Ghent University

Evaluation of CO_2-absorbents for Use in Sevoflurane Based Closed-circuit Anesthesia through GC-MS Determination of Compound A

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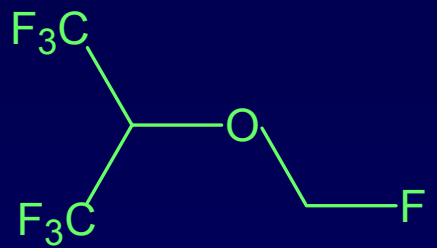
Outline

- Introduction
- Aim
- Development of the analytical procedure
- Method validation
- Application in clinical trial
- Conclusions

Introduction

- Sevoflurane: widespread use as inhalational anesthetic

  - Volatile, non-flammable, non-explosive
  - Pleasant smell
  - Low blood and tissue solubility



→ smooth induction of anesthesia
→ rapid recovery from anesthesia
→ more precise control of anesthesia

  - Ideal anesthetic ?

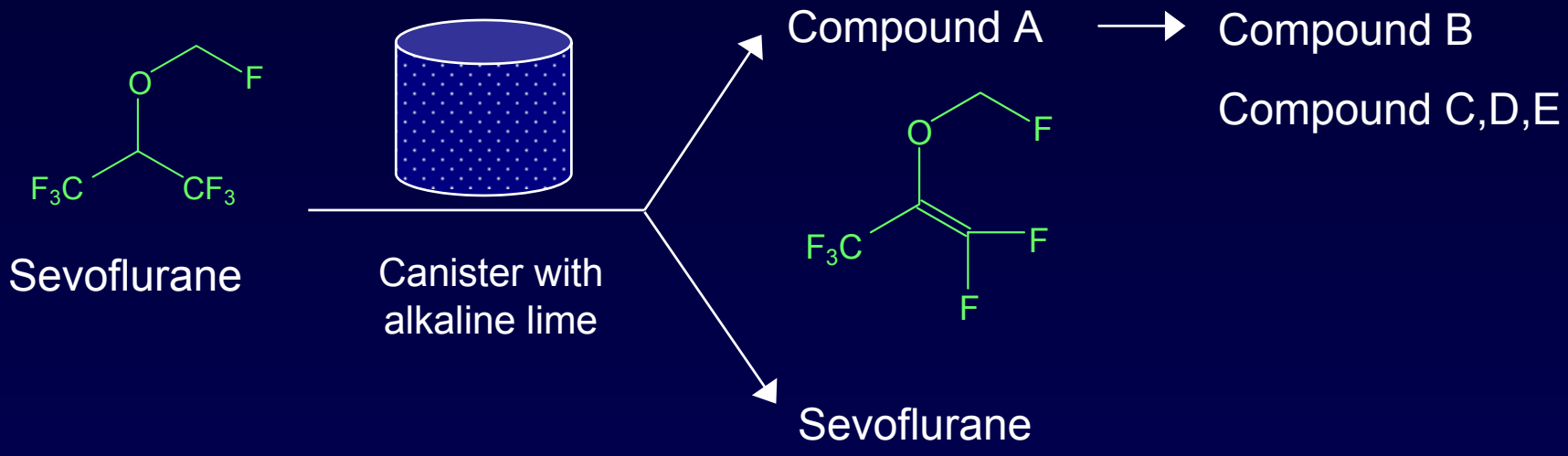
- However:
  - Metabolization in-vivo (2-5%)
    - → HFIP
    - → Inorganic F^-: nephrotoxic
  - Interaction and degradation by CO_2-absorbents

    e.g. sodalime, Baralyme®

    Used in low-flow and closed-circuit anesthesia to sequester CO_2 exhaled by the patient

    ⇒ Formation of potentially toxic by-products

- Compound A:



  - Known nephrotoxic in rats
    - LC_{50}: 120-1100 ppm
    - Threshold for nephrotoxicity: 50-114 ppm

- Parameters affecting compound A production
  - Sevoflurane concentration
  - Chemical composition of absorbent
  - Fresh gas flow rate
  - Temperature $\rightarrow$ CO_2 production
  - Duration of anesthesia
  - Water content
- FDA restriction: fresh gas flows < 2 L/min
- Reports in the literature:
  - 20-30 ppm
  - compound A controversy

$\Rightarrow$ Is sevoflurane administration safe ?

