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The hurdles of developing an LC-MS/MS assay for desmosine, a biomarker for elastin degradation

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Introduction

- Desmosine is a promising biomarker for estimating elastin degradation activity in diseases like chronic obstructive pulmonary disease (COPD) and cystic fibrosis and for monitoring the effect of therapeutic interventions.

- The objective of this study was to develop and validate a stable isotope dilution LC-MS/MS method for measurement of desmosine (DES) and isodesmosine (IDS) in urine and plasma.

- What was not anticipated were the many hurdles in the developmental process taking years before the assay could be introduced in daily practice.

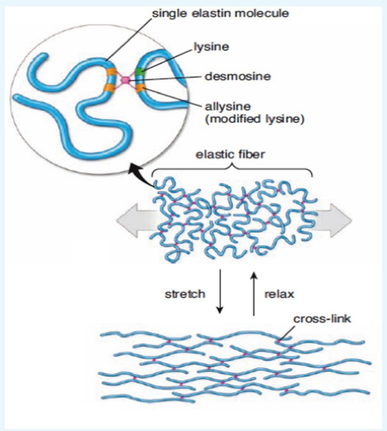


Fig. 1. Schematic representation of elastin fiber

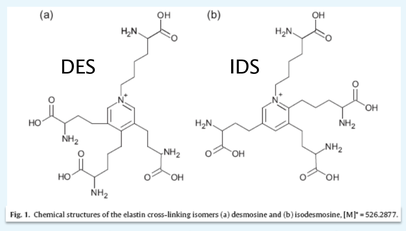
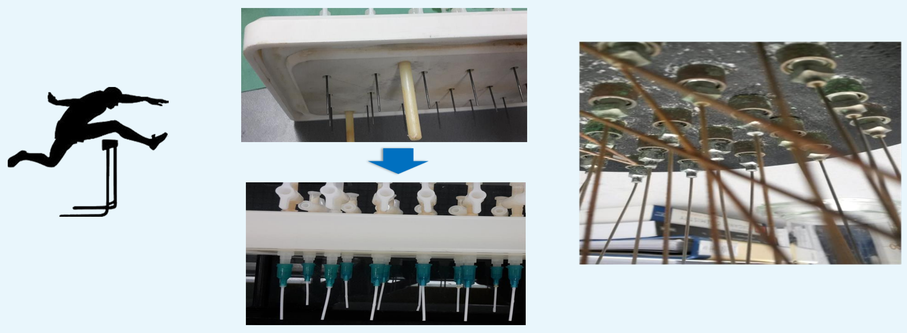


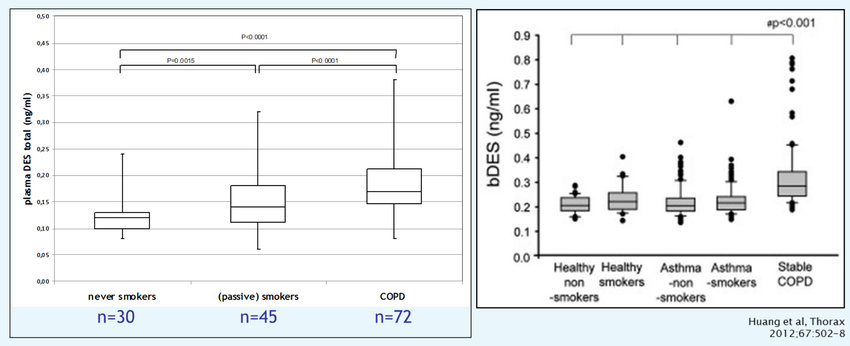
Fig. 2. Chemical structures of the elastin cross-linking isomers (a) desmosine (DES) and (b) isodesmosine (IDS)

Hurdles



- Acidic conditions resulted in corrosion of stainless steel needles in SPE manifold and dry down heat block -> loss of peaks.

