Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica

LA SPETTROMETRIA DI MASSA: APPLICAZIONI E INNOVAZIONI DIAGNOSTICHE

Milano 13/05/2019

Silvia Persichilli

Stesura di un documento per la validazione dei metodi in LC-MS/MS


DOCUMENTS DOCUMENTI

Validazione dei metodi quantitativi bioanalitici in spettrometria di massa: regole e protocolli sperimentali

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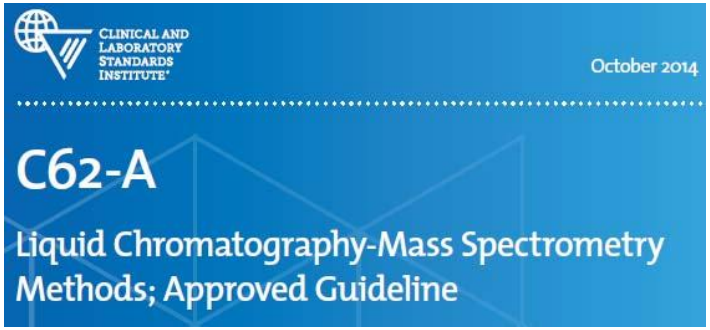
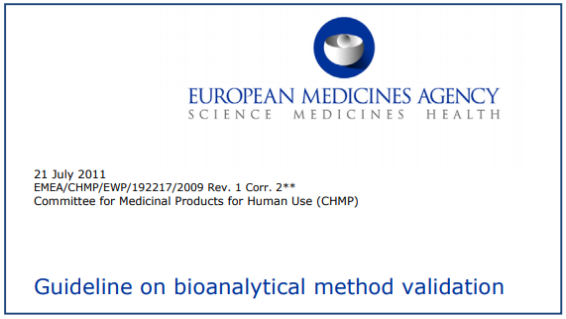
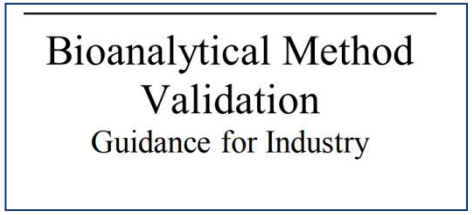
Bioanalytical method validation of quantitative mass spectrometry based assay: experimental protocols and regulations.







What Is the Current Status of Harmonization of Clinical LC-MS Methods?

Clinical Chemistry 62:1
24–29 (2016)

Perspective

CLSI C62-A: A New Standard for Clinical Mass Spectrometry

Kara L. Lynch[1*]

- ✓ Laboratory-to-laboratory variability for the same analyte, measured by LC-MS, has been observed as evidenced by results from external quality assessment programs and published reports.
- ✓ There are very few higher-order reference measurement procedures and certified reference materials (CRMs) available for metrological traceability of analytes routinely monitored by clinical LC-MS methods.
- ✓ A variety of guidance documents exist that aid clinical laboratories in the evaluation and routine use of clinical methods, but they are not specific to MS

What Is the Current Status of Harmonization of Clinical LC-MS Methods?

Table 1. Comparison of CLSI C62-A to FDA and European Medicines Agency (EMA) Bioanalytical Method Validation Guidelines.