Aim

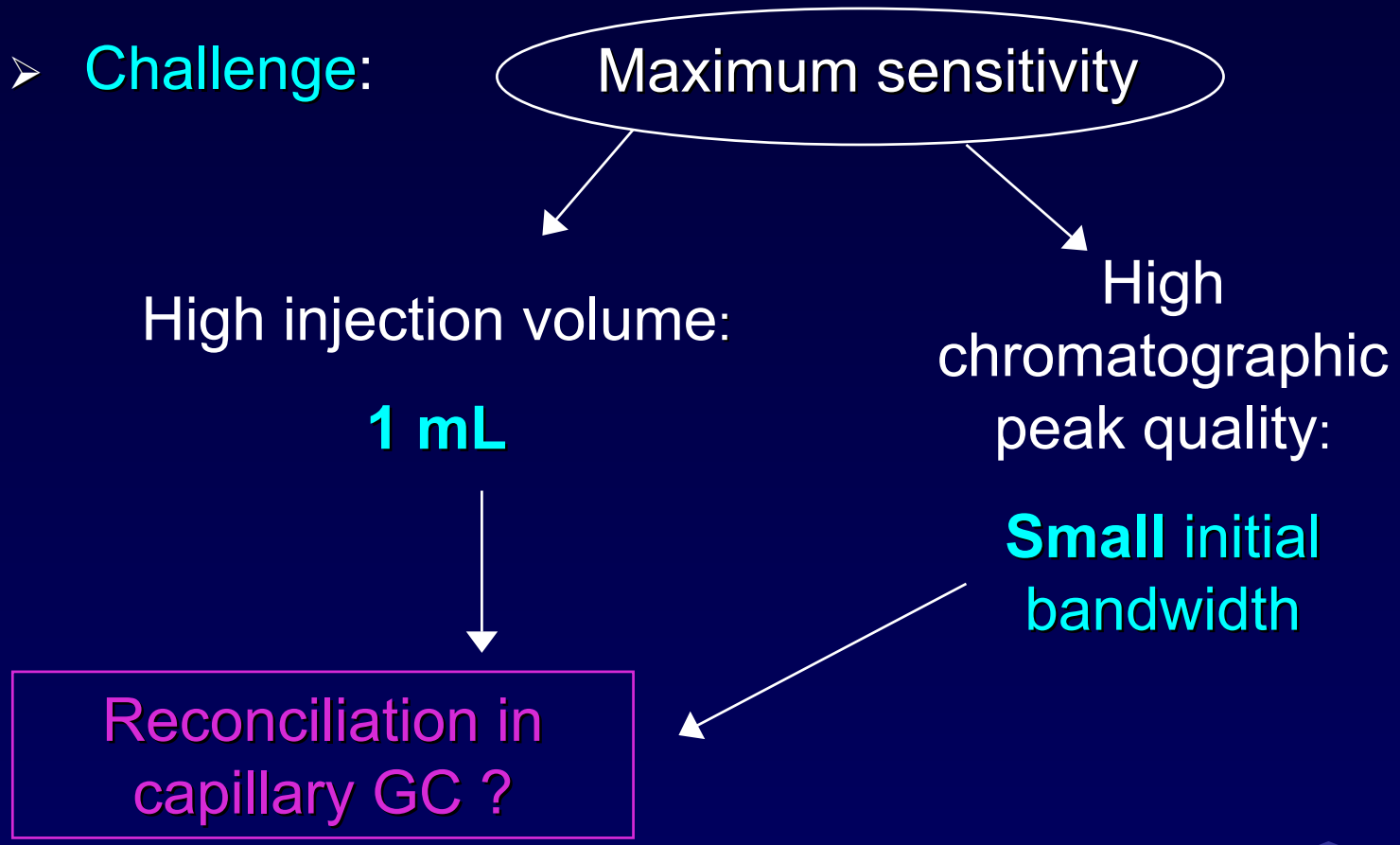
1. To assess “safe” sevoflurane administration in present day low-flow and closed-circuit anesthesia

