



# Current and Future Applications of Mass Spectrometry to the Clinical Laboratory

慈濟大學醫技系 林惠茹副教授  
2015/12/13 醫學檢驗品質研討會

# Who am I ?



- 林惠茹副教授
- 慈濟大學 醫學檢驗生物技術學系
- 經歷

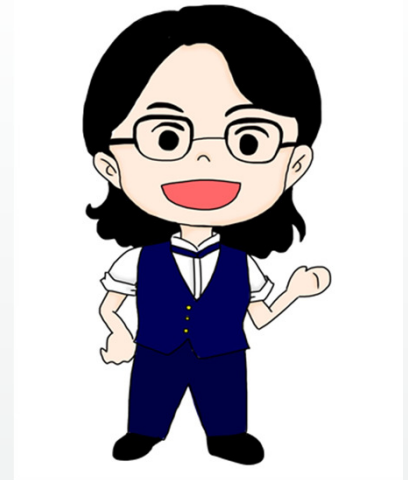
慈濟大學 殘留農藥檢驗中心 品質主管

中研院基因體研究中心 博士後研究員

慈濟大學 濫用藥物檢驗中心 技術員

# Who am I ?

- 林惠茹副教授
- 慈濟大學 醫學檢驗生物技術學系
- 現代柯南 跟著瑪斯一起探索檢驗世界
  - 磨課師線上課程
  - 榮獲教育部獎助&認定醫檢師繼續教育學分(6學分)







## 醫事檢驗師





# Mass Spectrometry in the Clinical Laboratory



液相層析串聯質譜儀  
(LC/MS/MS)

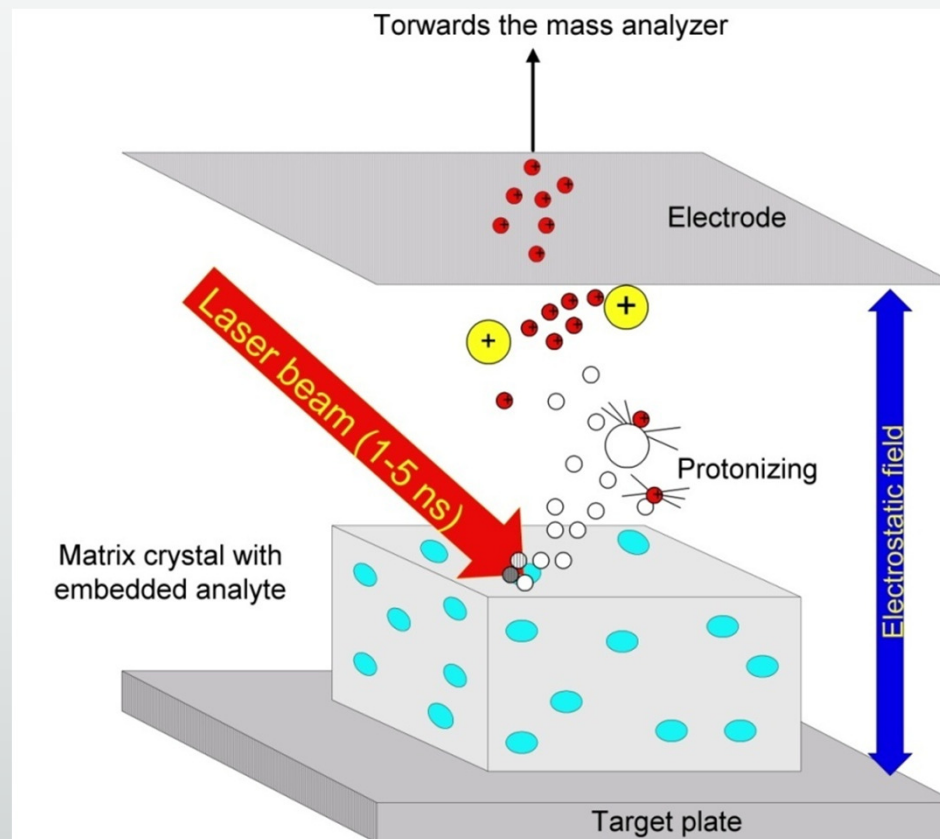


基質輔助雷射脫附游離  
飛行時間質譜儀  
(MALDI/TOF)

# Application

- MALDI/TOF
- bacteria identification

- Matrix-assisted laser desorption/ionization (MALDI)



# Matrix Assisted Laser Desorption/Ionization (MALDI)

Detector



TOF = Time of Flight

The positively charged proteins drift in flight tube; smaller proteins drift faster

