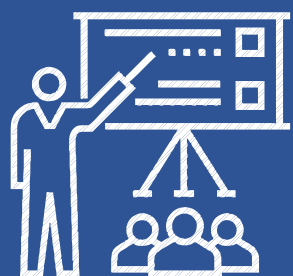


Chinese Medicine Regulatory Office
Department of Health
衛生署中醫藥規管辦公室



Analysis of Chemical Markers of
Chinese Materia Medica (CMM) in
Medicinal Oil for External Use
外用藥油中中藥材指標成分的分析



內容



方法介紹



標準品及樣本制備



儀器設置及數據分析



Q & A

 GCMTI RD-1:2019

GCMTI method publi




Determination of α -Pinene, Eucalyptol, Menthol and Methyl Salicylate in Chinese Medicinal Oil for External Use by Gas Chromatography

 GCMTI RD-2:2020


GCMTI method publi



Determination of Borneol in Chinese Medicinal Oil for External Use by Gas Chromatography

 GCMTI RD-3:2020

GCMTI method publications



Identification of Cinnamaldehyde, Citronellal, Eugenol, Linalool, Linalyl Acetate and Thymol in Chinese Medicinal Oil for External Use by Gas Chromatography

<https://www.cmro.gov.hk/html/b5/GCMTI/acmcmmmoou.html>



給市民的資訊 給中醫業界的資訊 給中藥業界的資訊

- ▶ 主頁
- ▶ 重要資訊
- ▶ 關於我們
- ▶ 政府中藥檢測中心
- ▶ 世衛傳統醫藥合作中心
- ▶ 中成藥生產質量管理規範
- ▶ 網上資源
- ▶ 健康資訊及活動
- ▶ 相關網頁
- ▶ 聯絡我們



政府中藥檢測中心

研究成果

性狀及顯微鑒別

- 香港容易混淆中藥的性狀及顯微鑒別研究

生物科技及化學

- 外用藥油中中藥材指標成分的分析

測試方法

http://www.cmro.gov.hk/html/b5/GCMTI/results_index.html

外用藥油中中藥材指標成分的分析

	有效成分 Active ingredient	指標成分 Chemical markers
第一階段 Phase I <ul style="list-style-type: none"> 儀器: GC-FID 建立及確認定性和定量分析5種指標成分的方法 方法: GCMTI RD-1:2019 	樟腦 Camphor	樟腦 Camphor
	桉油 Eucalyptus oil	桉油精 Eucalyptol
	薄荷腦 Menthol	薄荷腦 Menthol
	冬青油 Methyl Salicylate	水楊酸甲酯 Methyl Salicylate
	松節油 Turpentine oil	α -蒎烯 α -Pinene
第二階段 Phase II <ul style="list-style-type: none"> 儀器: GC-FID, GC-MS or GC-MS/MS 建立及確認定性分析7種指標成分的方法 方法: GCMTI RD-2:2020 & RD-3:2020 	冰片 Borneolum	龍腦 Borneol
	肉桂 Cinnamon / 肉桂油 Cinnamon oil	桂皮醛 Cinnamic aldehyde
	香茅 Citronella / 香茅油 Citronella oil	香茅醛 Citronellal
	丁香 Clove / 丁香油 Clove oil	丁香酚 Eugenol
	薰衣草油 Lavender oil	芳樟醇 Linalool、 乙酸芳樟酯 Linalyl acetate
	百里香油 Thyme oil	百里香酚 Thymol

【中成藥註冊】

品質性資料

技術指引



外用藥油中中藥材指標成分的分析

GCMTI RD-1:2019 & RD-2:2020

- 儀器：GC-FID
- 建立及確認定性和定量分析6種指標成分的方法

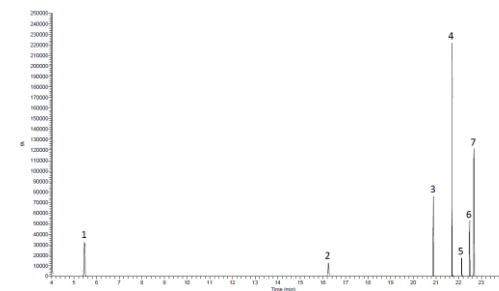
有效成分 Active ingredient	指標成分 Chemical markers
樟腦 Camphor	樟腦 Camphor
桉油 Eucalyptus oil	桉油精 Eucalyptol
薄荷腦 Menthol	薄荷腦 Menthol
冬青油 Methyl Salicylate	水楊酸甲酯 Methyl Salicylate
松節油 Turpentine oil	α -蒎烯 α -Pinene
冰片 Borneolum	龍腦 Borneol



【中成藥註冊】

品質性資料

技術指引

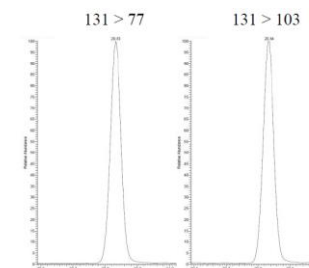
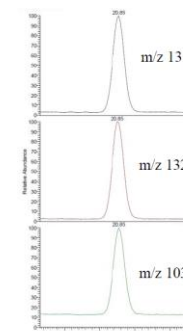
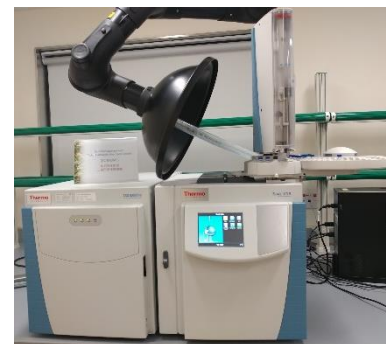


外用藥油中中藥材指標成分的分析

GCMTI RD-3:2020

- 儀器 : GC-MS or GC-MS/MS
- 建立及確認定性分析6種指標成分的方法

有效成分 Active ingredient	指標成分 Chemical markers
肉桂 Cinnamon / 肉桂油 Cinnamon oil	桂皮醛 Cinnamaldehyde
香茅 Citronella / 香茅油 Citronella oil	香茅醛 Citronellal
丁香 Clove / 丁香油 Clove oil	丁香酚 Eugenol
薰衣草油 Lavender oil	芳樟醇 Linalool、 乙酸芳樟酯 Linalyl acetate
百里香油 Thyme oil	百里香酚 Thymol