2. Development of an analytical assay for quantitative determination of trace levels of compound A in vapour phase samples

Development

- Requirements for the assay
  - Very limited sample volume (1-2 mL)
  - Automation
  - Complete resolution between compound A and sevoflurane
  - Sensitivity
  - Easy-to-perform calibration procedure

- Injection procedure
  - Challenge:



- Injection procedure
  - Based upon automated headspace sampling
  - PTV (Gerstel CIS-4)
  - Cryogenic condensation in the injector liner followed by flash desorption (250 °C)
    - Liquid N_2, -80 °C
    - Tenax TA

⇒ Simple, robust and automated injection approach

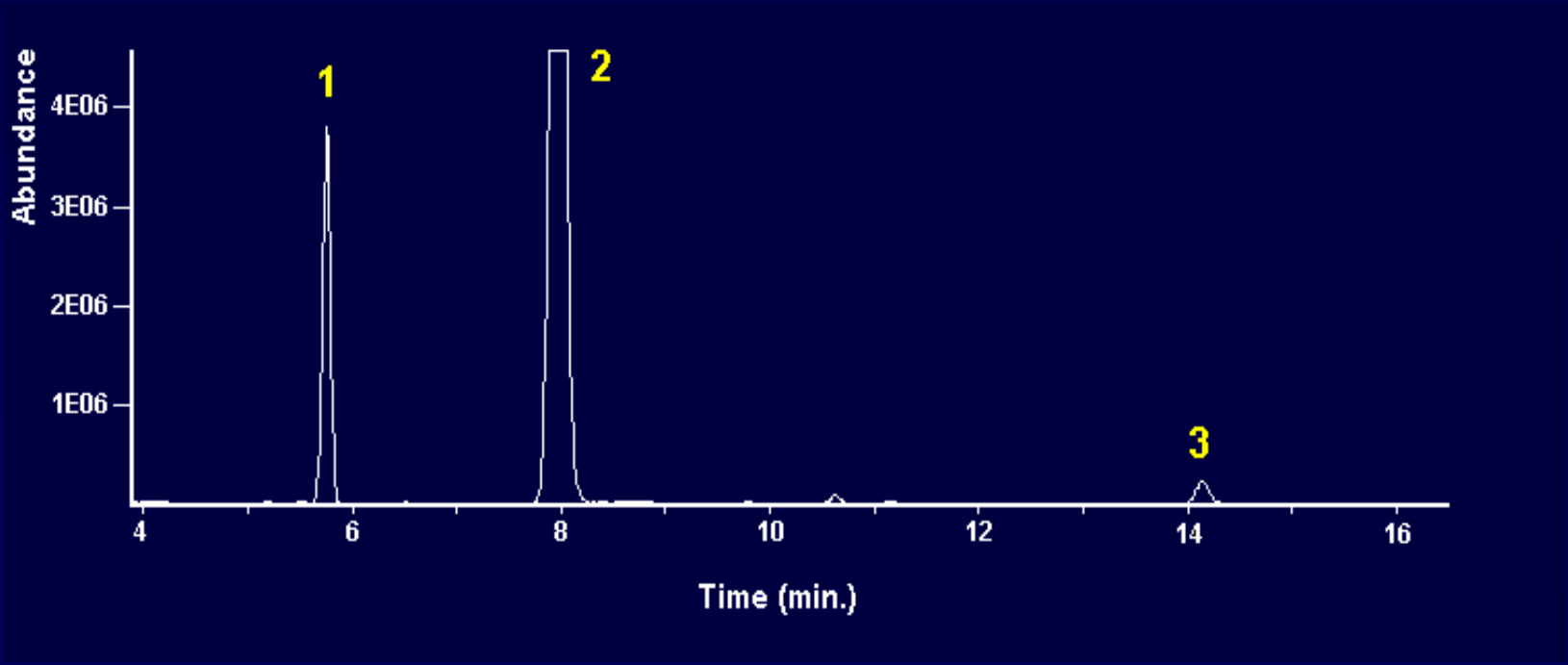
- Chromatographic separation
  - Adequate retention and separation