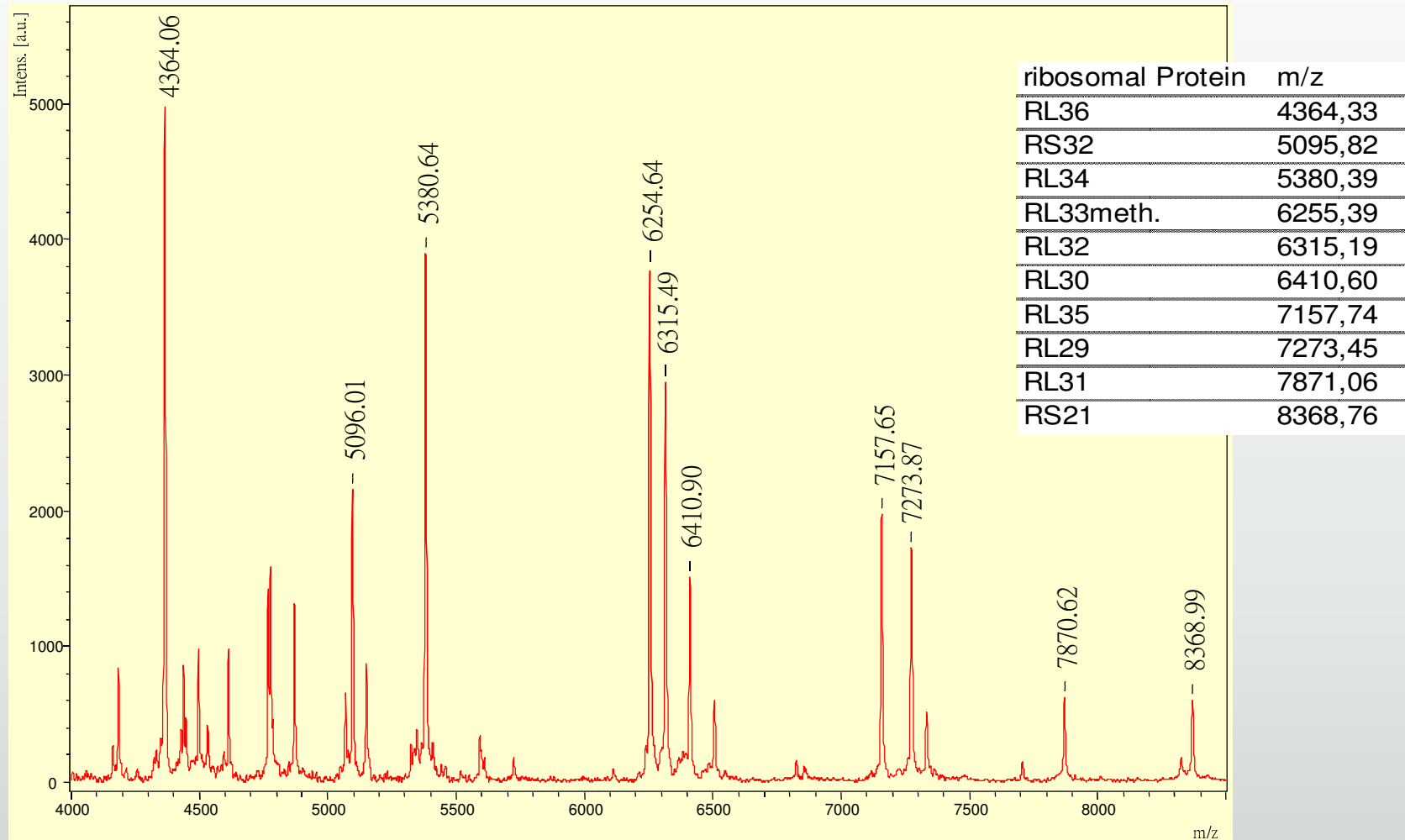
Drift region

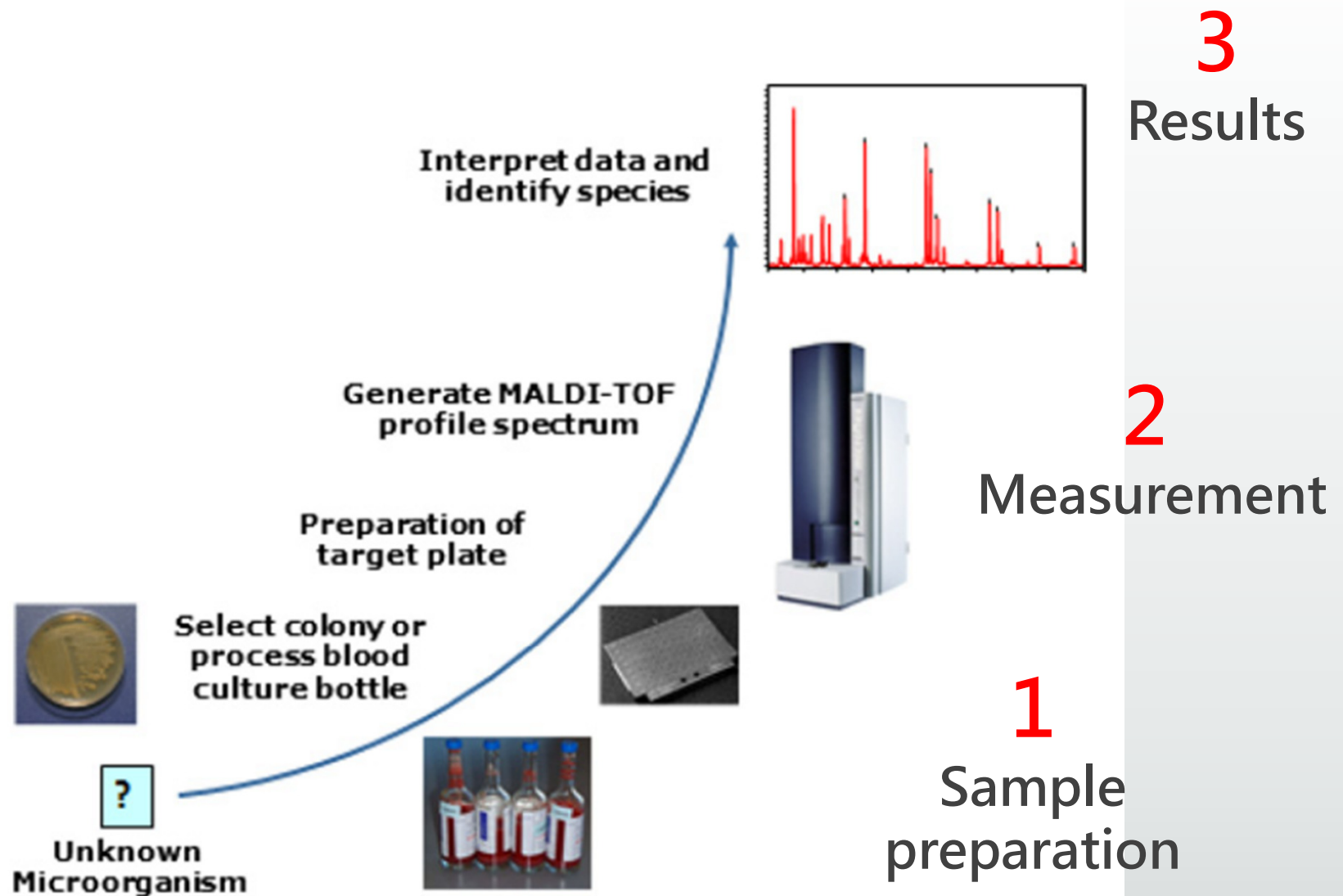




# MALDI-TOF

Mass spectra: characteristic proteins signal





**Figure 1.** MALDI-TOF MS for microorganism identification.

The Journal of Molecular Diagnostics, Vol. 14, No. 5, September 2012

# Mass Spectrometry in the Clinical Laboratory



液相層析串聯質譜儀  
(LC/MS/MS)



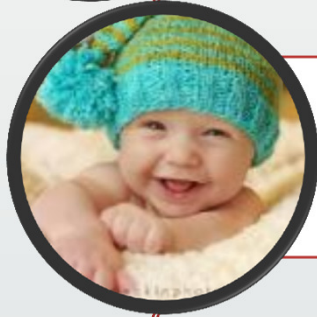
基質輔助雷射脫附游離  
飛行時間質譜儀  
(MALDI/TOF)



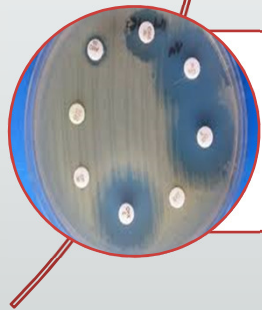
# Application -LC/MS/MS



Drug abuse/TDM



Newborn screening

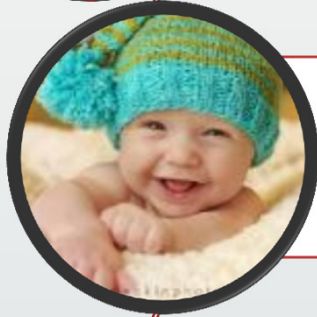


(bacteria) resistant strain

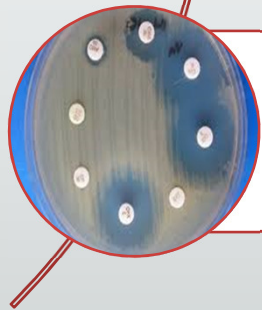
# Application -LC/MS/MS



Drug abuse/TDM

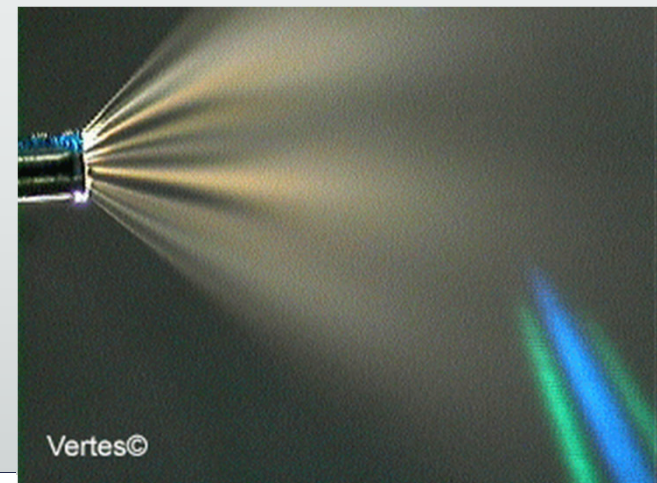
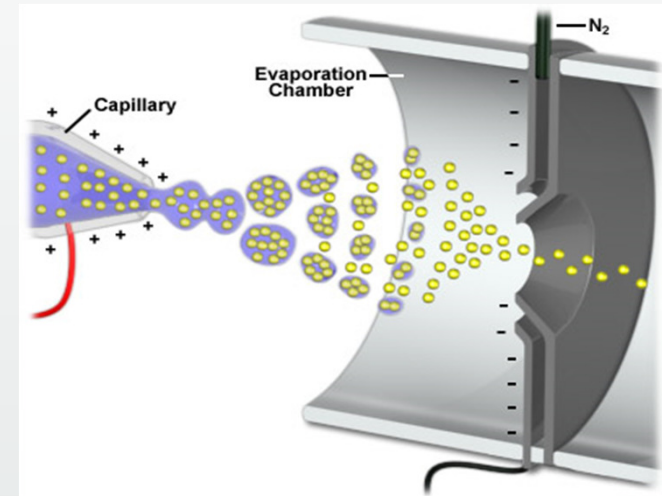
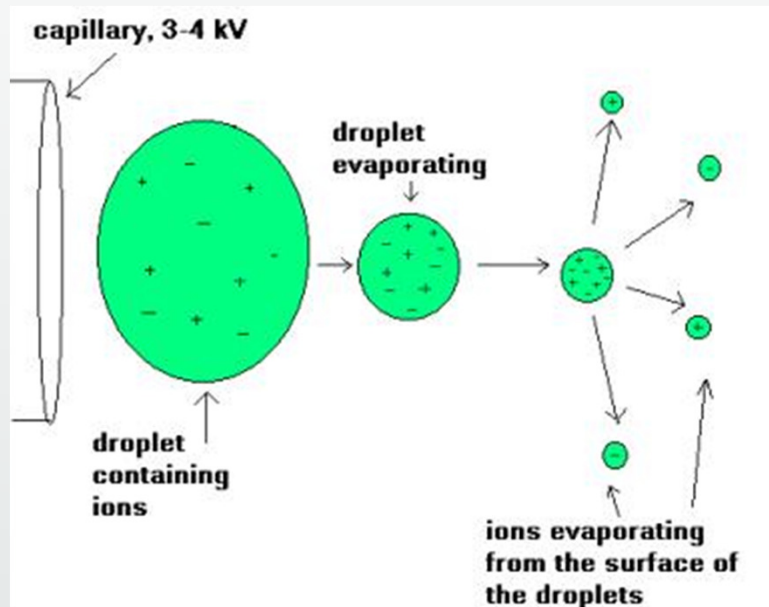


Newborn screening



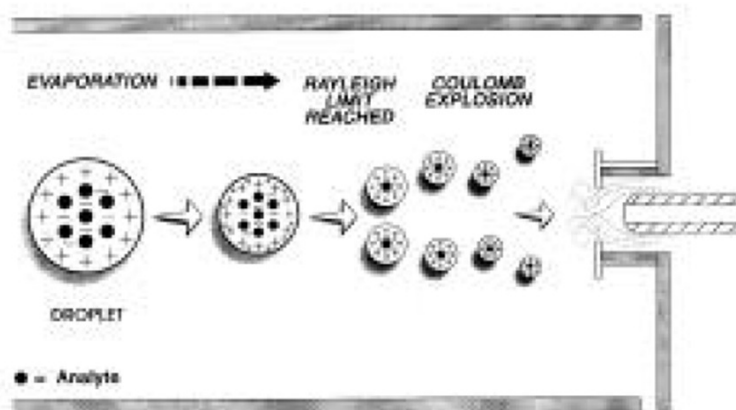
(bacteria) resistant strain

# Electrospray ionization (ESI)



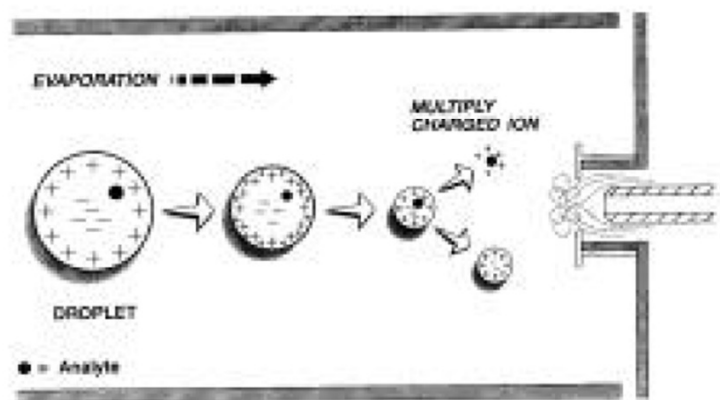


# Ionization Mechanisms (ESI)



- Coulomb Fission :

- Assumes that the increased charge density, due to solvent evaporation, causes large droplets to divide into smaller droplets eventually leading to single ions.