【中成藥註冊】

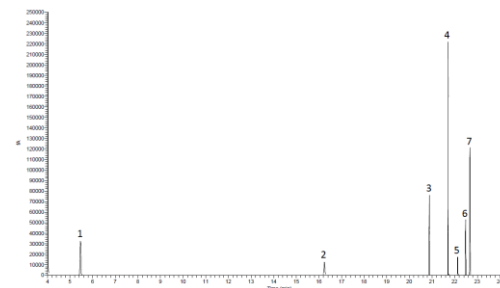
品質性資料

技術指引

外用藥油中中藥材指標成分的分析

• 特點

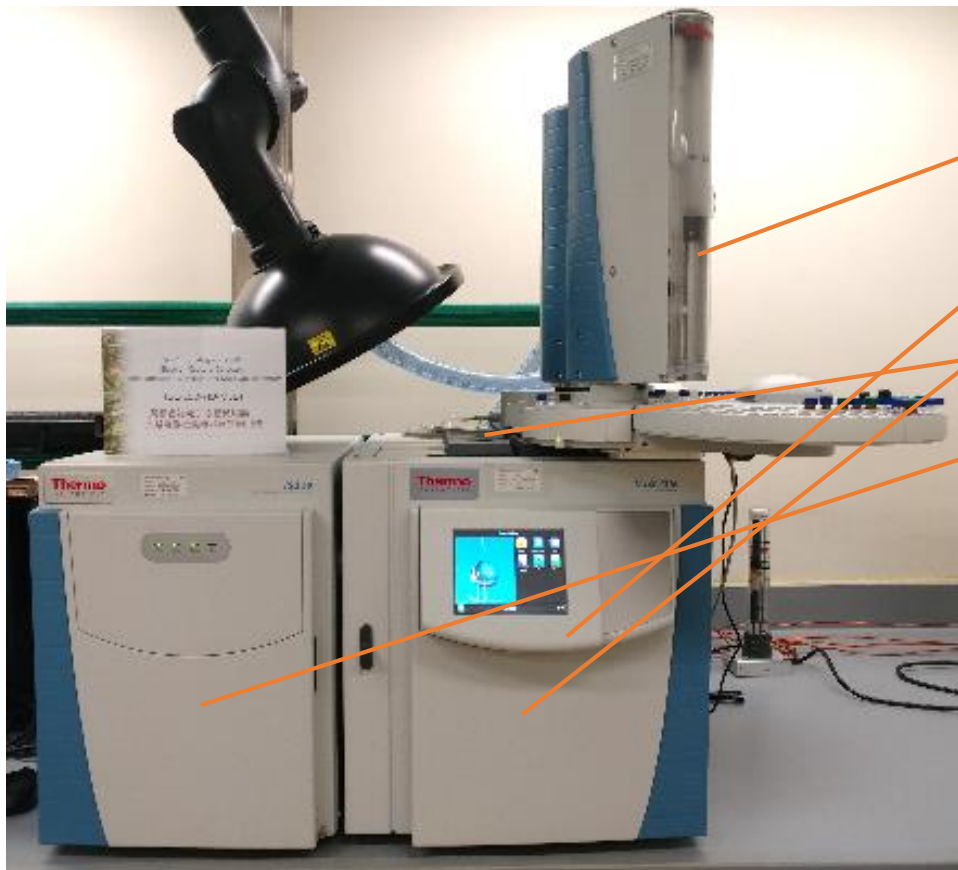
- ✓ 經驗證的一測多檢方法；
- ✓ 方法方便，簡單易用和普及；
- ✓ 提供雙色譜柱方法作選擇，可在分析外用藥油中化學指標成分時，在無法取得陰性對照樣本的情況下，證明方法的專屬性；
- ✓ 用先進儀器測試含量少於一個百分比的化學指標成分



氣相色譜火焰電離檢測器 GC-FID

氣相色譜質譜儀 GC-MS

氣相色譜串聯三重四極桿質譜儀 GC-MS/MS



部件

- 注射器 Injector
- 溫箱 Oven
- 色譜柱 Column
- 火焰電離檢測器 FID
- 質譜檢測器 MS or 三重四極桿質譜MS/MS

應用

- 分離與測定揮發性化學成分
- 藥物分析、食物檢測、環境監測.....

