Analytical Platform for *Radiopharmaceuticals*

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行政院原子能委員會核能研究所

Organization of INER

Organization of INER

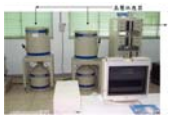
- Director-General: Dr. Yeh, Tao-Rjan
  - Deputy Diretor-General: Dr. Shen, Lie-Hang
    - Chemical Analysis Division (CAD): Dr. Men, Lee-Chung; Dr. Liu, Kung-Tien
    - Chemical Division: Dr. Men, Lee-Chung; Dr. Lian, Chung-San
  - Deputy Diretor-General: Dr. Chen, Chia-Chieh
    - Isotope Application Division: Dr. Lin, Wuu-Jyh; Dr. Lee, Te-Way
  - Deputy Diretor-General: Mr. Hsia Acety
    - 10 Divisions

Organization of CAD

- Director & Deputy Director: Dr. Men, Lee-Chung; Dr. Liu, Kung-Tien
  - Secretary
  - Project & Quality
  - Radio-Chemical Analysis Lab
    - Low level radioactive waste
    - Radionuclides, chemical components
  - Radio-Pharmaceutical Characterization Lab
    - Purity, impurity, forced degradation
    - Metabolites, reference materials (COA)
  - Biomass Material & Products Analysis Lab
    - Chemical components of biomass, including materials and products
  - Material & Nanomaterial Analysis Lab
    - Material of nuclear power plant
    - Surface
    - Water

Bulding of CAD

Core Equipments of CAD


- Gamma Spectrometer


- Liquid Scintillation Analyzer

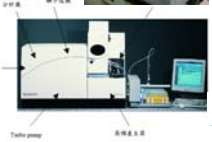

- SEM/EDX


- Surface Area/Pore Size Analyzer


- LC-MS/MS


- Q-TOF MS


- GC-MS

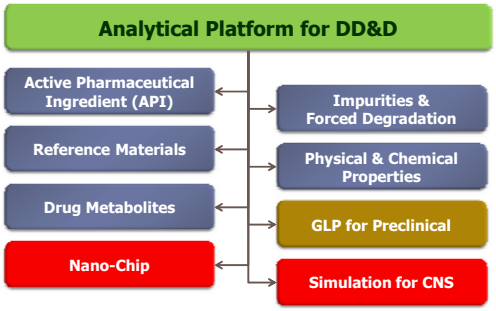

- ICP-MS

Member of RPCL

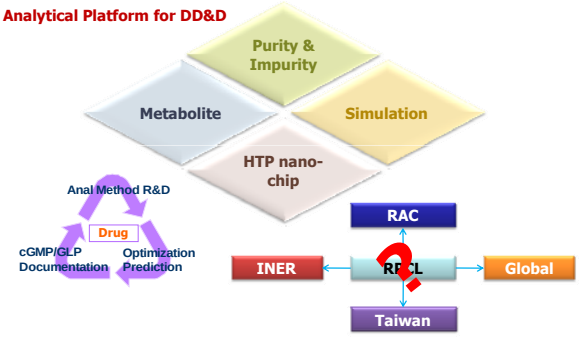
Radiopharmaceutical Characterization Laboratory (RPCL)
5 Scientists + 2 technicians

Dep. of Chem., Nat'l Taiwan Univ.,
Dep. of Biotech., Nat'l Taipei Tech. Univ.,
Dep. of Med., Taipei Med. Univ.,
Dep. of Pharm., UCSF, USA...

