

水體中 11 種抗生素的測定 Online SPE-LC-MS/MS 質譜聯用法

聯合層析（上海） 徐佳杭

抗生素(antibiotics)是生物包括微生物、植物和動物在內在其生命活動過程中所產生的(或由其他方法獲得的)，能在低微濃度下有選擇地抑制或影響它種生物功能的有機物質。抗生素的生態影響主要表現在兩方面：一方面將導致環境中耐藥菌的出現，並大量繁殖和傳播，最終人類健康造成影響；另一方面通過影響水生生物、動植物的營養傳遞方式和種群結構以及微生物的種群數量，生態環境中固有的食物鏈將會遭到破壞，進而打破生態系統的平衡。本文介紹了採用線上SPE的方法測定水體中11種抗生素的含量。

1、實驗儀器與耗材

1.1 AB QTRAP 5500 LC/MS/MS

1.2 聯和層析雙SPE柱串聯多維Online SPE系統HighSPEed

1.3 線上SPE柱：HLB 2.1*20mm, 2.5um；

1.4 11種抗生素標準品：阿奇黴素，紅黴素，羅紅黴素，克拉黴素，林可黴素，維吉尼黴素M1，維吉尼黴素S1，克林黴素，奧美普林，竹桃黴素，替米考星，純度 $\geq 95\%$ 。

2、聯和層析雙SPE柱串聯多維Online SPE系統HighSPEed（單柱線上SPE）

2.1 進樣量：1mL

2.2 取樣速度：200uL/s

2.3 SPE柱活化條件：用純甲醇以1.0mL/min的流速活化2min

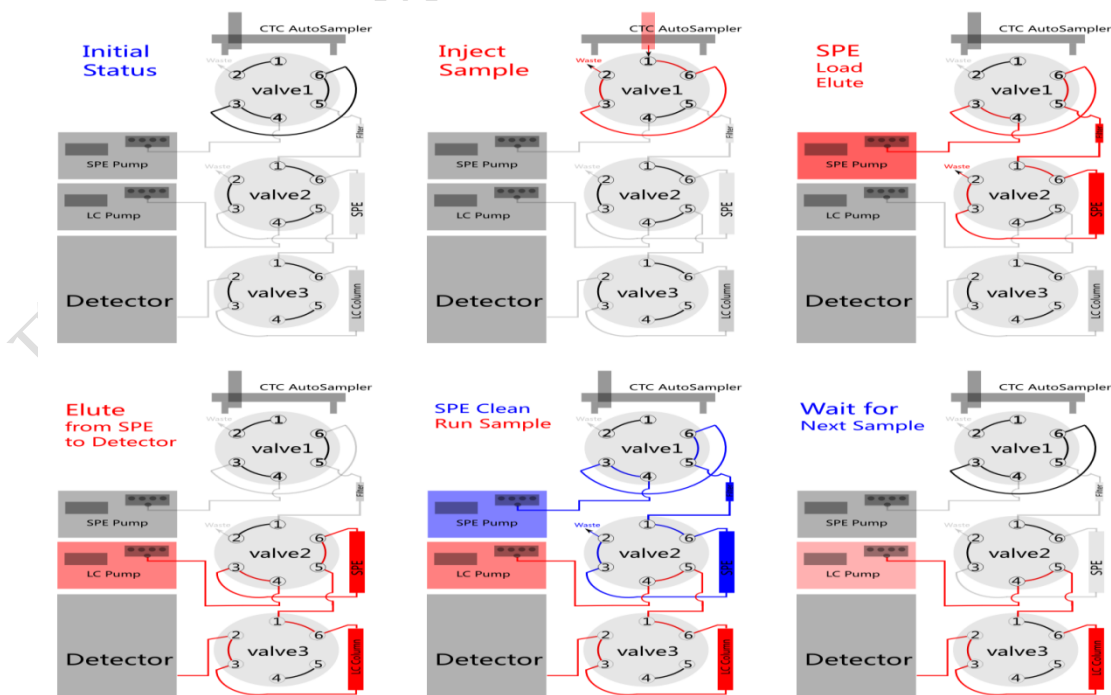
2.4 SPE柱平衡條件：用純水以1.0mL/min的流速活化1min

2.5 SPE柱上樣條件：用純水以2.0mL/min的流速上樣3min

2.6 SPE柱淋洗條件：用純水以0.5mL/min的流速淋洗1min

2.7 SPE柱再生條件：用純甲醇以1.0mL/min的流速再生1min

2.8 管路清洗條件：用純甲醇以1.0mL/min的流速清洗1min



3、色譜-質譜條件

3.1 色譜柱：Kineter 50*3.0mm 2.6um；

3.2 流動相：A為0.1%甲酸，5mmol/L甲酸銨水溶液，B為乙腈；

3.3 流動相洗脫程式：

表1 液相色譜流動性梯度洗脫程式

時間 (min)	流速 (mL/min)	0.1%甲酸/5mmol/L 甲酸銨水溶液 (%)	乙腈 (%)
0.00	0.5	80.0	20.0
5.00	0.5	5.0	95.0
11.00	0.5	5.0	95.0
11.50	0.5	80.0	20.0

備註：在流動相中加入適量甲酸和甲酸銨可以增加抗生素的保留，並改善峰形，促進離子化。因此本實驗採用 0.1%甲酸和 5 mmol/L 甲酸銨混合溶液和乙腈作為流動相，大部分化合物都得以較好分離，峰形良好。

3.4 質譜條件：電噴霧電離源 (ESI)，多反應監測 (MRM) 離子模式，11種抗生素定量離子對、裂解電壓、碰撞能量見表2。

表2 11種抗生素的相關質譜參數

化合物名稱	離子對 (m/z)	裂解電壓 (v)	碰撞能量 (v)
替米考星	869.5/174.1	110	62
Tilmicosin	869.5/132.1		70
竹桃黴素	688.4/158.0	141	39
Oleandomycin	688.4/544.3		23
紅黴素	734.4/158.0	141	39
Erythromycin	734.4/576.2		23
林可黴素	407.2/126.0	121	37
Lincomycin	407.2/359.1		25
克林黴素	425.1/126.0	121	39
Clindamycin	425.1/377.0		27
羅紅黴素	837.5/679.2	156	29
Roxithromycin	837.5/158.0		47
阿奇黴素	749.6/591.5	120	38
Azithromycin	749.6/158.1		46
維吉尼黴素 M1	526.2/508.3	90	18
VirginiamycinsM1	526.2/355.1		25
維吉尼黴素 S1	824.3/205.0	130	57
VirginiamycinsS1	824.3/663.2		36
克拉黴素	748.5/590.4	40	29
Clarithromycin	748.5/158.0		40
奧美普林	275.2/259.1	40	38
Ometoprim	275.2/123.0		31

4、試驗圖譜

- Calibration for AQMS1: $y = 1.51544e5 x + -6.40925e4$ ($r = 0.99921$) (weighting: $1/x$)
- Calibration for HMS1: $y = 1.04023e5 x + -2926.88056$ ($r = 0.99986$) (weighting: $1/x$)
- Calibration for LHMS1: $y = 4.10076e5 x + 14530.08957$ ($r = 0.99953$) (weighting: $1/x$)
- Calibration for KLMS1: $y = 8.12895e4 x + -8565.10721$ ($r = 0.99889$) (weighting: $1/x$)
- Calibration for HMS-H2O1: $y = 1.61697e5 x + -1962.61792$ ($r = 0.99981$) (weighting: $1/x$)
- Calibration for LKMS1: $y = 6.78096e4 x + -12347.18425$ ($r = 0.99899$) (weighting: $1/x$)
- Calibration for VJMS-M1-1: $y = 5.97348e4 x + 713.09919$ ($r = 0.99958$) (weighting: $1/x$)
- Calibration for VJMS-S1-1: $y = 6.57415e4 x + -11361.95289$ ($r = 0.99966$) (weighting: $1/x$)
- Calibration for EJYJJBAMD1: $y = 2.59997e5 x + -24047.05416$ ($r = 0.99939$) (weighting: $1/x^2$)
- Calibration for LSZTMS1: $y = 5.06984e4 x + -6952.26460$ ($r = 0.99935$) (weighting: $1/x$)
- Calibration for KLMS-1: $y = 3.36167e5 x + 3.36839e4$ ($r = 0.99915$) (weighting: $1/x$)
- Calibration for TMKX1: $y = 3.00749e4 x + -7405.51793$ ($r = 0.99801$) (weighting: $1/x^2$)