氣相色譜火焰電離檢測器 GC-FID

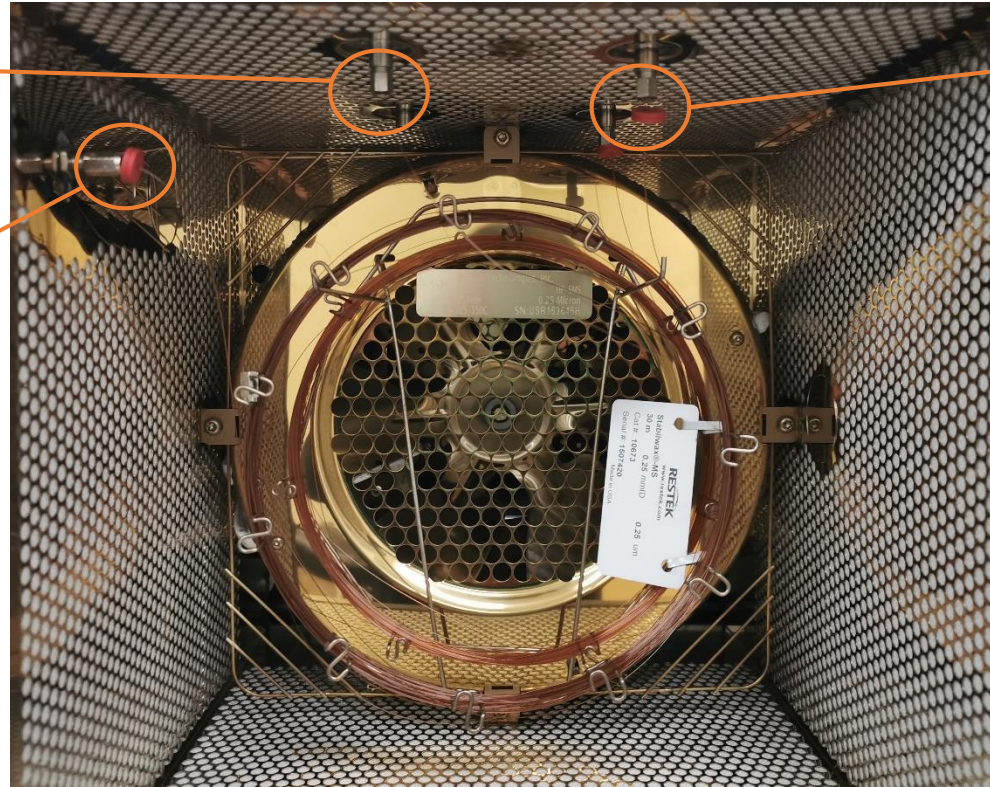
氣相色譜質譜儀 GC-MS

氣相色譜串聯三重四極桿質譜儀 GC-MS/MS

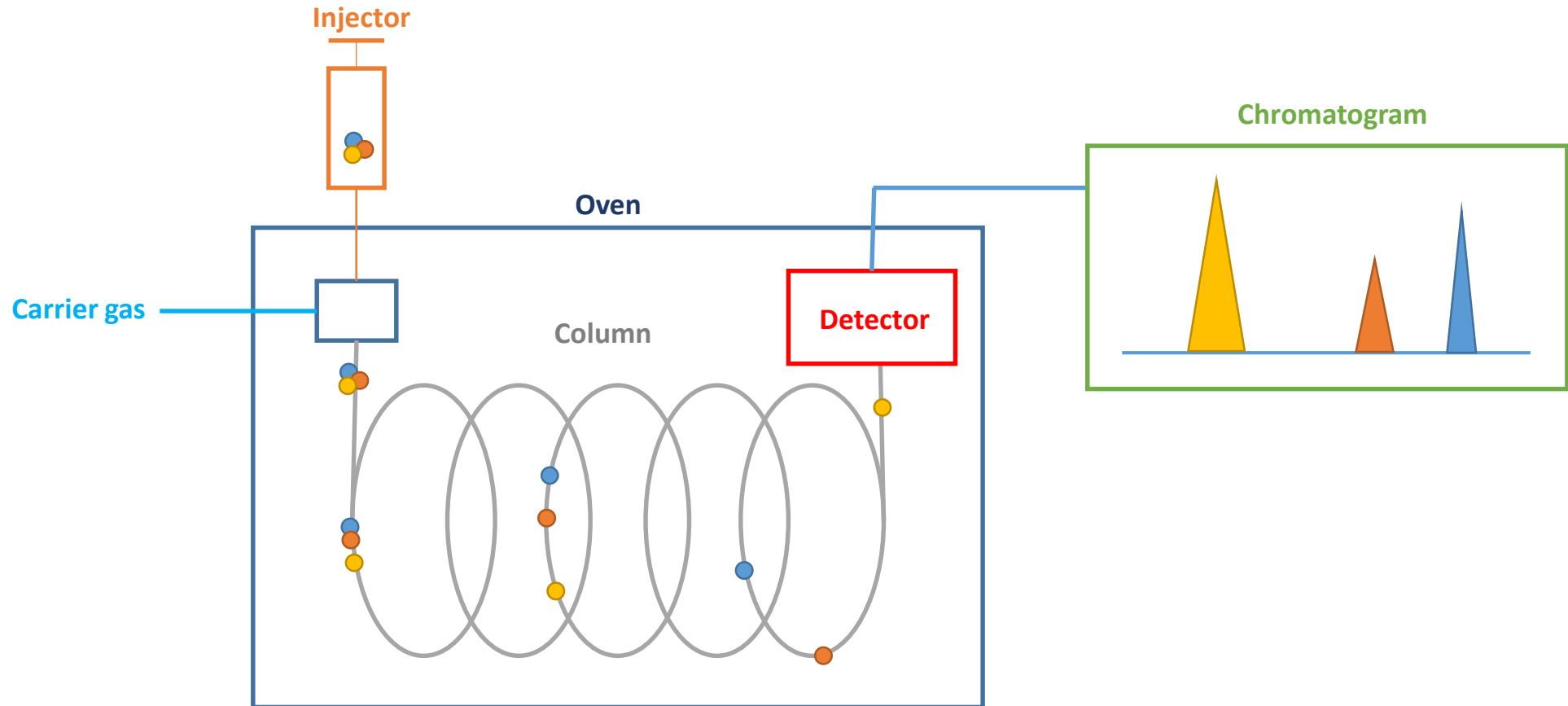
To FID

From inlet 進樣口

To MS or
MS/MS



GC 的原理



外用藥油中中藥材指標成分的分析

GCMTI RD-1:2019 & RD-2:2020

- 儀器：GC-FID
- 建立及確認定性和定量分析6種指標成的方法

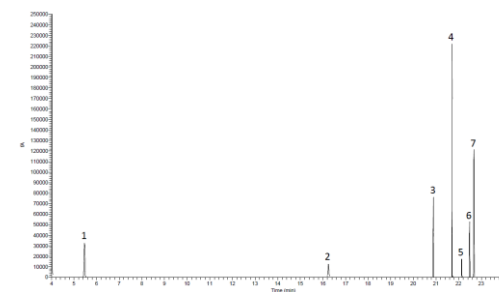
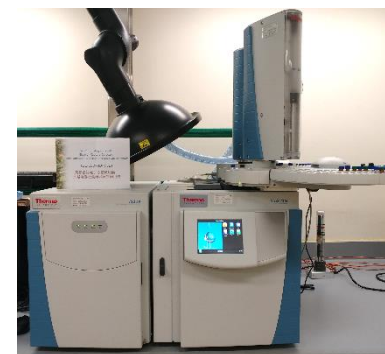