    ⇒ Custom made CP-Select 624

    Isothermal: 38 °C

- Mass spectrometric detection
  - Full-scan mode: 40 – 300 amu
  - Identification based on T_R + Mass spectrum
  - Quantification based on reconstructed mass fragmentograms (compound A, *m/z* 128)

- Internal standard
  - To enhance the precision
  - Choice:
    - Physicochemical properties
    - Halogenated → xenobiotic
    - Good retention profile
    - Typical mass spectrum
  - 1,1,1-trifluoro-2-iodoethane
  - Addition: 0.5 µL injected into every vial



Peak identification:

1. Compound A
2. Sevoflurane
3. Internal standard

- Calibration procedure
  - Easy-to-perform: static method
  - No dedicated gas instrumentation
  - 7 calibrators + zero-calibrator
  - Range: 0 – 75 ppm or µL/L compound A
  - Departing from liquid solutions of compound A and sevoflurane in EtAc

Liquid
stock solutions

Sevoflurane in EtAc

Compound A in EtAc

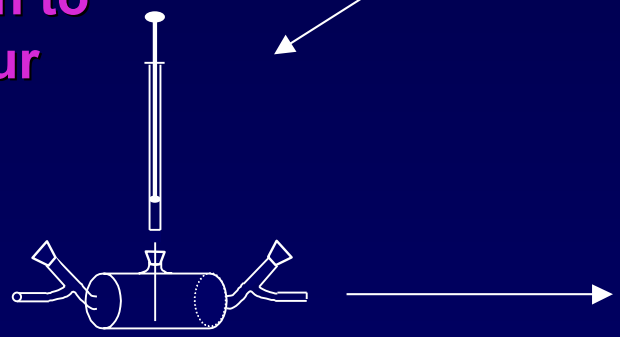
Liquid
working dilutions

Liquid
standard solutions

Zero calibrator

Varying concentrations of
Compound A

Transition to the vapour phase



5 mL-headspace vial

Validation

- Linearity
  - Range: 0 – 75 ppm or µL/L compound A
  - Peak area ratio ~ compound A concentration
  - Weighted linear regression (1/y)

n^1 = 10	R square	Slope	Intercept
mean	0.996	0.048	0.018
SD	0.003	0.003	0.003
CV² (%)	0.3	6.9	16.2

[1] ***n,*** **number of determinations**
[2] **CV, coefficient of variation**

- Other parameters

Validation parameter	Concentration added (ppm or µL/L air)		
	0.5	10	75
Precision (CV[1], %)			
within-day (n = 6)[2]	9.6	9.9	5.7
total (n = 10)[2]	10.0	7.1	4.1
Accuracy (recovery, %)			
mean	101.16	101.27	99.00
SD	10.09	6.57	4.10
CV[1] (%)	9.97	6.49	4.14
LOD (ppm)	0.1		
LOQ (ppm)	0.3		

