The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation and its use in clinical applications.

Amedeo De Nicolò

Laboratory of Clinical Pharmacology and Pharmacogenetics

Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy

Amedeo De Nicolò
Disclosure/Conflict of Interest

Grant/Research Support: none

Consultant/Advisor: Shimadzu

Sponsored Lectures: Merck-Millipore

Sponsored Writing: Novartis

Shareholder Company: CoQua Lab srl

from last 5 years to date

Liquid chromatography-mass spectrometry (LC-MS)

Tandem mass spectrometry (LC-MS/MS)

Spectrum of clinical chemistry applications

becoming

Standard tool for the analysis of endogenous and exogenous substances in both biological and environmental research laboratories, as well as in many clinical laboratories.

LC-MS

Very high sensitivity
Very high specificity
Very high analytical accuracy.

Process of ionization: degradation/ fragmentation of molecules.

"Matrix effect" (ME) (alteration of ionization efficiency caused by components of the matrix sample into the ion source) that have a differential impact on target analytes and internal standard compounds.

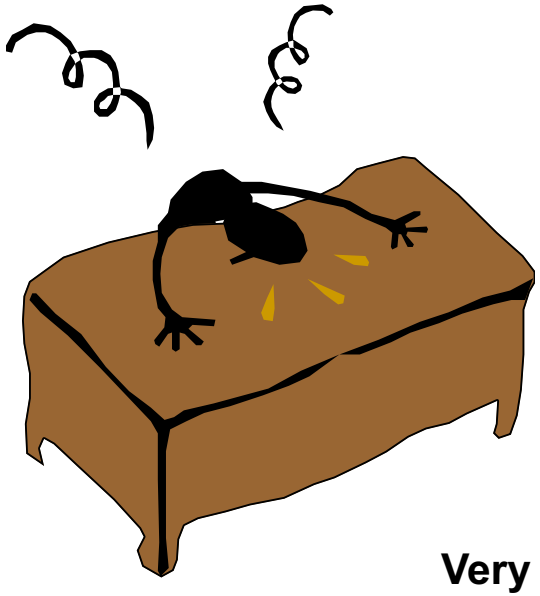
[1] M. Vogeser, C. Seger, Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory, Clin Chem, 56 (2010) 1234-1244.

Clinical Chemistry 56:8
1234–1244 (2010)

Reviews

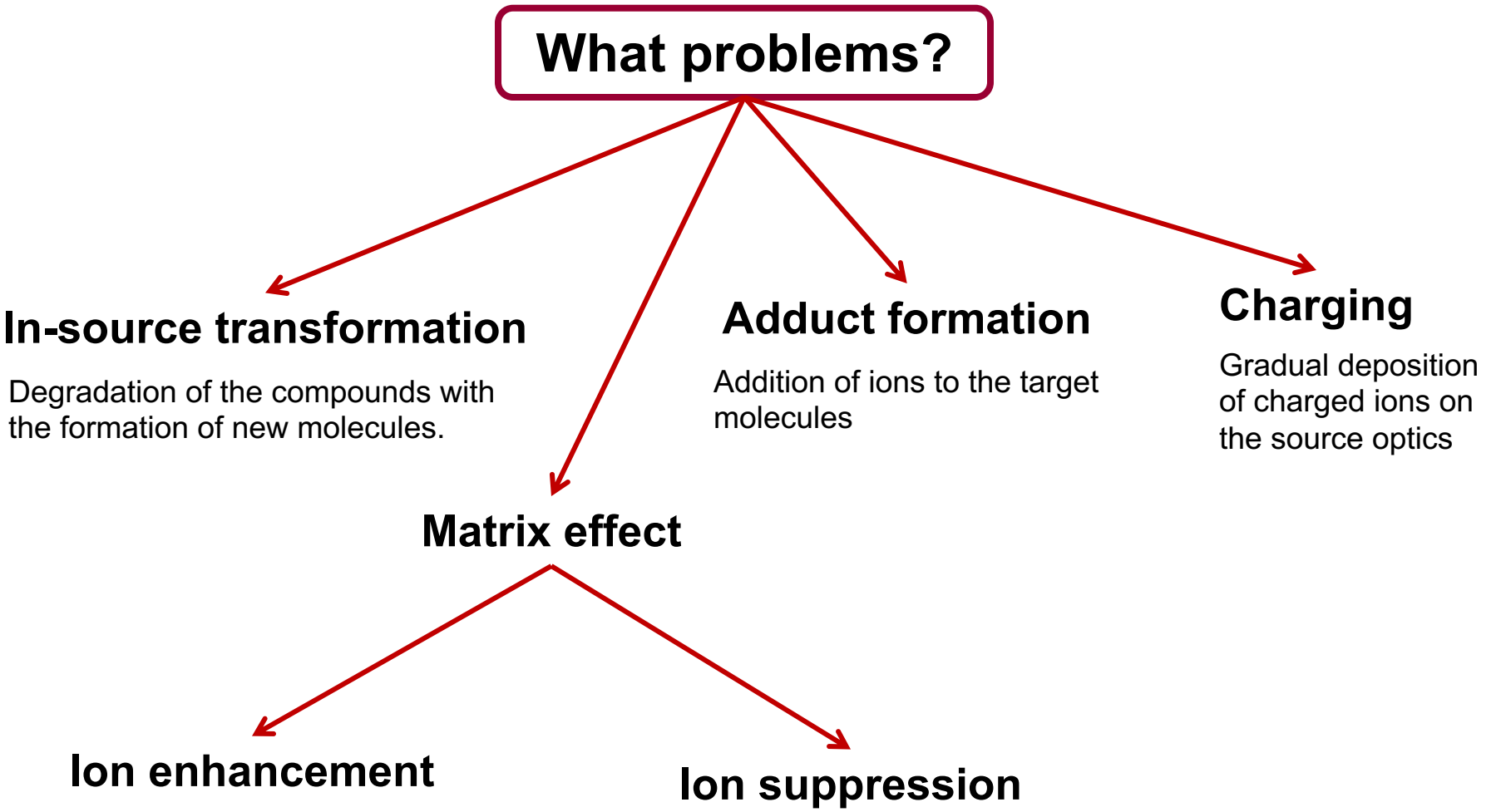
Pitfalls Associated with the Use of Liquid Chromatography–Tandem Mass Spectrometry in the Clinical Laboratory

Michael Vogeser[1*] and Christoph Seger[2*]



Tip: Read this paper!!!

Very interesting overview of the issues associated with MS/MS


What problems?
In-source transformation
Degradation of the compounds with the formation of new molecules.
Adduct formation
Addition of ions to the target molecules
Charging
Gradual deposition of charged ions on the source optics
Matrix effect
Ion enhancement
Ion suppression

COUNTERMEASURES?

- **Optimization of extraction procedures** (eg. solid phase extraction).

Against matrix effect, charging and adduct formation

- **Optimization of tune methods and ionization parameters** (eg. injection volume, chromatographic separation, curtain gas, right probe).

Against matrix effect and charging

- Engineering of mass spectrometers.

Against matrix effect

- **Use of stable-isotope-labeled internal standards.**

Against EVERYTHING

- **Thorough evaluation of matrix effect**, as suggested in many international guidelines [1-6] for LC-MS bio-analytical method validation, especially when it is used in a clinical routine.

[1] M. Vogeser, C. Seger, Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory, Clin Chem, 56 (2010) 1234-1244.
[2] EMA, Guideline on bioanalytical method validation, (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf), (2011).
[3] FDA, Guidance for Industry: Bioanalytical Method Validation (Rev. 1), in, 2013.
[4] SWGTOX, Standard practices for method validation in forensic toxicology, (http://www.swgtox.org/documents/Validation3.pdf), (2013).
[5] CLSI, C62-A - Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline, in, 2014.
[6] O. Gonzalez, M.E. Blanco, G. Iriarte, L. Bartolome, M.I. Maguregui, R.M. Alonso, Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect, J Chromatogr A, 1353 (2014) 10-27.

Scientific Working Group for
SWGTOX
Forensic Toxicology

SWGTOX Doc 003
Revision 1
Published May 20, 2013

Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2011
EMEA/CHMP/EWP/192217/2009
Committee for Medicinal Products for Human Use (CHMP)

Guideline on bioanalytical method

Guidance for Industry

Bioanalytical Method Validation

DRAFT GUIDANCE

s guidance document is being distributed for comment purposes only.

and suggestions regarding this draft document should be submitted within 90 days of in the *Federal Register* of the notice announcing the availability of the draft Submit electronic comments to http://www.regulations.gov. Submit written to the Division of Dockets Management (HFA-305), Food and Drug Administratio rs Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with number listed in the notice of availability that publishes in the *Federal Register*.

