Method development strategy for derivatization LC/ESI/MS

Riin Rebane[1], Maarja-Liisa Oldekop, Koit Herodes

University of Tartu, Ravila 14a, 50411 Tartu, Estonia

1. riin.rebane@ut.ee

LC/ESI/MS with derivatization used for low detection limits

Prerequisites for sensitive LC/ESI/MS analysis are:

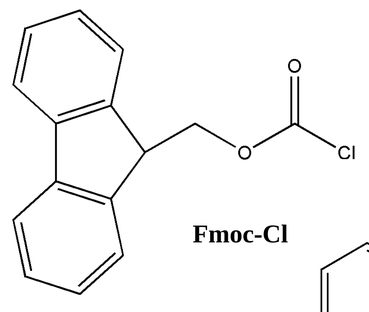
1) Chromatographic separation
2) Good ionization of the analytes

Derivatization is applied for improving separation and ionization

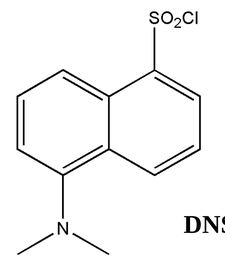
BUT

most methods apply classical ultraviolet detection derivatization reagents and develop methods accordingly.

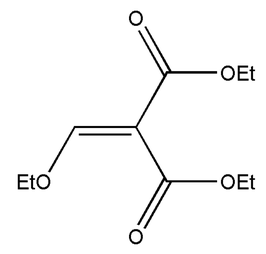
Classical reagents



Fmoc-Cl



DNS

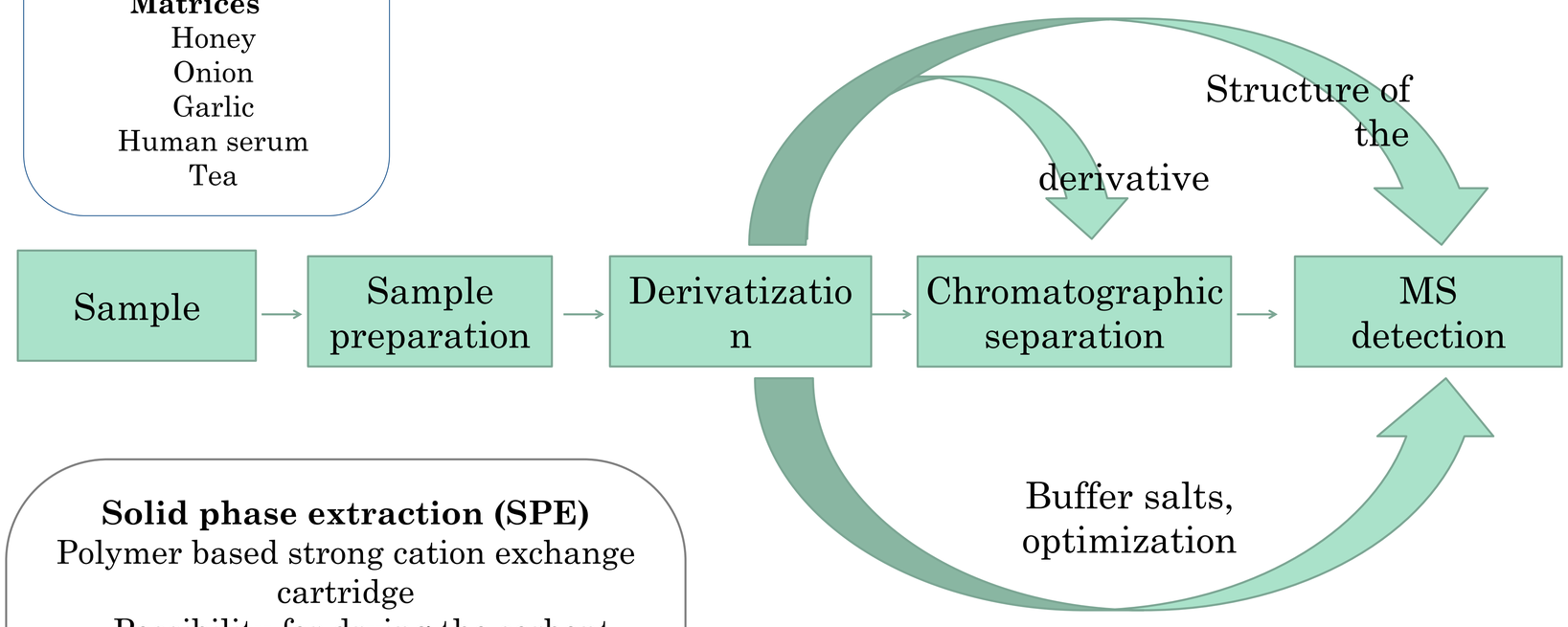


Deemm

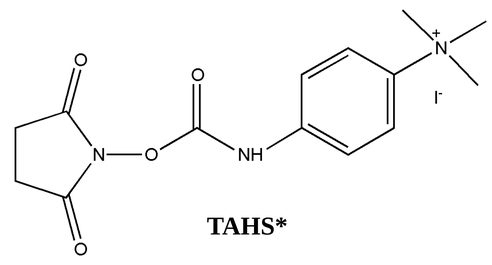
Analytes
23 amino acids
Selenoamino acids

Matrices
Honey
Onion
Garlic
Human serum
Tea

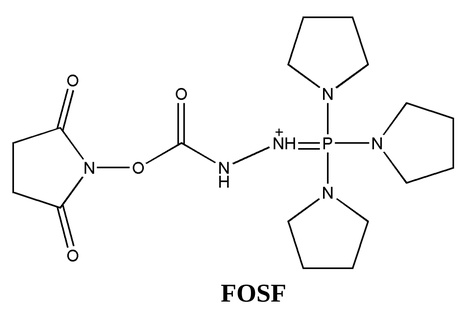
Amino acid analysis is chosen as a model system.



Novel reagents



TAHS*



FOSF

synthesized in this work

Solid phase extraction (SPE)
Polymer based strong cation exchange cartridge
Possibility for drying the sorbent
Tolerable of low and high pH solvents
Reusable cartridges
Possibility to change a solvent for derivatization
Suitable for LC/ESI/MS analysis

Recoveries for honey, onion and human serum around 100%
Lower recoveries for oilier samples

Interesting finding!
