全程自动化TDM色谱技术研究与应用

Total Solution automation chromatograph technology of TDM

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中南大学湘雅二医院临床药学部

全程自动化色谱技术

一. 研究背景与意义

二. 全程自动化色谱技术概念与技术特点

三. 实现路径与关键问题

四. 应用现状与扩展计划

11:00:20

研究背景 等级医院拥有大量HPLC










11:00:22

HPLC进行TDM自动化意义

我国三级甲等医院拥有HPLC系统超过1000以上台（套），并且在总数持续增长中，总价值高达5.0亿元。

缺乏高饱和运行能力；

不能按照CLIAI88、15189等实现质量管理

HPLC进行TDM自动化意义



三、感受

经济效益是TDM健康、持续发展的保证，所以必须保证TDM能够获得必要的经济利润，但是如果只强调经济效益，就可能导致人为增加不必要的TDM，从而让患者的权益受到损害。特定药剂治疗管理料体系，可以寻找一个良好的平衡点，首先通过付费体制确保了TDM的经济效益，这实质上也避免了医院因为自己赚不到钱甚至亏本而导致病人失去进行合理TDM的情况，但是通过保险付费的方式，又限制了不合理的TDM，从而实现了，提高医疗质量的社会效益与减少医疗开支，获得服务报酬的经济效益的双赢！

