Practical comparison of LC-MS/MS-based workflows for quantitation of desmosine and isodesmosine in urine – potential lung damage biomarkers in premature infants

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Overview

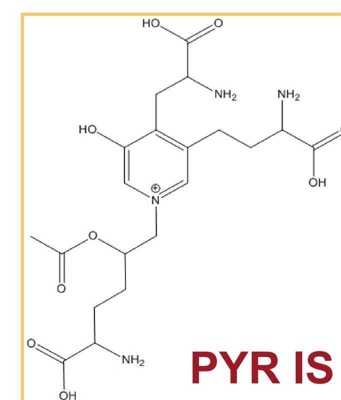
- This work compares the feasibility of three LC-MS/MS-based workflows for the quantitation of desmosine (DES) and isodesmosine (IDES) in urine samples
- Each of the tested methods, with its advantages and disadvantages, proves to be suitable for quantitative analysis

Introduction

Desmosine (DES) and isodesmosine (IDES) are lysine-derived pyridinium amino acids, byproducts of the destruction of elastin. Release of these two compounds to body fluids serves as an indicator of elastin degradation associated with lung damage in bronchopulmonary dysplasia (BPD), a significant cause for morbidity and mortality among infants born prematurely. This study evaluates three LC-MS/MS based workflows – differing mainly in chromatography – for quantitation of DES and IDES. Comparison criteria include sensitivity, chromatographic resolution, matrix interference, robustness, and total speed of analysis. Key goals were reduction of sample preparation time and use of volatile LC eluents. This study illustrates practical choices which must be made in developing and selecting a fit-for-purpose analytical method.

Sample preparation

Standards:
Powdered desmosine (DES) and isodesmosine (IDES) were purchased from Elastin Product Company.
Acetylated pyridinoline was obtained from Quidel as a 3.8 µM solution (in 90% acetic acid) and used as an Internal Standard (PYR IS).



Stock solution: 1 mM in 50% acetonitrile/0.1% formic acid
Working solutions: 50 µM; prepared by dilution of stock solution with water
Calibration curve: 0.1 nM – 5000 nM prepared by serial dilutions in matrix-free solvent or urine
Biological samples: Urine samples were collected from 33 human infants: 15 normal controls and 18 premature babies. Of the premature babies, 7 developed lung disease and 11 did not. Creatinine levels were used for normalization purposes.

All biological samples were injected as-is, without any sample cleanup or dilution.

