药物分析

第二十一章

药品质量控制中的现代分析方法与技术

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- 现代分析方法与技术，为药学的发展提供了适时而有效的手段与动力。
  - 色谱及其联用技术：药学研究——分子水平
  - 手性分析：毛细管电泳及手性色谱技术——药物研究与质量控制提供了保障
  - 现代光谱技术：药物结构鉴定、 微量杂质检定

Capillary electrophoresis，CE

主要分离模式：

- 毛细管区带电泳（CE）
- 胶束电动毛细管色谱（MECC或MEKC）
- 毛细管凝胶电泳（CGE）
- 毛细管等速电泳（CITP）
- 毛细管等电聚焦电泳（CIEF）
- 毛细管电色谱（CEC）
- 微芯片毛细管电泳（MEC）
- 毛细管阵列电泳（CAE）

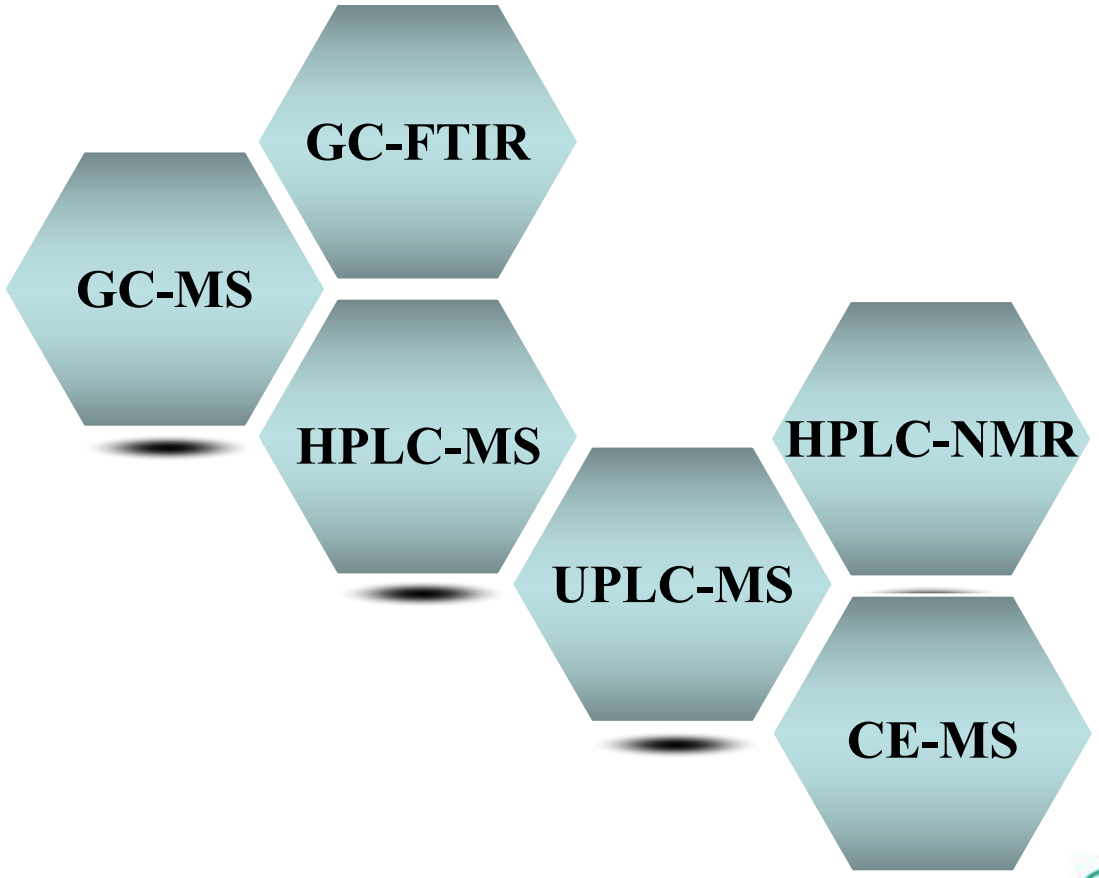
UPLC——基于小颗粒填料的液相色谱技术

- UPLC概念的引入是基于Van Deemter的著名理论：Van Deemter 曲线及其方程式。

- 其中，*A*项反映了颗粒度和柱床填装的优良程度；*B*项代表了轴向扩散；而*C*项则代表了传质。

现代联用技术及其应用

Hyphenated Techniques in Chromatography



液质联用技术与应用

质量分析器

检测离子

离子源

离子识别

- Quadrapole
- Time of Flight
- Fourier Transform
- …

离子检测

样品与溶剂脱离及电离

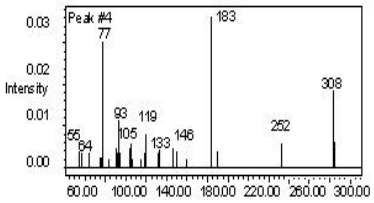
- EI
- ESI
- APCI

LC/MS 接口

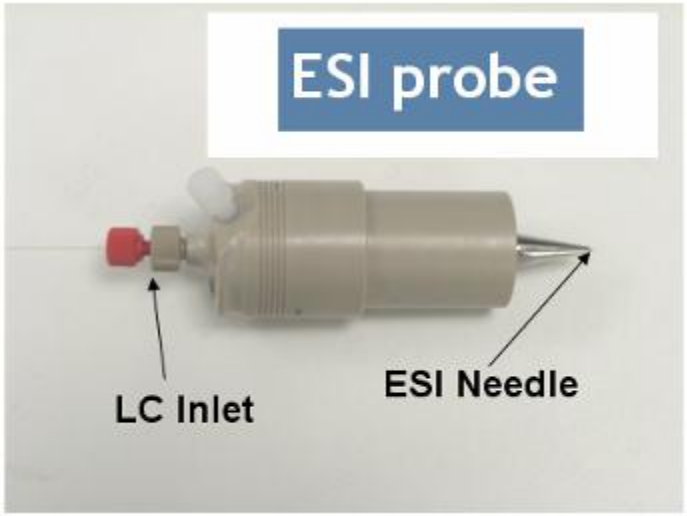
数据系统

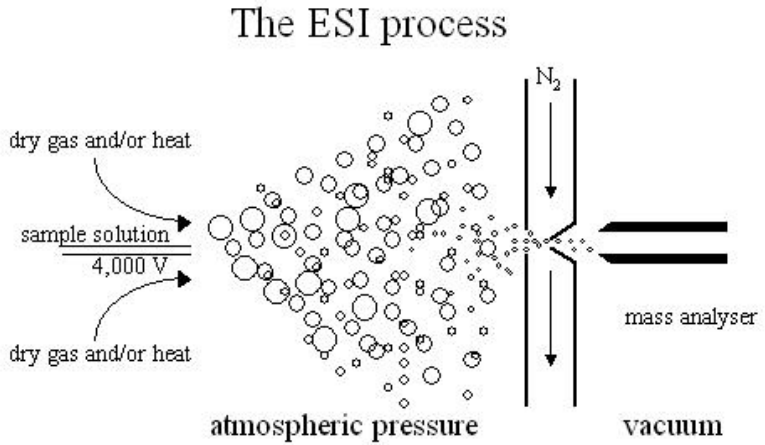
HPLC

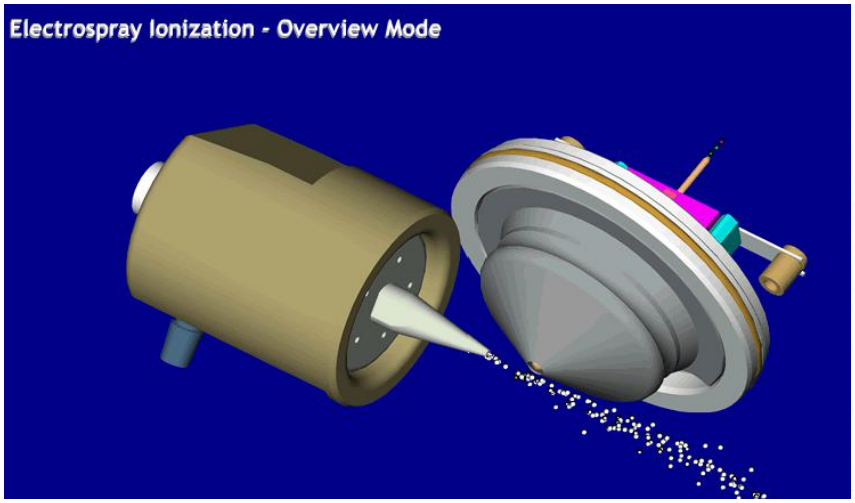
质谱

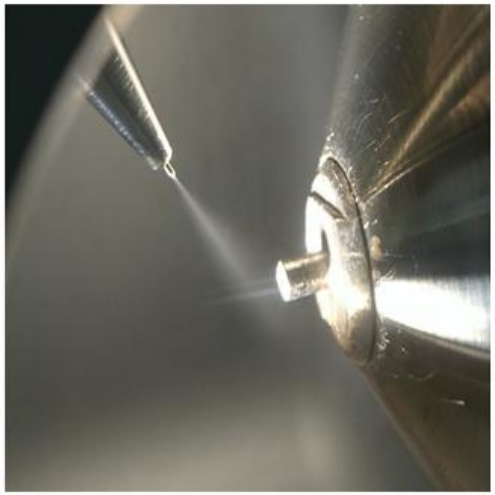


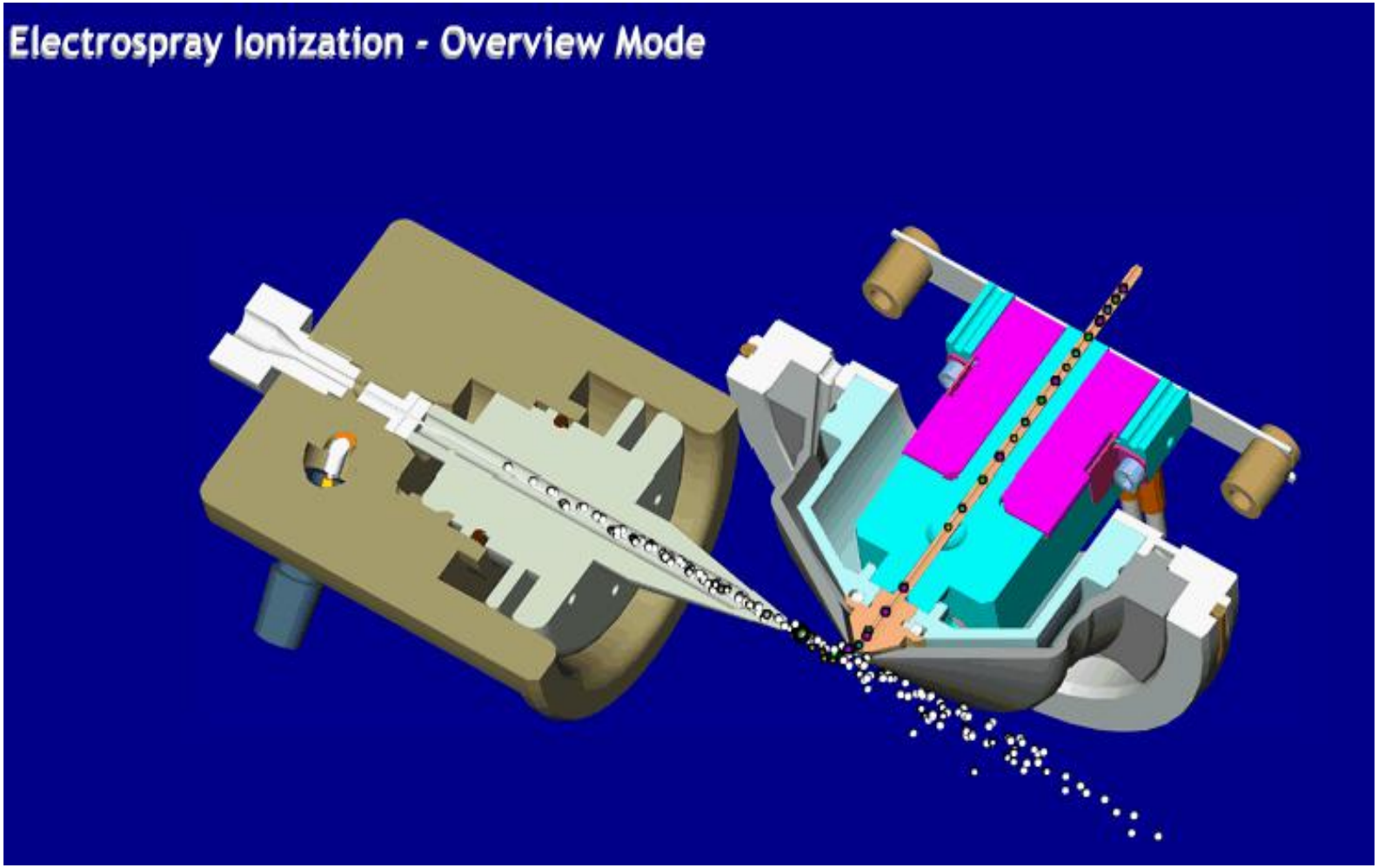
离子化方式











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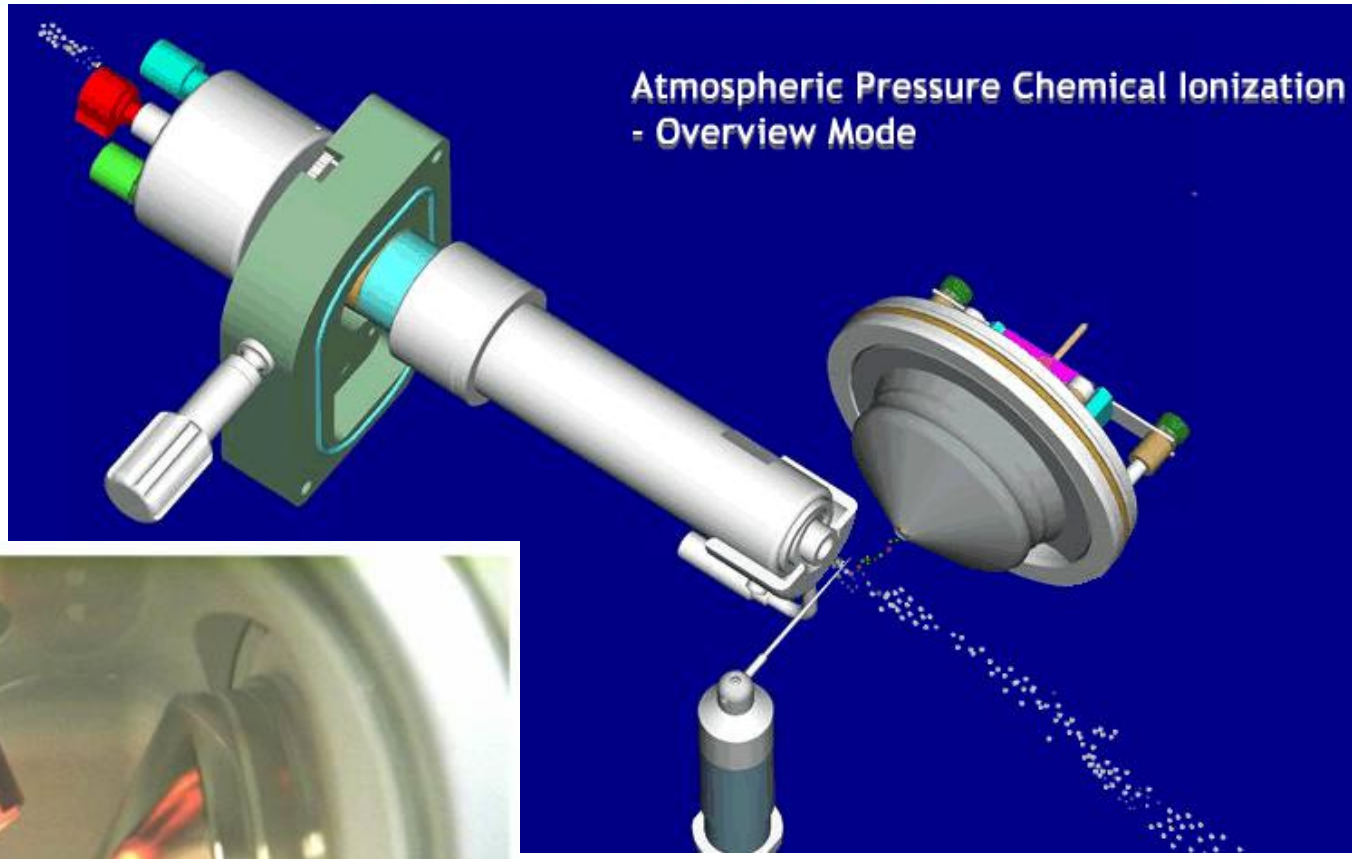

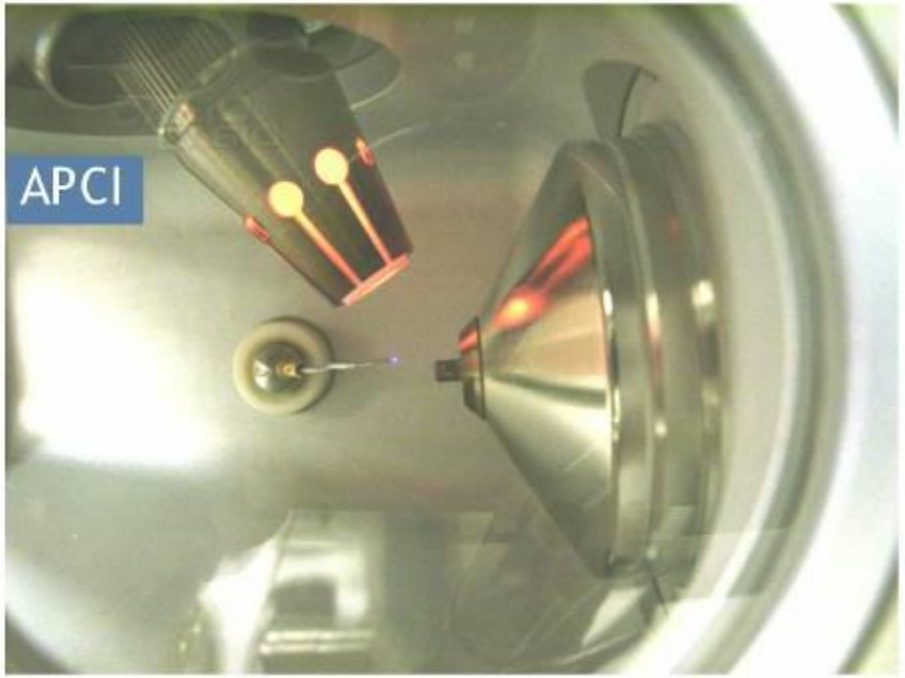

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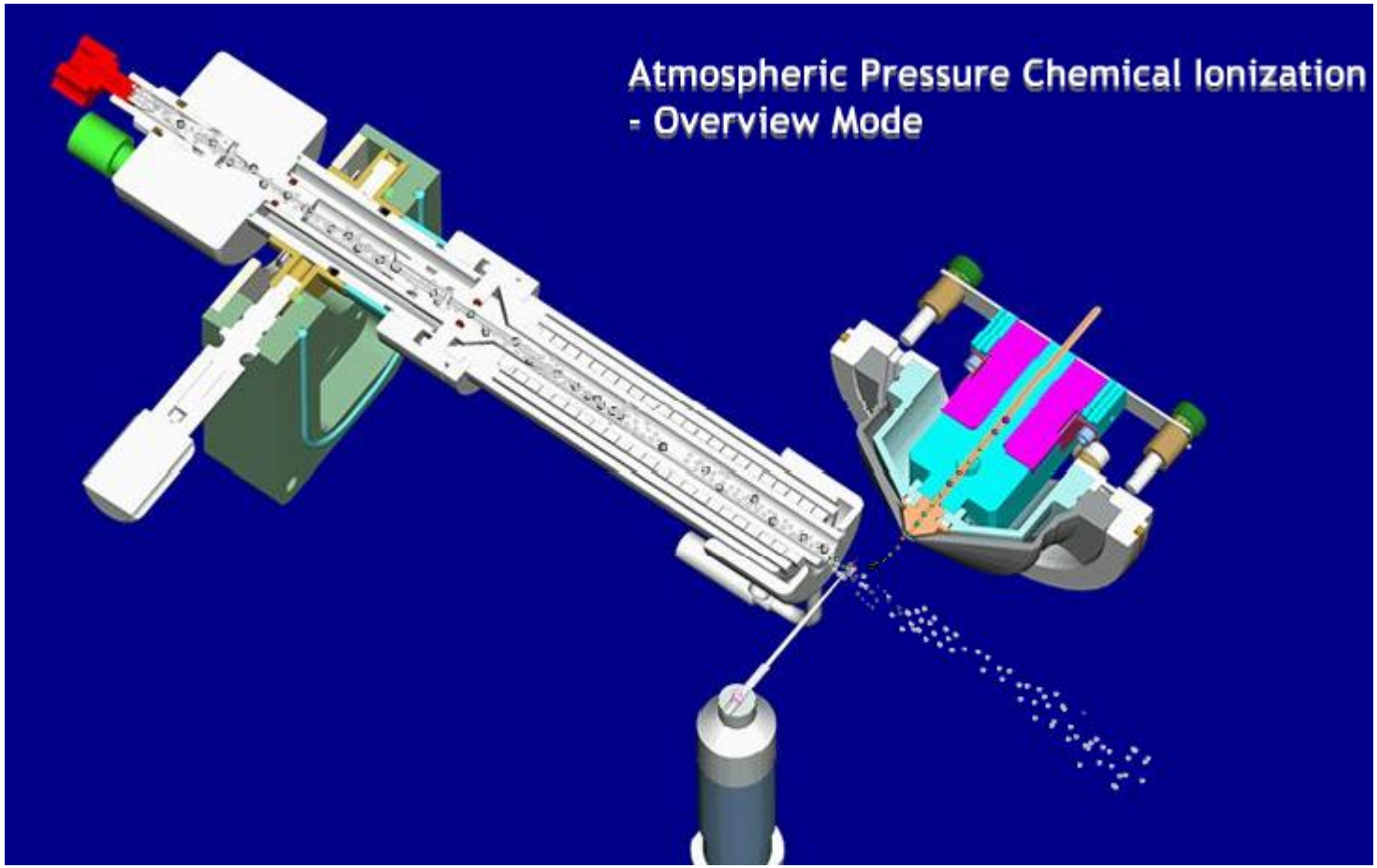

