Microphysiological models for the assessment of pulmonary concentration of inhaled aerosols

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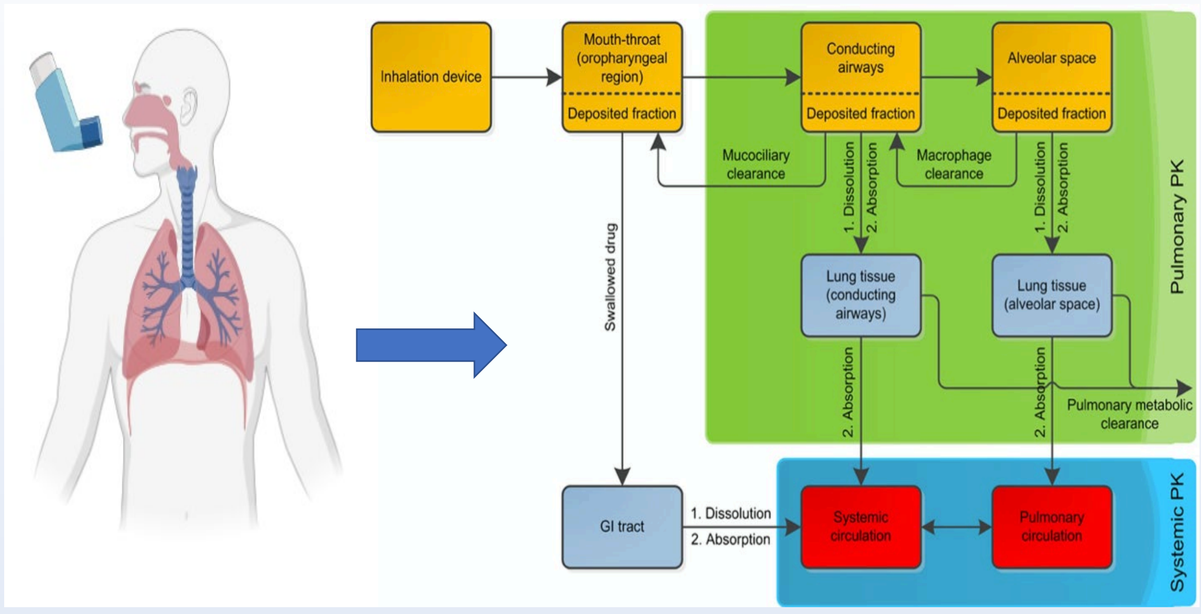
INTRODUCTION

- Lungs are a sensitive target, due to pollution and airborne infection, however, inhalation represents a valid route of administration for therapeutic aerosols.
- Topical bioavailability represents active local concentration profile of the drug, that is of relevance to clinical effect. However, for drug delivery to the lungs, this is uncertain, as key factors contributing to the pharmacokinetics (PK) have not been assessed.
- Dissolution rate and permeation rate have not been validated, as well as relevant pulmonary concentration.
- The aim of this is to build an *in vitro* model, to address such questions.



RESEARCH HYPOTHESIS AND OBJECTIVES

Given the gaps, the investigative tool proposed is lungs-on-chip. Here, the environment of the lungs is reproduced on a microscale. The idea is to expose this device to aerosolized compounds and after a varying period of time, they will have crossed the barrier and be present in the internal compartment. The assessment of permeation rate is the key for elucidating absorption and, consequently, pulmonary exposure to aerosol treatment.

- **Objective 1** Validation of a methodology for compounds bio-analysis via Liquid Chromatography-Mass Spectroscopy.
- **Objective 2** Validation of Solid Phase Micro Extraction methodology to assess airway lining fluid concentration.
- **Objective 3** Development of microphysiological model, reflecting lungs conditions.
- **Objective 4** Cell cultures characterisation and evaluation of relative inflammation.