LC-MS/MS platforms

Instrumentation

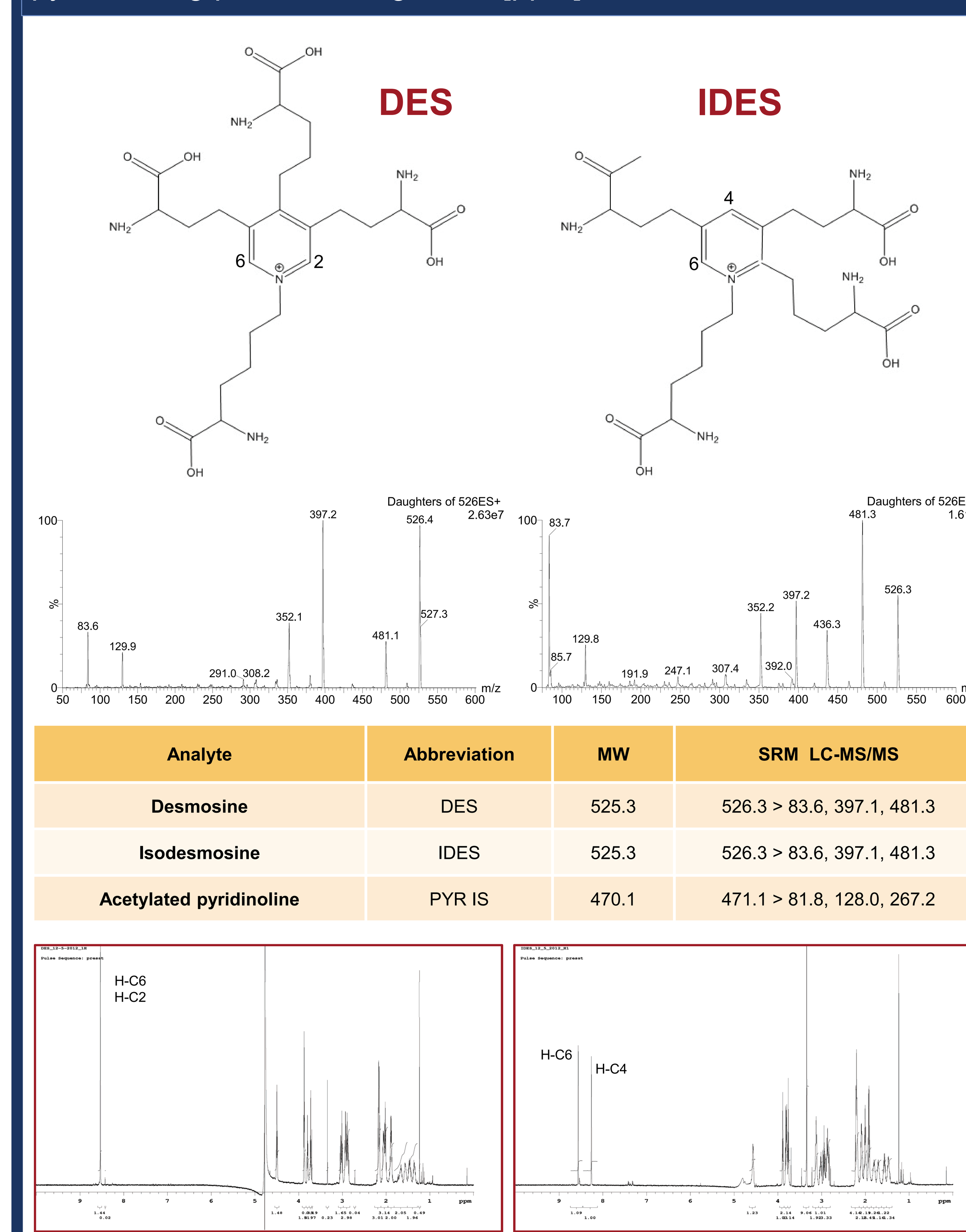
LC system: Agilent HP 1100
MS detection: Waters Quattro Premier triple quadrupole MS
Ionization mode: positive ESI
Scanning mode: Selected Reaction Monitoring (SRM)

LC conditions

	A	B	C
Column	**Hypercarb** (Thermo Fisher Scientific) 100 mm x 2.1 mm, 3 µm	**Atlantis T3** (Waters) 2.1 mm x 100 mm, 3 µm	**Hypercarb** (Thermo Fisher Scientific) 100 mm x 2.1 mm, 3 µm
Mobile Phase A	0.1% FA	5 mM HFBA/ 5 mM AA	0.5% TFA
Mobile Phase B	0.1% FA in acetonitrile	5 mM HFBA/ 5mm AA in 80% acetonitrile	0.5% TFA
Flow rate	250 µL/min	250 µL/min	300 µL/min

FA: formic acid; HFBA: heptafluorobutyric acid; AA: ammonium acetate; TFA: trifluoroacetic acid

FIGURE 1. Analytes. Desmosine (DES) and Isodesmosine (IDES) are positional isomers which generate nearly identical CID fragmentation patterns. Due to this similarity, MS analysis is not sufficient to distinguish between these two isomers. The H-NMR spectra of DES and IDES reveal significant differences for the pyridine ring protons at high field [ppm].



Analyte	Abbreviation	MW	SRM LC-MS/MS
Desmosine	DES	525.3	526.3 > 83.6, 397.1, 481.3
Isodesmosine	IDES	525.3	526.3 > 83.6, 397.1, 481.3
Acetylated pyridinoline	PYR IS	470.1	471.1 > 81.8, 128.0, 267.2

Results

FIGURE 2. Comparison of LC conditions. Three mobile phase modifiers were evaluated for DES/IDES separation in matrix free samples: 0.1% formic acid (A), 5 mM HFBA/5 mM AA (B) and 0.5% TFA (C). With each eluent system, several gradient, temperature and flow rate settings were tested. The best results for each modifier and its respective column are presented below.