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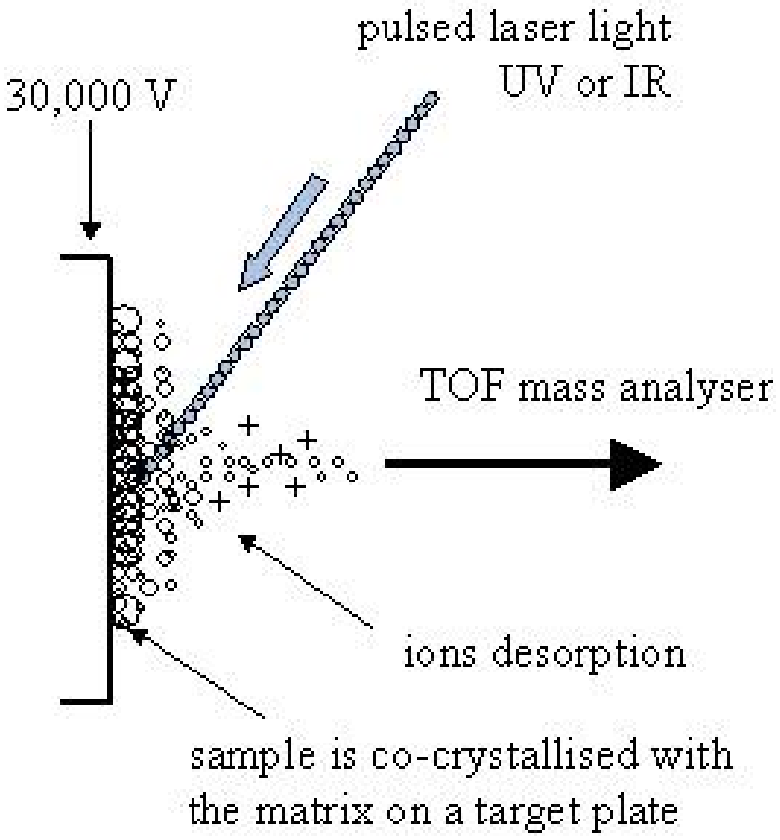
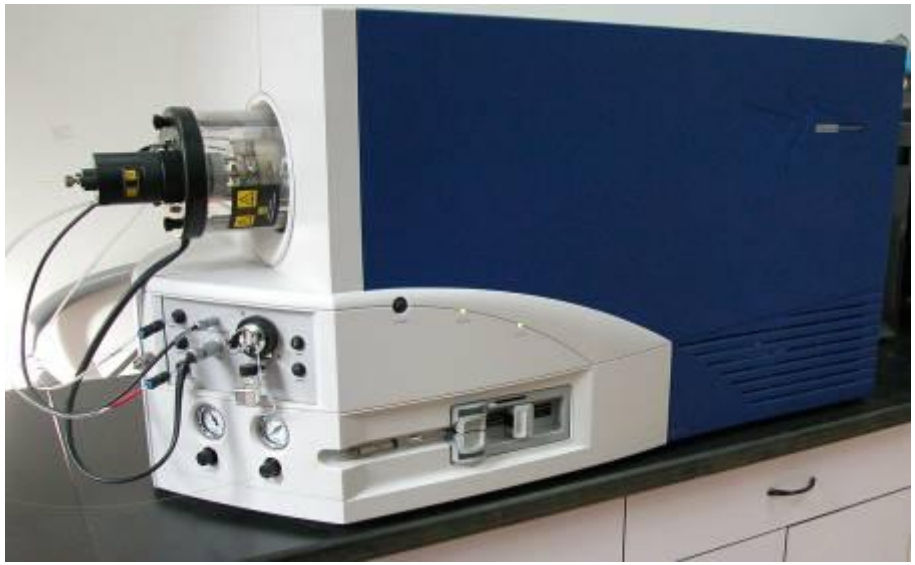
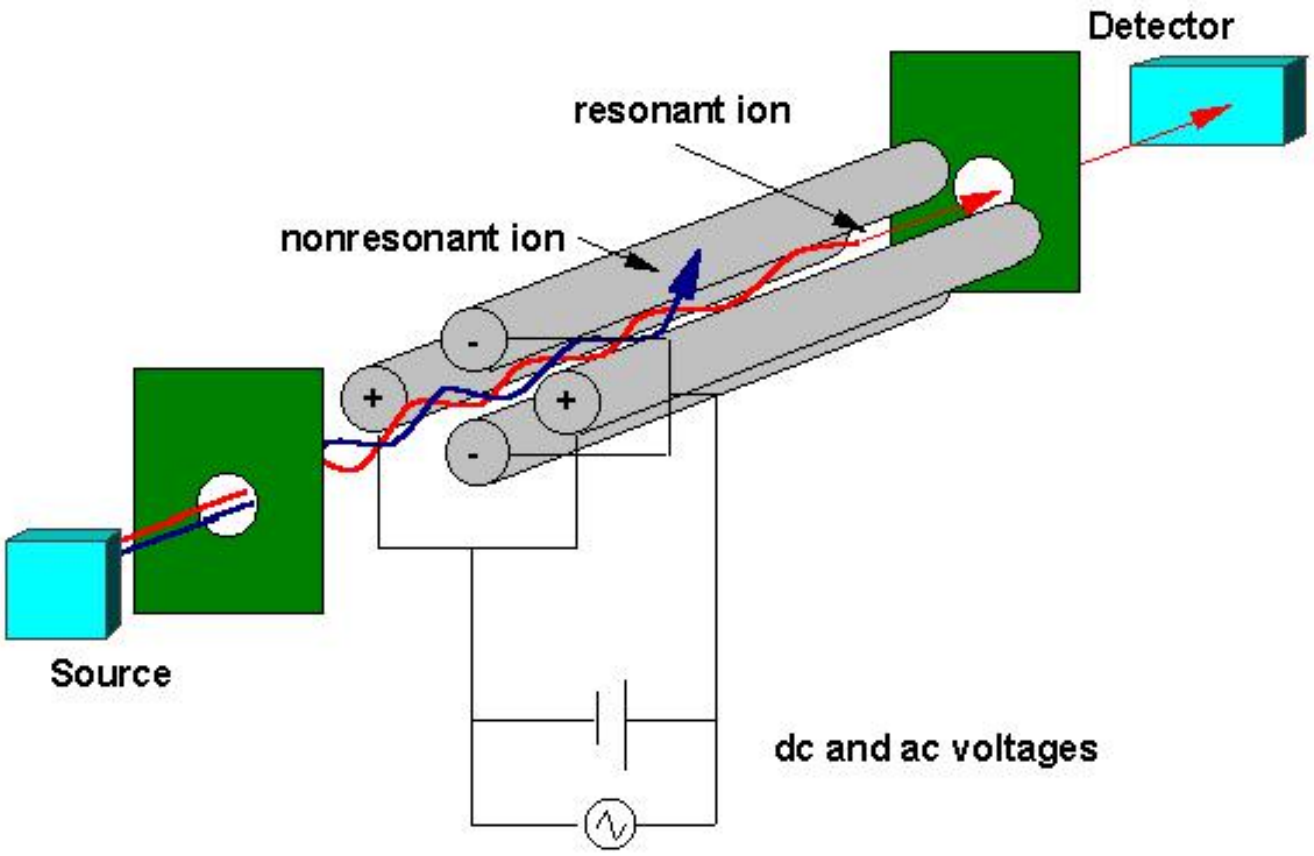
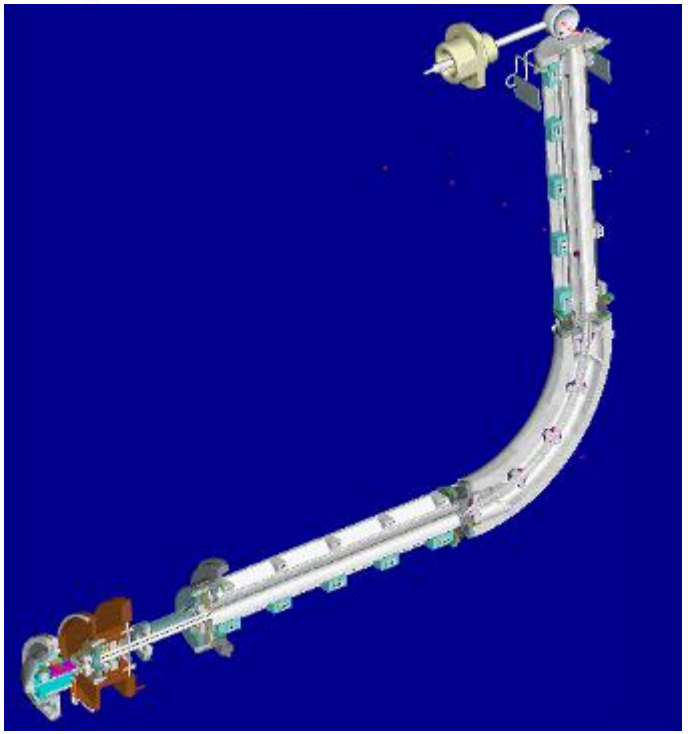
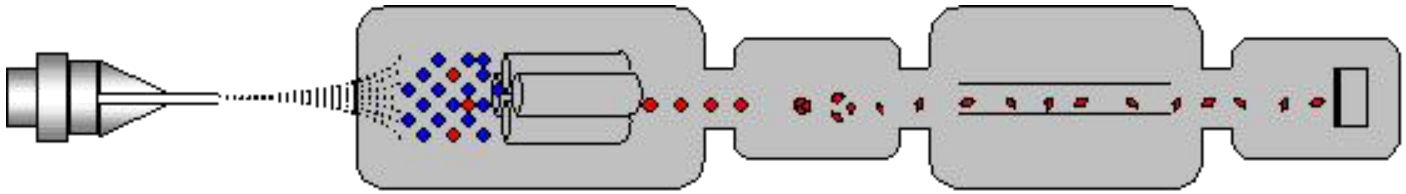




离子分离与测定模式







ESI MS-1 Collision cell MS-2 Detector

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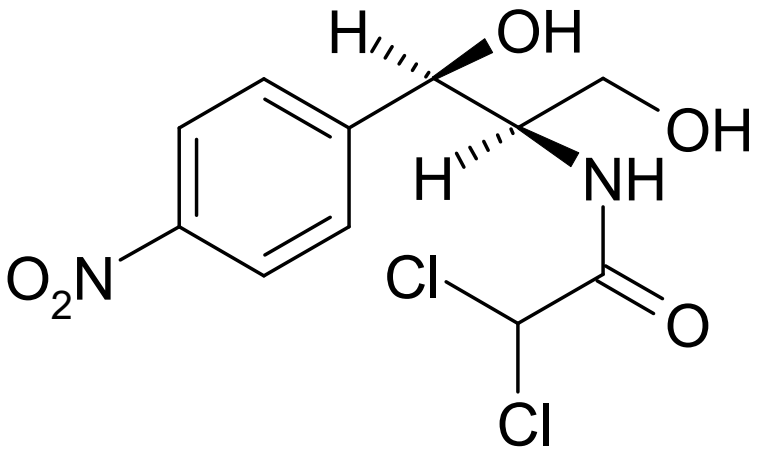
药物分析中的典型应用

- **Chlroamphenicol（氯霉素，CAP）残留测定**
- 黄杨生物碱成分鉴定
- 苯甲酸利扎曲普坦人体药代动力学研究

（1） Chlroamphenicol（氯霉素，CAP）残留测定



$C_{11}H_{12}Cl_2N_2O_5$

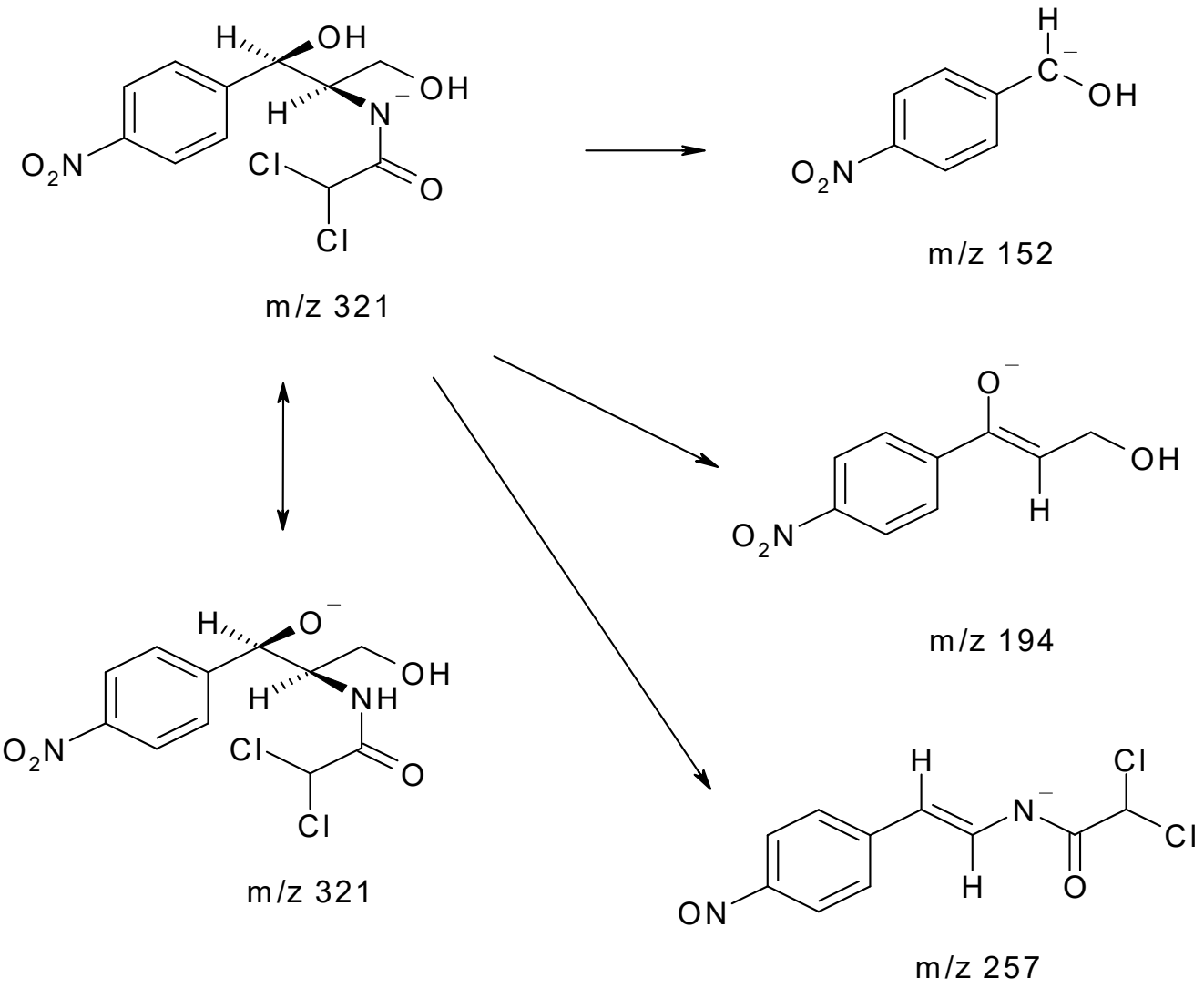
FMW=323.13

【类别】酰胺醇类抗生素

【适应证】

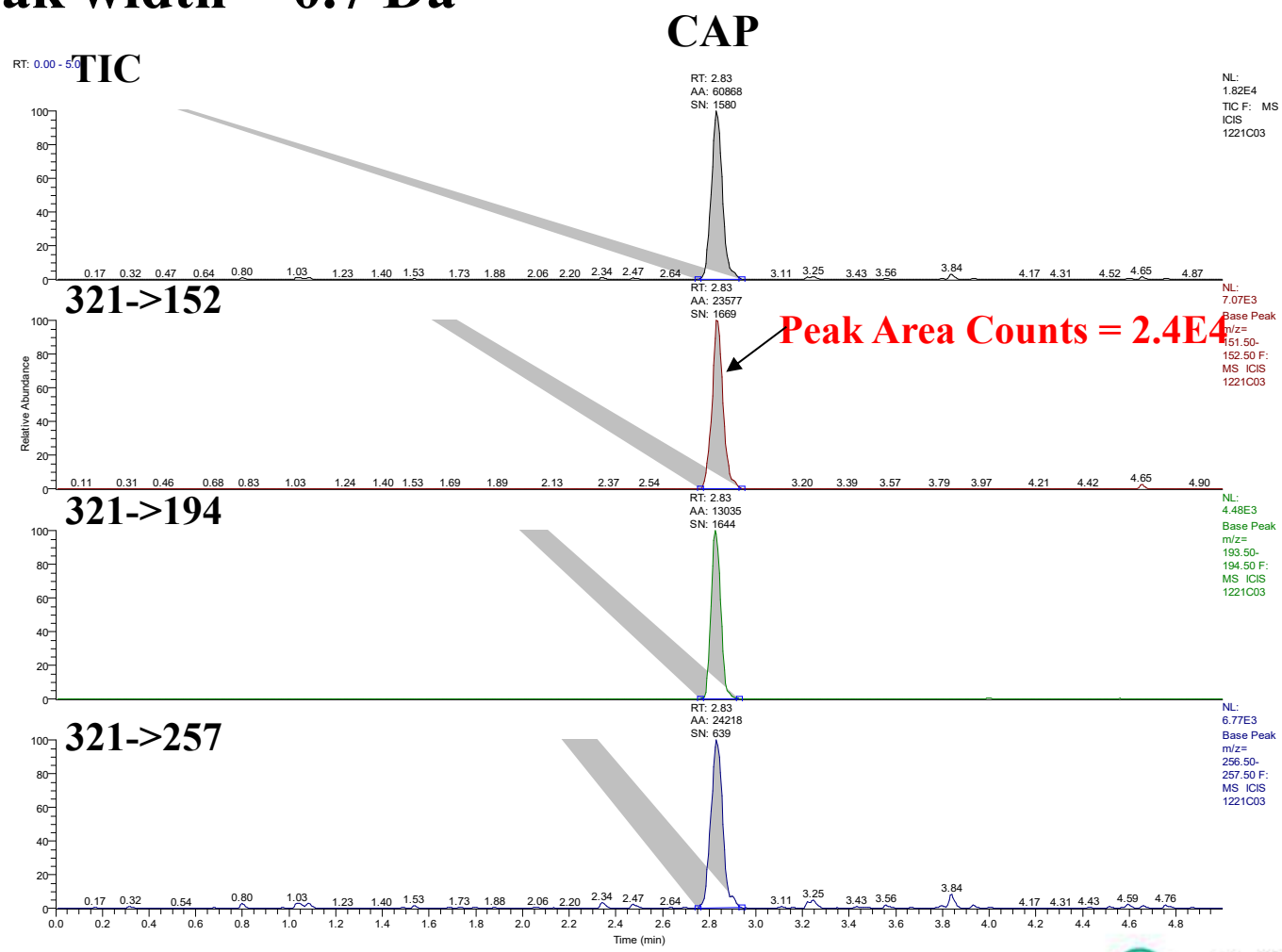
- 本品是治疗伤寒、副伤寒的首选药物，外用可治疗沙眼。因脑脊液浓度高，故常用于治疗细菌性脑膜炎和脑脓肿。此外，尚可外用治疗痤疮、酒糟鼻、脂溢性皮炎等。