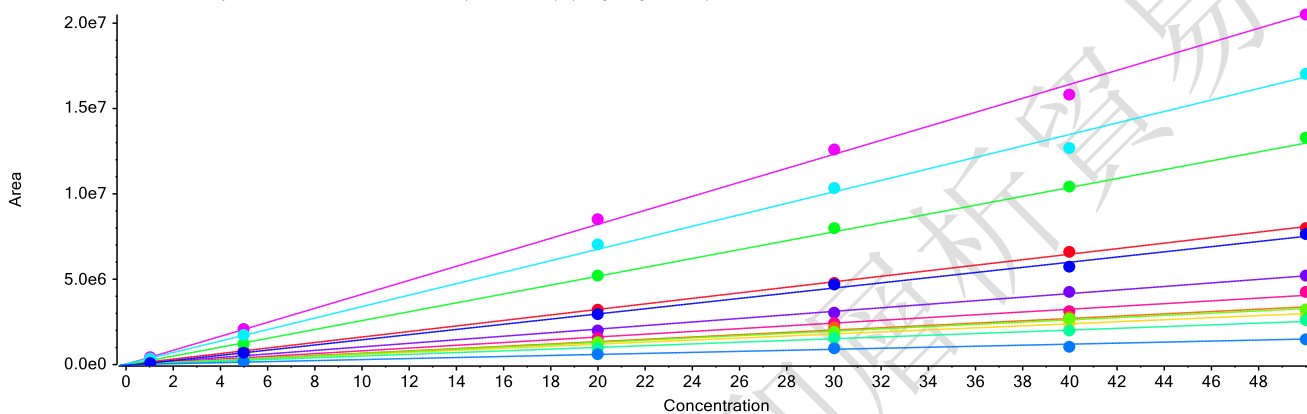


圖1 11種抗生素標準曲線以及線性匯總圖

Calibration for AQMS1: $y = 1.51544e5 x + -6.40925e4$ ($r = 0.99921$) (weighting: $1/x$)

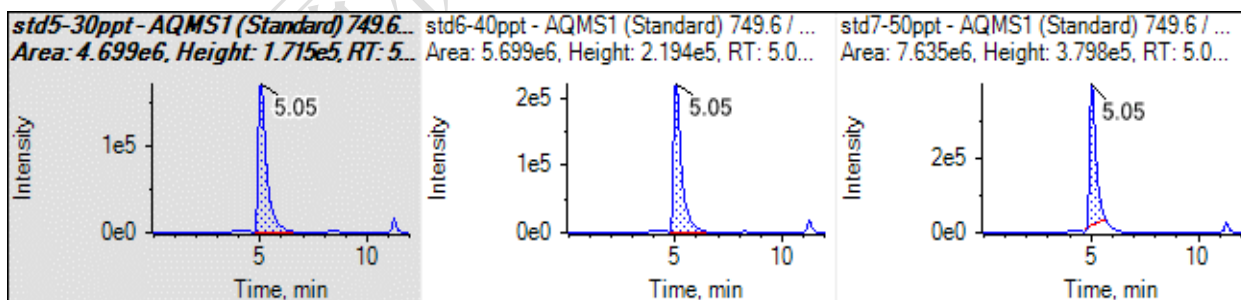
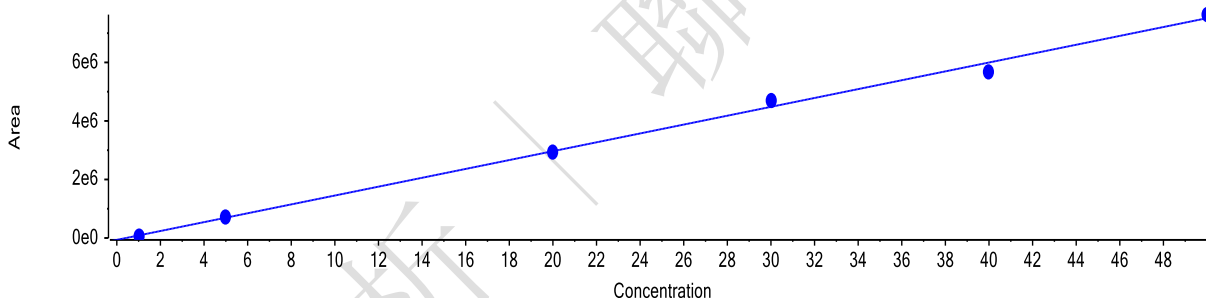


圖2 阿奇黴素標準曲線以及MRM圖

Calibration for HMS1: $y = 1.04023e5 x + -2926.88056$ ($r = 0.99986$) (weighting: $1/x$)

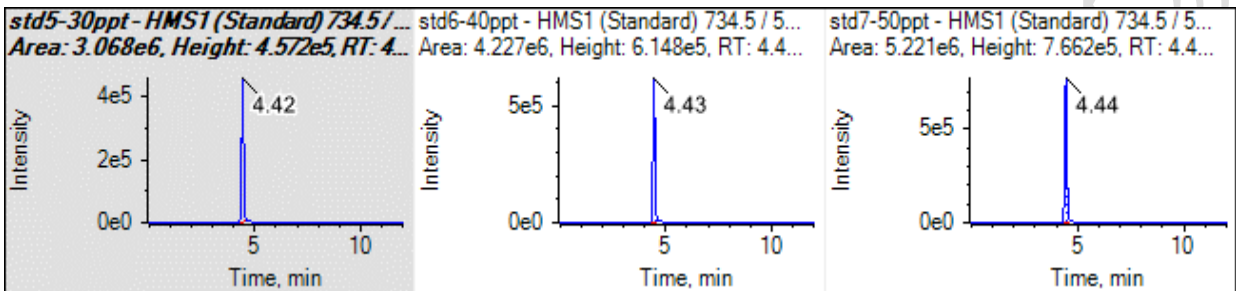
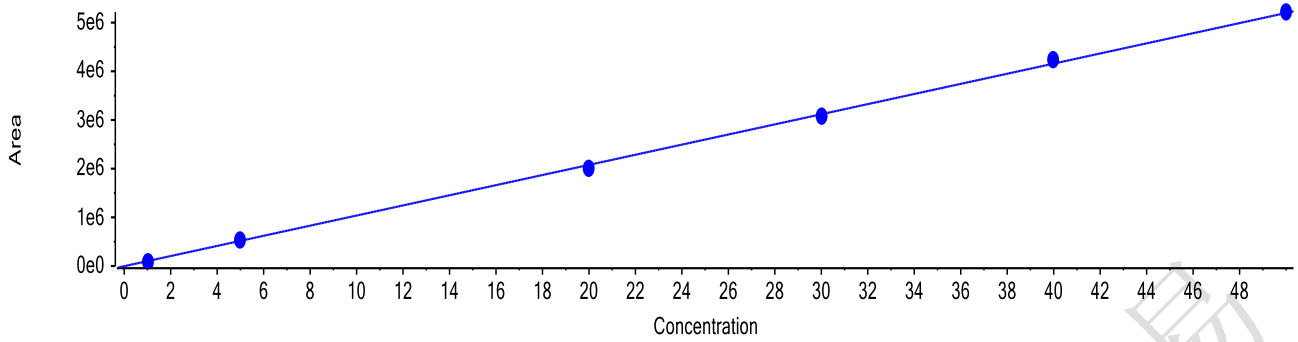


圖3 紅黴素標準曲線圖以及MRM圖

Calibration for LHMS1: $y = 4.10076e5 x + 14530.08957$ ($r = 0.99953$) (weighting: $1/x$)

