Critérios de validação e identificação em análises por LC-MS/MS

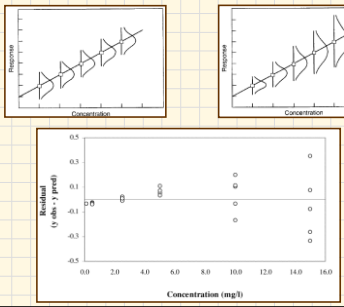
Álvaro J. Santos Neto

Validação em LC-MS(/MS)

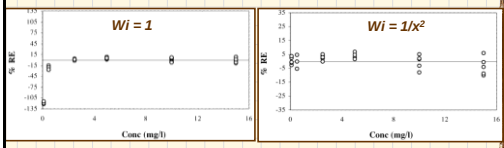
- **Mesmos critérios que outras técnicas (ex. HPLC):**
  - Linearidade; Exatidão e Precisão; LD e LQ; Estabilidade; Robustez...
  - Legislação, exemplos:
    - FDA, **Guidance for industry**: bioanalytical method validation, Drug information branch, 2001
    - ANVISA, Guia para validação de métodos analíticos e bioanalíticos - RE n° 899, de 29 de maio de 2003
- **Especial atenção em LC-MS(/MS):**
  - **Calibração**
  - **Recuperação / Efeito de Matriz**

Calibração ponderada

- Dados homoscedásticos / heteroscedásticos

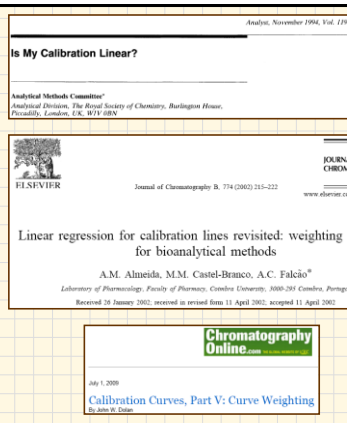


Calibração ponderada



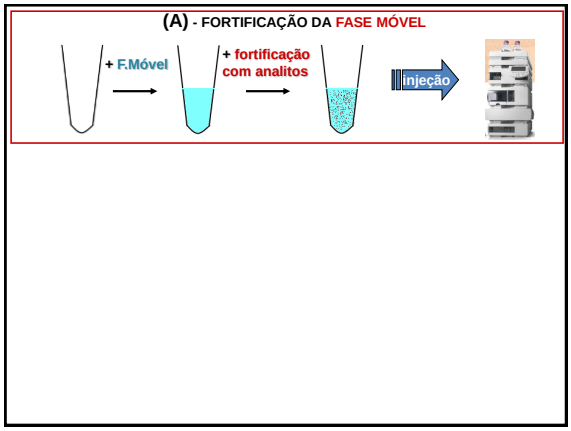
Parâmetros de regressão para curvas de calibração com diferentes ponderações

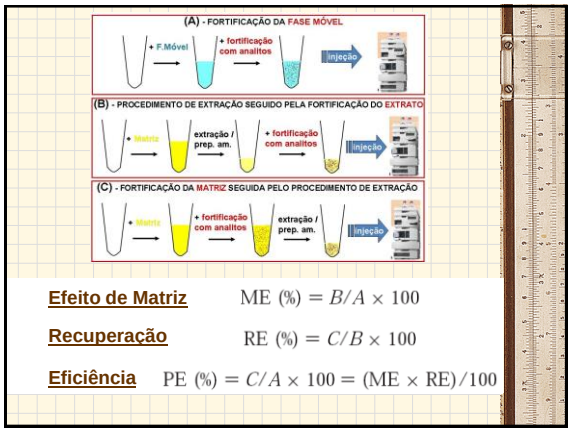
Model	w_i	b	a	r	Σ%RE
1	1	0.2713	+0.0281	0.997	788.90
2	$1/y^{1/2}$	0.2743	+0.0033	0.998	264.31
3	$1/y$	0.2762	−0.0034	0.998	133.48
4	$1/y^2$	0.2789	−0.0048	0.998	116.47
5	$1/x^{1/2}$	0.2747	+0.0037	0.998	271.34
6	$1/x$	0.2770	−0.0033	0.998	133.71
7	$1/x^2$	0.2804	−0.0049	0.999	112.38