被农业养殖滥用！ 肉食品中严格检查。

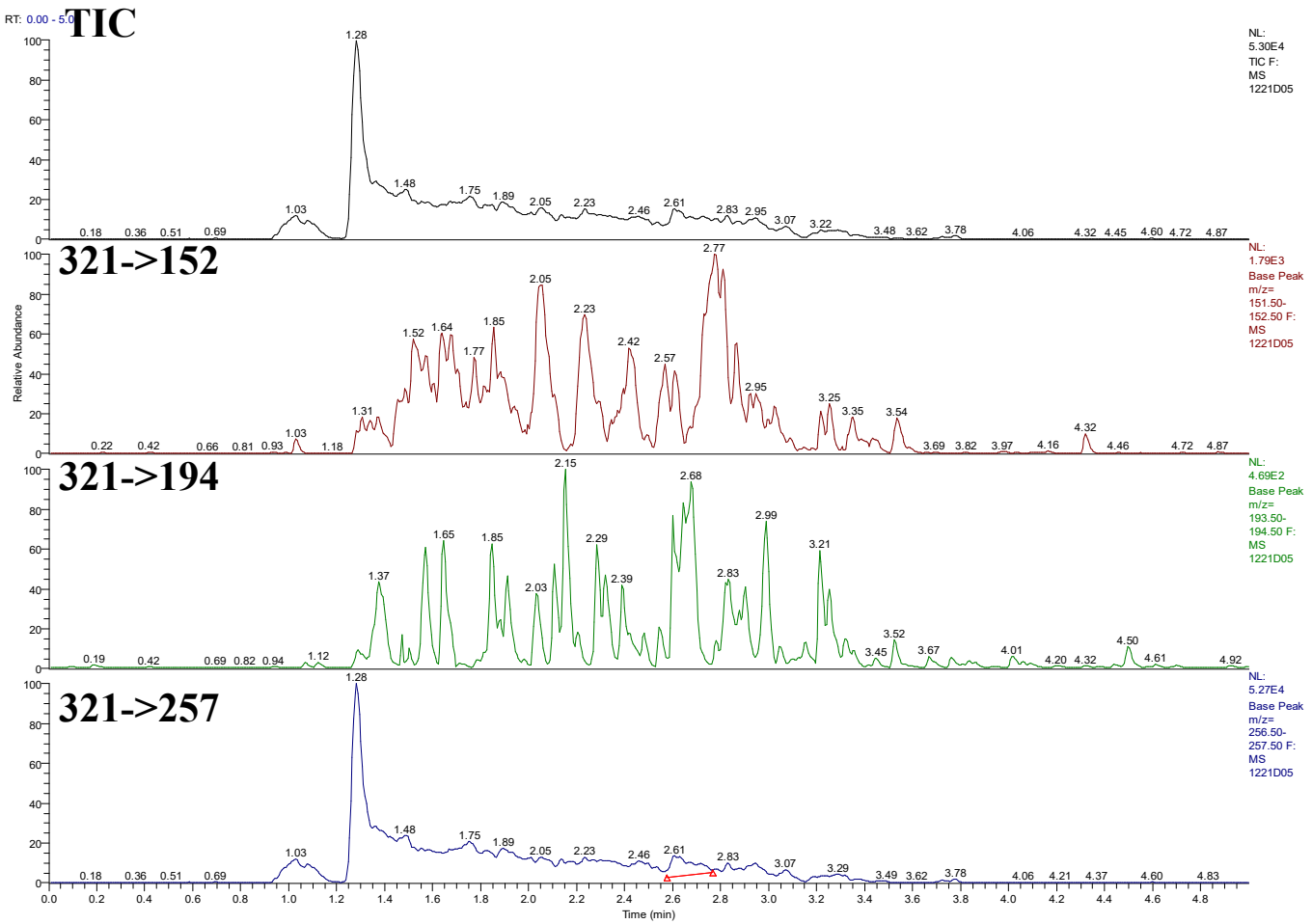


CAP SRM Result: CAP Standard

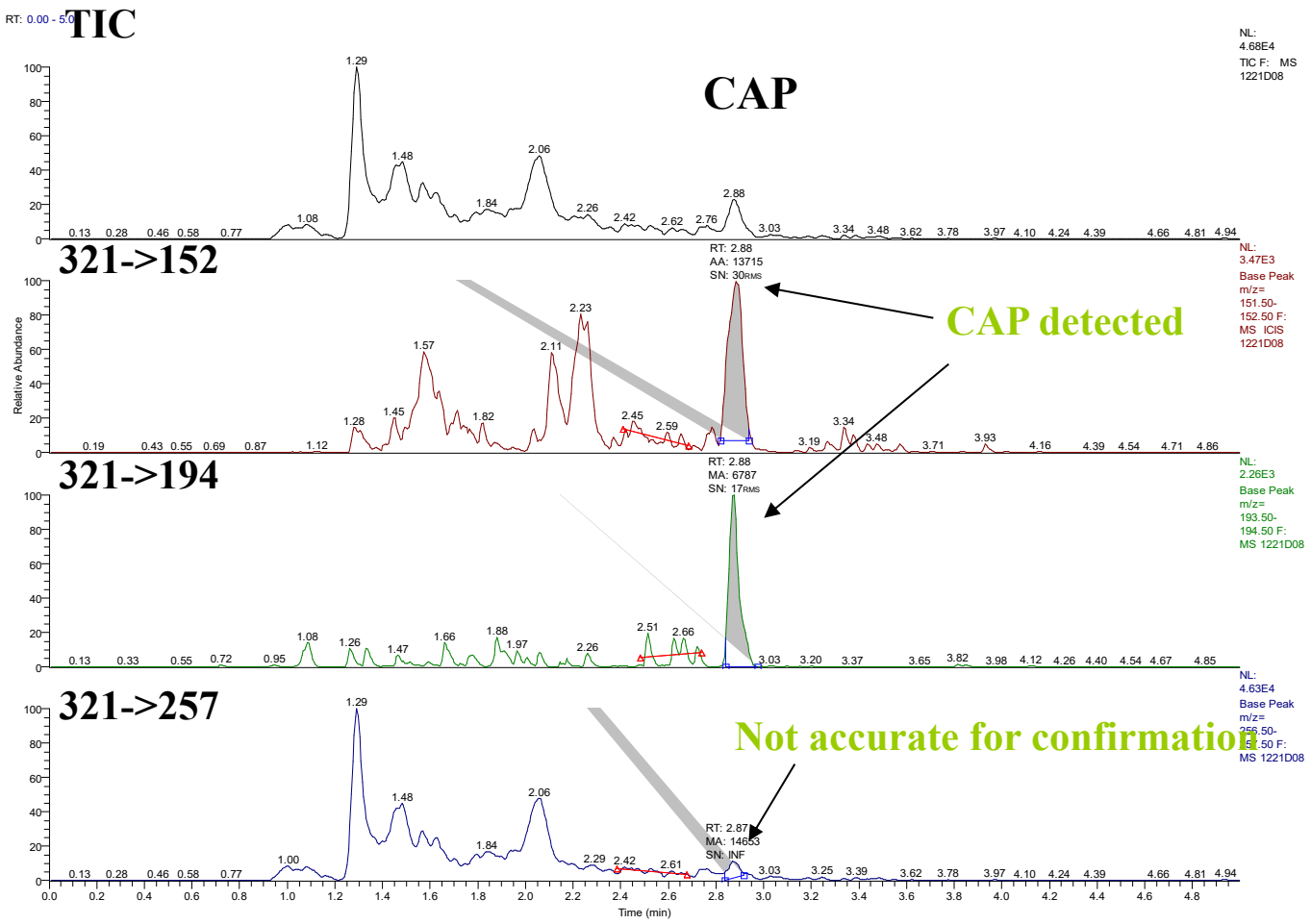
Q1 peak width = 0.7 Da



CAP SRM Result: Kidney Blank

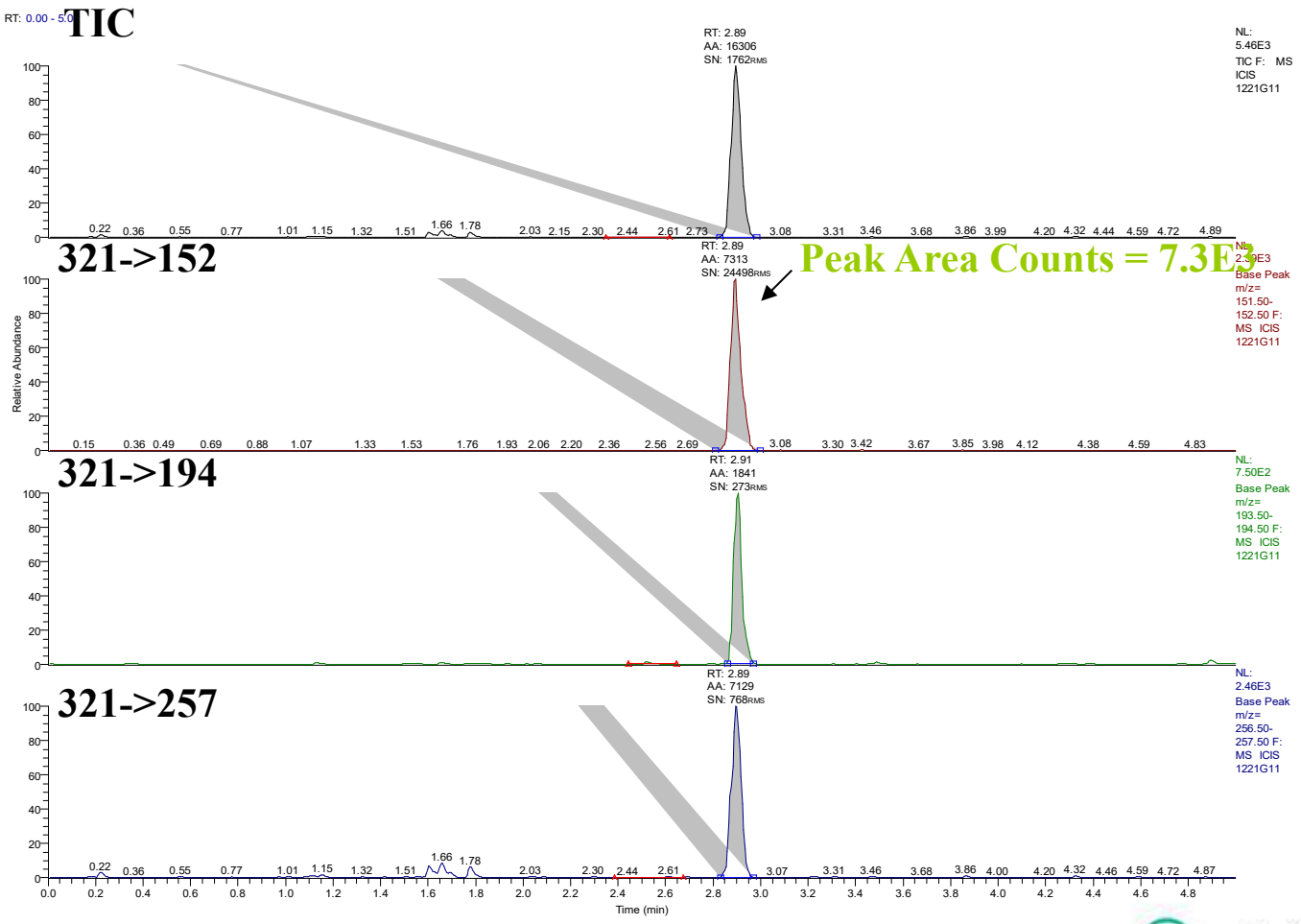


CAP SRM Result: Kidney Spiked （0.5ng/g）

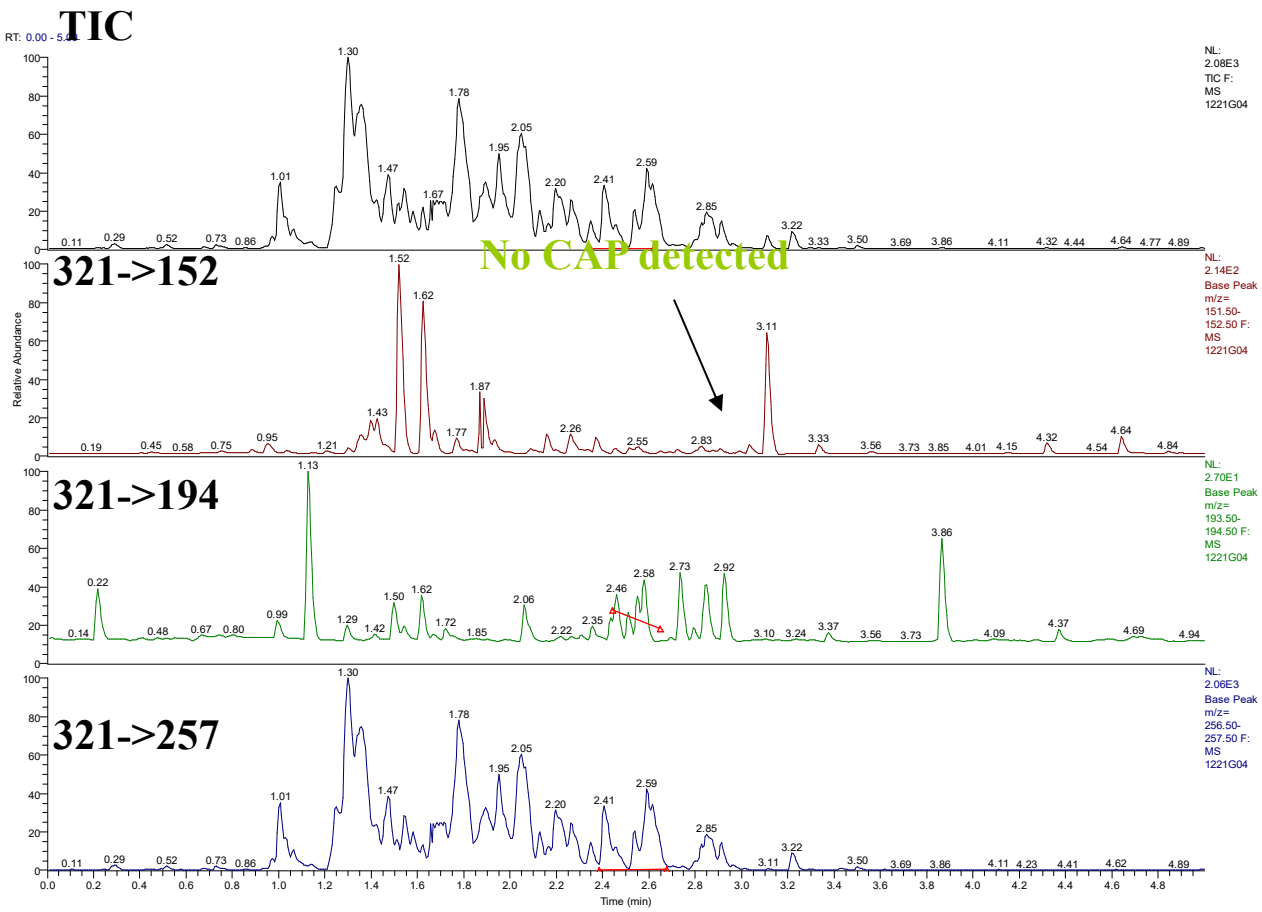


CAP H-SRM Result: CAP Standard

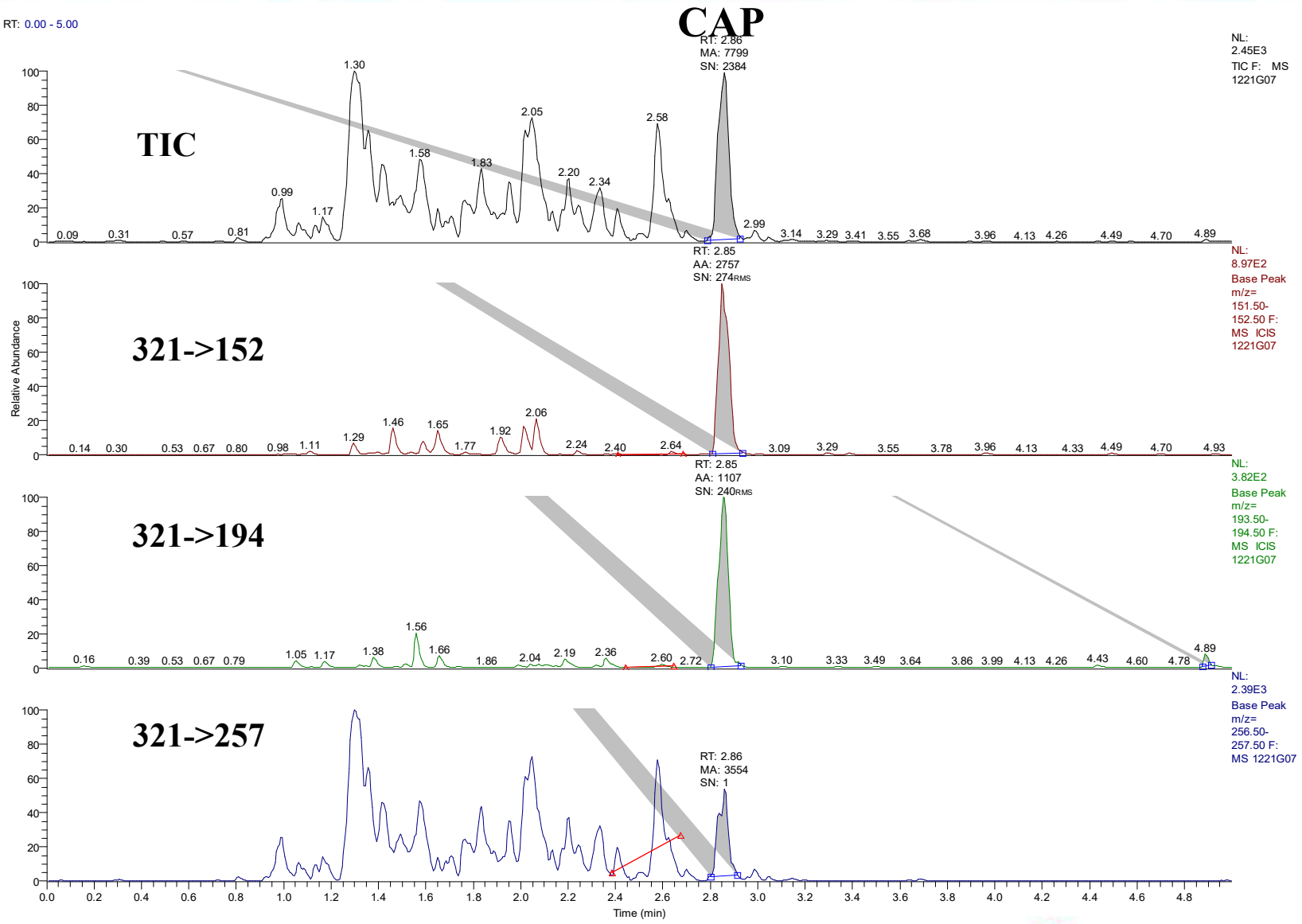
Q1 peak width = 0.2 Da



CAP H-SRM Result: Kidney Blank

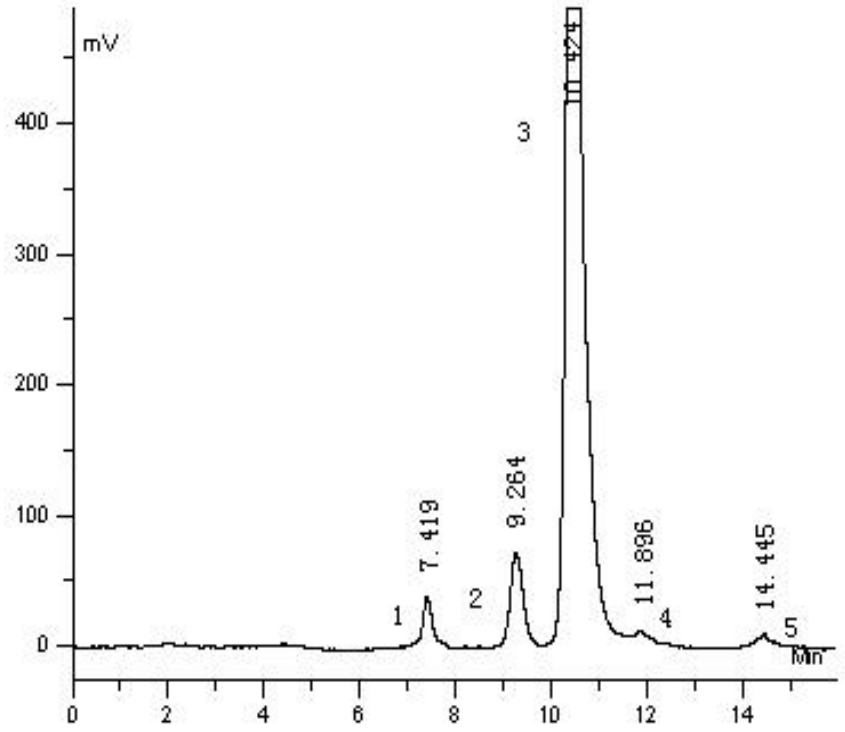


CAP H-SRM Result: Kidney Spiked （0.5ng/g）



（2） 黄杨生物碱HPLC-MS联用鉴定

- 黄杨科植物小叶黄杨*Buxus microphlla* Sieb. et. Zucc. var. *sinica* Rehd.et Wils中含有具有较强心血管疾病治疗活性的孕甾烷生物碱，主要含环维黄杨星D、环黄杨碱D和环常绿黄杨碱C等生物碱成分。



黄杨生物碱HPLC-ELSD色谱图

色谱条件

- 色谱柱：Lichrospher SiO_2（250mm×4.6mm，5 μm）
- 流动相：四氢呋喃-甲醇-乙腈-氨水（32：50：13：3）
- 流速：1ml/min
- 柱温：30℃
- ELSD参数：漂移管温度70℃
  雾化气体（N_2）
  流速：1.5 L/min

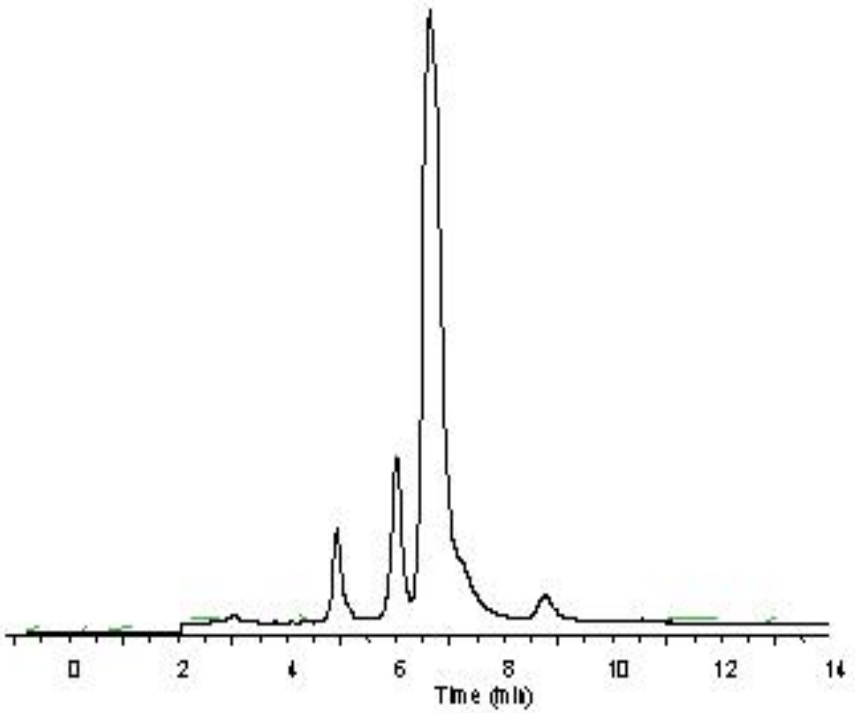
环维黄杨星D和有关生物碱含量测定结果

次数	环维黄杨星D含量%	峰1生物碱含量%	峰2生物碱含量%	峰4生物碱含量%	峰5生物碱含量%	有关生物碱总含量%
1	86.85	3.57	8.09	0.79	0.92	13.37
2	87.08	3.84	8.54	0.80	0.98	14.15
3	85.83	3.46	8.82	0.81	0.95	14.03
4	85.70	3.54	8.76	0.87	0.93	14.10
5	85.05	3.52	8.64	0.74	0.85	13.75
6	84.84	3.74	9.03	0.79	0.97	14.53
Mean	85.89	3.61	8.65	0.80	0.93	13.99
RSD%	1.07	3.65	3.38	4.62	4.63	2.8

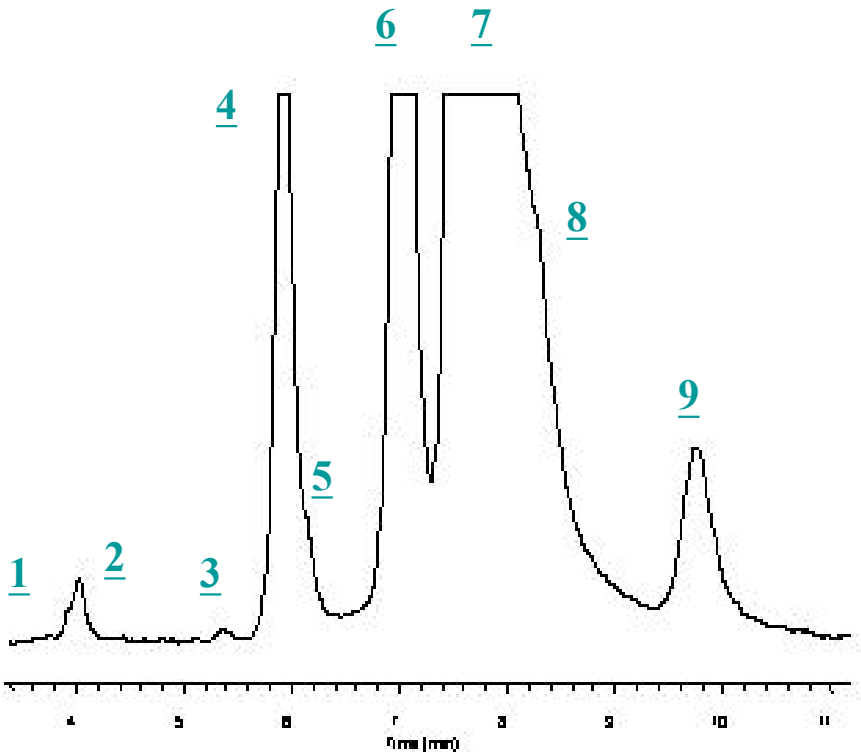
环维黄杨星D及其有关生物碱的鉴别

- 质谱条件
  - 电喷雾离子化正离子检测
  - 喷口电压5000V
  - 雾化气压35psi
  - 辅助气压力5psi
  - 毛细管温度350℃
  - 碰撞气氩气压力1.5mTorr

- 色谱条件
  - 色谱柱：Lichrospher SiO_2（250mm×4.6mm，5 μm）
  - 流动相：四氢呋喃-甲醇-乙腈-氨水 （32：50：13：3）
  - 流速：1ml/min
  - 柱温：30℃