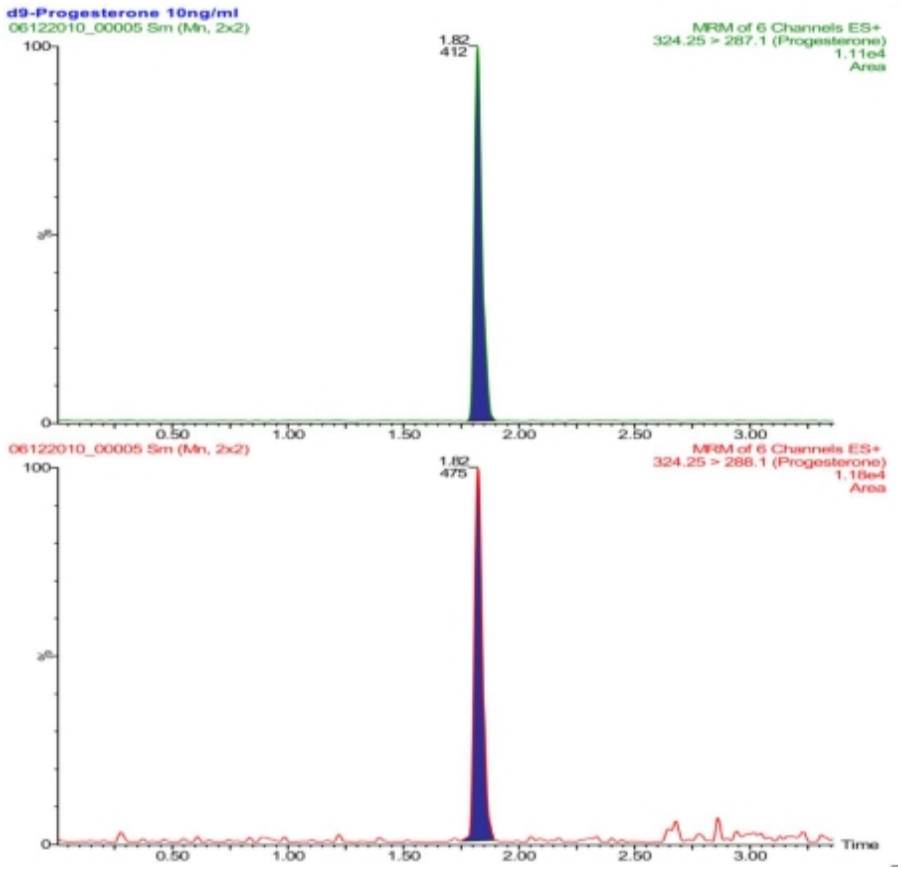
ns regarding this draft document contact (CDER) Brian Booth, 301-796-1508 or n Kadavil, John.Kadavil@fda.hhs.gov

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

September 2013
Biopharmaceutics

Revision 1

A Representative Example of Deuterium Scrambling on Tandem Quad MS: Progesterone-D_9 (324->287); Progesterone (315->279)



Review

For reprint orders, please contact reprints@future-science.com

Bioanalysis

Alternative matrices for therapeutic drug monitoring of immunosuppressive agents using LC–MS/MS

Mwlod Ghareeb[1] & Fatemeh Akhlaghi*[,1]

[1]Clinical Pharmacokinetics Research Laboratory, Department of Biomedical & Pharmaceutical Sciences, 495A College of Pharmacy, University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, USA

*Author for correspondence:
Tel.: +1 401 874 9205
Fax: +1 401 874 5787
fatemeh@uri.edu

Key terms

Matrix effect: Interference of matrix components with ionization of the analyte of interest at the ionization site in LC–MS/MS, resulting in ion enhancement or suppression.

Deuterated IS: Isotopic analogue that co-elutes with the analyte of interest and therefore would have similar chromatographical conditions.

Same chemical structure and same RT => Same behavior

http://www.sigmaaldrich.com/technical-documents/articles/biology/mitigation-of-deuterium-scrambling-in-stable-labeled-internal-st.html

• However, sometimes, the use of stable-isotope-labeled internal standards is not possible…

- the analyte does not exist on the market
- In multiplexed methods buying all the isotope-labeled IS would be too expensive.
- both above or other reasons…

Journal of Pharmaceutical and Biomedical Analysis 129 (2016) 535–541

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

UHPLC–MS/MS method with protein precipitation extraction for the simultaneous quantification of ten antihypertensive drugs in human plasma from resistant hypertensive patients

Amedeo De Nicolò (BSc, MSc)[a,*,1], Valeria Avataneo[a,1], Franco Rabbia[b], Gabriele Bonifacio[a], Jessica Cusato[a], Cristina Tomasello[a], Elisa Perlo[b], Paolo Mulatero[b], Franco Veglio[b], Giovanni Di Perri[b], Antonio D'Avolio[a]

Different chemical structure and RT => Different behavior

Internal Standard

Different Matrix Effects

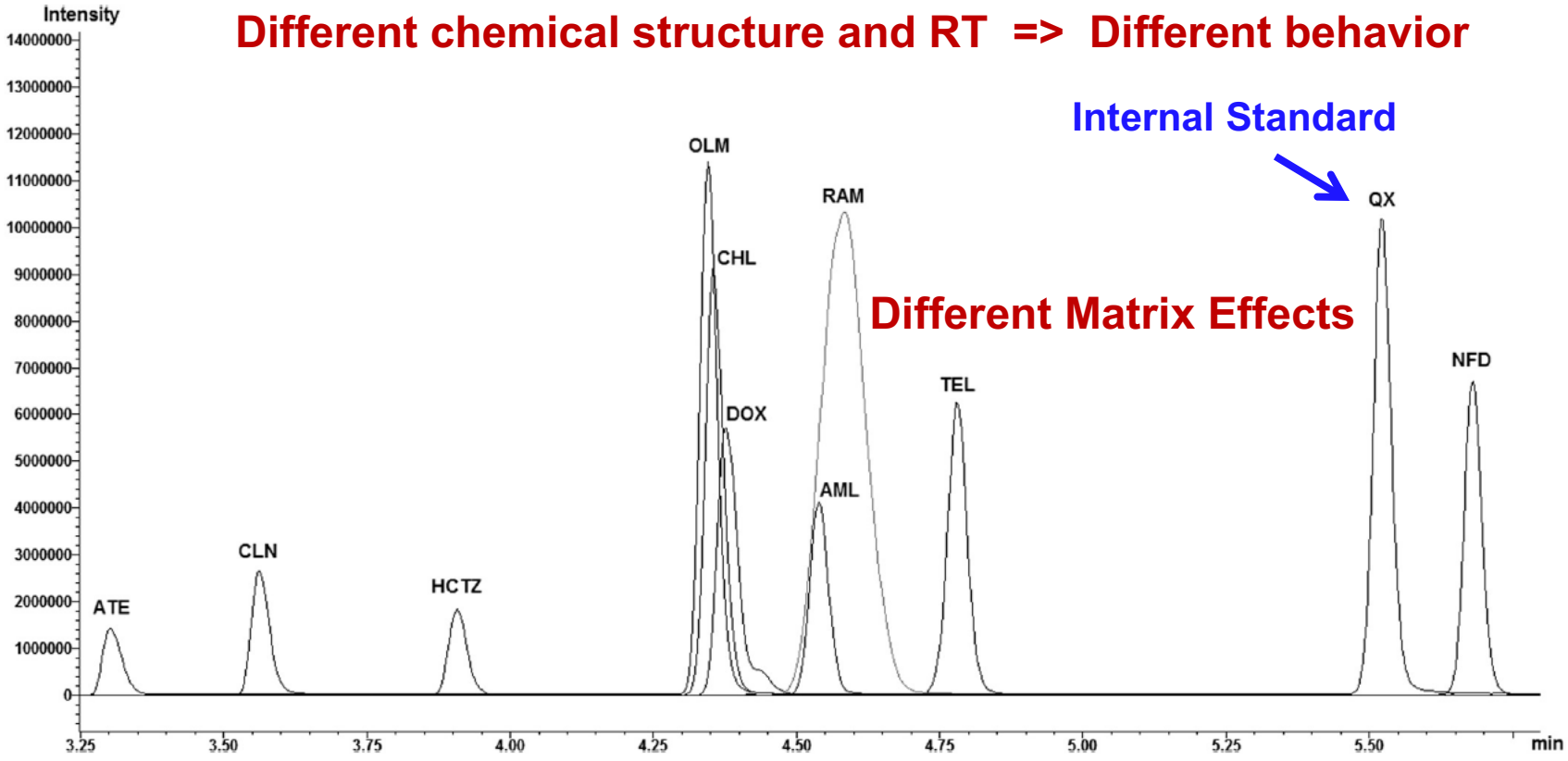


Fig. 1. Overlaid chromatograms of each analyte from direct injection of a chemical mix.

- **Matrix effects could be both positive and negative.**

- **Sometimes matrix effects are impossible to eliminate.**

- **Do you know if IS correct your analites from recoveries and matrix effects point of view?**

No labeled IS…. Different RT…. Matrix effect….

How do you know that your results are acceptable?

Guidelines are not so clear about indications or rules

TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]

The ME have to be calculated for each lot of matrix and for each analyte(s) and IS(s) as in equation 1:

$$ME\% = \left[\left(\frac{Peak\ area\ analyte\ in\ matrix}{Peak\ area\ analyte\ in\ neat\ sample}\right) - 1\right] * 100$$

Equation 1: standard equation for the calculation of the matrix effect. The same equation is applied to both analytes and ISs.

