler
    2. PTV injector
    3. GC
- Optimization ?
  - ✓ Classical approach: 2^{16} experiments
  - ✓ Chemometrical approach: Experimental design

- Experimental design: ≠ steps

1. Defining instrumental parameters and experimental domain
2. Defining responses
3. « Screening design »
   - Evaluation of all instrumental parameters at 2 levels (high, low)
   - Identification of significant instrumental parameters
4. « Optimization design »
   - Final optimization: only significant parameters
     more levels

Result: optimal injection parameters
optimal injection efficiency

→ optimal quantitation quality (peak shape and height)

- Experimental design: ≠ steps

1. Defining parameters and experimental domain

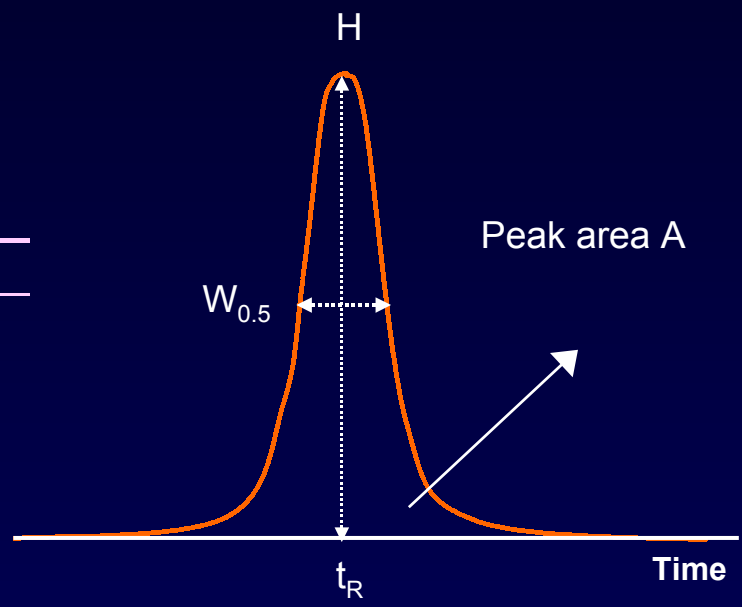
	Parameter	Low	High	Unit
1	Prewarm temperature	30	60	°C
2	Prewarm time	4	20	min
3	Fill speed	1000	10000	µl/min
4	Equilibrum pressurization time	0.2	5	min
5	Injection volume	100	1000	µl
6	Injection speed	1000	10000	µl/min
7	Adsorbent type	Tenax TA	Chromosorb W	
8	Adsorbent quantity	5	30	mg
9	Adsorbent particle size	20/35	80/100	mesh
10	Trap temperature	-110	-30	°C
11	Trap time	0	2	min
12	Trap split	5	20	(ratio)
13	Vent pressure	60	100	kPa
14	Desorption time	0.1	2	min
15	Desorption split	0.1	5	(ratio)
16	Flush split	20	60	(ratio)

- Experimental design: ≠ steps

1. Defining parameters and experimental domain
2. Defining responses

« Ideal peak » for compound A and IS

Response	Symbol
Area Compound A	A_{CA}
Area Internal Standard	A_{IS}
Peak Height Compound A	H_{CA}
Peak Height Internal Standard	H_{IS}
Peak Width at half height Compound A	$W_{0.5\ CA}$
Peak Width at half height Internal Standard	$W_{0.5\ IS}$
Peak Shape Compound A	PS_{CA}
Peak Shape Internal Standard	PS_{IS}
Baseline Asymmetry Factor Compound A	$BAsF_{CA}$
Baseline Asymmetry Factor Internal Standard	$BAsF_{IS}$