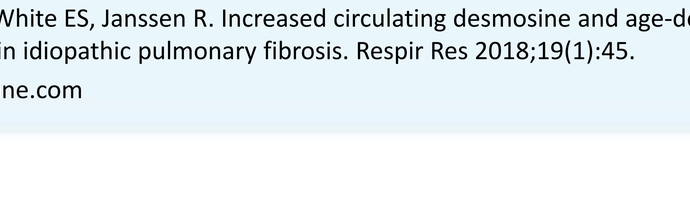
- Discontinuation of critical SPE material by the manufacturer led to a long-lasting search for suitable alternatives.



Huang et al, Thorax 2012;67:502–8

Fig. 4. Plasma total DES levels in healthy non-smokers (n=30), (passive) smokers (n=45) and patients with COPD (n=72).

- A two-fold difference was observed in measured concentrations of total DES when compared to data obtained from literature.



*"Through forgetfulness the last batch was made with **1uM** of each component. We forgot that the catalog stated **0.5 uM** of each".*

- Response from the supplier lasted half a year, and ultimately it was traced back to an error in designation of the desmosine standard concentration.

Methods

Sample hydrolysis: Add 200 µl plasma or urine and 100 µl d4-DES IS solution to 300 µl 37% HCL and heat to 110°C for 24 h.

SPE: Perform cellulose SPE to extract total DES/IDS from plasma or urine

Drying and re-suspension: Dry down in heating block and re-constitute in 100 µl 5mM NH4COOH + 0.1% HFBA

HPLC: 20 µl of each sample injected, separated in a C18 column using a gradient of mobile phases A (5mM NH4COOH + 0.1% HFBA) and B (methanol)

MS/MS: Monitor transitions 526/481 for DES, 526/397 for IDS and 530/485 for d4-DES

HFBA: heptafluorobutyricacid

Results

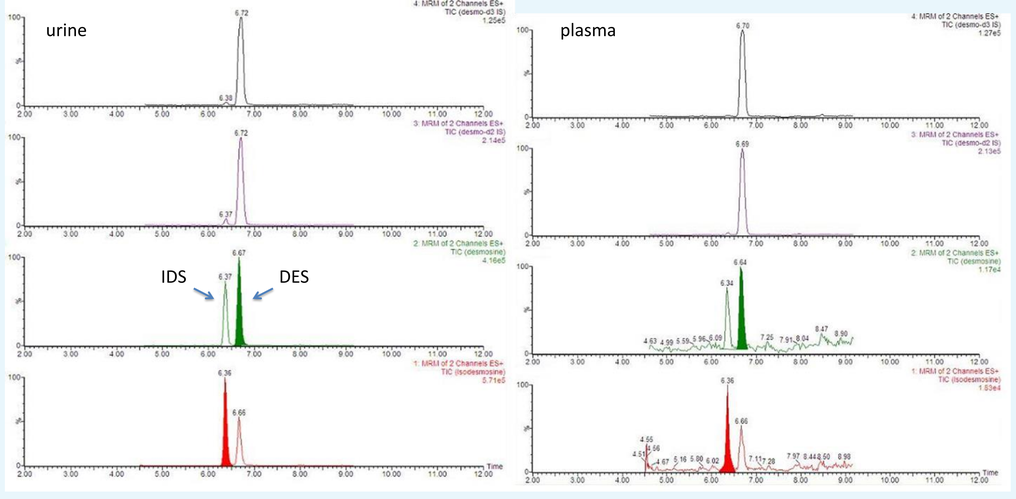


Fig. 3. Chromatographic separation of DES and IDS in a urine (left) and plasma (right) sample

Conclusion

- We have successfully developed and validated a sensitive and specific assay for the measurement of DES and IDS in human urine and plasma.
- This method can be used to assess the potential of DES and IDS as biomarkers for estimating disease activity in COPD and the effect of therapeutic interventions.

References

1. De Brouwer B, Drent M, van den Ouweland JMW, Wijnen PA, van Moorsel CHM, Bekers O, Grutters JC, White ES, Janssen R. Increased circulating desmosine and age-dependent elastinolysis in idiopathic pulmonary fibrosis. Respir Res 2018;19(1):45.
2. www.demosine.com

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