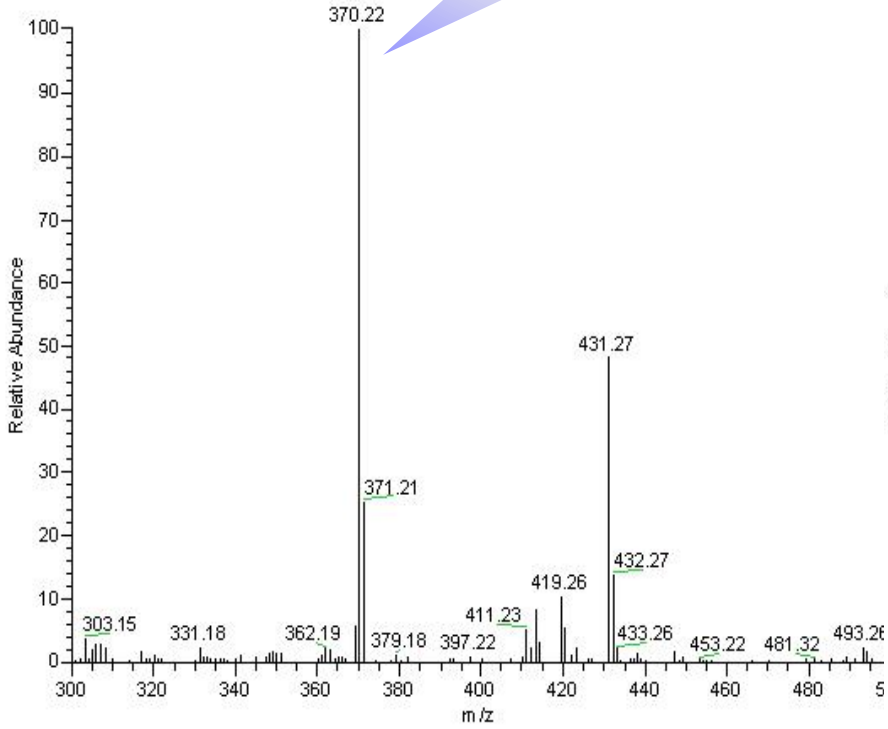


黄杨宁LC-MS/MS全扫描色谱图

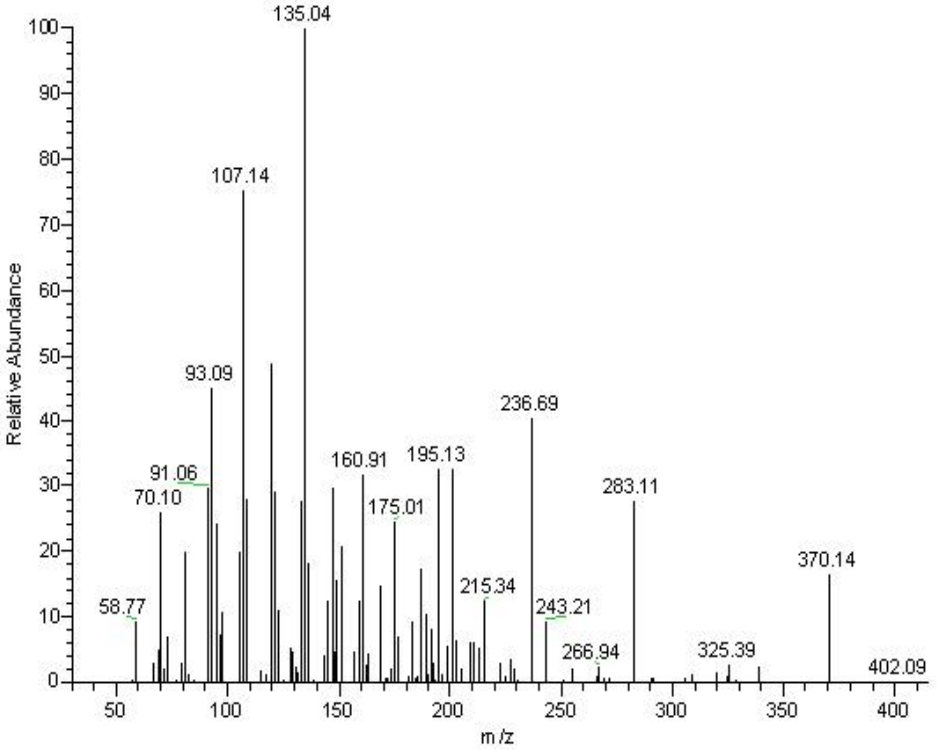


黄杨宁LC-MS/MS全扫描色谱放大图

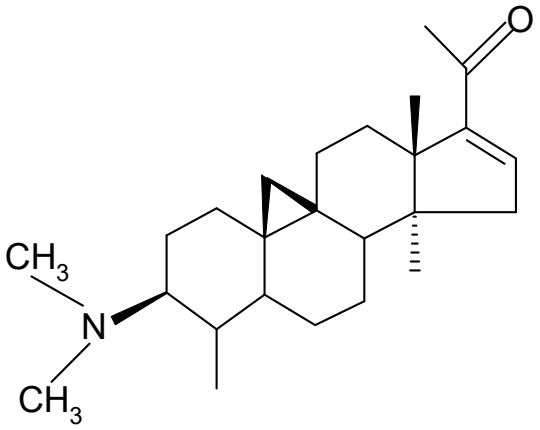
$[M+H]^+=370$



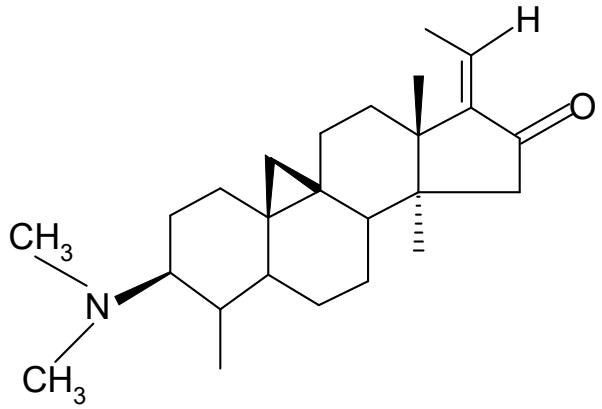
峰1母离子质荷比



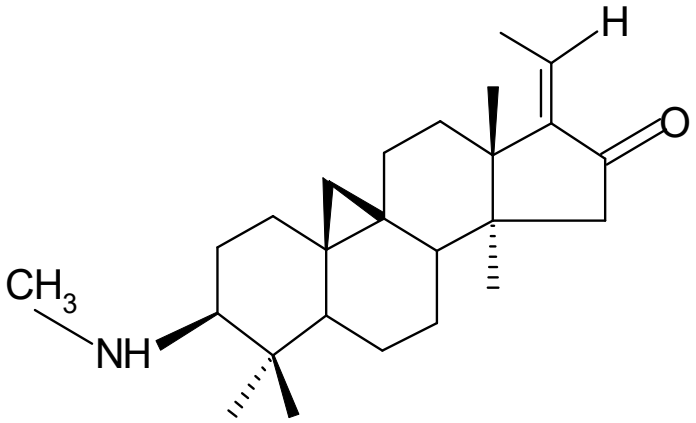
峰1二级质谱图



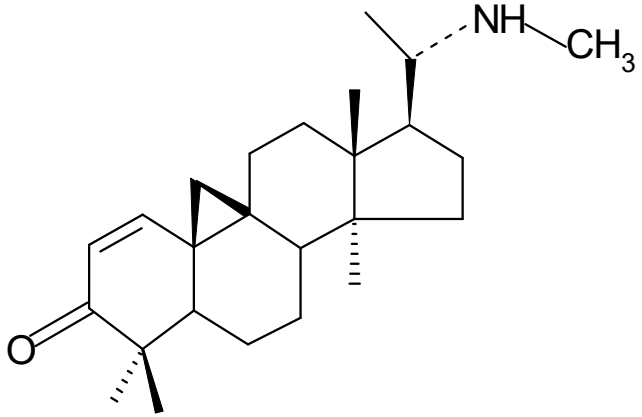
Cyclobuxomicreine K



Cyclobuxosuffrine K



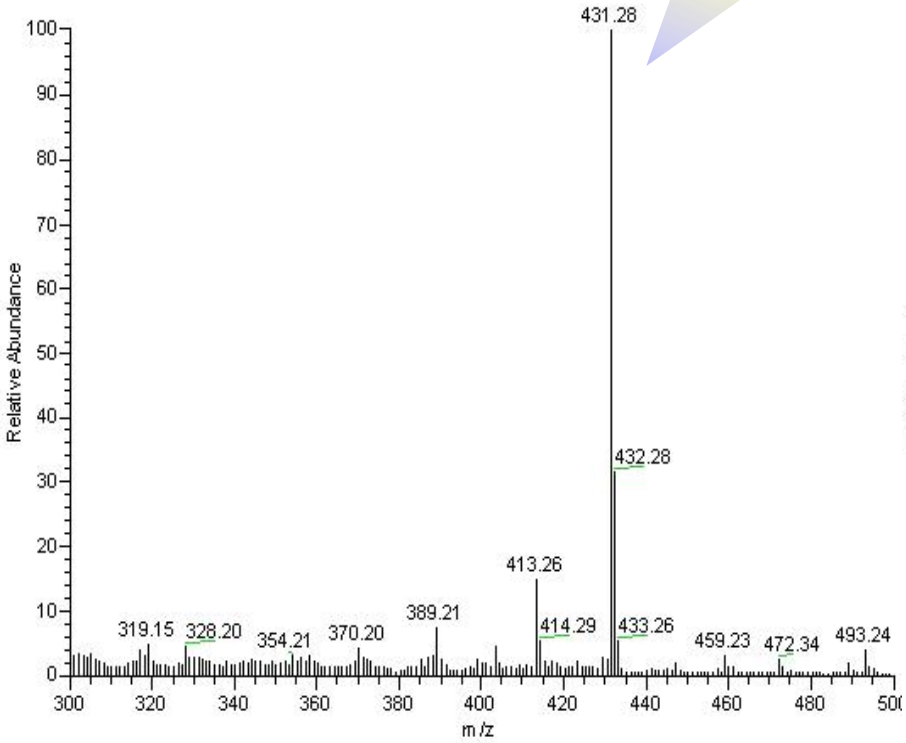
Buxenone M



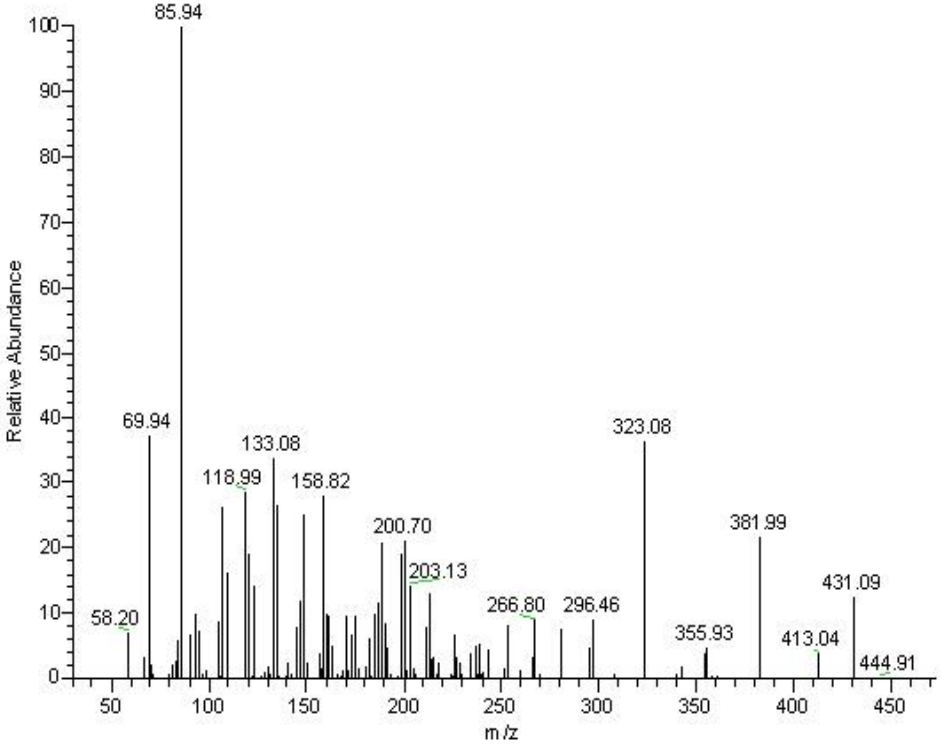
Cyclobuxoviridine B

可能为峰1的黄杨宁有关生物碱

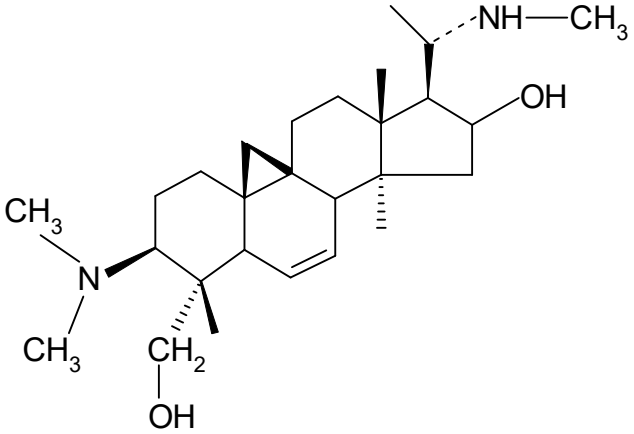
$[M+H]^+ = 431$



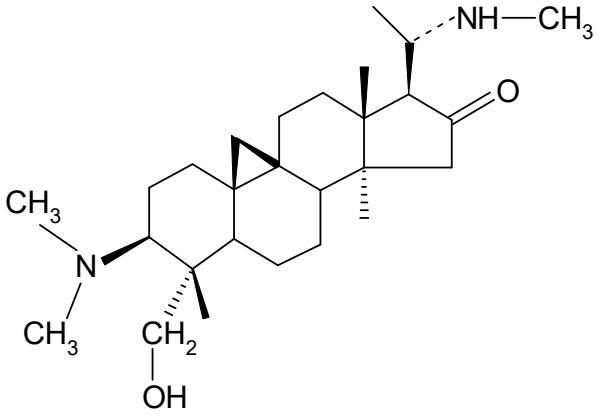
峰2母离子质荷比



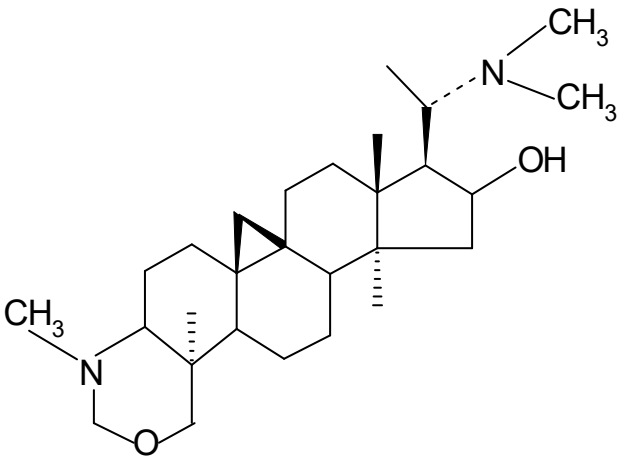
峰2二级质谱图



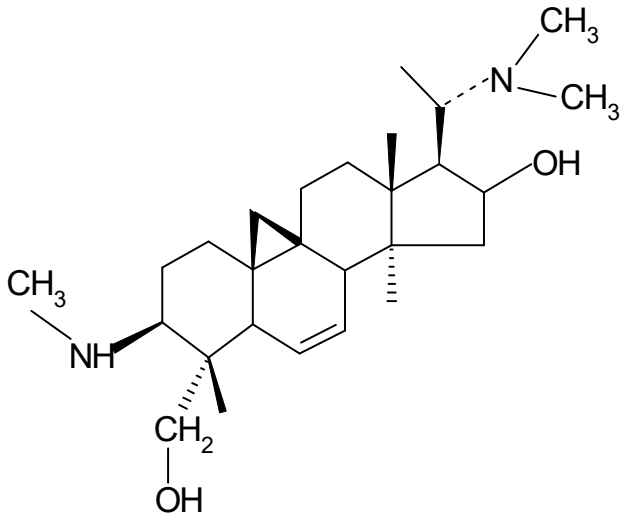
Cyclomicrophylline B



Buxazidine B



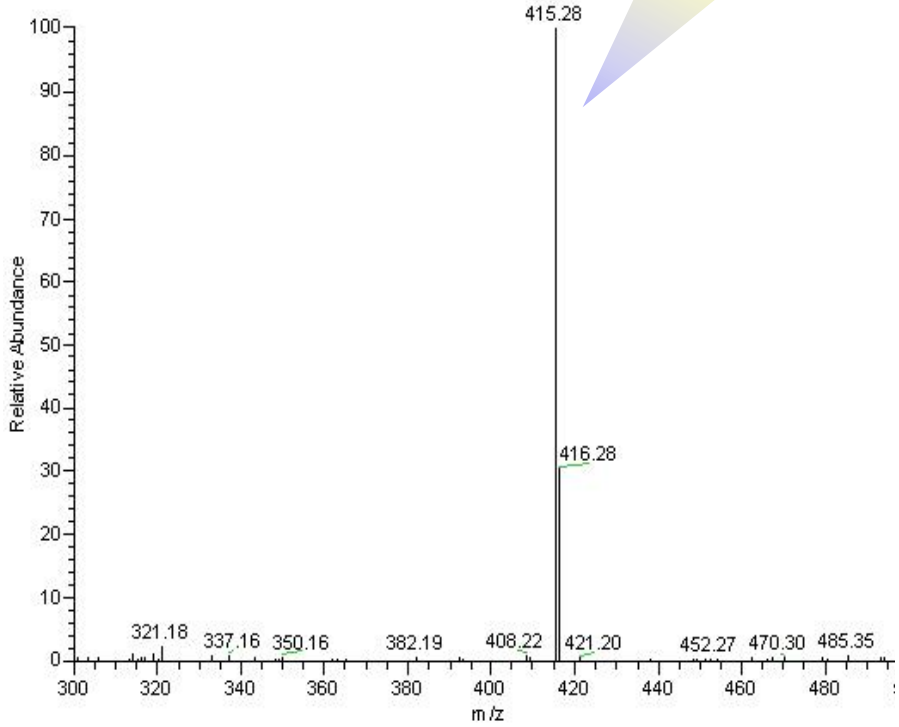
Cyclobuxoxazine C



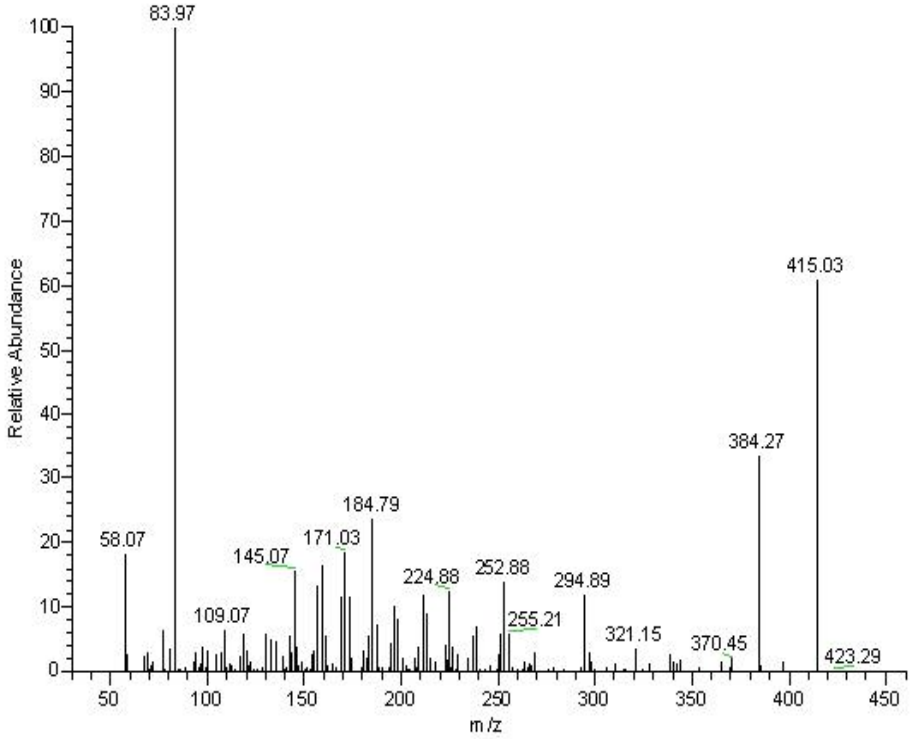
Cyclomicrophylline C

可能为峰2的黄杨宁有关生物碱

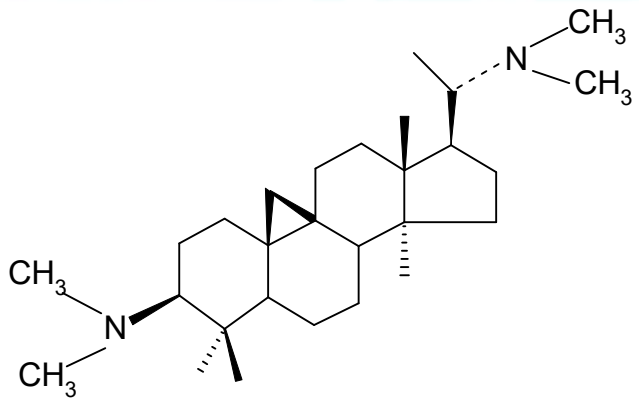
$[M+H]^+ = 415$



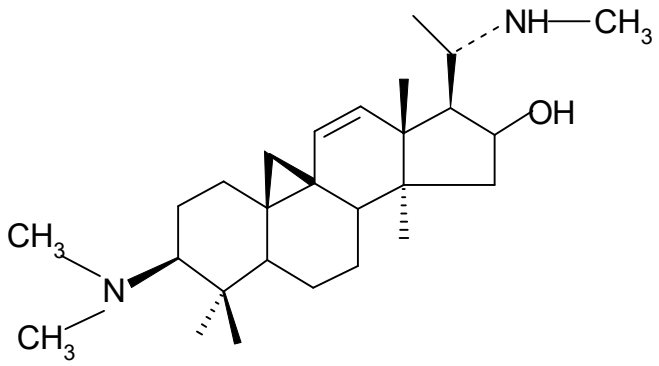
峰3母离子质荷比



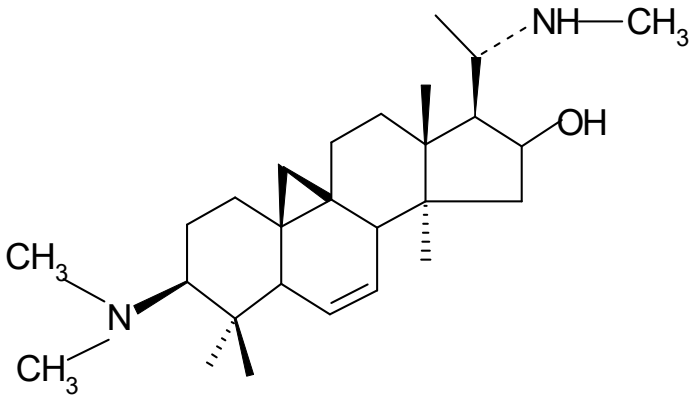
峰3二级质谱图



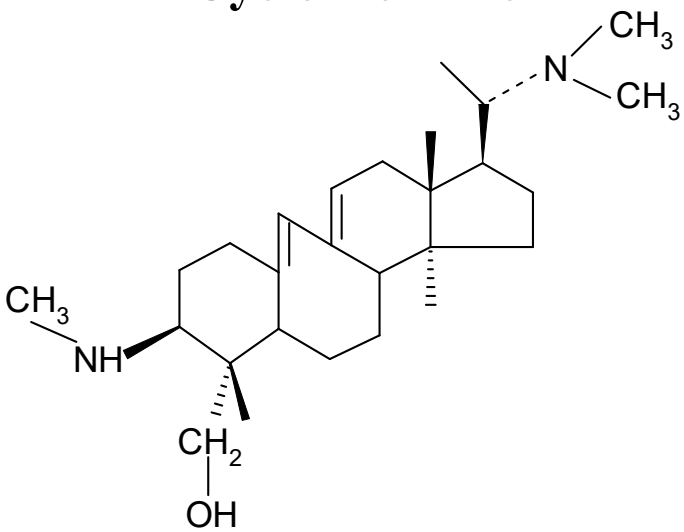
Cycloprotobuxine A



Cyclokreanine B



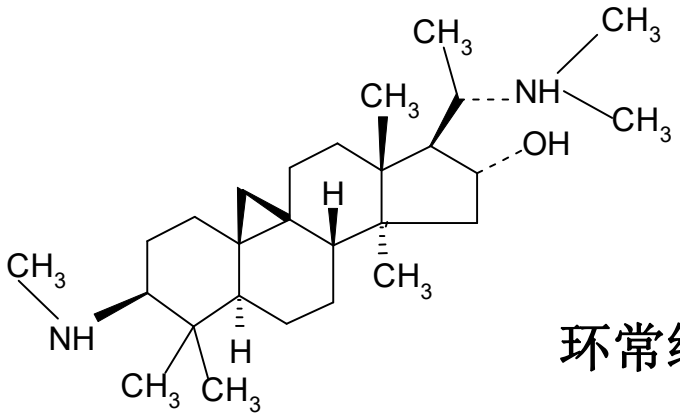
Cyclovirobuxeine B



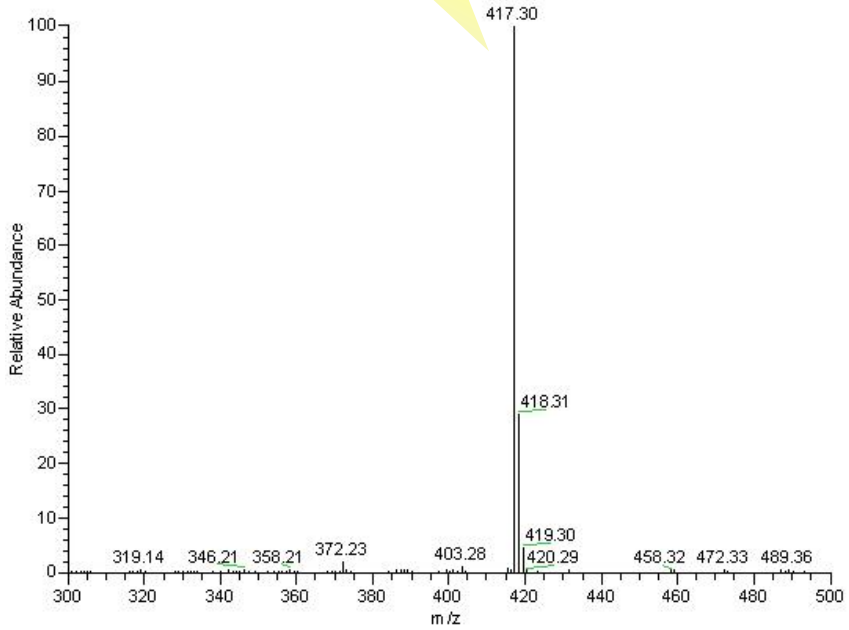
16-deoxybuxidienine C

可能为峰3的黄杨宁有关生物碱

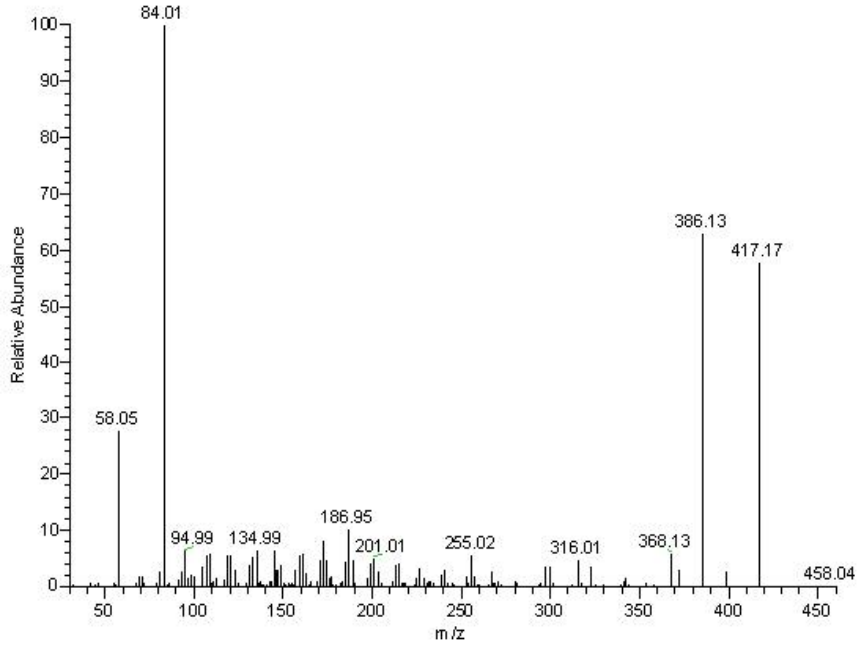
$[M+H]^+ = 417$



环常绿黄杨碱C

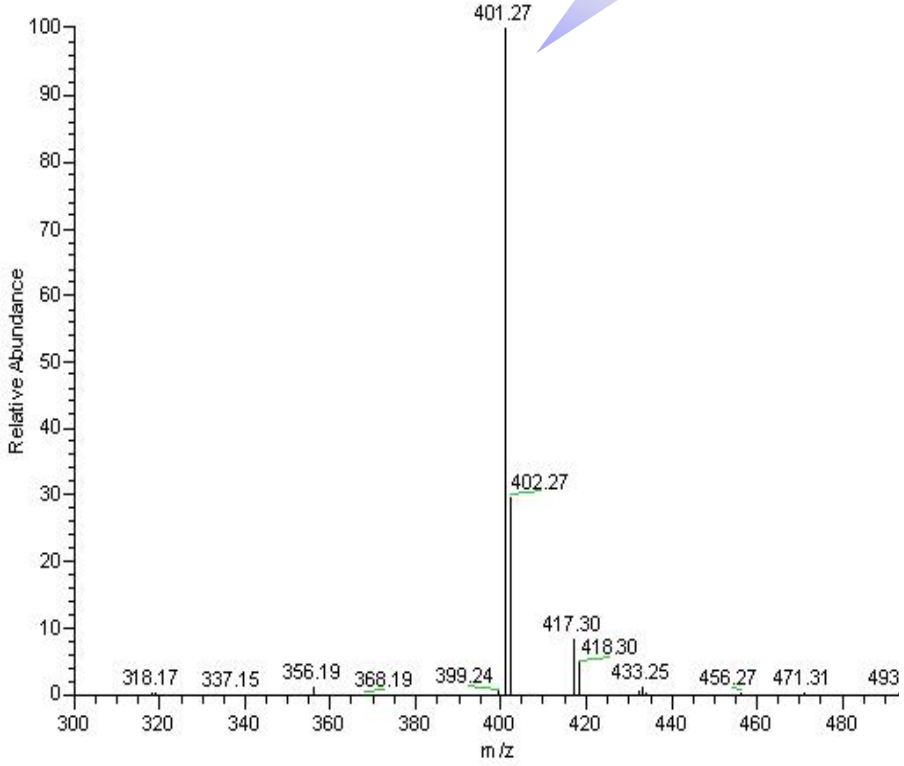


峰4母离子质荷比

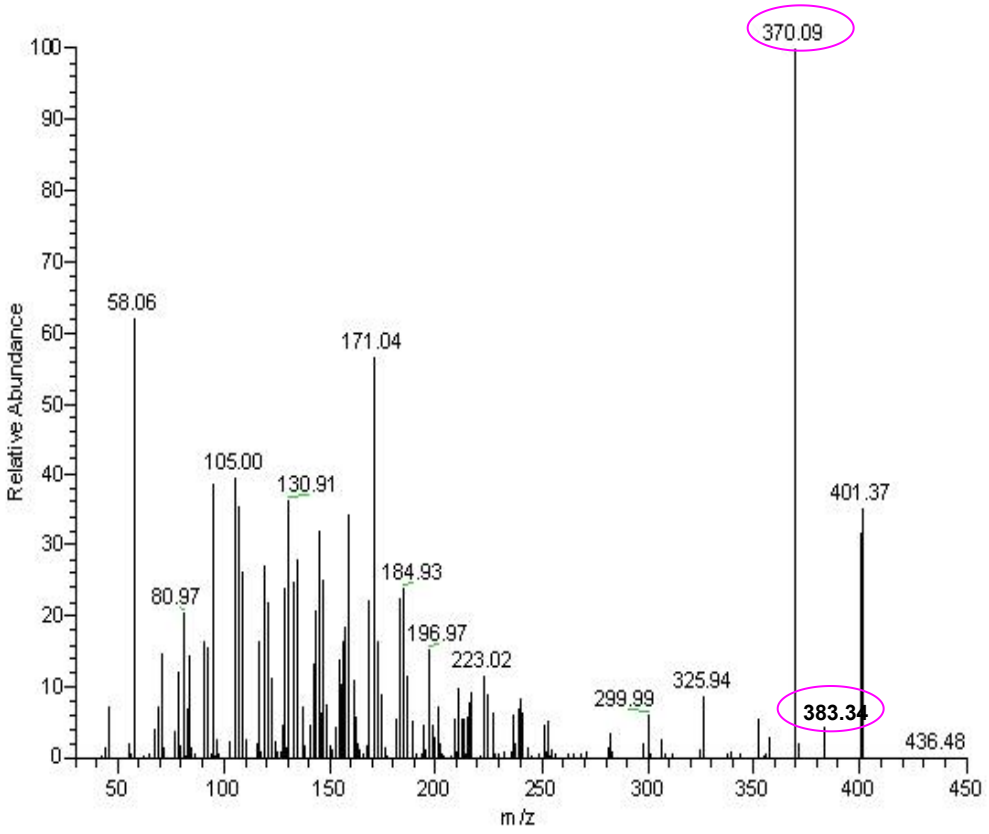


峰4二级质谱图

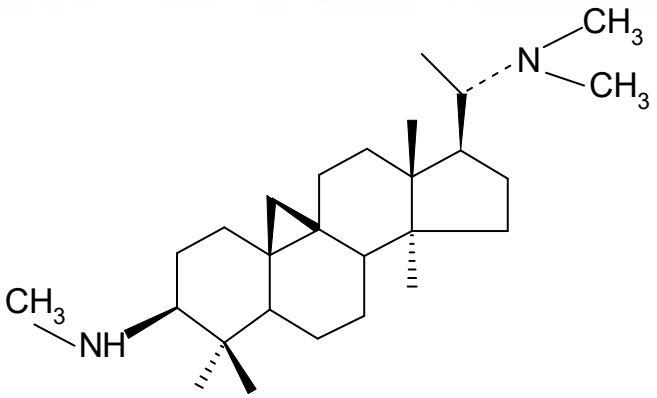
$[M+H]^+ = 401$



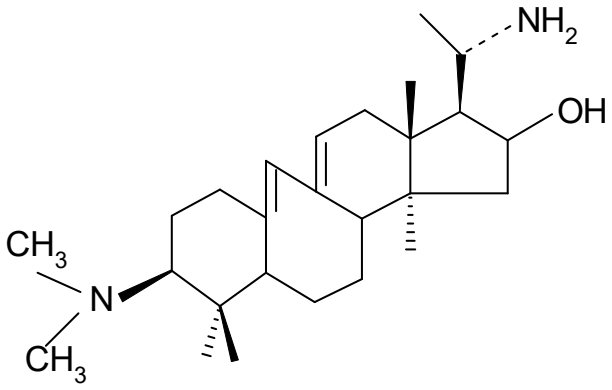
峰5母离子质荷比



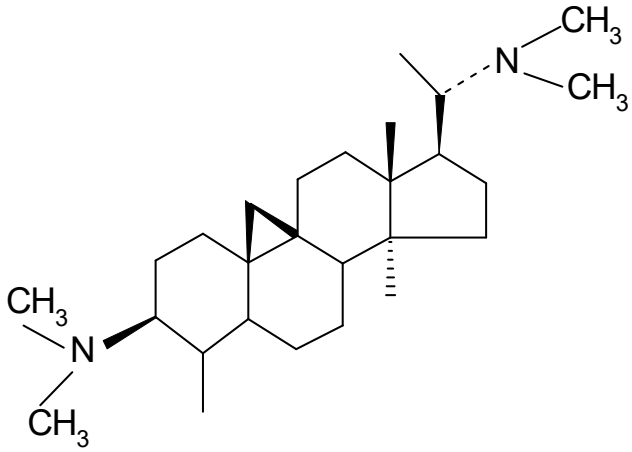
峰5二级质谱图