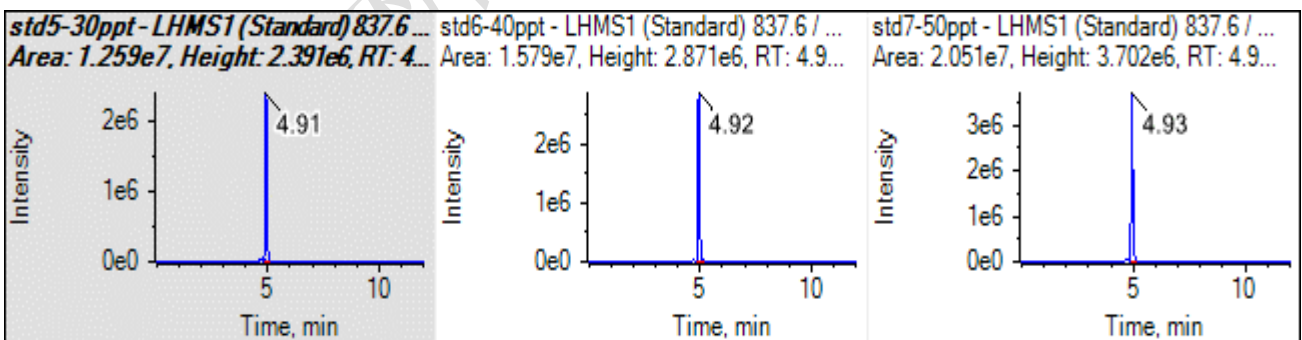
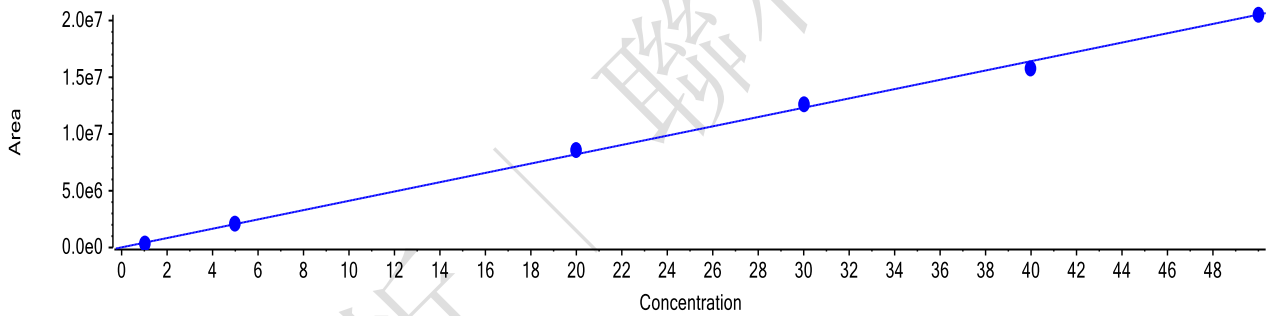


圖4 羅紅黴素標準曲線圖以及MRM圖

Calibration for KLMS1: $y = 8.12895e4 x + -8565.10721$ ($r = 0.99889$) (weighting: $1/x$)

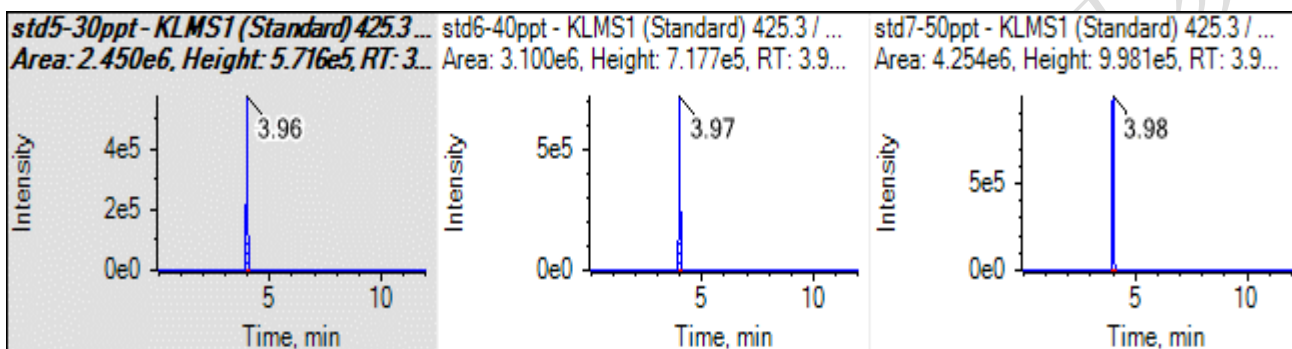
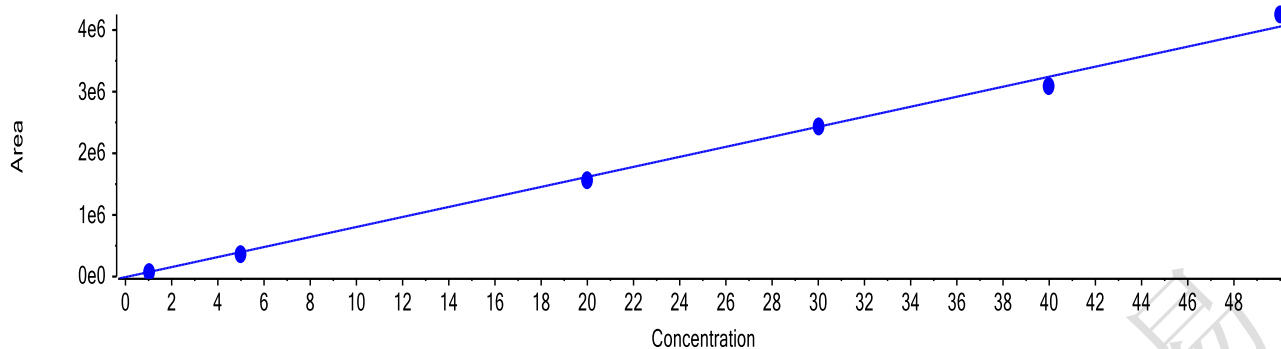


圖5 克拉黴素標準曲線圖以及MRM圖

Calibration for LKMS1: $y = 6.78096e4 x + -12347.18425$ ($r = 0.99899$) (weighting: $1/x$)

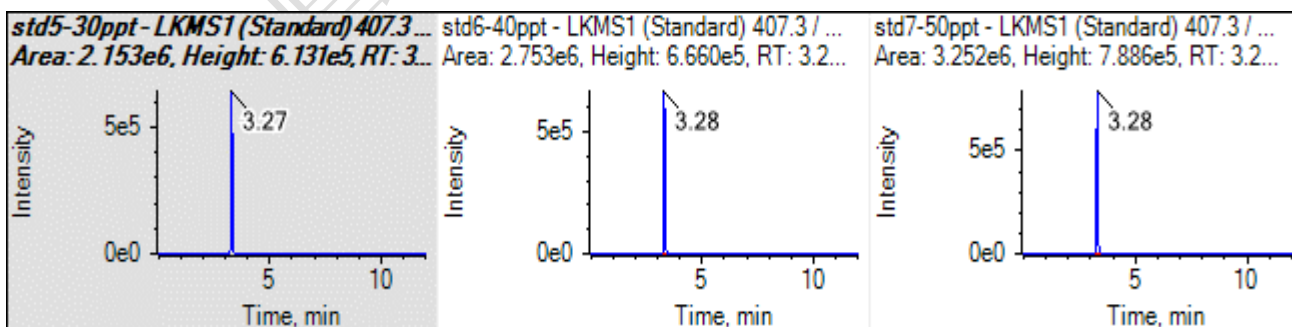
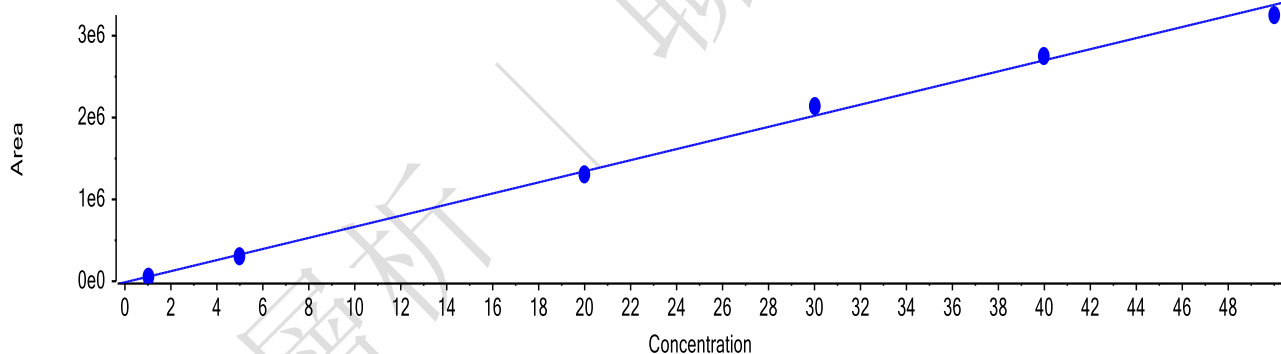