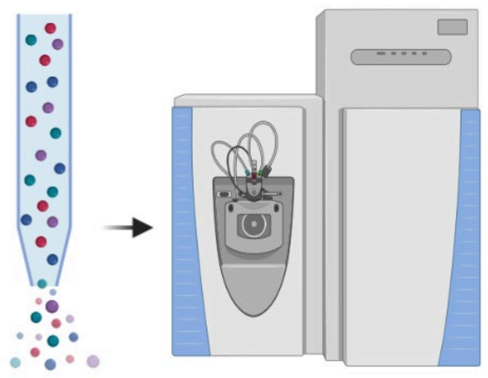
PROGRAMME AND METHODOLOGY

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

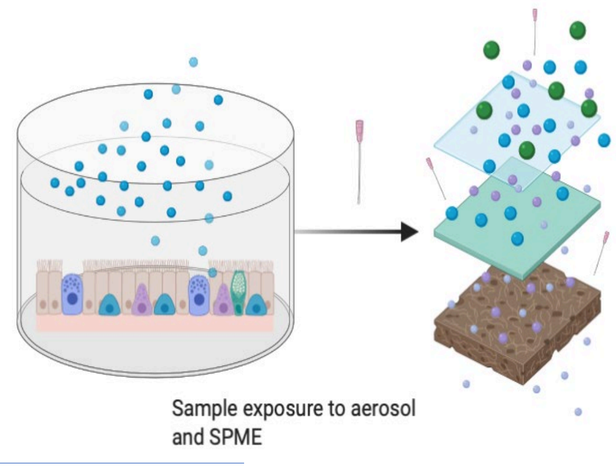
Principle of operation:

1. Separation of components upon retention time.
2. Ionisation of eluted mobile phase.
3. Fragment analysis and detection.

Well-established technique, supplying info in terms of numbers of components, their molecular mass and relative abundance.



Liquid chromatography separation tandem mass spectroscopy analysis



Sample exposure to aerosol and SPME

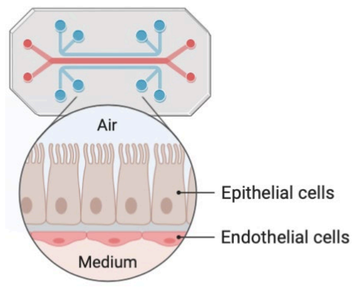
SOLID PHASE MICROEXTRACTION (SPME)

Principle of operation:

1. Distribution of aerosolised molecules within layers.
2. A coated fibre carries out the extraction and samples are collected from different layers .
3. Desorption into LC liquid and following analysis.

Non exhaustive sample preparation technique, where only a small portion of the total analyte concentration is removed.

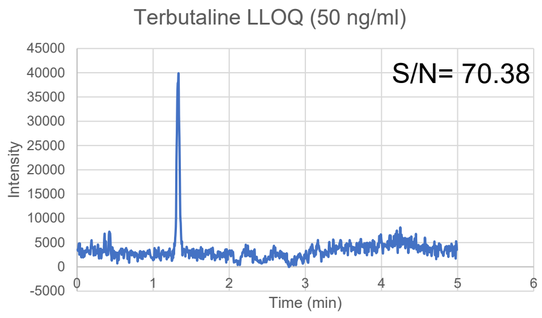
LUNG-ON-A-CHIP



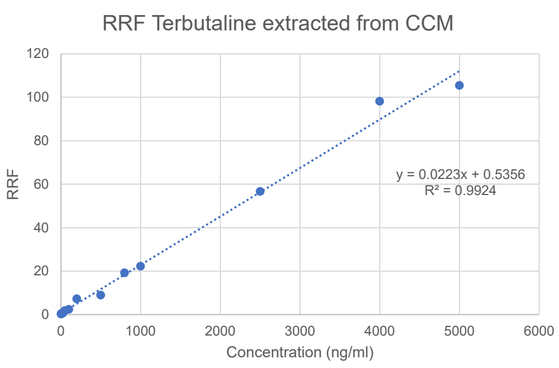
Lung-on-a-chip

- In the device the geometry typical of the respiratory system is reproduced.
- Cell cultures are grown onto a poly-dimethyl-siloxane support and the upper part interacts with air, miming lungs particular interface.
- Cells are chosen according to the capacity of presenting tight junction at air liquid interface, therefore barrier-like.
- Bronchiolar region will be represented by Calu-3 and 16HBE14o- and alveolar region by NCI-H441

PRELIMINARY RESULTS



Graph 1, Chromatogram of Terbutaline at Lower Limit of Quantification (LLOQ= 50ng/ml). S/N as signal to noise ratio



Graph 3, Relative Response Factor (RRF) of Terbutaline, extracted from Cell Culture Media (CCM).
RRF as the ratio between area of the analyte and area of the internal standard (IS= Salbutamol D3)