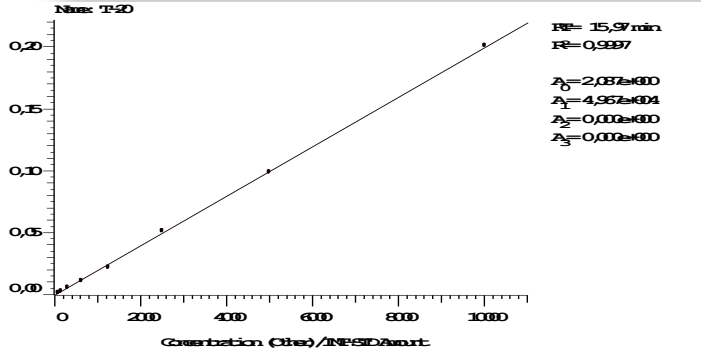
MIX injection at the same concentration in neat solvent

The mean of ME% is commonly used to describe the ME. Usually this is the minimum parameter required that should be evaluated; however, this does not always correctly describe the impact of ME on the analytical results, that originate by a ratio between analyte and IS.

D'Avolio A. *et. al.* Submitted 2016

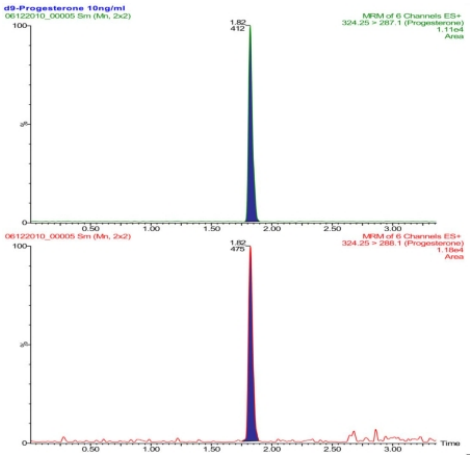
TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]



$$Response = \frac{Peak\ area\ analyte}{Peak\ area\ IS}$$

Equation 2: Definition of the chromatographic response.



Ratio between peak area (or height) of analyte and its Internal Standard

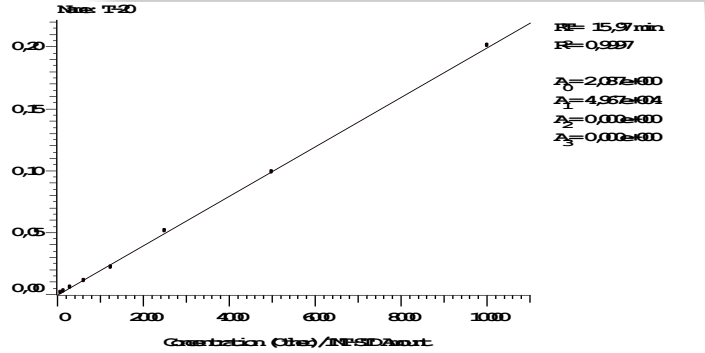
Same chemical structure and same RT => <u>Same</u> behavior

<u>The mean of ME% is commonly used to describe the ME</u>. Usually this is the minimum parameter required that should be evaluated; <u>however, this does not always correctly describe the impact of ME on the analytical results, that originate by a ratio between analyte and IS.</u>

D'Avolio A. et. Al. Submitted 2016

TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]



$$Response = \frac{Peak\ area\ analyte}{Peak\ area\ IS}$$

Equation 2: Definition of the chromatographic response.

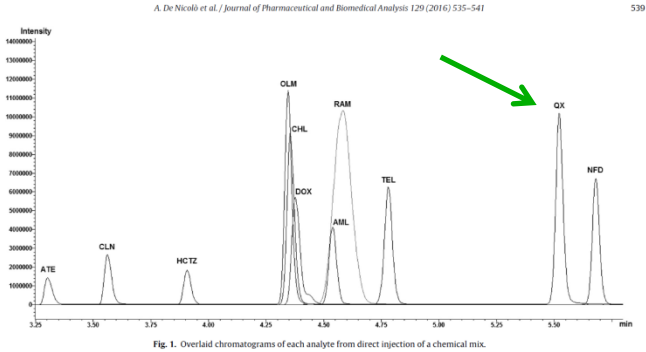


Fig. 1. Overlaid chromatograms of each analyte from direct injection of a chemical mix.

Ratio between peak area (or height) of analyte and its Internal Standard

Different chemical structure and RT => Different behavior

The mean of ME% is commonly used to describe the ME. Usually this is the minimum [illegible] evaluated, however, it does not always correctly describe the impact of ME on the analytical results that is appropriately by a ratio between analyte and IS.

We should evaluate the ME for each analyte relatively to its IS: an IS-normalized ME (IS-nME)

D'Avolio A. et. Al. Submitted 2016

What is the "internal standard normalized matrix effect" (IS-nME)?

TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]

$$Response = \frac{Peak\ area\ analyte}{Peak\ area\ IS}$$

Equation 2: Definition of the chromatographic response.

Since the processing of results is based on the ratio between the peak of the analyte and the one of IS (equation 2), the IS-nME is defined by the ratio between the response in matrix and the one in the standard neat sample (equation 3).

$$IS - nME\% = \left[\left(\frac{Response\ in\ matrix}{Response\ in\ neat}\right) - 1\right] * 100$$

$$= \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}}\right) - 1\right] * 100$$

Equation 3: Equation for the correct estimation of the IS-nME in each sample.

D'Avolio A. et. Al. Submitted 2016

TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]

$$IS - nME\% = \left[\left(\frac{Response\ in\ matrix}{Response\ in\ neat}\right) - 1\right] * 100$$

$$= \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}}\right) - 1\right] * 100$$

Equation 3: Equation for the correct estimation of the IS-nME in each sample.

The mean IS-nME% should possibly not exceed the ±15%.

ANYWAY

The mean value is well counterbalanced by the adoption of a calibration curve prepared in matrix!!

In these cases, the main source of error is its variability!!!!!

D'Avolio A. et. Al. Submitted 2016

TITLE: The Internal Standard Normalized Matrix Effects (IS-nME): a new tool for the mass spectrometry method validation.

AUTHORS: Antonio D'Avolio[1], Amedeo De Nicolò[1], Marco Cantù[2]

The coefficient of variation (CV%) or the relative standard deviation (RSD%) of the IS-nME should be evaluated on at least 6 different matrix lots!!

The value shoud not exceed the 15%!!

$$CV\%\ of\ IS-nME = \frac{Standard\ Deviation\ of\ IS-nME\%}{Mean\ IS-nME\% + 100}$$

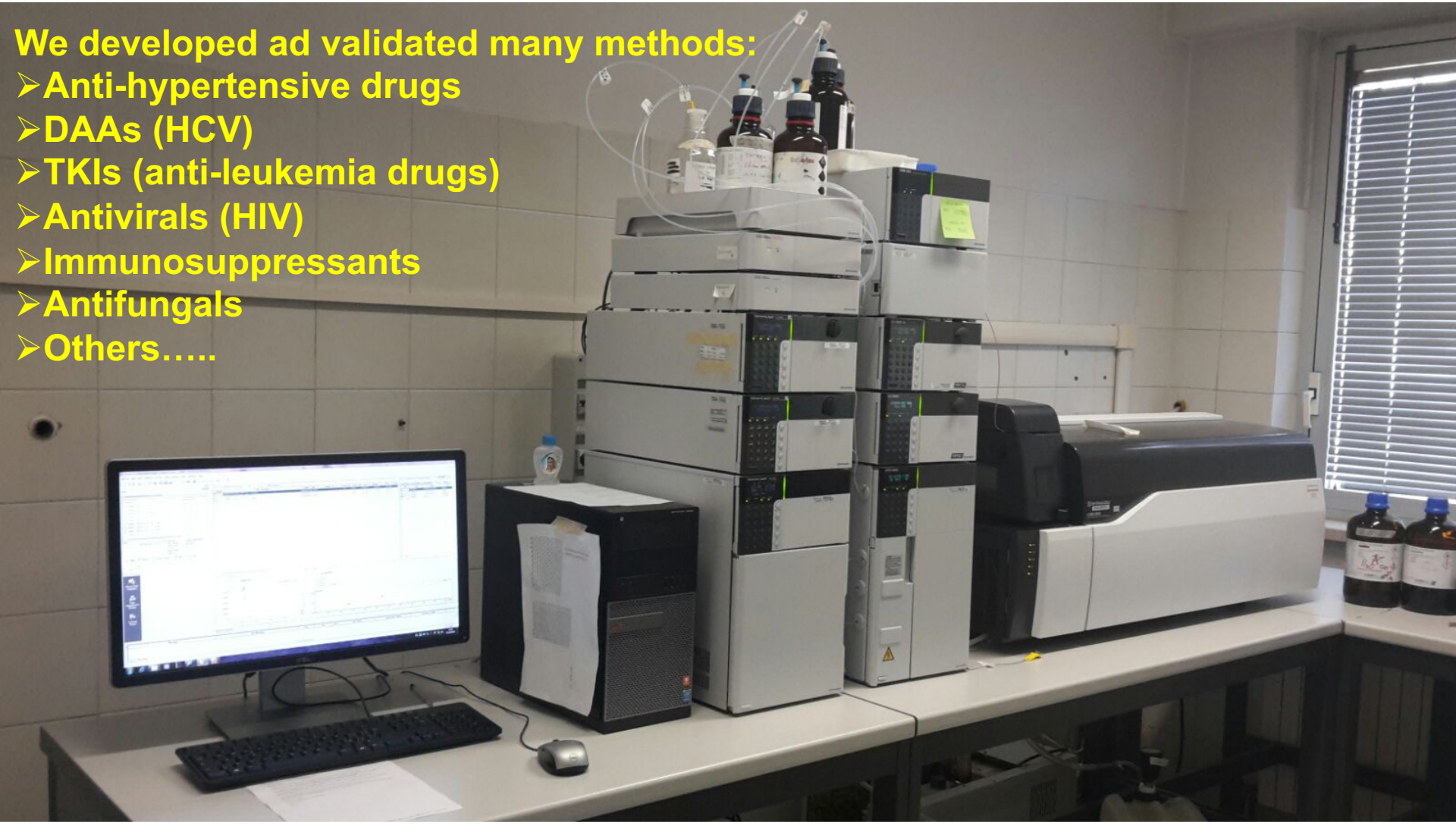
Equation 4: Equation for the evaluation of the reproducibility of IS-nEM.

