HOW TO DEAL WITH THE MATRIX EFFECT?

Bioequivalence and Development Workshop
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QUINTA-ANALYTICA

DEFINITION OF MATRIX EFFECT

« The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample »

V.P. Shah et al., "Bioanalytical method validation – a revisit with decade of progress", Pharm. Res. *17*, 1551, **2000**

Guidance for Industry, Bioanalytical Method Validation, US FDA, CDER and CVM, Washington, DC, **2001.**

EMEA/CHMP/EWP/192217/2009 Rev.1 Corr., Committee for Medicinal Products for Human Use (CHMP) "Guideline on bioanalytical method validation", **2012**

FDA, EMA REQUIREMENTS

FDA: « Matrix effects on ion suppression or enhancement or on extraction efficiency should be addressed »

FDA: « Appropriate steps should be taken to ensure the lack of matrix effects throughout the application »

EMA: The whole Chapter 4.1.8. is devoted to the matrix effect and detailed instructions for its evaluation are given.

EVALUATION OF MATRIX EFFECT (EMA)

1. Preparation of two solution sets:

   a) neat solution of the analyte
   b) neat solution of the internal standard

2. Preparation of plasma blank extracts from 6 independent plasma donors including plasma haemolysed and hyperlipidaemic samples
3. Spiking of plasma blanks extracts with the defined quantity of both analyte and internal standard
4. Evaluation of Matrix Factors (MF) of both analyte (MFa) and internal stantard (MFs) as a ratio responses of the analyte (internal standard) from the spiked extracts to the neat solutions
5. Evaluation of Normalised Matrix Factor as MFa/MFs
6. Determination of **CV of Normalised Matrix Factor** of (MF norm) in six different plasma sources
7. Acceptance criterion: **CV<15%**

EXAMPLE – ION ENHANCEMENT

OH-PGZ (M-IV)

(EMA EVALUATION)

Standard solution			
Conc. (ng/mL)	1250.0		
Replicate	M-IV (peak area)	dM-IV (peak area)	y (M-IV/dM-IV)
1	1210388	197394	6.131836
2	1069706	167129	6.400493
3	1001176	153005	6.543398
4	1048084	159659	6.564513
5	1082675	161246	6.714411
6	1099991	161346	6.817568
Mean	**1085337**	**166630**	**6.528703**
CV (%)	**6.5**	**9.4**	**3.7**

Standard added to the extract of blank plasma				
Conc. (ng/mL)	1250.0			
Replicate	M-IV (peak area)	dM-IV (peak area)	y (M-IV/dM-IV)	Normalized MF
1	7514954	1112860	6.752831	1.034
2	6581614	944935	6.965150	1.067
3	7226609	1091906	6.618340	1.014
4	7464284	1166792	6.397269	0.980
5	7693520	1146435	6.710818	1.028
6	2352208	368049	6.391022	0.979
Mean	**6472198**	**971830**	**6.639239**	**1.017**
CV (%)	**31.8**	**31.5**	**3.3**	**3.3**
Matrix Factor (MF)	**5.963**	**5.832**		

EXAMPLE– ION SUPRESSION

ENTECAVIR

(EMA EVALUATION)

Standard solution			
Conc. (ng/mL)	16.000		
Replicate	ETV (peak area)	dETV(peak area)	Peaks ratio (ETV/dETV)
1	551 258	115 343	4.779308
2	576 771	117 856	4.893880
3	555 762	112 595	4.935950
4	548 101	114 426	4.790015
5	555 892	114 141	4.870240
6	490 370	100 648	4.872107
Mean	**546 359**	**112 501**	**4.856917**
CV (%)	**5.35**	**5.39**	**1.25**

Standard added to the extract of blank plasma				
Conc. (ng/mL)	16.000			
Replicate	ETV (peak area)	dETV (peak area)	y (ETV/dETV)	Normalizoed MF
1	187 233	38 396	4.876322	1.004
2	191 552	38 337	4.996520	1.029
3	208 954	40 926	5.105623	1.051
4	175 945	35 517	4.953795	1.020
5	181 459	36 652	4.950896	1.019
6	196 500	38 122	5.154576	1.061
Mean	**190 274**	**37 992**	**5.006289**	**1.031**
CV (%)	**6.14**	**4.83**	**2.09**	**2.09**
MF	**0.348**	**0.338**		

EMA REQUIREMENTS
EFFECT OF EXCIPIENTS

If a formulation for injection to be administered to the subjects or animals contains excipients known to be responsible for matrix effects, for instance polyethylene glycol or polysorbate, matrix effects should be studied with matrix containing these excipients, in addition to blank matrix.

The matrix used for this evaluation should be obtained from subjects or animals administered the excipient, unless it has been demonstrated that the excipient is not metabolised or transformed in-vivo.

The effect of the excipients can be studied by the determination of the MF or by a dilution study of a study sample with a high concentration with blank matrix not containing the excipient.

EMA REQUIREMENTS
SPECIAL PLASMA SOURCES

In addition to the normal matrix it is recommended to investigate matrix effects on other samples *e.g.* **haemolysed and hyperlipidaemic plasma samples.**

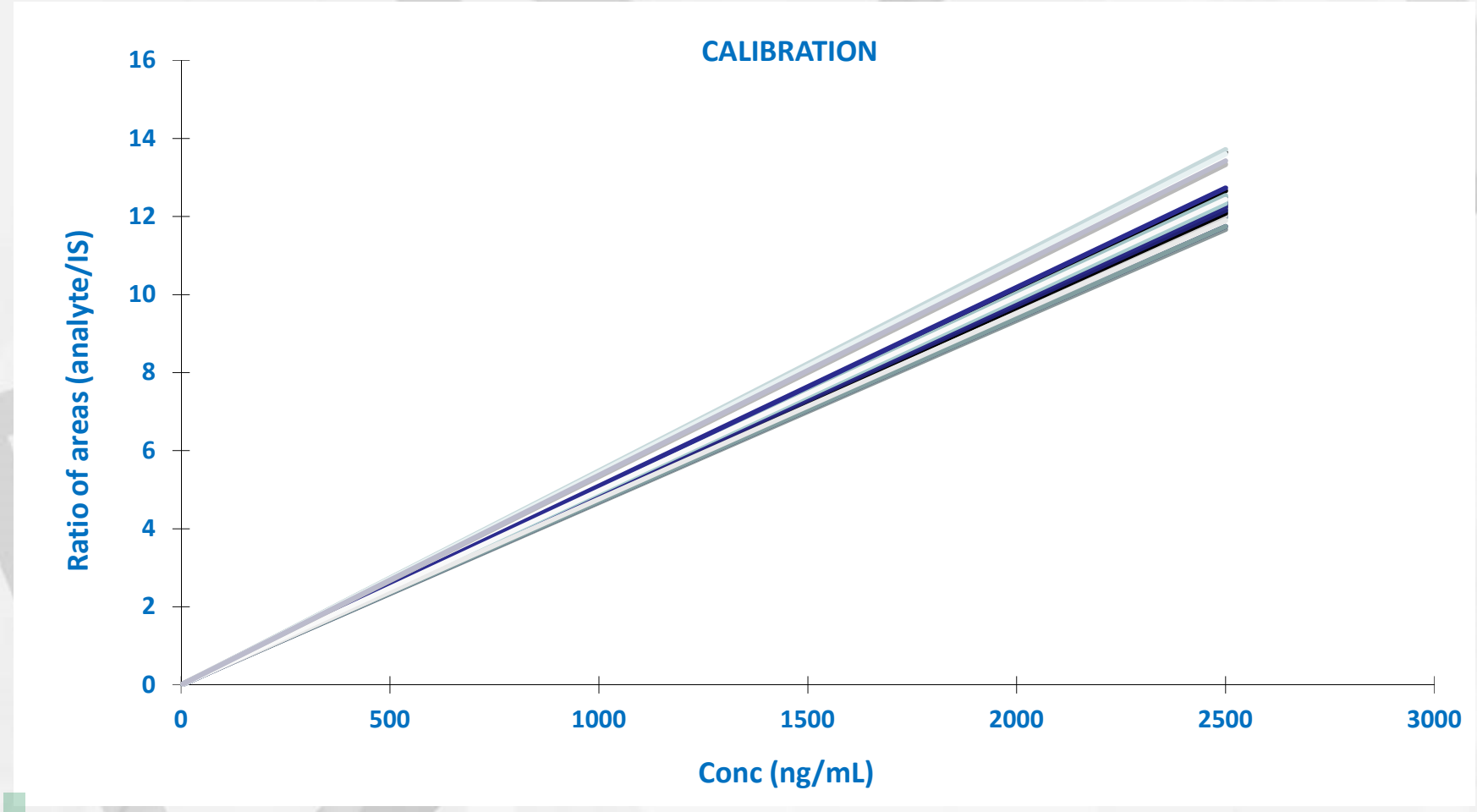
If samples from special populations (such as renally or hepatically impaired populations) are to be analyzed it is also recommended to study matrix effects using matrix from such populations.

RATIONALE FOR CV OF MF NORM CV < 15%

- Why not use values of MFnorm itself as a criterion?
- MFa/MFs may be generally influenced by matrix components
- EMA as well as FDA guidelines require to prepare calibration (standard) curve and independent QCs for each individual strictly defined analytical lot of samples
- **MFa/MFs value reflects** the impact of the matrix on the slope of calibration curves of each individual lot of samples. As the calibration is done for each lot individually, **the accuracy** of the measurements is not directly influenced. Assuming the constant amount of internal standard is added to the samples, the calibration curve may be plotted as a function **y = a + bx, where y = Analyte response/IS response at the concentration x** of the analyte
- **CV (%) of MFnorm is reflecting the precission**
- Acceptable limit for the **precission** is determined as **15%**

CALIBRATION CURVES – 39 ANALYTICAL LOTS

OH-PGZ (M-IV)



PROCESSES RESPONSIBLE FOR MATRIX EFFECT

Physical

Deposits of inorganic and organic impurities (salts) in the ion source, capillary, skimmer, orifice, resulting in partial block of the ion beam, ion beam deflection etc.

Physico-chemical

- overlapping with other non-resolved peaks (role of metabolites)
- influence of ionization efficiency by **altering the surface tension of droplets (ion suppression in ESI)**. Components from matrix may influence the **viscosity of droplets and thus their surface tension**

Chemical

Participation of matrices components in **ion-molecule** ionization **reactions**

PHYSICAL AND PHYSICO-CHEMICAL FACTORS

Physical factors in our understanding **should not be considered** as a real matrix effect. The way of their elimination is a mechanical cleaning of ion source and avoiding the use of inorganic salts in the mobile phases, sample processing etc.

Physico-chemical factors generally may be minimized by **efficient chromatography and thorough clean-up processing of sample extracts**.