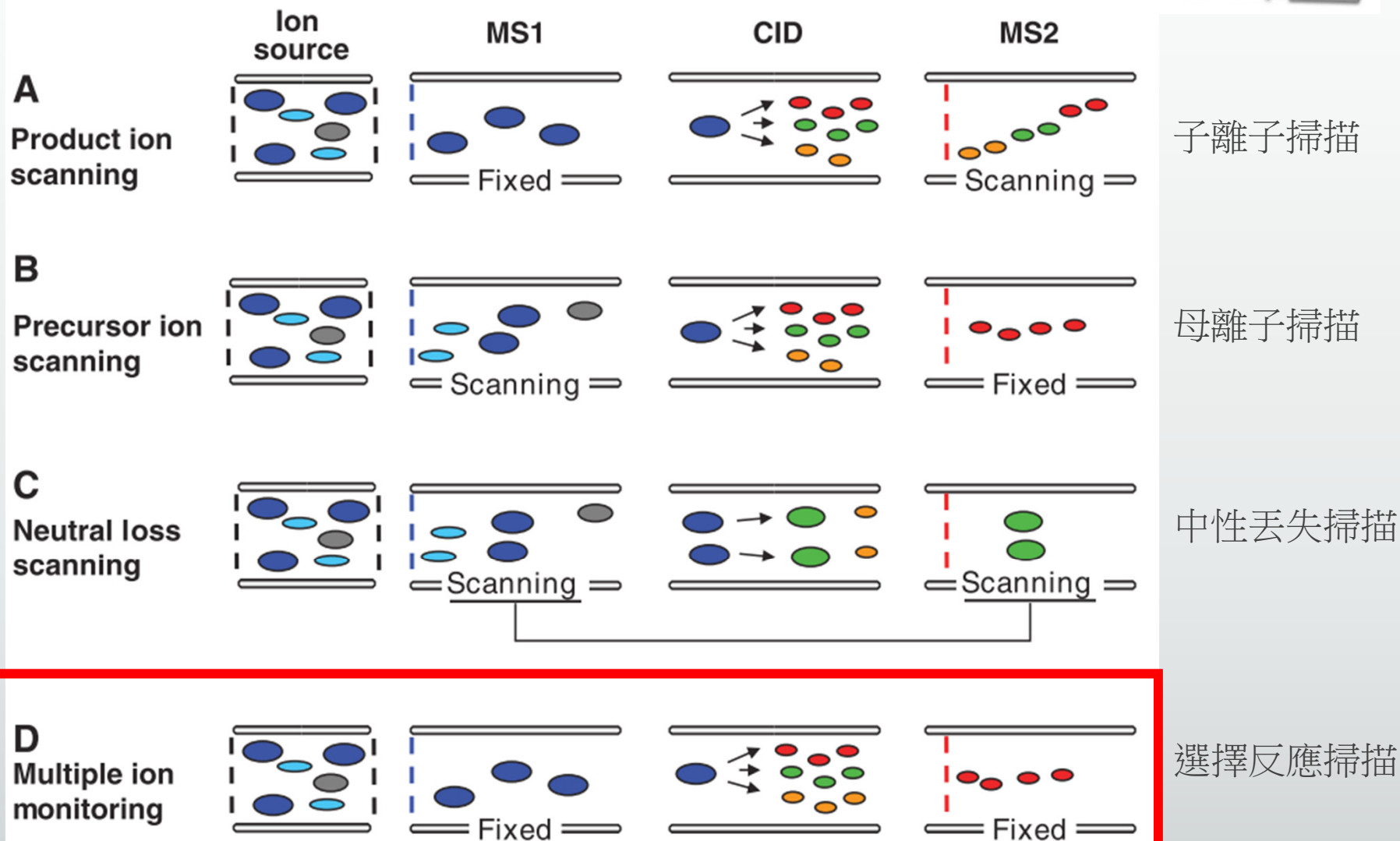


- Ion Evaporation:

- Assumes the increased charge density that results from solvent evaporation causes Coulombic repulsion to overcome the liquid's surface tension, resulting in a release of ions from droplet surfaces

# Tandem mass spectrometry

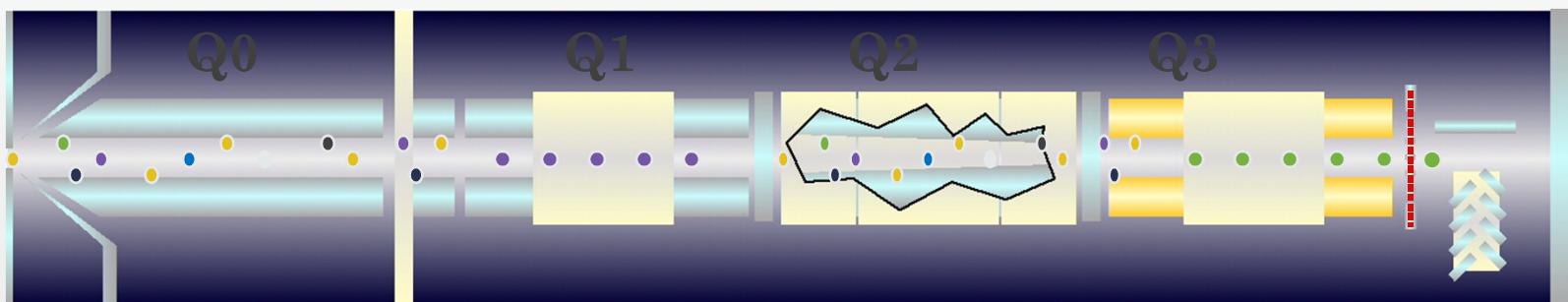
Science. 2006 Apr 14;312(5771):212-7.