11:00:22

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11:00:22

色谱自动化基本原理（LCxLC）

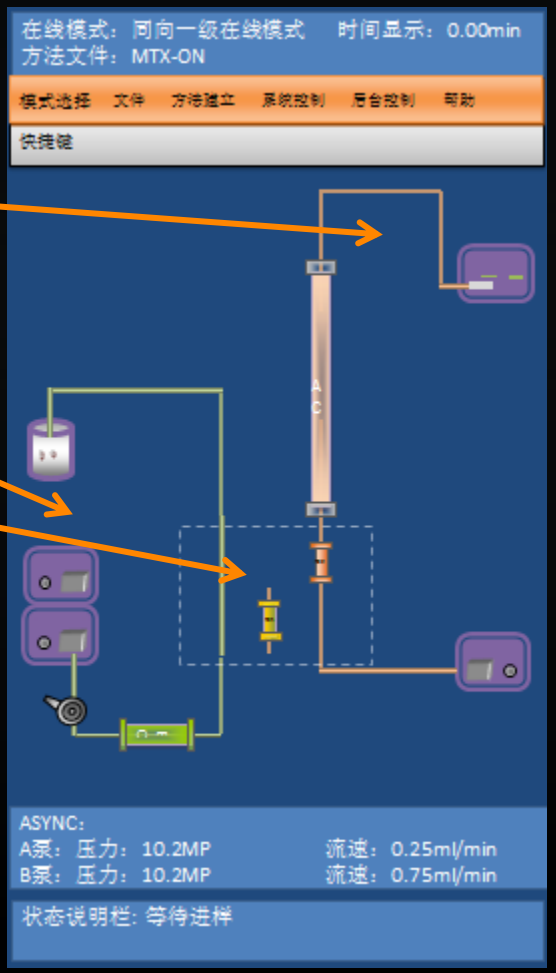
系统组成：

LC1（分析系统）

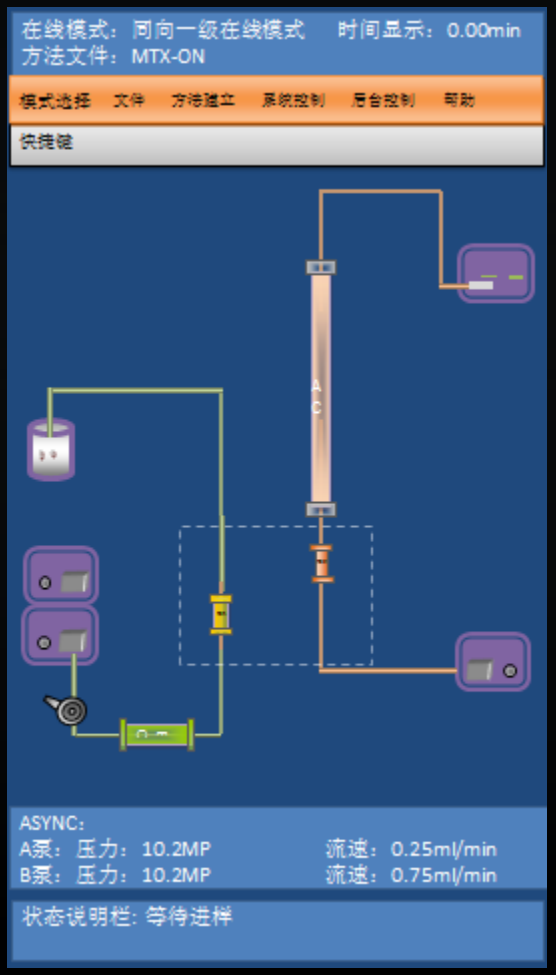
LC2（全自动处理系统）

INTERFACE系统

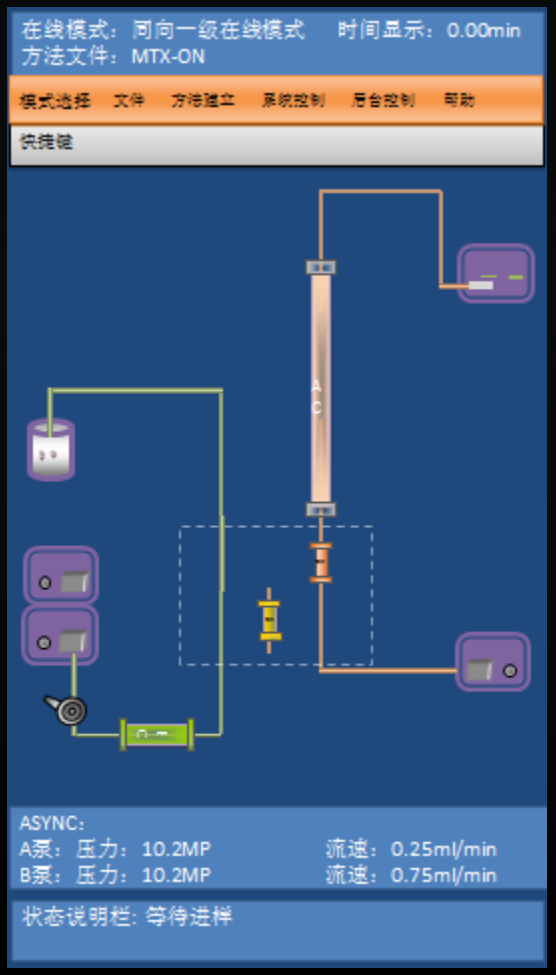
控制系统



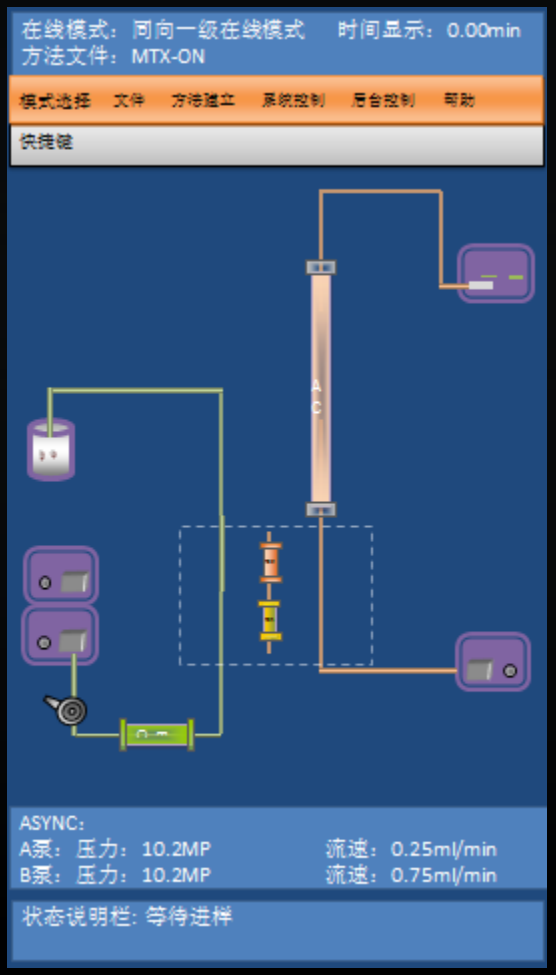
待机状态


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

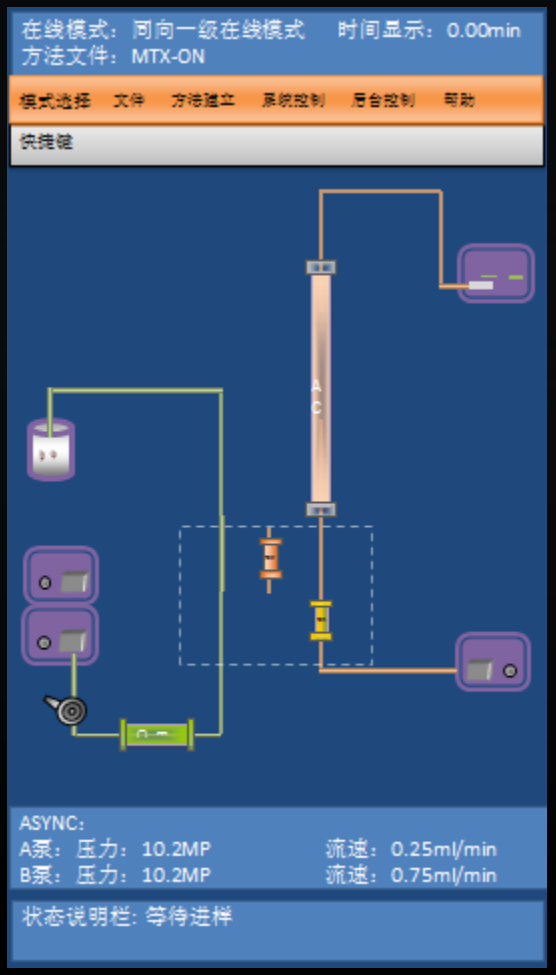
第一次F1装载样品


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

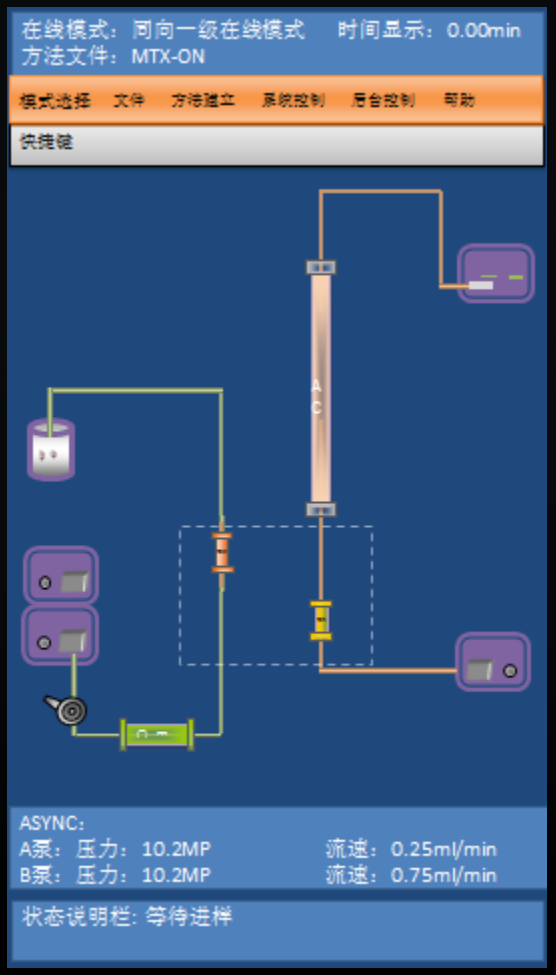
第一次F2载样完成


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

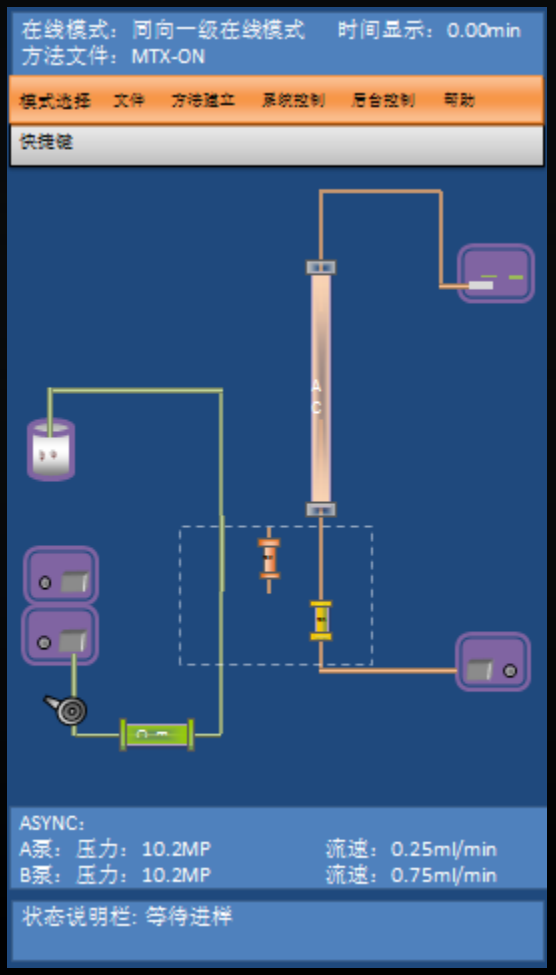
等待进样


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

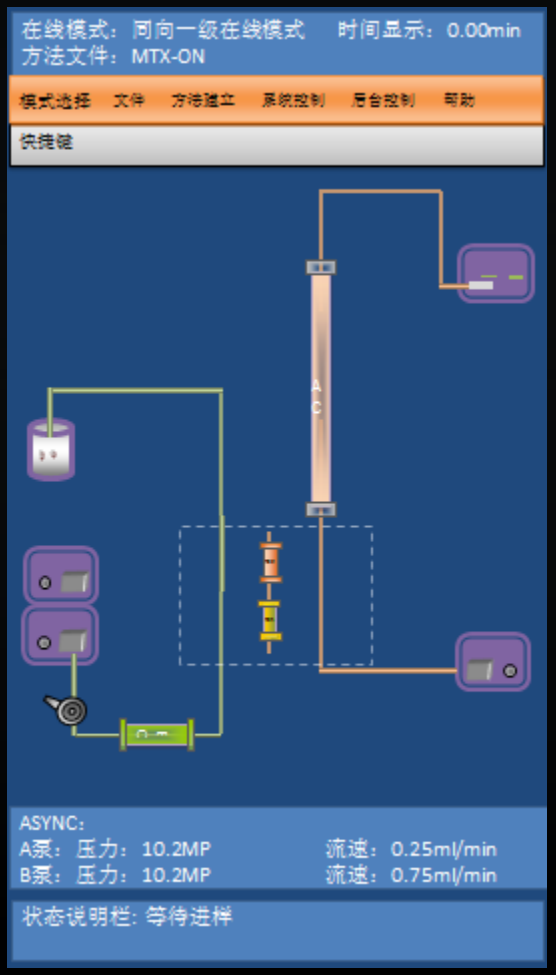
进样开始



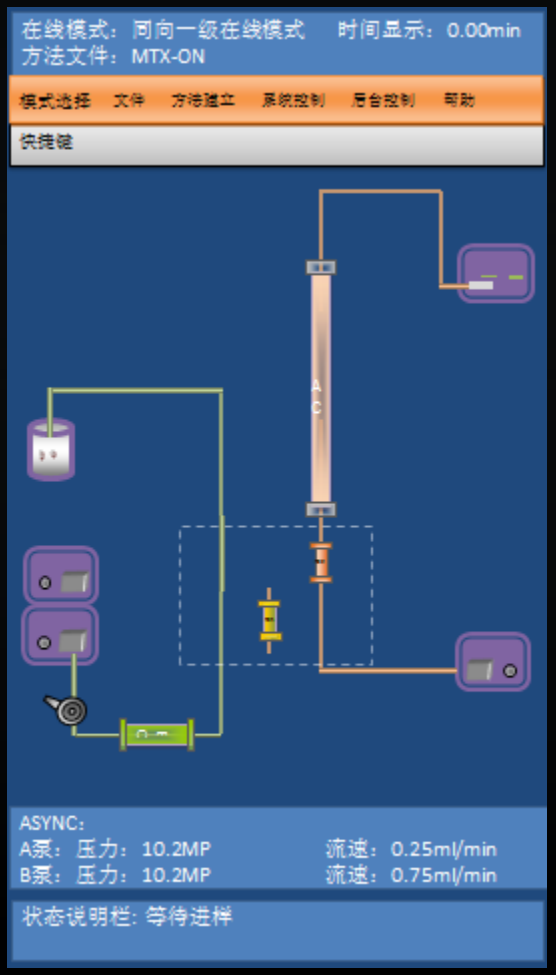
第二次F2载样同步进行


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

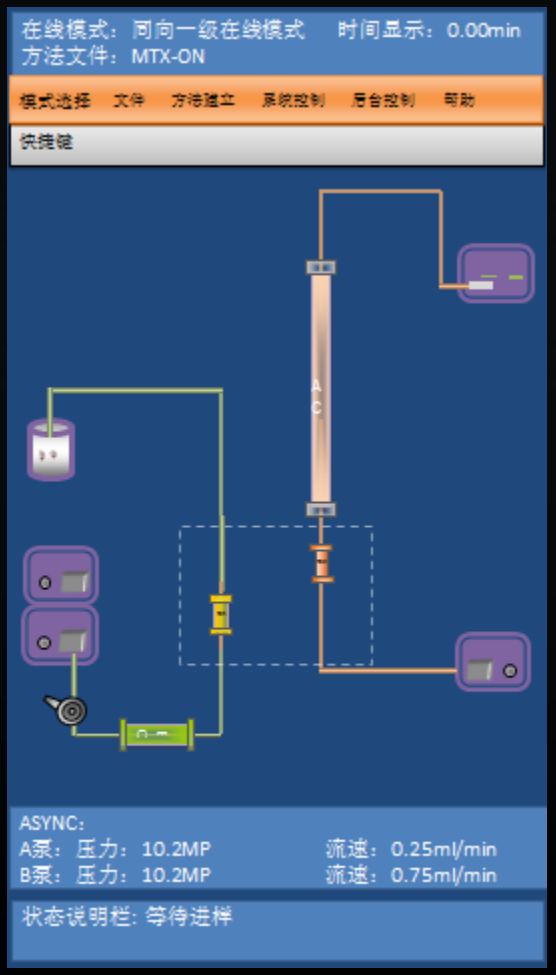
F2载样完成
等待进样



F1进样完成
等待第二次进样


在线模式：同向一级在线模式　时间显示：0.00min
方法文件：MTX-ON
模式选择　文件　方法建立　系统控制　后台控制　帮助
快捷键
ASYNC:
A泵：压力：10.2MP　流速：0.25ml/min
B泵：压力：10.2MP　流速：0.75ml/min
状态说明栏：等待进样

第二次F2进样开始

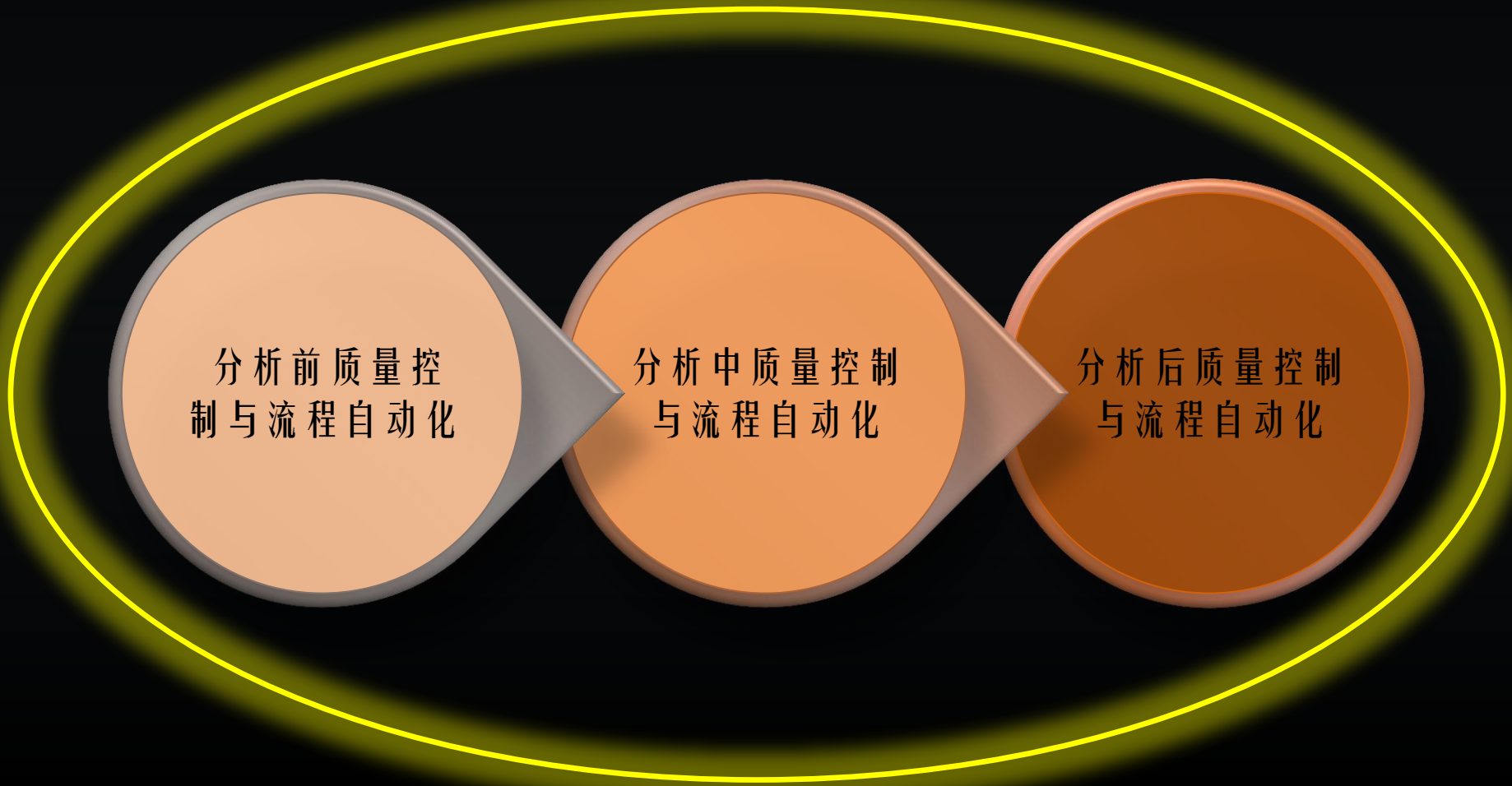


第二次F2进样开始

第三次F1载样开始

流程自动化=全程自动化?

全程自动化——流程与质量的结合



中南大学湘雅二医院药学TDM

- 两种自动分析方法用于TDM日常工作

1、LLE（液液萃取）

样品准备（Vial）

调节PH

加入内标

加入破乳试剂

加入萃取溶剂

振荡提取

离心/静止分层

分取提取液

挥干溶液

溶解残留溶液

通过0.22过滤膜

分析结果(File)

2、SPE（固相萃取）

样品准备(Vial)

调节PH

加入内标

活化固相萃取柱

饱和固相萃取柱

上样

清洗杂质

洗脱目标物

挥干洗脱溶液

溶解残留溶液

通过0.22过滤膜

分析结果(File)

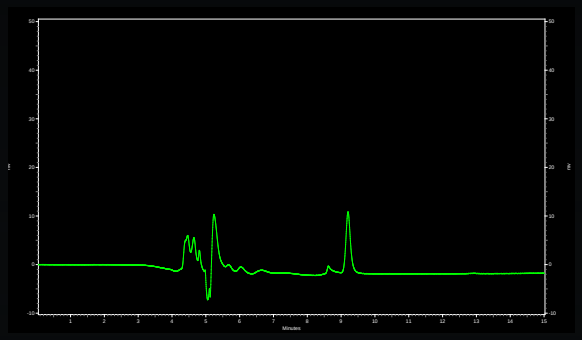
3自动化TDM系统

样品准备(Vial)

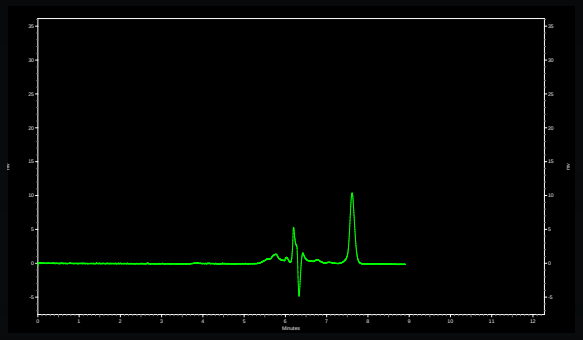
DBS或去蛋白

分析结果(File)