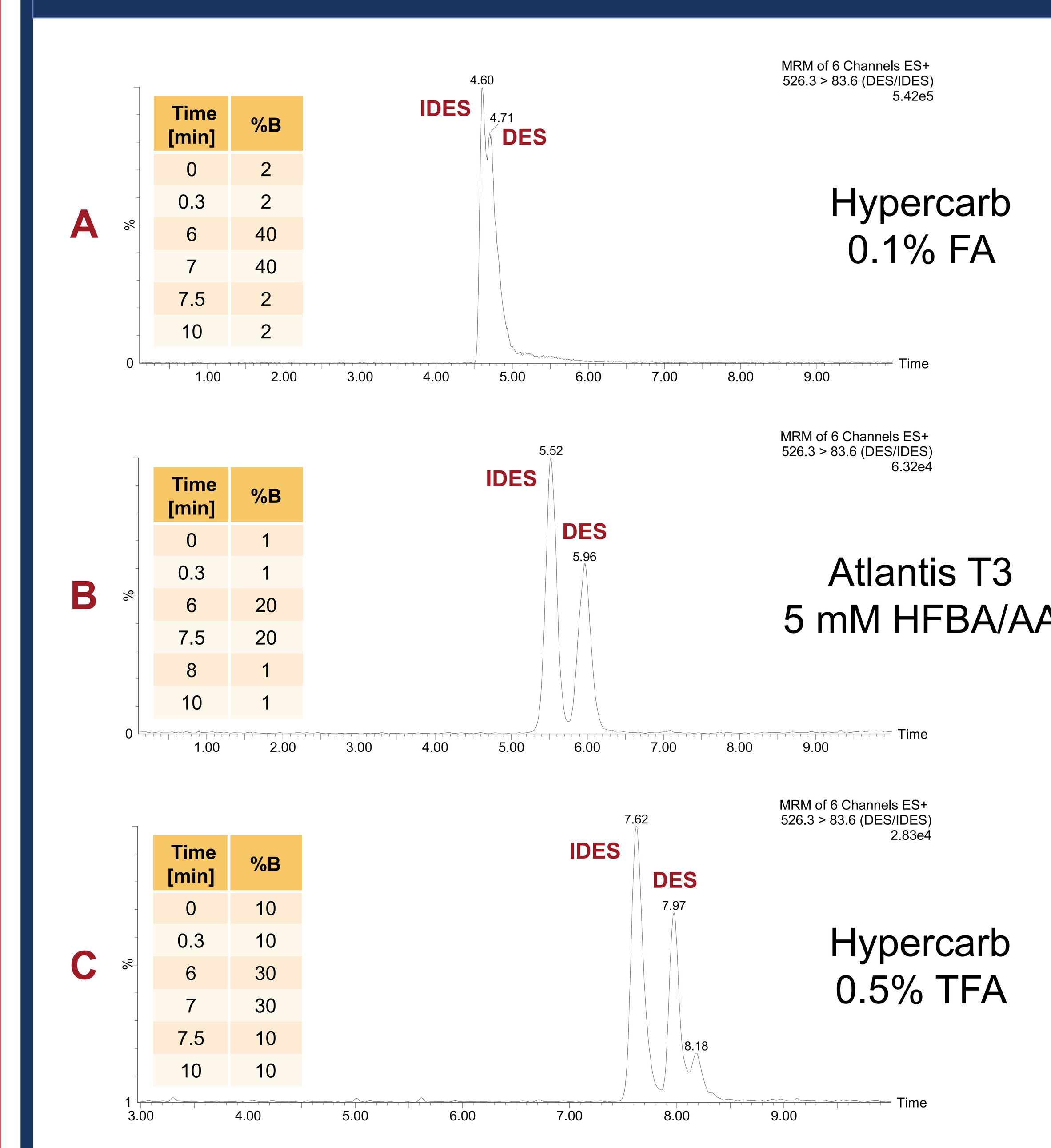


FIGURE 3. Analysis of human urine samples. Urine samples from 33 infants were analyzed using method A. The cohort includes 18 premature and 15 full term newborns. Of the premature babies, 7 developed bronchopulmonary dysplasia (BPD) and 11 did not. No obvious correlation among disease state, age and prematurity was observed in this set of samples.