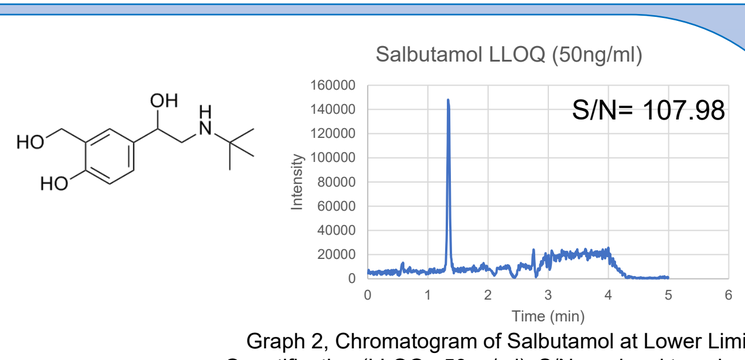
LC-MS method for the bioanalysis of hydrophilic aerosol-wise compounds of interest Terbutaline (chemical structure on the left side) and Salbutamol (chemical structure on the right side).

Ionisation	Electrospray
HPLC Column (type/size)	HILIC 50 x 2.1mm i.d. Acquity BEH 1.7 µm, Waters
Column temp (°C)	30
Flow rate (mL/min)	0.4
Run time (min)	5.00
Solvent A	95:5 Acetonitrile:Water, 10 mM Ammonium Acetate, 0.1% Formic Acid
Solvent B	50:50 Acetonitrile:Water, 10 mM Ammonium Acetate, 0.1% Formic Acid

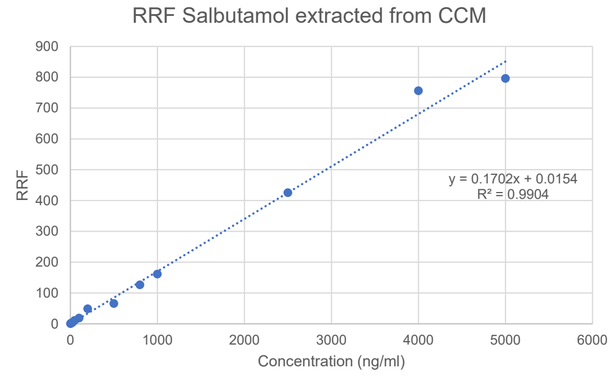
Table 1, Bioanalytical parameters for the analysis of Terbutaline and Salbutamol

From a 2nd calibration curve	Accuracy (%)		Precision (%)	
Concentration (ng/ml)	Terbutaline	Salbutamol	Terbutaline	Salbutamol
15	-13.90	27.80	8.56	21.69
50	82.30	88.90	32.52	30.86
500	93.20	98.70	7.05	8.20
2500	101.36	109.38	6.41	7.34
5000	107.60	113.80	7.15	6.46

Table 2, Accuracy obtained as comparison with an independent calibration curve. Precision obtained as n=6 independent repeated extractions



Graph 2, Chromatogram of Salbutamol at Lower Limit of Quantification (LLOQ= 50ng/ml). S/N as signal to noise ratio.



Graph 4, Relative Response Factor (RRF) of Salbutamol extracted from Cell Culture Media (CCM).
RRF as the ratio between area of the analyte and area of the internal standard (IS= Salbutamol D3)

FUTURE WORK AND REMARKS

- Method validation for hydrophilic compounds (Terbutaline and Salbutamol), afterwards for hydrophobic compounds (Fluticasone Propionate and Budesonide).
- Innovative elements proposed:

1. Dissolution and permeation rate will be measured at the same time thanks to SPME and correlated to specific regions of the lungs.
2. Knowing their relative contribution, would lead to dose reduction with equivalent therapeutic effect.
3. If the assays are accepted in R&D, this would lead to reduction of animal testing.

REFERENCES: J.M. Borghardt, B. Weber, A. Staab, C. Kloft, Pharmacometric Models for Characterizing the Pharmacokinetics of Orally Inhaled Drugs, AAPS Journal. 17 (2015) 853–870. https://doi.org/10.1208/s12248-015-9760-6. B. Forbes, P. Bäckman, D. Christopher, M. Dolovich, B. v. Li, B. Morgan, In Vitro Testing for Orally Inhaled Products: Developments in Science-Based Regulatory Approaches, AAPS Journal. 17 (2015) 837–852. https://doi.org/10.1208/s12248-015-9763-3. K. Zscheppang, J. Berg, S. Hedtrich, L. Verheyen, D.E. Wagner, N. Suttorp, S. Hippenstiel, A.C. Hocke, Human Pulmonary 3D Models For Translational Research, Biotechnology Journal. 13 (2018). https://doi.org/10.1002/biot.201700341.
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