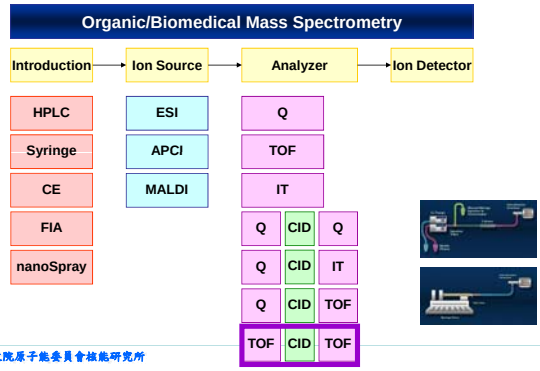
Core Tech. of RPCL



Core Tech. of RPCL



Core Equipments of RPCL



Core Equipments of RPCL

HPLC-QqQ/Linear Ion Trap MS

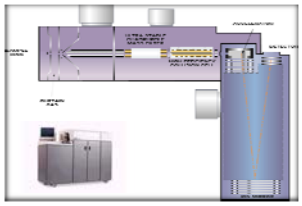
Core Equipments of RPCL

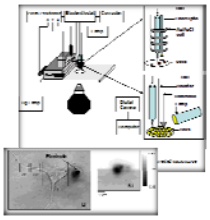
3Q Validation

Core Equipments of RPCL

ABI LC-MS/MS (QqQ-LIT)

Core Equipments of RPCL





- Q-TOF MS
- SECM for neuron
- Biosample concentration workstation
- UV-Visible spectrometer

Core Equipments of RPCL

ABI Q-TOF MS

Preparation HPLC & column

Goals of 2008

	Category	Pharm	Detail
1.1	Precursor: purity, impurity, stability, forced degradation ...	ECD	a. LC-MS/MS structure determination R&D b. HPLC purity assay method R&D c. Method validation: forced degradation, stability d. Structure & pathway of FD (evaluation) e. Impurities (residual solvents, metal, isomers)(evaluation) f. Reference material (COA) g. CMC documentation (SOP, project, report...)
1.2		BZM	
1.3		Sn-ADAM	
1.4		TsDDNP	
1.5		SMPY	
1.6		N2S2-DWAY	
1.7		MBPP	
1.8		Sn-Epidepride	
2	Cold kit: uniformity, stability...	ECD cold kit	a. LC-MS/MS by C-13 isotope replacement internal standard b. Method validation: c. Reference material (COA) d. CMC documentation (SOP, project, report...) e. Instrumentation (3Q validation, SOP)

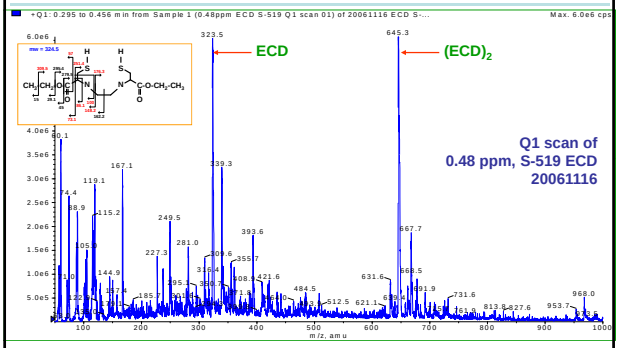
Goals of 2008

	Category	Pharm	Detail
3	Metabolite & Bioassay	Iodine-ADAM	a. Biosample: samplin, pretreatment, extraction... b. Biosample stability c. Cell culture d. Metabolic stability: blood, urine, tissue e. Metabolite ID & pathway f. Bioassay method validation g. C-13-replacement MS internal RMs & COA h. CMC documentation (SOP, validation) i. Factors of species, sex, age, p450, drug-drug interaction, mass balance (evaluation)

Criteria of Purity Assay

- Follow the guideline of ICH and FDA for the criteria of method development of ECD, BZM, and SnADAM purity assay (ICH Q2A, ICH Q2B, and CDER & CBER, FDA, 1994 & 2000)
  1. Stress test (acid, base, oxidation, heat, UV): R ≥ 1.0
  2. 7 points for linearity test
  3. Calibration curve: r ≥ 0.995
  4. Precision (repeatability, reproducibility and intermediate): RSD ≤ 2 %
  5. Accuracy (recovery): 95-105 %
  6. LOQ of impurity ≥ 0.2 %; RSD ≤ 15 %
  7. Bench-top solution stability of 3 days: t_R, RSD ≤ 1 %
  8. Robustness(pH, flow rate, temp.): RSD ≤ 1 %
  9. Suitability: capacity 2-8, selectivity 1.05-2, resolution ≥ 1.0, no. plate ≥ 3,000, tailing factor 0.9-2.5