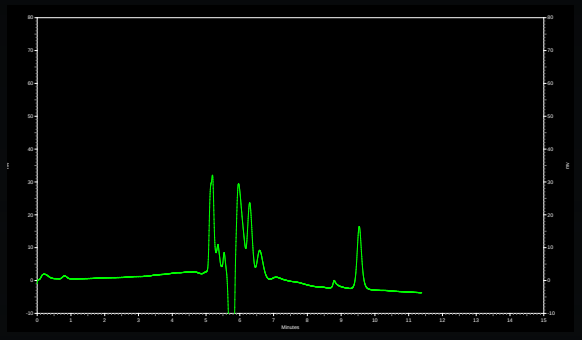
色谱图一览



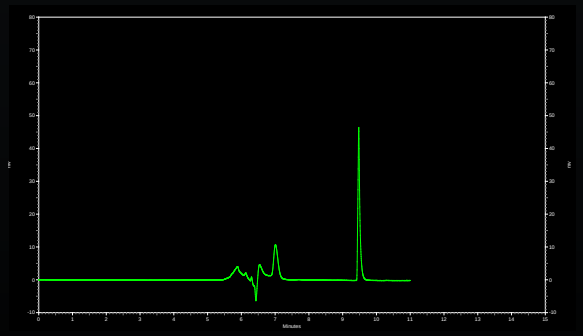
帕利哌酮（TDM）



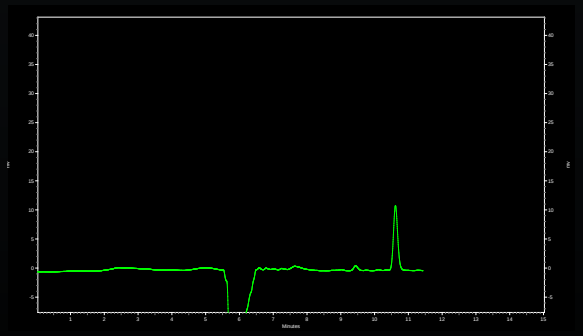
奥氮平（TDM）



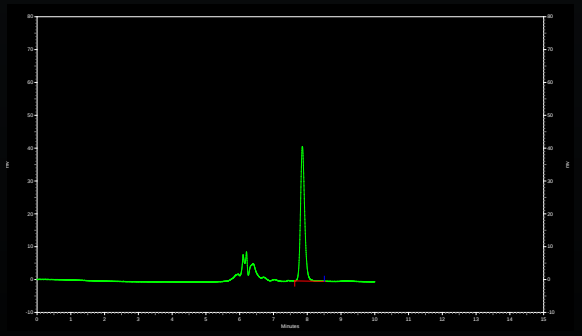
利培酮（TDM）



氯氮平（TDM）

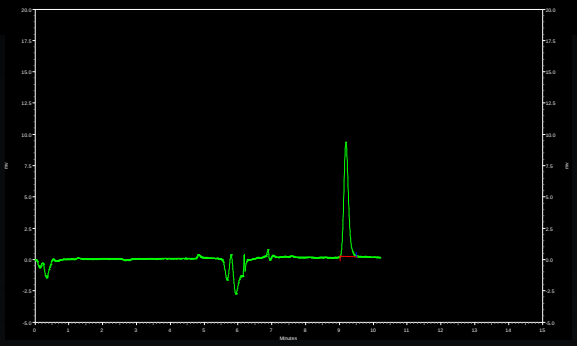


氯硝安定

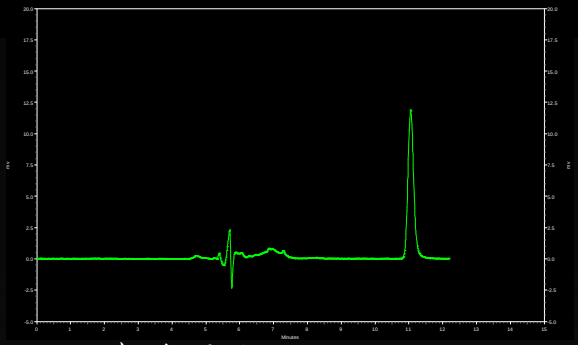


喹硫平（重点专科研究）

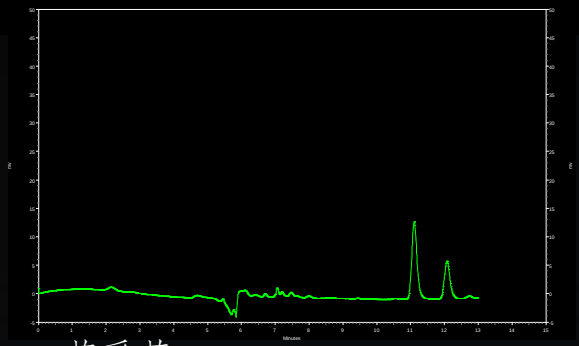
11:00:32



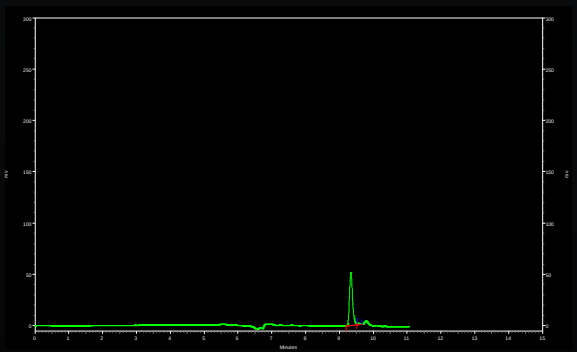
MTX



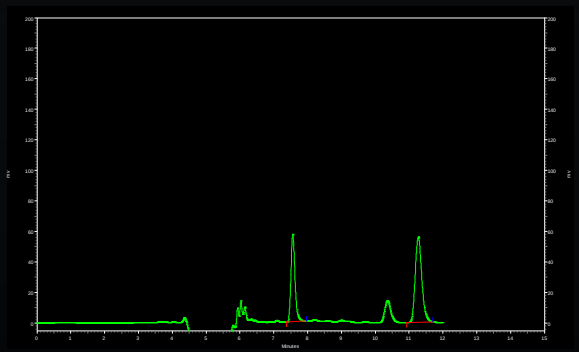
卡马西平



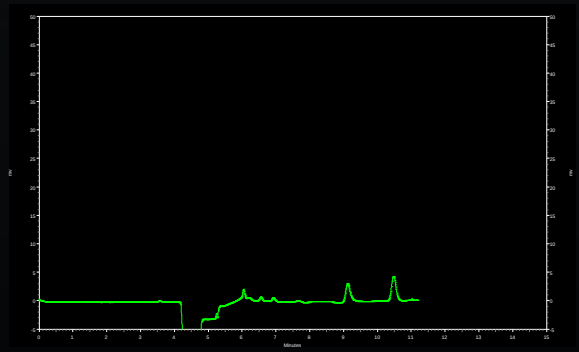
苯妥英



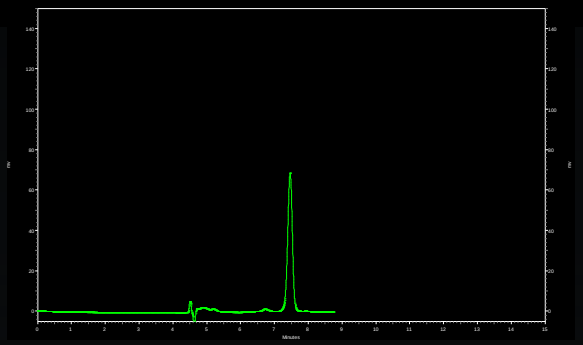
苯巴比妥



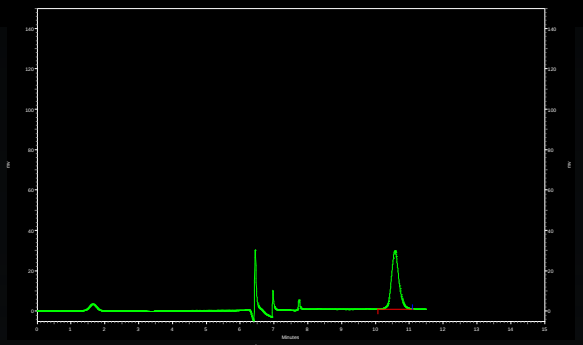
卡马西平、苯妥英、苯巴比妥 同时分析



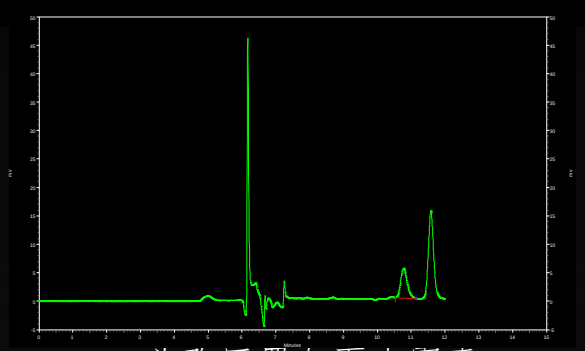
茶碱



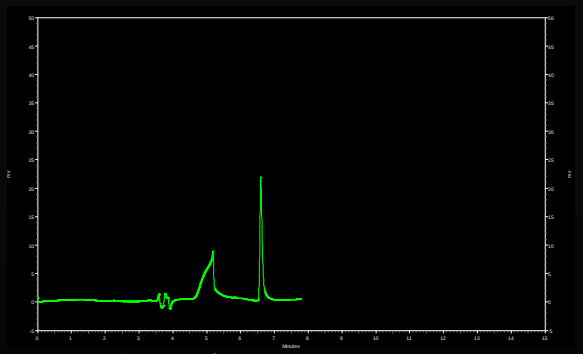
霉酚酸



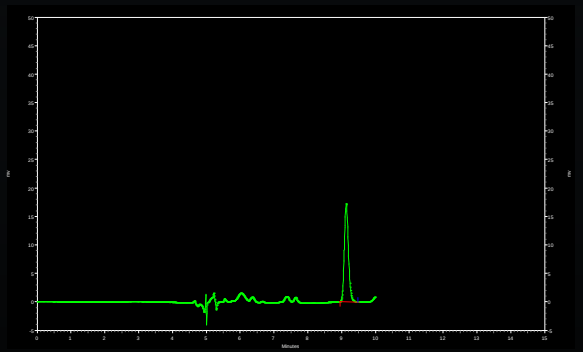
万古霉素



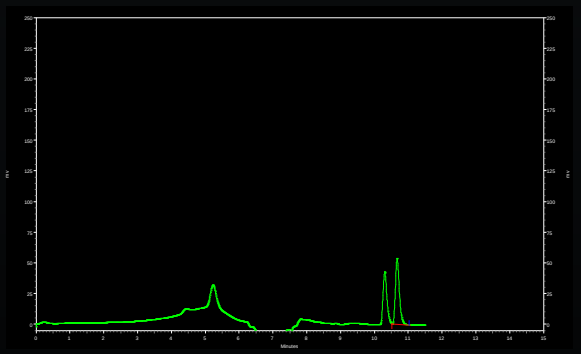
头孢匹罗与万古霉素



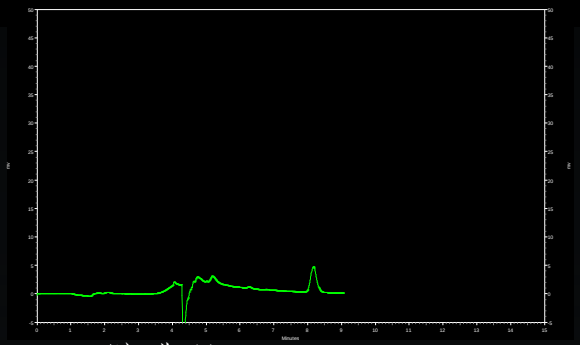
异烟肼



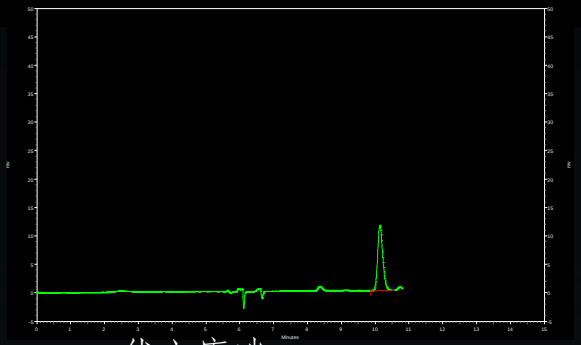
水杨酸



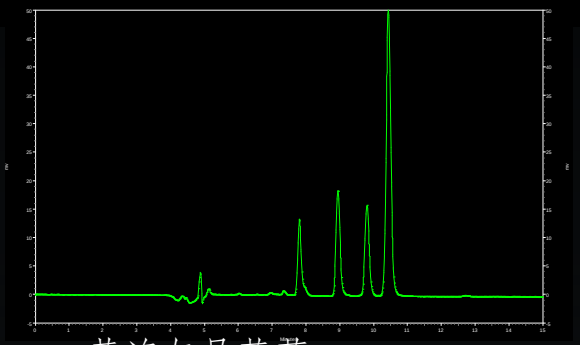
5-FU



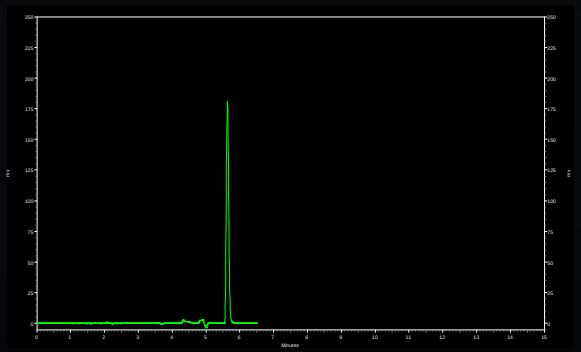
胺碘酮



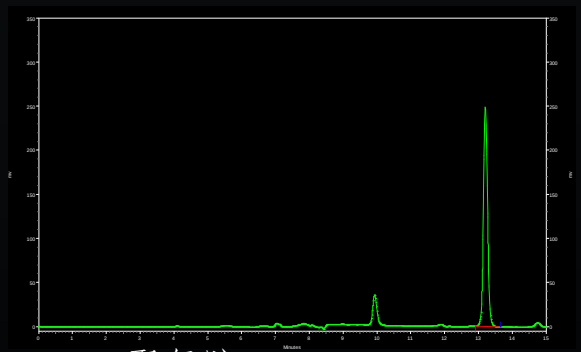
伏立康唑



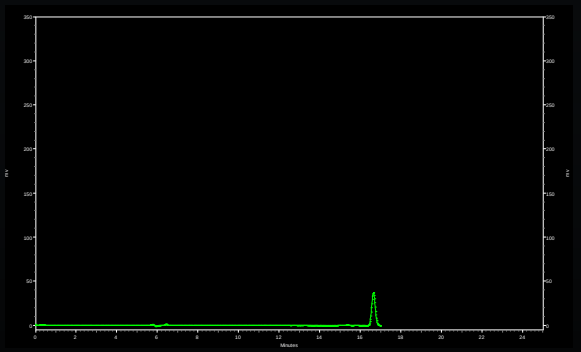
黄连与吴茱萸



美罗培南

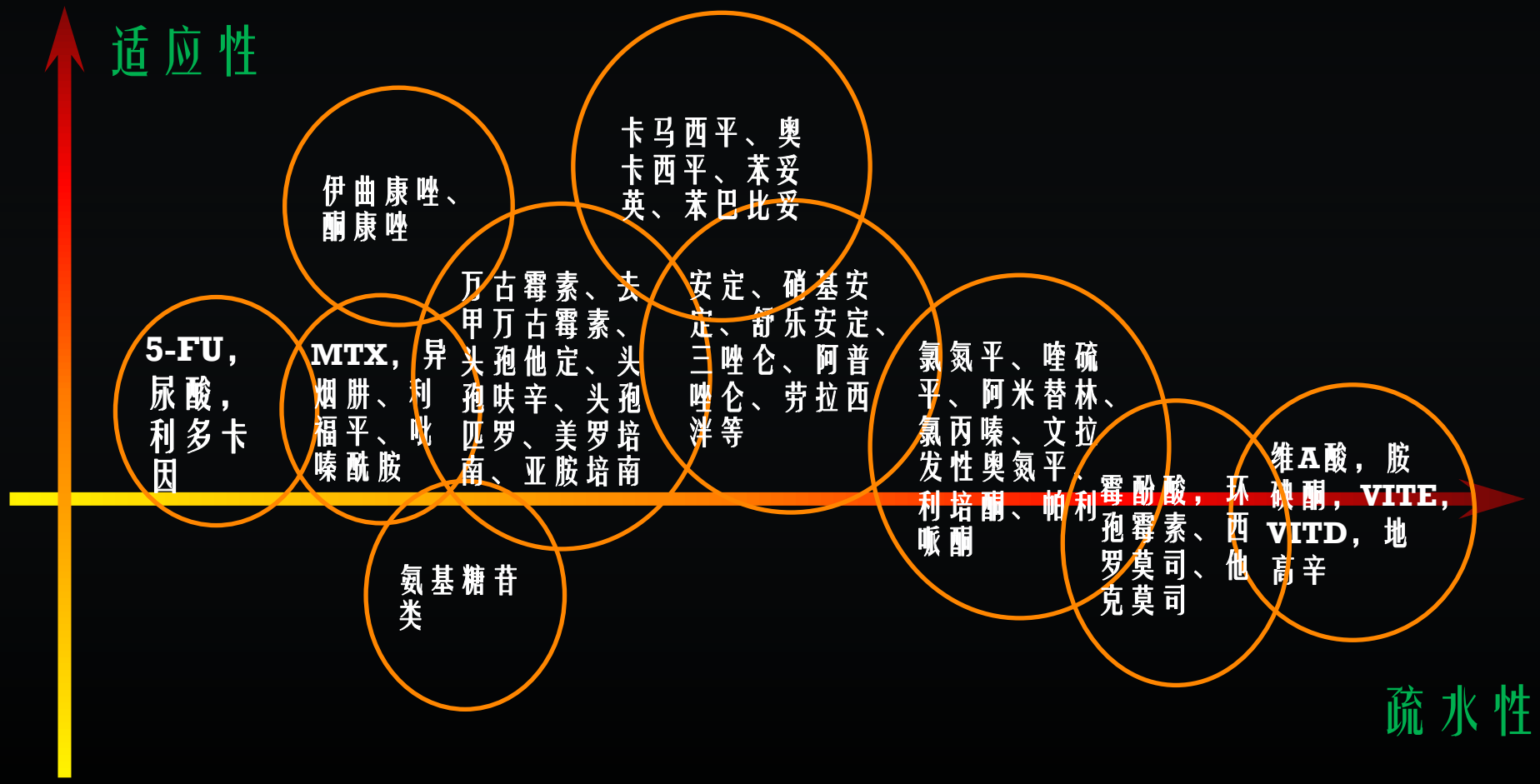


三聚氰胺



VIT A

全程自动化系统可分析范围谱



- 去内标运行
- 二维自动多级去干扰
- 异态扩散控制

……



- 结果极准确、质控偏移极小
- 几乎无样品处理过程
- 色谱峰稳定、纯净无干扰

……

- 实验室仅需要20m²
- 附属设备和步骤急剧减少
- 仪器效率急剧提高，一机多用，培训时间缩短
- 突破TDM技术与盈利瓶颈
- ……

全程自动化色谱技术

一. 研究背景与指导方针

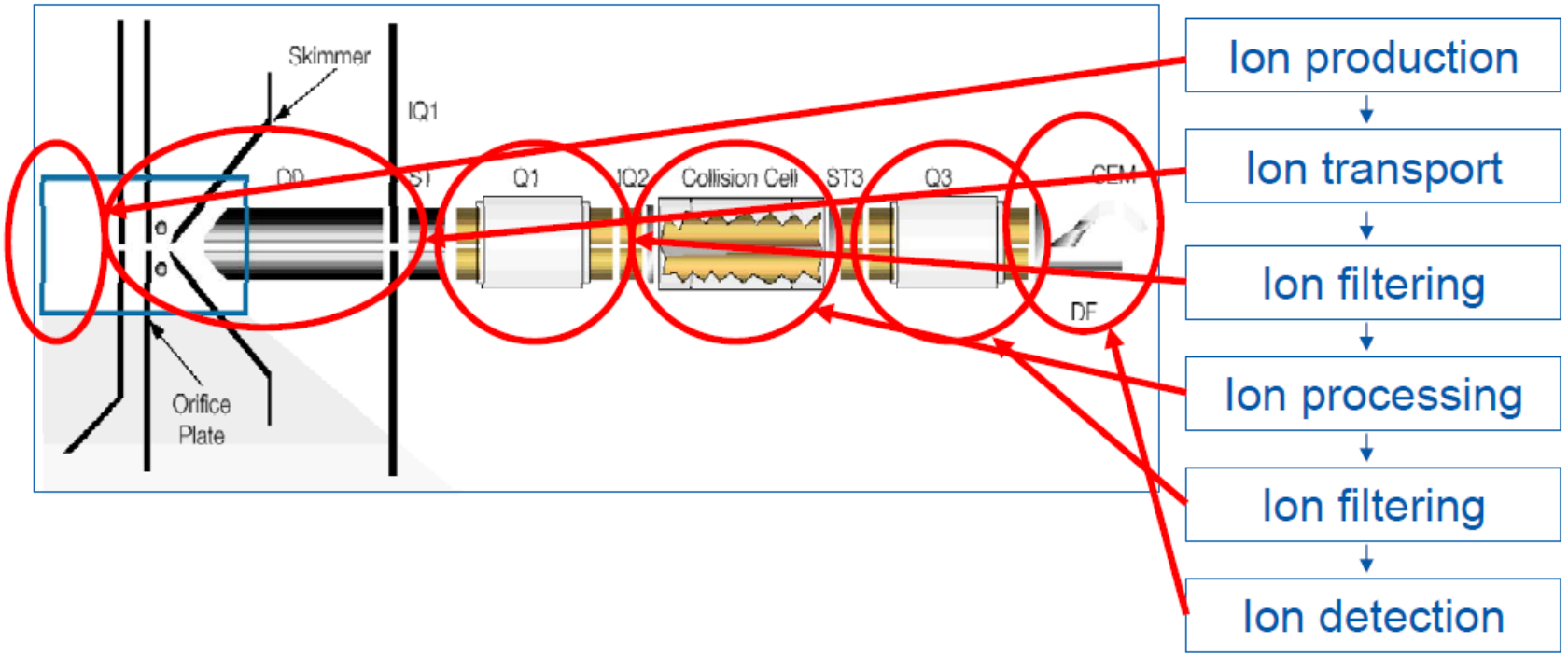
二. 全程自动化色谱技术概念与技术特点

三. 实现路径与关键问题

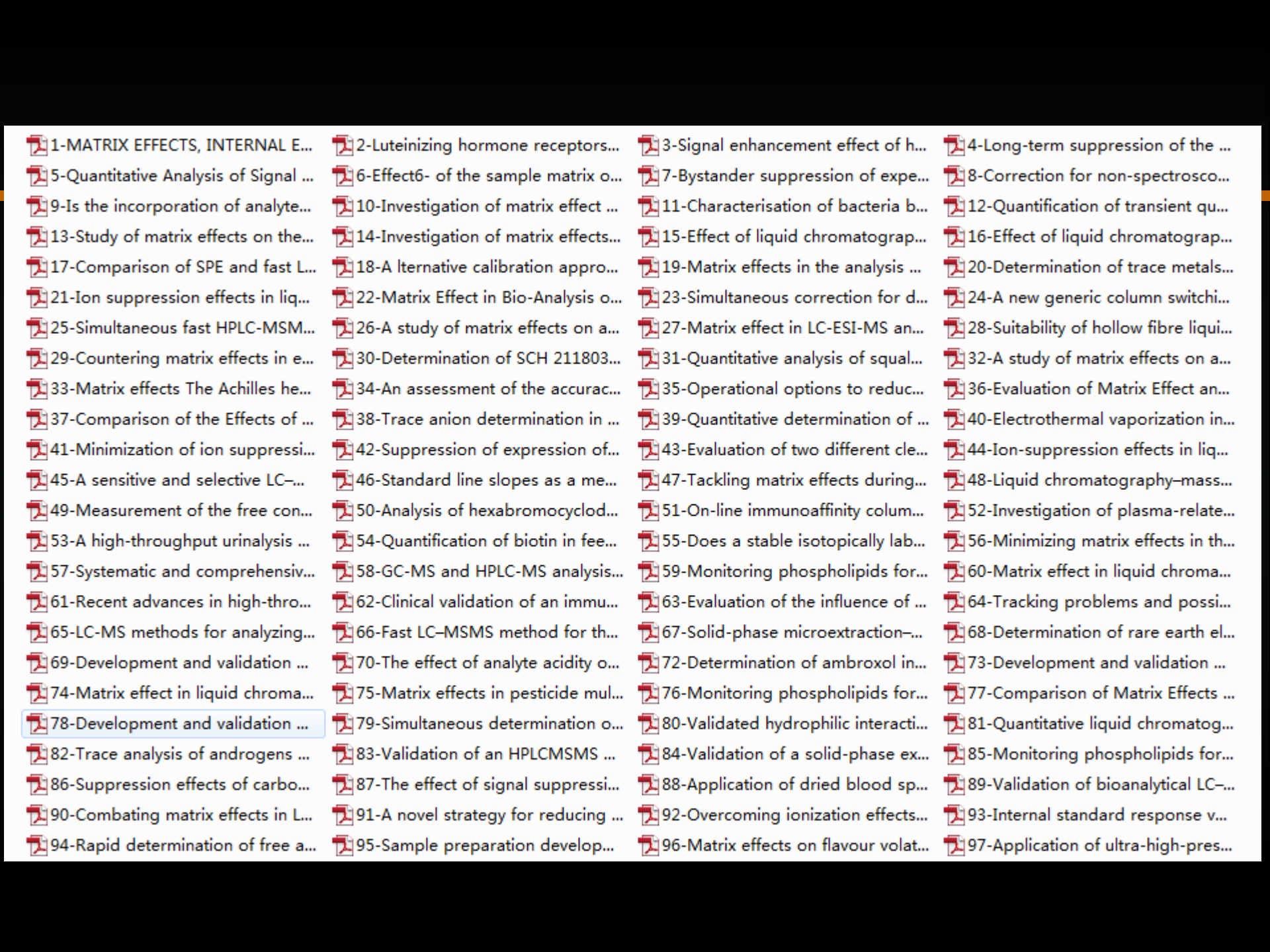
四. 应用现状与扩展计划

11:00:32

LC-MS否用于全程自动化TDM分析?



临床检验设备从不采用此原理


1-MATRIX EFFECTS, INTERNAL E...
2-Luteinizing hormone receptors...
3-Signal enhancement effect of h...
4-Long-term suppression of the ...
5-Quantitative Analysis of Signal ...
6-Effect6- of the sample matrix o...
7-Bystander suppression of expe...
8-Correction for non-spectrosco...
9-Is the incorporation of analyte...
10-Investigation of matrix effect ...
11-Characterisation of bacteria b...
12-Quantification of transient qu...
13-Study of matrix effects on the...
14-Investigation of matrix effects...
15-Effect of liquid chromatograp...
16-Effect of liquid chromatograp...
17-Comparison of SPE and fast L...
18-A lternative calibration appro...
19-Matrix effects in the analysis ...
20-Determination of trace metals...
21-Ion suppression effects in liq...
22-Matrix Effect in Bio-Analysis o...
23-Simultaneous correction for d...
24-A new generic column switchi...
25-Simultaneous fast HPLC-MSM...
26-A study of matrix effects on a...