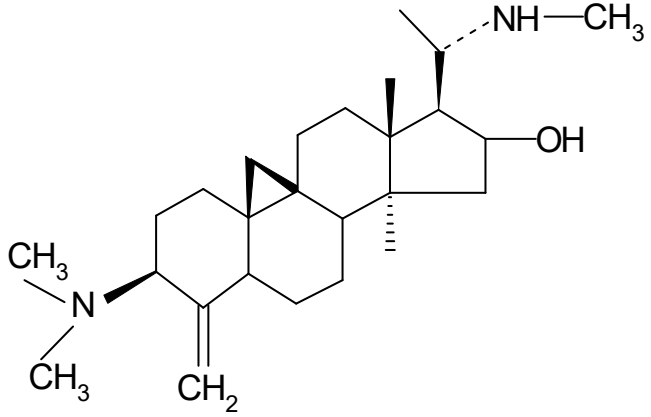
Cycloprotobuxine C



Buxaminol E



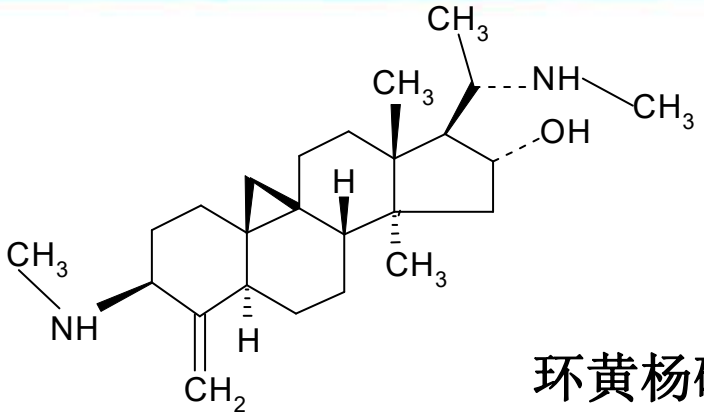
Buxocyclamine A



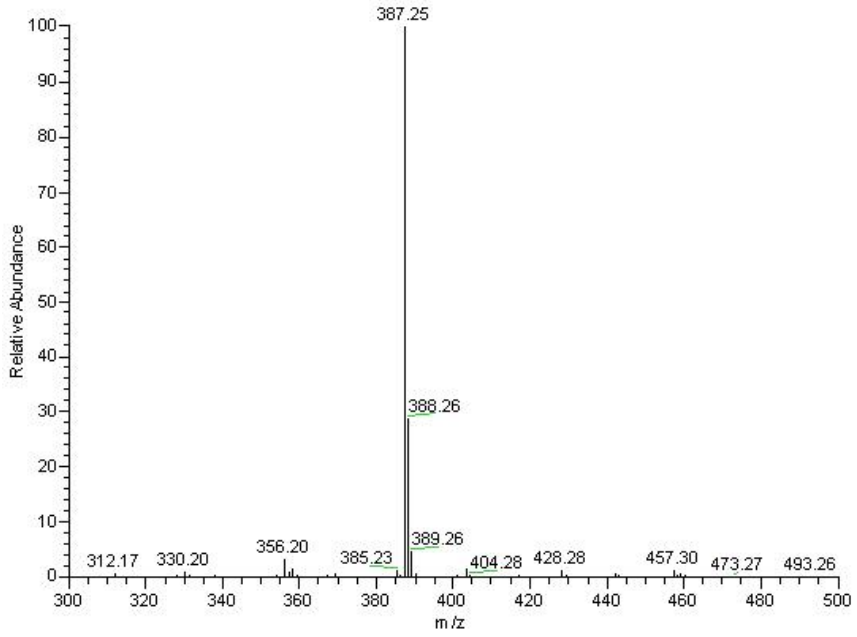
Cyclobuxine B

可能为峰5的黄杨宁有关生物碱

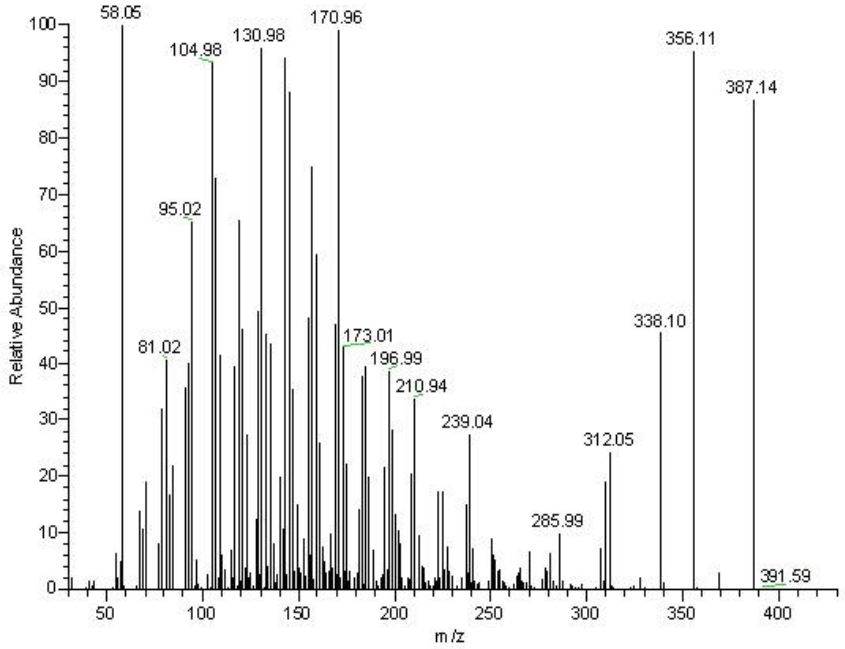
$[M+H]^+ = 387$



环黄杨碱D

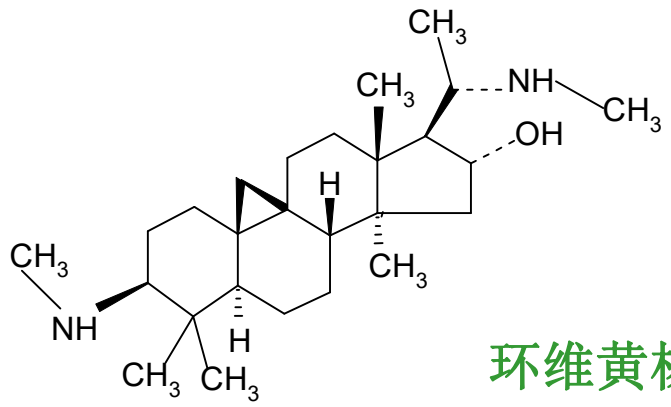


峰6母离子质荷比

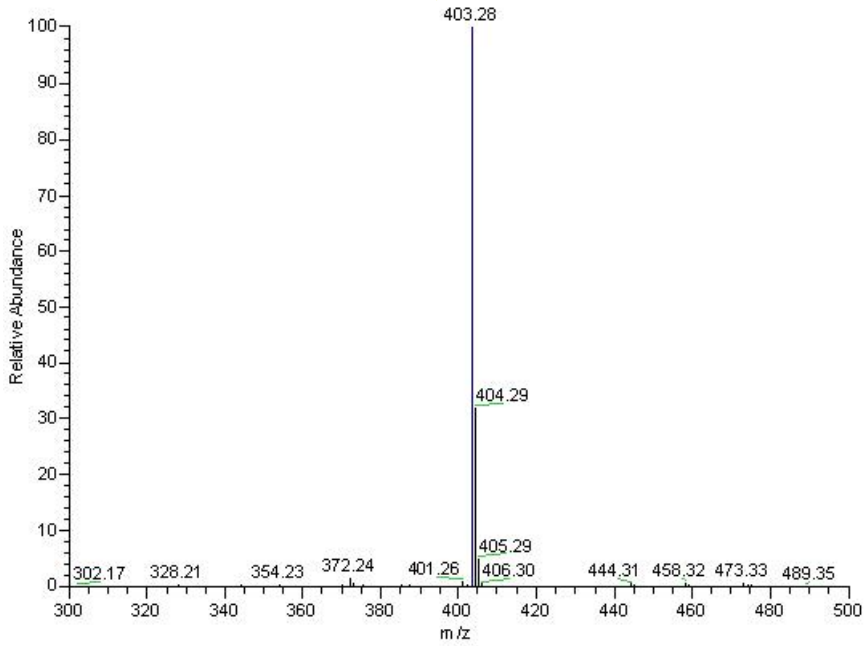


峰6二级质谱图

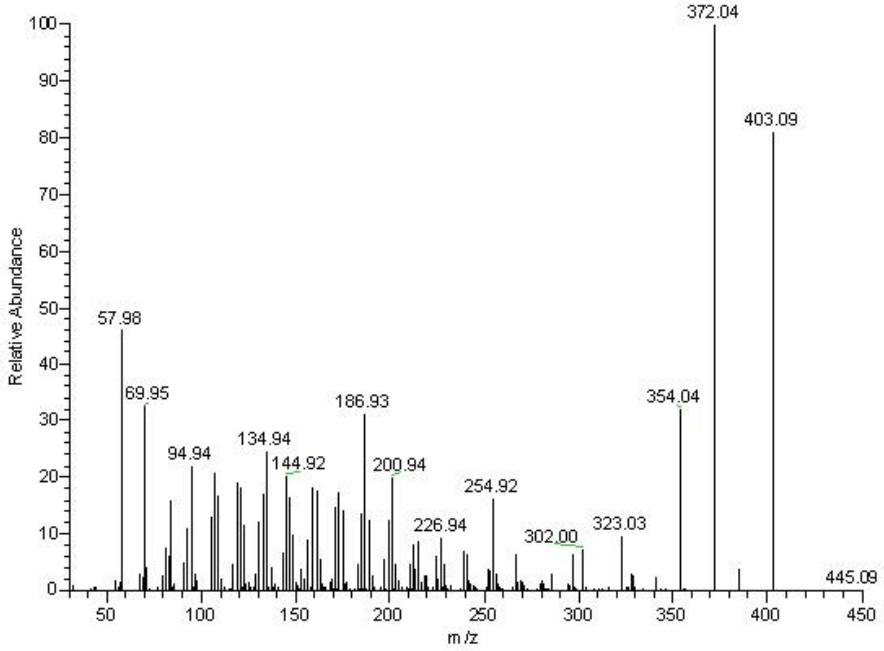
$[M+H]^+ = 403$



环维黄杨星D

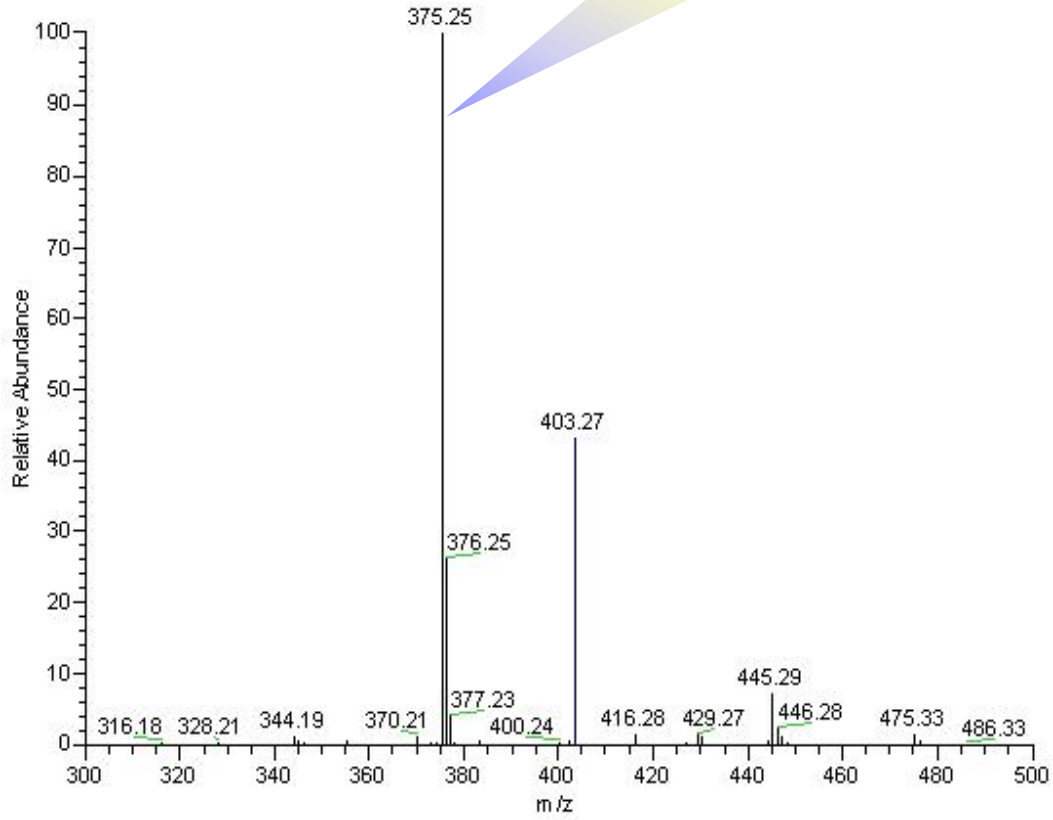


峰7母离子质荷比

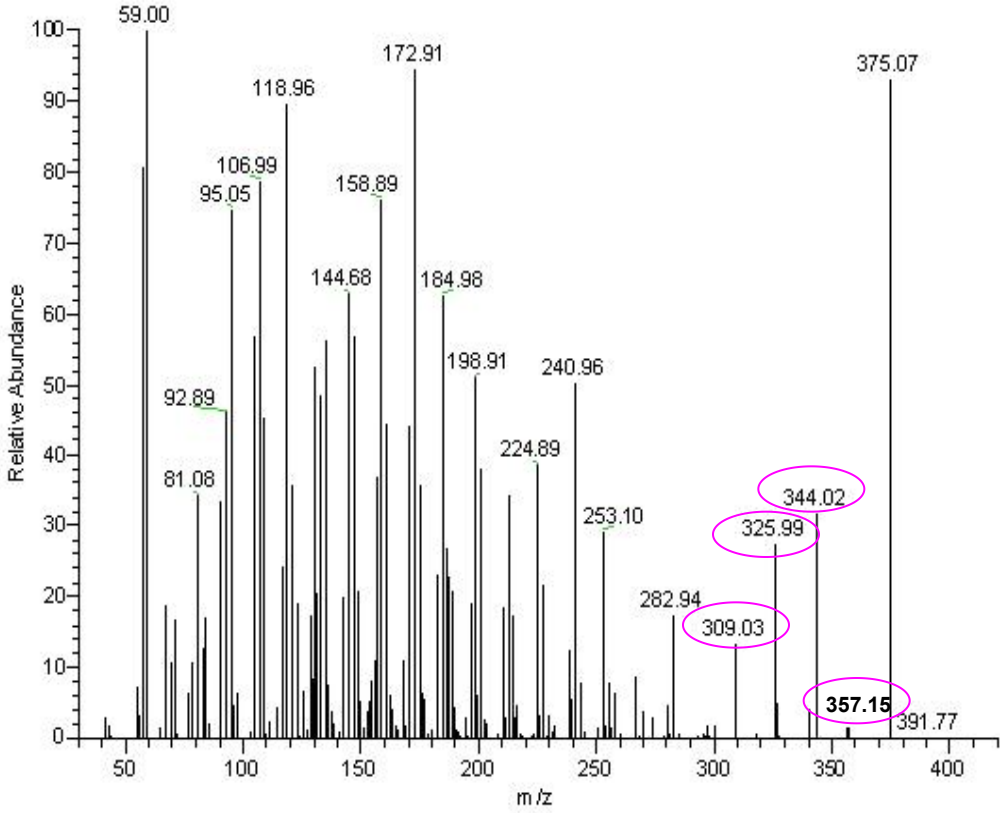


峰7二级质谱图

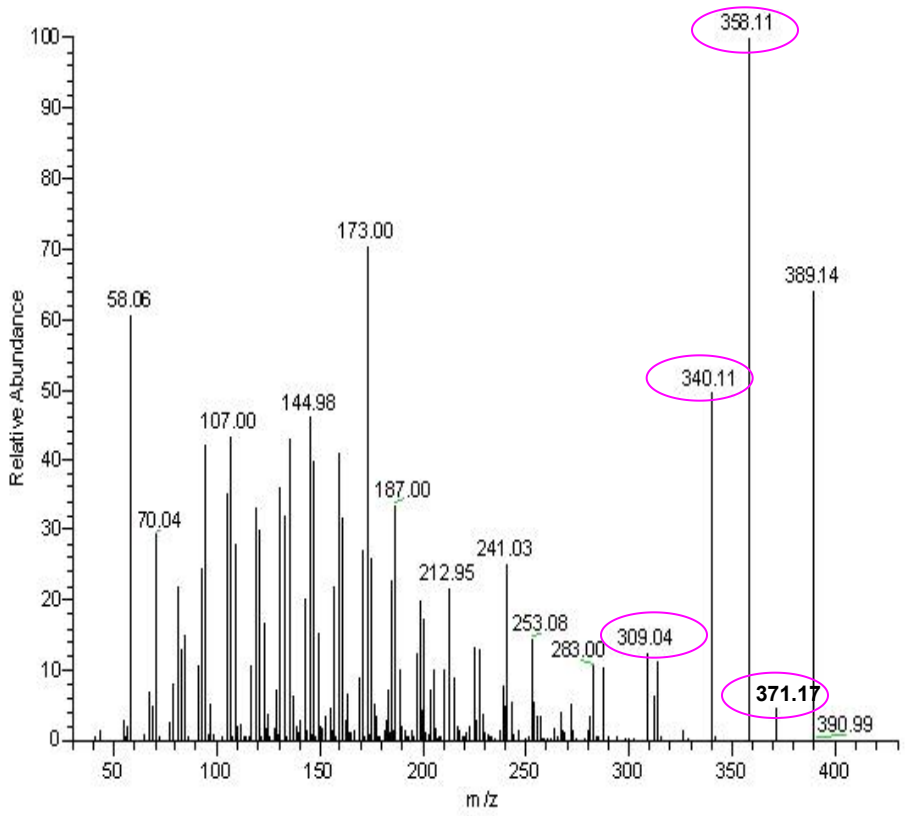
$[M+H]^+ = 375$



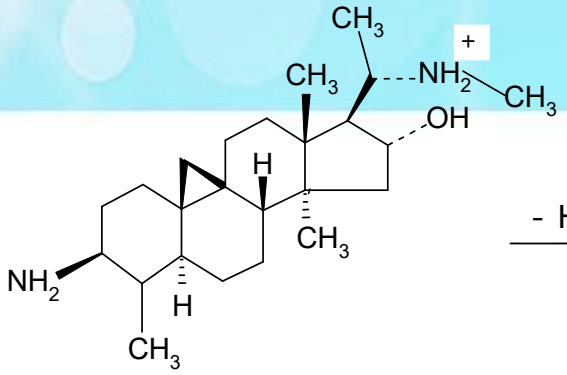
峰8母离子质荷比



峰8二级质谱图

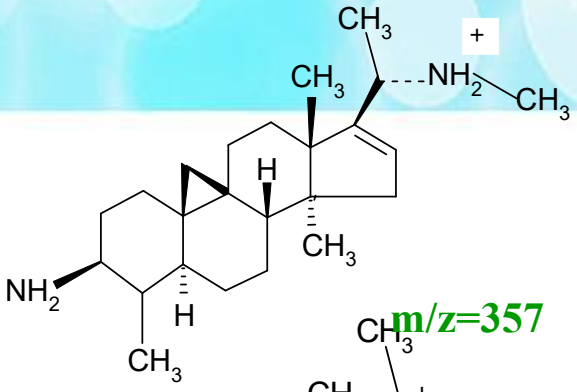


峰9二级质谱图



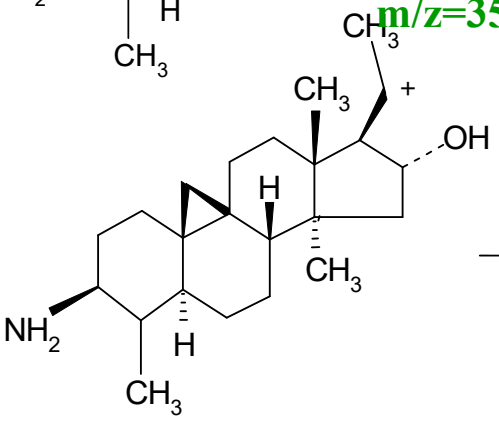
m/z=375

- H_2O



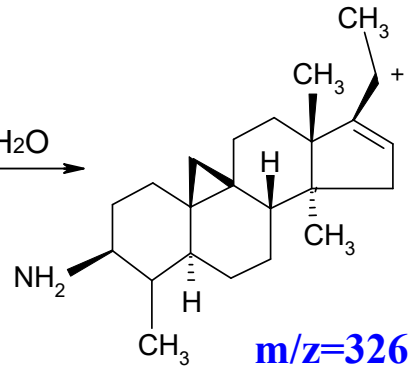
m/z=357

- NH_2CH_3



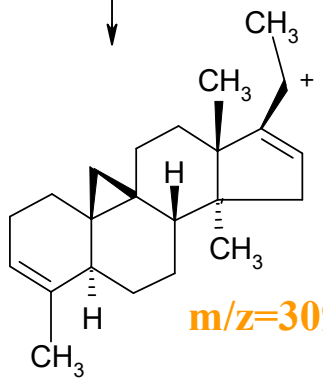
m/z=344

- H_2O

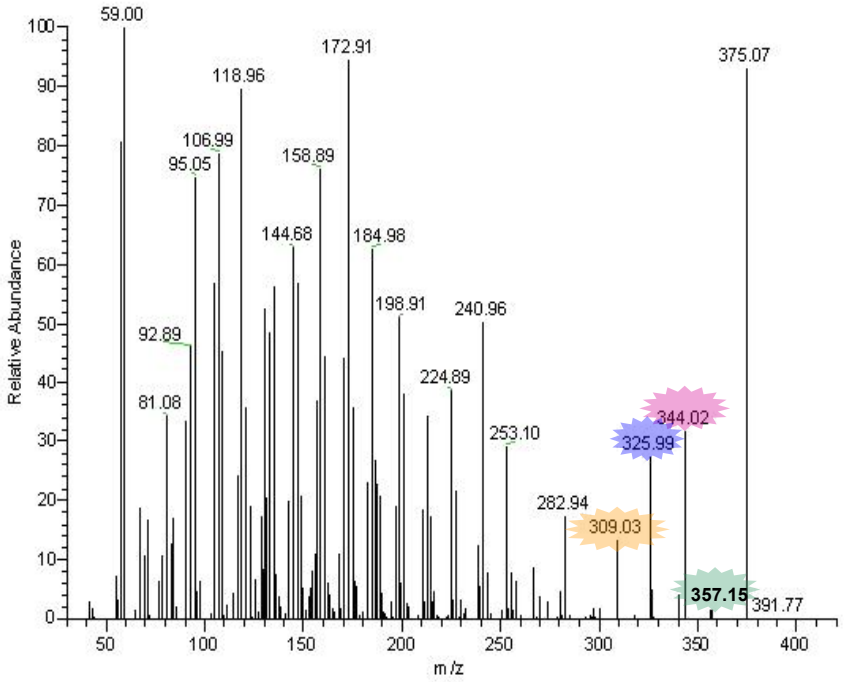


m/z=326

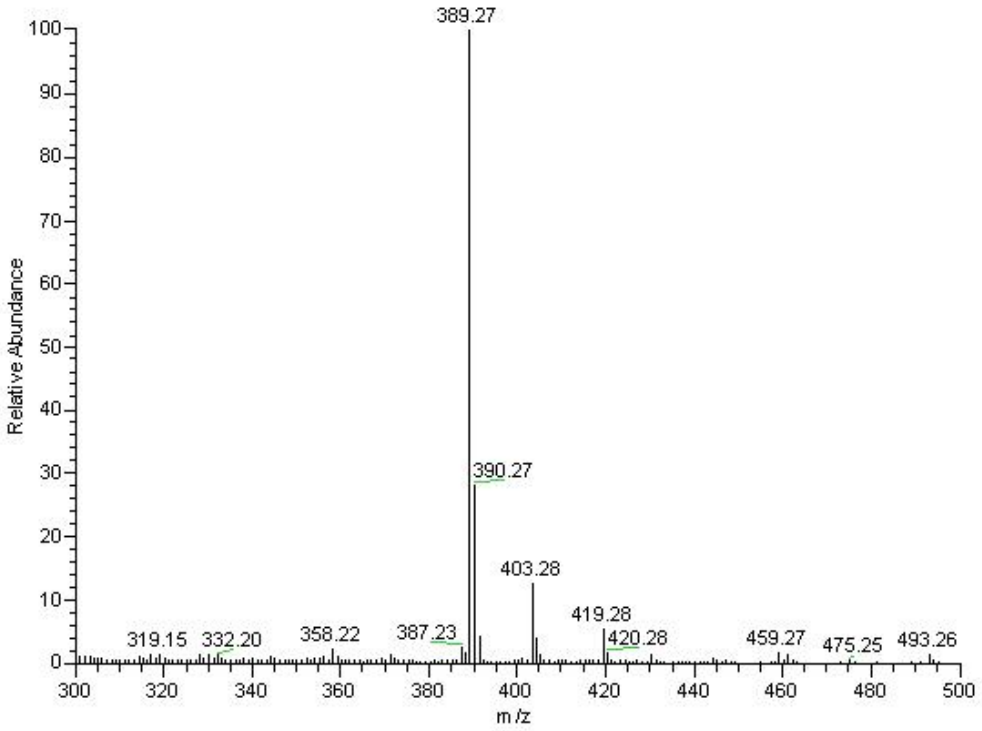
- NH_3

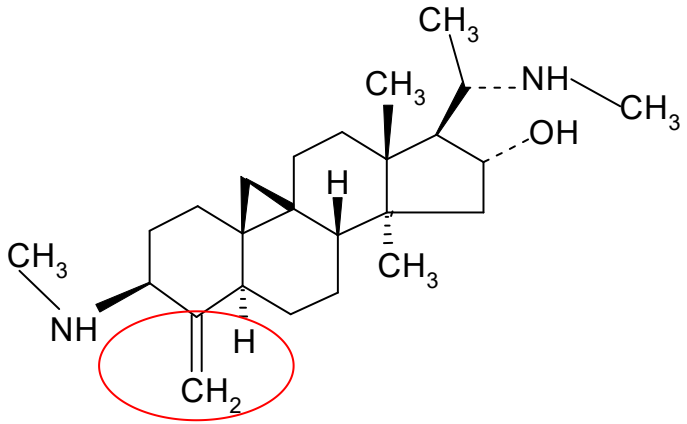


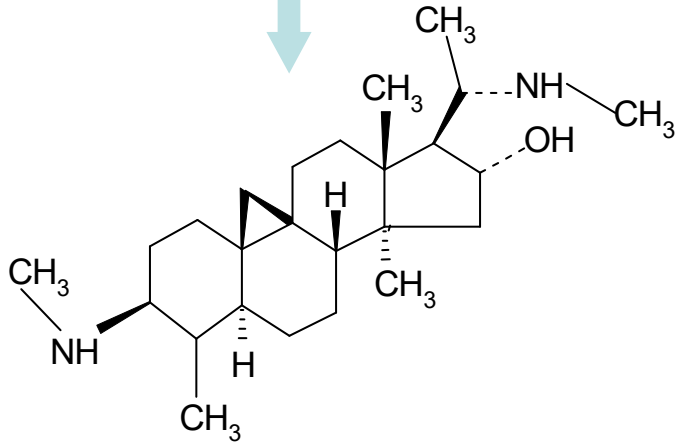
m/z=309



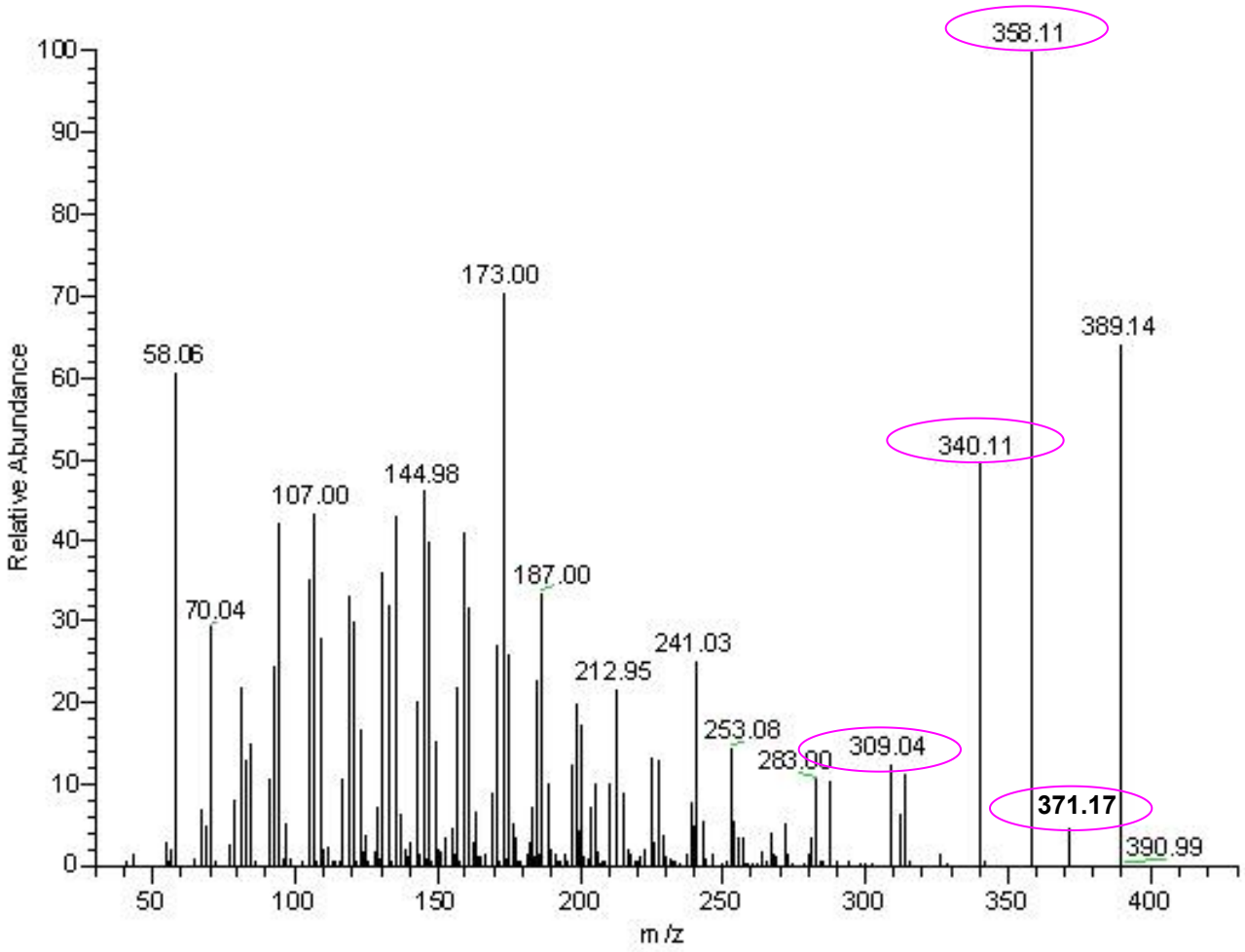
$[M+H]^+ = 389$



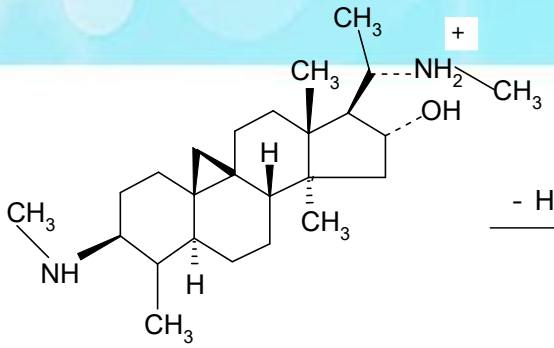




峰9母离子质荷比

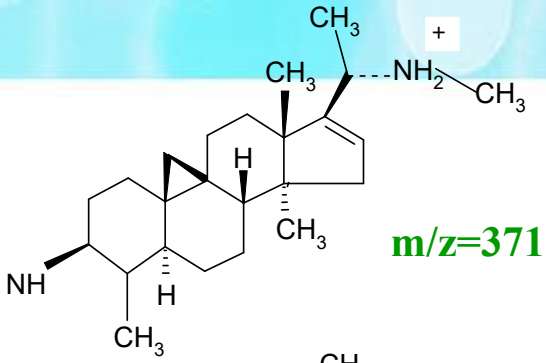


峰9二级质谱图



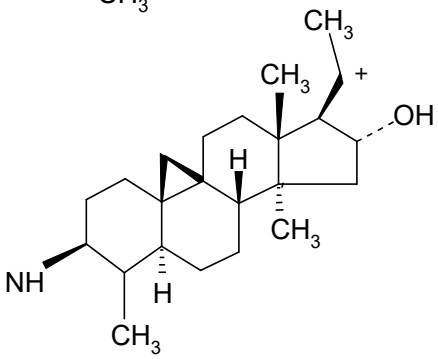
m/z=389

- H_2O



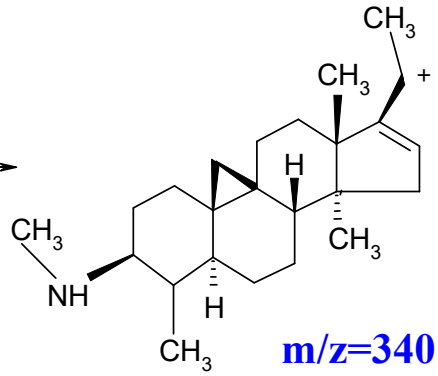
m/z=371

- NH_2CH_3



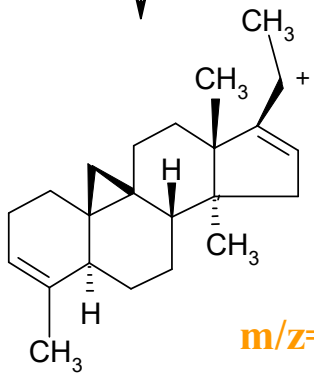
m/z=358

- H_2O

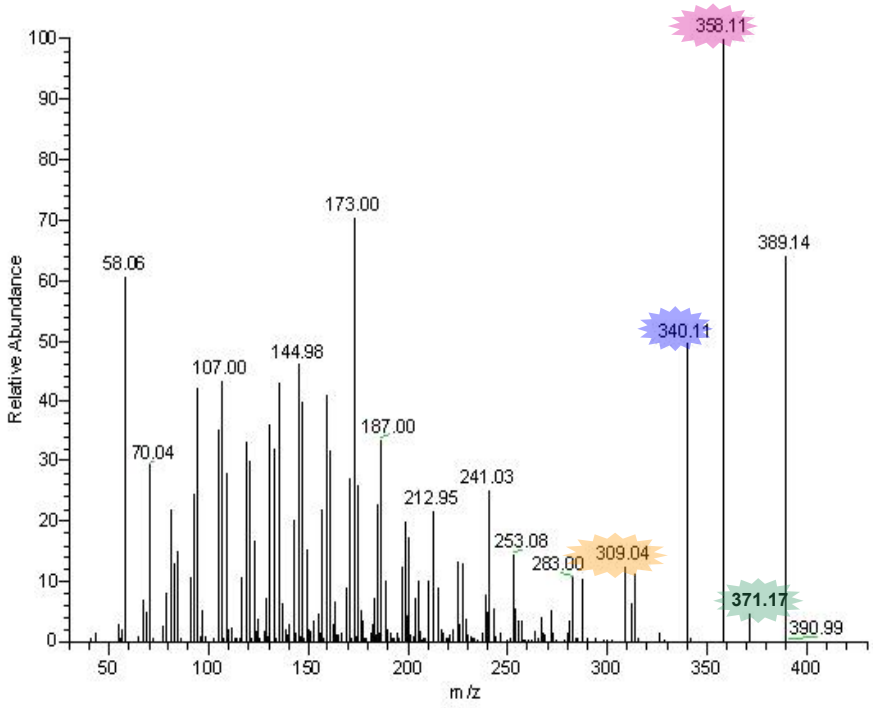


m/z=340

- NH_2CH_3



m/z=309



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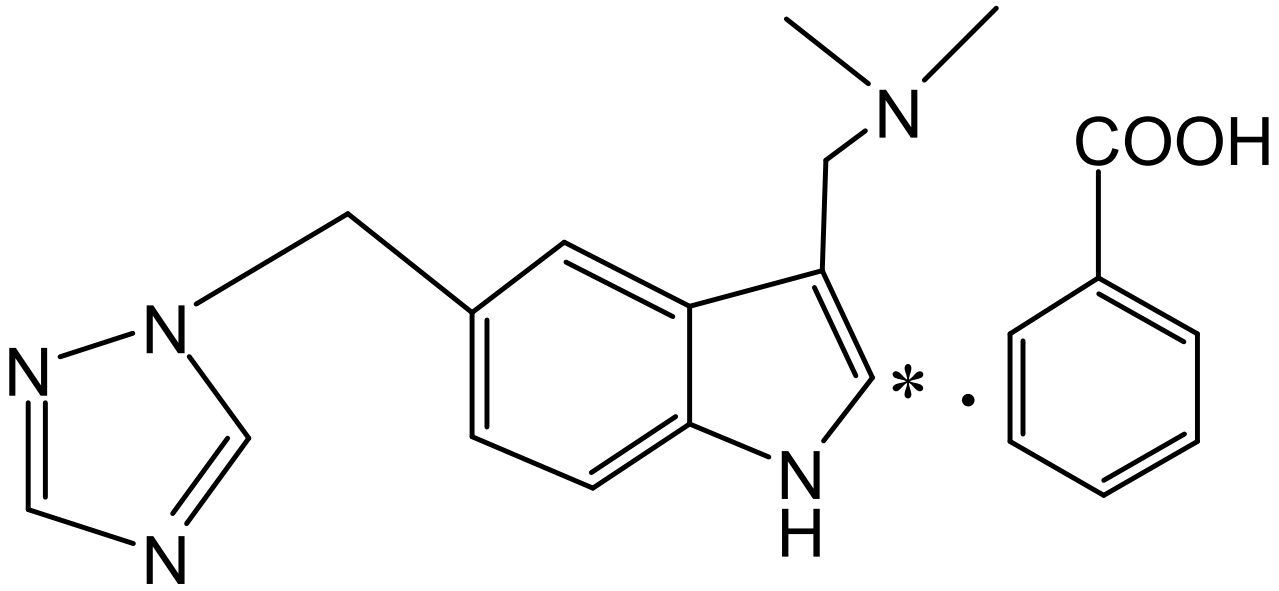