圖6 林可黴素標準曲線圖以及MRM圖

Calibration for VJMS-M1-1: $y = 5.97348e4 x + 713.09919$ ($r = 0.99958$) (weighting: $1/x$)

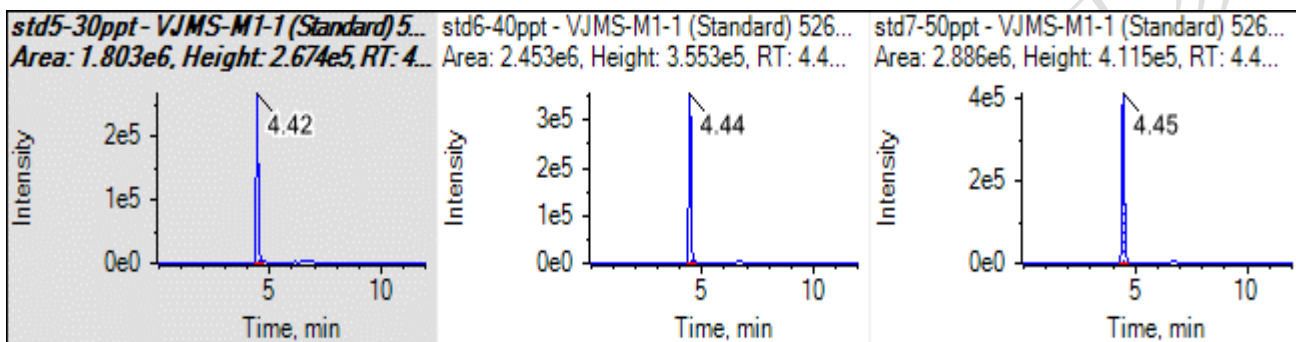
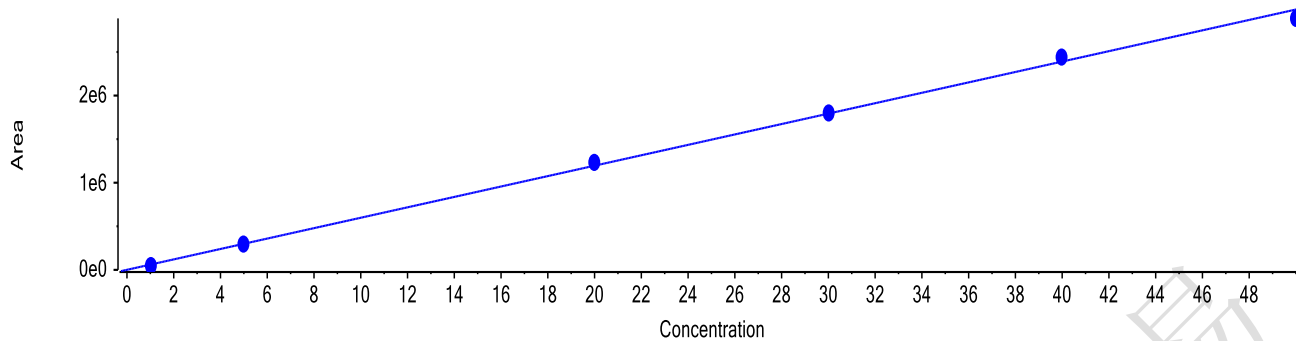


圖7 維吉尼黴素M1標準曲線圖以及MRM圖

Calibration for VJMS-S1-1: $y = 6.57415e4 x + -11361.95289$ ($r = 0.99966$) (weighting: $1/x$)

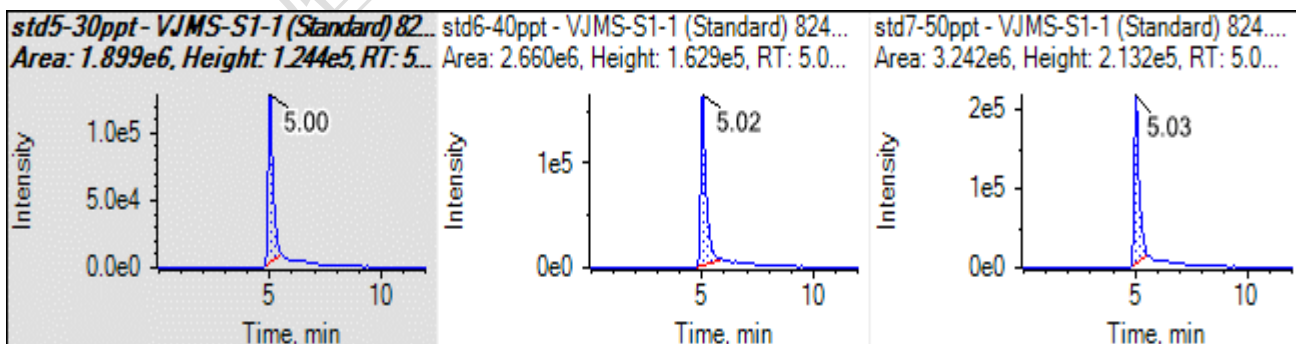
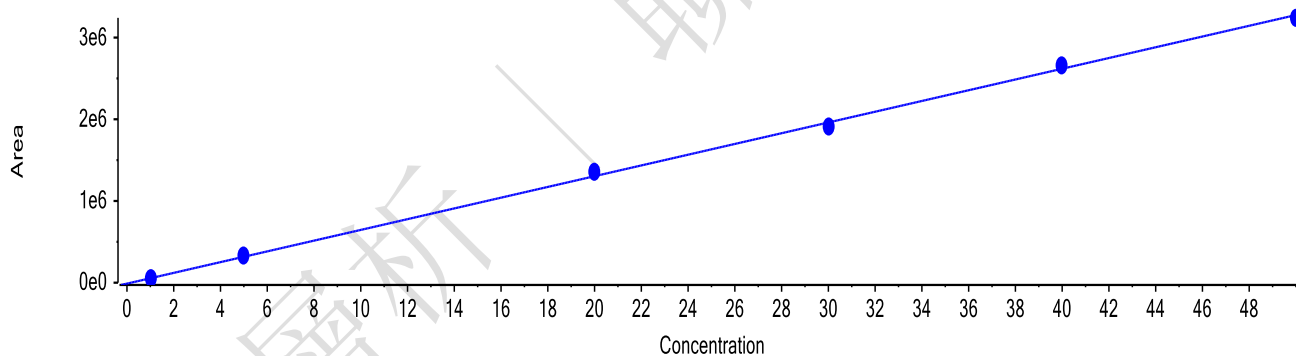


圖8 維吉尼黴素S1標準曲線圖以及MRM圖

Calibration for EJYJJBAMD1: $y = 2.59997e5 x + -24047.05416$ ($r = 0.99939$) (weighting: $1 / x^2$)