27-Matrix effect in LC-ESI-MS an...
28-Suitability of hollow fibre liqui...
29-Countering matrix effects in e...
30-Determination of SCH 211803...
31-Quantitative analysis of squal...
32-A study of matrix effects on a...
33-Matrix effects The Achilles he...
34-An assessment of the accurac...
35-Operational options to reduc...
36-Evaluation of Matrix Effect an...
37-Comparison of the Effects of ...
38-Trace anion determination in ...
39-Quantitative determination of ...
40-Electrothermal vaporization in...
41-Minimization of ion suppressi...
42-Suppression of expression of...
43-Evaluation of two different cle...
44-Ion-suppression effects in liq...
45-A sensitive and selective LC–...
46-Standard line slopes as a me...
47-Tackling matrix effects during...
48-Liquid chromatography–mass...
49-Measurement of the free con...
50-Analysis of hexabromocyclod...
51-On-line immunoaffinity colum...
52-Investigation of plasma-relate...
53-A high-throughput urinalysis ...
54-Quantification of biotin in fee...
55-Does a stable isotopically lab...
56-Minimizing matrix effects in th...
57-Systematic and comprehensiv...
58-GC-MS and HPLC-MS analysis...
59-Monitoring phospholipids for...
60-Matrix effect in liquid chroma...
61-Recent advances in high-thro...
62-Clinical validation of an immu...
63-Evaluation of the influence of ...
64-Tracking problems and possi...
65-LC-MS methods for analyzing...
66-Fast LC–MSMS method for th...
67-Solid-phase microextraction–...
68-Determination of rare earth el...
69-Development and validation ...
70-The effect of analyte acidity o...
72-Determination of ambroxol in...
73-Development and validation ...
74-Matrix effect in liquid chroma...
75-Matrix effects in pesticide mul...
76-Monitoring phospholipids for...
77-Comparison of Matrix Effects ...
78-Development and validation ...
79-Simultaneous determination o...
80-Validated hydrophilic interacti...
81-Quantitative liquid chromatog...
82-Trace analysis of androgens ...
83-Validation of an HPLCMSMS ...
84-Validation of a solid-phase ex...
85-Monitoring phospholipids for...
86-Suppression effects of carbo...
87-The effect of signal suppressi...
88-Application of dried blood sp...
89-Validation of bioanalytical LC–...
90-Combating matrix effects in L...
91-A novel strategy for reducing ...
92-Overcoming ionization effects...
93-Internal standard response v...
94-Rapid determination of free a...
95-Sample preparation develop...
96-Matrix effects on flavour volat...
97-Application of ultra-high-pres...