有效成分 Active ingredient	指標成分 Chemical markers
樟腦 Camphor	樟腦 Camphor
桉油 Eucalyptus oil	桉油精 Eucalyptol
薄荷腦 Menthol	薄荷腦 Menthol
冬青油 Methyl Salicylate	水楊酸甲酯 Methyl Salicylate
松節油 Turpentine oil	α -蒎烯 α -Pinene
冰片 Borneolum	龍腦 Borneol



【中成藥註冊】

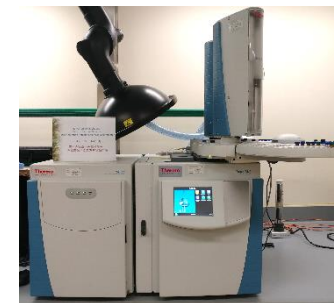
品質性資料

技術指引



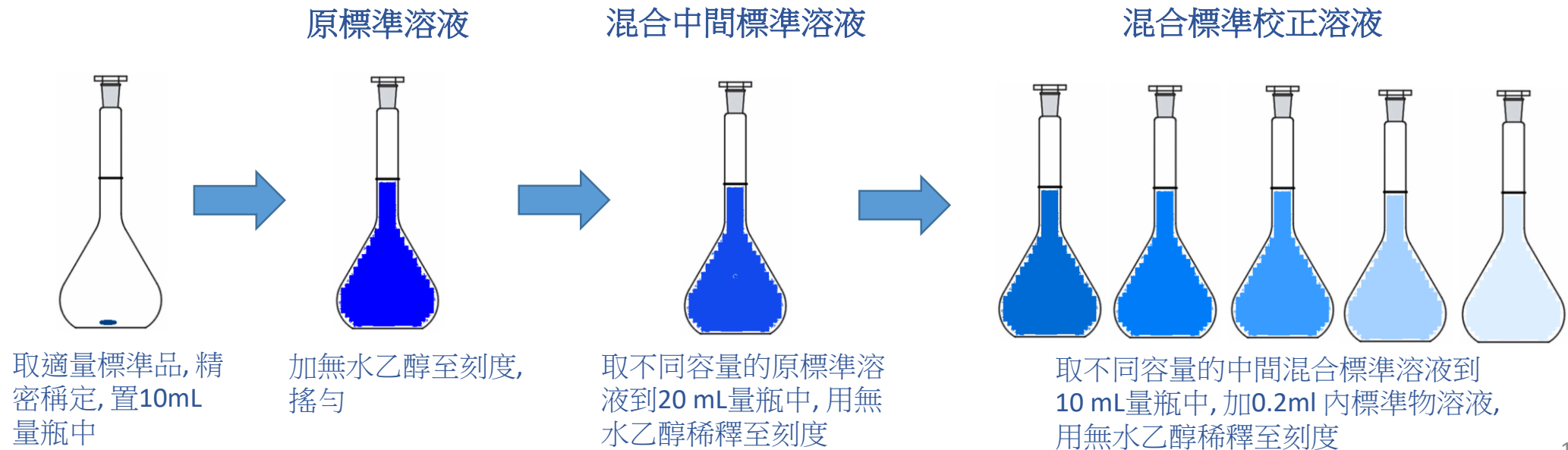
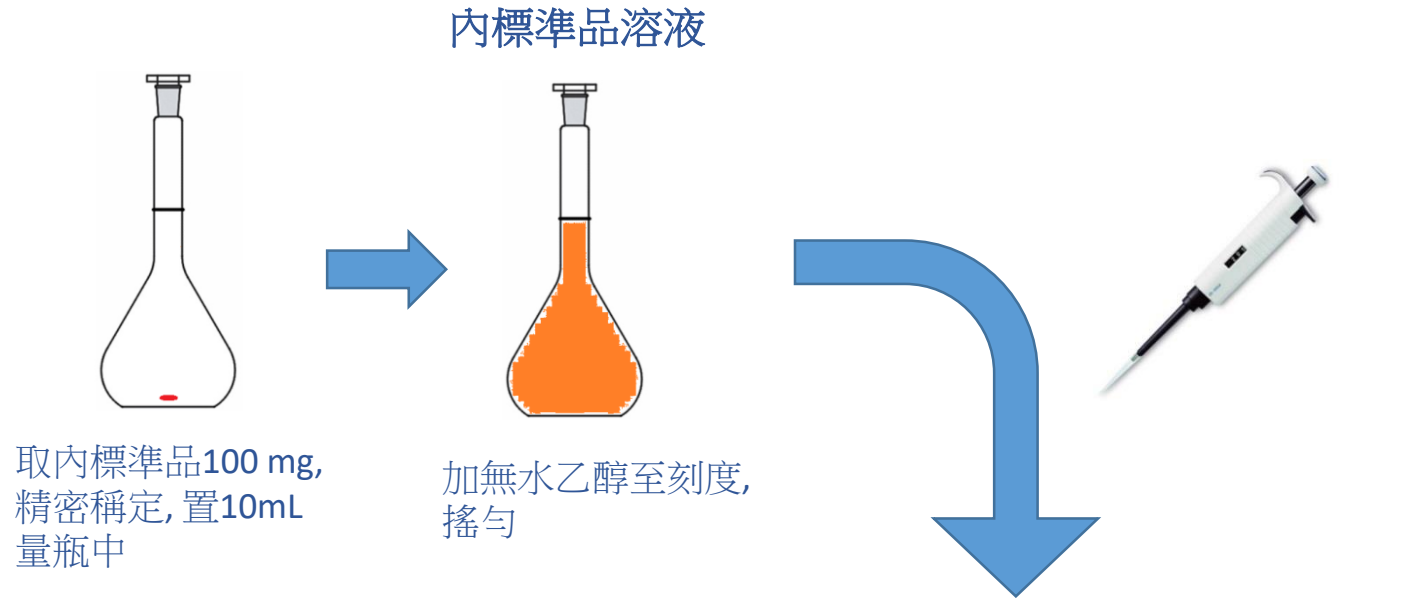
儀器 Apparatus