[1] **CV, coefficient of variation**
[2] ***n*, number of determinations**

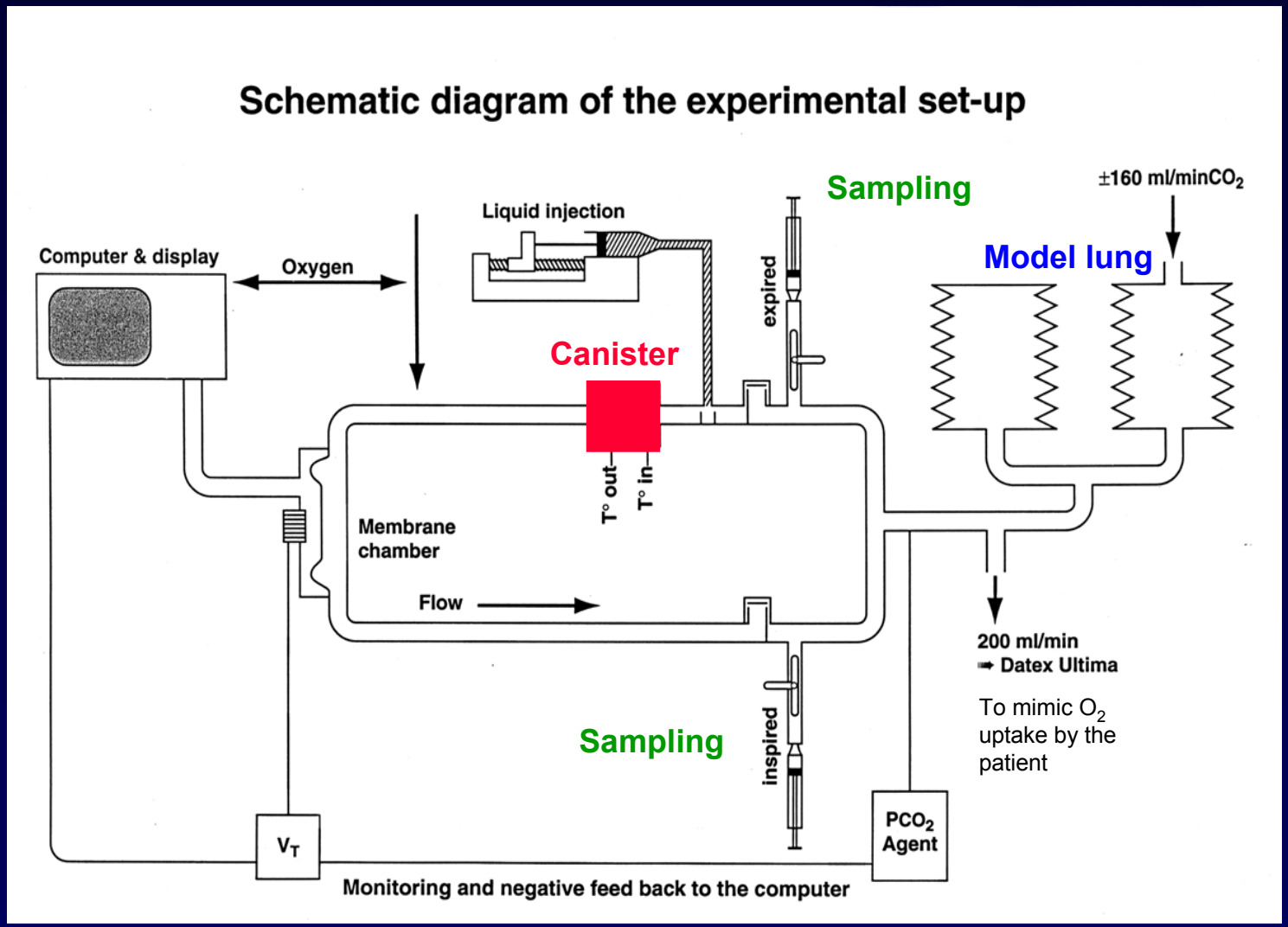
Application

- Aim of the investigation
  - In-vitro closed-circuit anesthesia
  - Use of different CO_2-absorbents