Results and Discussion



Results and Discussion

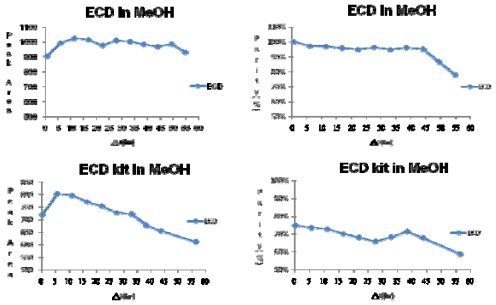


Fig. Stability test of ECD cold kit (DP)

Results and Discussion

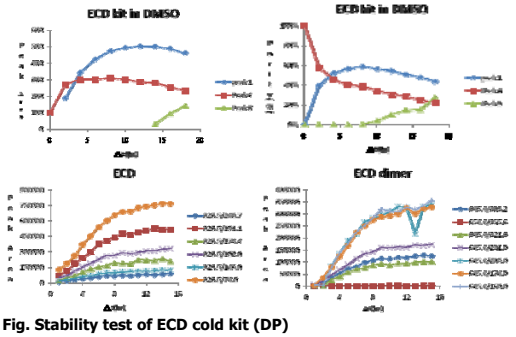


Fig. Stability test of ECD cold kit (DP)

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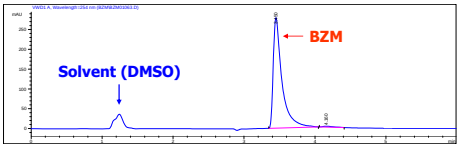
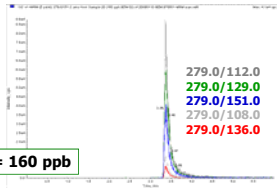


[BZM] = 650.9 ppm
Purity: 99.02 % ± 0.03 %, n = 3

Fig. Purity and LC-MRM of BZM



[BZM] = 160 ppb

Results and Discussion

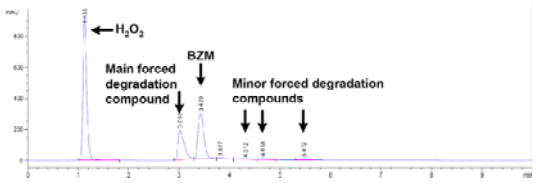
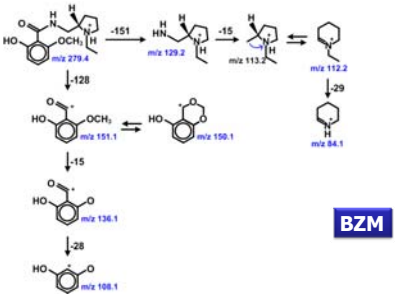


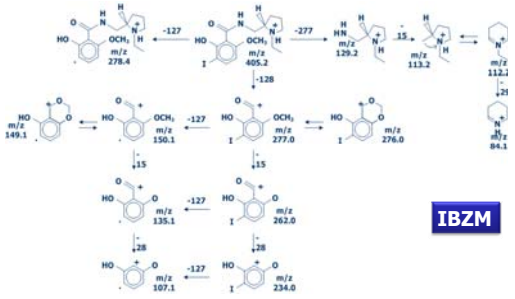
Fig. Forced degradation study with 3 % hydrogen peroxide. 0.63 mg of BZM was dissolved in 1.2 mL of 3 % hydrogen peroxide for 24 hrs at room temperature (25 °C) and analyzed by HPLC.