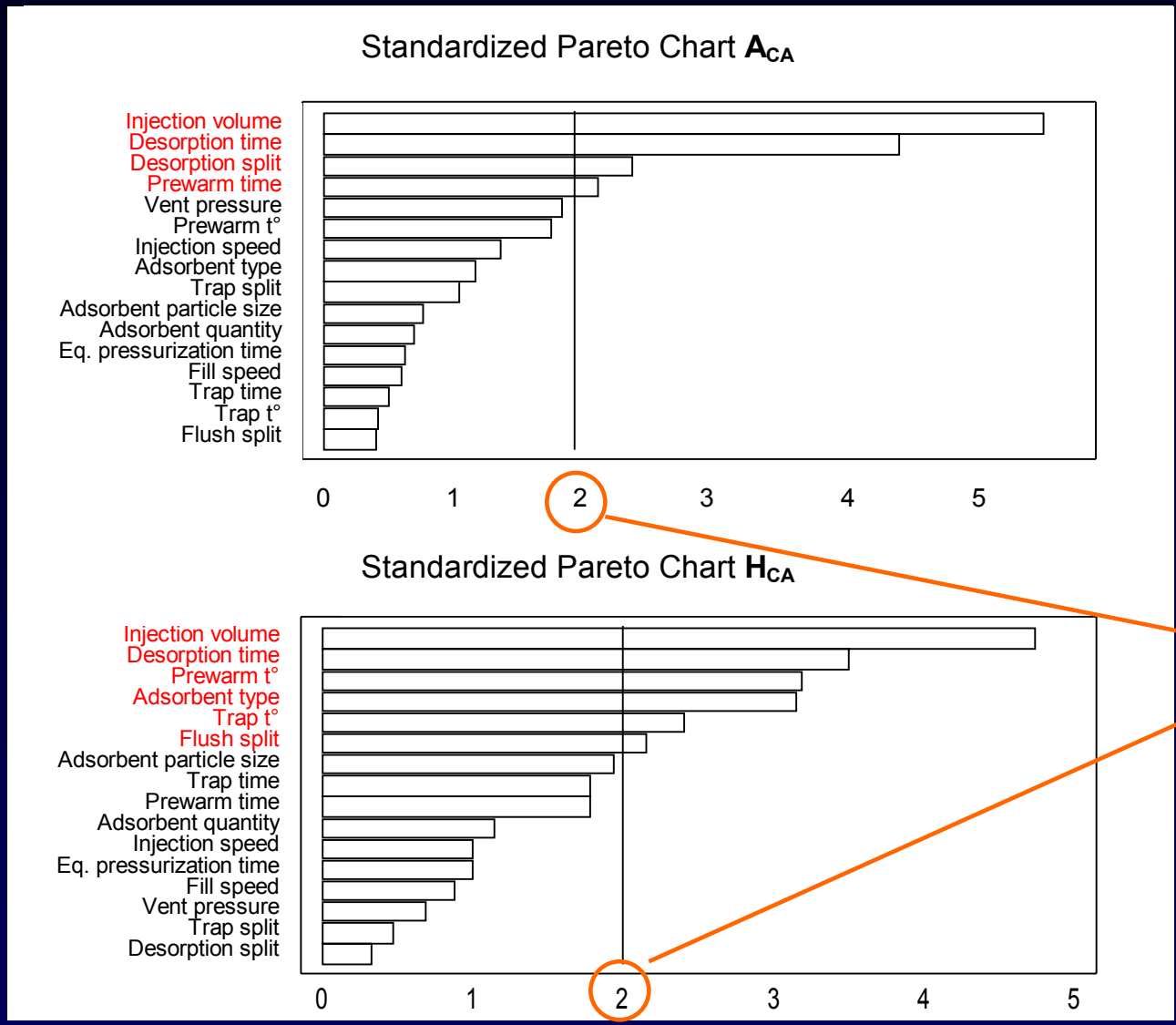
- Experimental design: ≠ steps

1. Defining parameters and experimental domain
2. Defining responses
3. « Screening design »

    - Plackett-Burman design with fold-over
    - 16 instrumental parameters: 2 levels → (-) en (+), 40 ≠ combinations
    - 80 experiments
    - For each response:

        - Numerical: ANOVA → $p < 0.05$
        - Graphical: Pareto chart

⇒ Identification of significant instrumental parameters



Length of bar ~ effect of parameter on the response

Threshold for statistical significance

		A_{CA}	A_{IS}	H_{CA}	H_{IS}	$W_{0.5\ CA}$	$W_{0.5\ IS}$	PS_{CA}	PS_{IS}	$BAsF_{CA}$	$BAsF_{IS}$
	Desired outcome	↑	↑	↑	↑	↓	↓	↓	↓	↓	↓
1	Prewarm temperature			-							
2	Prewarm time	+									-
3	Fill speed										
4	Equilibrum press. time									-	-
5	Injection volume	+	+	+	+	-	-				
6	Injection speed										
7	Adsorbent type			-		-	-	-	-	-	
8	Adsorbent quantity				+						
9	Adsorbent particle size										
10	Trap temperature			+	+						-
11	Trap time										
12	Trap split				+						
13	Vent pressure							+	+		
14	Desorption time	+	+	+	+	-	-	-	-	-	-
15	Desorption split	-	-			+	+	+	+		+
16	Flush split			-	-						

		A_{CA}	A_{IS}	H_{CA}	H_{IS}	$W_{0.5\ CA}$	$W_{0.5\ IS}$	PS_{CA}	PS_{IS}	$BAsF_{CA}$	$BAsF_{IS}$
	Desired outcome	↑	↑	↑	↑	↓	↓	↓	↓	↓	↓
1	Prewarm temperature			-							
2	Prewarm time	+									-
3	Fill speed										
4	Equilibrum press. time									-	-
5	Injection volume	+	+	+	+	-	-				
6	Injection speed										
7	Adsorbent type			-		-	-	-	-	-	
8	Adsorbent quantity				+						
9	Adsorbent particle size										
10	Trap temperature			+	+						-
11	Trap time										
12	Trap split				+						
13	Vent pressure							+	+		
14	Desorption time	+	+	+	+	-	-	-	-	-	-
15	Desorption split	-	-			+	+	+	+		+
16	Flush split			-	-						