Recuperação / Efeito de Matriz

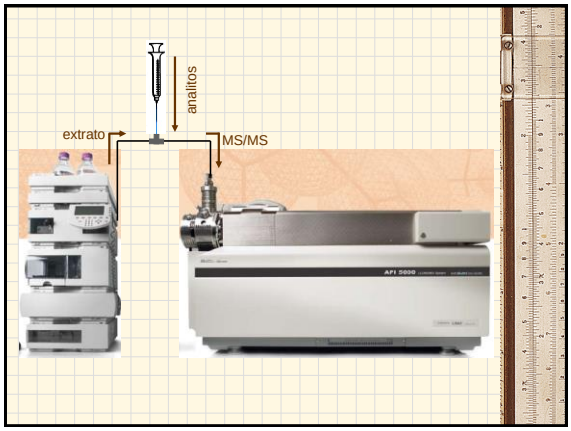
- **Modos de avaliação:**
  - **(1) Clássico / Quantitativo**
    - **Avalia Efeito de matriz, Recuperação e Eficiência do processo**
  - (2) Infusão em "Te" / Qualitativo



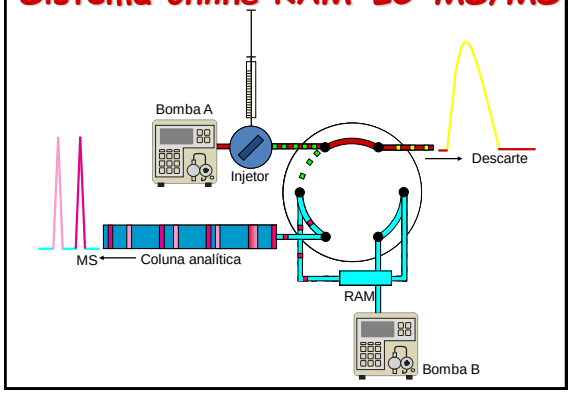


Recuperação / Efeito de Matriz

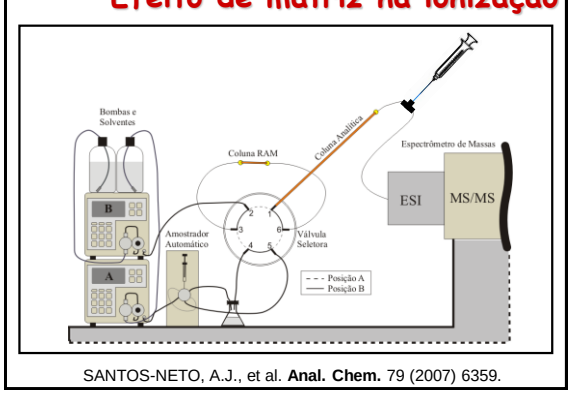
- **Modos de avaliação:**
  - (1) Clássico / Quantitativo
  - **(2) Infusão em "Te" / Qualitativo**
    - **Opção para sistemas *online***
    - **Avalia o efeito de matriz**
    - **A recuperação geralmente é calculada como "recuperação relativa" em sistemas *online***



Sistema *online* RAM-LC-MS/MS

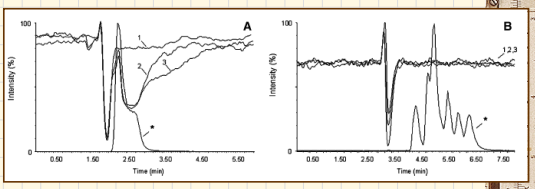


Efeito de matriz na ionização



SANTOS-NETO, A.J., et al. **Anal. Chem.** 79 (2007) 6359.