# Multiple Reaction Monitoring (MRM)



*Ion accumulation*



*Precursor ion selection*   *Fragmentation*  
N<sub>2</sub> CAD Gas

AB Applied Biosystems

MDS SCIEX

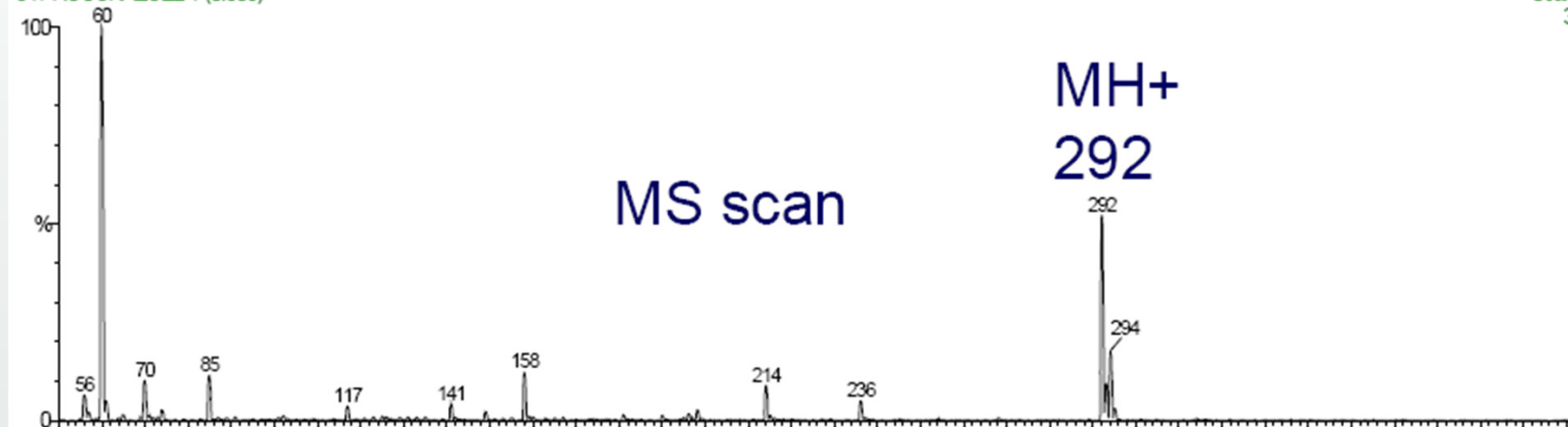


# Cyproconazole

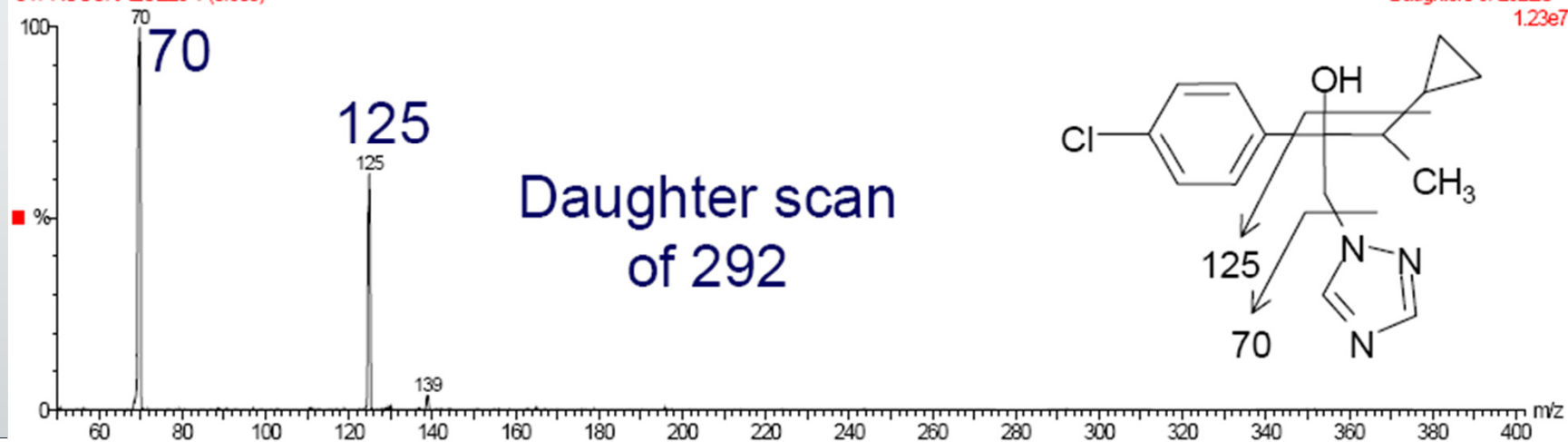
292>70  
292>125

Cyproconazole, 10 µg/ml, 10 µl/min, cone 25 V; Collision 30V

CYPROCONAZOLE 1 (0.505)



CYPROCONAZOLE 3 1 (0.505)





Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

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Analytica Chimica Acta 492 (2003) 69–82



Review

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

Laurent Rivier\*

15/2011

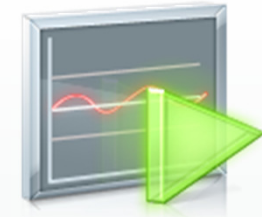
World Anti-Doping Agency (WADA), International standard for doping control, ver 2.0,

**EVIDENCE**

1. Retention time criteria
2. Identification point (1 precursor+2 transition product)
3. Relative MRM ion-intensity criteria

# 濫用藥物尿液檢驗作業準則

## 第四章 品質管制及品質保證



- 每一批確認檢驗檢體，應包括下列各品管尿液：
  - 一、在閾值濃度之單點校正檢體。
  - 二、一個以上不含待測藥物或其代謝物之尿液。
  - 三、一個以上在待測藥物或其代謝物閾值濃度25%內之陽性品管尿液。
  - 四、一個以上在待測藥物或其代謝物閾值濃度25%內之陰性品管尿液。
  - 五、一個以上品管檢體為實驗室內部之盲品管尿液。
- 每一批確認檢驗尿液檢體中，至少應含百分之十之品管尿液。
- 檢驗機構確認檢驗方法之線性、精密度及準確性，應至少每年評估一次。



# 濫用藥物尿液檢驗及醫療機構認可管理辦法

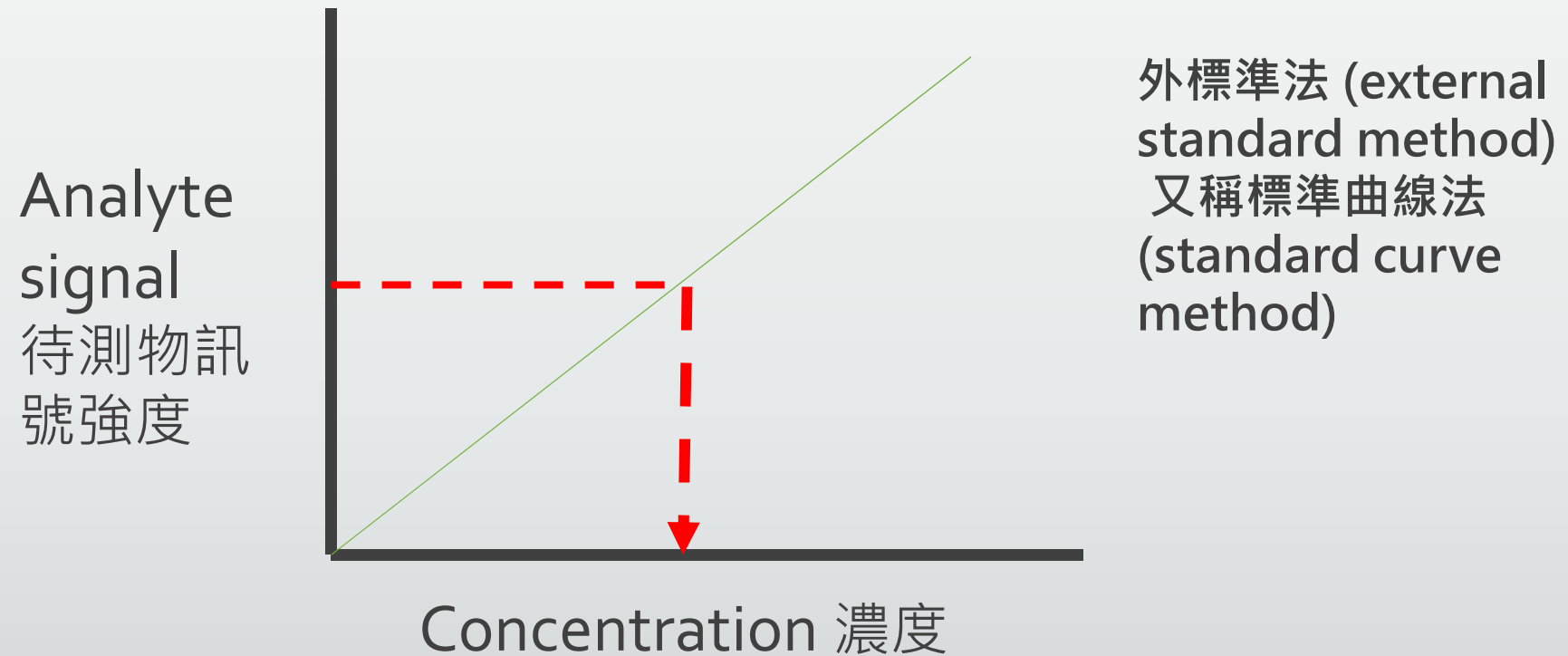
每三個月應接受執行  
機關績效監測一次



每年一次實地訪評



# 定量



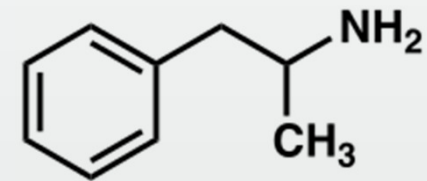
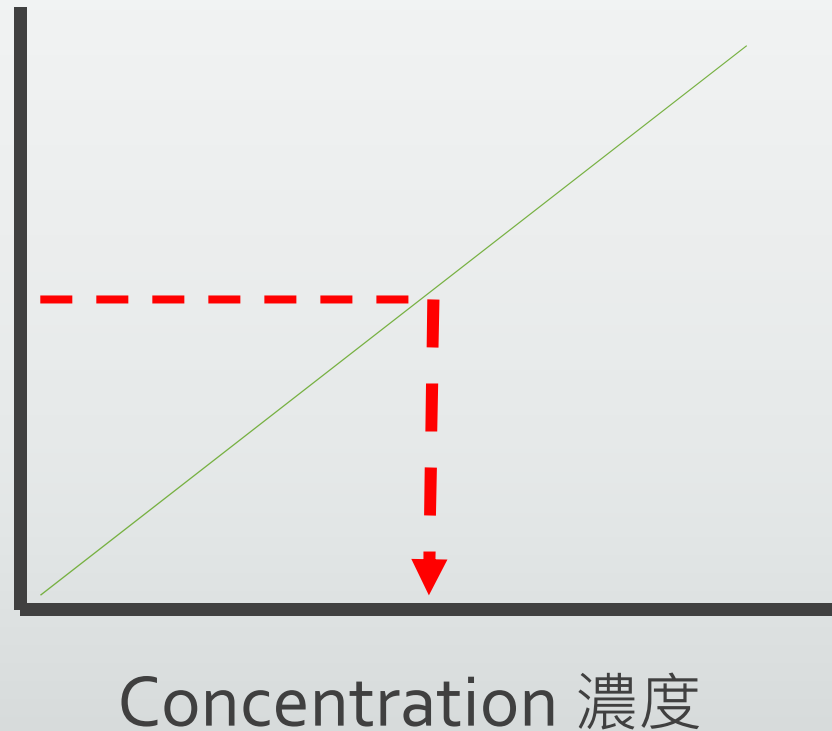