Results and Discussion

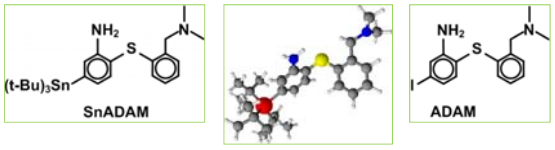


K. T. Liu et al., J Food & Drug Analysis, 2008, (Publishing).

Results and Discussion



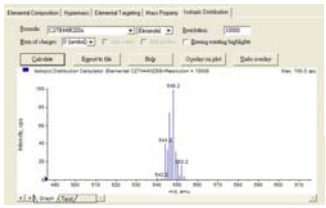
K. T. Liu et al., J Food & Drug Analysis, 2008, (Publishing).



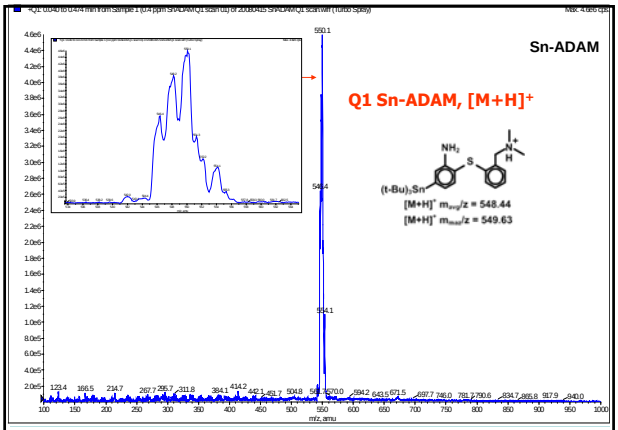
- **ADAM** [2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine], $C_{15}H_{17}N_2SI$, mw_{avg} = 384.28; $[M+H]^+$ = 385.29 (計算值); 385.1 (實驗值)
- **SnADAM** [2-((2-((Dimethylamino)methyl)phenyl)thio)-5-(tri-n-butyltin)-phenylamine], $C_{27}H_{44}N_2SSn$, mw_{avg} = 547.43; mw_{max} = 548.63
- **SnADAM**, $[M+H]^+$ = 549.6 (計算值); 549.7 (實驗值)
- Sn_{Avg} = 118.71; Sn_{max} = 119.90; **Δ = 1.19**