⇒ 4 parameters not significant

12 parameters significant

		A_{CA}	A_{IS}	H_{CA}	H_{IS}	$W_{0.5\ CA}$	$W_{0.5\ IS}$	PS_{CA}	PS_{IS}	$BAsF_{CA}$	$BAsF_{IS}$
	Desired outcome	↑	↑	↑	↑	↓	↓	↓	↓	↓	↓
1	Prewarm temperature			-							
2	Prewarm time	+									-
3	Fill speed										
4	Equilibrum press. time									-	-
5	Injection volume	+	+	+	+	-	-				
6	Injection speed										
7	Adsorbent type			-		-	-	-	-	-	
8	Adsorbent quantity				+						
9	Adsorbent particle size										
10	Trap temperature			+	+						-
11	Trap time										
12	Trap split				+						
13	Vent pressure							+	+		
14	Desorption time	+	+	+	+	-	-	-	-	-	-
15	Desorption split	-	-			+	+	+	+		+
16	Flush split			-	-						

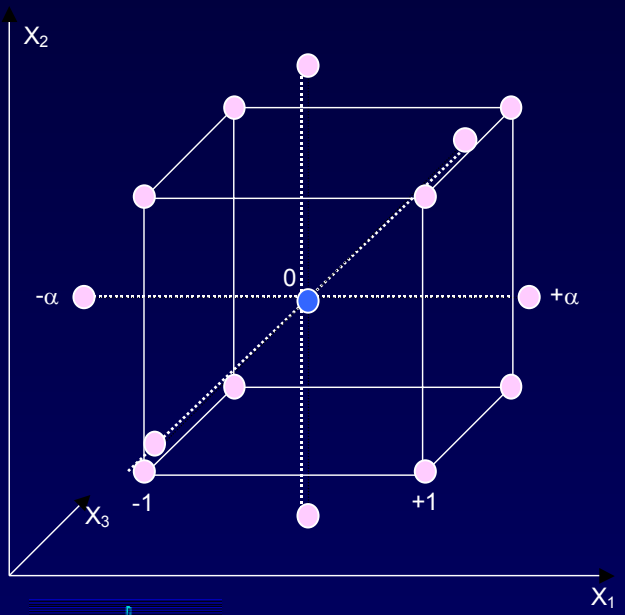
⇒ 8 parameters: « best result » value

		A_{CA}	A_{IS}	H_{CA}	H_{IS}	$W_{0.5\ CA}$	$W_{0.5\ IS}$	PS_{CA}	PS_{IS}	$BAsF_{CA}$	$BAsF_{IS}$
	Desired outcome	↑	↑	↑	↑	↓	↓	↓	↓	↓	↓
1	Prewarm temperature			-							
2	Prewarm time	+									-
3	Fill speed										
4	Equilibrum press. time									-	-
5	Injection volume	+	+	+	+	-	-				
6	Injection speed										
7	Adsorbent type			-		-	-	-	-	-	
8	Adsorbent quantity				+						
9	Adsorbent particle size										
10	Trap temperature			+	+						-
11	Trap time										
12	Trap split				+						
13	Vent pressure							+	+		
14	Desorption time	+	+	+	+	-	-	-	-	-	-
15	Desorption split	-	-			+	+	+	+		+
16	Flush split			-	-						