HPLC-ELSD法黄杨宁有关生物碱归属表

峰位号	t_R（min）	[M+H]+（m/z）	特征碎片离子（m/z）	可能生物碱名称
1	3.92	370	339，325，283，135，70，58	Cyclobuxomicreine K， Cyclobuxosuffrine K，Buxenone M， Cyclobuxoviridine B
2	4.03	431	413，382，323，86，70，58	Buxazidine B， Cyclomicrophylline B，Cyclobuxoxazine C， Cyclomicrophylline C
3	5.35	415	384，84，58	Cycloprotobuxine A，Cyclokreanine B，Cyclovirobuxeine B， 16-deoxybuxidienine C
4	5.92	417	399，386，368，84，58	Cylcyclovirobuxine C
5	6.11	401	370，352，326，171，58	Cycloprotobuxine C， Buxaminol E，Buxocyclamine A， Cyclobuxine B
6	7.03	387	369，356，338，171，58	Cyclobuxine D
7	7.63	403	385，372，354，70，58	Cyclovirobuxine D
8	8.28	375	357，344，326，309，58	未有相关文献报道
9	9.77	389	371，358，340，173，70，58	可能为Cyclobuxine D双键加氢还原产物

（3）苯甲酸利扎曲普坦人体药代动力学研究



苯甲酸利扎曲普坦　（Rizatriptan Benzoate）

MW: 391.47　分子式：$C_{15}H_{19}O_5 \cdot C_7H_6O_2$

5-HT受体阻断剂

药理作用

- 刺激大脑血管壁的后接点5-HT_{1B}受体，收缩血管，降低颅内血管通透性；
- 刺激三叉神经前突触5-HT_{1D}受体，调节神经递质的释放，抑制硬膜的神经原性炎症反应和血浆外渗；
- 阻止血管肽的释放，使血管口径正常化，通过收缩颅内血管并抑制神经炎症；
- 刺激脑干5-HT_{1B}或5-HT_{1D}受体，抑制三叉神经核兴奋；
- 减少颈动脉血流；
- 透过血脑屏障，增加脑血流量。

实验内容

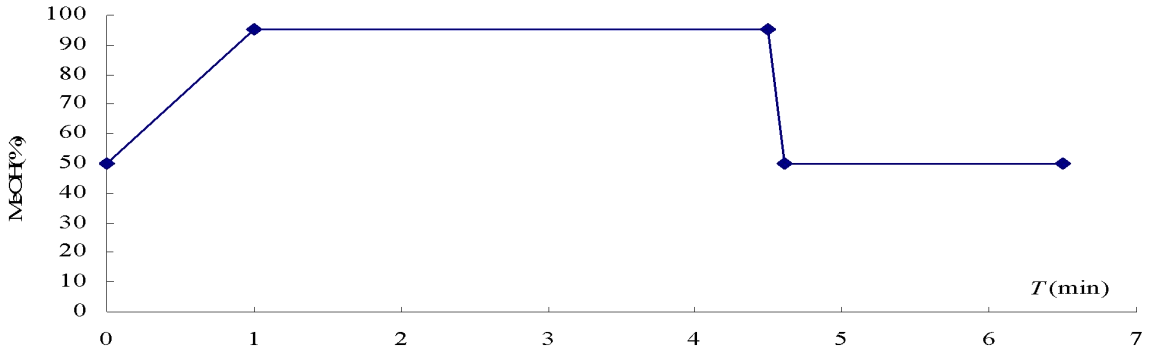
- 建立苯甲酸利扎曲普坦在血浆、尿样浓度的LC-MS/MS测定方法
- 苯甲酸利扎曲普坦分散片和胶囊进行生物等效性试验
- 苯甲酸利扎曲普坦片体内药代动力学研究
  - 单剂量 （5、10、15 mg）
  - 多次给药（10 mg）
  - 稳态（10 mg）

LC-MS/MS测定方法建立

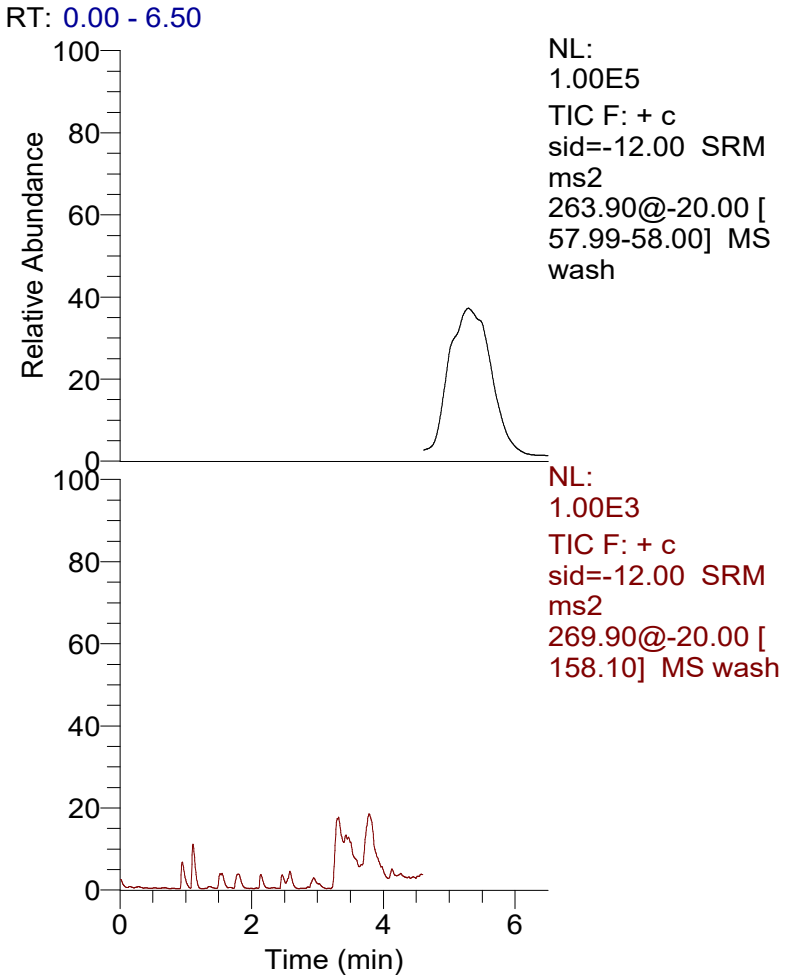
- 测定方法选择 — LC-MS/MS
- 质谱条件优化 — m/z 270→m/z 158
- 色谱条件选择
  - 色谱柱选择 — Phenomenx PFP
  - 缓冲盐选择 — 1%冰醋酸， 0.2%醋酸铵
- 血浆处理方法
  - 液液萃取法
  - 蛋白沉淀法 — 蛋白沉淀法
- 内标选择 — 盐酸曲马多

色谱条件

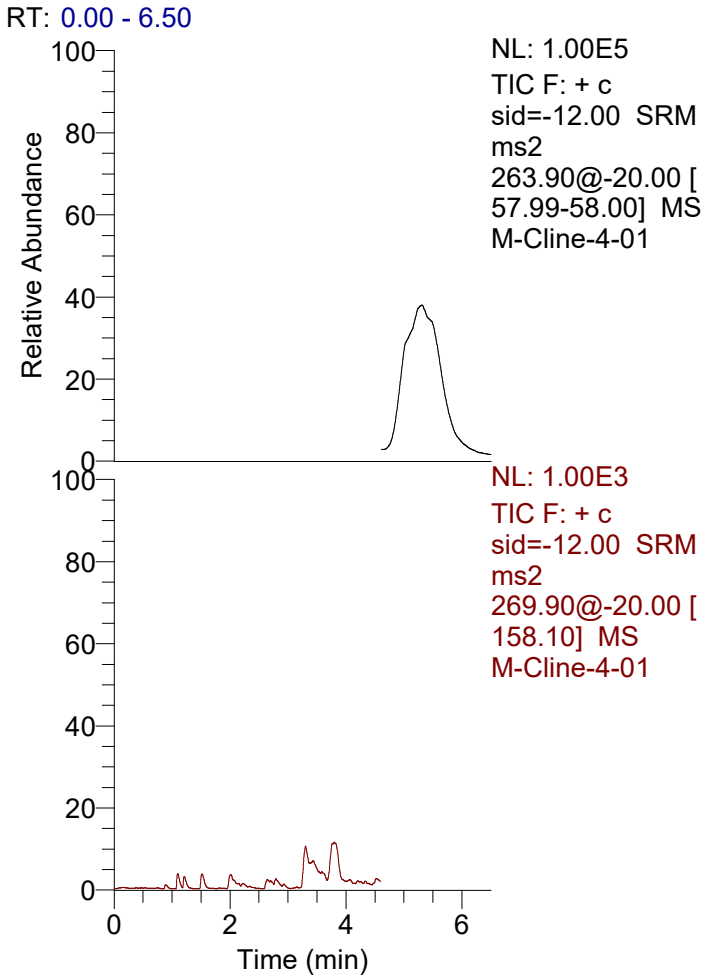
- 流动相A：醋酸盐缓冲液（1%冰醋酸，2%醋酸铵；pH 3.5）
- 流动相B：甲醇（0.1%甲酸）
- 梯度条件过程：0 min （B50%）→1.0 min （B95%）→4.5 min （B95%）→4.6 min （B50%） →6.5 min （B50%）