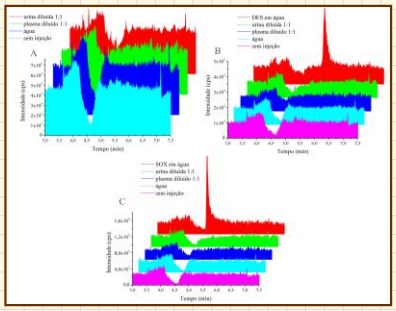
Demonstração do Efeito de Matriz



1- água
2- plasma
3- urina
*- cromatograma

K. Georgi, K. Boos, Chromatographia 63 (2006) 523.

Ausência do Efeito de Matriz



A: TIC

B: MRM da desipramina

C: MRM do albendazol

SANTOS-NETO, A.J., et al. **J. Chromatogr. A**, *1189 (2008) 514.*

Anal. Chem. 2003, *75*, 3019−3030

Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS

B. K. Matuszewski,* M. L. Constanzer, and C. M. Chavez-Eng

Merck Research Laboratories, West Point, Pennsylvania 19486

Além do efeito de matriz

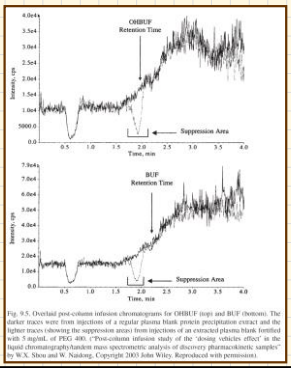


Fig. 9.5. Overlaid post-column infusion chromatograms for OHBUF (top) and BUF (bottom). The darker traces were from injections of a regular plasma blank protein precipitation extract and the lighter traces (showing the suppression areas) from injections of an extracted plasma blank fortified with 5 ng/mL of PEG 400. ("Post-column infusion study of the 'dosing vehicles effect' in the liquid chromatography/tandem mass spectrometric analysis of discovery pharmacokinetic samples" by W.X. Shou and W. Naidong. Copyright 2003 John Wiley. Reproduced with permission).

Além do efeito de matriz

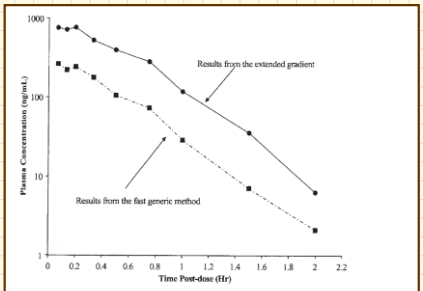


Fig. 9.6. Comparison of analysis results obtained from the same set of dog IV plasma samples containing PEG 400 when two different gradient LC/MS/MS methods were used. ("Post-column infusion study of the 'dosing vehicles effect' in the liquid chromatography/tandem mass spectrometric analysis of discovery pharmacokinetic samples" by W.X. Shou and W. Naidong. Copyright 2003 John Wiley. Reproduced with permission).

Além do efeito de matriz

CONCENTRATIONS OF ACIDIC AND BASIC DRUGS USED AS TEST ANALYTES FOR SUPPRESSION/ENHANCEMENT EVALUATION

Compound	$[M-H]^-$	[ ] (ng/μL)	Compound	$[M+H]^+$	[ ] (ng/μL)
Fumaric acid	115.0	1.0	Propranolol	260.2	1.0
Malic acid	133.1	1.0	Trimethoprim	291.3	1.0
Etidronic acid	205.2	8.0	Pipenzolate	354.4	1.0
Clodronic acid	243.2	1.0	Resperidone	411.4	0.5
Niflumic acid	281.4	6.0	Terfenadine	472.6	1.0
Canrenoic acid	357.6	6.0	Methoxyverapamil	485.6	1.0
Cholic acid	407.7	10.0	Benextracmin	591.6	7.0
Raffinose	503.4	10.0	Reserpine	609.6	5.0

Source: From Ref [12]. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. Copyright 2003 John Wiley. Reproduced with permission.

Além do efeito de matriz

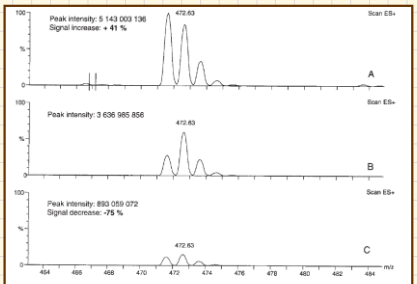


Fig. 9.11. Examples of ion suppression and enhancement on terfenadine. (A) Terfenadine mixed with 50/50 MeOH/H_2O + 0.5% NH_4OH; (B) terfenadine mixed with 50/50 MeOH/H_2O + no additive and (C) terfenadine mixed with 50/50 MeOH/H_2O + 0.5% TFA. ("A study of ion suppression effects in ESI from mobile phase additives and solid-phase extracts" by C.R. Mallet *et al.* Copyright 2003 John Wiley. Reproduced with permission).