D'Avolio A. et. Al. Submitted 2016

...practical examples...

Laboratory of Clinical Pharmacology and Pharmacogenetics

Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy

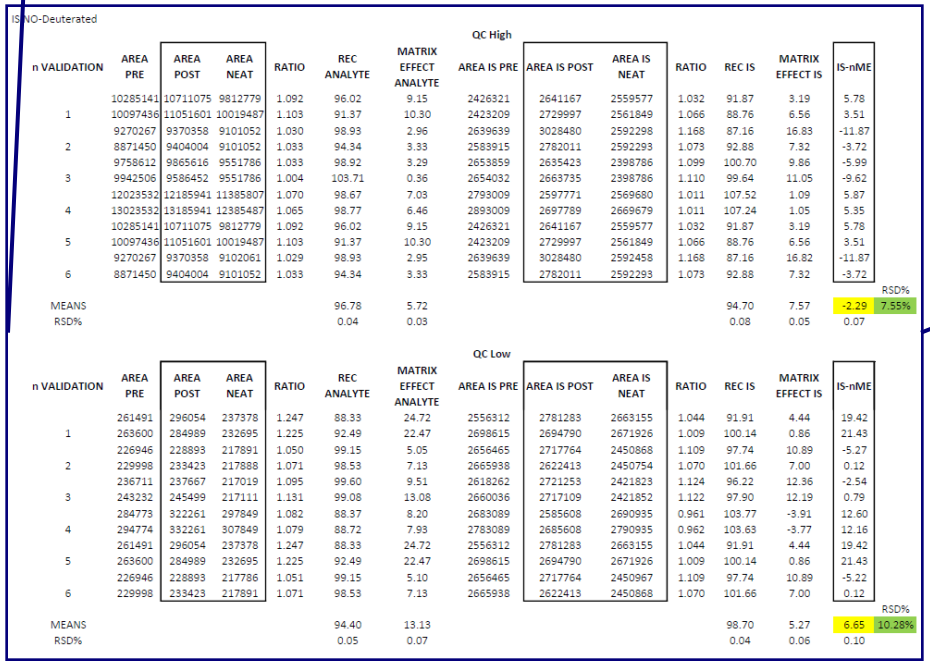


UHPLC Shimadzu Nexera + SPE on line + LC-8050 Mass Spec. Detector

IS NO-Deuterated

QC High

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

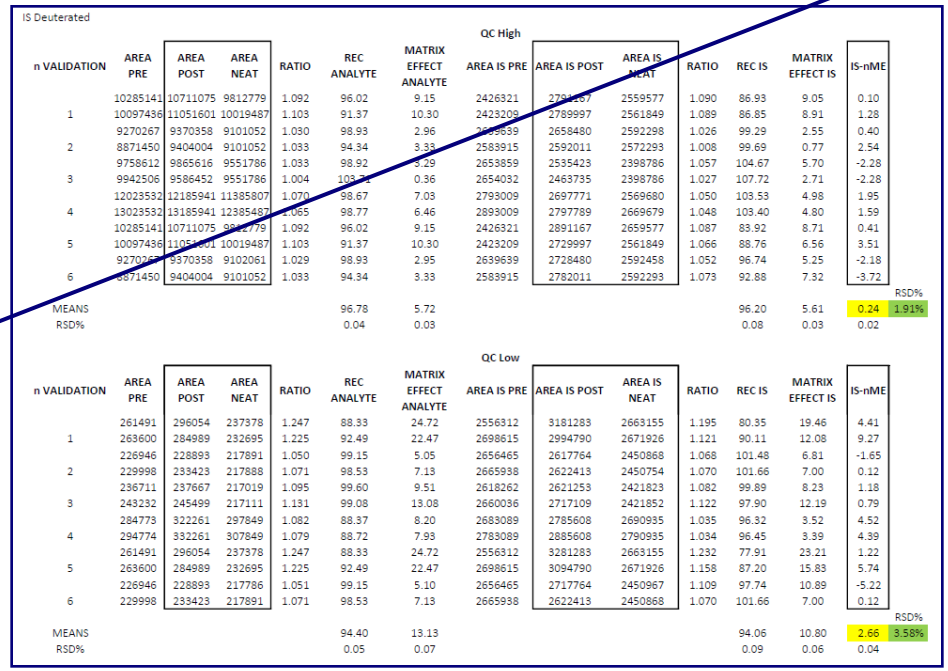

IS NO-Deuterated

QC High

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

QC Low

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	261491	296054	237378	1.247	88.33	24.72	2556312	2781283	2663155	1.044	91.91	4.44	19.42
1	263600	284989	232695	1.225	92.49	22.47	2698615	2694790	2671926	1.009	100.14	0.86	21.43
	226946	228893	217891	1.050	99.15	5.05	2656465	2717764	2450868	1.109	97.74	10.89	-5.27
2	229998	233423	217888	1.071	98.53	7.13	2665938	2622413	2450754	1.070	101.66	7.00	0.12
	236711	237667	217019	1.095	99.60	9.51	2618262	2721253	2421823	1.124	96.22	12.36	-2.54
3	243232	245499	217111	1.131	99.08	13.08	2660036	2717109	2421852	1.122	97.90	12.19	0.79
	284773	322261	297849	1.082	88.37	8.20	2683089	2585608	2690935	0.961	103.77	-3.91	12.60
4	294774	332261	307849	1.079	88.72	7.93	2783089	2685608	2790935	0.962	103.63	-3.77	12.16
	261491	296054	237378	1.247	88.33	24.72	2556312	2781283	2663155	1.044	91.91	4.44	19.42
5	263600	284989	232695	1.225	92.49	22.47	2698615	2694790	2671926	1.009	100.14	0.86	21.43
	226946	228893	217786	1.051	99.15	5.10	2656465	2717764	2450967	1.109	97.74	10.89	-5.22
6	229998	233423	217891	1.071	98.53	7.13	2665938	2622413	2450868	1.070	101.66	7.00	0.12
													RSD%
MEANS					94.40	13.13					98.70	5.27	6.65	10.28%
RSD%					0.05	0.07					0.04	0.06	0.10	


IS Deuterated

QC High

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2791167	2559577	1.090	86.93	9.05	0.10
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2789997	2561849	1.089	86.85	8.91	1.28
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	2658480	2592298	1.026	99.29	2.55	0.40
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2592011	2572293	1.008	99.69	0.77	2.54
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2535423	2398786	1.057	104.67	5.70	-2.28
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2463735	2398786	1.027	107.72	2.71	-2.28
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2697771	2569680	1.050	103.53	4.98	1.95
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2797789	2669679	1.048	103.40	4.80	1.59
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2891167	2659577	1.087	83.92	8.71	0.41
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	2728480	2592458	1.052	96.74	5.25	-2.18
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					96.20	5.61	0.24	1.91%
RSD%					0.04	0.03					0.08	0.03	0.02	

QC Low

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	261491	296054	237378	1.247	88.33	24.72	2556312	3181283	2663155	1.195	80.35	19.46	4.41
1	263600	284989	232695	1.225	92.49	22.47	2698615	2994790	2671926	1.121	90.11	12.08	9.27
	226946	228893	217891	1.050	99.15	5.05	2656465	2617764	2450868	1.068	101.48	6.81	-1.65
2	229998	233423	217888	1.071	98.53	7.13	2665938	2622413	2450754	1.070	101.66	7.00	0.12
	236711	237667	217019	1.095	99.60	9.51	2618262	2621253	2421823	1.082	99.89	8.23	1.18
3	243232	245499	217111	1.131	99.08	13.08	2660036	2717109	2421852	1.122	97.90	12.19	0.79
	284773	322261	297849	1.082	88.37	8.20	2683089	2785608	2690935	1.035	96.32	3.52	4.52
4	294774	332261	307849	1.079	88.72	7.93	2783089	2885608	2790935	1.034	96.45	3.39	4.39
	261491	296054	237378	1.247	88.33	24.72	2556312	3281283	2663155	1.232	77.91	23.21	1.22
5	263600	284989	232695	1.225	92.49	22.47	2698615	3094790	2671926	1.158	87.20	15.83	5.74
	226946	228893	217786	1.051	99.15	5.10	2656465	2717764	2450967	1.109	97.74	10.89	-5.22
6	229998	233423	217891	1.071	98.53	7.13	2665938	2622413	2450868	1.070	101.66	7.00	0.12
													RSD%
MEANS					94.40	13.13					94.06	10.80	2.66	3.58%
RSD%					0.05	0.07					0.09	0.06	0.04	

$$IS - nME\% = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}}\right) - 1\right] * 100$$