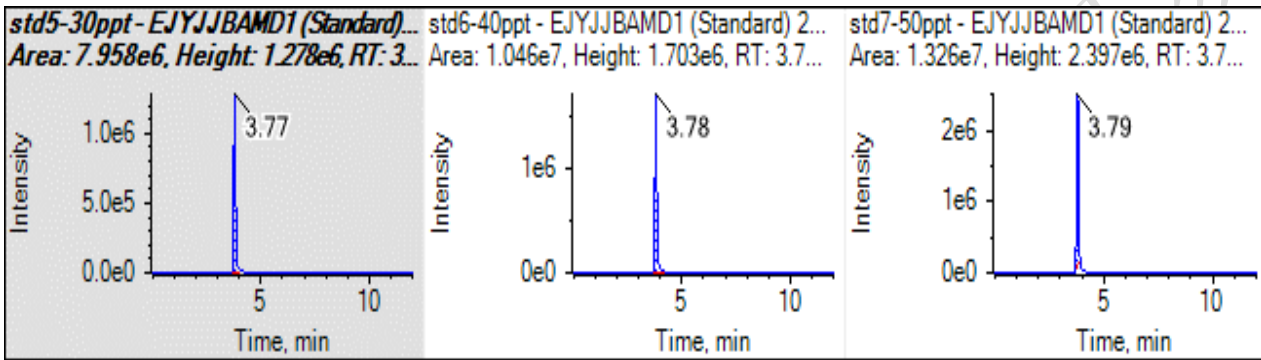
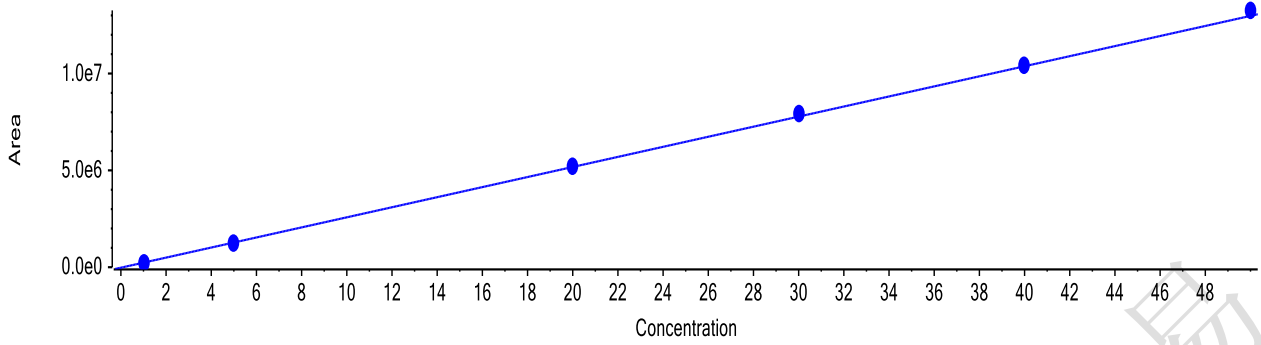


圖9 奧美普林標準曲線圖以及MRM圖

Calibration for LSZTMS1: $y = 5.06984e4 x + -6952.26460$ ($r = 0.99935$) (weighting: $1 / x$)

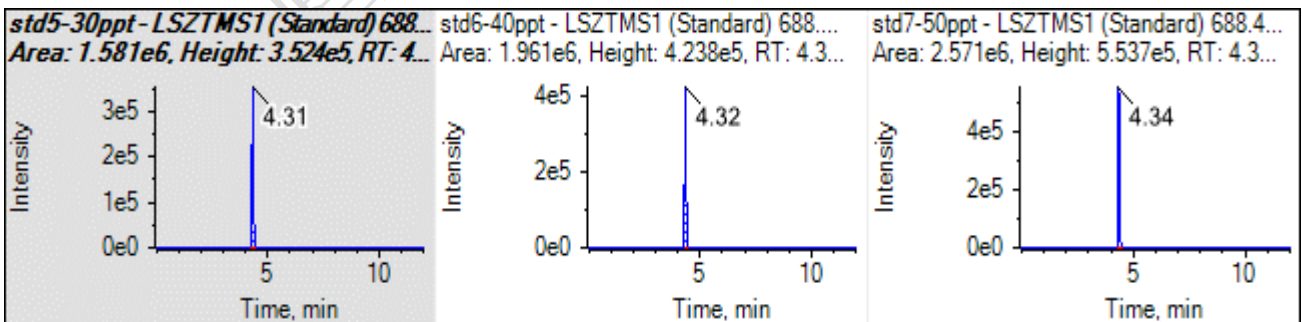
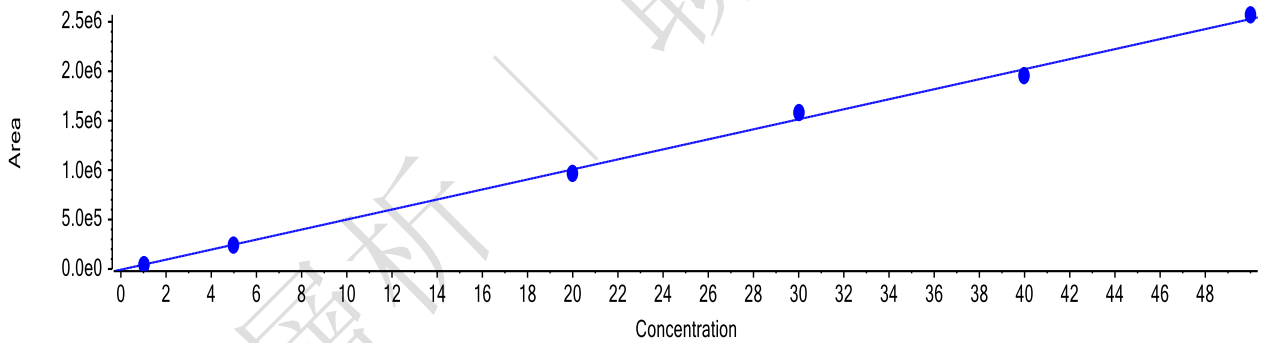


圖10 竹桃黴素標準曲線圖以及MRM圖

Calibration for KLMS-1: $y = 3.36167e5 x + 3.36839e4$ ($r = 0.99915$) (weighting: $1/x$)

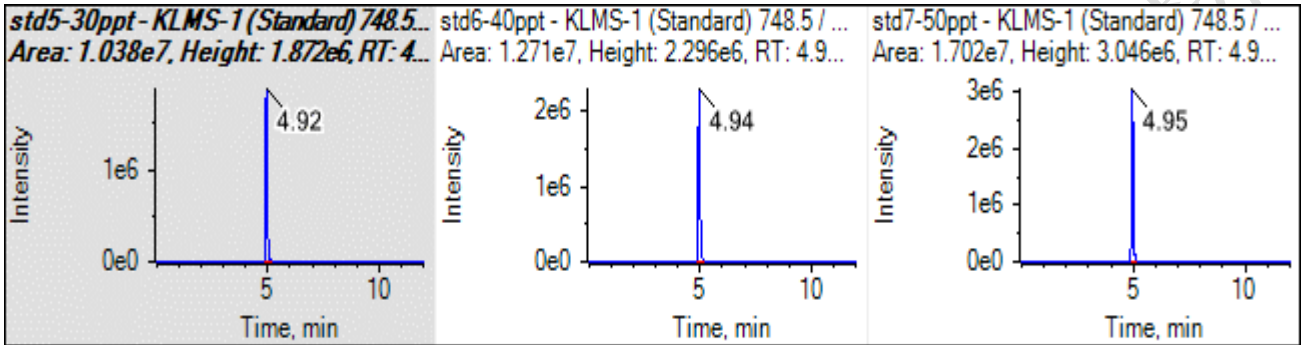
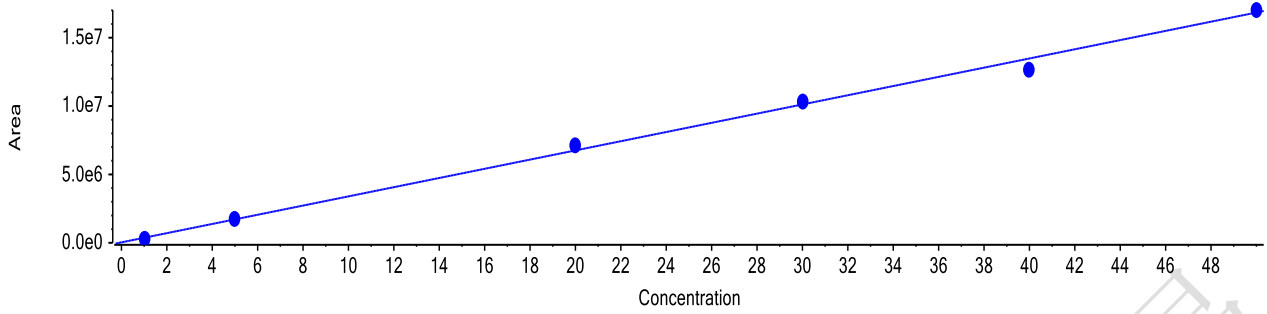