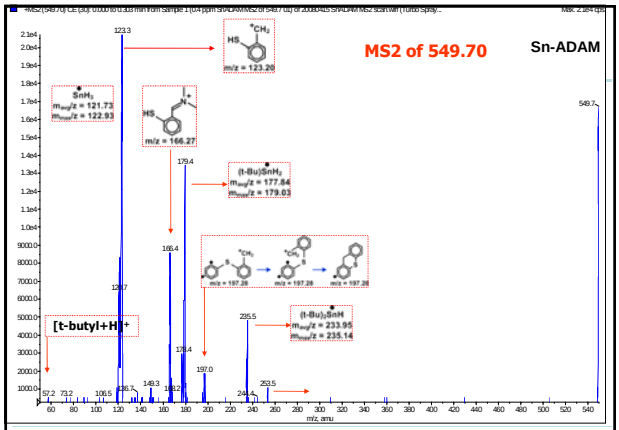
Atomic Weights & Isotopic Compositions

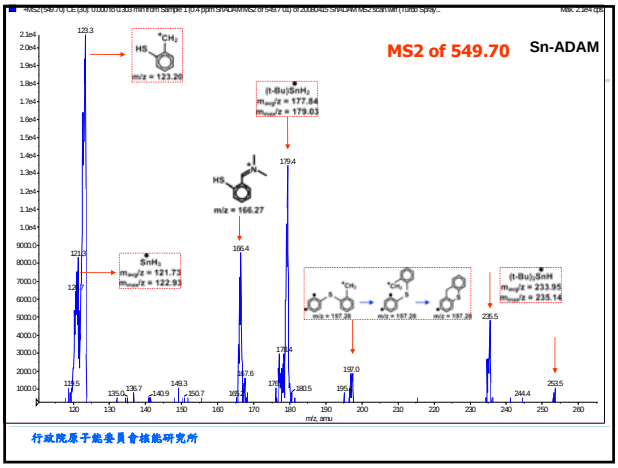
	Isotope		Relative Atomic Mass	Isotopic Composition	Standard Atomic Weight
6	C	12	12.0000000	98.93	12.0107
		13	13.0033548	1.07	
7	N	14	14.0030740	99.632	14.006 74
		15	15.0001089	0.368	
8	O	16	15.9949146	99.757	15.9994
		17	16.9991315	0.038	
		18	17.9991604	0.205	
16	S	32	31.9720707	94.93	32.066(6)
		33	32.9714585	0.76	
		34	33.9678668	4.29	
		36	35.9670809	0.02	
50	Sn	112	111.904821	0.97	118.710(7)
		114	113.902782	0.66	
		115	114.903346	0.34	
		116	115.901744	14.54	
		117	116.902954	7.68	
		118	117.901606	24.22	
		119	118.903309	8.59	
		120	119.902197	32.58	
		122	121.903440	4.63	
		124	123.905275	5.79	



Molecular Formula = $C_{27}H_{44}N_2SSn$
Formula Weight = 547.42666
Composition = C(59.24%) H(8.10%) N(5.12%) S(5.86%) Sn(21.69%)
Monoisotopic Mass = 548.224718 Da
Nominal Mass = 548 Da
Average Mass = 547.4267 Da







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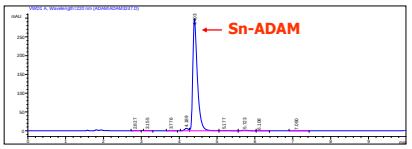
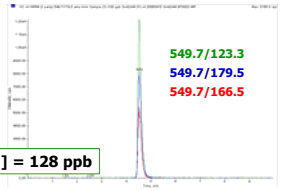
Results and Discussion



[Sn-ADAM] = 317.8 ppm,
Purity: 96.71 % ± 0.05 %, n = 3

圖1. Sn-ADAM純度分析與結構分析



[Sn-ADAM] = 128 ppb

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MRM	L eq.	r
235.3/197.2	Y= -0.0439X + 11	-0.1489
291.0/197.2	Y= 0.137X – 87.7	0.1400
549.7/197.2	Y= 143X – 611	0.9943
165.9/123.0	Y= -0.0678X + 26.5	-0.1068
178.9/123.0	Y= 1110X + 11300	0.9960
137.3/123.0	Non	Non
197.2/123.0	Non	Non
164.8/123.0	Y= 0.979X + 686	0.1305
549.7/123.0	Y= 927X – 3010	0.9990
149.1/121.0	Y= 113X + 1950	0.9931
392.4/121.0	Y= 0.198X – 894	0.0395
165.9/121.0	Y= -0.012X + 64.1	-0.0144
177.1/121.0	Y= 1050X + 8170	0.9962
178.9/121.0	Y= 203X + 2180	0.9947
549.7/121.0	Y= 271X – 1150	0.9991

[SnADAM] =
5, 10, 20, 40, 80, 160 and 320 ppb
Date: 20080715

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MRM	L eq.	r
549.7/179.5	Y= 551X + 911	0.9997
549.7/166.5	Y= 374X + 638	0.9996
549.7/123.3	Y= 847X + 1540	0.9996

[SnADAM] =
1, 4, 8, 16, 32, 64, 128 and 256 ppb
Date: 20080415

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MRM	L eq.	r
385.0/340.0	Y= 2.45E+4 X + 3.47E+5	0.9970
385.0/212.5	Y= 4.48E+4 X + 9.55E+5	0.9914
385.0/196.5	Y= 2.32E+4 X + 3.20E+5	0.9965
385.0/184.5	Y= 2.25E+3 X + 2.05E+4	0.9992
385.0/180.5	Y= 5.91E+3 X + 7.67E+4	0.9968
385.0/165.6	Y= 5.01E+3 X + 6.84E+4	0.9948
385.0/152.5	Y= 1.22E+4 X + 1.48E+5	0.9982