# 精準定量 穩定性同位素稀釋法 (stable isotope dilution)

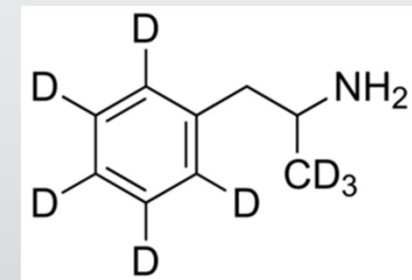
- 內標準法 (internal standard method)

compensates for  
variability  
(1)chemical  
derivatization  
(2)sample extraction  
(3)LC/MS/MS analysis

Analyte  
signal/internal  
standard  
signal  
待測物訊號強度  
/內標訊號強度



amphetamine



amphetamine-d<sub>8</sub>



Review

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

Laurent Rivier\*

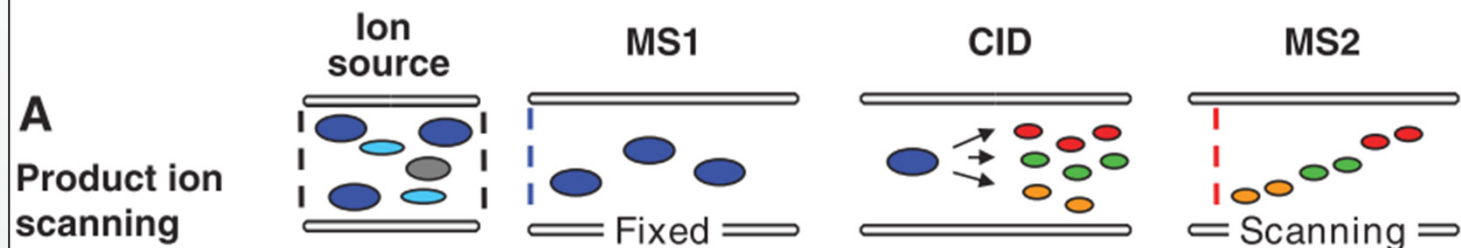
1. SANCO/12495/2011
2. World Anti-doping Agency (WADA), International standard for laboratories, ver 2.0,

1. Retention time criteria
2. Identification point (1 precursor+2 transition product)
3. Relative MRM ion-intensity criteria

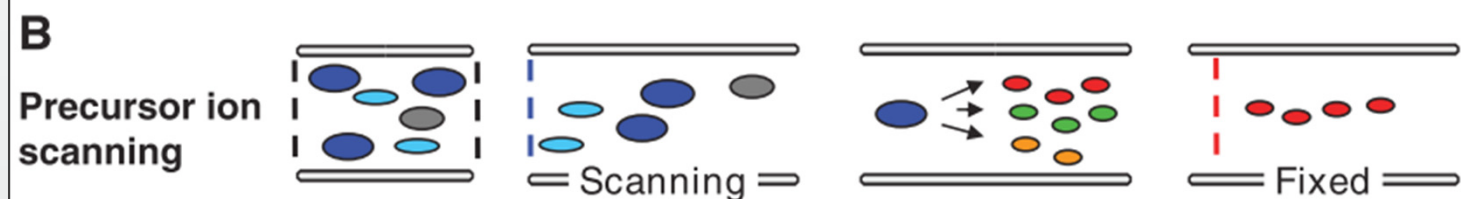
Regulation	R.T.	MRM transitions to be monitored	Ion ratio (% of base peak)	Maximum tolerance window for relative ion ratio (%)
EU (SANCO 12571/2013)	±0.2 min	2	all	±30 (relative)
WADA (TDR2010)	±2% or ±0.1 min	2	>50	±10 (absolute)
			25-50	±20 (relative)
			5-<25	±5 (absolute)
			<5	±50 (relative)
SOFT/AAFS (2006)	±1-2% <sup>a</sup>	2	all	±25 or 30 (relative)
FDA <sup>b</sup> (2003)	±5%	2	all	±10 (relative)
		3 or more	all	±20 (relative)

# Tandem mass spectrometry

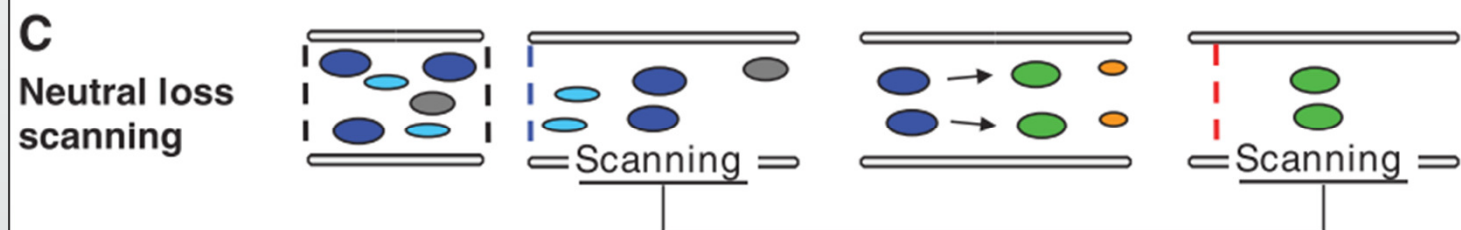
Science. 2006 Apr 14;312(5771):212-7.



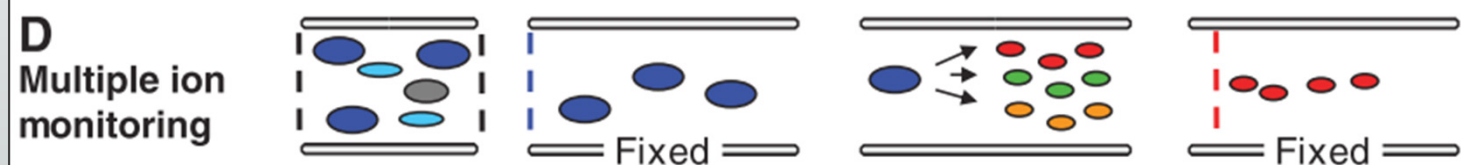
子離子掃描



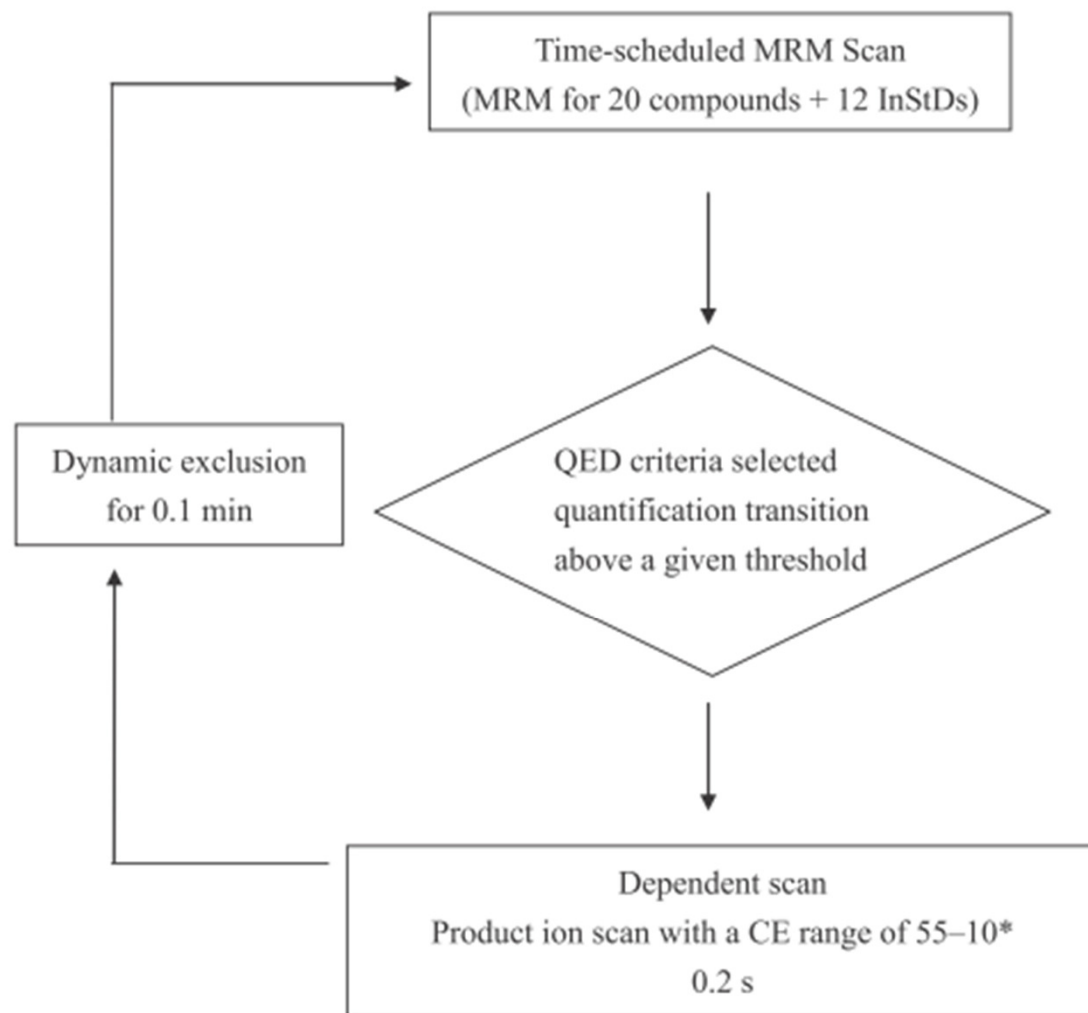
母離子掃描



中性丢失掃描



選擇反應掃描



**Figure 1.** Flow diagram of the LC/MS/MS scan mode using MRM as the survey scan and the product ion scan as the dependent scan in QED acquisition. \*The CE range for MOR and COD was 55–50.