IS NO-Deuterated

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	QC High AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME	RSD%
	1028514	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
1	1009743	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87	
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99	
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62	
	1202353	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87	
4	1302353	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35	
	1028514	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
5	1009743	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87	
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

$$IS - nME\% = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}} \right) - 1 \right] * 100$$

IS NO-Deuterated

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	QC High AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME	RSD%
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87	
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99	
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62	
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87	
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35	
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87	
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

$$IS - nME\% = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}}\right) - 1\right] * 100$$

IS NO-Deuterated

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	QC High AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME	
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87	
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99	
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62	
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87	
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35	
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87	
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
														RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

$$IS - nME\% = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}} \right) - 1 \right] * 100$$

IS NO-Deuterated

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	QC High AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME	
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87	
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99	
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62	
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87	
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35	
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78	
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51	
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87	
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72	
														RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29	7.55%
RSD%					0.04	0.03					0.08	0.05	0.07	

$$IS - nME\% = \left[\left(\frac{\frac{Peak\ area\ analyte\ matrix}{Peak\ area\ IS\ matrix}}{\frac{Peak\ area\ analyte\ neat}{Peak\ area\ IS\ neat}} \right) - 1 \right] * 100$$

IS NO-Deuterated

							QC High						
n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29 7.55%
RSD%					0.04	0.03					0.08	0.05	0.07

Assay for Anti-HCV drugs
Analyte: Daclatasvir

IS NO-Deuterated

QC High

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	3028480	2592298	1.168	87.16	16.83	-11.87
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2635423	2398786	1.099	100.70	9.86	-5.99
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2663735	2398786	1.110	99.64	11.05	-9.62
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2597771	2569680	1.011	107.52	1.09	5.87
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2697789	2669679	1.011	107.24	1.05	5.35
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2641167	2559577	1.032	91.87	3.19	5.78
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	3028480	2592458	1.168	87.16	16.82	-11.87
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					94.70	7.57	-2.29 7.55%
RSD%					0.04	0.03					0.08	0.05	0.07

QC Low

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	261491	296054	237378	1.247	88.33	24.72	2556312	2781283	2663155	1.044	91.91	4.44	19.42
1	263600	284989	232695	1.225	92.49	22.47	2698615	2694790	2671926	1.009	100.14	0.86	21.43
	226946	228893	217891	1.050	99.15	5.05	2656465	2717764	2450868	1.109	97.74	10.89	-5.27
2	229998	233423	217888	1.071	98.53	7.13	2665938	2622413	2450754	1.070	101.66	7.00	0.12
	236711	237667	217019	1.095	99.60	9.51	2618262	2721253	2421823	1.124	96.22	12.36	-2.54
3	243232	245499	217111	1.131	99.08	13.08	2660036	2717109	2421852	1.122	97.90	12.19	0.79
	284773	322261	297849	1.082	88.37	8.20	2683089	2585608	2690935	0.961	103.77	-3.91	12.60
4	294774	332261	307849	1.079	88.72	7.93	2783089	2685608	2790935	0.962	103.63	-3.77	12.16
	261491	296054	237378	1.247	88.33	24.72	2556312	2781283	2663155	1.044	91.91	4.44	19.42
5	263600	284989	232695	1.225	92.49	22.47	2698615	2694790	2671926	1.009	100.14	0.86	21.43
	226946	228893	217786	1.051	99.15	5.10	2656465	2717764	2450967	1.109	97.74	10.89	-5.22
6	229998	233423	217891	1.071	98.53	7.13	2665938	2622413	2450868	1.070	101.66	7.00	0.12
													RSD%
MEANS					94.40	13.13					98.70	5.27	6.65 10.28%
RSD%					0.05	0.07					0.04	0.06	0.10

No Isotope Labeled IS (QX)

RSD%	
-2.29	7.55%

RSD%	
6.65	10.28%

Higher RSD% => Sub-optimal robustness in terms of IS-nME

Isotope-labeled IS

RSD%	
0.24	1.91%

nME is close to “0” with lower RSD% => High robustness

RSD%	
2.66	3.58%

IS Deuterated

QC High

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2791167	2559577	1.090	86.93	9.05	0.10
1	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2789997	2561849	1.089	86.85	8.91	1.28
	9270267	9370358	9101052	1.030	98.93	2.96	2639639	2658480	2592298	1.026	99.29	2.55	0.40
2	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2592011	2572293	1.008	99.69	0.77	2.54
	9758612	9865616	9551786	1.033	98.92	3.29	2653859	2535423	2398786	1.057	104.67	5.70	-2.28
3	9942506	9586452	9551786	1.004	103.71	0.36	2654032	2463735	2398786	1.027	107.72	2.71	-2.28
	12023532	12185941	11385807	1.070	98.67	7.03	2793009	2697771	2569680	1.050	103.53	4.98	1.95
4	13023532	13185941	12385487	1.065	98.77	6.46	2893009	2797789	2669679	1.048	103.40	4.80	1.59
	10285141	10711075	9812779	1.092	96.02	9.15	2426321	2891167	2659577	1.087	83.92	8.71	0.41
5	10097436	11051601	10019487	1.103	91.37	10.30	2423209	2729997	2561849	1.066	88.76	6.56	3.51
	9270267	9370358	9102061	1.029	98.93	2.95	2639639	2728480	2592458	1.052	96.74	5.25	-2.18
6	8871450	9404004	9101052	1.033	94.34	3.33	2583915	2782011	2592293	1.073	92.88	7.32	-3.72
													RSD%
MEANS					96.78	5.72					96.20	5.61	0.24 1.91%
RSD%					0.04	0.03					0.08	0.03	0.02

QC Low

n VALIDATION	AREA PRE	AREA POST	AREA NEAT	RATIO	REC ANALYTE	MATRIX EFFECT ANALYTE	AREA IS PRE	AREA IS POST	AREA IS NEAT	RATIO	REC IS	MATRIX EFFECT IS	IS-nME
	261491	296054	237378	1.247	88.33	24.72	2556312	3181283	2663155	1.195	80.35	19.46	4.41
1	263600	284989	232695	1.225	92.49	22.47	2698615	2994790	2671926	1.121	90.11	12.08	9.27
	226946	228893	217891	1.050	99.15	5.05	2656465	2617764	2450868	1.068	101.48	6.81	-1.65
2	229998	233423	217888	1.071	98.53	7.13	2665938	2622413	2450754	1.070	101.66	7.00	0.12
	236711	237667	217019	1.095	99.60	9.51	2618262	2621253	2421823	1.082	99.89	8.23	1.18
3	243232	245499	217111	1.131	99.08	13.08	2660036	2717109	2421852	1.122	97.90	12.19	0.79
	284773	322261	297849	1.082	88.37	8.20	2683089	2785608	2690935	1.035	96.32	3.52	4.52
4	294774	332261	307849	1.079	88.72	7.93	2783089	2885608	2790935	1.034	96.45	3.39	4.39
	261491	296054	237378	1.247	88.33	24.72	2556312	3281283	2663155	1.232	77.91	23.21	1.22
5	263600	284989	232695	1.225	92.49	22.47	2698615	3094790	2671926	1.158	87.20	15.83	5.74
	226946	228893	217786	1.051	99.15	5.10	2656465	2717764	2450967	1.109	97.74	10.89	-5.22
6	229998	233423	217891	1.071	98.53	7.13	2665938	2622413	2450868	1.070	101.66	7.00	0.12
													RSD%
MEANS					94.40	13.13					94.06	10.80	2.66 3.58%
RSD%					0.05	0.07					0.09	0.06	0.04

MSACL 2016 EU, 12-15 September, Salzburg, Austria

Poster #K1

UHPLC-MS/MS method with on-line SPE to quantify tacrolimus and everolimus in peripheral blood mononuclear cells: application of "IS-normalized matrix effect".

Amedeo De Nicolò[1], Debora Pensi[1], Michele Pinon[2], Clarissa Pisciotta[1], Pier Luigi Calvo[2], Antonello Nonnato[4], Renato Romagnoli[3], Francesco Tandoi[3], Giovanni Di Perri[1] and Antonio D'Avolio[1].