The back-up conversion of some metabolites (glucuronides, N-oxides) must be studied and documented (importance of incurred samples reanalyses)

CHEMICAL FACTORS AS A CORE

Electron Impact (EI) and Photon Ionization

Monomolecular ionization mechanism in gas phase

No matrix effect

Chemical ionization (EI)

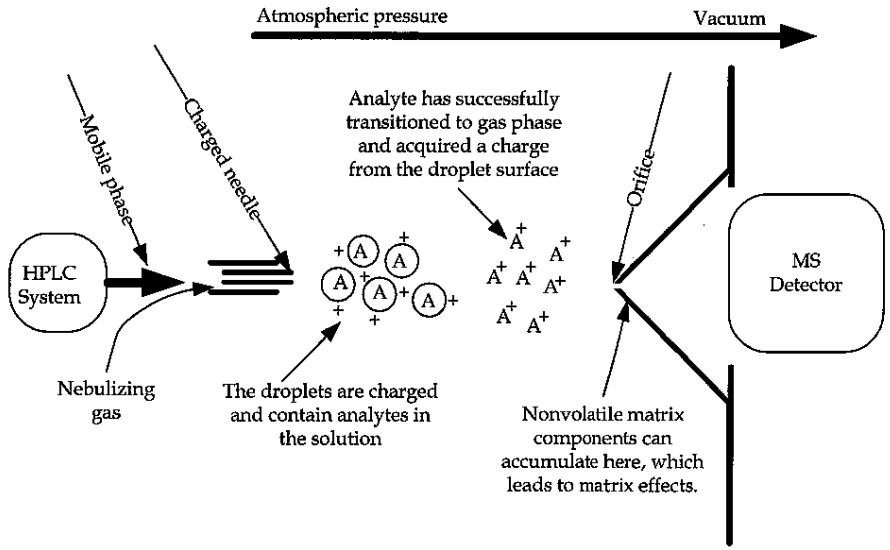
Bimolecular (Brønsted acid-base reactions) in gas phase

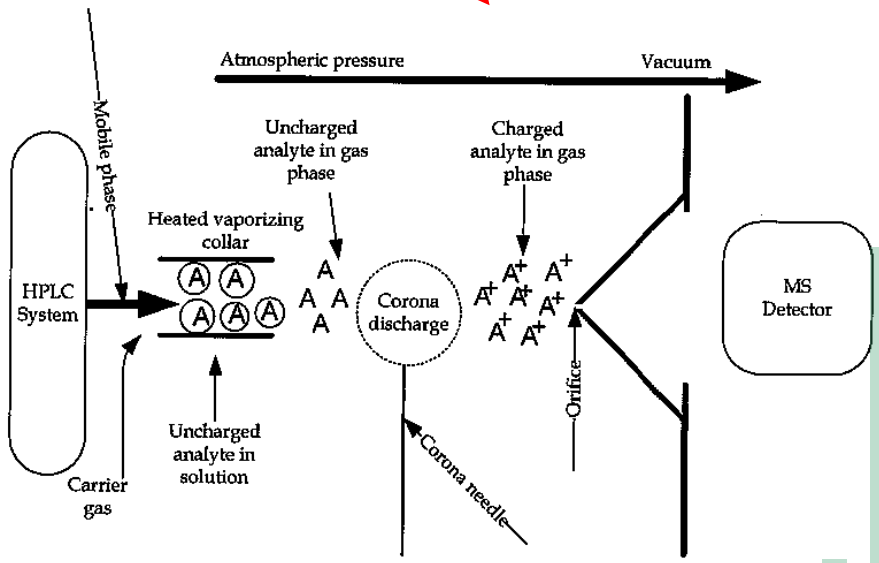
Matrix effect may be observed

ESI and APCI ionization techniques

- mostly used in LC/MS, LC/MS/MS
- ESI - ions in liquid phase evaporated by high electric field
- APCI - thermal evaporation of solvents → ionization under atmospheric pressure by electric discharge

ESI AND APCI PRINCIPLES





T. G. Hall et al, **2012**

BASIC IONIZATION PROCESSES IN LC/MS – simplified (both in APCI and ESI)

Ion source as a chemical reactor
BRØNSTED ACID – BASE **(ION-MOLECULE REACTIONS)**

Positive ion mode:

- **Transfer (addition) of proton, $[M + H]^+$ ions formation**
- Charge transfer (ion addition) $[M]^+$, $[M + NH_4]^+$, $[M + Na]^+$
- Formation of clusters $[M + M_2 + H]^+$
- Hydride anion splitting off $[M - H]^+$

Negative ion mode:

- **Molecule deprotonation $[M – H]^-$ ions formation**
- Addition of negative ions: $[M + Cl]^-$, $[M + HCOO]^-$, $[M + CH_3COO]^-$

REACTIONS TAKE PLACE IN PARALLEL OR COMPETITIVE MODE

- **High proton affinity compounds (amines and bases generally)**

  protonation of the molecule is a dominating process, abundance of **[M + H]$^+$ ions** is very high

- **Lower proton affinity compounds**

  Addition of ammonium ions and alkali metal ions exceeds the protonation. **Cluster ion formation often occurs**

TRACES OF CATIONS FROM SOLUTION OR ADSORBED IN ION SOURCE

(NH_4^+, Na^+, and K^+)

Typical well known ion multiplets: $[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$, and $[M + K]^+$ with typical mass difference **m/z [M + 1] + 17 + 5 + 16** allow unequivocal molecular weight determination

In general this clusters formation **cannot be avoided**.

Presence of traces of ammonium and alkali metal cations is sufficient for such clusters formation.

These traces may be either present **in mobile phases** or as **adsorbed in the ion source**.

No purification and cleaning procedures are effective enough for getting rid of those cations.

Strong adsorption effects of cations and polar substances (e.g. amines) resulted in matrix effect in our extended definition.

ADSORPTION EFFECTS

Experience (**good** and **bad**) from our own practice:

☺ **Methyl amine** adsorption phenomenon

☹ **Ion pair reagent** adsorption phenomenon

METHYL AMINE PHENOMENON

Example 1

Unexpected differences in mass spectra of the same compound obtained on two instruments:

(M+1), (M+18), (M+23) on LCQ **(O.K.)**

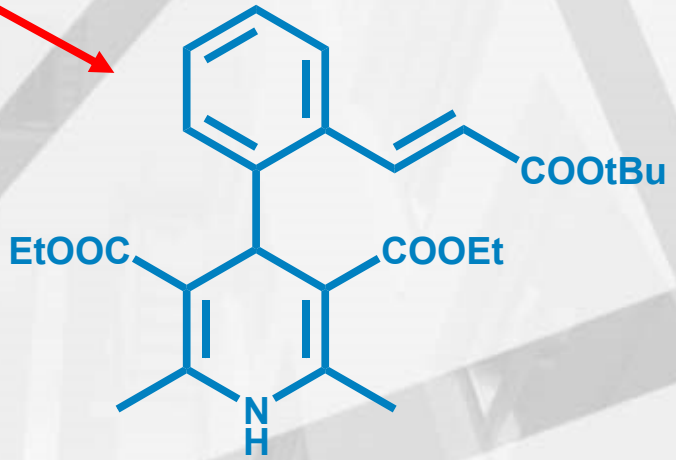
vs

Unexpected (M+32) on QQQ **(???)**

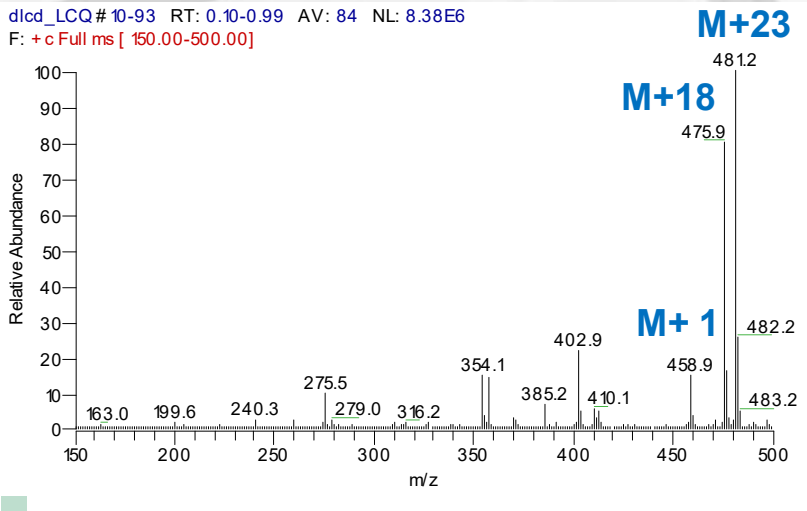
The difference cannot be assigned to the different type of instrument design, but to the **history of measurements** on both instruments.

Methyl amine was used in mobile phase in mmol concentration **half a year ago** on QQQ → **STRONG ADSORPTION OF METHYL AMINE !!!**

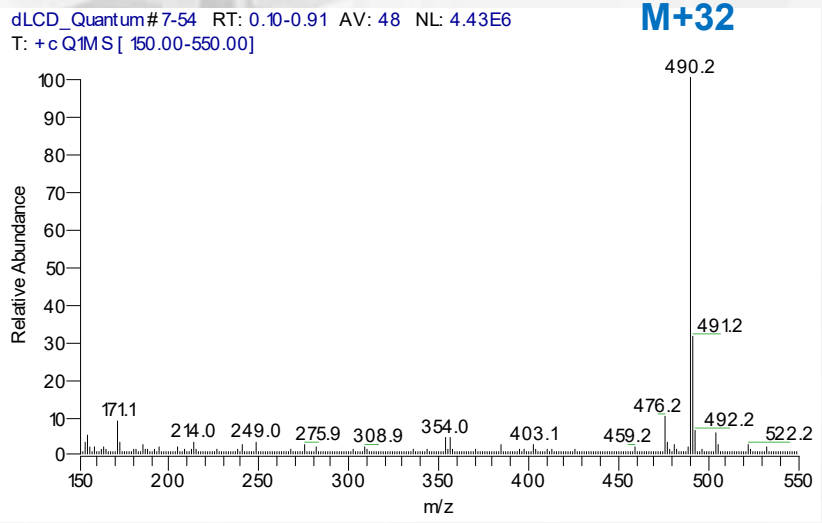
Lacidipine C^2H_3
Ion cluster formation
$[M + H + CH_3NH_2]^+$