[I-ADAM] = 10, 20, 50, 100, 200 and 400 ppb
Date: 20080416
Eluent: ACN:MeOH:NH_4Ac = 80:20:0.4 (v/v/v)

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MRM	L eq.	r
385.0/340.0	Y= 1.88E+5 X + 3.08E+5	0.9960
385.0/212.5	Y= 3.55E+5 X + 7.08E+5	0.9951
385.0/196.5	Y= 1.85E+5 X + 2.86E+5	0.9955
385.0/184.5	Y= 1.80E+4 X + 1.86E+4	0.9978
385.0/180.5	Y= 4.83E+4 X + 4.84E+4	0.9985
385.0/165.6	Y= 4.27E+4 X + 4.49E+4	0.9960
385.0/152.5	Y= 9.82E+4 X + 1.18E+5	0.9984

[I-ADAM] = 10, 20, 50, 100, 200 and 400 ppb
Date: 20080416
Eluent: ACN:MeOH:NH_4Ac = 80:20:0.6 (v/v/v)

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MRM	L eq.	r
385.0/340.0	Y= 1.62E+4 X – 4.79E+3	0.9996
385.0/212.5	Y= 3.28E+4 X – 5.16E+3	0.9997
385.0/196.5	Y= 1.54E+4 X + 1.30E+3	0.9994
385.0/184.5	Y= 1.42E+3 X – 1.24E+3	0.9996
385.0/180.5	Y= 3.98E+3 X – 5.18E+3	0.9999
385.0/165.6	Y= 3.38E+3 X – 6.46E+3	1.0000
385.0/152.5	Y= 8.18E+3 X – 1.03E+4	0.9999

[I-ADAM] = 10, 20, 50 and 100 ppb (與PBS混合)
Date: 20080416
Eluent: ACN:MeOH:NH_4Ac = 80:20:0.4 (v/v/v)

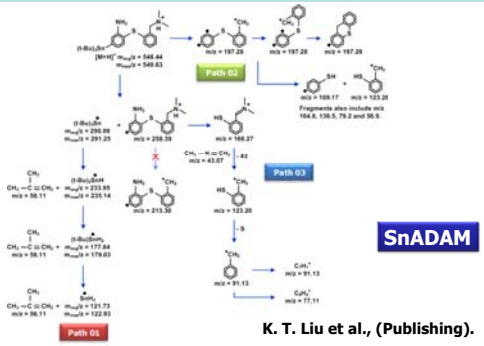
Results and Discussion

- Sn-ADAM MRM: (5 – 320 ppb)
  - 549.7/197.2, 549.7/179.5, 549.7/166.5, 549.7/123.0, 549.7/121.0
  - 178.9/123.0, 178.9/121.0
  - 177.1/121.0
  - 149.1/121.0
- I-ADAM MRM: (10 – 400 ppb)
  - 385.0/340.0, 385.0/212.5, 385.0/196.5, 385.0/184.5, 385.0/180.5, 385.0/165.6, 385.0/152.5

Results and Discussion

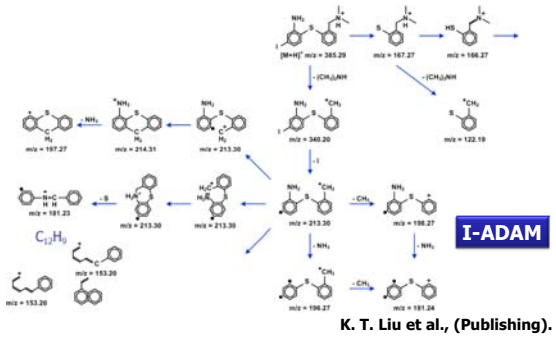
- CID fragmentation pathway of Sn-ADAM and I-ADAM is different.
  - Sn-ADAM首先斷裂(t-butyl)$_3$Sn-Φ鍵，然後斷裂Φ-S-Φ鍵，不會進一步斷裂NH(CH_3)$_2$鍵，得不到m/z = 213.3碎片。
  - I-ADAM首先斷裂NH(CH_3)$_2$鍵，然後斷裂I-Φ鍵，得到m/z = 213.3碎片。
- Similar CID fragments of Sn-ADAM and I-ADAM
  - 197.2:196.5, 179.5:180.5, 166.5:165.6