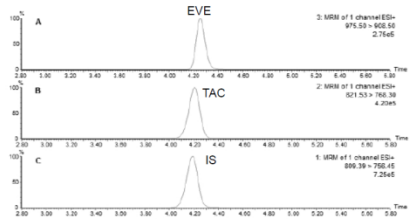
1. Department of Medical Sciences, Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin.
2. Unit of Pediatric Gastroenterology and Hepatology, University of Turin, "Città della Salute e della Scienza" Hospital, Turin, Italy
3 . Liver Transplantation Center and General Surgery, "Cittàdella Salute e della Scienza" Hospital, University of Turin, Turin, Italy.
4 .Clinical Biochemistry Unit, Dept. Diagnostic Laboratory, A.O.U. "Città della Salute e della Scienza" Hospital, Turin, Italy

Author info:
Amedeo De Nicolò
amedeo.denicolo@unito.it
website: www.tdm-torino.org

Table 1: Intra/inter-day precision and accuracy (n=5) at 3 QCs concentrations

Tacrolimus	Accuracy	Precision	
		Intra-day (RSD%)	Inter-day (RSD%)
QC H	95,8	4,9	4,3
QC M	102,6	5,3	5,8
QC L	102,7	6,3	10
Everolimus	**Accuracy**	**Precision**	
		Intra-day (RSD%)	Inter-day (RSD%)
QC H	93,8	5,5	6
QC M	96,1	4,1	7,2
QC L	101,1	9,2	10,7

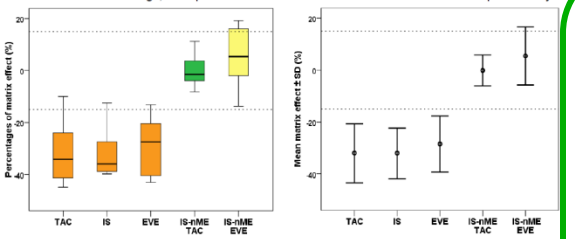
Figure 1 : Chromatograms from direct injection of a standard sample.



Equation 1: Arithmetical expression of the percentage of "IS-normalized matrix effect". The relative standard deviation has to be calculated on the same value + 100%. PAA=peak area of the analyte; PAIS =peak area of IS; neat = extraction solvents spiked with drugs without matrix.

$$IS-nME=\left[\frac{(PAA-matrix/PAIS-matrix)}{(PAA-neat/PAIS-neat)}-1\right]*100$$

Figure 2: graphical representation of the observed percentages of matrix effect both in the "standard" form and "normalized" based on the IS. Is clear as the IS is capable of reducing the mean value of ME for both drugs, but it performs better for TAC rather than EVE in terms of reproducibility.



RESULTS

Intra-day and inter-day precision and accuracies resulted all within the limits of acceptance indicated by FDA and EMA guidelines (table 1). The lower limit of detection (LLOD) for TAC and EVE were 0.0195 ng and 0.0190 ng, respectively, while the lower limit of quantification (LLOQ) were 0.0975 ng and 0.039 ng, respectively.
Recoveries for TAC, EVE and IS were all above 91% and stable for all compounds (RSD <4.70%). Mean matrix effects, determined on samples from $3x10^6$ to $24x10^6$ cell/mL, for TAC, EVE and IS -32% (RSD 15.8%), - 28.5% (RSD 15.1%) and -32% (RSD 14.24%), respectively (Figure 2). Considering only population lower than $12x10^6$cell/mL, these values became -29.48% (RSD 3.35%), -14.87% (RSD 1.67%) and -31.71% (RSD 8.00%), respectively, allowing a reliable quantification. Therefore, the validation was made with samples of $12x10^6$ cell/mL and samples were diluted to this cells number.
Considering all the cell numbers, the "IS-normalized matrix effect" resulted -0.1% (RSD 6.0%) and +5.0% (RSD 9.8%) for TAC and EVE, respectively.
All tested samples from patients resulted within the ranges of the calibration curve. Interestingly, TAC and EVE concentrations within PBMCs resulted respectively 19.23 and 218.61 times higher than the hematic ones.

CONCLUSIONS

In this work an analytical method was developed capable of successfully quantifying TAC and EVE concentrations in PBMC extracts. The adoption of on-line SPE allowed to quantify very low drugs concentrations, limiting the manual preparation steps to the spiking of standards and QCs and the addition of IS. Furthermore, in this work we introduced a model for evaluating the "IS-normalized matrix effect": this parameter considers the effect of matrix effect in each sample for both IS and target analyte and represents the capability of the IS of successfully counterbalancing the variability in analytical performance due to matrix effect.
In this case, being the calibration curve prepared in matrix,the bias due to ME in mainly dependent by its variability (different samples and different blank PBMCs from healthy donors), expressed as the RSD%.
In our method "IS-normalized matrix effect" resulted very low and highly reproducible for TAC, confirming ascomycin as a very good IS for TAC. Although the corrective effect of the IS on the mean nME for EVE, the reproducibility in this case was less good, in accordance with previous reports. However, its RSD% was yet acceptable, indicating that the calibration curve prepared in matrix is capable of correcting the error due to matrix effect.

First UHPLC MS/MS method coupled with automated on-line SPE for quantification both of tacrolimus and everolimus in peripheral blood mononuclear cells and its application on samples from co-treated pediatric patients

Debora Pensi, Amedeo De Nicolò, Michele Pinon, Clarissa Pisciotta, Pier Luigi Calvo, Antonello Nonnato, Renato Romagnoli, Francesco Tandoi, Giovanni Di Perri, Antonio D'Avolio

Submitted 2016

In collaboration with CoQua Lab srl
Web Site: www.coqualab.it
Mail: info@coqualab.it

MSACL 2016 EU, 12-15 September, Salzburg, Austria

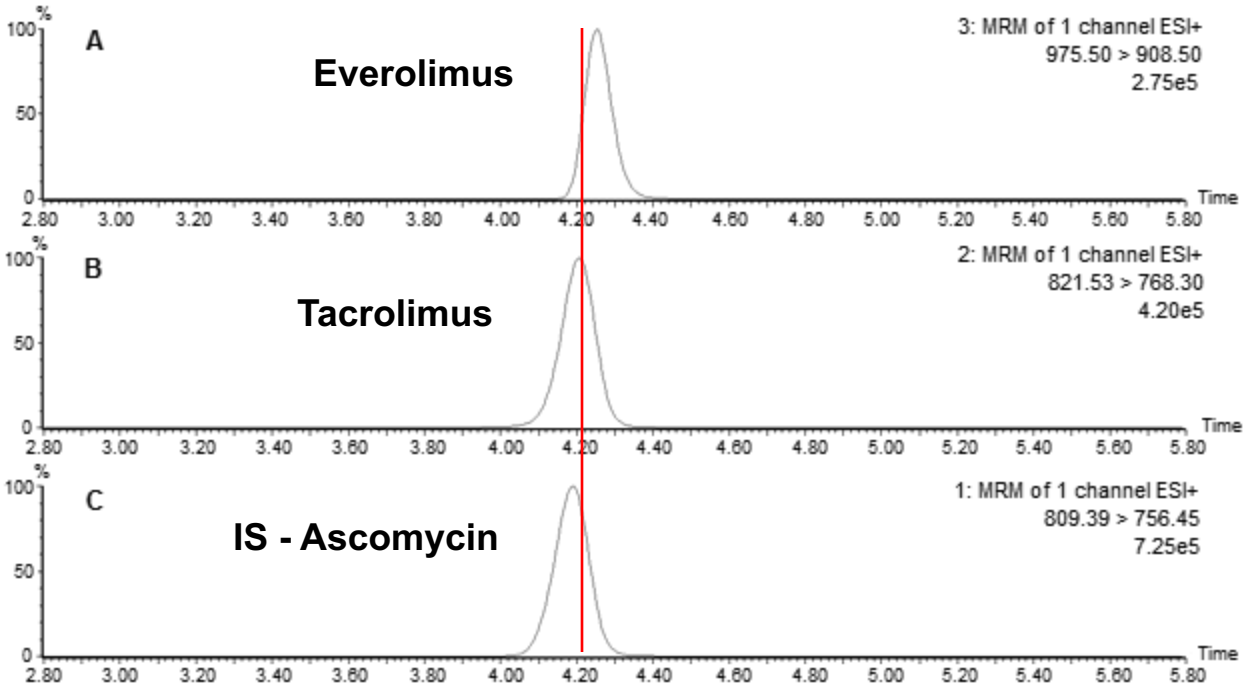
Poster #K1

UHPLC-MS/MS method with on-line SPE to quantify tacrolimus and everolimus in peripheral blood mononuclear cells: application of "IS-normalized matrix effect".

Amedeo De Nicolò[1], Debora Pensi[1], Michele Pinon[2], Clarissa Pisciotta[1], Pier Luigi Calvo[2], Antonello Nonnato[4], Renato Romagnoli[3], Francesco Tandoi[3], Giovanni Di Perri[1] and Antonio D'Avolio[1].