空白溶剂色谱图



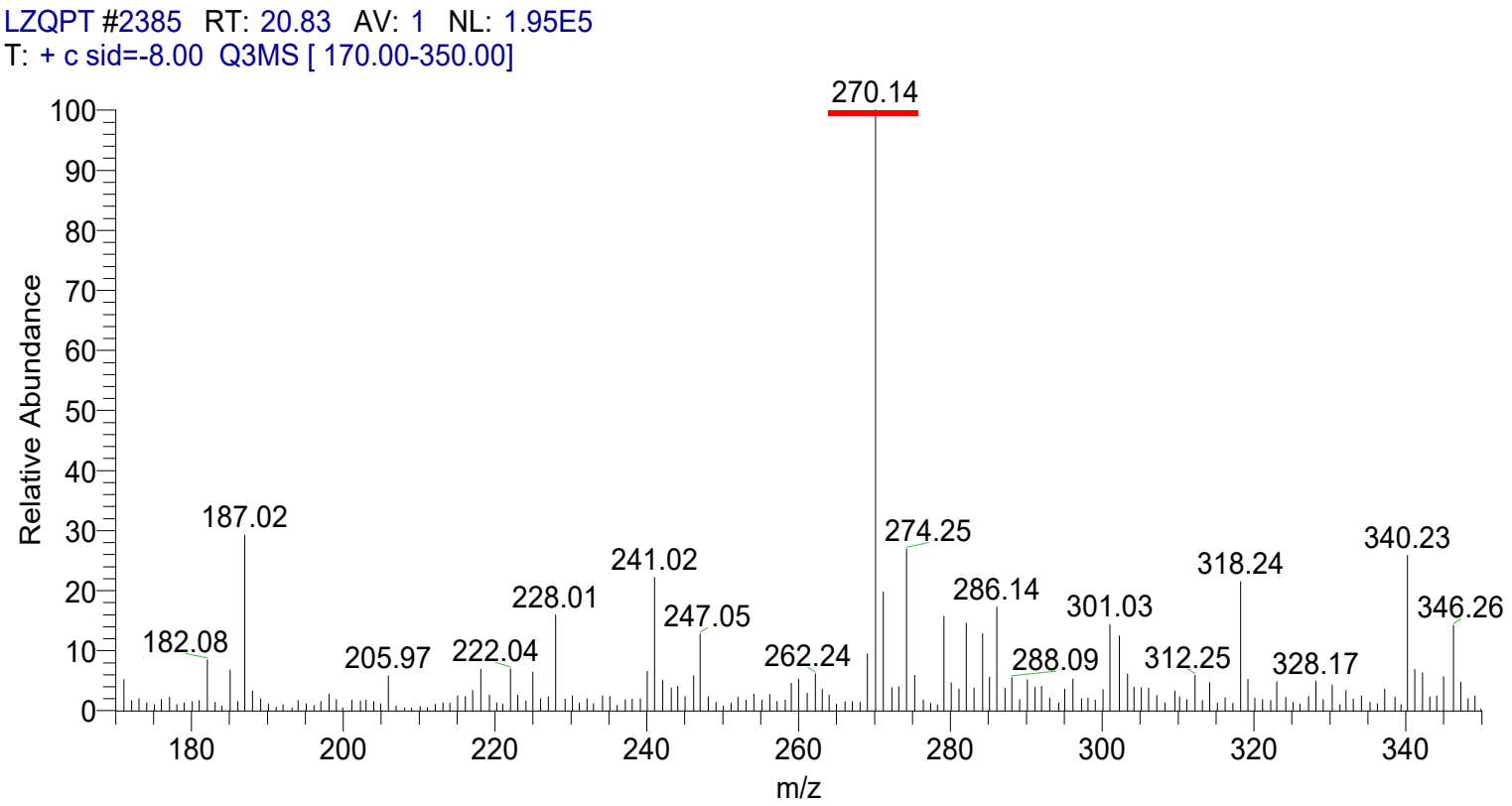
预处理的空白血浆色谱图



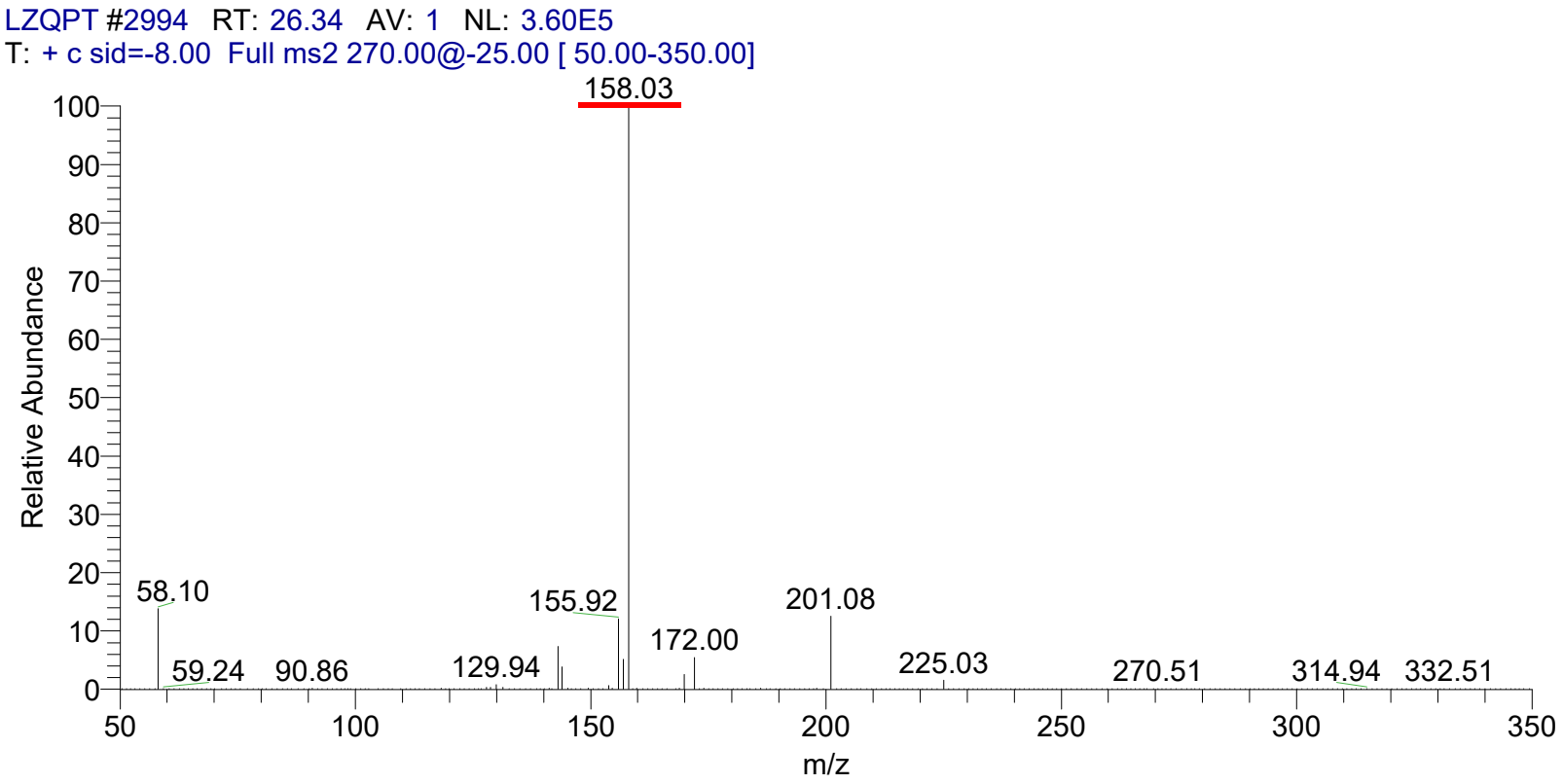
质谱条件

- 离子检测方式：ESI^+ SRM
- 检测对象： 利扎曲普坦 m/z 269.9→158.1
  曲马多 m/z 263.9→58.0
- 喷口电压：5000 V
- 雾化气压： 35 psi
- 辅助气压力： 5 psi
- 毛细管温度：350℃
- 碰撞气氩气压力： 1.3mTorr
- 碰撞能量： 20 eV

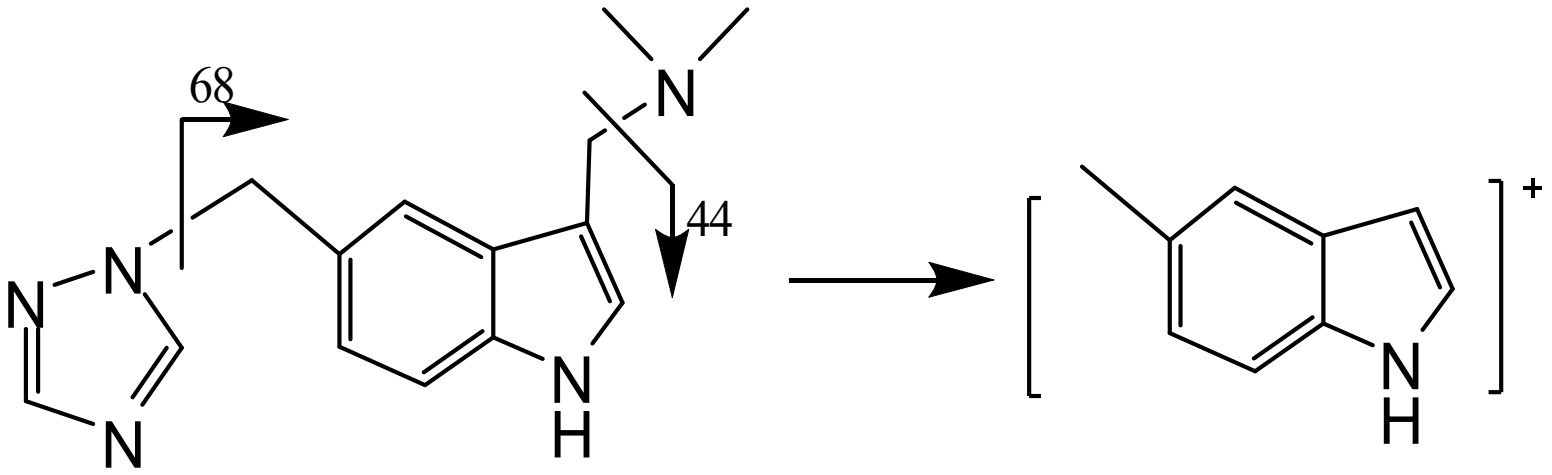
质谱条件优化



利扎曲普坦LC-MS质谱扫描图（m/z =270）



利扎曲普坦LC-MS/MS质谱扫描图（m/z=158）



m/z 270　　m/z 158

利扎曲普坦质谱裂解图 m/z 270→m/z 158

（碰撞能量：20ev）

色谱条件选择

- 缓冲盐条件（0.1%HCOOH，*v/v*; 0.2%醋酸铵，*w/v*; pH3.0）

色谱柱
- Intersil-ODS C_{18}
- Lichrospher-C_{18}
- Zorbak-ODS C_{18}
- Lichrospher-CN
- Phenomenx Curosil-PFP

- 采用PFP（五氟苯基）柱，样品的保留适当，峰形良好，有机相和水相的比例也较为适当。

甲醇：水=（50：50）

醋酸铵（*w/v*）
- 0.05%
- 0.1%
- 0.2%

→ 0.2%（*w/v*）醋酸铵样品峰响应高，拖尾得到一定程度的改善。

- 0.1% HCOOH
- 0.1% HAC
- 0.2% HAC
- 0.5% HAC
- 1.0% HAC

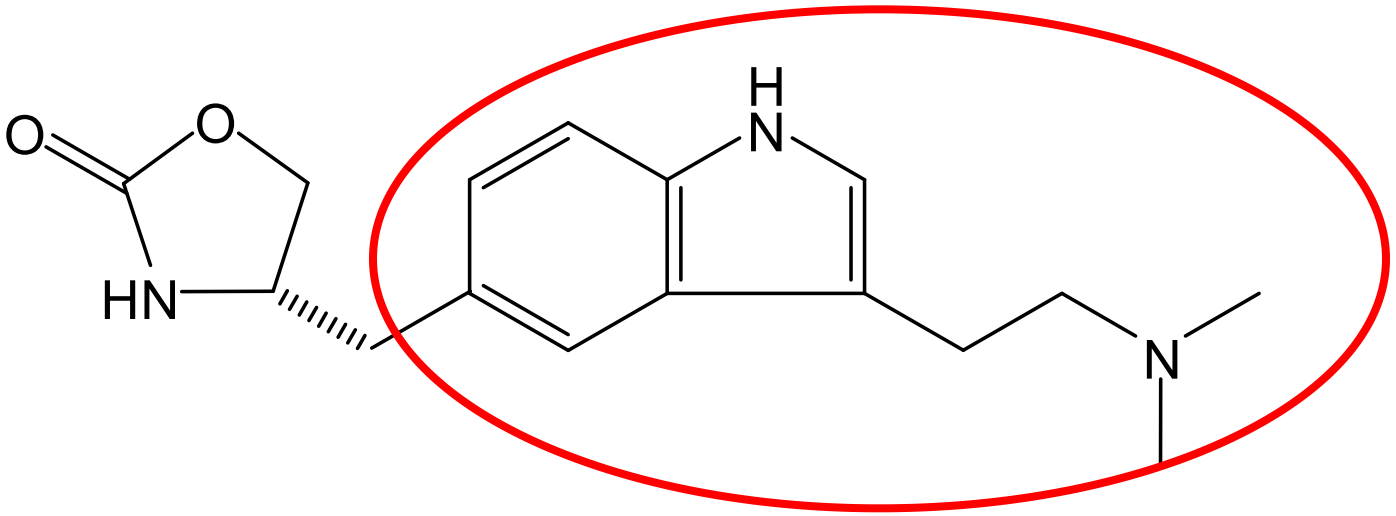
→ 1%HAC水相pH为3.5，样品保留时间合适，峰形良好。

血浆处理方法

提取溶剂	碱化液种类	碱化液体积(μl)	样品回收率(%)	结论
乙酸乙酯	1M NaOH	100	71.8	空白血浆有明显干扰
乙醚	1M NaOH	100	70.9	空白血浆略有干扰
三氯甲烷	1M NaOH	100	45.9	无干扰
二氯甲烷	1M NaOH	100	58.4	空白血浆干扰，
特丁基醚	1M NaOH	100	89.9	空白血浆无明显干扰
特丁基醚	0.1M NaOH	50	76.8	无干扰
特丁基醚	0.1M NaOH	100	70.8	空白血浆有干扰
特丁基醚	0.1M NaOH	200	75	无干扰
特丁基醚	1M NaOH	50	88.2	无干扰
特丁基醚	**1M NaOH**	**200**	**92.4**	**无干扰**
特丁基醚	浓氨水	50	88.5	无干扰
特丁基醚	浓氨水	100	83.8	无干扰
特丁基醚	浓氨水	200	81.7	无干扰