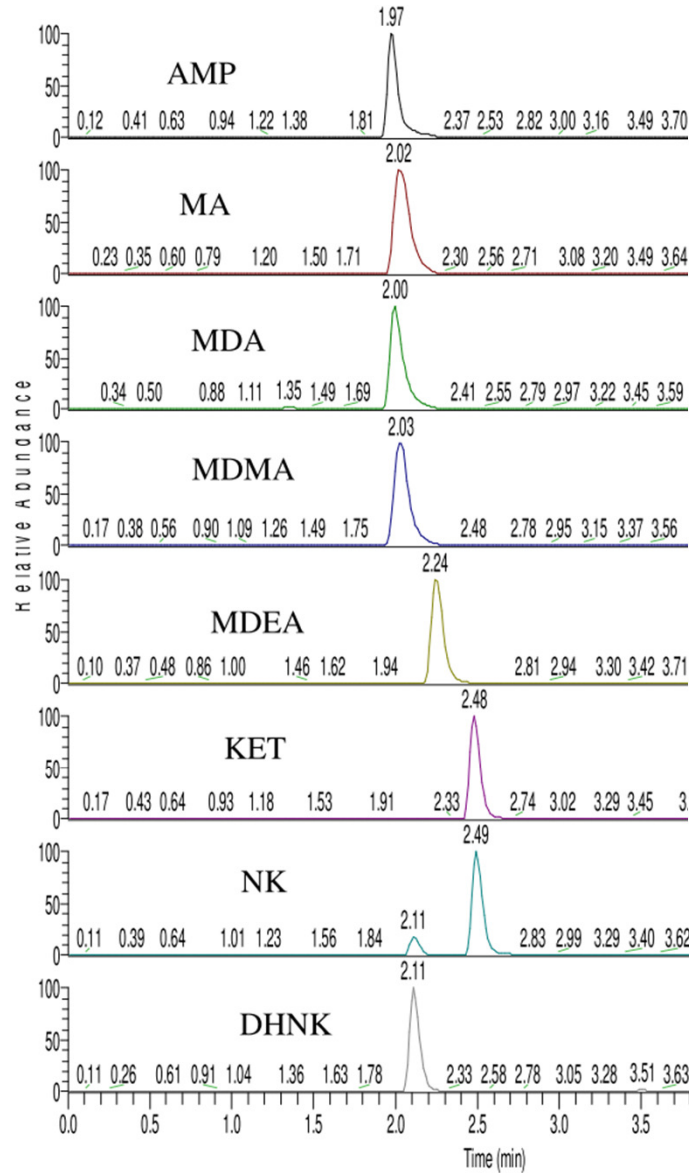


液相層析串聯質譜儀 (LC/MS/MS)

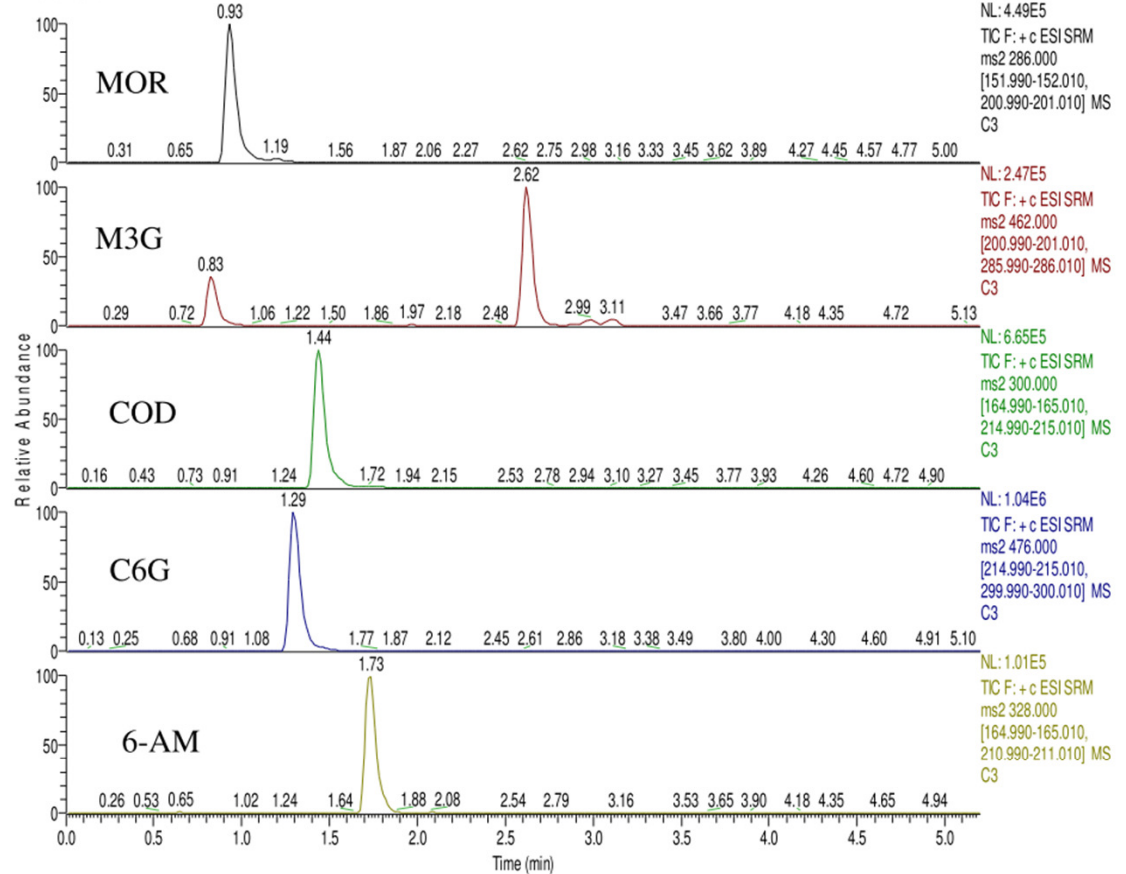
Rapid Commun Mass Spectrom 2014, 28, 2043-2053



RT: 0.00-5.20



RT: 0.00-5.20



以液相層析串聯質譜儀建立在尿液中多種濫用藥物之確認檢驗分析

SPP column (Agilent poroshell 120 EC-C<sub>18</sub>, 2.1 mm × 100 mm, 2.7 μm).