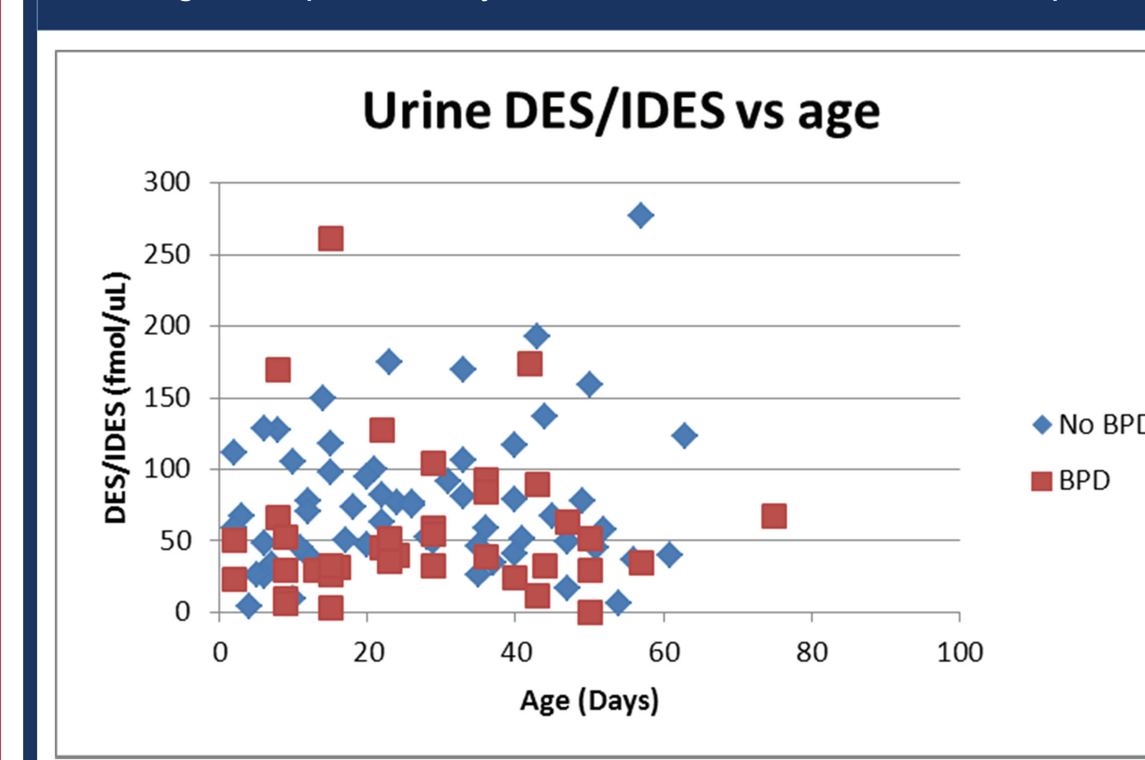


TABLE 1. Comparison of technical features. Analysis of DES and IDES in straight urine is only possible with methods A and C. Strong matrix effect and poor sensitivity has been observed when using method B. Of all three, method A utilizes the most mass spectrometry compatible LC mobile phases but does not allow baseline resolution of DES and IDES.

Comparison criterion	A	B	C
LLOQ in solvent / urine	5 / 10 fmol	5-10 / 200*) fmol	50 / 5 fmol
Time – Sample analysis	10 min	10 min	10 min
Chromatographic resolution (DES/IDES separation	Not sufficient for quantitation	baseline	baseline
Matrix interference (urine – no sample prep)	Minor	Significant**)	Not observed
Column	Hypercarb	Atlantis T3	Hypercarb
Mobile phase modifier	0.1% FA	5 mM HFBA/5 mM AA	0.5% TFA
Flow rate [µl/min]	250	250	300

*) matrix interferences made sensitivity assessment inaccurate and irreproducible
**) method B is not feasible for analysis of straight urine. In order to achieve good sensitivity and reproducibility biological sample need extensive sample preparation (solid phase extraction) as described by Ma *et al.* [1]

Conclusions

Each of the tested methods can be applied to the quantitative analysis of DES and IDES in urine samples – however, each has advantages and disadvantages which must be considered in context of project requirements:

- Methods B and C achieve baseline resolution of two isomeric analytes. However, current clinical data do not support the necessity of quantifying DES and IDES individually – so chromatographic separation, while satisfying analytically, may be extraneous.
- Method A is the most MS compatible and would be best suited for large scale measurement of combined DES and IDES, as its run time can be significantly shortened to provide high-throughput analysis.
- Both methods B and C utilize ion paring agents; of the two, TFA is more MS friendly than HFBA, in that it is more volatile, easier to wash out, and causes less ionization suppression.
- Successful application of Method B requires extensive, time consuming sample preparation, while methods A and C work well with straight urine.
- Due to its sensitivity, chromatographic resolution and lack of matrix interferences, Method C is our choice for this ongoing project.

Future work

- The measured levels of DES/IDES in infant urine did not support the use of DES/IDES as biomarkers for BPD. Future work will concentrate on the analysis of plasma and airway fluid samples using the established LC-MS/MS workflow.

References

1) Ma,S.; Turino,G.; Lin,Y.Y. *J.Chrom.B* **2011**, 879: 1893-1898

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