1. Department of Medical Sciences, Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin.
2. Unit of Pediatric Gastroenterology and Hepatology, University of Turin, "Città della Salute e della Scienza" Hospital, Turin, Italy
3 . Liver Transplantation Center and General Surgery, "Cittàdella Salute e della Scienza" Hospital, University of Turin, Turin, Italy.
4 .Clinical Biochemistry Unit, Dept. Diagnostic Laboratory, A.O.U. "Città della Salute e della Scienza" Hospital, Turin, Italy

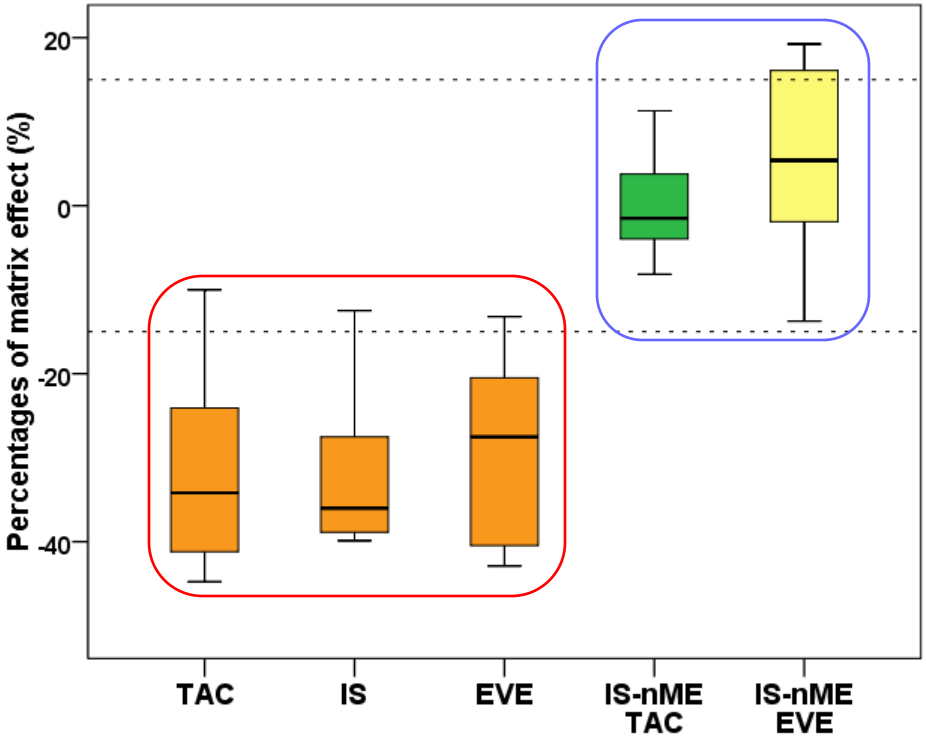
Author info:
Amedeo De Nicolò
amedeo.denicolo@unito.it
website: www.tdm-torino.org

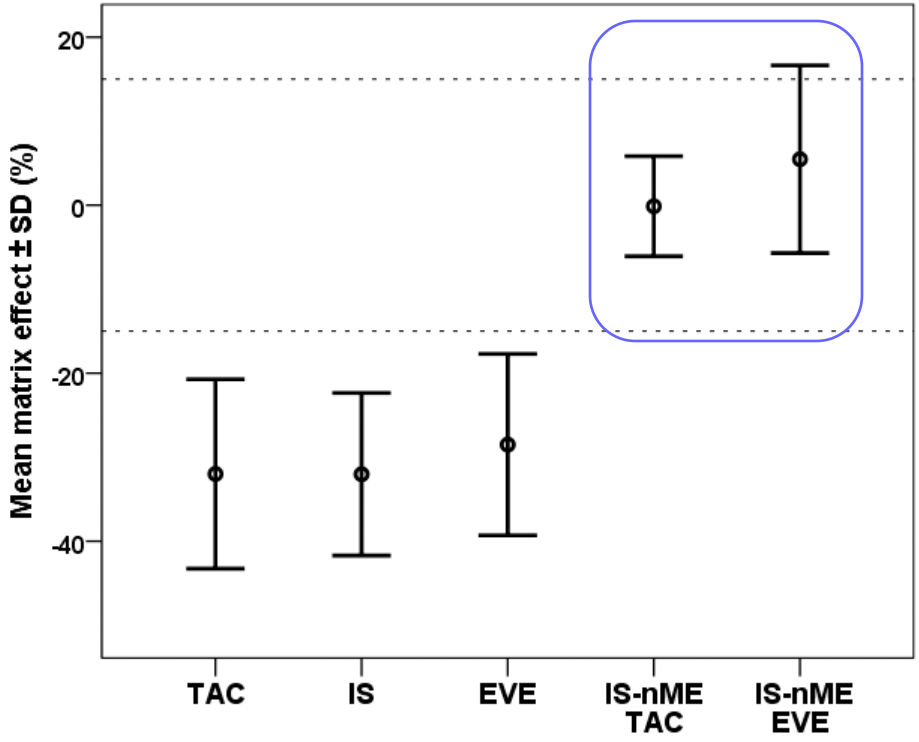


IS=Ascomycin; TAC=Tacrolimus; EVE=Everolimus

$$CV\%\ of\ IS-nME = \frac{Standard\ Deviation\ of\ IS-nME\%}{Mean\ IS-nME\% + 100}$$

Equation 4: Equation for the evaluation of the reproducibility of IS-nEM.





TAC and EVE intra-PBMCs determination; The matrix effect was affected by the cell number. Different number of cells from each patient.

TAC, IS and EVE elute with higher percentage of organic mobile phase (with PL?); they have matrix effect!

Ascomycin is the "historical" IS for TAC, and it does not perfectly correct EVE (high SD%)

Pensi D. et Al. JPBA 2015; D'Avolio A. et. Al. Submitted 2016; Pensi D. et Al. Submitted 2016; De Nicolò et Al. MSACL 2016 #K1

- **The model explained the already known good performance of ascomycin as IS for TAC.**

- **The model confirmed the already reported sub-optimal performance of ascomycin as IS for EVE.**

- **Although the method is valid (the RSD of IS-nME for EVE was lower than 15%), the use of an isotope-labeled-IS is suggested for EVE (and not for TAC).**

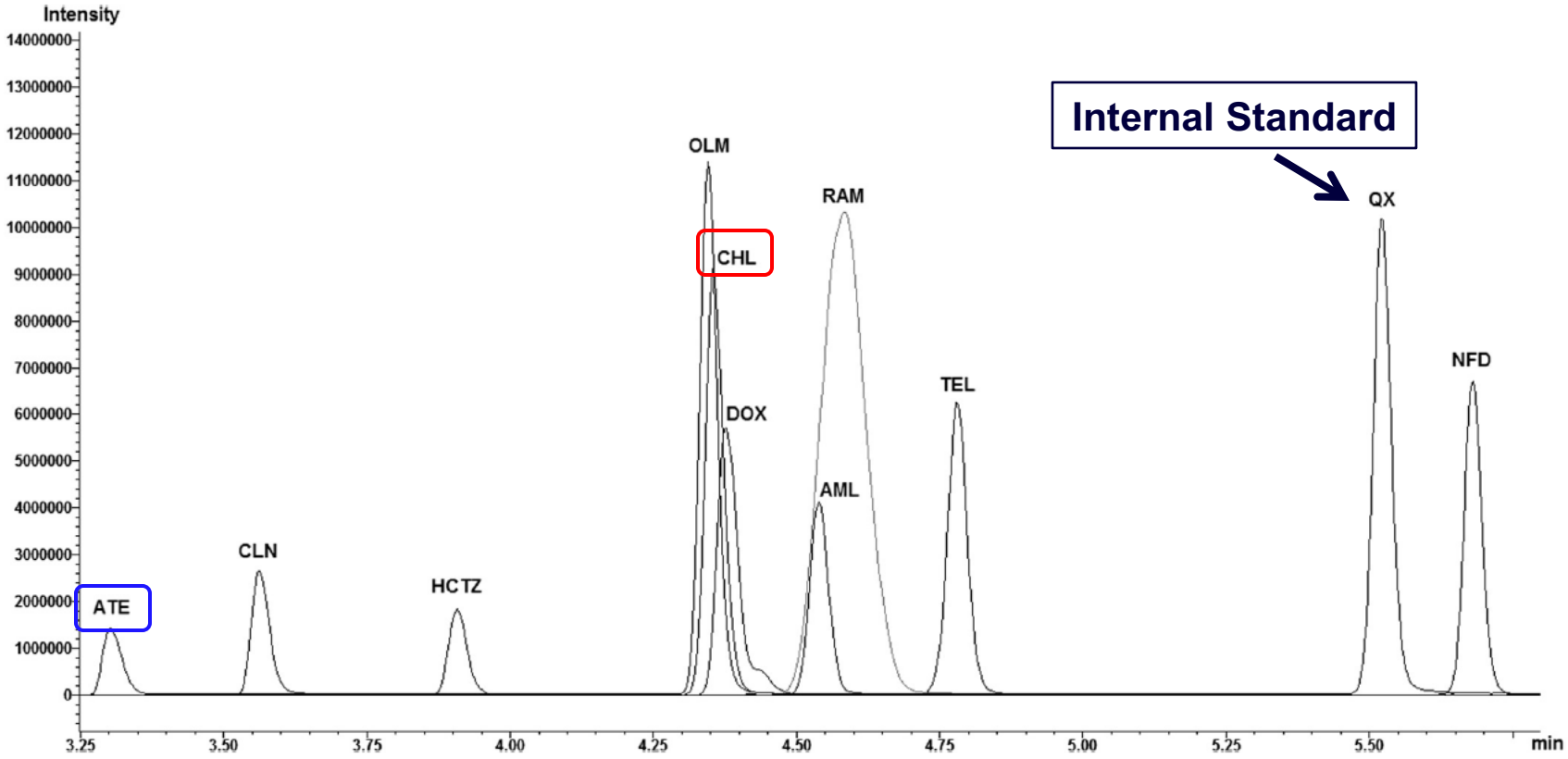


Fig. 1. Overlaid chromatograms of each analyte from direct injection of a chemical mix.