78-Development and validation ...
79-Simultaneous determination o...
80-Validated hydrophilic interacti...
81-Quantitative liquid chromatog...
82-Trace analysis of androgens ...
83-Validation of an HPLCMSMS ...
84-Validation of a solid-phase ex...
85-Monitoring phospholipids for...
86-Suppression effects of carbo...
87-The effect of signal suppressi...
88-Application of dried blood sp...
89-Validation of bioanalytical LC–...
90-Combating matrix effects in L...
91-A novel strategy for reducing ...
92-Overcoming ionization effects...
93-Internal standard response v...
94-Rapid determination of free a...
95-Sample preparation develop...
96-Matrix effects on flavour volat...
97-Application of ultra-high-pres...
98-Sensitive method for the dete...
99-A modified method for the d...
100-Determination of opiates an...
101-Quantitative UPLC-MSMS an...
102-Study of dried blood spots t...
103-Determination of low ppm le...
104-Measurement of mycophen...
105-Investigation of the effects o...
106-Matrix EffectsdA Challenge T...
107-Strategies for the eliminatio...
108-Study on the matrix effect in ...
109-Characterization and classifi...
110-Investigation of endogenous...
111-Determination of benzene in...
112-Evaluation of Alternate Isoto...
113-Validated hydrophilic interac...
114-Fast chiral chromatographic ...
115-Simultaneous HPLC–MS–MS...
116-Simple, sensitive and rapid ...
117-Analytical issues in HPLCMS...
118-Signal suppressionenhance...
119-An improved on-line solid p...
120-Analysis of llicit and illicit dru...
121-Simultaneous determination ...
122-Development and validation ...
123-Potential bias and mitigation...
124-Signal suppressionenhance...
125-LC–MSMS coupled with imm...
126-Development and validation ...
127-Simultaneous UPLC–MSMS q...
128-Determination of nucleoside...
129-Assessment of sample clean...
130-Overcoming matrix effects u...
131-Determination of resveratrol...
132-Low temperature followed b...
133-The use of human plasma as...
134-Development of an LC–MSM...
135-Comparative study of the LC...
136-Matrix matching in liquid chr...
137-Determination of carboplati...
138-Planar solid phase extractio...
139-An accurate quantitative LCE...
140-Development of a fast instru...
141-LC-MSMS quantitation of pl...
142-Liquid chromatography cou...
143-Development and validation ...
144-Determination of benzodiaz...
145-Development and validation ...
146-Theory-guided efficient strat...
147-Optimisation of pressurised ...
148-13C labelled internal standar...
149-Identification and eliminatio...
150-Profiling signatures of ovari...
151-A high-throughput U-HPLC–...
152-Monitoring of organophosp...
153-Comprehensive investigation...
154-UPLC–MSMS quantification ...

11:00:32

全程自动化理论基础：数模建造

$$H = A + \frac{B}{\mu} + C\mu$$



H——塔板数；　A——涡流扩散；$\frac{B}{\mu}$——分子扩散项；$C\mu$——传质阻力项

常规HPLC系统扩散表征

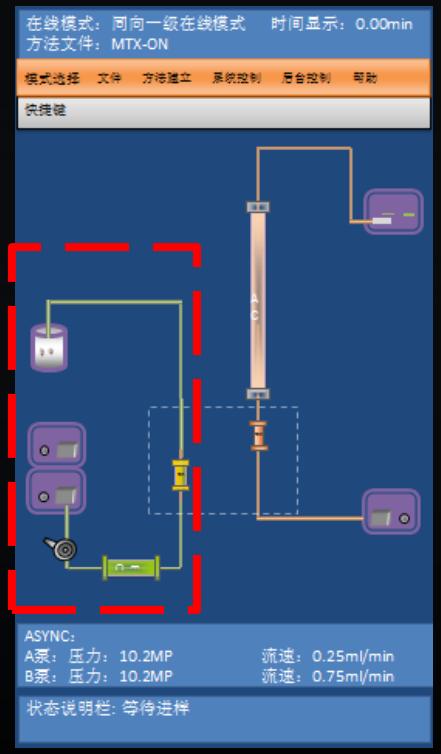


$$\delta_{ext}^{2}=\left(K_{inj}\frac{V_{INJ}^{2}}{12}\right)+\left(K_{CELL}\frac{V_{CELL}^{2}}{12}+\pi^{2}F^{2}\right)+\left(\frac{r_{c}^{4}l_{c}F}{7.6D_{m}}\right)$$

δ_{ext}^{2}——扩散程度；　K——常数；　V_{inj}——进样体积；　V_{cell}——流通池体积；

F——流速；　r_c——管道半径；　l_c——管道长度；　D_m——在流动相中的扩散系数

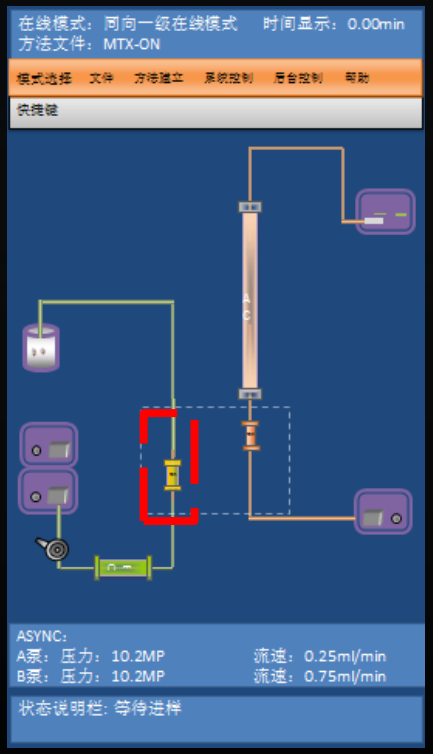
LC×LC系统表征数模（LC1）



$$\delta_{ext}{}^2=\left(K_{inj}\frac{V_{INJ}{}^2}{12}\right)+\left(K_{CELL}\frac{V_{CELL}{}^2}{12}+\pi^2F^2\right)+\left(\frac{r_c{}^4 l_c F}{7.6D_m}\right)$$

$$\delta_{ext}{}^2=\left(K_{inj}\frac{V_{INJ}{}^2}{12}\right)+\pi^2F^2+\left(\frac{r_c{}^4 l_c F}{7.6D_m}\right)$$

LC×LC系统表征数模（F1）



$$\delta_{\mathrm{ext}}{}^{2}=\left(K_{inj}\frac{V_{INJ}{}^{2}}{12}\right)+\pi^{2}F^{2}+\left(\frac{r_{c}{}^{4}l_{c}F}{7.6D_{m}}\right)$$

$$\mathrm{f}(dx)=\frac{d_{\delta_{ext1}}}{dx}\cdot x$$

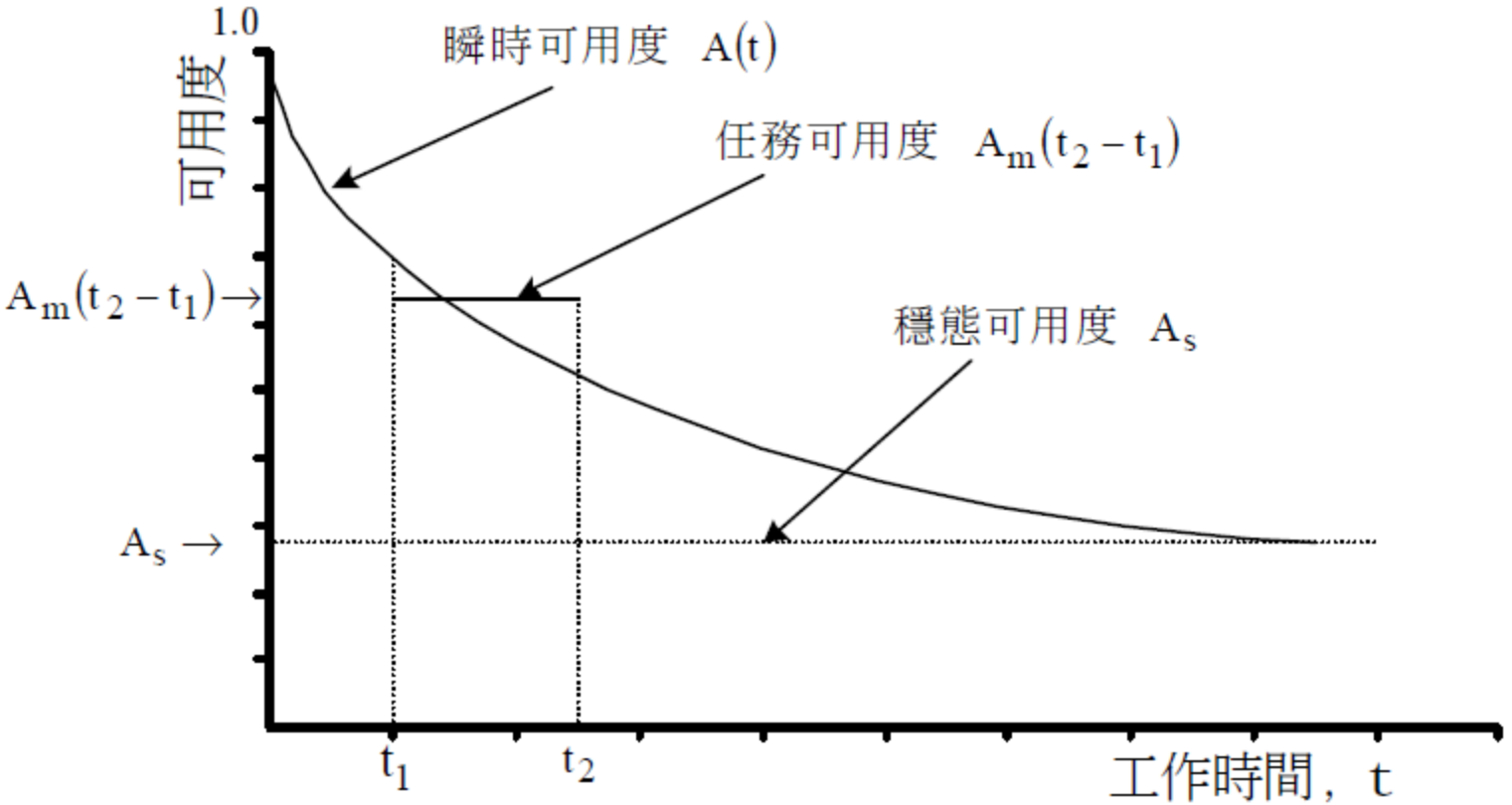
固有可用性(A_I)——可靠性技术的表征指标

$$\mathrm{Ai} = \frac{MTBF}{MTBF+MTTR} = \frac{1}{1+\alpha} \qquad \alpha = \frac{MTTR}{MTBF}$$

MTBF(mean time between failure):平均无故障工作时间
MTTR（ mean time to restoration ）： 平均修復時間 IEC 61508