⇒ 4 instrumental parameters: further optimization

- Experimental design: ≠ steps

1. Defining parameters and experimental domain
2. Defining responses
3. « Screening design »
4. « Optimization design »

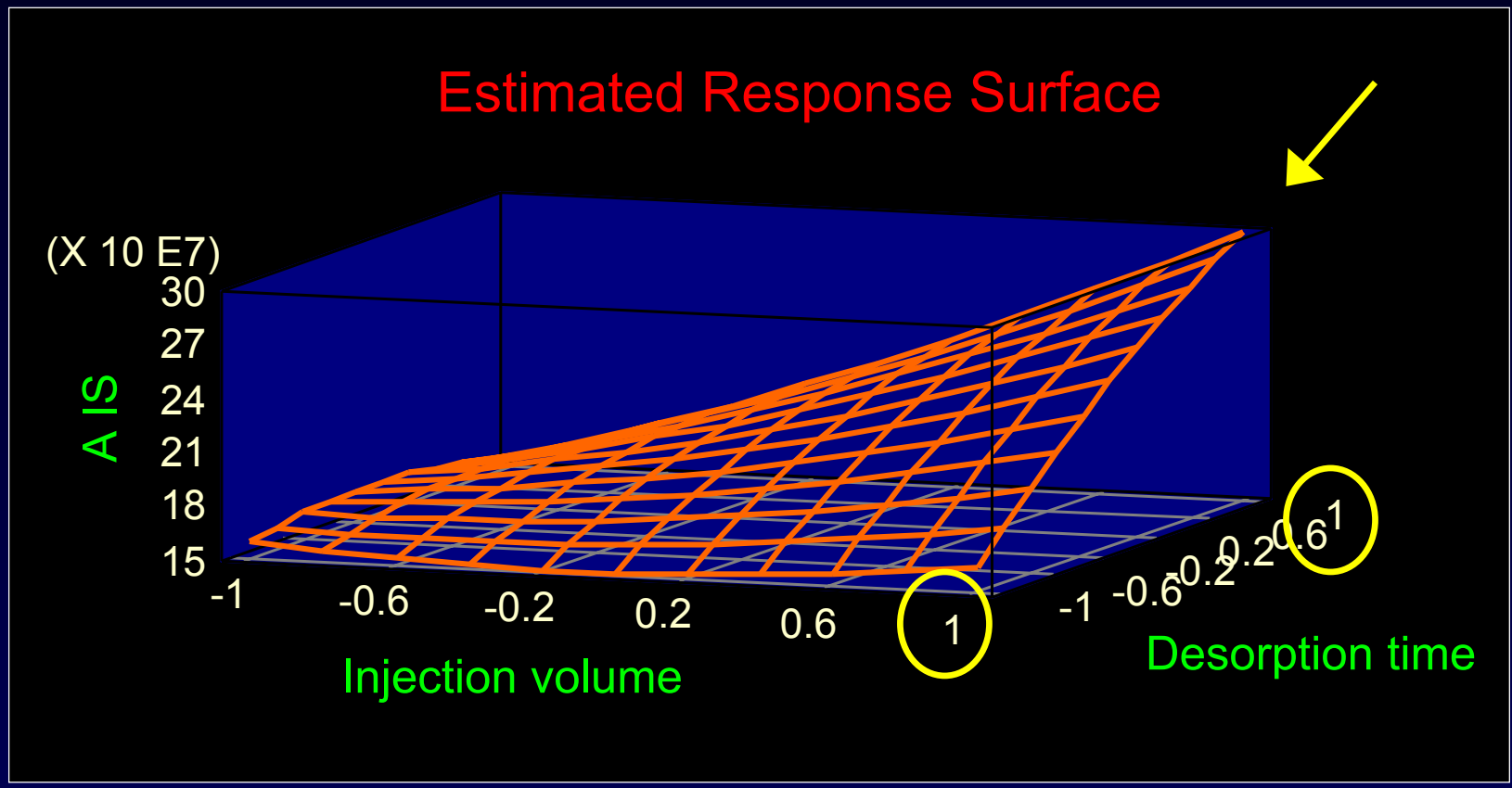


- Central composite design
- 4 instrumental parameters: 5 levels
- 54 experiments
- For each response:

  Significant parameters and interactions

  - Numerical: ANOVA → $p < 0.05$
  - Graphical: pareto + response surface

⇒ Optimal value of 4 sign. parameters


Estimated Response Surface
(X 10 E7)
30
27
24
21
18
15
A IS
-1
-0.6
-0.2
0.2
0.6
1
Injection volume
-1
-0.6
-0.2
0.2
0.6
1
Desorption time

Optimal values for all responses:

⇒ Single response function (SRF)
= weighed sum of the 10 response functions

	Injection parameter	Value		Injection parameter	Value
1	Prewarm temperature	30 °C	11	Trap time	0.3 min
2	Prewarm time	10 min	12	Trap split	5:1 (ratio)
3	Fill speed	5000 µl/min	13	Vent pressure	100 kPa
4	Equilibrum pressurization time	0.2 min	14	Desorption time	2 min
5	Injection volume	1000 µl	15	Desorption split	0.7:1 (ratio)
6	Injection speed	5000 µl/min	16	Flush split	20:1 (ratio)
7	Adsorbent type	Tenax TA	17	Depth of injection	20 mm
8	Adsorbent quantity	Full liner	18	Fill volume	1000 µl
9	Adsorbent particle size	20/35 mesh	19	Syringe temperature	39 °C
10	Trap temperature	-30°C	20	Desorption temperature	250 °C

Conclusion

- Chemometrical approach:
  - ✓ Optimal injection efficiency: sound analytical quantitation
  - ✓ Identification and optimization of relevant factors
  - ✓ ↓ lab time, ↓ lab cost, ↑ productivity
  - ✓ Other volatile poisons

Fast development of dedicated quantitative methods

⇓

Useful tool for both **forensic** and **clinical toxicology**