Além do efeito de matriz

SUPPRESSION AND ENHANCEMENT EFFECTS FOR THE pH ADDITIVES

	0.05%	0.10%	0.50%	1.00%	0.05%	0.10%	0.50%	1.00%
	Formic acid				Ammonium hydroxide			
Positive test solution								
Propranolol	36.5	28.8	4.5	−8.3	−2.2	2.02	10.2	11.4
Trimethoprim	41.7	30.1	−5.3	−17.5	−5.4	−5.4	4.2	8.9
Pipenzolate	−0.1	−0.2	−5.5	−9.5	0.02	0.02	0.02	0.01
Resperidone	−27.5	−37.1	−54.2	−59.4	6.1	9.6	16.1	16.8
Terfenadine	17.3	11.6	−7.9	−16.5	10.8	21.3	57.9	66.6
Methoxyverapamil	22.8	17.1	−1.8	−10.7	38.8	41.1	46.6	49.1
Benextramine	−39.77	−44.1	−52.7	−52.8	22.1	30.7	37.9	38.3
Reserpine	21.4	21.4	17.2	8.9	−12.1	−11.9	−6.2	−3.2
Negative test solution								
Fumaric acid	−11.9	−29.5	−64.7	−68.1	−38.4	−41.1	−45.8	−57.8
Malic acid	−11.2	−27.9	−62.2	−63.9	−35.5	−38.8	−42.4	−53.4
Etidronic acid	29.8	17.8	−17.2	−30.9	−61.9	−63.5	−75.9	−70.3
Clodronic acid	5.7	−15.7	−58.3	−66.6	0.3	1.3	−5.3	−27.7
Niflumic acid	−0.28	−21.4	−60.9	−64.5	14.1	11.1	5.3	−11.6
Canrenoic acid	13.8	−11.1	−51.6	−57.6	196.1	202.5	201.9	127.3
Cholic acid	31.9	3.7	−40.8	−44.7	420.5	454.9	403.1	352.8
Raffinose	−4.6	−26.3	−39.4	−43.7	60.9	61.9	66.6	32.1

Além do efeito de matriz

SUPPRESSION AND ENHANCEMENT EFFECTS FOR THE pH ADDITIVES

	0.05%	0.10%	0.50%	1.00%	0.05%	0.10%	0.50%	1.00%
	Trifluoroacetic acid				Acetic acid			
Positive test solution								
Propranolol	−54.8	−62.8	−74.7	−77.1	25.5	25.8	17.3	−0.2
Trimethoprim	−40.1	−58.1	−73.9	−76.6	18.3	10.4	−0.4	−7.1
Pipenzolate	−27.5	−37.4	−43.9	−43.7	0.01	−0.01	−0.4	−1.7
Resperidone	−53.7	−62.3	−68.2	−69.3	−2.1	−16.8	−37.7	−44.2
Terfenadine	−24.4	−44.6	−61.5	−64.8	15.9	11.9	7.5	−2.8
Methoxyverapalmil	−59.9	−57.3	−70.2	−72.6	19.5	16.6	8.9	−4.8
Benextramine	−29.4	−41.8	−42.7	−38.7	−21.9	−28.9	−29.9	−27.8
Reserpine	−32.5	−52.8	−71.7	−75.7	19.3	15.6	12.4	11.1
Negative test solution								
Fumaric acid	−87.4	−89.7	−91.1	−91.2	−15.1	−29.1	−51.3	−59.5
Malic acid	−84.1	−86.9	−88.4	−88.1	−14.5	−27.6	−48.3	−58.04
Etidronic acid	−71.9	−73.1	−71.6	−65.9	29.2	26.8	10.1	−17.8
Clodronic acid	−95.6	−97.4	−98.8	−98.8	4.9	−4.9	−36.5	−49.6
Niflumic acid	−91.7	−94.8	−98.2	−98.1	38.5	20.6	−22.1	−34.7
Canrenoic acid	−93.8	−96.1	−96.8	−96.1	−16.7	−33.8	−67.4	−59.4
Cholic acid	−95.2	−97.5	−99.5	−99.6	−18.9	−33.1	−48.5	−63.9
Raffinose	−84.1	−91.2	−96.5	−97.6	−5.7	−19.4	−26.3	−63.6

Source: From Ref. [12]. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. Copyright 2003 John Wiley. Reproduced with permission.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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

Adoption by CHMP ..... 21 July 2011
Date for coming into effect ..... 1 February 2012

Guideline on bioanalytical method validation

"4.1.8 Matrix effect

...

If this approach cannot be used, for instance ***in the case of on-line sample preparation***, the ***variability of the response from lot to lot*** should be assessed by analysing at least ***6 lots of matrix***, spiked at a ***low*** and at a ***high level*** of concentration (maximum of 3 times the LLOQ and close to the ULOQ). The validation report should include the peak areas of the analyte and of the IS and the calculated concentration for each individual sample. The overall ***CV calculated for the concentration should not be greater than 15%***."