Most commonly used buffer in derivatization mixture is boric acid and it was found to **suppress/enhance signal** in ESI source.
For FOSF, diverting boric acid away from the ESI source helped, and for Deemm:

Optimization for MS analysis

	Deemm	Fmoc-Cl	DNS	TAHS	FOSF
m/z	$[M+Na]^+$	$[M+Na]^+$	$[M+H]^+$	$[M+H]^+$	$[M+H]^+$
Ammonium acetate as buffer component	Suitable for some	Not suitable	Better without	Suitable	-
MeOH or MeCN	MeCN	MeOH	MeCN	both suitable	MeCN

Post column flow splitting in range 60-80% provides better signal for some cases.

MS^2 parameters are better to optimize with chromatographic eluent flow

Comparison of derivatization buffers

Fmoc-Cl, FOSF, TAHS – **no alternative**

Carbonate as a possible **alternative**

*! For DNS, boric acid acts as a signal enhancer and infusion of boric acid into ESI source raised detection limits up to **4 times** !*

Novel FOSF proved to be more similar to very sensitive TAHS than to classical derivatization reagents, but **chromatographic separation** was much better.

With right method development for LC/ESI/MS analysis, detection limits as low as 0.1 pmol can be achieved.

LoQ (fmol on column)

	DEEMM	FMOC-Cl	DNS	TAHS	FOSF
Arg	84	259	365	81	[c]
Asp	154	943	[b]	117	96
Gly	384	3615	3887	61	168
β-Ala	227	1687	377	101	54
Pro	[a]	174	1381	31	130
Trp	53	164	55	92	7
Phe	26	193	252	22	41

[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/LoQ values obtained not reliable.

Acknowledgement
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