	CLSI C62-A assay verification[a]		FDA bioanalytical method validation		EMA bioanalytical method validation	
Factor	Performance specification	Validation practices	Performance specification	Validation practices	Performance specification	Validation practices
Accuracy/trueness	Acceptable criteria should be defined based on biological variation, clinical guidelines established by expert groups, and local or regional regulatory requirements	• Reference to CLSI documents EP32, EP09, EP15, and C50 • Assessment of trueness should include >1 approach: (a) comparison to an RMP[b] using ≥40 samples (preferably patient samples) covering the AMI; (b) analysis of commutable CRMs; and/or (c) spike and recovery analysis	Mean values should be within 15% of the nominal value except at the LLOQ, where it should be within 20%	Accuracy should be assessed using ≥5 determinations for ≥3 concentrations in the AMI using a spike and recovery analysis	Mean values should be within 15% of the nominal value except at the LLOQ, where it should be within 20%	• Within-run accuracy should be assessed using ≥5 determinations for ≥4 concentrations in the AMI using a spike and recovery analysis • Between-run accuracy should be assessed using LLOQ and low, medium, and high QC samples from ≥3 runs analyzed on ≥2 different days
Imprecision	• Imprecision should be <15% CV except at the LLMI, where it should be <20% • Imprecision goals should be set based on predetermined total allowable error, biological variation, clinical guidelines by expert groups, and local or regional regulatory requirements	• Reference to CLSI EP05 • Imprecision should be assessed using 20 determinations at 110% of the LLMI, 90% of the ULMI, and (LLMI + ULMI)/2; samples at and within 25% of any medical decision points should also be evaluated	Imprecision should be <15% CV except at the LLOQ, where it should be <20%	Imprecision (within-run and between-run) should be assessed using ≥5 determinations at ≥3 concentrations in the AMI	Imprecision should be <15% CV except at the LLOQ, where it should be <20%	• Within-run imprecision should be assessed using ≥5 determinations at 4 concentrations (LLOQ and low, medium, and high QC) • Between-run imprecision should be assessed at 4 concentrations (LLOQ and low, medium, and high QC) from ≥3 runs analyzed on ≥2 different days
Sensitivity	• S/N >20:1 at the LLMI • Imprecision should be <20% CV and trueness within 15%	• Reference to CLSI EP17 • LLMI should be established using ≥40 replicates from 3-5 different samples close to the predetermined limit of detection, over ≥5 runs	• S/N >5:1 at the LLOQ • Imprecision should be <20% CV and trueness within 20%	LLOQ should be established using ≥5 samples independent of standards	• S/N > 5:1 at the LLOQ • Imprecision should be <20% CV and trueness within 20%	• Within-run LLOQ should be established using ≥5 samples independent of standards • Between-run LLOQ should be established using samples from 3 runs on ≥2 different days
Matrix Effects	• Imprecision due to matrix effects should be <15% • Matrix effects should be evaluated in the context of total allowable error limits for specific analytes	• Reference to CLSI documents EP07, EP21, and C50 • Comparison of the peak area for 5 native matrix samples spiked with analyte postextraction vs analyte spiked into neat solution tested at all points of the calibration curve	• No specific performance criteria specified • Matrix effects should be investigated to ensure that precision, selectivity, and sensitivity will not be compromised	• Comparison of the calibration curve spiked into matrix vs neat solution • Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects	Imprecision due to matrix effects should be <15%	Comparison of the peak area for 6 native matrix samples spiked with analyte postextraction vs analyte spiked into neat solution tested at 2 concentrations (3× the LLOQ and close to the ULOQ)
Specificity/selectivity/interferences	Potential interferents should not alter the calculated concentration of analyte or the ion ratio; if the signal of the qualifier ion is >50% that of the quantifier ion, the ion ratio in the patient samples should be within 20% from the mean ratio of the standards	• Reference to CLSI EP07 • Evaluate a high concentration of potential interferent in matrix (preferably a patient sample) with and without analyte present	• No specific performance criteria specified • Selectivity should be ensured at the LLOQ	Same as matrix effects experiments	Response from a potential interferent should be <20% of the LLOQ for the analyte and <5% for the IS	Same as matrix effects experiments

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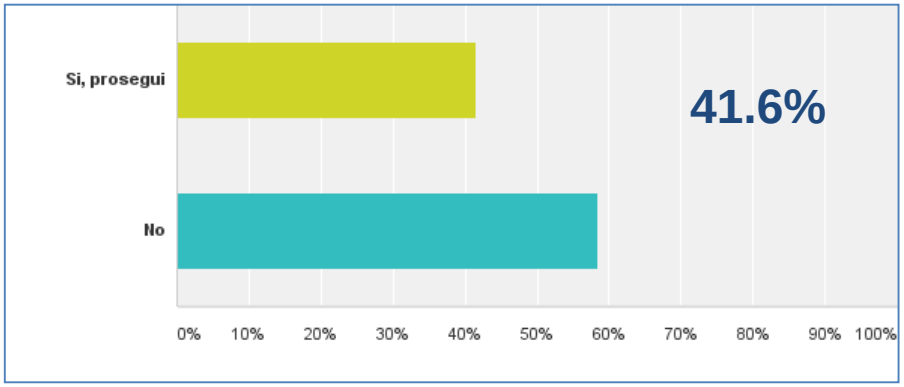
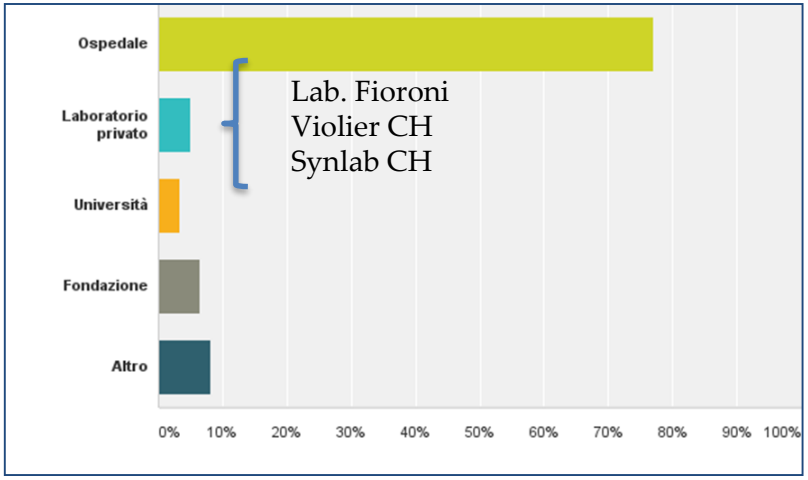
SONDAGGIO