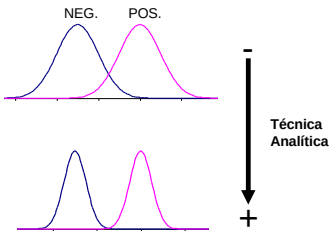
Critérios de Identificação por LC-MS(/MS)

Algumas regulamentações

- **WADA – World Anti-Doping Agency**
- **SANCO – Directorate General Health and Consumers**
- **Council Directive European Communities**
- **Department of Health and Human Services – Federal Workplace Drug Testing Programs - USA**
- **CCA – Codex Alimentarius, WHO**

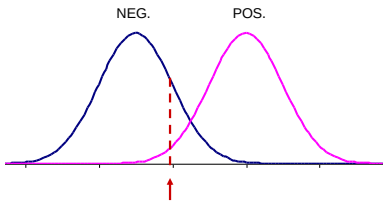
Qualidade da identificação

Falso positivo vs. Falso negativo



Qualidade da identificação

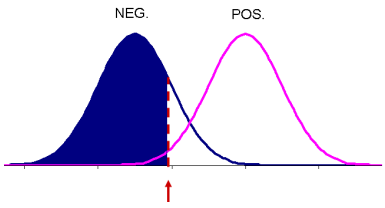
Falso positivo vs. Falso negativo



CRITÉRIO RÍGIDO

Qualidade da identificação

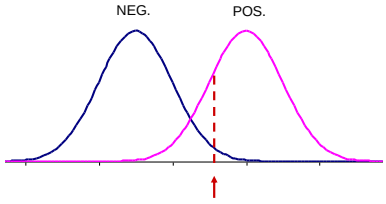
Falso positivo vs. Falso negativo



CRITÉRIO RÍGIDO

Qualidade da identificação

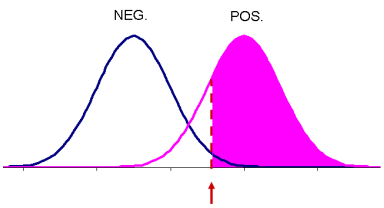
Falso positivo vs. Falso negativo



CRITÉRIO TOLERANTE

Qualidade da identificação

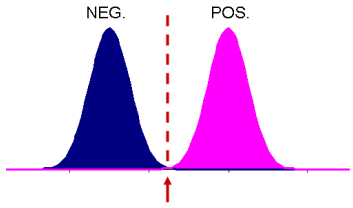