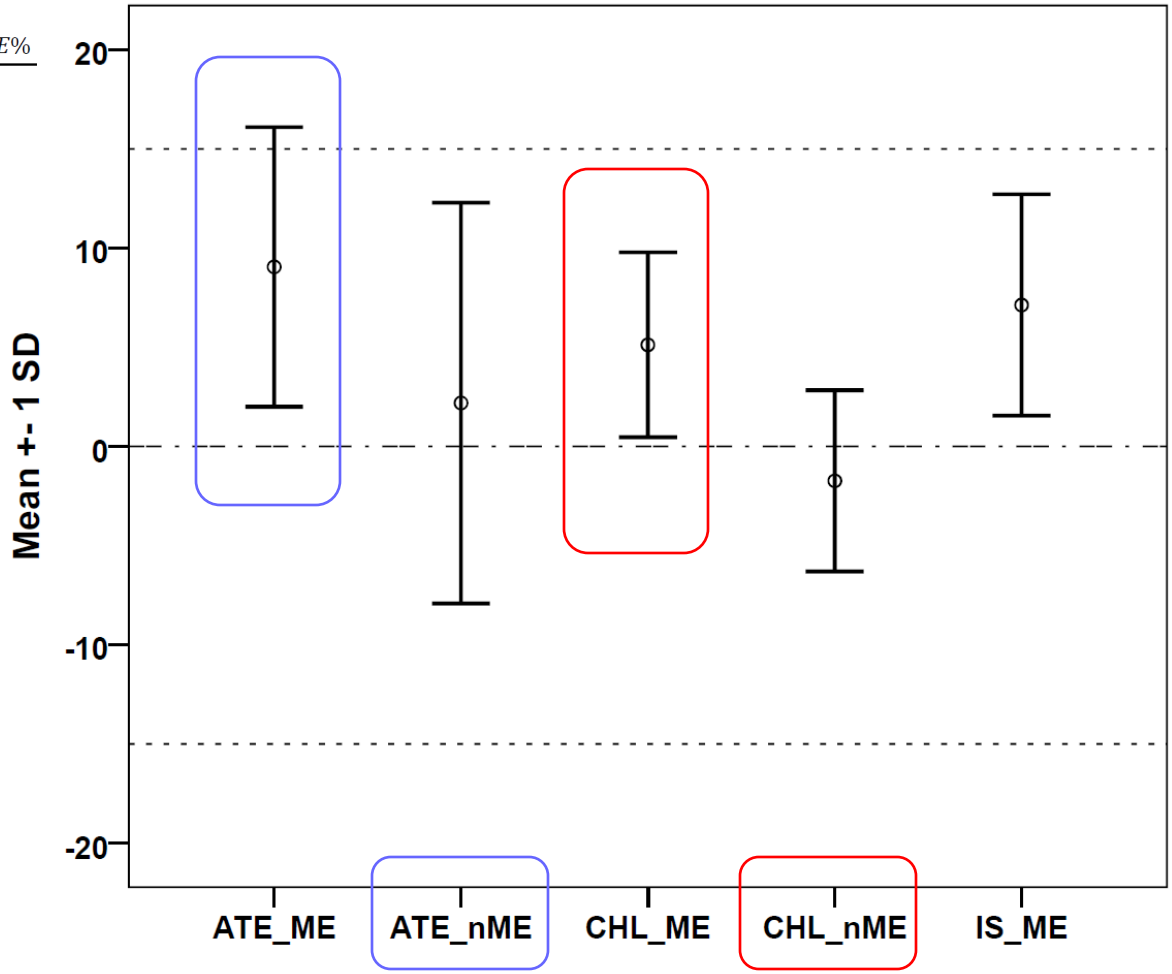
Atenolol

Chlortalidone

$$CV\%\ of\ IS-nME = \frac{Standard\ Deviation\ of\ IS-nME\%}{Mean\ IS-nME\% + 100}$$

Equation 4: Equation for the evaluation of the reproducibility of IS-nEM.

Atenolol has higher IS-nME Standard Deviation than chlortalidone



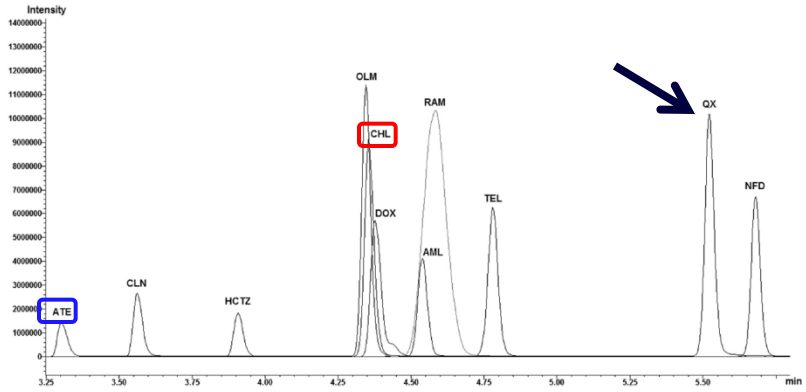


Fig. 1. Overlaid chromatograms of each analyte from direct injection of a chemical mix.

Atenolol has a lower lipid solubility (for example) than chlortalidone and quinoxaline.

But we were lucky!!!
IS-nME is close to "zero" with RSD% <15!
The method resulted barely OK!!!

Conclusion

- **This examples highlighted how analytes with high ME could have a low IS-nME, considering their relation with IS, if they share similar chemical properties.**

- **The evaluation of the CV% (RSD%) of IS-nME allowed to correctly describe the overall reproducibility of the response factor, directly affecting the accuracy and the reproducibility of the method.**

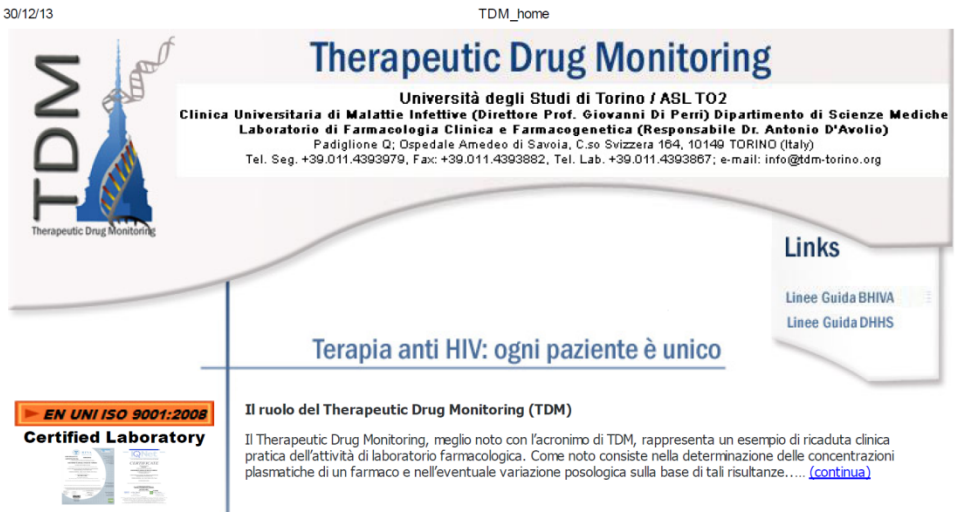
- **IS-nME could be considered a tool to evaluate the performances during the validation, obtaining accuracy, intraday and interday precisions results within the limits suggested by FDA and EMA guidelines.**

Pensi D. et Al. JPBA 2015; D'Avolio A. et. Al. Submitted 2016; Pensi D. et Al. Submitted 2016

Acknowledgement

University of Turin

Prof. Giovanni Di Perri
Prof. Stefano Bonora
Prof. Francesco Giuseppe De Rosa
Dott. Andrea Calcagno
Dott. ssa Letizia Marinaro
Dott. Lucio Boglione



The Laboratory Team

Dott. Antonio D'Avolio
Dott. ssa Jessica Cusato
Dott.ssa Valeria Avataneo
Mauro Sciandra
Dott. Marco Simiele
Dott. ssa Alessandra Ariaudo
Dott. ssa Debora Pensi
Dott. ssa Sarah Allegra
Dott. Fabio Favata
Dott. Giovanna Fatiguso
Dott. ssa Cristina Tomasello
Dott. ssa Chiara Carcieri
…and the students

www.tdm-torino.org

Laboratory of Clinical Pharmacology and Pharmacogenetics

UNI EN ISO 9001:2008 and 13485:2012

Certified Laboratory

CoQua LAB

www.coqualab.it