圖11 克拉黴素標準曲線圖以及MRM圖

Calibration for TMKX1: $y = 3.00749e4 x + -7405.51793$ ($r = 0.99801$) (weighting: $1/x^2$)

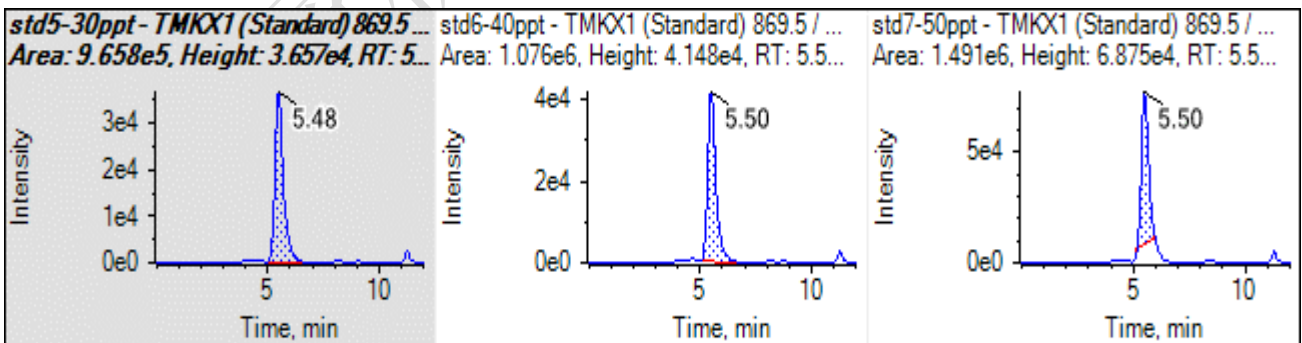
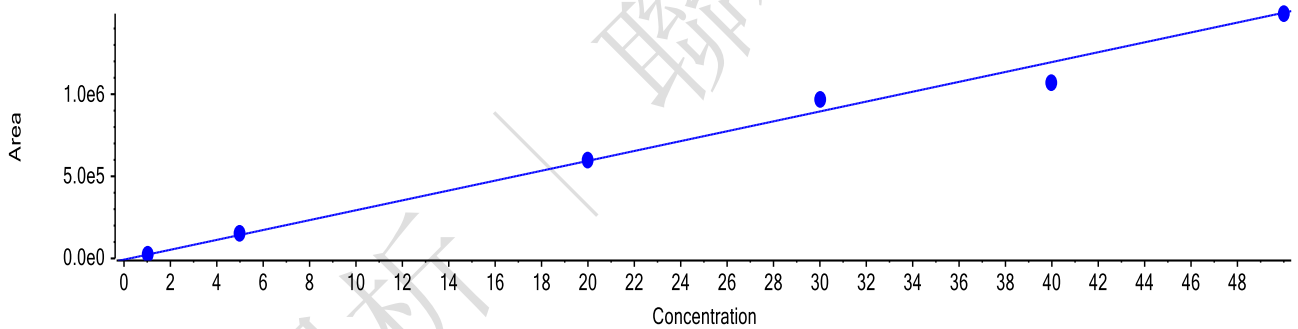