11:00:39

固有可用度理论在系统开发中的应用



11:00:39

全程自动化色谱技术

一. 研究背景与指导方针

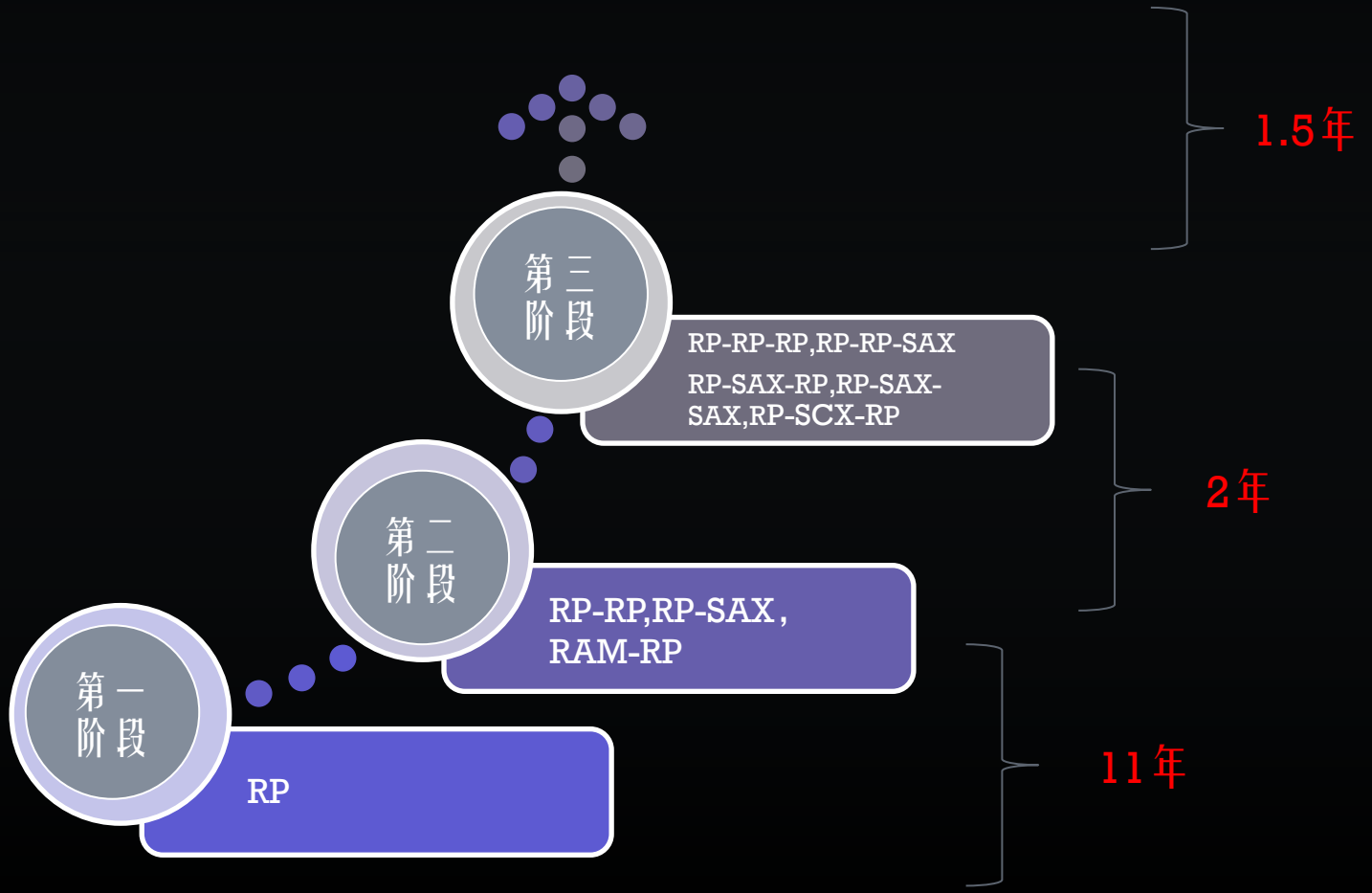
二. 全程自动化色谱技术原理与技术特点

三. 实现路径与关键问题

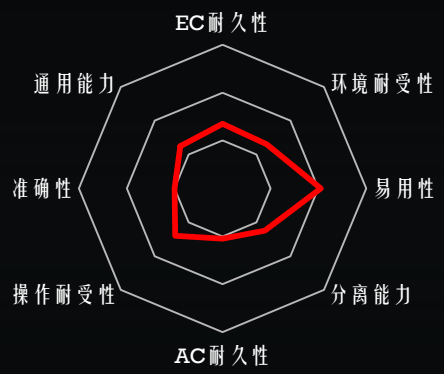
四. 应用现状与扩展计划

11:00:39

典型应用1：MTX测定自动化历程

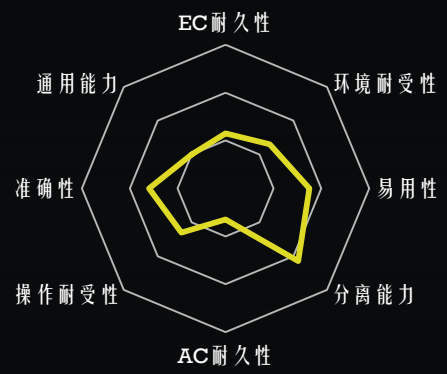


一级自动化MTX测定系统综合性能



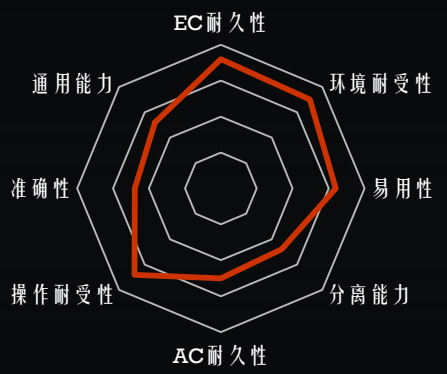
RP-RP

分离能力、耐受能力、准确性均非常差



RP-SAX

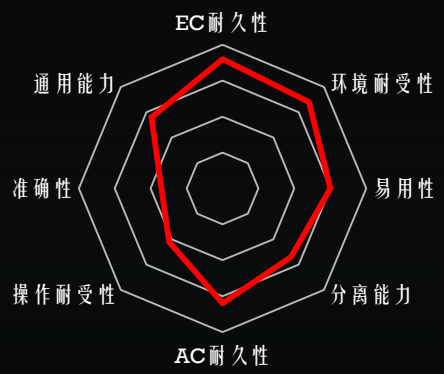
分析系统易损坏，环境忍受程度低



RP-SCX

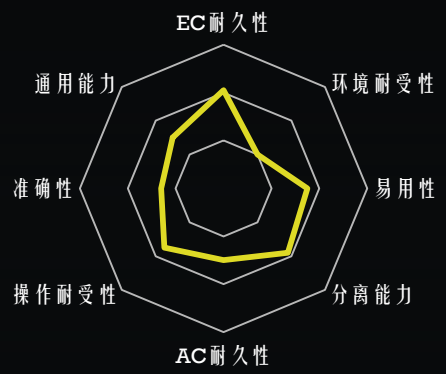
AC耐久性及准确性（易受干扰）

二级自动化MTX测定系统综合性能



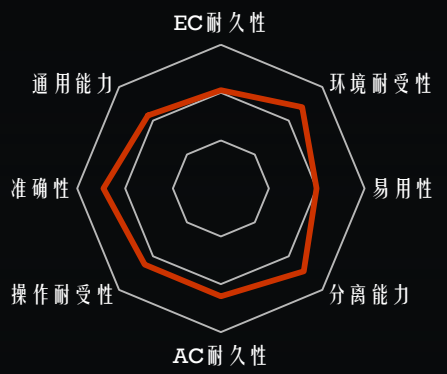
RP-SAX-SAX

操作耐受性非常差



RP-SAX-SCX

环境耐受性差



RP-SCX-RP

比较均衡

自动化基本条件

LC1色谱条件：

ASTON RG C_8 (50X4.6,5um，ANAX) 水相：有机相=70：30

交换材料：SCX（20X4.6,5um）

LC2 色谱条件：

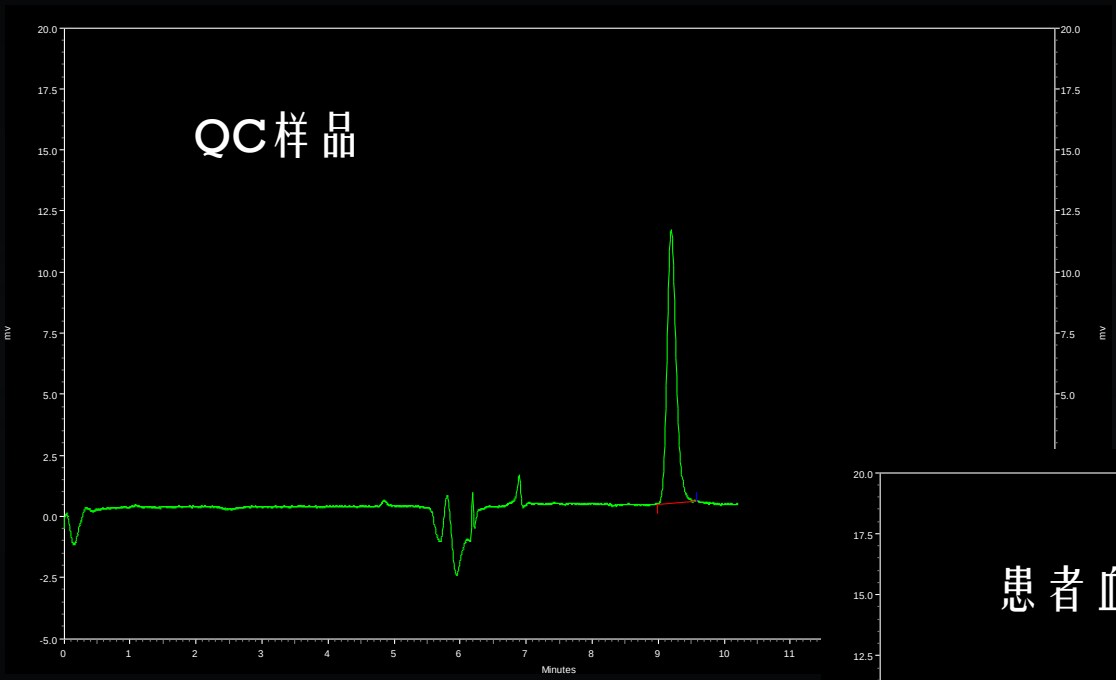
ASTON RG C_{18} (250X4.6,5um，ANAX) 水相：有机相=78：22

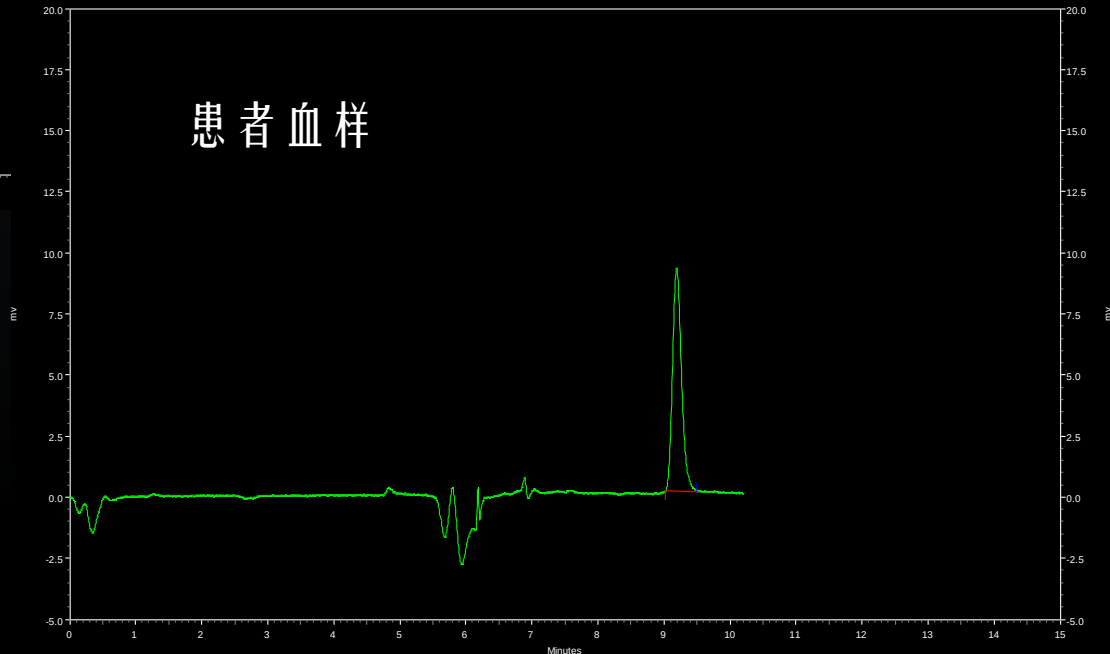
样品处理：

200ul血样加入600ul 蛋白沉淀剂,**进样**100ul

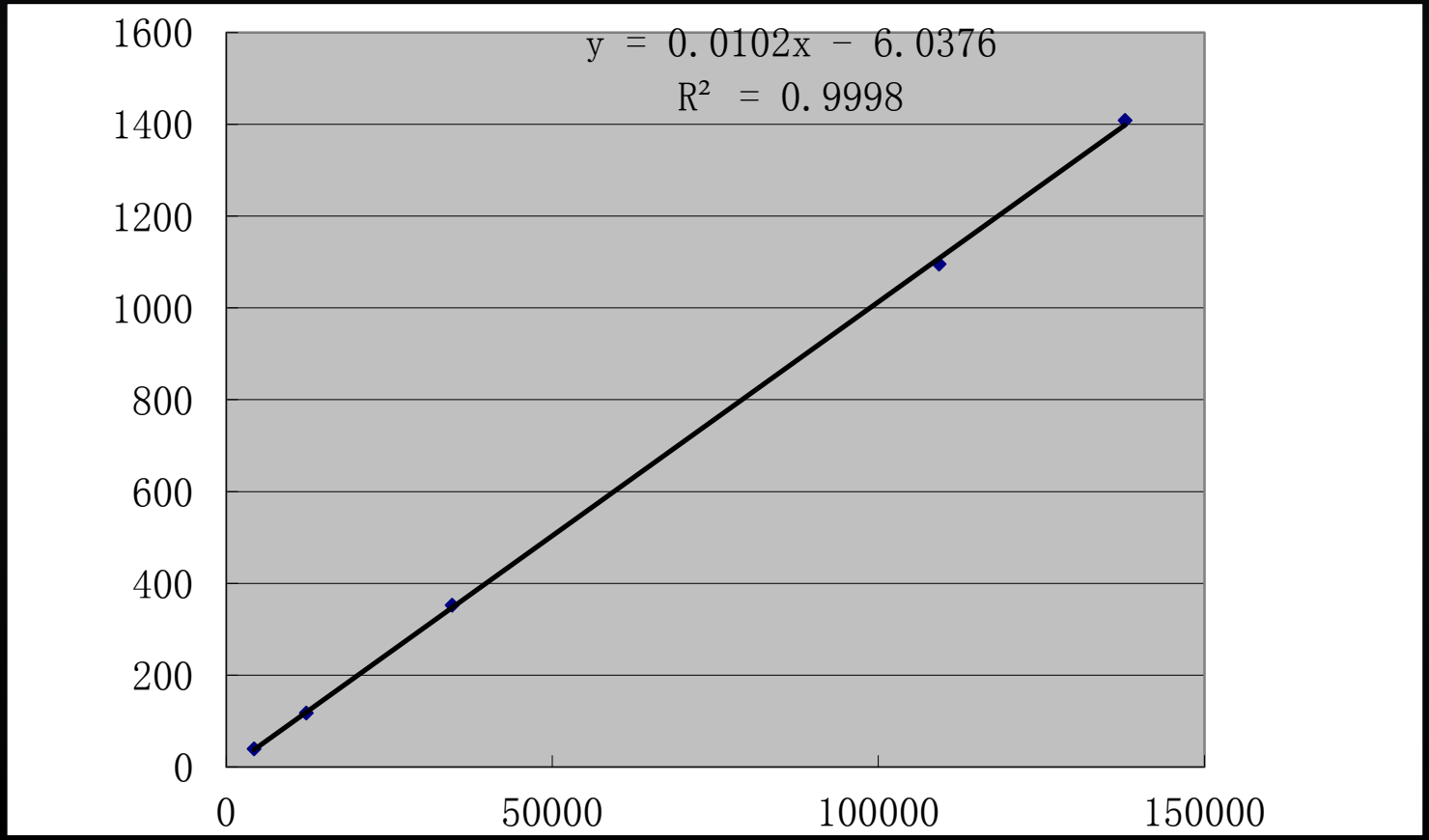
色谱柱温：35℃ **检测波长：**306nm **工作曲线范围：**50-1500ng/ml