- 分析天平 Analytical balance
- 容量瓶 Volumetric flasks
- 移液管 Pipettes
- PTFE過濾膜 PTFE membrane filters
- 石英毛細管柱 Fused silica capillary column
- 氣相色譜火焰電離檢測器 GC-FID



標準品制備

- 標準品溶液製備

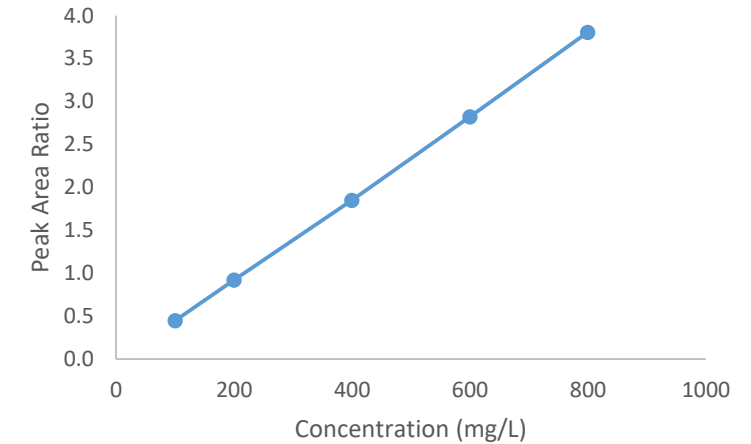


標準品制備

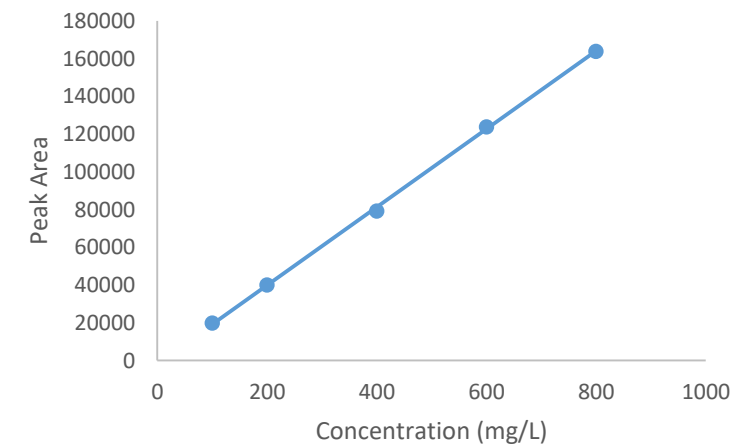
- 標準曲綫

Marker	Concentration (mg/L)				
	Level 1	Level 2	Level 3	Level 4	Level 5
α -Pinene	100	200	400	600	800
Eucalyptol	50	100	200	300	400
Camphor	100	200	400	600	800
Menthol	250	500	1000	1500	2000
Methyl salicylate	250	500	1000	1500	2000
Borneol	20	40	80	120	160

Internal standard calibration

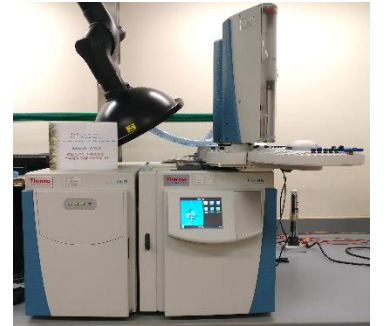
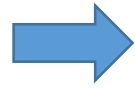
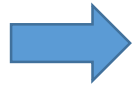