Results and Discussion



K. T. Liu et al., (Publishing).

Results and Discussion



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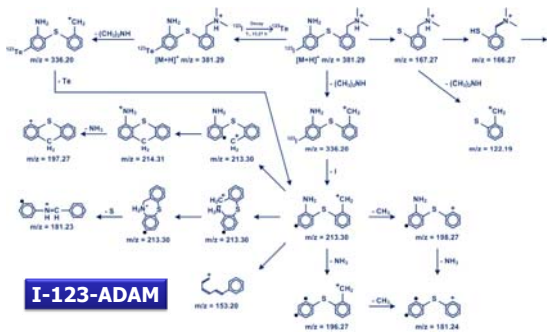
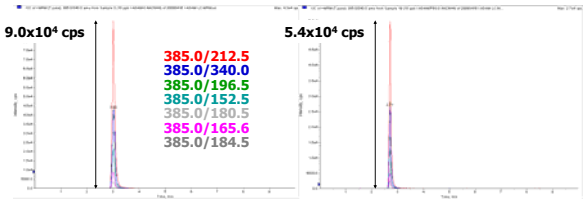


Results and Discussion

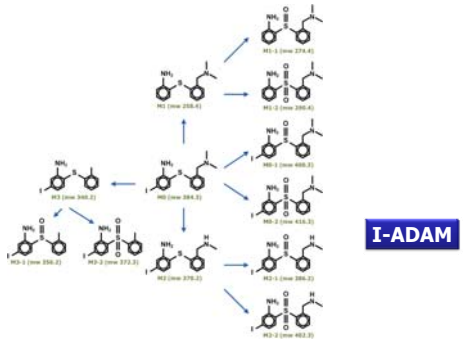


[I-ADAM] = 10 ppb/MeOH　　[I-ADAM] = 10 ppb/PBS+ACN

基質因子(matrix factor，MF) = {分析物波峰信號}基質 / {分析物波峰信號}無基質

圖1. I-ADAM代謝產物研究

藥物代謝鑑定



Results and Discussion

ICH Q3C- Impurities: Guideline for Residual Solvents

分類	溶劑	濃度限值 (ppm)	PDE值 (mg/day)
第一類	苯、四氯化碳、1,2-二氯乙烷、1,1-二氯乙烯、1,1,1-三氯乙烷	2-1,500	無法接受
第二類	硝基甲烷、氯仿、1,1,2-三氯乙烯、吡啶、己烷、氯苯、乙腈、二氯甲烷、乙二醇、甲苯、1,2-二氯乙烯、二甲苯、甲酚…	50-4,840	0.5-48.4
第三類	醋酸、丙酮、苯甲醚、丁醇、乙酸丁酯、乙醇、DMSO、乙酸乙酯、二乙醚、甲酸乙酯、甲酸、庚烷、乙酸異丁酯、乙酸異丙酯、乙酸甲酯、戊烷、丙醇、乙酸丙酯、四氫呋喃…	● 無特定上限PDEs，可以超過50 mg/day ● 濃度必須受GMP規範	

Results and Discussion

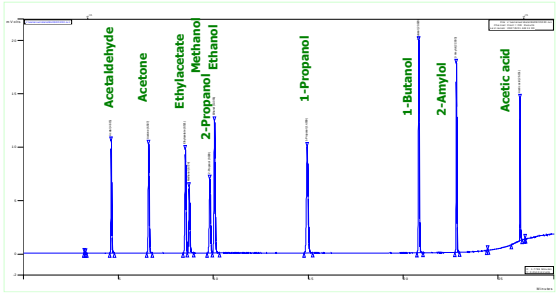


圖1. 常用有機溶劑標準品GC-FID圖譜，LOD = 2 ppm

恭請指導