    Classical soda lime ↔ alternate products

    Sofnolime
    Amsorb
    LiOH
  - Evaluation of **compound A formation**

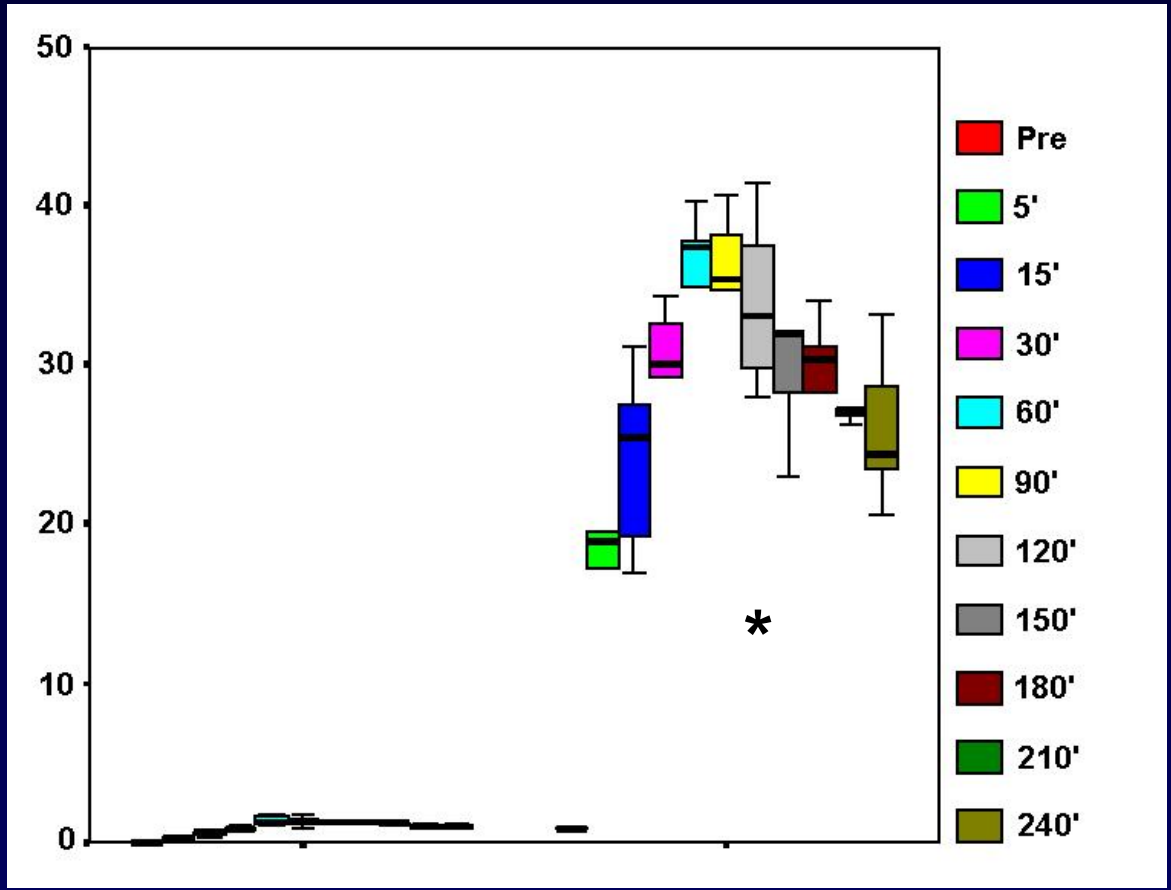
- In-vitro set-up
  - Closed-circuit Physioflex ® with model lung
  - 2 valves to mimic classical circuit set-up
  - Incorporated fan: off
  - $Sevoflurane_{insp}$: 2.2 %
  - Duration: 240 min
  - 2 mL gas samples for compound A determination in duplicate, at 0, 15, 30, 60, 90, 120, 150, 180, 210 and 240 min


Schematic diagram of the experimental set-up
Sampling
±160 ml/minCO2
Liquid injection
Computer & display
Oxygen
Model lung
expired
Canister
T° out
T° in
Membrane chamber
Flow
200 ml/min
➡ Datex Ultima
To mimic O2 uptake by the patient
Sampling
inspired
VT
PCO2 Agent
Monitoring and negative feed back to the computer

- Results

Compound A inspiratory concentrations

(ppm)