MTX色谱信号图

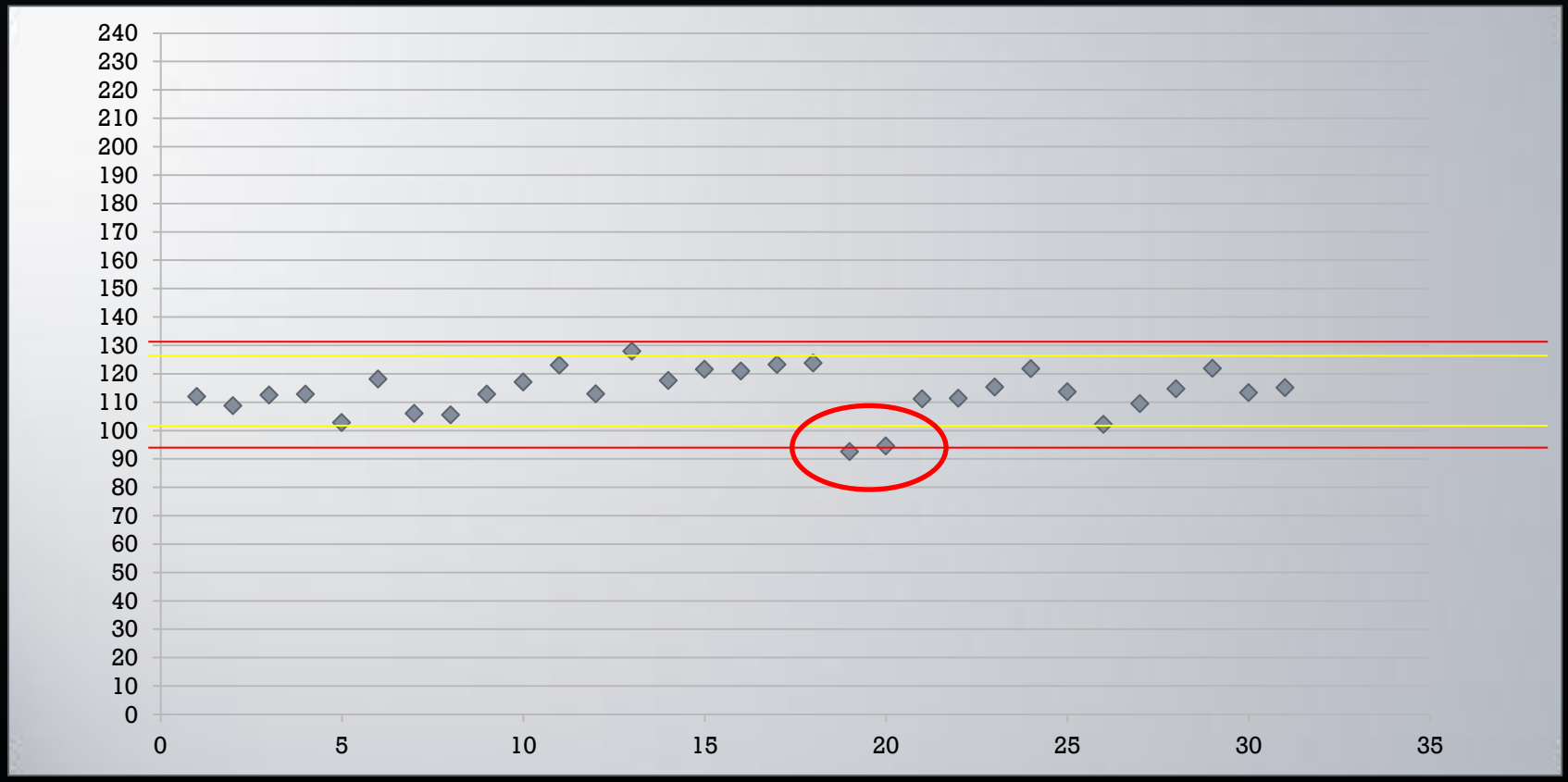




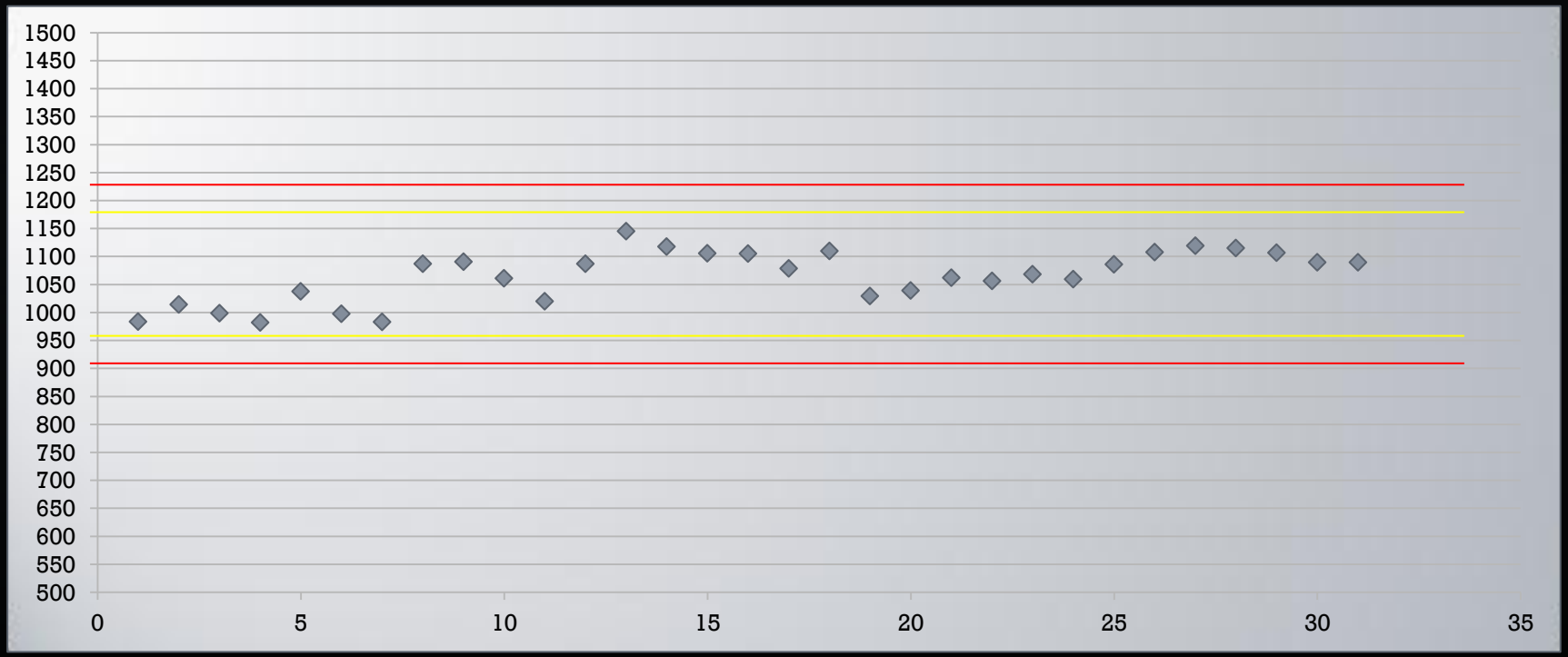
3月3号MTX色谱信号图



3月3日-4月10日低浓度质控（117.0ng/ml）

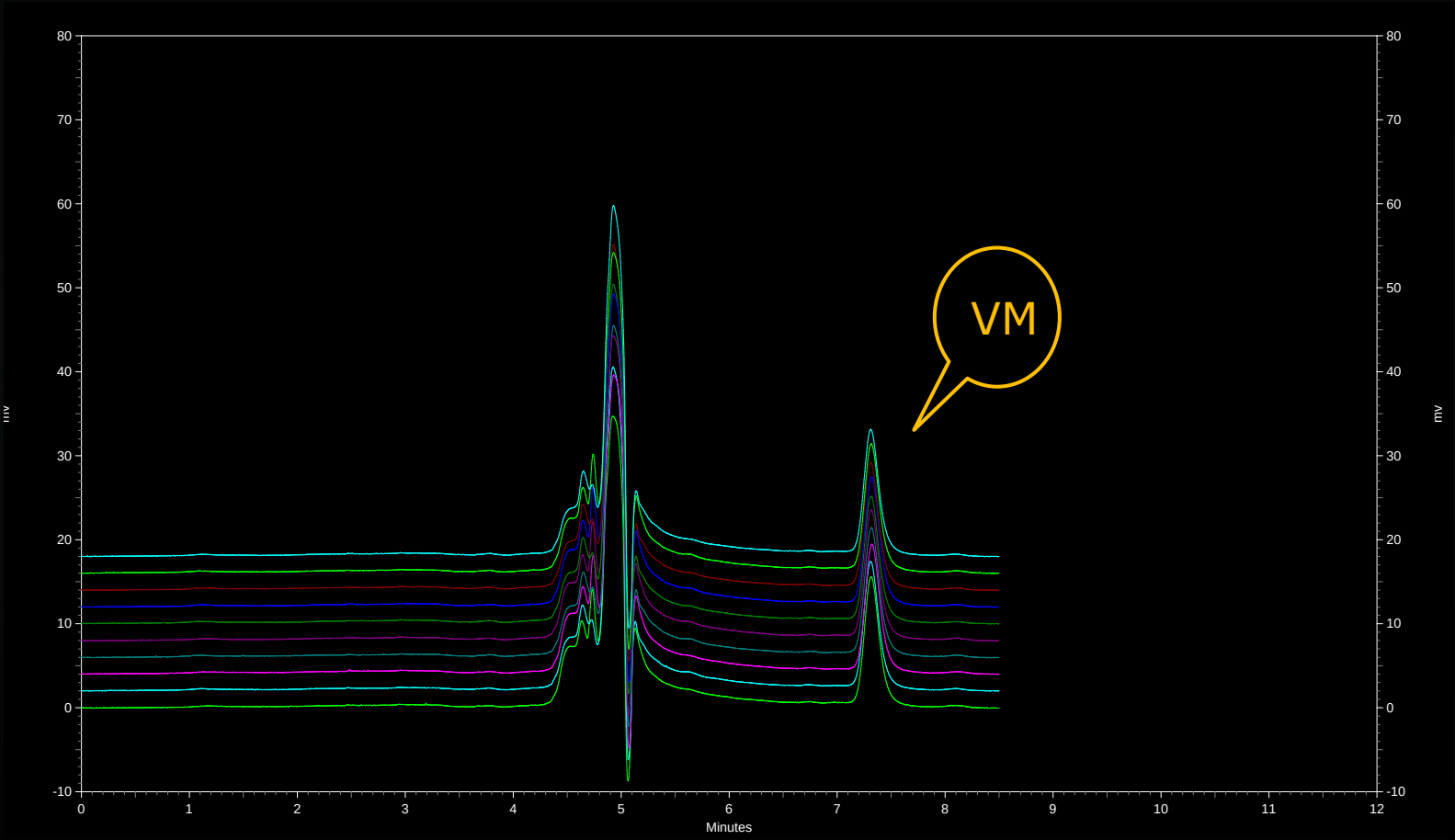


3月3日-4月10日高浓度质控（1076ng/ml）

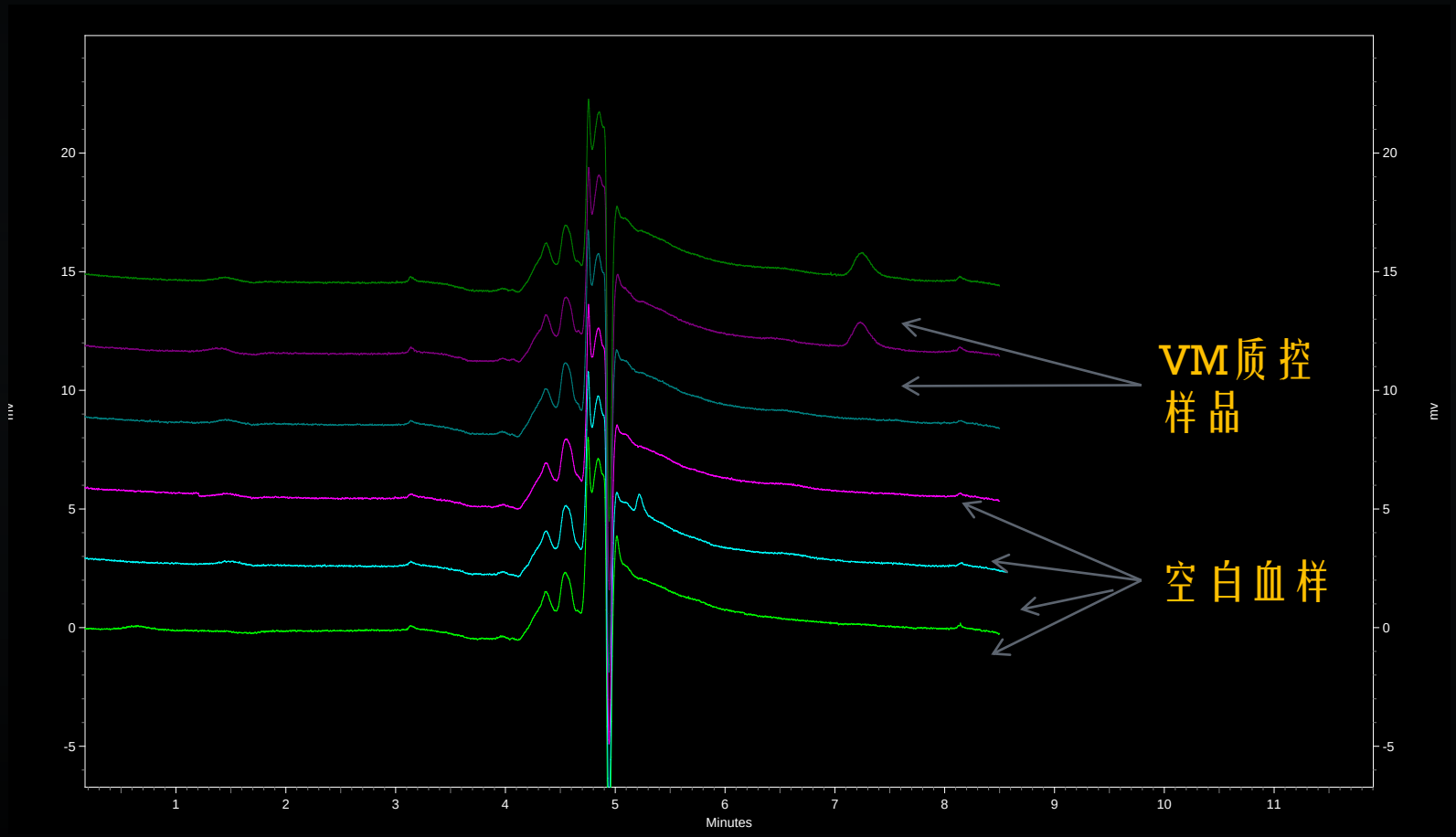


11:00:20

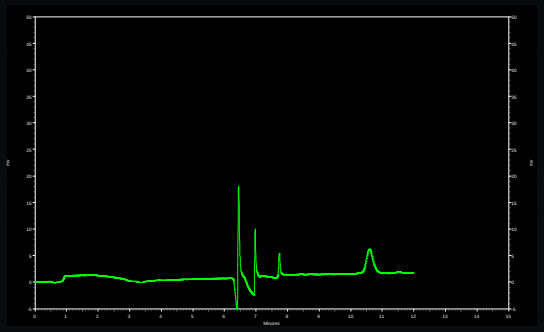
典型应用二：万古霉素TDM



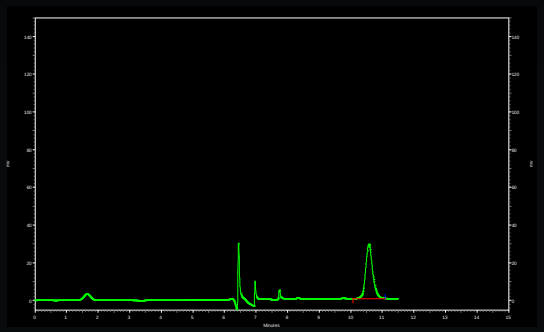
血样中几乎完全没有干扰



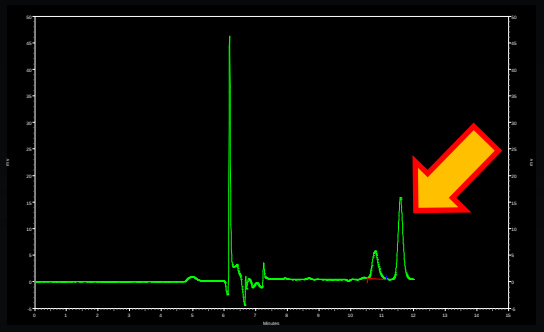
更改条件后的万古霉素TDM



标准对照品



空白血样+标准



临床样品（头孢匹罗）

自动化系统：
RP-RP-MIXSCX系统

色谱柱温： 35℃
检测波长： 282nm
浓度范围： 0.2-60ug/ml

样品处理：
300ul血样加入600ul沉淀剂，进样200ul

11:00:43

实例三：超越离线DBS

采用干血斑技术结合超高效液相色谱和增强型离子生成 ESI 技术简化药代动力学分析

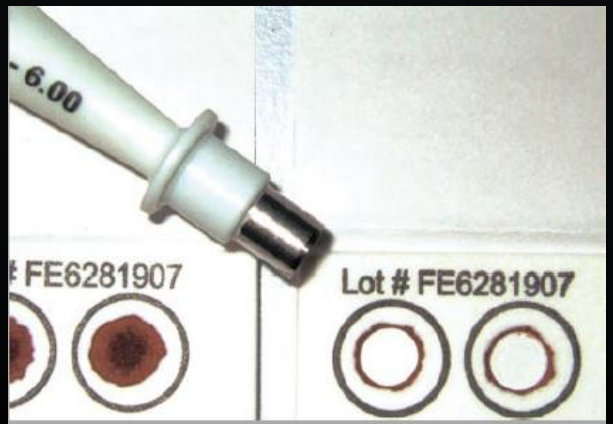
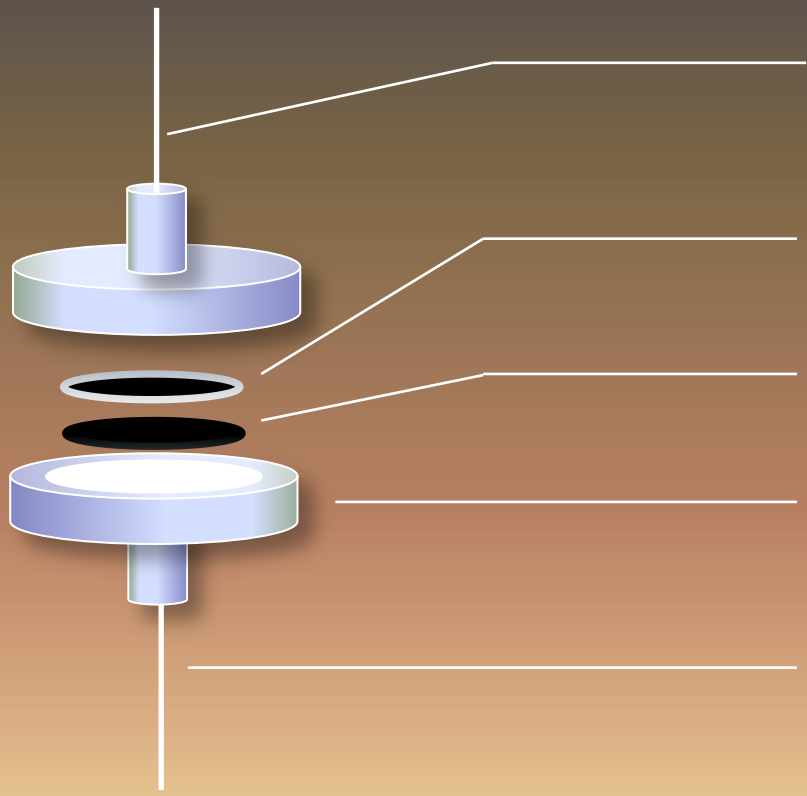