Falso positivo vs. Falso negativo



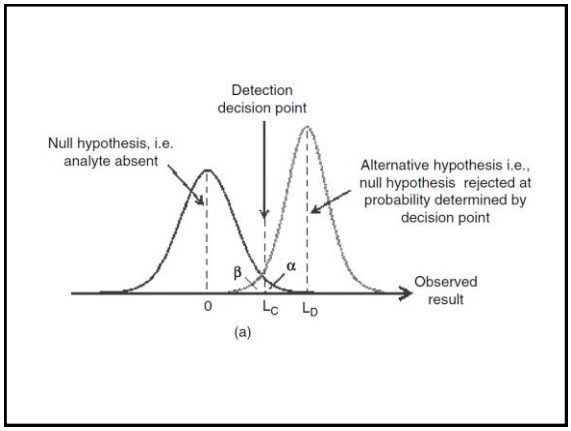
CRITÉRIO TOLERANTE

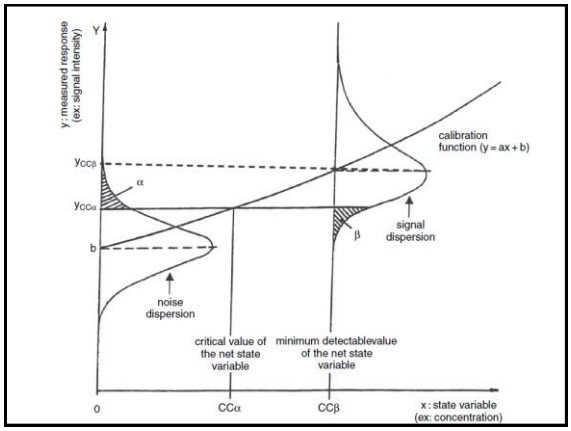
Qualidade da identificação

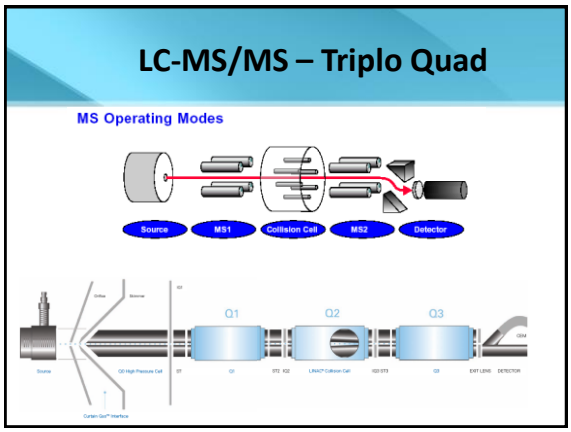
Falso positivo vs. Falso negativo

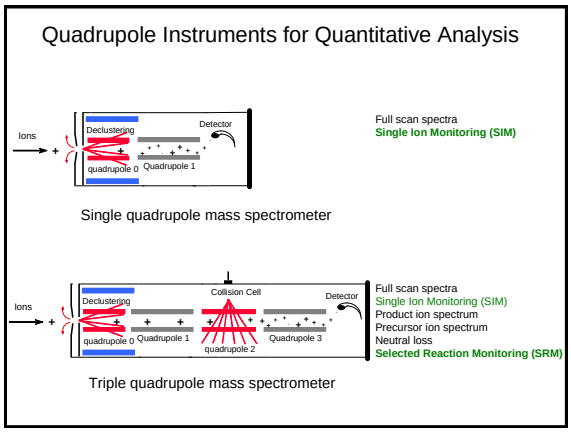


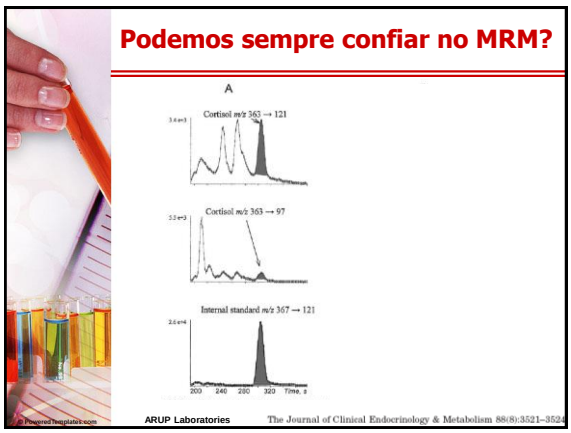
IDEAL

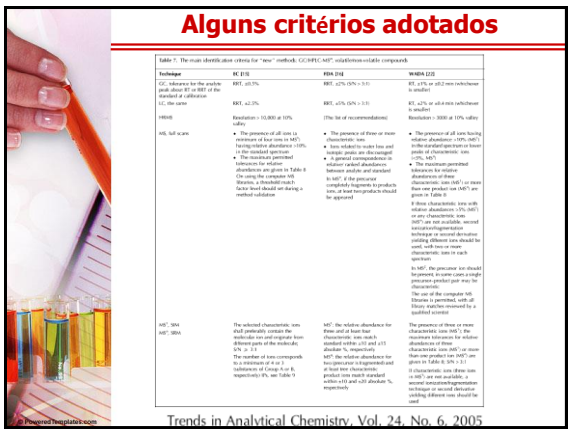

Detection
decision point
Null hypothesis, i.e.
analyte absent
Alternative hypothesis i.e.,
null hypothesis rejected at
probability determined by
decision point
β
α
Observed
result
0
L_C
L_D
(a)


y: measured response
(ex: signal intensity)
Y
$y_{CC\beta}$
$y_{CC\alpha}$
b
α
β
calibration
function (y = ax + b)
signal
dispersion
noise
dispersion
critical value of
the net state
variable
minimum detectablevalue
of the net state
variable
0
CCα
CCβ
x: state variable
(ex: concentration)


LC-MS/MS – Triplo Quad
MS Operating Modes
Source
MS1
Collision Cell
MS2
Detector
Q1
Q2
Q3