External standard calibration



樣品制備

- 樣品溶液製備



取樣本 100 mg, 精密稱定, 置 10 mL 量瓶中

加無水乙醇至刻度, 搖勻

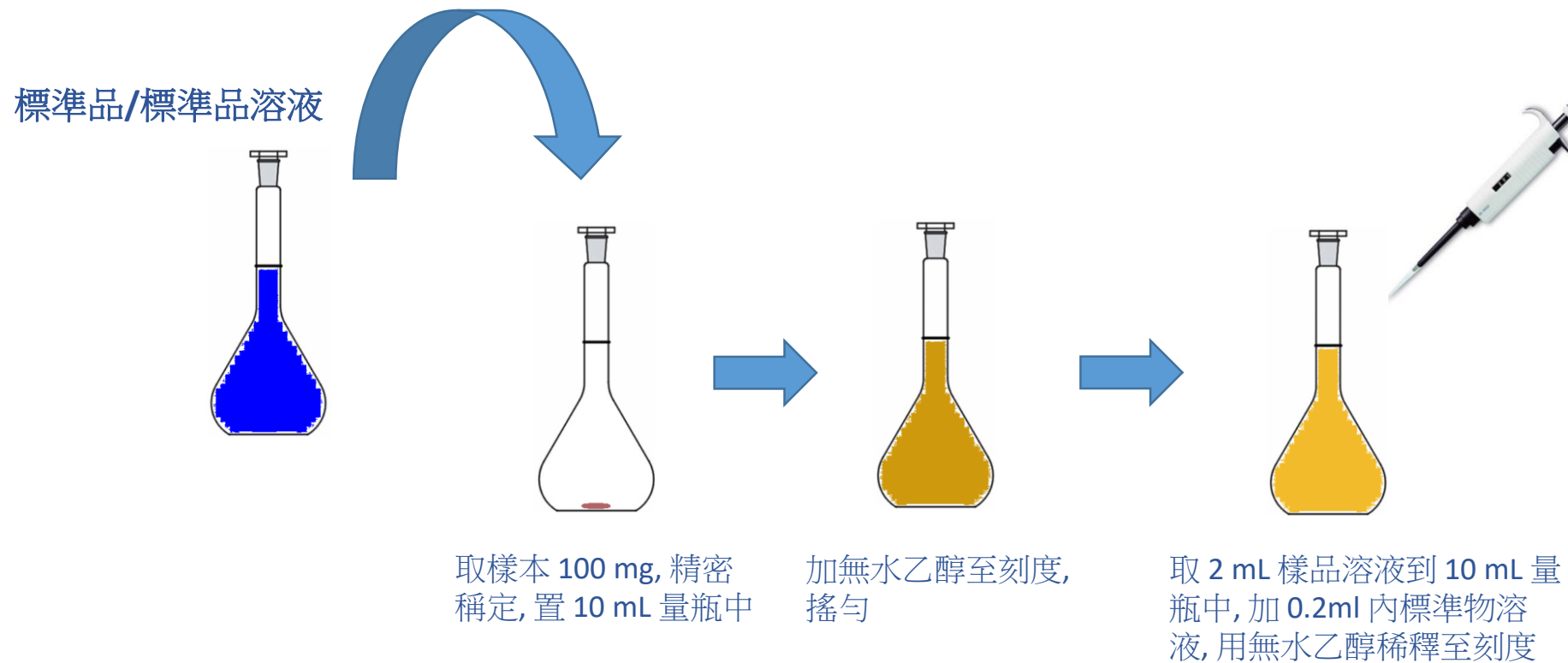
取 2 mL 樣品溶液到 10 mL 量瓶中, 加 0.2ml 內標準物溶液, 用無水乙醇稀釋至刻度

經 0.45 μm 微膜過濾至 GC vial

GC-FID 分析

樣品制備

- 樣品加標溶液製備



儀器設置



- 色譜柱

	Column	Dimension	Stationary Phase
1	Restek Stabilwax-MS	0.25 mm ID x 30 m x 0.25 μ m	Polyethylene glycol (PEG)
2	Agilent Technologies HP-5MS	0.25 mm ID x 60 m x 0.25 μ m	5% Phenyl Methylpolysiloxane

- GC-FID設置

Injection volume	1 μ L	
Injection mode	Split mode, split ratio 50:1	
Column flow rate	1.5 mL/min	
Temperature programme	Column 1: 40°C for 15 min, then 20°C/min to 190°C for 4 min (Total run time 26.5 min)	Column 2: 65°C for 5 min, 1°C/min to 80°C for 0 min, 20°C/min to 190°C for 3 min (Total run time 28.5 min)

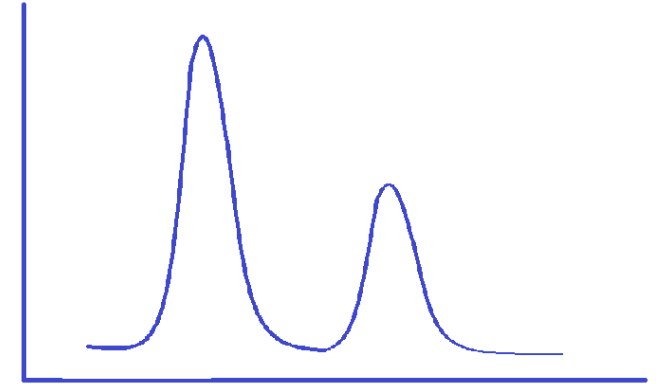
數據分析

- 定性分析

$$RRT = \frac{\text{Retention time (RT) of analyte peak}}{\text{Retention time (RT) of naphthalene peak}}$$

- 定量分析

$$\text{Content (mg/g) of the analyte in sample} = \frac{C \times V \times D}{1000 \times W}$$