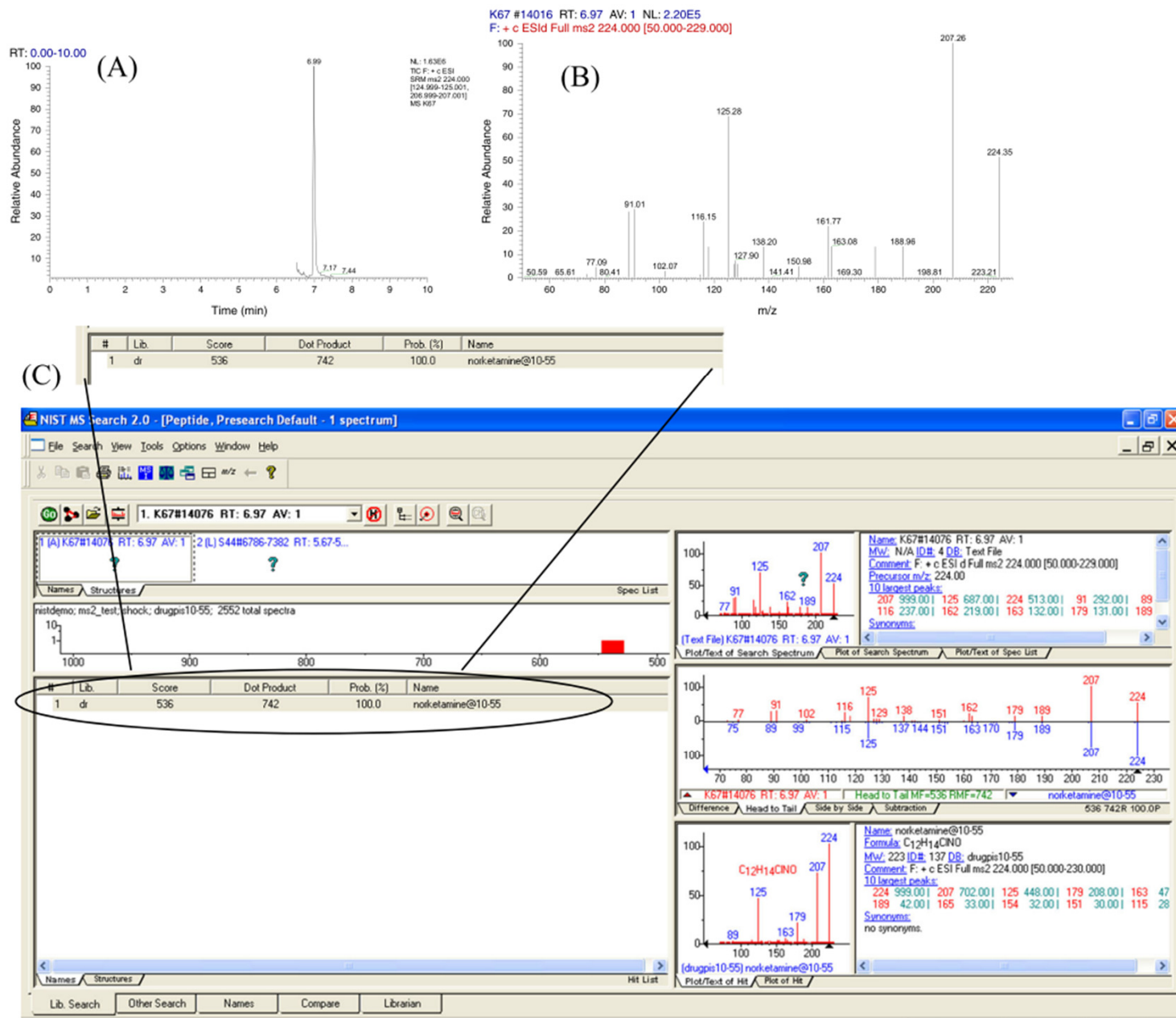
total analysis time: 6.7 min/sample

Lin HR et al. Journal of chromatography B

**Table 1.** LC/MS/MS parameters

Analyte	RT (min)	Quantification transition (CE, eV)	Confirmatory transition (CE, eV)	Relative intensity ratio (%)	Characteristic product ions
AMP-d <sub>8</sub>	5.68	144>97 (16)			
AMP	5.78	136>91 (16)	136>119 (5)	27.3	91, 136, 119, 65
MA-d <sub>8</sub>	6.04	158>93 (20)			
MA	6.11	150>91 (20)	150>119 (8)	26.2	150, 91, 65, 119
MDA-d <sub>5</sub>	6.14	185>168 (8)			
MDA	6.17	180>163 (8)	180>135 (19)	26.1	163, 135, 133, 105
MDMA-d <sub>5</sub>	6.32	199>165 (13)			
MDMA	6.34	194>163 (13)	194>135 (21)	25.5	194, 163, 135, 105
MDEA-d <sub>5</sub>	6.74	213>163 (13)			
MDEA	6.75	208>163 (13)	208>105 (25)	28.0	208, 163, 135, 105
KET-d <sub>4</sub>	7.04	242>129 (25)			
KET	7.06	238>125 (27)	238>220 (16)	75.9	238, 125, 220, 207
NK-d <sub>4</sub>	7.04	228>211 (14)			
NK	7.05	224>207 (14)	224>125 (24)	59.5	224, 207, 125, 179
DHMK-d <sub>4</sub>	6.57	226>209 (12)			
DHMK	6.59	222>205 (13)	222>142 (28)	39.8	222, 205, 142, 177
MOR-d <sub>6</sub>	2.93	292>152 (56)			
MOR	2.95	286>152 (59)	286>201 (25)	96.0	286, 152, 165, 128
M3G-d <sub>3</sub>	1.99	465>289 (29)			
M3G	2.00	462>286 (29)	462>201 (41)	4.3	462, 286, 201
COD-d <sub>3</sub>	5.21	303>215 (30)			
COD	5.23	300>215 (25)	300>165 (44)	75.3	300, 215, 199, 225
C6G	5.08	476>300 (30)	476>215 (29)	2.2	476, 300, 282, 215
6-MAM-d <sub>6</sub>	6.07	334>165 (38)			
6-MAM	6.11	328>165 (38)	328>211 (27)	78.5	328, 165, 211, 193
Phentermine	6.83	150>91 (21)	–		91, 150, 133, 65
phenylpropanolamine	3.97	152>134 (7)	–		134, 117, 115, 91
pseudoephedrine	4.74	166>148 (12)	–		148, 166, 133, 115
hydromorphone	1.11	286>185 (29)	–		286, 185, 157, 199
hydrocodone	5.71	300>199 (28)	–		300, 199, 283, 183
oxycodone	1.67	316>298 (19)	–		316, 298, 214, 256
norcodeine	1.58	286>268 (20)	–		286, 165, 268, 215

RT: retention time; CE: collision energy.



**Figure 6.** Analysis of an authentic sample using the MRM mode and data-dependent analysis. (A) MRM chromatography of NK. (B) The RER product ion spectra of NK. (C) Results obtained using the library search engine NIST with a product ion spectrum of NK in the authentic sample (#67).

**Table 5.** Profile of the authentic samples with variation in relative ion ratio greater than 10%

No#	Analyte	Ion ratio <sup>a</sup> (%)	Bias of ion ratio <sup>b</sup> (%)	Product ion spectra match probability (%)	Quantification conc. (ng/mL)
01	MA	32.14	17.90	98.8	1146
03	MA	30.40	11.52	98.6	1781
07	MA	33.82	24.06	98.2	152
14	MA	35.31	29.53	98.3	3358
15	AMP	19.72	-25.86	94.0	97
19	MA	30.62	12.33	95.0	1430
21	MA	32.77	20.21	94.7	2477
24	MA	32.81	20.36	98.4	552
27	MA	31.24	14.60	98.8	964
28	MA	32.34	18.64	98.8	486
29	MA	32.49	12.03	98.7	10136
54	M3G	3.14	14.6	100.0	67
55	M3G	3.29	20.51	100.0	1979
57	M3G	3.32	21.61	100.0	1233
67	NK	84.56	17.15	100.0	75
78	KET	32.62	-18.88	100.0	30
87	NK	82.57	14.39	100.0	79
88	C6G	3.56	67.14	100.0	53
91	M3G	3.32	21.61	100.0	1811
94	M3G	3.25	18.61	100.0	64
95	C6G	3.05	11.31	100.0	107
97	M3G	3.30	20.44	100.0	127
99	M3G	2.29	-16.12	100.0	1349
100	M3G	3.32	21.61	100.0	1799
100	C6G	1.44	32.39	100.0	504
102	M3G	3.33	21.98	100.0	2018
106	C6G	1.52	28.64	100.0	237
107	M3G	3.36	23.08	100.0	2161

<sup>a</sup>The ratio of two MRM abundances.<sup>b</sup> $[(\text{Ion ratio of samples}/\text{ion ratio of standards}) - 1] \times 100$ .



# Published paper



## Research Article



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(wileyonlinelibrary.com) DOI: 10.1002/rcm.6997

## Improved identification of multiple drugs of abuse and relative metabolites in urine samples using liquid chromatography/triple quadrupole mass spectrometry coupled with a library search

Huei-Ru Lin<sup>1,2,3\*</sup>, Chao-Chuan Liao<sup>1</sup> and Tzu-Chieh Lin<sup>3</sup>

<sup>1</sup>Institute of Medical Biotechnology, Tzu Chi University, Hualien, Taiwan

<sup>2</sup>Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan

<sup>3</sup>Center for Drug Analysis, Tzu Chi University, Hualien, Taiwan

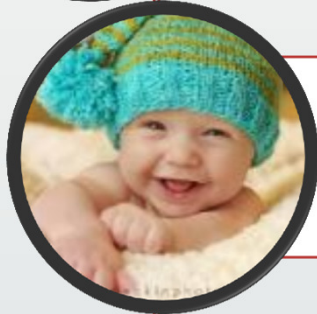
The proposed LC/QqQ-MS/MS, which is method based on a set of criteria including **retention time, MRM transitions, and product ion spectra**, enabled the identification and confirmation of **20 target analytes** by using a QqQ MS analyzer.