LCQ ion trap

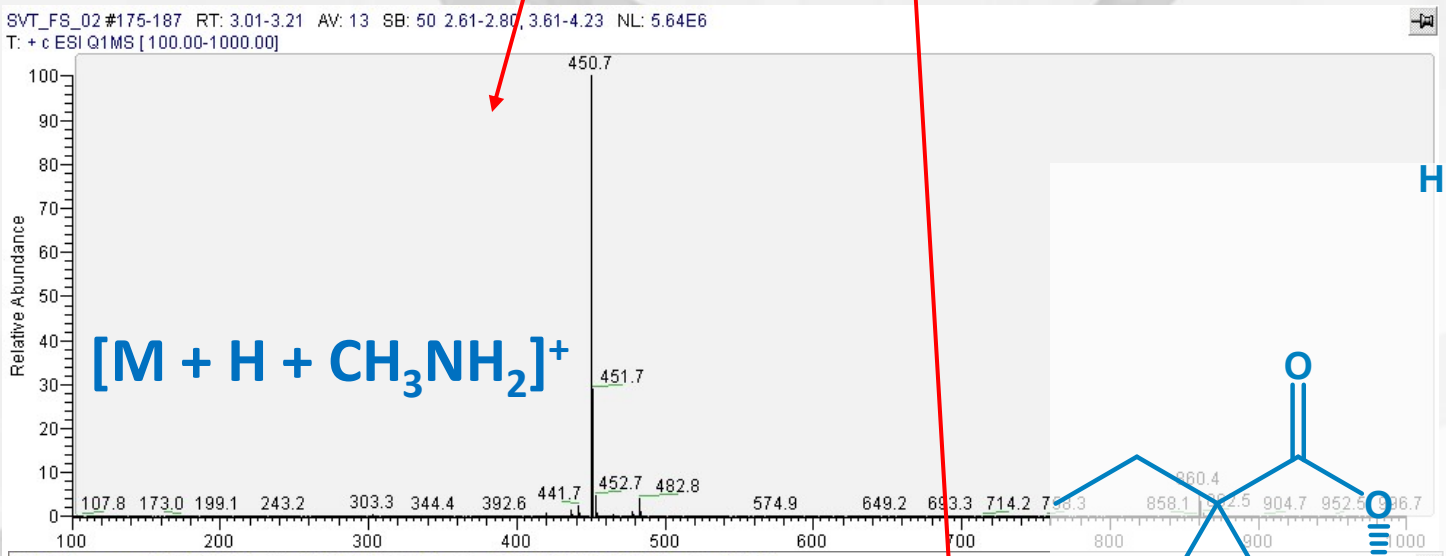


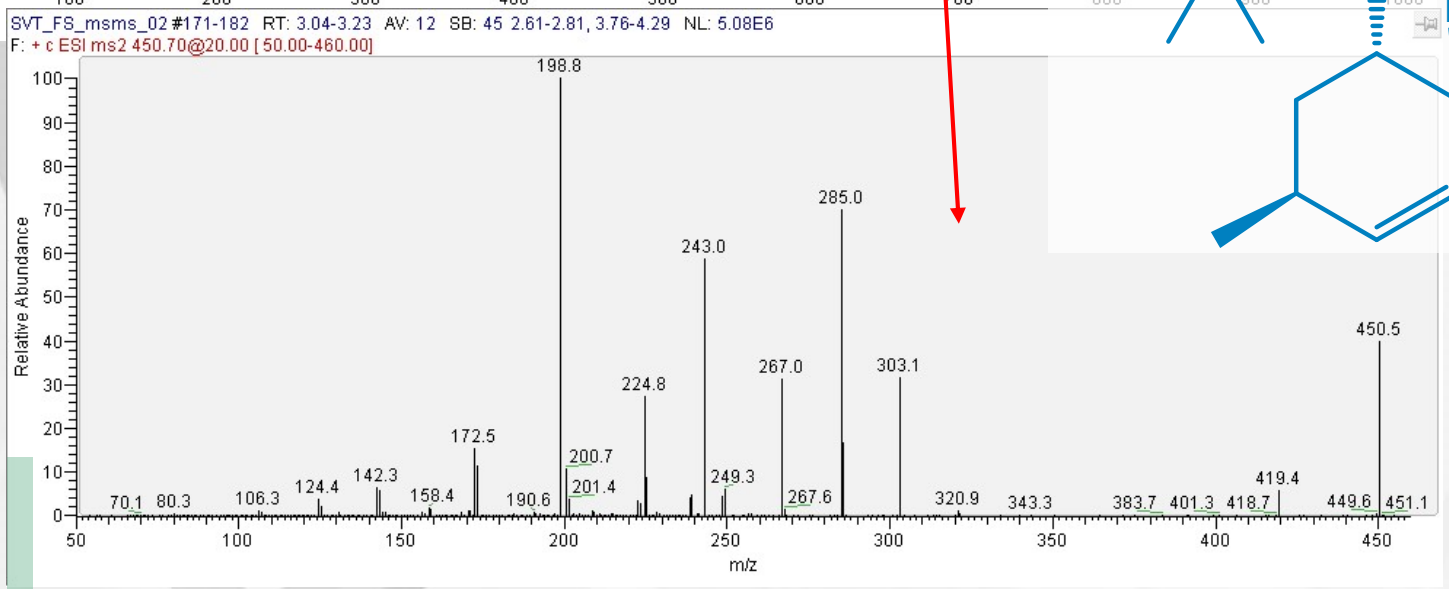
QQQ Quantum

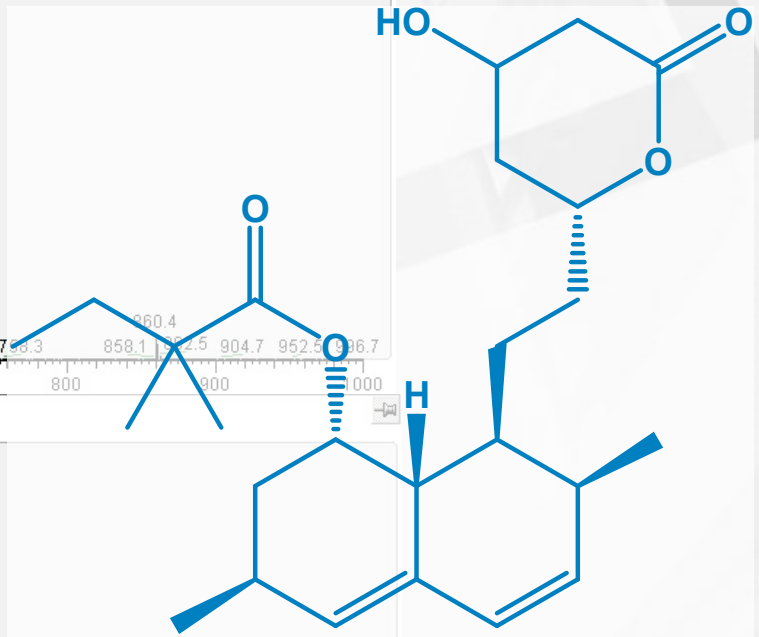


SIMVASTATIN

Full-scan and MS/MS spectrum

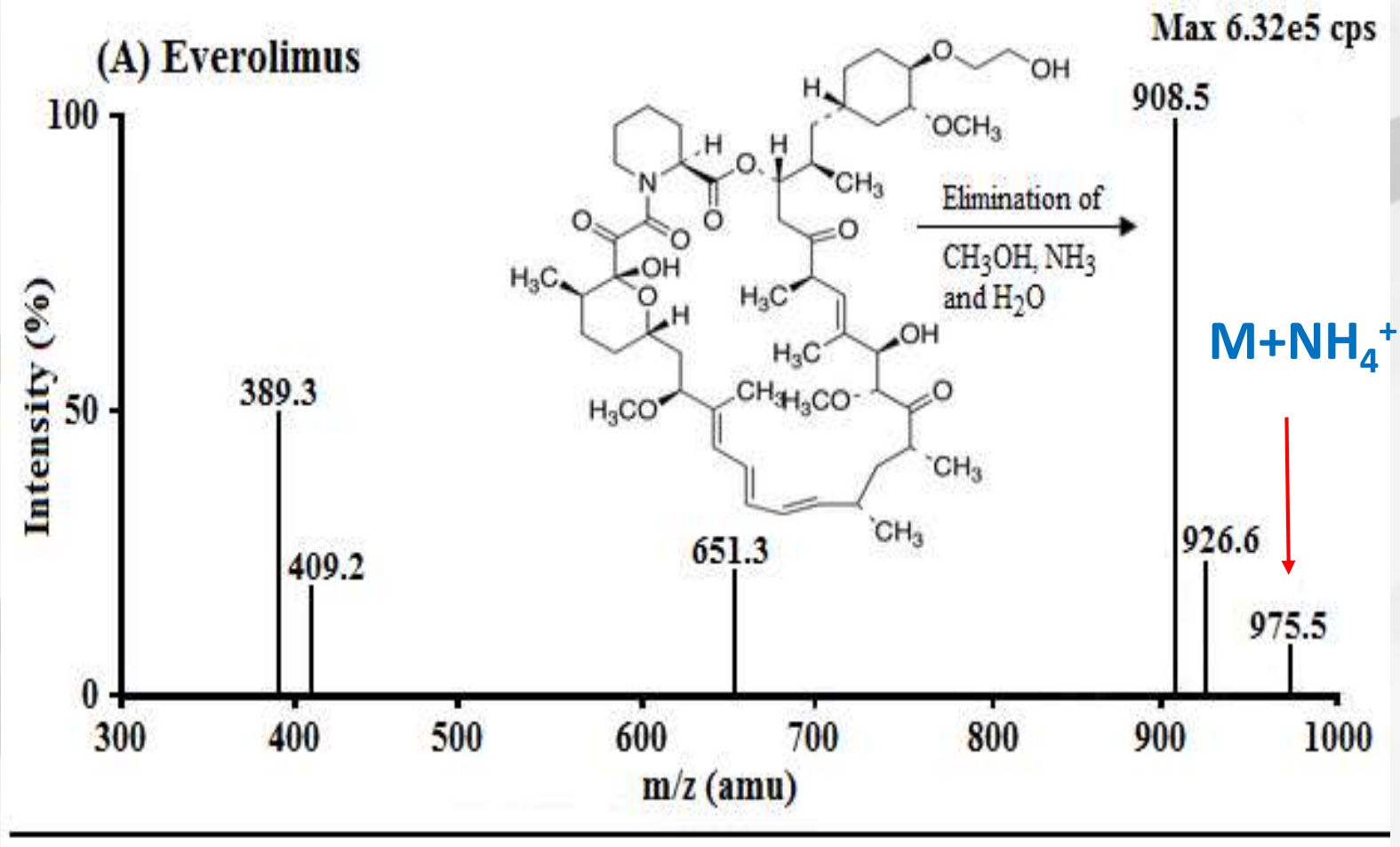






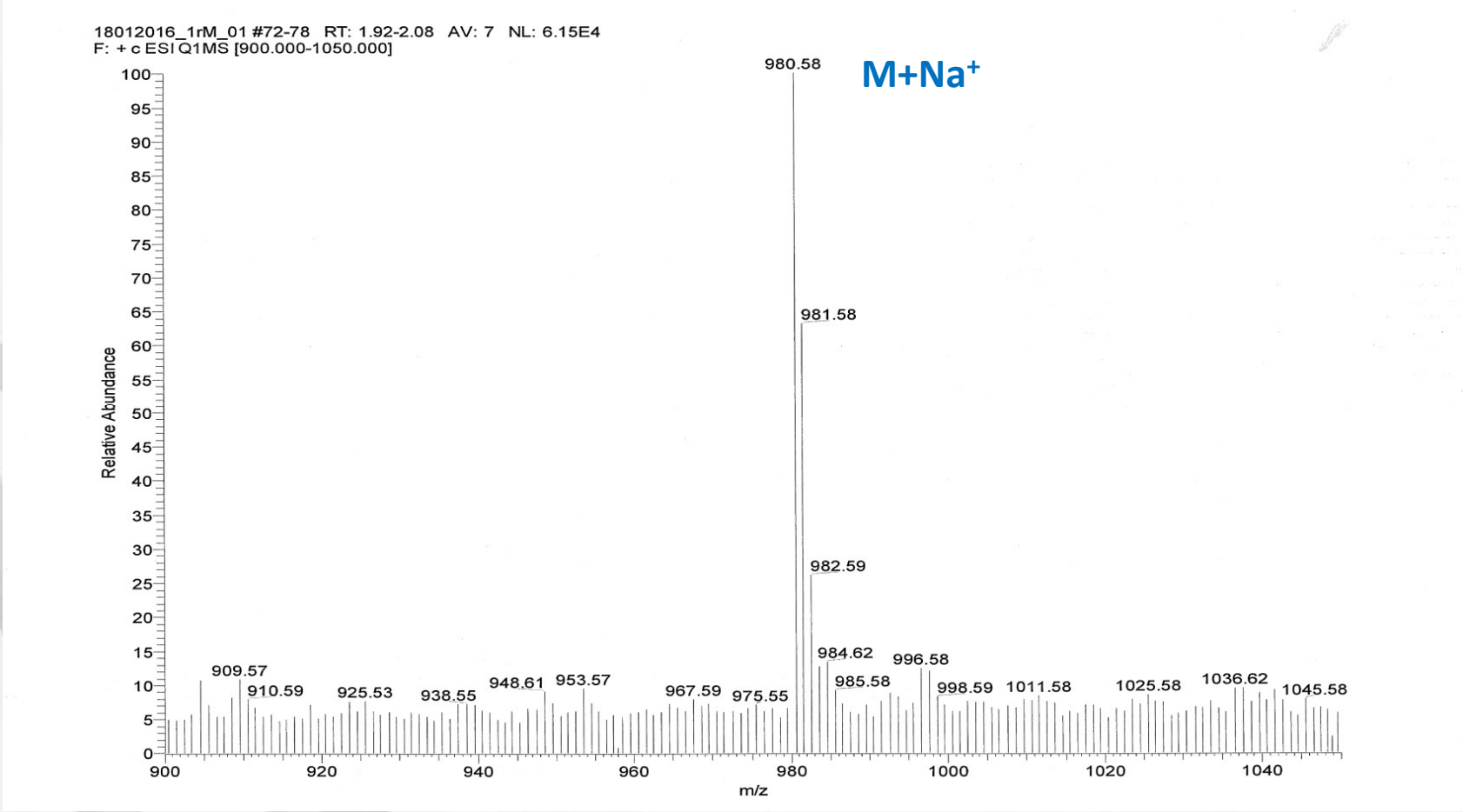
EVEROLIMUS
MS/MS spectrum

(V. Upadhyay et al)