雙色譜柱

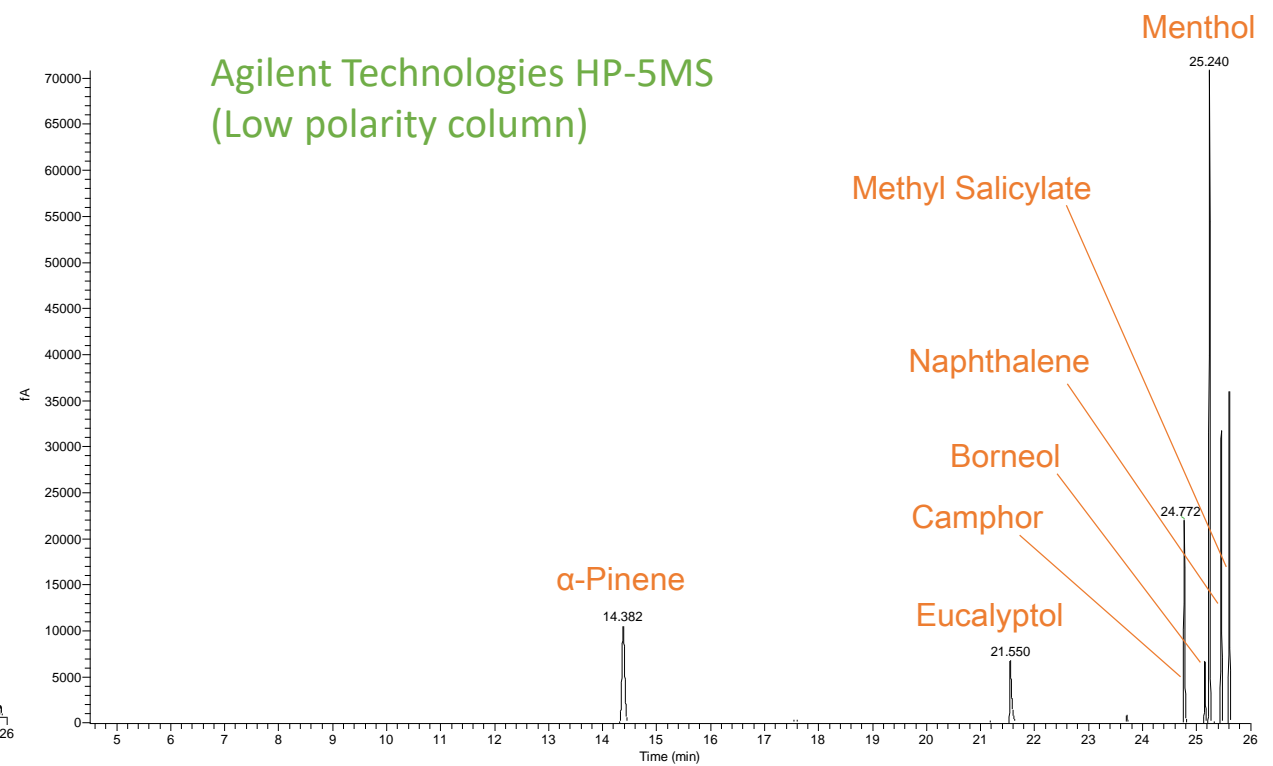
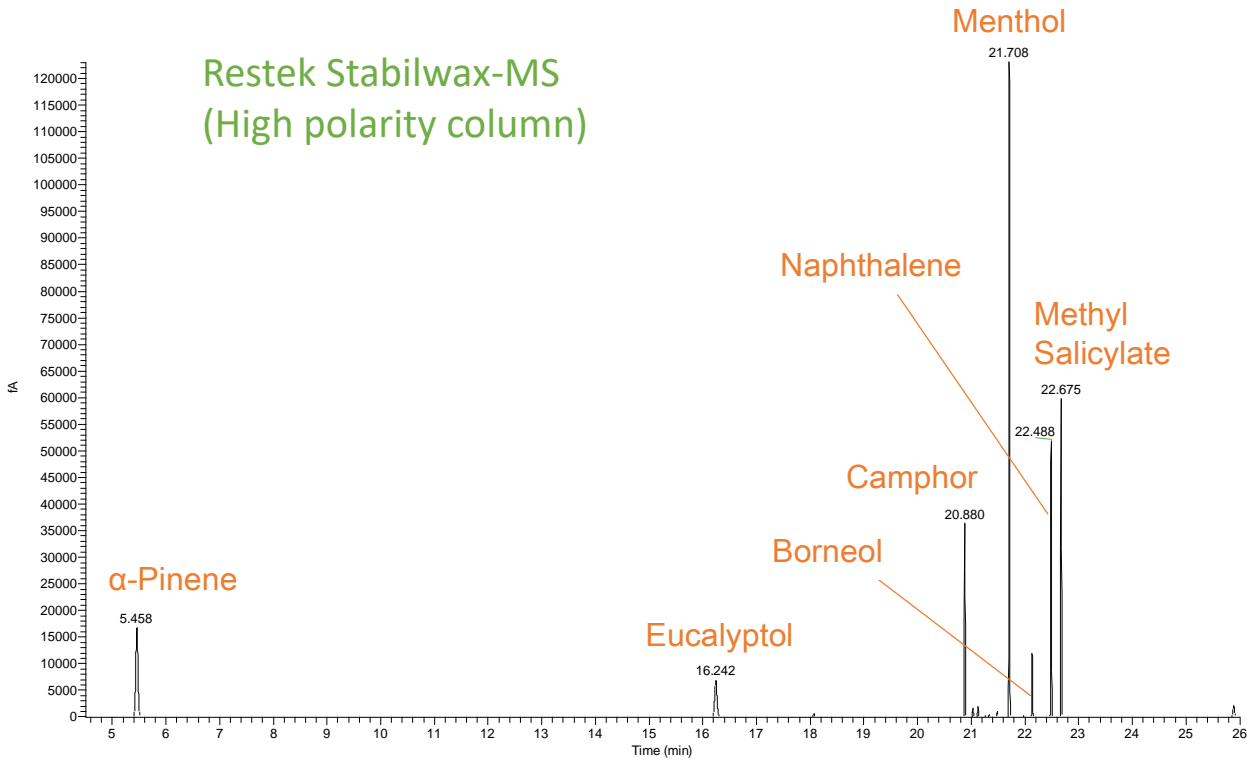
- 提供另一方法選擇
- 證明方法專屬性
 - ✓ 不同色譜柱，不同極性 → 指標成分出峰時間不同
 - ✓ 不同色譜柱測試結果一致



	Column	Dimension	Stationary Phase	Polarity
1	Restek Stabilwax-MS	0.25 mm ID x 30 m x 0.25 μ m	Polyethylene glycol (PEG)	High
2	Agilent Technologies HP-5MS	0.25 mm ID x 60 m x 0.25 μ m	5% Phenyl Methylpolysiloxane	Low

雙色譜柱

兩條色譜柱的出峰時間不同



方法適用性

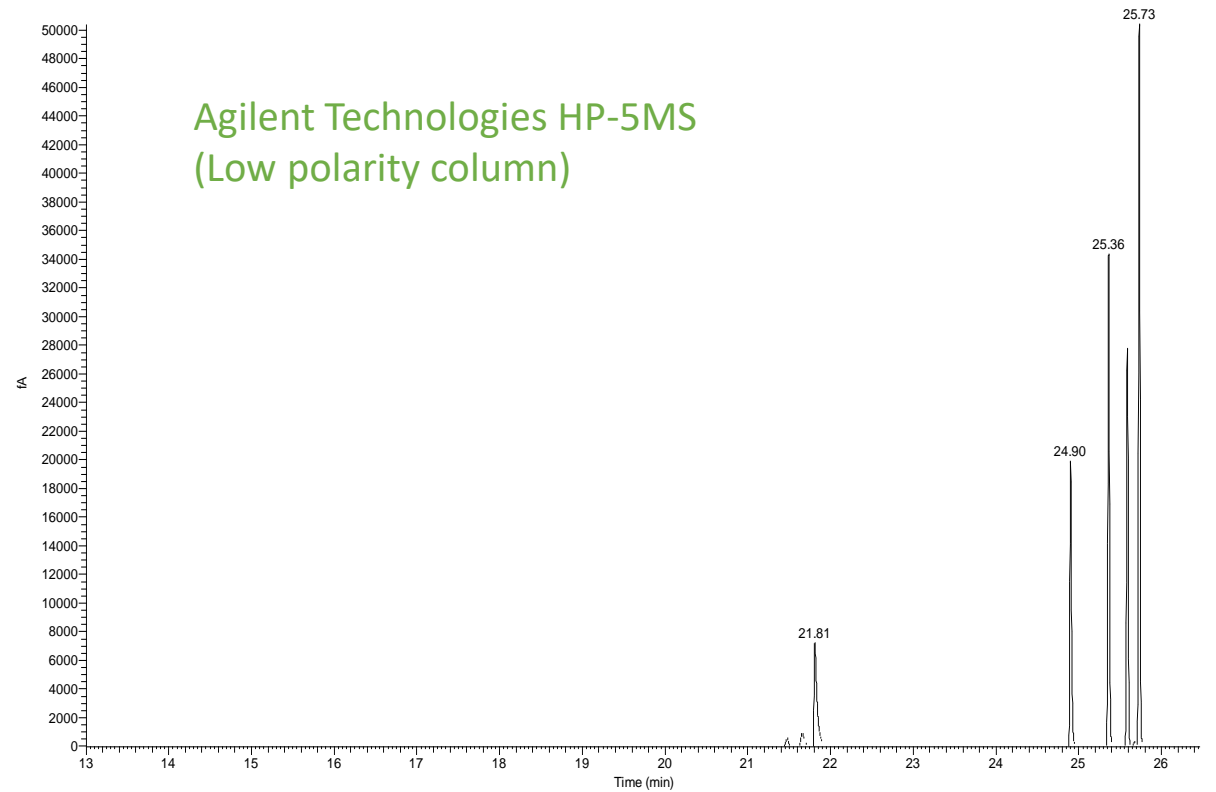
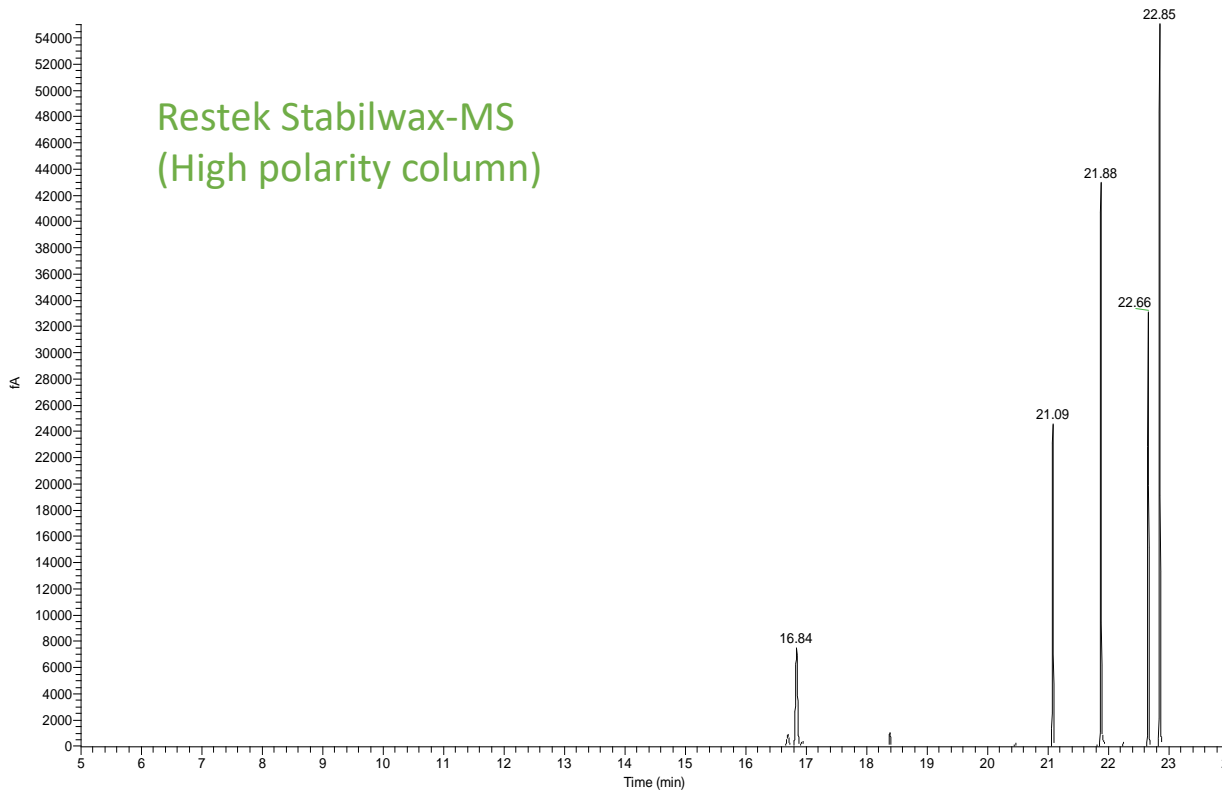
- 目的
 - ✓ 購買市面上已獲中成藥註冊的藥油，用建立的方法進行分析，測試方法對不同樣本的適用性
- 結果: 不同色譜柱的結果比較