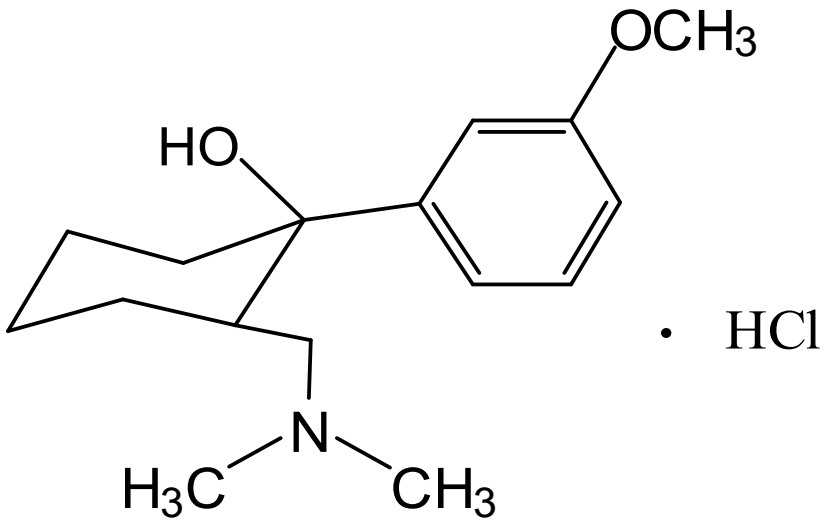
苯甲酸利扎曲普坦血浆提取条件试验

内标选择



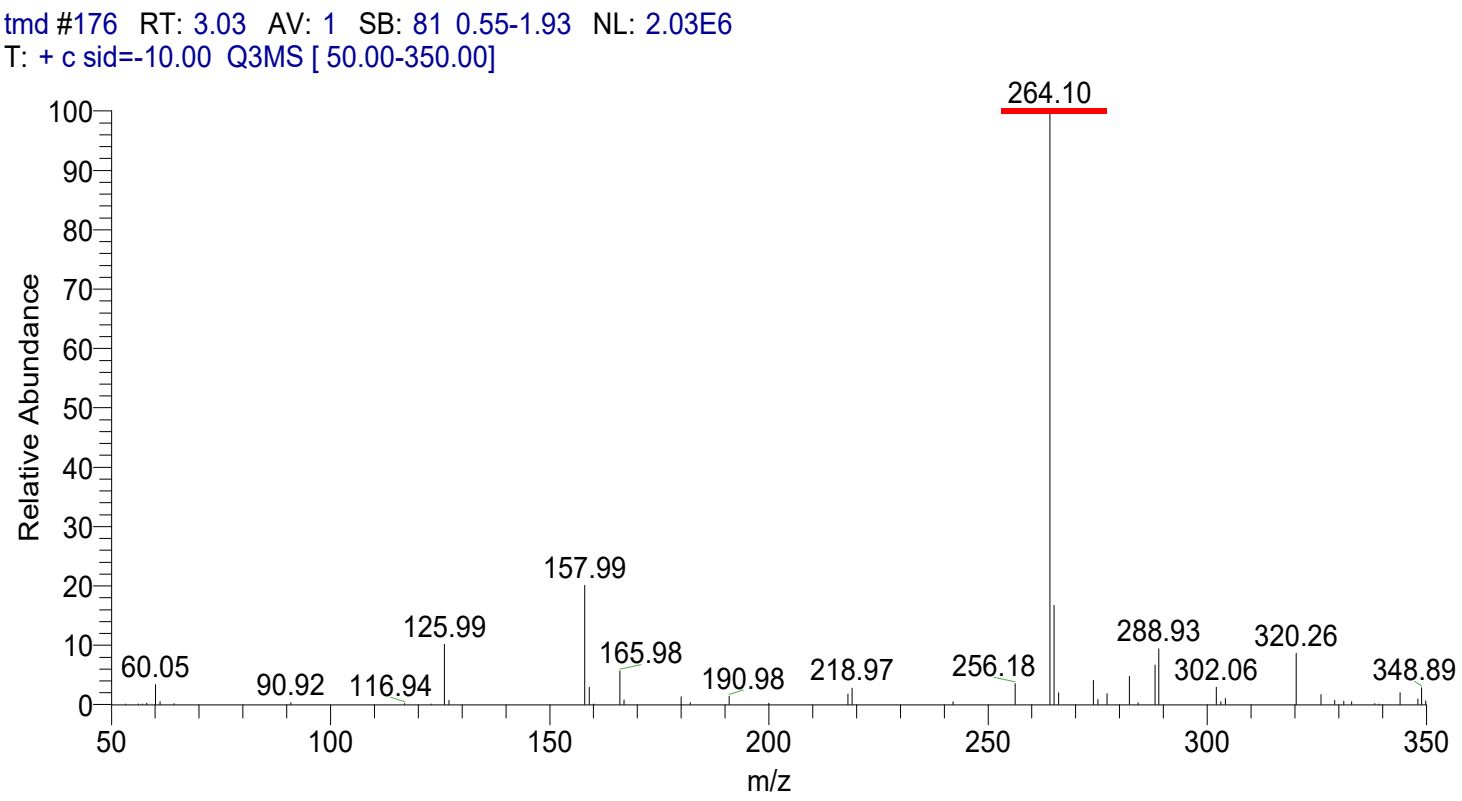
佐米曲普坦（Zolmitriptan）

MW：287 分子式：$C_{16}H_{21}N_3O_2$

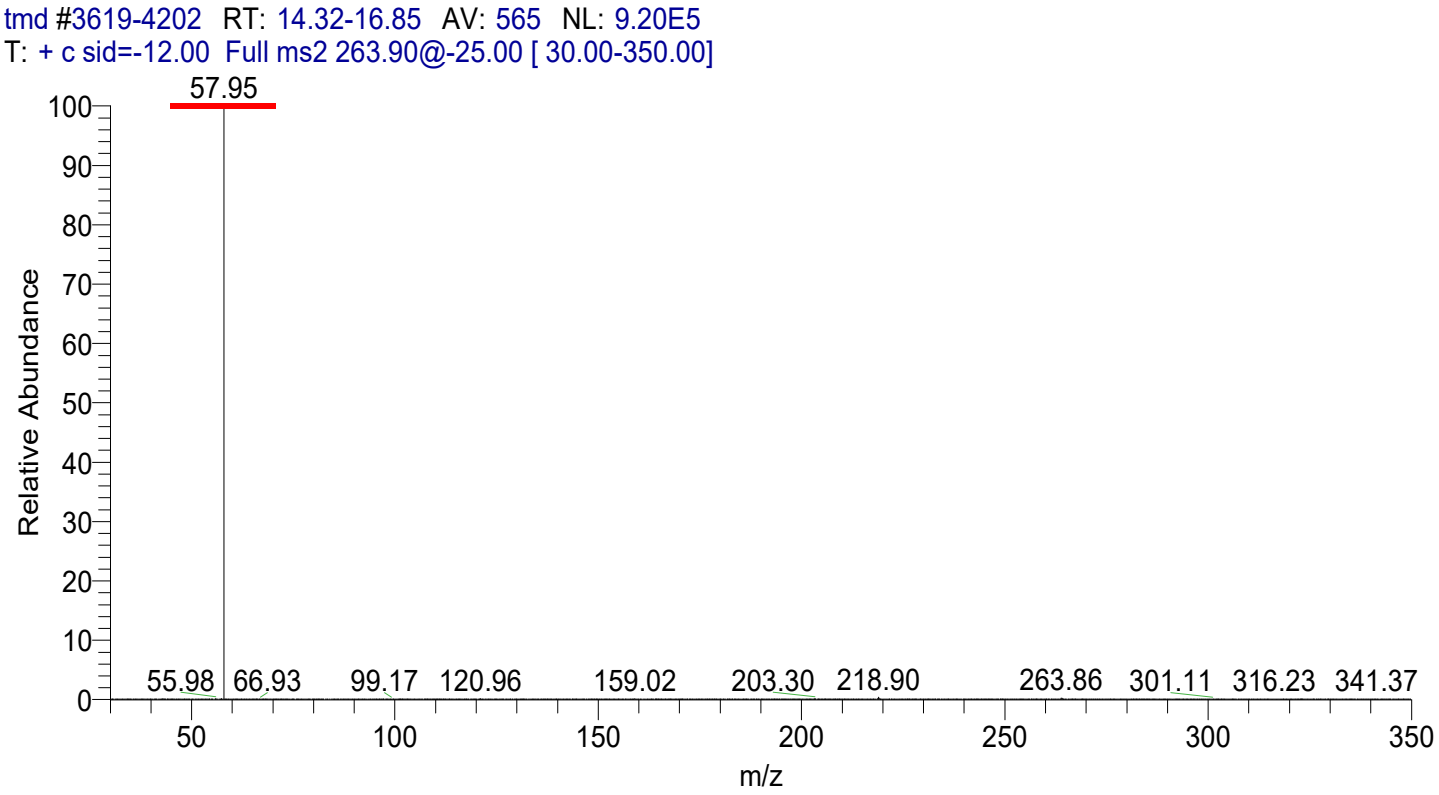


盐酸曲马多（Tramadol hydrochloric）

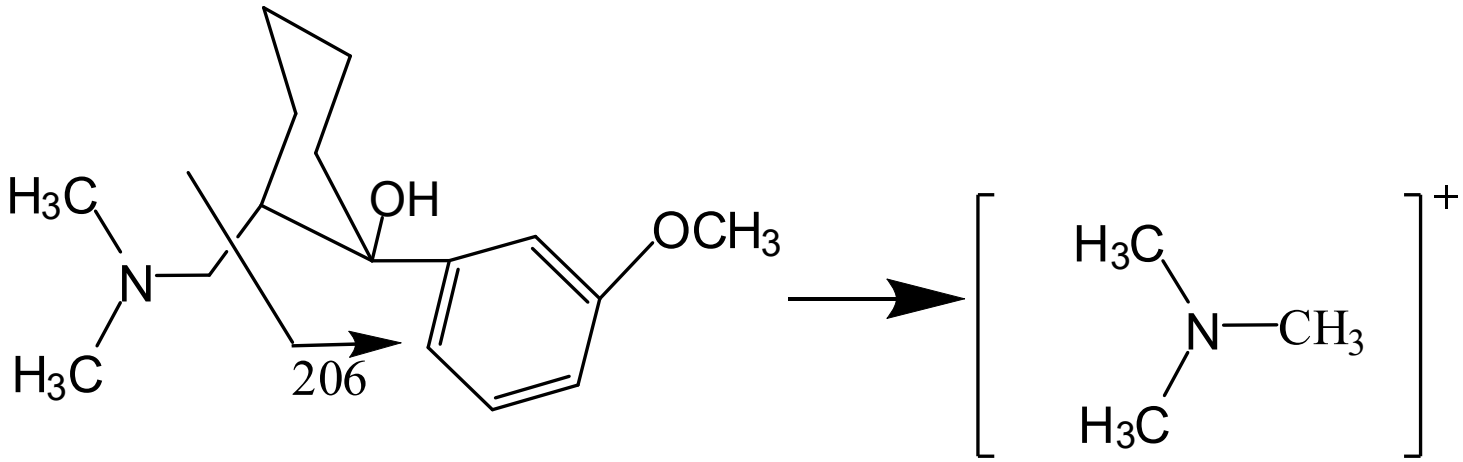
MW：299.8 分子式：$C_{16}H_{25}NO_2$



曲马多LC-MS质谱扫描图（m/z=264）



曲马多LC-MS/MS质谱扫描图（m/z=58）

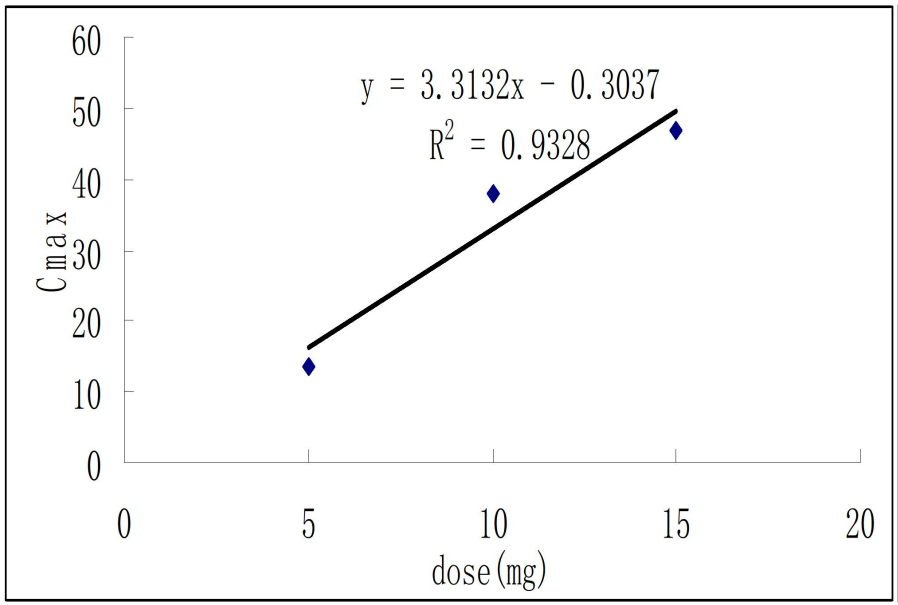


m/z 264　　m/z 58

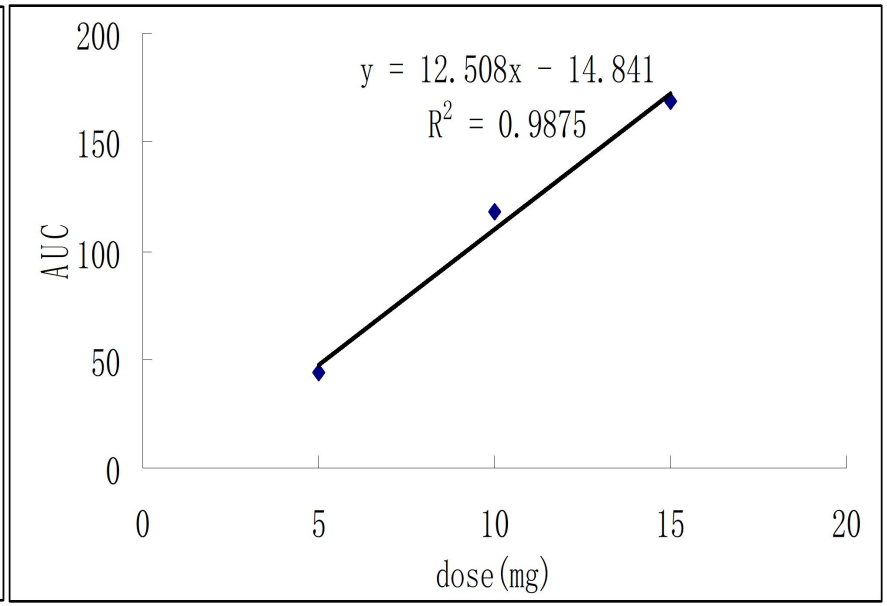
曲马多质谱裂解图 m/z 264 →m/z 58

单次给药

- 受试者单次口服苯甲酸利扎曲普坦片后，苯甲酸利扎曲普坦的C_{max}和AUC与服药剂量成正比关系。

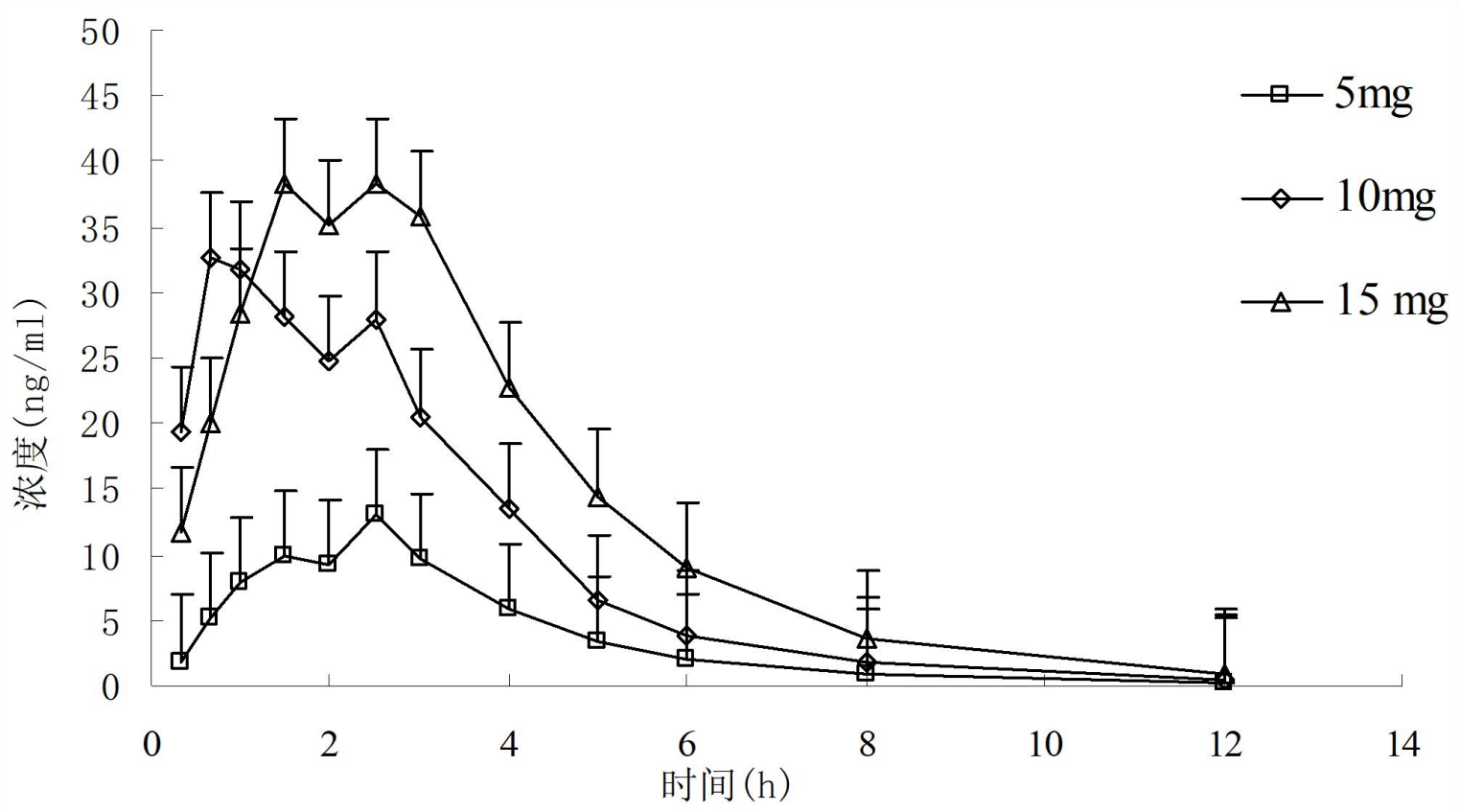


C_{max}—Dose

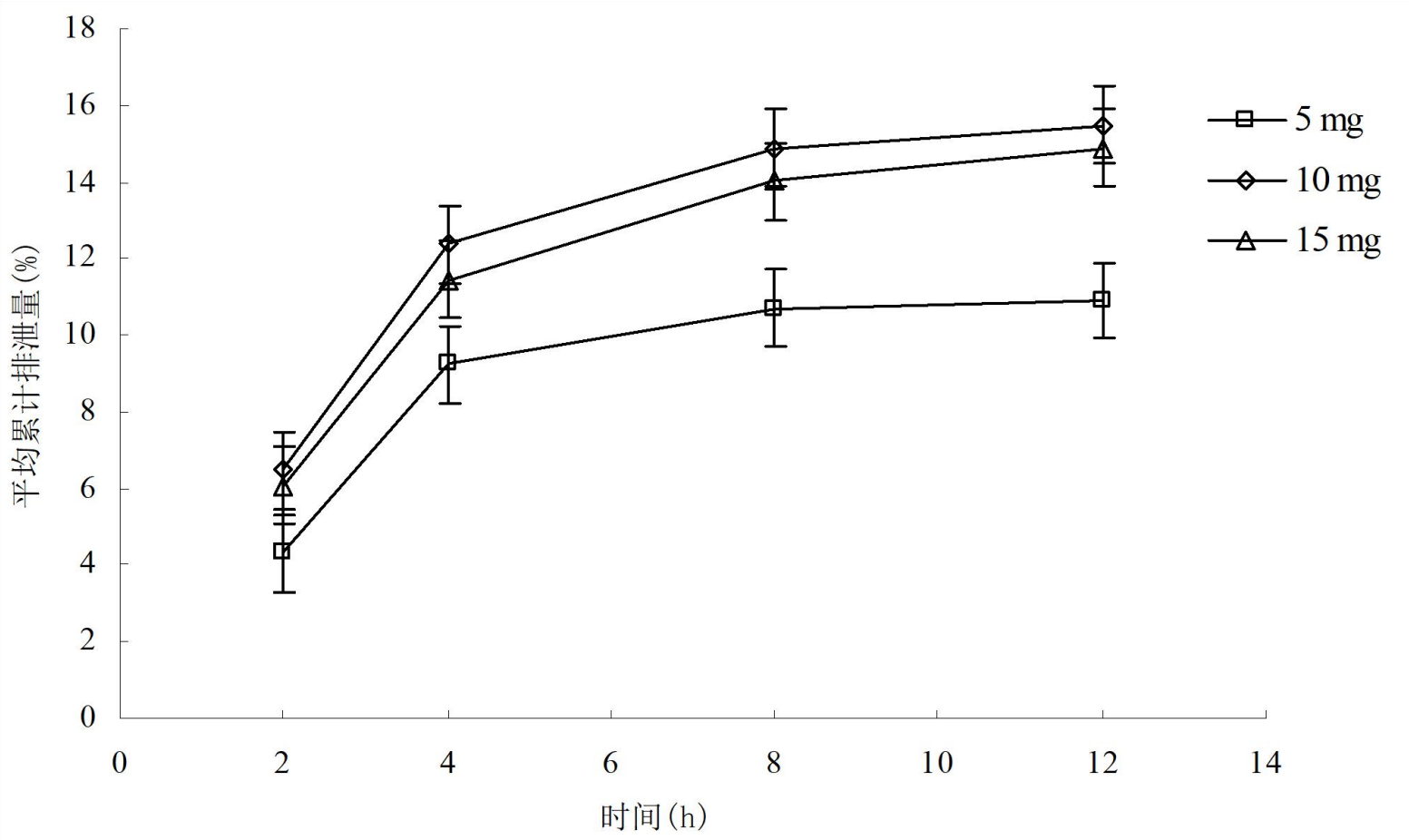


AUC—Dose

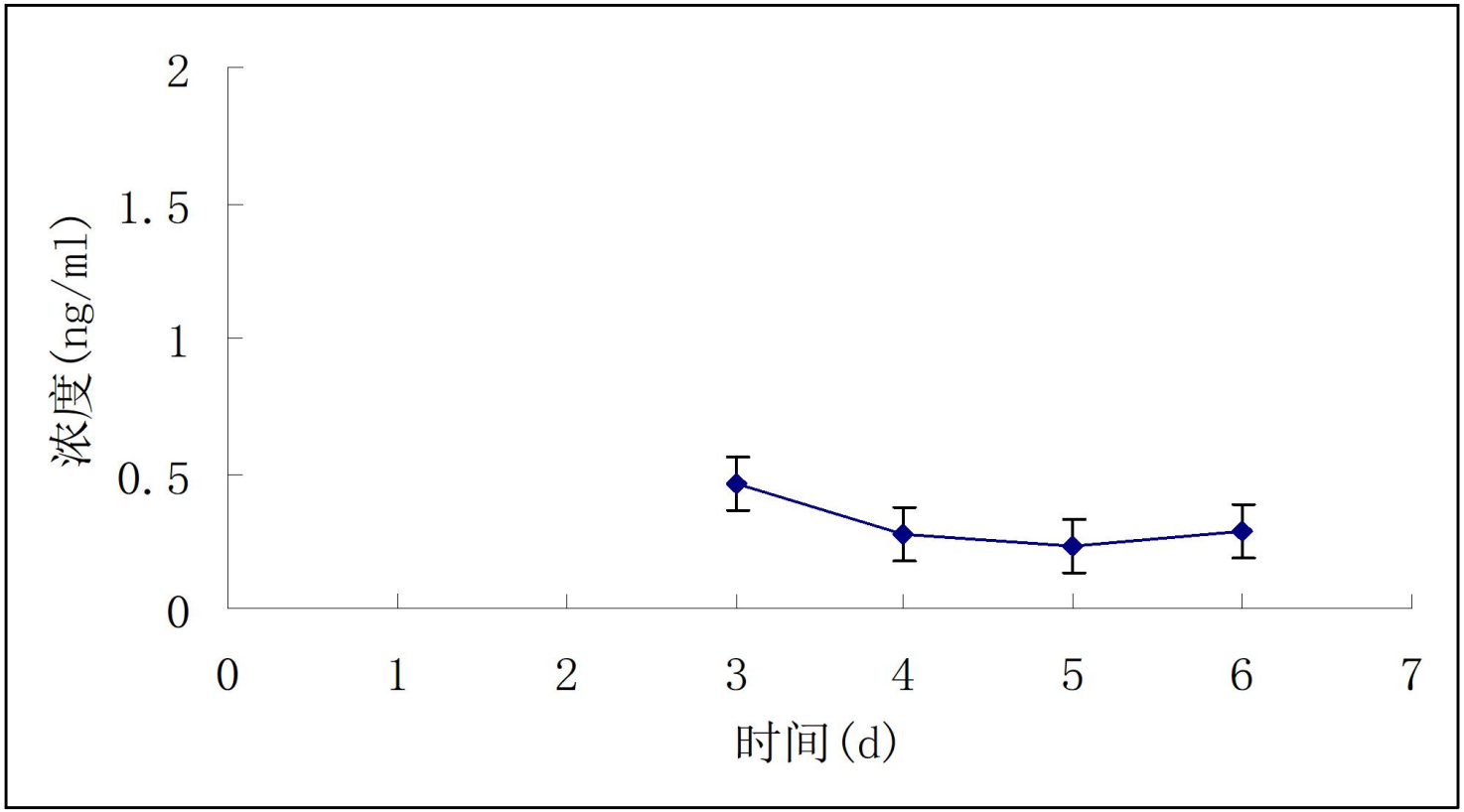
数据分析



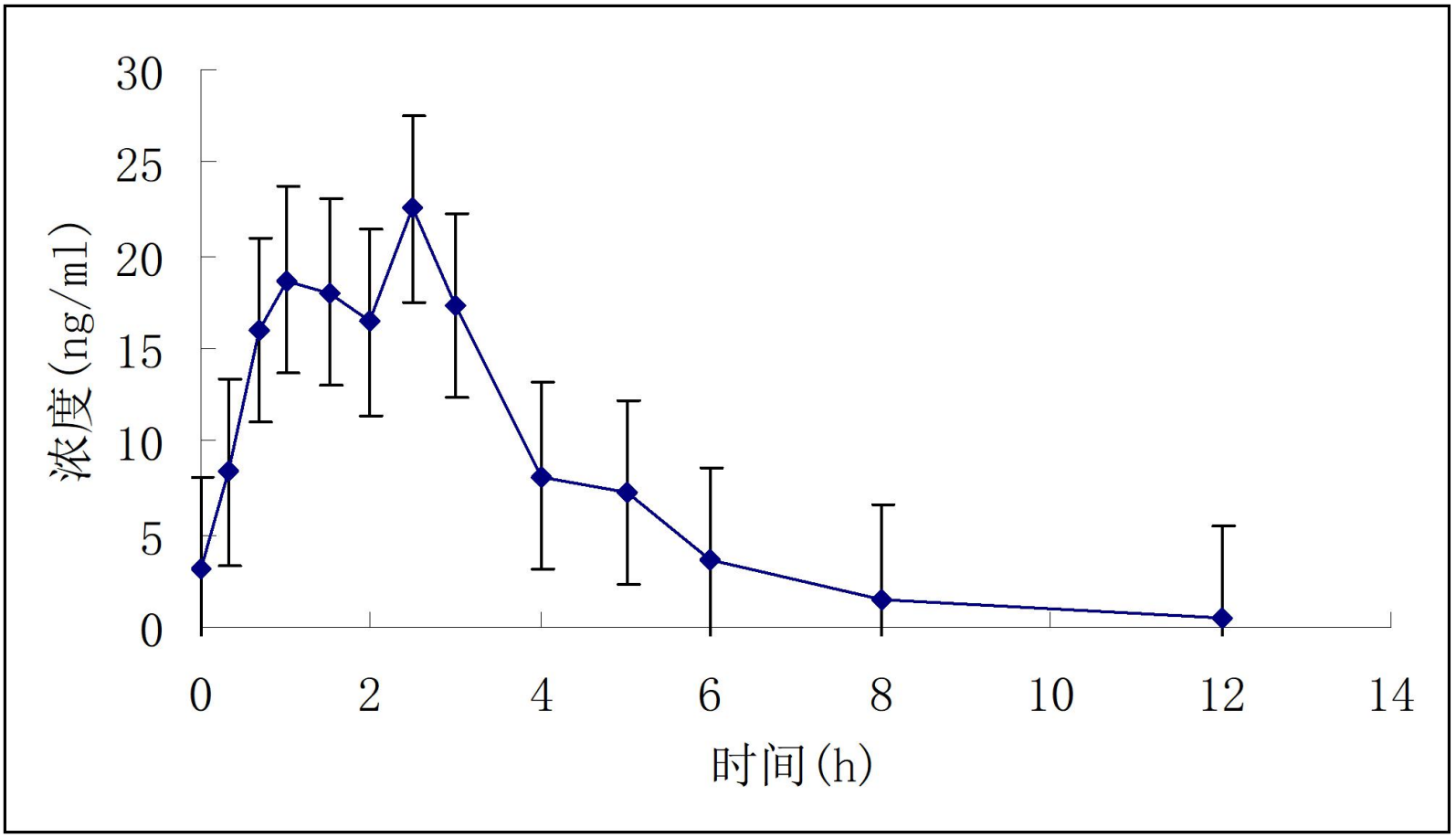
受试者单剂量口服低中高剂量后的平均血药浓度-时间曲线



受试者单剂量口服低中高剂量后的
平均累积排泄量-时间曲线

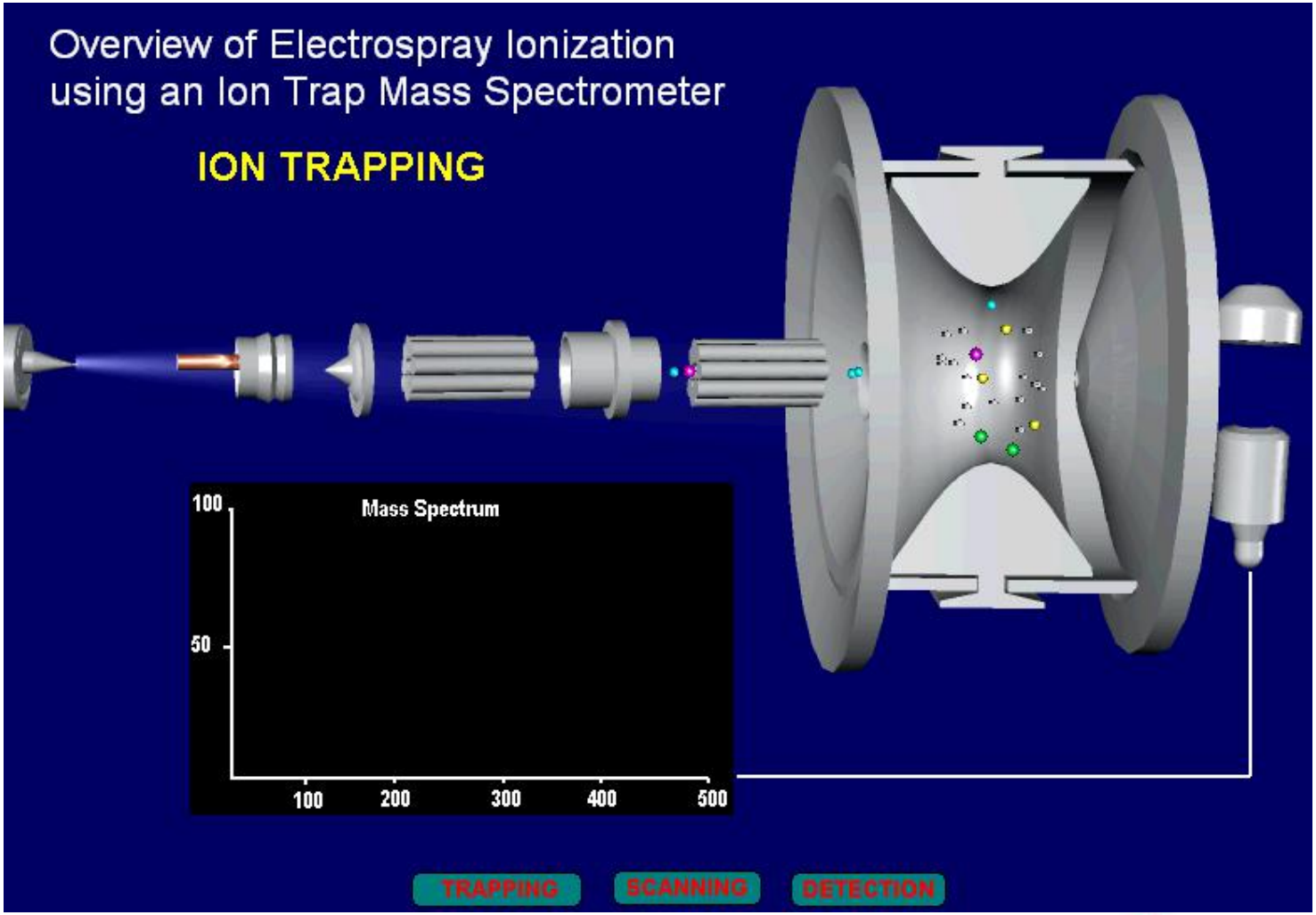


受试者多次服药第3～6天时平均谷时血药浓度-时间曲线



受试者多次口服达稳态后的平均药时曲线

其他质谱技术与特点



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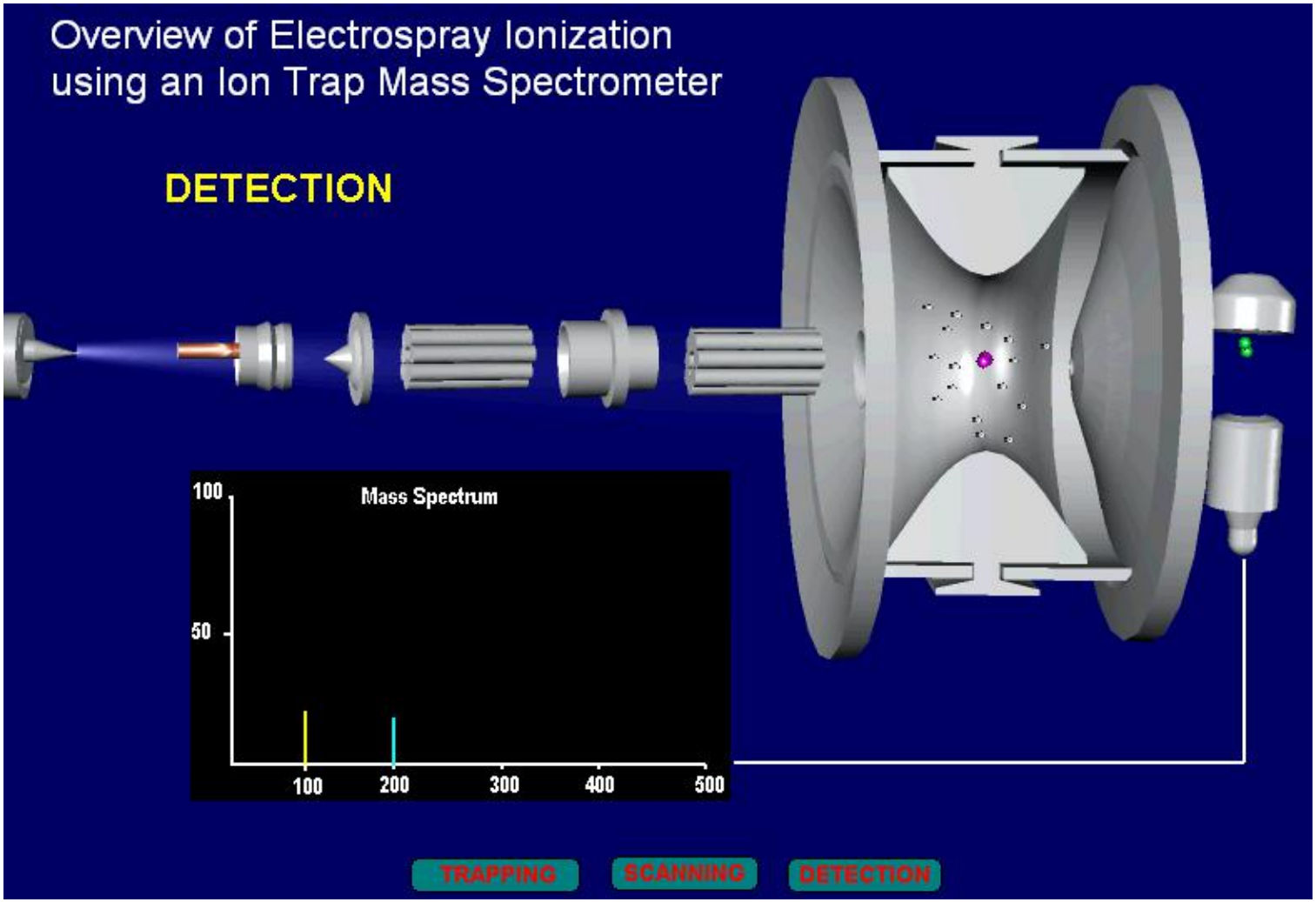

Thank you very much!

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