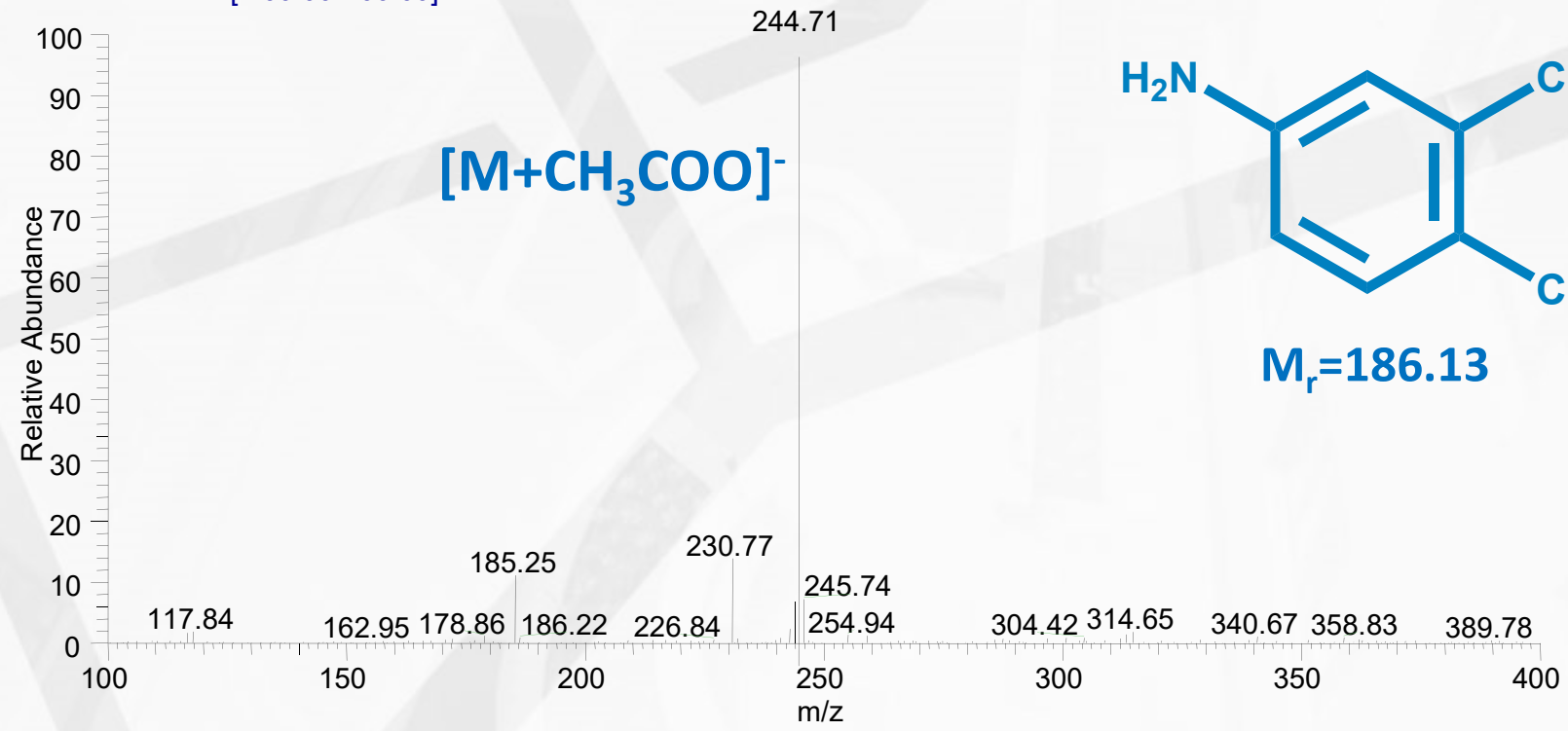
EVEROLIMUS
Full-scan spectrum

(Our lab)

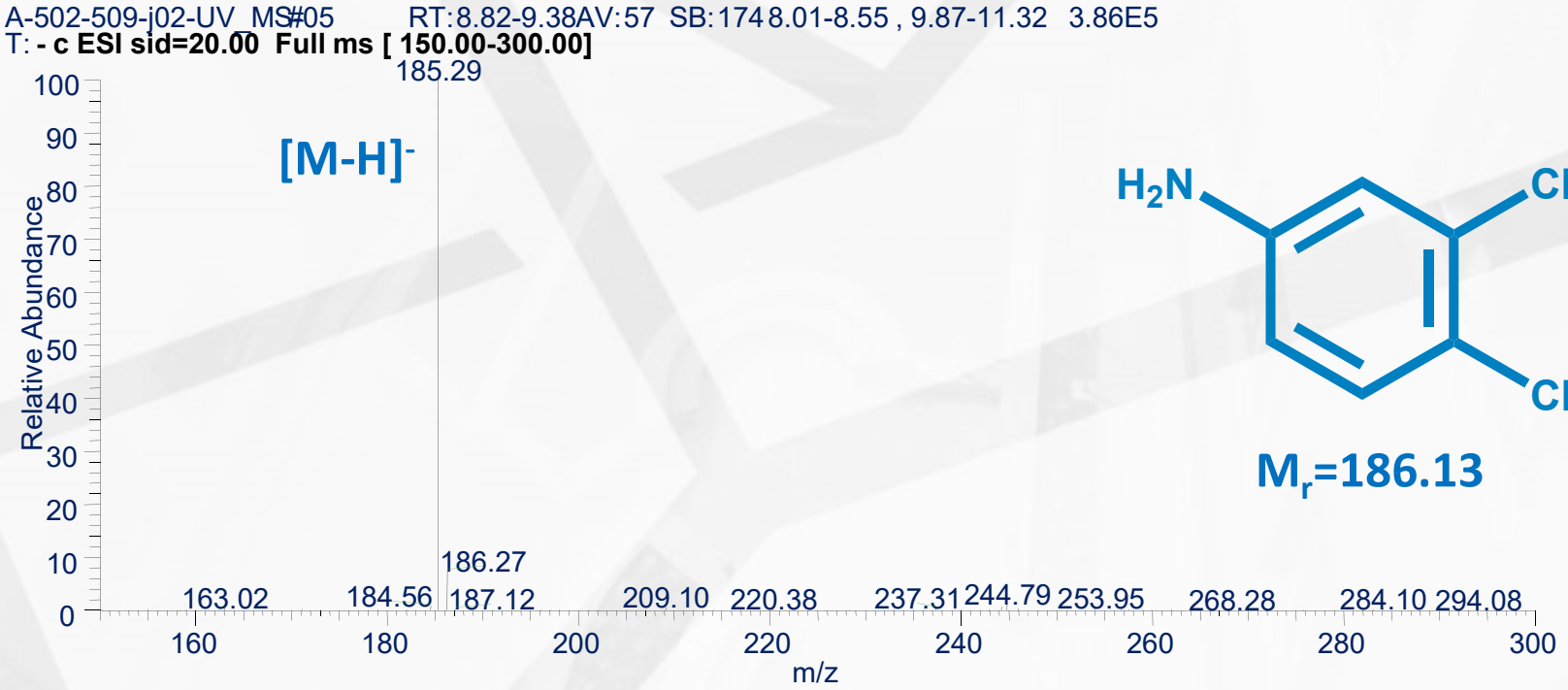


EXAMPLE – CLUSTER FORMATION

negative ion mode



MS/MS spectrum - $[M - H]^-$



M_r=186.13

ION PAIR REAGENT - ADSORPION PHENOMENON

Example 2

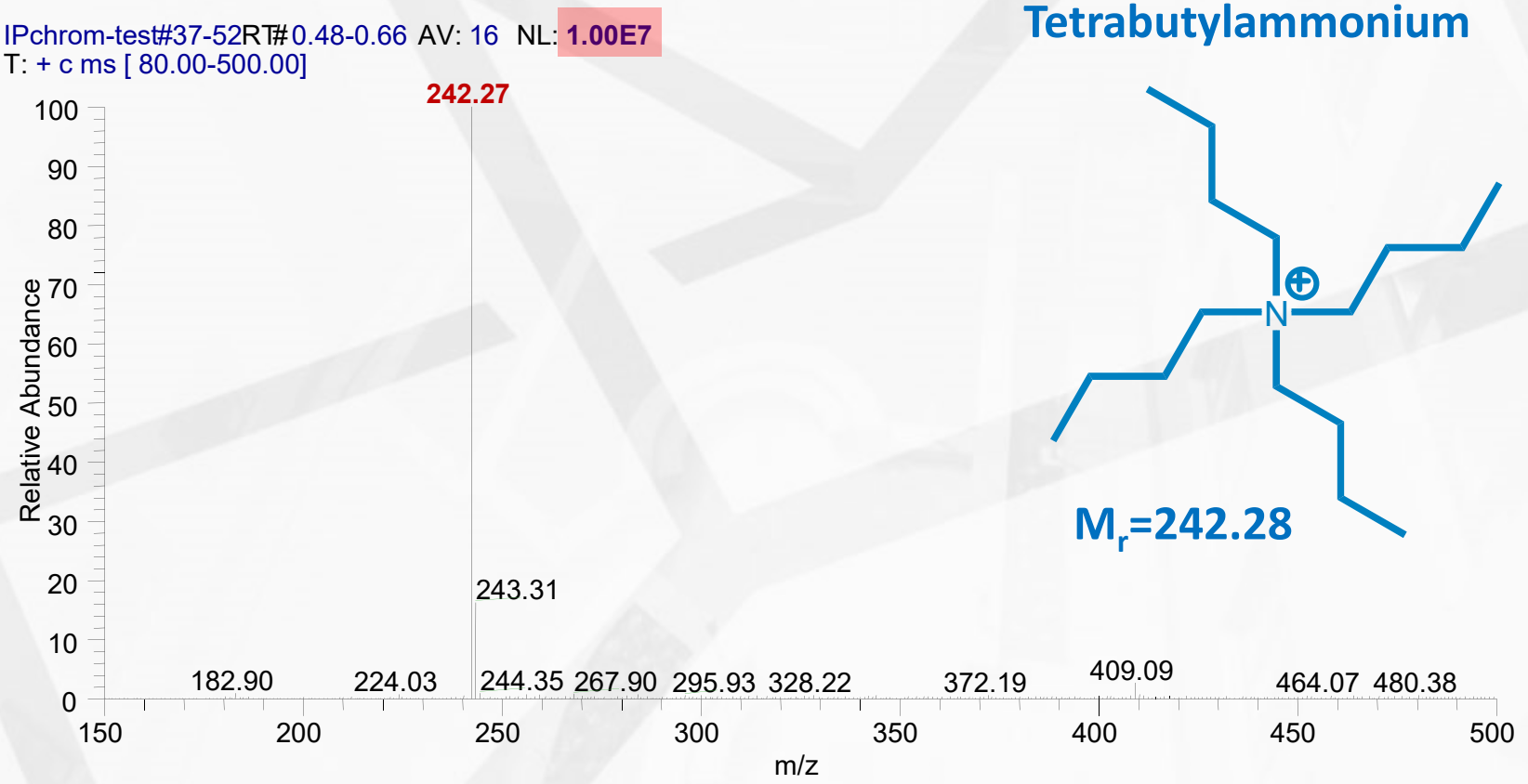
Ion pair reagents must not be used in mobile phases. The memory (adsorption) effects remain for years.