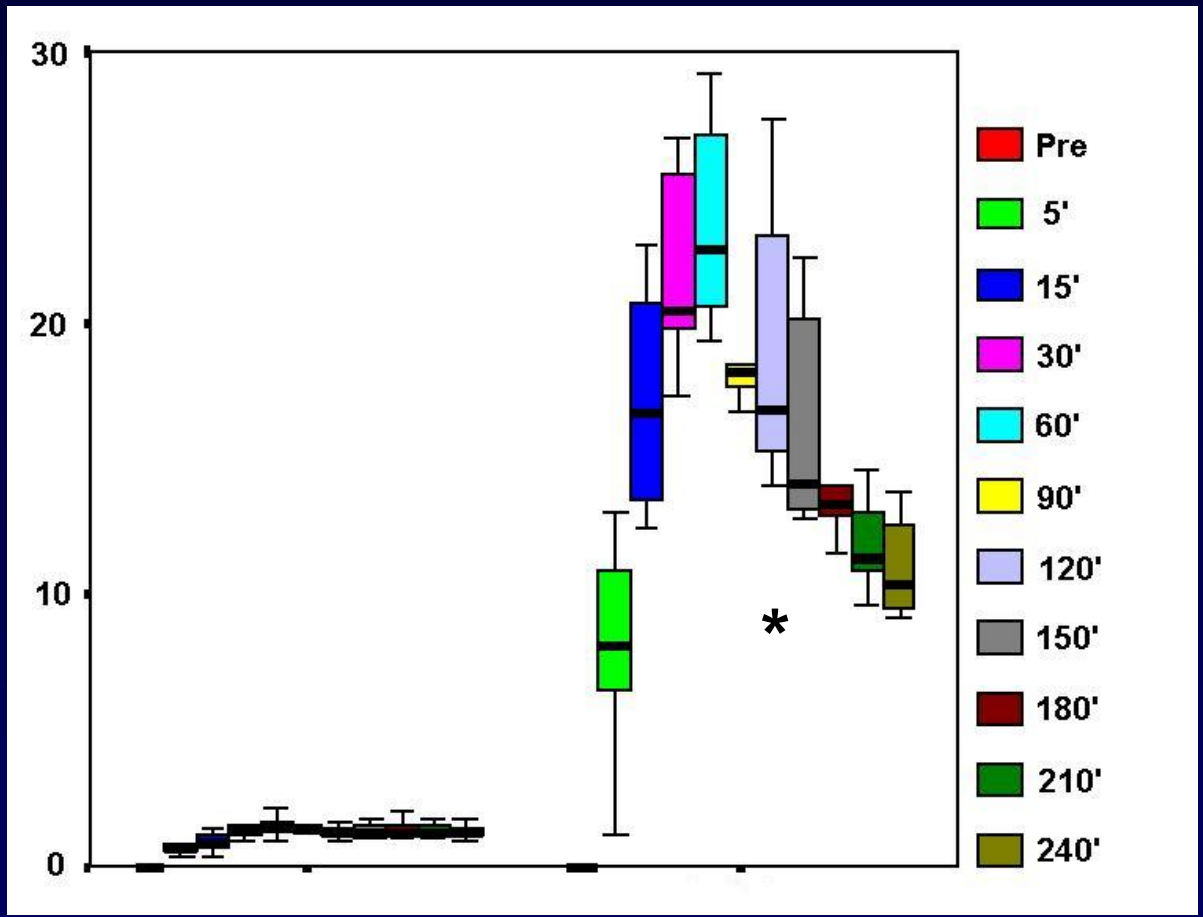
Amsorb® (*n* = 7) Sofnolime (*n* = 5)

* $p < .05$ at all assessments between groups

- Results

Compound A inspiratory concentrations

(ppm)



LiOH (n = 7) Sodalime (n = 6)

* $p < .05$ at all assessments between groups

- Results

Sodalime: NaOH + KOH

Sofnolime: NaOH, KOH-free

→ $[\text{Compound A}]_{insp}$

10 – 35 ppm

Amsorb®: $Ca(OH)_2 + CaCl_2$

LiOH

→ Practically NO compound A formation

➢ Best choice: Amsorb®

Conclusions

- Novel, highly sensitive and fully validated assay for vapour phase determination of compound A
- Closed-circuit sevoflurane anesthesia = safe

provided the use of modern anesthesia equipment and products

Acknowledgments

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