圖12 替米考星標準曲線圖以及MRM圖

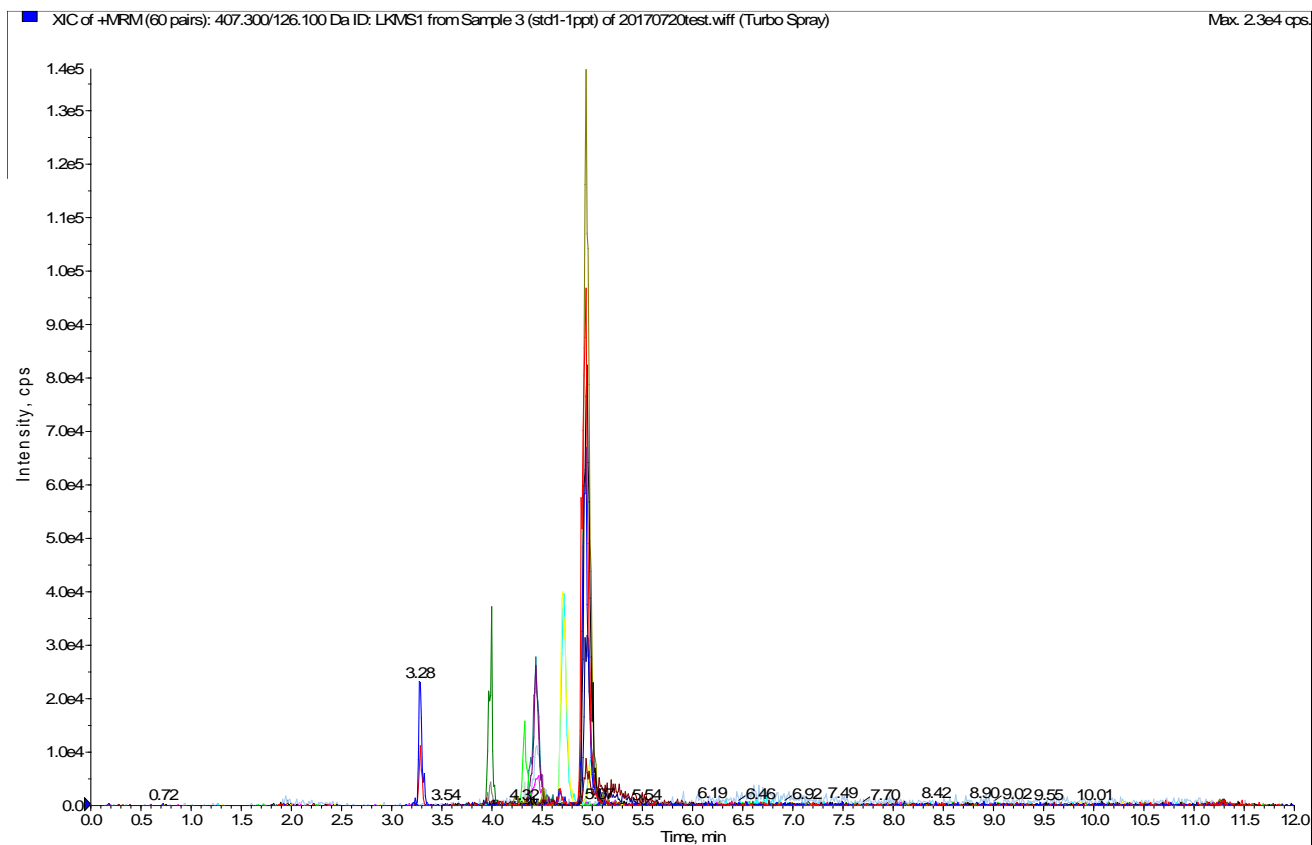


圖13 11種抗生素混標 (1.0ng/L) MRM圖

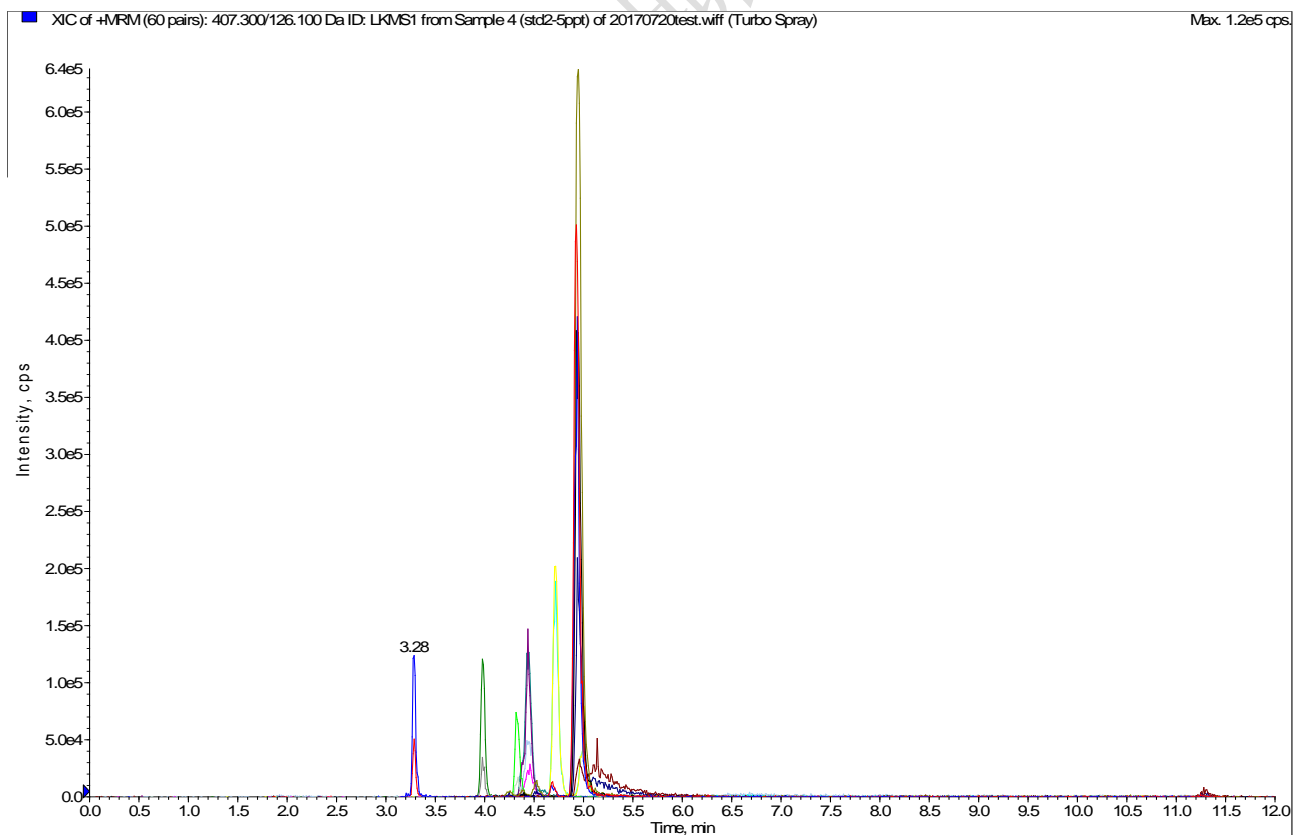


圖14 11種抗生素混標 (5.0ng/L) MRM圖

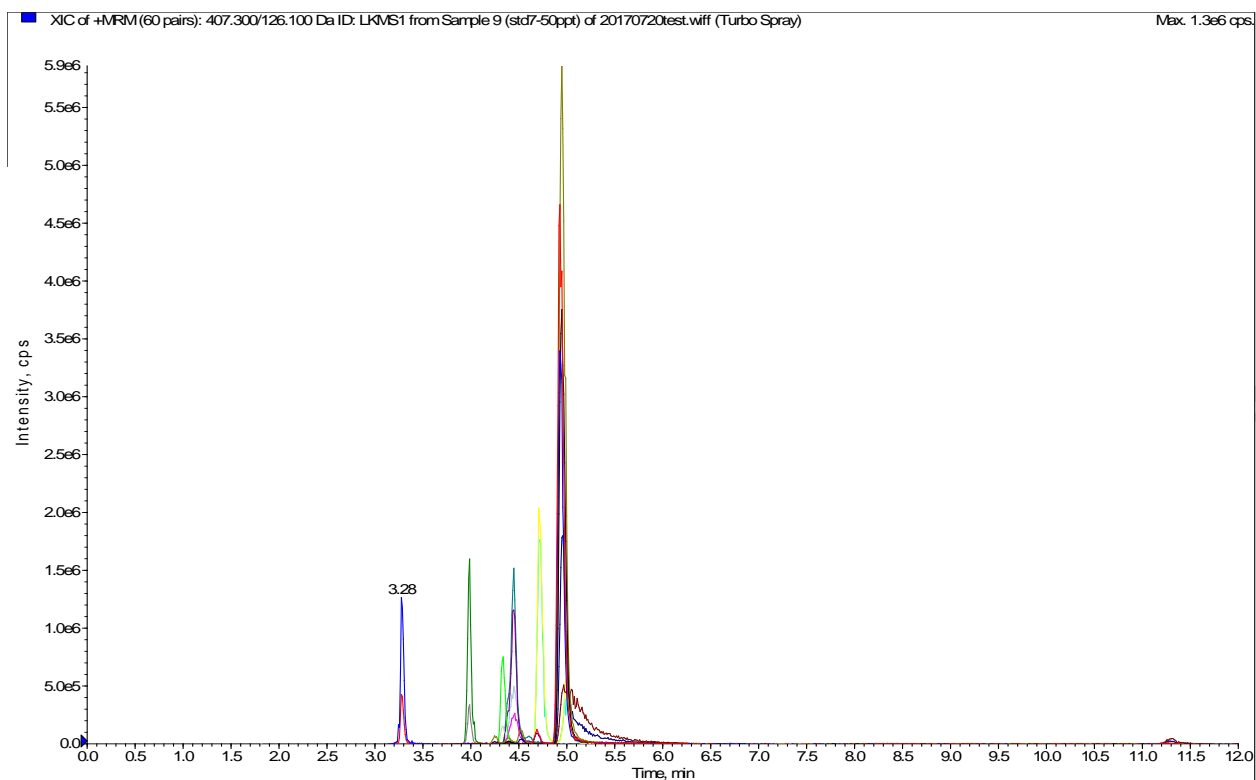


圖15 11種抗生素混標 (50.0ng/L) MRM圖

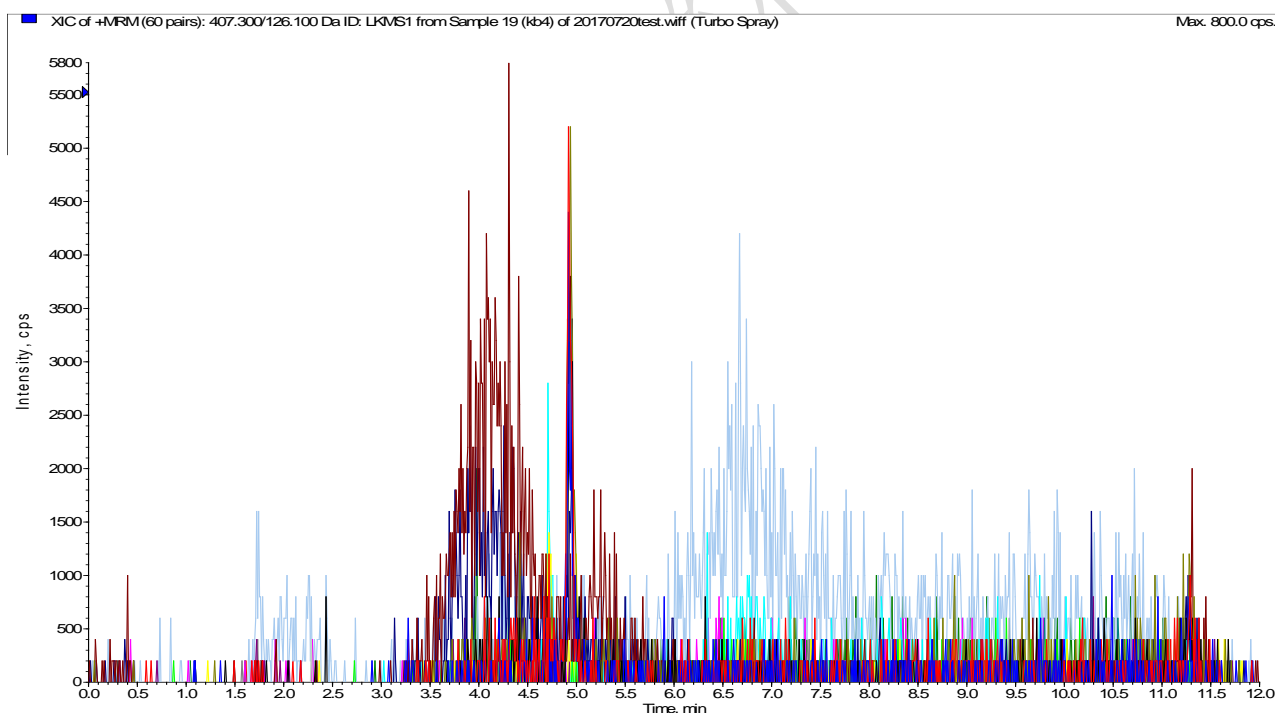


圖16 某空白水樣MRM圖

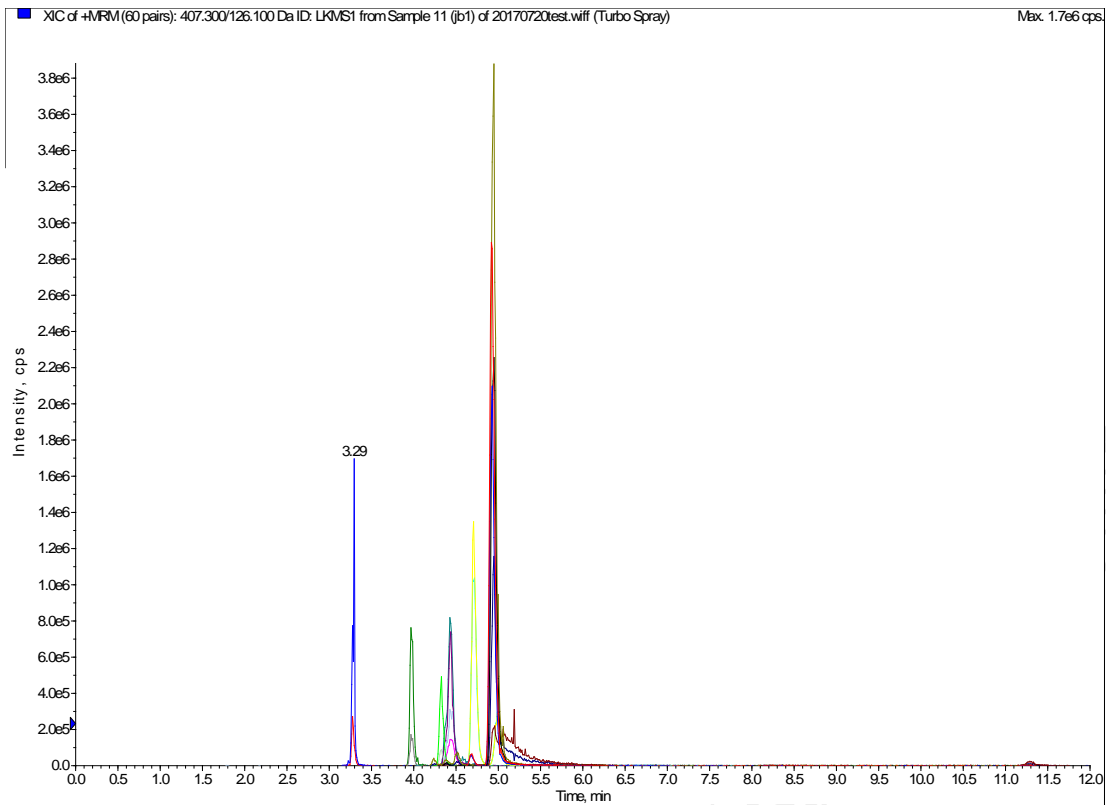


圖17 某空白水樣11種抗生素加標 (30.0ng/L) MRM圖

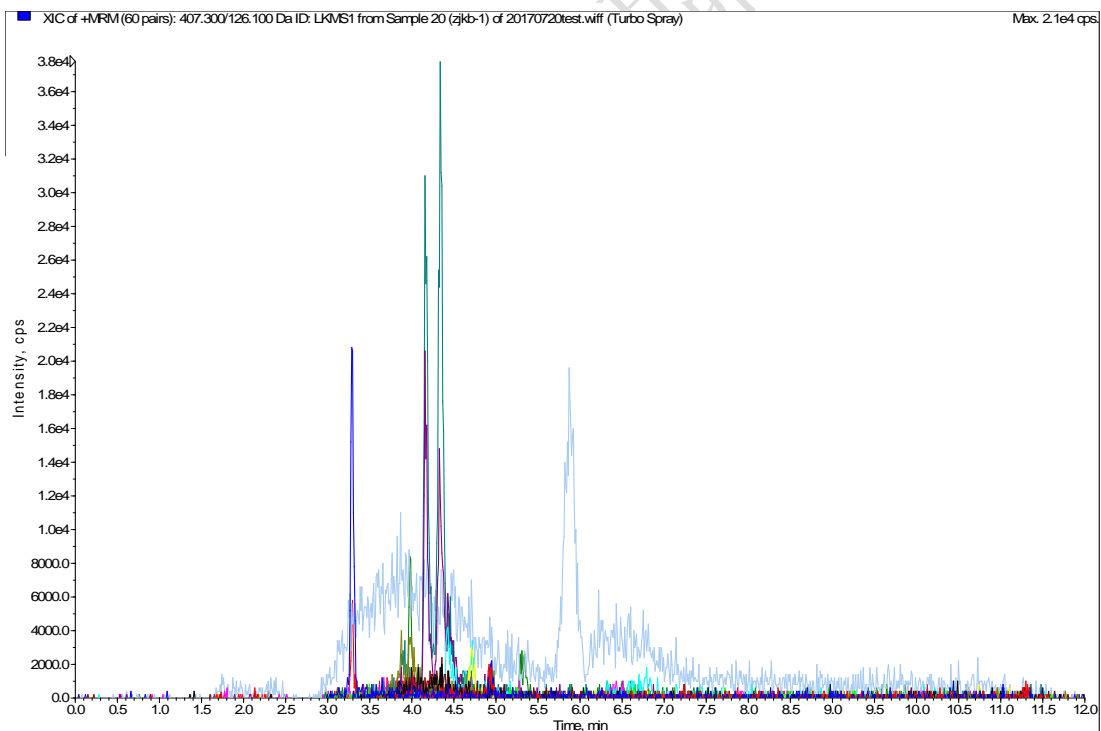


圖18 某基質陽性水樣MRM圖

(其中檢出阿奇黴素: 0.4ng/L, 紅黴素0.83ng/L, 克林黴素0.38ng/L)

5、重複性、檢出限、回收率

表3 某空白水樣加標11種抗生素的回收試驗 (n=6)

Compounds	Linear Range (ng/L)	Correlation coefficients	Added (ng /L)	Recoveries (%)	RSD (%)	LOD (ng/L)
Azithromycin	1.0-50.0	0.9992	30.0	96.52	3.17	0.1
Clarithromycin	1.0-50.0	0.9988	30.0	90.18	6.03	0.1
Erythromycin	1.0-50.0	0.9998	30.0	97.60	2.23	0.1