No cleaning procedure is effective enough to remove the contamination.

Even the exchange of chromatographic system does not help.

Do not use ion pairs reagents in LC/MS, unexpected ion reactions might occur

MS spectrum obtained after the use…

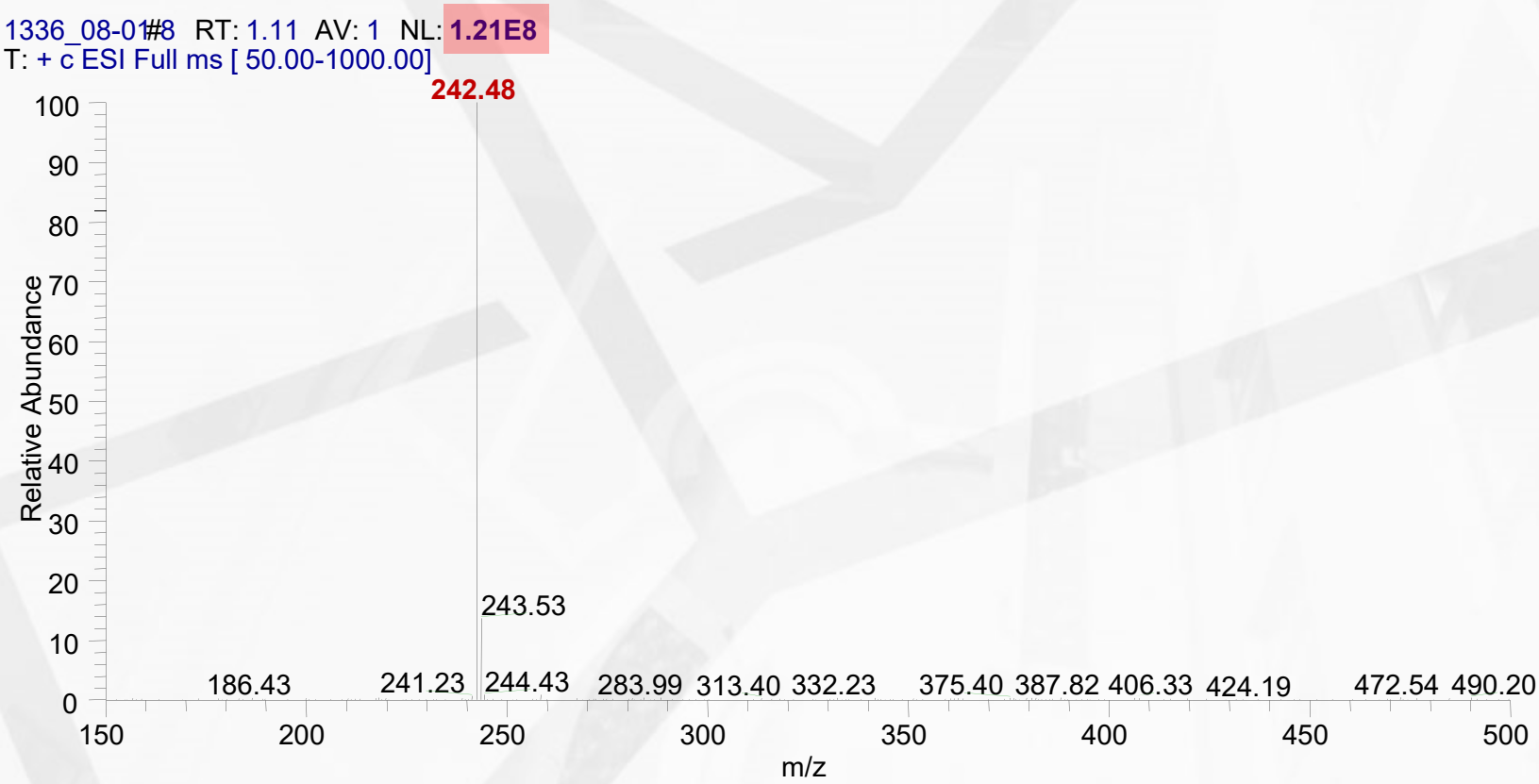


Tetrabutylammonium

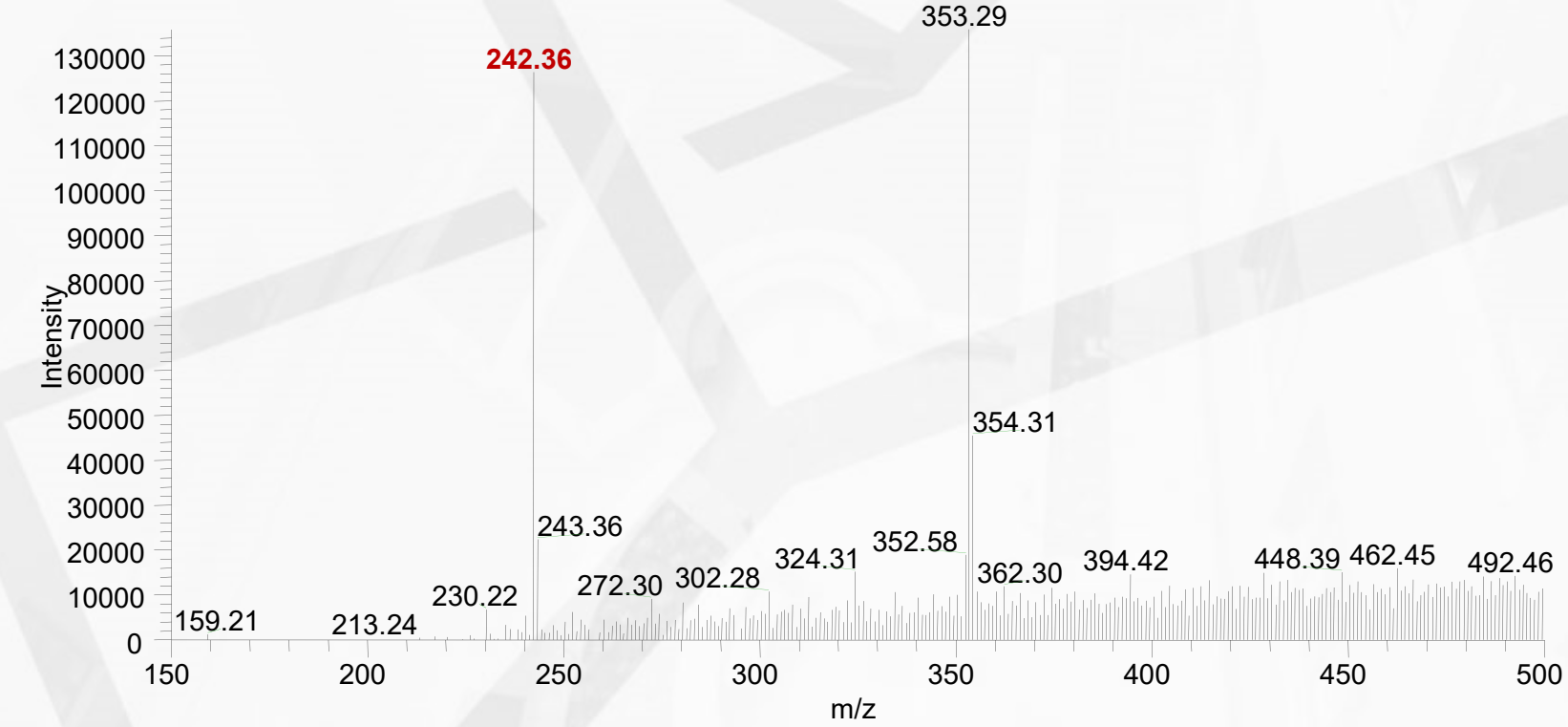
M_r=242.28

… 2 months later…

(after the intense cleaning of the whole system and replacement of LC)



… three years later



HOW TO AVIOD PROBLEMS WITH MATRIX EFFECT ?

How to **remove, avoid, minimize or compensate** the matrix effect?

TWO STRATEGIES

1.**Separation of all impurities**

use more effective chromatographic methods and techniques like LLE, SPE, UPLC, HILIC…and/or **Sample dilution** frequent strategy in literature

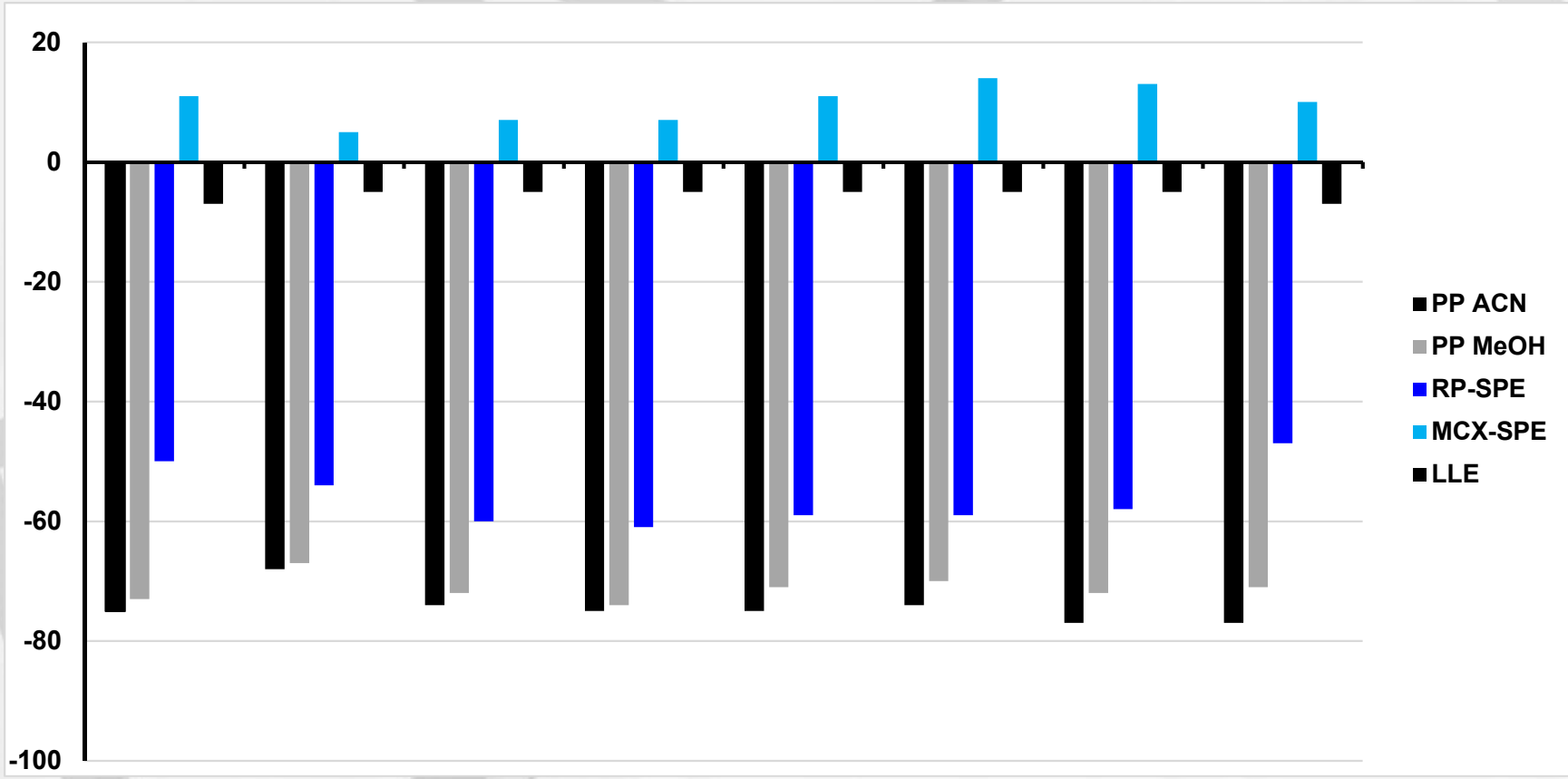
2. **Accepting the matrix effect as unavoidable**

compensate or exclude negative impacts on quantitative results

use of internal standards especially of those labelled by stable isotopes (isotope dilution technique)