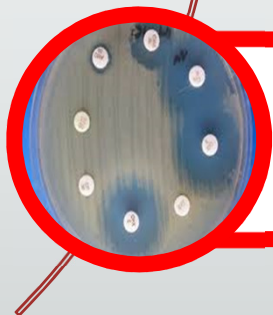
# Application -LC/MS/MS



Drug abuse/TDM



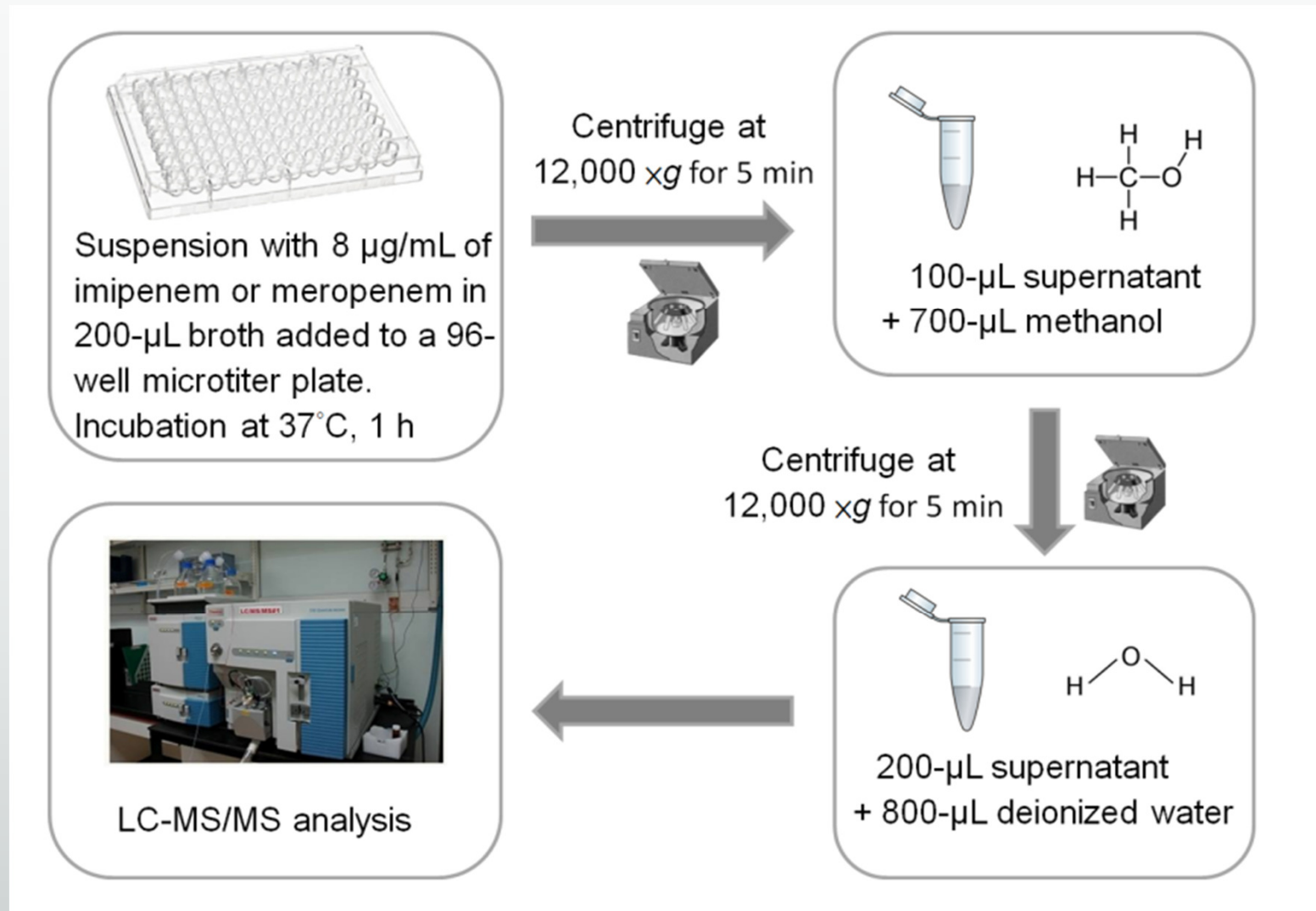
Newborn screening



(bacteria) resistant strain

# Rapid and Sensitive Detection of carbapenemase-producing *A. baumannii* Using Superficially Porous liquid Chromatography-Tandem Mass Spectrometry Method

液相層析串聯質譜儀

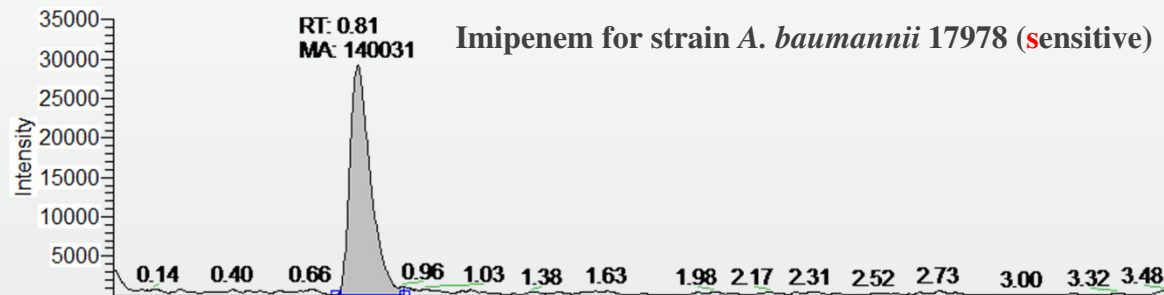


# LC-MS/MS chromatograms for imipenem

Imipenem 300>142

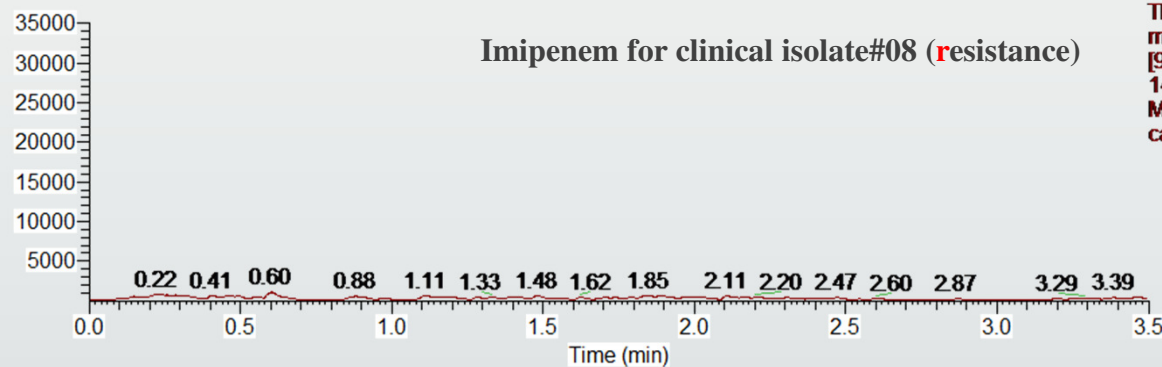
S

RT: 0.00 - 3.50



NL: 3.50E4  
TIC F: + c ESI SRM  
ms2 300.100  
[98.190-98.210,  
142.090-142.110]  
MS  
Carbapenemase\_13

R



NL: 3.50E4  
TIC F: + c ESI SRM  
ms2 300.100  
[98.190-98.210,  
142.090-142.110]  
MS  
carbapenemase\_21

- The strain has ability to hydrolyze carbapenem since the base peak decrease
- Hydrolysis rates =  $\frac{\text{Decrease of the base peak in test strains}}{\text{Remaining base peak in ATCC 17978}}$

# Optimization and validation of the methods

Relationship between incubation condition and hydrolysis rates										
A. baumannii strain	MIC (range) (µg/mL)		LC-MS/MS hydrolysis rate (%)							
Different concentration and incubation period			OD <sub>600</sub> = 2, 1 h		OD <sub>600</sub> = 2, 2 h		OD <sub>600</sub> = 4, 1 h		OD <sub>600</sub> = 4, 2 h	
Antibiotics	IPM	MEM	IPM	MEM	IPM	MEM	IPM	MEM	IPM	MEM
ATCC 17978 (carbapenem-sensitive)	<2	<2	<1	<1	<1	<1	<1	<1	<1	<1
ATCC 17978 IPM-2m (carbapenem-borderline-susceptible)	2	2	75	33	97	56	100	66	100	100
ATCC 17978 IPM-8m (carbapenem-resistant)	>8	>8	100	100	100	100	100	100	100	100

The relationship between culture condition and hydrolysis rate

# Imipenem hydrolysis rate of clinical isolates

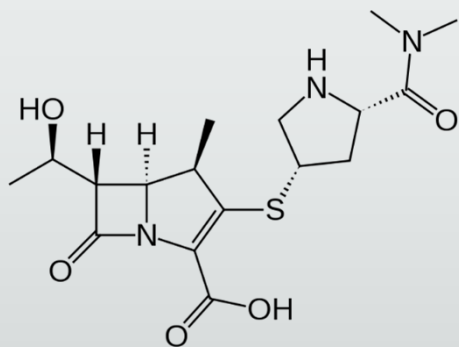
No. of isolates	Strain info.	Description	MIC ( $\mu\text{g/mL}$ ) detected	IPM hydrolysis rate (%)
7	<i>A. baumannii</i>	ATCC strains	<2	0
1	17978 IPM-2m	carbapenem-borderline-susceptible	2	100
1	17978 IPM-8m	carbapenem-resistant	>8	100
35	Clinical isolates	carbapenem-susceptible strains	<2	0-56
67	Clinical isolates	carbapenem-resistant strains	>8	84-100
1	Clinical isolates	carbapenem-resistant strains	>8	19

A total of 112 *A. baumannii* strains were used in this study, including 103 clinical isolates with 68 carbapenem-resistant strains and 35 carbapenem-susceptible strains, seven ATCC strains and two selected mutants. The results of the superficially porous LC-MS/MS assay showed

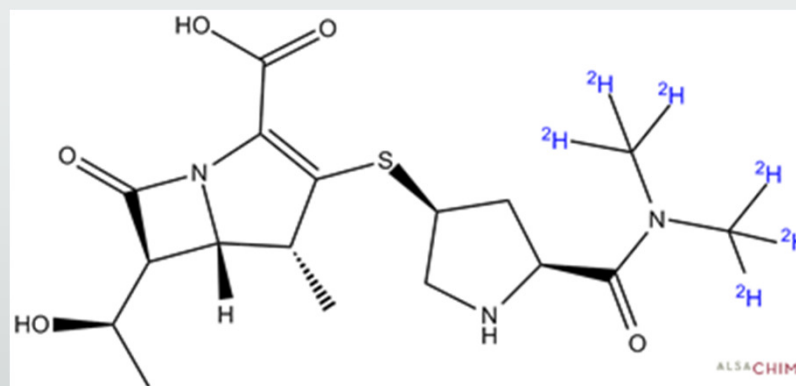


# Monitoring compound Quality control

- 300.1  $m/z$  to 142.2  $m/z$  for imipenem
- 383.9  $m/z$  to 141.2  $m/z$  for meropenem
- 390.1  $m/z$  to 147.1  $m/z$  for the internal standard (meropenem- $d_6$ )



meropenem



meropenem- $d_6$

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ORIGINAL ARTICLE

## Rapid and sensitive detection of carbapenemase activity in *Acinetobacter baumannii* using superficially porous liquid chromatography-tandem mass spectrometry

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