Quadrupole Instruments for Quantitative Analysis
Ions
Declustering
Detector
quadrupole 0
Quadrupole 1
Full scan spectra
Single Ion Monitoring (SIM)
Single quadrupole mass spectrometer
Ions
Declustering
Collision Cell
Detector
quadrupole 0
Quadrupole 1
quadrupole 2
Quadrupole 3
Full scan spectra
Single Ion Monitoring (SIM)
Product ion spectrum
Precursor ion spectrum
Neutral loss
Selected Reaction Monitoring (SRM)
Triple quadrupole mass spectrometer


Podemos sempre confiar no MRM?
A
Cortisol m/z 363 → 121
Cortisol m/z 363 → 97
Internal standard m/z 367 → 121
200
240
280
320
Time, s
ARUP Laboratories
The Journal of Clinical Endocrinology & Metabolism 88(8):3521–3524


Alguns critérios adotados
Trends in Analytical Chemistry, Vol. 24, No. 6, 2005

Alguns critérios adotados

INTERVALOS DE TOLERÂNCIA PARA A ABUNDÂNCIA RELATIVA DOS PICOS DE MS

Table 8. Tolerance for abundance of mass peaks in new methods

Relative abundance	EI-GC–MS		CI-GC–MS, LC–MS, GC/LC–MS^n	
	EC [15]	WADA [22]	EC [15]	WADA [17]
>50%	±10% (relative)	±10% (absolute) ±20% (relative)	±20% (relative)	±15% (absolute) ±25% (relative)
25–50%				
>20–50%	±15% (relative)		±25% (relative)	
>10–20%	±20% (relative)		±30% (relative)	
<25%		±5% (absolute)		±10% (absolute)
≤10%	±50% (relative)		±50% (relative)	

Ex.: betametasona vs. dexametasona

(m/z 345; 49% e 92%, respectivamente)

Trends in Analytical Chemistry, Vol. 24, No. 6, 2005

Alguns critérios adotados

CRITÉRIO DOS 4 PONTOS

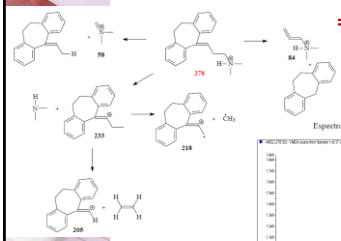
Table 9. IP for different MS techniques [15]

MS technique	IP/ion
LRMS, LRMS^n precursor ion	1.0
LRMS^n transition products	1.5
HRMS, HRMS^n precursor ion	2.0
HRMS^n transition products	2.5

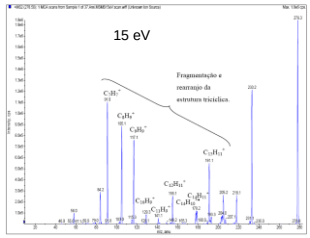
Obs: = 4 pontos para moléculas pequenas
> 4 pontos para peptídeos e proteínas

Trends in Analytical Chemistry, Vol. 24, No. 6, 2005

Alguns critérios adotados

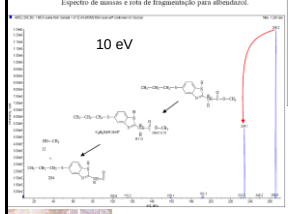


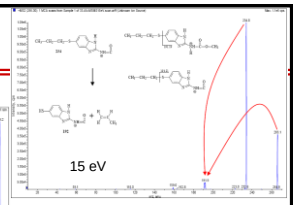
Espectro de massas e rota de fragmentação para amitriptilina.

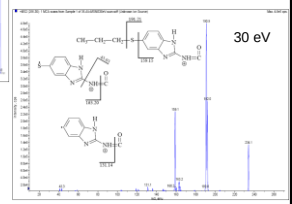


Alguns critérios adotados

Espectro de massas e rota de fragmentação para albendazol.







Alguns critérios adotados

Table 4
Examples of the number of IP earned for a range of MS techniques and combinations thereof (n: an integer, adapted from [29])

Technique(s)	Number of ions	IP
GC–MS (EI or CI)	n	n
GC–MS (EI and CI)	2 (EI) + 2 (CI)	4
GC–MS (EI or CI), two derivatives	2 (derivative A) + 2 (derivative B)	4
LC–MS	n	n
GC–MS^2 or LC–MS^2	1 precursor and 2 daughters	4
GC–MS^2 and LC–MS^2	2 precursor ions, each with 1 daughter	5
LC–MS^3	1 precursor, 1 daughter and 2 grand-daughters	5.5
HR-MS	n	$2n$
GC–MS and LC–MS	2 + 2	4
GC–MS and HR-MS	Same 2 ions for each (2 IPs each for the HRMS, no additional IPs for the LRMS, same 2 ions).	4