No thorough clean-up mostly needed

SAMPLE PROCESSING (PURIFICATION) OF 8 DRUGS IN PLASMA



E. Chambers et al., J. Chromatog.B, 852, 22–34
Graphics L. Nováková, **2007**

IMPORTANCE OF IS LABELLED BY STABLE ISOTOPES

- Requirements for the exact position of labelling in the molecule of an analyte usually are not very strict as a rule
- Isotope labelling may be mostly chosen:
- By synthesis availability but the presence of non-labelled analyte must be excluded. Isotope exchange reactions during sample processing must be avoided (position of non exchangable deuterium atoms is important)
- **^{13}C and ^{15}N isotopes** have low isotope effect, they are **optimal** for use but expensive. The availability of such IS is limited
- **Deuterium** easily available but exhibits a high isotope effect
- 3 deuterium atoms are optimal, **high number of deuterium atoms and the labeling close to some functional groupes may considerably increase the kinetic isotope effect**

DETERMINATION OF MATRIX EFFECT BY EMA

133 methods of drugs determination in plasma

MF > 1.15 - (ion enhancement)

MF < 0.85 - (ion suppression)

(EMA EVALUATION)

	Drug assayed	IS labelling	Ion. mode	SP	MFa	MFs	MF norm.	CV MF norm. %	Note
1	Alendronate	$^{2}H_6$	HESI (+)	PP-D	**12.615**	14.151	0.892	**2.400**	
2	Hydroxypioglitazon	$^{2}H_4$	APCI (+)	PP	**5.963**	5.832	1.017	**3.300**	
3	Esomeprazol	$^{2}H_3$	HESI (+)	PP	**5.789**	5.937	0.975	**1.190**	
4	Pioglitazon	$^{2}H_4$	APCI (+)	PP	**2.663**	2.610	1.020	**1.300**	
5	Fosfomycin	$^{13}C_3$	HESI (-)	PP	**2.464**	2.475	0.998	**1.710**	
6	S-Bicalutamide	$^{2}H_4$	APCI (-)	SPE	**1.827**	1.833	1.080	**1.520**	
7	Gliclazide	$^{2}H_4$	APCI (+)	PP	**1.821**	2.030	0.897	**3.800**	
8	Atorvastatin	$^{2}H_5$	HESI (+)	LLE	**1.622**	1.572	1.032	**1.490**	
9	Moxifloxacin	$^{2}H_4$	HESI (+)	PP	**1.611**	1.580	1.020	**0.860**	
10	Bosentan	$^{2}H_4$	HESI (+)	PP	**1.607**	1.664	0.965	**2.020**	
11	Naproxen	$^{13}C^{2}H_3$	ESI (-)	PP	**1.530**	1.558	0.982	**1.500**	
12	Propafenone	$^{2}H_5$	ESI (+)	PP	**1.438**	1.454	0.990	**0.780**	
13	Milnacipran	$^{2}H_4$	HESI (+)	PP	**1.406**	1.363	1.029	**1.940**	
14	Olmesartan	$^{2}H_4$	ESI (+)	PP	**1.306**	1.303	1.003	**2.900**	
15	Ponatinib	$^{2}H_8$	ESI (+)	PP	**1.285**	0.810	1.606	**8.220**	rat plasma
16	Ritonavir	$^{2}H_6$	APCI (+)	PP	**1.280**	1.275	1.004	**0.960**	
17	Fenspiride	$^{2}H_5$	ESI (+)	PP	**1.272**	1.269	1.004	**2.500**	

18	Ticagrelor	$^{2}H_{7}$	HESI (-)	PP	**1.218**	1.227	0.993	**2.750**	
19	(-)Tianeptine	$^{2}H_{6}$	ESI (+)	SPE	**1.217**	1.215	1.002	**0.900**	
20	(+)Tianeptine	$^{2}H_{6}$	ESI (+)	SPE	**1.211**	1.202	1.007	**1.000**	
21	Pramipexol	$^{2}H_{5}$	HESI (+)	LLE	**1.173**	1.172	1.001	**0.580**	
22	Drospirenon	$^{2}H_{4}$	HESI (+)	PP	**1.159**	1.204	0.962	**0.570**	
23	Propiverine	$^{2}H_{7}$	HESI (+)	PP	**1.156**	1.126	1.026	**1.300**	
24	R-Bicalutamide	$^{2}H_{4}$	APCI (-)	SPE	**1.156**	1.157	0.999	**1.280**	
25	Drotaverin	$^{2}H_{5}$	HESI (+)	PP	**1.140**	1.135	1.003	**3.040**	
26	Torasemide	$^{2}H_{7}$	HESI (-)	SPE	**1.108**	1.111	0.997	**0.500**	
27	Levothyroxine	$^{13}C_{6}$	HESI (+)	SPE	**1.103**	0.721	1.020	**0.820**	
28	4-Hydroxyatorvastatin	$^{2}H_{5}$	HESI (+)	LLE	**1.097**	1.086	1.010	**2.030**	
29	Tolterodine	$^{2}H_{5}$	HESI (+)	PP	**1.094**	0.991	1.104	**1.300**	
30	Prednisolone	$^{2}H_{6}$	HESI (-)	PP	**1.093**	1.093	1.000	**0.760**	
31	Nevirapine	$^{2}H_{3}$	HESI (+)	PP	**1.086**	0.910	1.194	**4.400**	
32	Amlodipin	$^{2}H_{4}$	HESI (+)	SPE	**1.081**	1,085	1.000	**1.280**	
33	*p*-Acetamidobenzoic Acid	$^{2}H_{3}$	HESI (-)	PP	**1.077**	1.088	0.991	**1.240**	
34	Ornidazol	$^{2}H_{5}$	HESI (+)	PP	**1.071**	1.089	0.984	**0.940**	
35	Everolimus	$^{2}H_{4}$	HESI (+)	BP	**1.063**	1.151	0.925	**3.410**	**Full blood**
36	Sildenafil	$^{2}H_{8}$	HESI (+)	PP	**1.047**	1.084	1.011	**2.760**	
37	Paracetamol	$^{2}H_{3}$	HESI (-)	PP	**1.043**	1.080	0.966	**2.120**	
38	Bendroflumethiazide	$^{2}H_{5}$	HESI (-)	PP	**1.043**	1.053	0.990	**0.710**	
39	2-Hydroxyatorvastatin	$^{2}H_{5}$	HESI (+)	LLE	**1.038**	1.050	0.988	**1.200**	
40	Fingolimod	$^{2}H_{4}$	HESI (+)	BP	**1.037**	1.047	0.990	**0.740**	**Full blood**
41	Imatinib	$^{2}H_{8}$	APCI (+)	PP	**1.029**	1.042	0.988	**1.900**	
42	Tenofovir	$^{2}H_{7}$	HESI (+)	PP	**1.029**	1.028	1.001	**0.810**	
43	Letrozol	$^{2}H_{4}$	APCI (+)	LLE	**1.024**	1.040	0.985	**2.100**	
44	Celecoxib	$^{2}H_{7}$	ESI (+)	PP	**1.020**	0.985	1.036	**4.000**	
45	Olanzapin	$^{2}H_{3}$	ESI (+)	LLE	**1.017**	0.003	1.024	**2.170**	
46	Rasagiline	$^{13}C_{3}$	HESI (+)	SPE	**1.014**	1.023	0.991	**0.920**	

47	Chlorambucil	$^{2}H_{8}$	HESI (+)	PP	1.011	0.999	1.012	**0.330**	
48	Pacritinib-M1 (SB1518)	$^{2}H_{6}$	HESI (+)	PP	1.011	1.017	0.994	**0.820**	**Mouse plasma**
49	Aciclovir	$^{2}H_{4}$	ESI (+)	PP	1.010	1.035	0.976	**1.500**	
50	Anastrozol	$^{2}H_{12}$	APCI (+)	LLE	1.009	1.010	1.000	**1.100**	
51	CT1812	$^{2}H_{4}$	HESI (+)	PP	1.009	1.013	0.996	**0.640**	**Rat plasma**
52	Linezolid	$^{2}H_{3}$	APCI (+)	PP	1.001	0.996	1.004	**1.150**	
53	Atomoxetine	$^{2}H_{5}$	HESI (+)	PP	1.001	0.977	1.025	**1.200**	
54	Azithromycin	$^{13}C^{2}H_{3}$	ESI (+)	PP	1.000	0.997	1.003	**1.930**	
55	Paliperidone	$^{2}H_{4}$	ESI (+)	PP	1.000	1.015	0.985	**1.100**	
56	Levetiracetam	$^{2}H_{6}$	APCI (+)	PP	0.995	1.021	0.974	**0.220**	
57	Montelukast	$^{2}H_{8}$	HESI (+)	PP	0.994	0.933	1.066	**2.000**	
58	Acetazolamide	$^{2}H_{3}$	ESI (-)	PP	0.993	1.003	0.990	**1.540**	
59	Voriconazol	$^{2}H_{3}$	APCI (+)	PP	0.993	0.985	1.009	**1.500**	
60	Ezetimibe Phenoxy Glucuronide	$^{2}H_{4}$	HESI (-)	LLE	0.990	1.008	0.982	**1.000**	
61	Losartan	$^{2}H_{4}$	HESI (+)	PP	0.989	0.985	1.004	**1.520**	
62	Agomelatine	$^{2}H_{6}$	HESI (+)	PP	0.989	1.001	0.988	**0.820**	
63	Cefprozil	$^{2}H_{4}$	HESI (+)	PP	0.981	0.964	1.018	**2.530**	
64	Pacritinib-M1 (SB1518)	$^{2}H_{6}$	HESI (+)	PP	0.981	0.967	1.015	**0.720**	**Rat plasma**
65	Ivabradine	$^{2}H_{6}$	ESI (+)	PP	0.980	0.956	1.025	**0.460**	
66	Ranolazine	$^{2}H_{5}$	APCI (+)	PP	0.980	0.977	1.003	**1.080**	
67	Abiraterone	$^{2}H_{4}$	HESI (+)	PP	0.979	0.978	1.002	**0.740**	
68	Hydrocortisone	$^{2}H_{4}$	HESI (+)	PP	0.977	0.963	0.995	**0.950**	
69	Bisoprolol	$^{2}H_{7}$	ESI (+)	PP	0.974	0.969	1.005	**1.300**	
70	Lenalidomide	$^{13}C_{5}$	HESI (+)	PP	0.971	0.977	0.994	**0.540**	
71	Aripiprazol	$^{2}H_{8}$	HESI (+)	PP	0.970	0.929	1.044	**2.600**	
72	Ganciclovir	$^{2}H_{5}$	HESI (+)	PP	0.968	0.949	1.020	**0.550**	
73	Pacritinib (SB1518)	$^{2}H_{4}$	HESI (+)	PP	0.968	0.951	1.018	**0.690**	**Rat plasma**
74	Metronidazol	$^{2}H_{4}$	APCI (+)	PP	0.967	0.971	0.996	**1.500**	
75	Paraxanthine	$^{2}H_{3}$	HESI (+)	PP	0.967	0.972	0.995	**0.390**	

