Design of amino acid DERIVATIZATION REAGENTS for LC-ESI-MS/MS.

Development and validation of derivatization LC-ESI-MS/MS method for amino acids at sub-femtomole level

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Methods

- design of reagent targeting high ionization efficiency, chromatographic retention and reactivity,
- synthesis and purification,
- matrix effect investigation,
- derivatization procedure optimization
- method validation (VaLChrom).

LC-ESI-MS AMINO ACID DERIVATIZATION REAGENTS

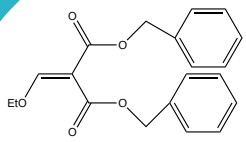
- **provide good reversed phase chromatographic separation,**
- **provide low detection limits, are easily synthesized**
- **produce derivatives which are less susceptible to matrix influences**
- **have convenient derivatization procedure with stable derivatives suitable for automatization.**

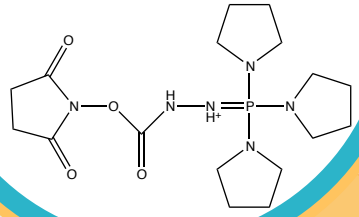
NOVEL DERIVATIZATION REAGENTS

- **should not carry a permanent charge in solution**
- **for efficient ESI ionization, should be able to chelate the charge carrier (H^+ or Na^+)**
- **for efficient ESI ionization should be large (hydrophobic).**

- One step synthesis
- **Attomole level** detection limits for selenoamino acids
- Good chromatographic separation
  - Fast reaction, HFIP buffer
  - suitable for automatization
- No matrix effect for onion samples
- Wide linear range

DBEMM





FOSF

- Novel for LC-ESI-MS/MS
- Multiple step synthesis
- Fast derivatization reaction
- Suitable for real samples
- Poor chromatographic separation
- Narrow linear range
- **Femtomole level** detection limits for amino acids

Limit of Quantifications

	DEEMM	FMOC-Cl	DNS	TAHS	FOSF	DBEMM	EBEMM
Arg	84	259	365	81	[c]	0.26	3.1
Asp	154	943	[b]	117	96	1.7	3.4
Gly	384	3615	3887	61	168	9.1	42
β-Ala	227	1687	377	101	54	1.6	20
Pro	[a]	174	1381	31	130	2.5	34
Trp	53	164	55	92	7	0.8	63
Phe	26	193	252	22	41	0.7	6.9

[a] – Pro was unstable for DEEMM analysis and not added to the comparison.
[b] – the signal of Asp was not obtained for DNS analysis
[c] – the signal of Arg for FOSF was not stable and LoD values obtained not reliable.

Matrix effects in onion samples

	TAHS	DNS	FMOC-Cl	DEEMM	DBEMM
Se-MeSeCys	22%	64%	14%	65%	111%
SeMet	17%	58%	25%	58%	101%

Results

- **Design principles for LC-ESI-MS/MS derivatization reagents were set**
- **Two novel derivatization reagents were designed, synthesized and validated**
- **FOSF and DBEMM**

References
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Rebane, R.; Oldekop, M-L.; Herodes, K. Journal of Chromatography A, 1390, 2015, 62–70.

VaLChrom

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