L. Rivier / Analytica Chimica Acta 492 (2003) 69–82

WADA Technical Document – TD2003IDCR

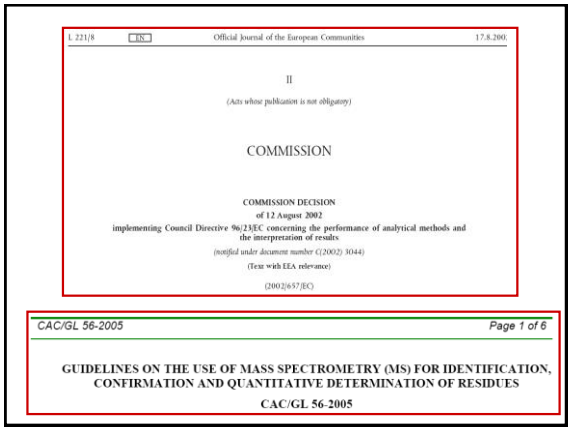
Document Number:	TD2003IDCR	Version Number:	1.2
Written by:	WADA Project Team	Approved by:	
Date:	May 11, 2003	Effective Date:	January 1, 2004

IDENTIFICATION CRITERIA FOR QUALITATIVE ASSAYS INCORPORATING CHROMATOGRAPHY AND MASS SPECTROMETRY

QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS

Document N° SANCO/10232/2006

24/March/2006



L 221/8 EN Official Journal of the European Communities 17.8.2002

II

(Acts whose publication is not obligatory)

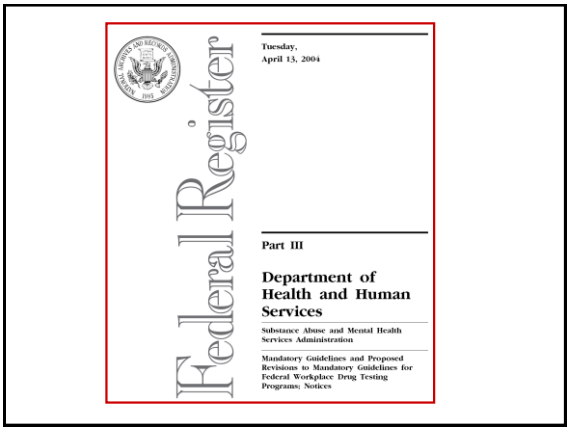
COMMISSION

COMMISSION DECISION
of 12 August 2002
implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results
(notified under document number C(2002) 3044)
(Text with EEA relevance)
(2002/657/EC)

CAC/GL 56-2005 Page 1 of 6

GUIDELINES ON THE USE OF MASS SPECTROMETRY (MS) FOR IDENTIFICATION, CONFIRMATION AND QUANTITATIVE DETERMINATION OF RESIDUES

CAC/GL 56-2005



Federal Register

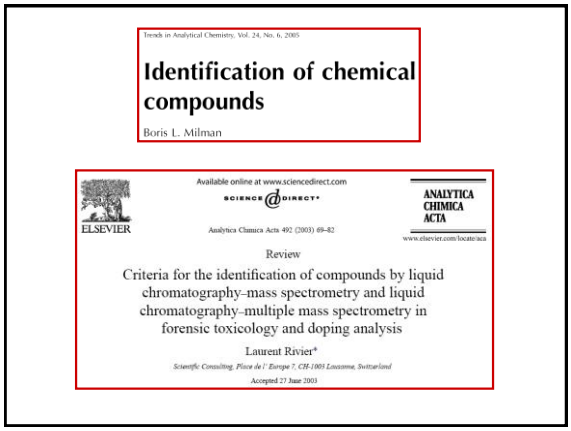
Tuesday,
April 13, 2004

Part III

Department of Health and Human Services

Substance Abuse and Mental Health Services Administration

Mandatory Guidelines and Proposed Revisions to Mandatory Guidelines for Federal Workplace Drug Testing Programs; Notices



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Identification of chemical compounds

Boris L. Milman

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Analytica Chimica Acta 492 (2003) 69–82
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Review

Criteria for the identification of compounds by liquid chromatography–mass spectrometry and liquid chromatography–multiple mass spectrometry in forensic toxicology and doping analysis

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Accepted 27 June 2003