76	CT1812	$^{2}H_{4}$	HESI (+)	PP	0.966	1.028	0.940	**2.040**	**Dog plasma**
77	Pacritinib (SB1518)	$^{2}H_{4}$	HESI (+)	PP	0.964	0.949	1.016	**0.540**	**Mouse plasma**
78	Indapamide	$^{2}H_{3}$	HESI (+)	BP	0.948	0.949	0.999	**1.850**	**Full blood**
79	Memantine	$^{2}H_{6}$	APCI (+)	SPE	0.946	0.942	1.004	**1.100**	
80	Ranolazine	$^{2}H_{5}$	HESI (+)	PP	0.942	1.009	0.934	**3.840**	
81	Valsartan	$^{2}H_{9}$	HESI (+)	PP	0.936	0.879	1.065	**1.760**	
82	Pacritinib-M1 (SB1518)	$^{2}H_{6}$	HESI (+)	PP	0.935	0.933	1.003	**0.710**	**Human plasma**
83	Cilostazol	$^{2}H_{4}$	HESI (+)	PP	0.934	0.891	1.048	**1.030**	
84	Anagrelide	$^{13}C_{3}{}^{2}H_{2}$	HESI (+)	PP	0.930	0.928	1.001	**0.660**	
85	Megestrol	$^{2}H_{3}$	ESI (+)	PP	0.920	0.922	0.995	**1.660**	
86	Vardenafil	$^{2}H_{5}$	ESI (+)	PP	0.917	0.922	0.995	**1.660**	
87	Duloxetine	$^{13}C^{2}H_{3}$	HESI (+)	PP	0.911	0.888	1.026	**0.500**	
88	Pacritinib (SB1518)	$^{2}H_{4}$	HESI (+)	PP	0.911	0.911	1.000	**0.660**	**Rabbit plasma**
89	Ethinylestradiol	$^{2}H_{4}$	HESI (+)	SPE-D	0.910	0.913	0.997	**0.330**	
90	Desloratadine	$^{2}H_{5}$	HESI (+)	PP	0.910	0.898	1.013	**5.300**	
91	Capecitabine	$^{2}H_{11}$	ESI (+)	PP	0.901	0.903	0.998	**0.390**	
92	Cefuroxim	$^{2}H_{3}$	HESI (-)	PP	0.901	0.899	1.002	**0.560**	
93	Ipidacrine	$^{2}H_{6}$	HESI (+)	PP	0.901	0.910	0.989	**0.340**	
94	Diclofenac	$^{2}H_{4}$	HESI (-)	PP	0.900	0.890	1.010	**2.600**	
95	Eplerenone	$^{13}C^{2}H_{3}$	HESI (+)	SPE	0.897	0.946	0.948	**0.960**	
96	Mycophenolic acid	$^{2}H_{3}$	HESI (-)	PP	0.894	1.000	0.892	**7.560**	
97	Solifenacin	$^{2}H_{5}$	HESI (+)	PP	0.891	0.845	1.050	**3.030**	
98	Tadalafil	$^{13}C^{2}H_{3}$	APCI (+)	PP	0.885	0.898	0.986	**3.100**	
99	Levothyroxine	$^{13}C_{6}$	HESI (-)	SPE	0.882	0.899	1.009	**3.500**	
100	Valganciclovir	$^{2}H_{5}$	HESI (+)	PP	0.865	0.848	1.020	**1.400**	
101	Nerofe	$^{13}C_{14}{}^{15}N_{2}$	HESI (+)	PP	0.864	0.936	0.921	**5.490**	
102	Toremifene	$^{2}H_{6}$	HESI (+)	PP	0.863	0.869	0.991	**0.940**	
103	Pacritinib (SB1518)	$^{2}H_{4}$	HESI (+)	PP	0.862	0.856	1.008	**1.310**	**Human plasma**
104	Hydrochlorothiazide	$^{13}C^{2}H_{2}$	HESI (-)	PP	0.859	0.840	1.022	**1.350**	

105	Raloxifen	$^{2}H_4$	ESI (+)	LLE	**0.857**	0.857	0.999	**5.810**	
106	Caffeine	$^{2}H_3$	HESI (+)	PP	**0.852**	0.851	1.002	**0.960**	
107	Ibuprofen	$^{2}H_3$	HESI (-)	PP	**0.848**	0.838	1.012	**3.290**	
108	Dexketoprofen	$^{2}H_3$	ESI (-)	PP	**0.848**	0.832	1.020	**0.800**	
109	Rosuvastatin	$^{2}H_7$	HESI (+)	LLE	**0.841**	0.831	1.012	**0.900**	
110	Zidovudine	$^{2}H_3$	HESI (+)	PP	**0.838**	0.826	1.015	**8.320**	
111	Escitalopram	$^{2}H_3$	ESI (+)	PP	**0.835**	0.822	1.020	**1.680**	
112	Dutasteride	$^{13}C_6$	HESI (-)	SPE	**0,830**	0.801	1.037	**1.160**	
113	Ezetimibe	$^{2}H_4$	HESI (-)	LLE	**0.825**	0.735	1.125	**2.160**	
114	Pentoxifylline	$^{2}H_6$	HESI (+)	PP	**0.788**	0.774	1.018	**2.780**	
115	2-OH-Metronidazol	$^{2}H_2$	APCI (+)	PP	**0.787**	0.792	0.994	**4.900**	
116	Liothyronine	$^{13}C_9{}^{15}N$	HESI (-)	SPE	**0.769**	0.771	1.002	**2.300**	
117	Flurbiprofen	$^{2}H_4$	ESI (-)	PP	**0.768**	0.740	1.036	**4.500**	
118	Cefditoren	$^{2}H_3$	HESI (+)	PP	**0.765**	0.758	1.010	**2.800**	
119	Candesartan	$^{2}H_4$	HESI (+)	PP	**0.764**	0.765	1.000	**1.480**	
120	Mebeverine acid	$^{2}H_5$	HESI (+)	PP	**0.763**	0.725	1.053	**2.240**	
121	Metformin	$^{2}H_6$	ESI (+)	PP	**0.728**	0.744	0.979	**0.820**	
122	Amlodipin	$^{2}H_4$	HESI (+)	DPI	**0.617**	0.607	1.018	**2.910**	
123	Mebeverine acid, O-desmethyl	$^{2}H_5$	HESI (+)	PP	**0.588**	0.523	1.127	**2.950**	
124	Ponatinib	$^{2}H_8$	ESI (+)	PP	**0.575**	0.568	1.012	**1.430**	**Dog plasma**
125	Pregabalin	$^{2}H_4$	HESI (+)	PP	**0.510**	0.514	0.993	**1.060**	
126	Ciprofloxacin	$^{2}H_8$	HESI (+)	PP	**0.481**	0.558	0.987	**2.570**	
127	Meloxicam	$^{2}H_3$	HESI (+)	PP	**0.408**	0.415	0.984	**0.880**	
128	Lisinopril	$^{2}H_5$	HESI (+)	SPE	**0.349**	0.363	0.963	**0.560**	
129	Entecavir	$^{2}H_2$	HESI (+)	PP	**0.348**	0.338	1.031	**2.090**	
130	Methyldopa	$^{2}H_3$	HESI (+)	PP	**0.341**	0.338	1.010	**0.400**	
131	Ramiprilat	$^{2}H_5$	HESI (+)	PP	**0.297**	0.285	1.043	**5.530**	
132	N,N-Dimethylamino-2-propanol	$^{2}H_6$	HESI (+)	PP	**0.280**	0.201	1.389	**2.050**	
133	Ramipril	$^{2}H_5$	HESI (+)	PP	**0.259**	0.257	1.008	**2.480**	
	MEAN				**1.152**	**1.146**	**1.012**	**1.852**	
	STDEV				**1.215**	**1.329**	**0.072**	**1.518**	
	MAX				**12.615**	**14.151**	**1.606**	**8.320**	
	GEOMEAN				**0.983**	**0.929**	**1.010**		

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LEGEND

- **APCI (+)** Atmospheric pressure chemical ionisation (positive)
- **ESI (+/-)** Electrospray ionisation (positive/negative)
- **HESI** Heated electrospray
- **PP** Protein precipitation
- **PP/D** Protein precipitation and subsequent derivatization
- **LLE** Liquid/liquid extraction
- **SPE** Solid phase extraction
- **SPE/D** Solid phase extraction and subsequent derivatization

SUMMARY OF MATRIX EFFECTS EVALUATION

Matrix effect has been evaluated according to EMA in a relatively high number of LC/MS/MS methods of quantitative determination of drugs using different:

- **biological matrices (mainly plasma)**
- **IS labelling**
- **ionization techniques**
- **sample processing**
- **instruments**
- **different laboratories**

• • •

133	validated methods in total
124	at QUINTA - ANALYTICA Prague
9	at QUINTA - ANALYTICA Yaroslavl (Russian Federation)
121	human plasma
2	dog plasma
4	rat plasma
2	mouse mlasma
1	rabbit plasma
3	full blood

118	$^{2}H_{n}$ IS labelling (n=2-10)
15	^{13}C and/or ^{15}N IS labelling
116	ESI or HESI either in pozitive or negative mode
17	APCI
102	protein precipitation (PP) for sample processing
1	PP with subsequent derivatization
11	LLE
15	SPE
1	SPE and subsequent derivatization
1	Direct on line injection
3	Full blood processing

...