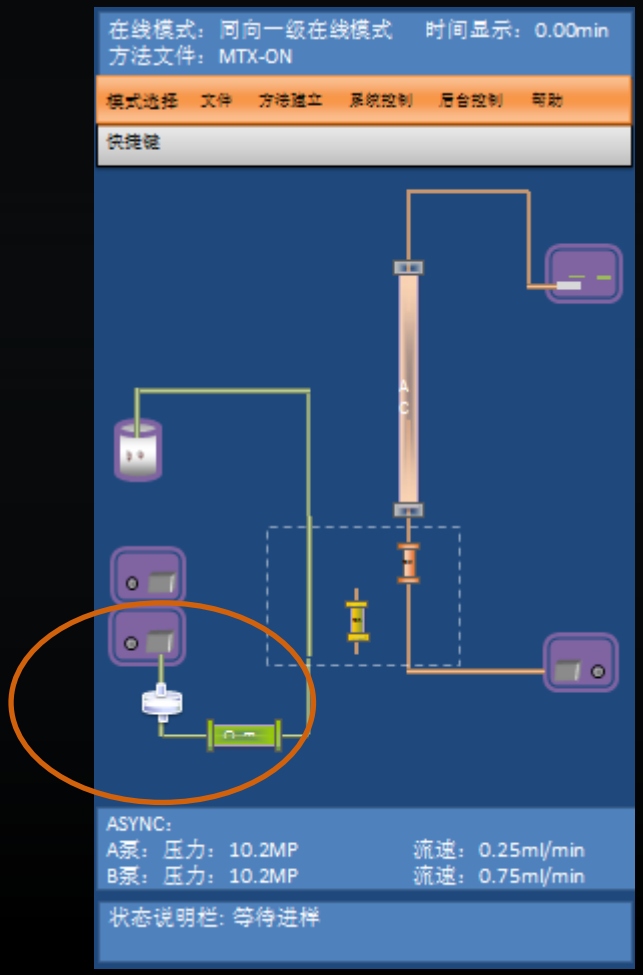


图 1：FTA 卡和 6 mm 直径打孔器图。左：15µL 的血斑。右：6 mm ID 打孔器提取后的干血斑。

5-FU 与在线DBS

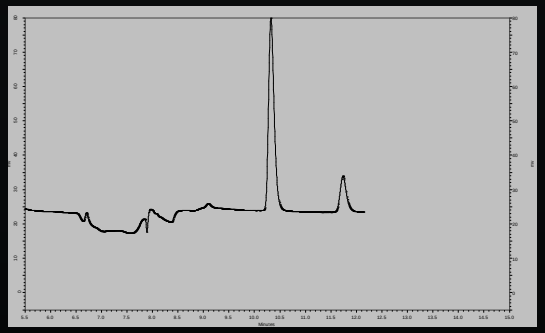


最新研制Online Solide Extrat—HPLC

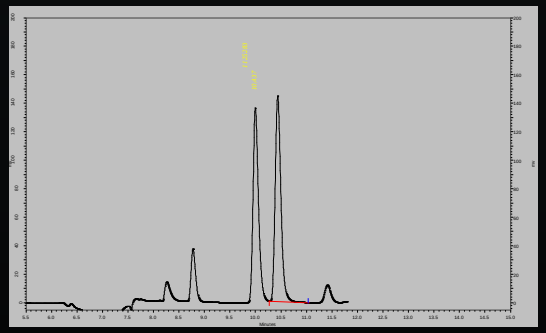


5-FU 与在线DBS

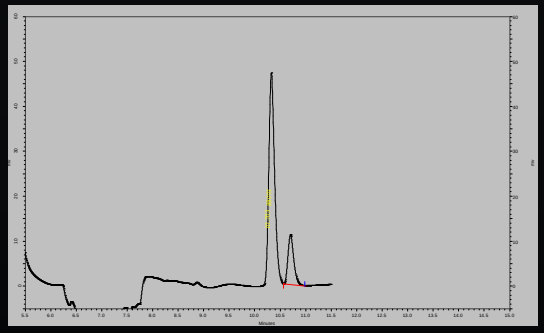
样品处理：取40ul血样点放干纸法上，将纸片置于微波炉，用480W的功率工作8min，即可使用。



空白血样

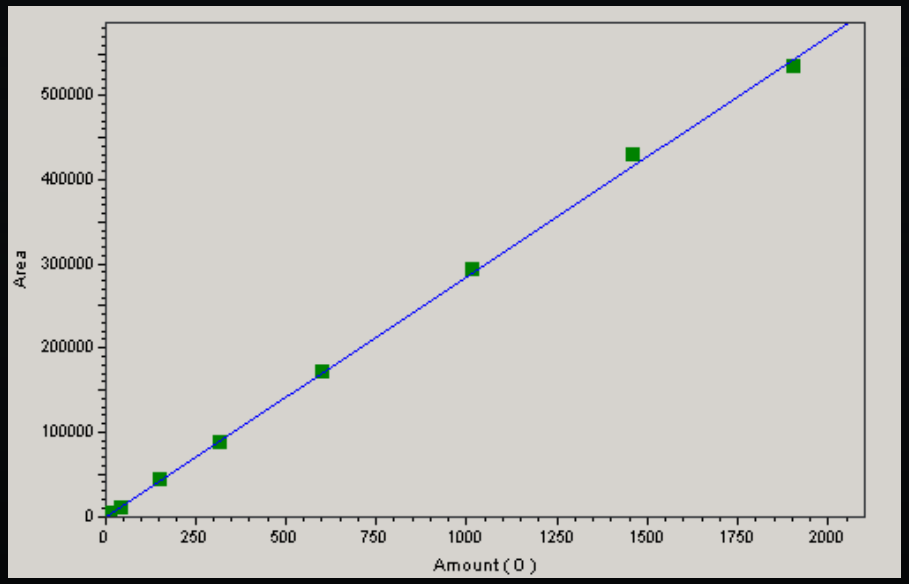


空白血样+标准



临床样品

外标法标准曲线结果



External Standard Curve

Average RF: 264.591
RF StDev: 39.3052
RF %RSD: 14.8551

Scaling: None
LSQ Weighting: None
Force Through Zero: Off
Replicate Mode: Replace

Linear Fit ax + b
a = 286.082
b = -887.627
Goodness of fit (r^2): 0.99904

11:00:43

小结

一. 研究背景与意义

二. 全程自动化色谱技术概念与技术特点

三. 实现路径与关键问题

四. 应用现状与扩展计划

11:00:43



Thanks