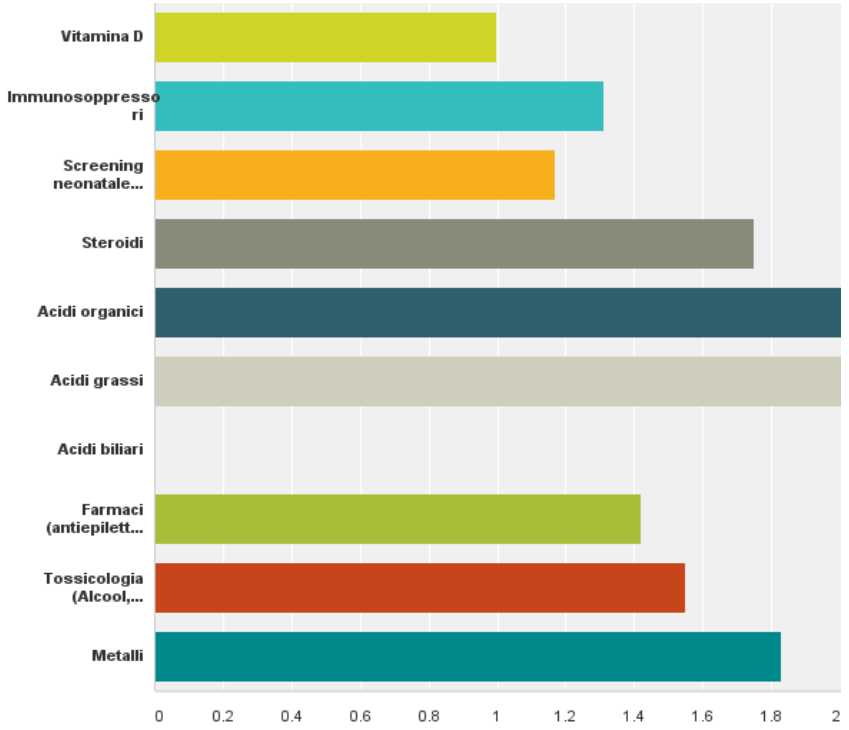
La spettrometria di massa nei laboratori italiani

Q1: Il vostro laboratorio fa uso di Spettrometria di massa?

- Hanno risposto: 221 Hanno saltato la domanda: 0







La spettrometria di massa nei laboratori italiani

ENTI PUBBLICI			LABORATORI PRIVATI	
Torino	Molinette		LAMAT	Torino
Torino	Amedeo Savoia		CEDAM	Milano
Orbassano	Centro Antidoping		BIANALISI	Carate Brianza
Alessandria	Ospedale		SYNLAB	Brescia
Novara	Ospedale		LIFEBRAIN	Padova
Genova	Gaslini		SAN RAFFAELE	Milano
Genova	Arenzano			
Imperia	Ospedale			
Varese	Ospedale			
Legnano	Ospedale			
Como	Ospedale			
Desio	Ospedale			
Lodi	Ospedale			
Milano	Niguarda			
Milano	Università Medicina Legale			
Milano	Ploclinico Medicina del Lavoro			
Milano	Ospedale Sacco-Malattie infettive			
Milano	Besta			
Pavia	San Matteo			
Pavia	Università Medicina Legale			
Bergamo	Ospedale			
Brescia	Spedali Civili			
Brescia	Università Medicina Legale			
Brescia	Università Medicina del Lavoro			
Verona	Ospedale Borgo Roma			
Trento	Ospedale			
Bolzano	Ospedale			
Vicenza	Ospedale			
Padova	Ospedale			
Padova	Università Med. Legale			
Venezia	Medicina Legale			
Treviso	Ospedale			
Pordenone	Ospedale			
Udine	Ospedale			
Trieste	Osp. Pediatrico Burlo			
Bologna	LUM			
Bologna	Università Endocrinologia			
Bologna	Università Medicina Legale			
Modena	Baggiovara			
Modena	Policlinico Universitario			
Modena	Univesità Medicna Legale			
Cesena	Ospedale			
Ferrara	Università Medicina Legale			
Parma	Universià Medicina Lavoro