Ion enhancement (MFa≥1.15) in 24 cases

Ion suppression (MFa≤0.85) in 27 cases

CV MFnorm MAX = 8.320% (average 1.852%)

100% COMPENSATION OF NEGATIVE IMPACT

SUMMARY STATISTICS

Method	Number	MFa			MF norm		CV MF norm %	
		Average	MAX	MIN	Average	MAX	Average	MAX
APCI	17	**1.490**	**5.963**	**0.787**	0.998	1.080	1.871	4.900
ESI+HESI	116	**1.102**	**12,615**	**0.259**	1.014	1.606	1.849	8.320
PP	102	**1.077**	**5.963**	**0,259**	1.016	1.606	1.925	8.320
LLE	11	**1.045**	**1.622**	**0.825**	1.014	1.125	1.867	5.810
SPE	15	**1.034**	**1.827**	**0.349**	1.004	1.080	1.209	3.500
^{13}C / ^{15}N	15	**1.077**	**2.464**	**0.769**	0.996	1.037	1.763	5.490
^{2}H	118	**1.161**	**12.615**	**0.277**	1.014	1.606	1.863	8.320
In total	**133**	**1.152**	**12.615**	**0.259**	**1.012**	**1.606**	**1.852**	**8.320**

MATRIX EFFECT OF PACRITINIB AND ITS METABOLITE IN DIFFERENT PLASMA SAMPLES

Drug assayed	Plasma	IS labelling	Ionisation mode	SP	MFa	MFs	MF norm.	CV MF norm. %
Pacritinib	rat	$^{2}H_4$	HESI (+)	PP	**0.968**	0.951	1.018	**0.690**
Pacritinib	mouse	$^{2}H_4$	HESI (+)	PP	**0.964**	0.949	1.016	**0.540**
Pacritinib	human	$^{2}H_4$	HESI (+)	PP	**0.862**	0.856	1.008	**1.310**
Pacritinib	rabbit	$^{2}H_4$	HESI (+)	PP	**0.911**	0.911	1.000	**0.660**
Pacritinib-M1	rat	$^{2}H_6$	HESI (+)	PP	**0.981**	0.967	1.015	**0.720**
Pacritinib-M1	mouse	$^{2}H_6$	HESI (+)	PP	**1.011**	1.017	0.994	**0.820**
Pacritinib-M1	human	$^{2}H_6$	HESI (+)	PP	**0.935**	0.933	1.003	**0.710**

NO DIFFERENCE IN MFa PARAMETERS !!!

MATRIX EFFECT OF PONATINIB IN TWO ANIMAL SPECIES

Drug assayed	Plasma	IS labelling	Ionisation mode	Sample processing	MFa	MFs	MF norm.	CV MF norm. %
Ponatinib	Rat	$^{2}H_{8}$	ESI (+)	PP	**1.285**	0.810	1.606	**8.220**
Ponatinib	Dog	$^{2}H_{8}$	ESI (+)	PP	**0.575**	0.568	1.012	**1.430**

MATRIX EFFECT OF MILNACIPRAM (MNC) IN TWO MS SYSTEMS OF THE SAME TYPE

	MFa	MF norm.	CV MF norm. %
Equipment 1	**1.406**	1.029	**1.94**
Equipment 2	**1.019**	1.013	**3.41**

Significant difference of MF, no difference of Mf norm and no difference of CV Mfnorm %

3 METHODS WITH
0.85 ≥ MFnorm ≥ 1.15

Drug assayed	IS labelling	Ionisation mode	SP	MFa	MFs	MF norm.	CV MF norm. %
Ponatinib in rat plasma	$^{2}H_{8}$	ESI (+)	PP	1.285	0.810	**1.606**	**8.220**
N,N-Dimethylamino-2-propanol	$^{2}H_{6}$	HESI (+)	PP	0.280	0.201	**1.389**	**2.050**
Nevirapine	$^{2}H_{3}$	HESI (+)	PP	1.09	0.91	**1.194**	**4.400**

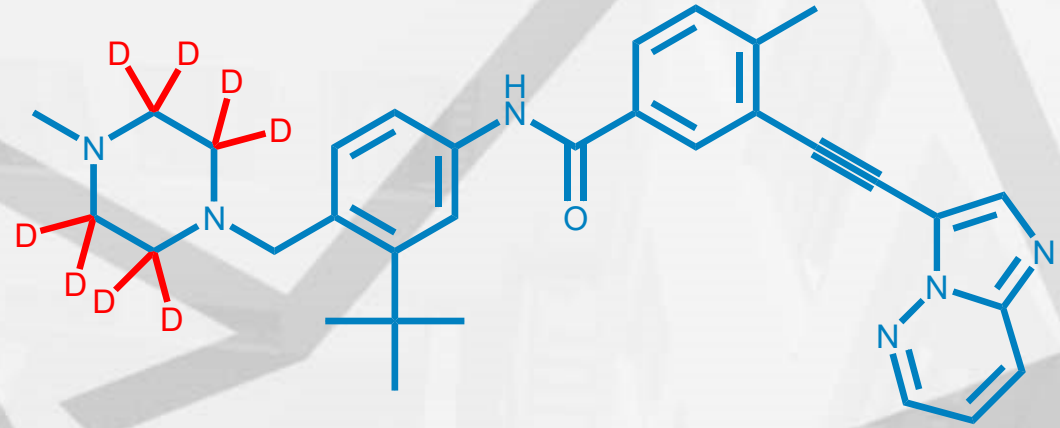
Possible explanation for high isotopic effect:
high number of deuterium atoms near the charge location

Significant influence on slope of calibration curve (cf Matuszewski), however no influence on accuracy of concentration determination when individual calibration curve for each analytical lot of samples is used.

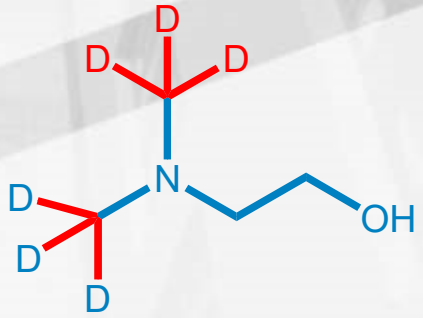
CV MF norm less than 15%, precision o.k.

POSSIBLE ISOTOPE EFFECTS

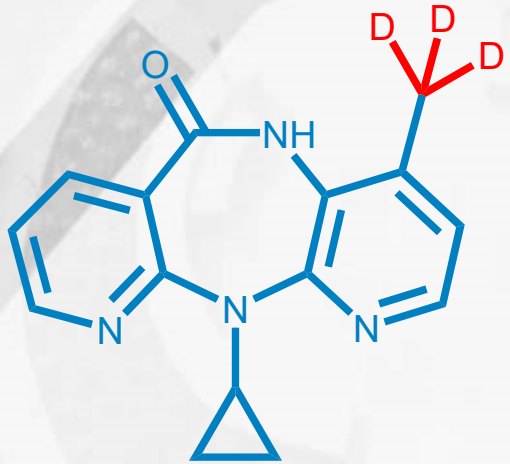
Ponatinib-D_8



N,N-Dimethylamino-2-propanol-D_6



Nevirapine-D_3



CONCLUSION

1) Important role of adsorption effects in the ionization proces has been shown. These effects cannot be simply removed/avoided and should be considered within our extended term of “Matrix effect” understanding (definition) in LC/MS

2) Matrix effect has been evaluated according to EMEA in 133 LC/MS/MS methods using isotopically labelled internal standards, 4 different methods of sample processing, 4 different ionization modes and 7 different LC/MS/MS instruments. EMA criterion **CV MFnorm <15%** was met by all methods used

3) The number of ion enhancements and ion suppressions cases were practically equal (24 and 27 resp.)

4) “Matrix effect” cannot be removed but its negative impacts on the quantitative determination of drugs in human and animal plasma may be 100% compensated when isotopically labelled internal standards are used

REFERENCES

- T. G. Hall et al, INTECH Open Access Publisher, **2012**
- E. Chambers et al, Journal of Chromatography B, 852, 22–34, **2007**
- P. Panuwet et al, Crit. Rev. Anal. Chem., 46(2), 93–105, **2016**
- L. Silvestro et al, Biochemistry, Genetics and Molecular Biology, "Tandem Mass Spectrometry – Molecular Characterization", **2013**
- B.K. Matuszewski , Journal of Chromatography B, 830, 293–300, **2006**
- V.P. Shah et al, Pharm. Res. 17, 15551, **2000**
- T. Sangster et al, Rapid Commun. Mass Spectrom., 18, 1361, **2004**
- B.K. Matuszevski et al, Anal Chem, 75, 3019, **2003**
- L. Nováková, 17th Summer School on Mass Spectrometry, Luhačovice, **2016**
- V. Upadhyay et al, Journal of Advancement in Medical and Life Science, 1, 1-11, **2014**
- M. Ryska, Eur. J. Mass Spectrom, 21, 423-432, **2O15**

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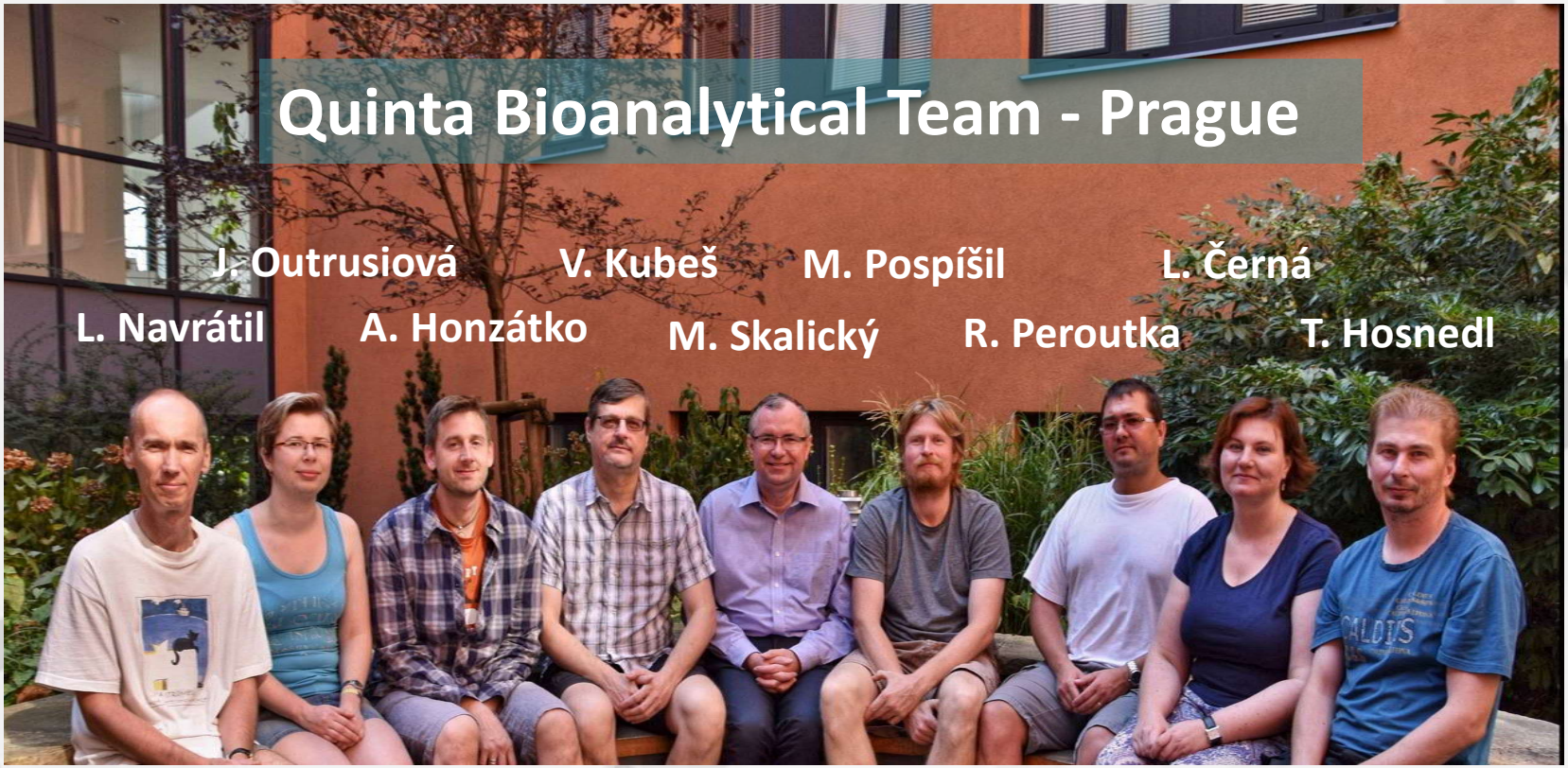

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