Lincomycin	1.0-50.0	0.9989	30.0	99.42	7.49	0.1
Roxithromycin	1.0-50.0	0.9995	30.0	98.45	2.87	0.1
VirginiamycinsM1	1.0-50.0	0.9995	30.0	98.86	2.81	0.5
VirginiamycinsS1	1.0-50.0	0.9996	30.0	101.4	4.98	0.5
Ometoprim	1.0-50.0	0.9993	30.0	96.49	3.52	0.1
Clindamycin	1.0-50.0	0.9991	30.0	99.06	2.09	0.1
Oleandomycin	1.0-50.0	0.9993	30.0	95.16	2.87	0.2
Tilmicosin	1.0-50.0	0.9980	30.0	94.31	7.48	0.5

本文採用線上SPE富集淨化的前處理、聯用LC-MS/MS檢測的方法，在優化後的實驗條件下，用基質空白水溶液配製系列濃度的標準溶液，以每種目標物定量例子的峰面積與濃度來繪製標準曲線。從表3可以看出11種抗生素的在對應的範圍內線性關係良好，相關係數均大於0.995；並對某空白水樣進行6次加標回收實驗，回收率在85%-110%之間，相對標準差皆小於10%，對應的11種抗生素檢出限（LOD，按S/N=3計算）在0.1-0.5ng/L之間，說明方法的靈敏度和精密度較好，適合水樣中多種抗生素殘留的同時檢測。

採用線上SPE的優點：A實現線上SPE和自動進樣功能，大大節省人力勞動；B實現線上富集，節省離線SPE耗材，節省成本；C上一個樣品在LC-MS/MS分析時，自動完成下一個樣品SPE柱的再生、上樣、淨化、富集，大大提高了系統的通量。