Marker	Precision RSD (%)	
	Internal standard calibration	External standard calibration
α -Pinene	2.11	2.91
Eucalyptol	0.89	1.14
Camphor	1.46	2.37
Menthol	1.20	2.69
Methyl salicylate	1.54	3.23
Borneol	0.90	2.88

方法適用性

- **Example 1**

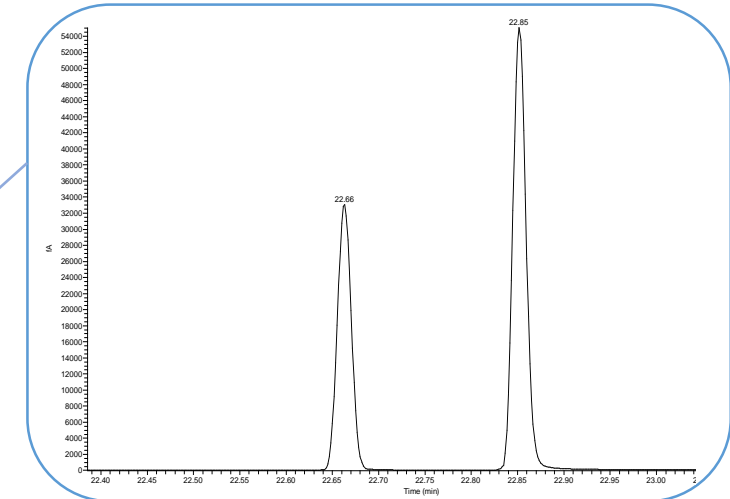
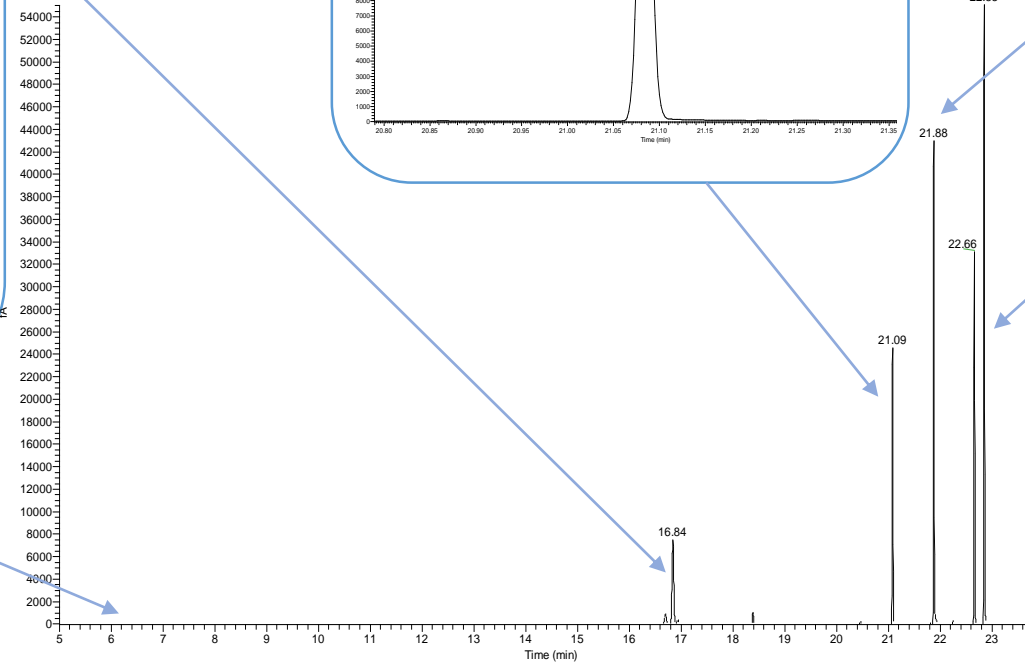
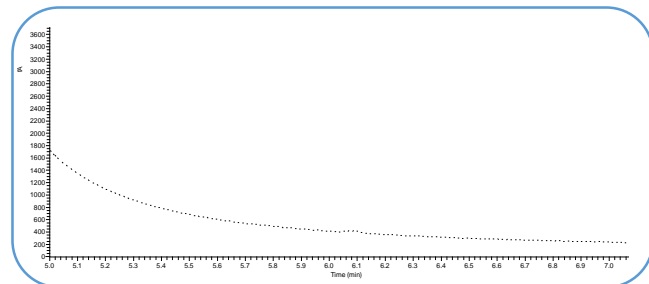
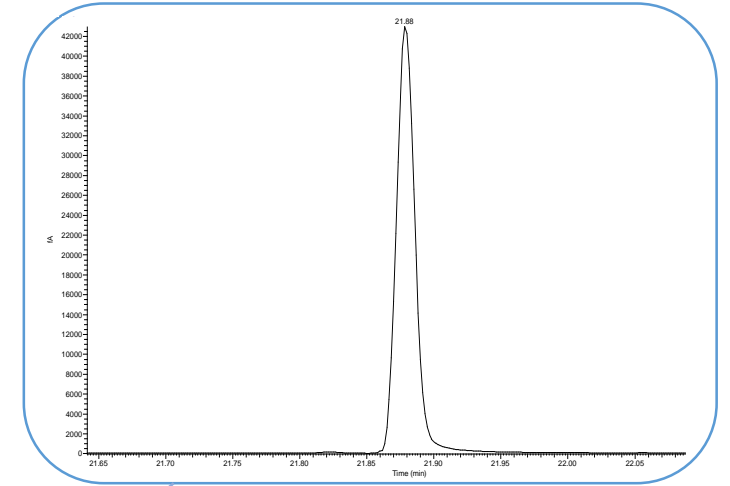
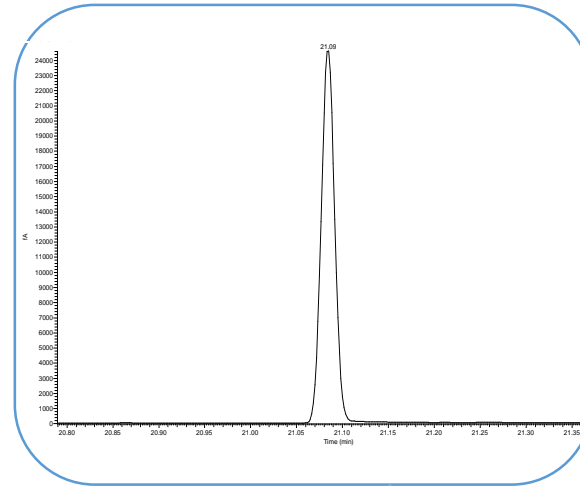
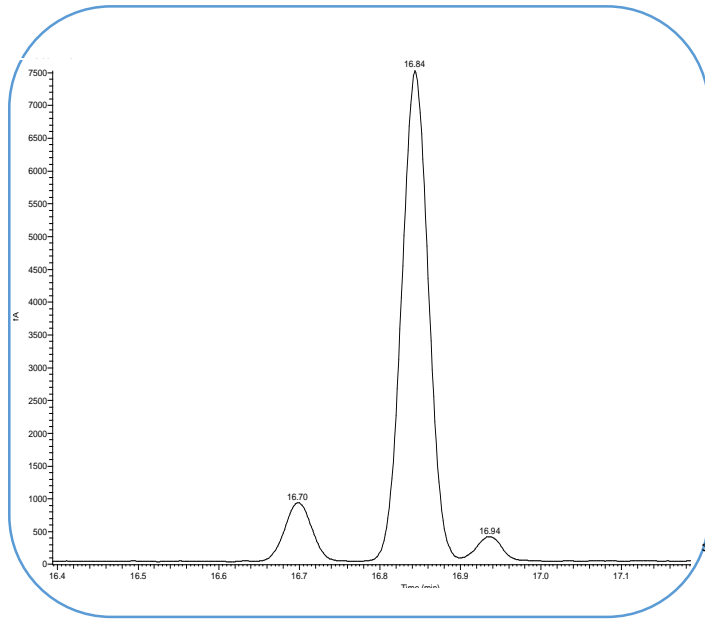
➤ 有效成分：乳香，當歸，沒藥，肉桂，桉油，大黃，生川烏，薄荷腦，紅花，連翹，莪朮，水楊酸甲酯等



方法適用性

- Example 1

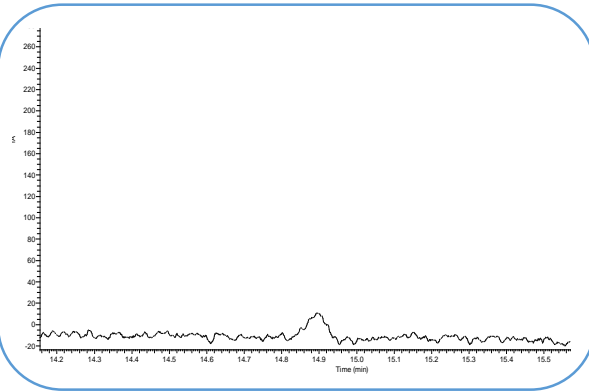
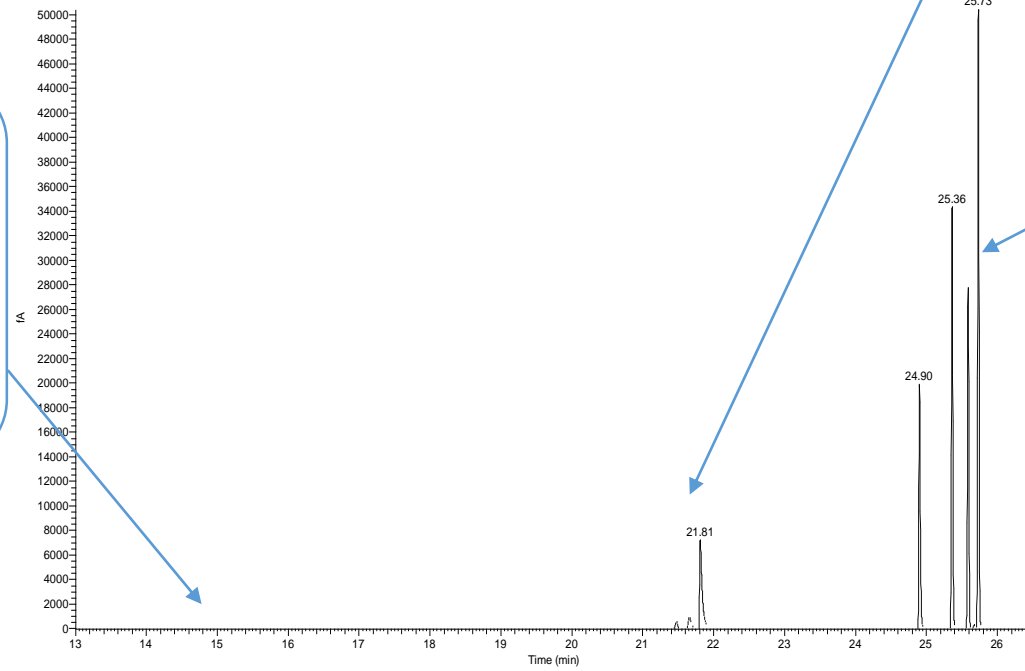
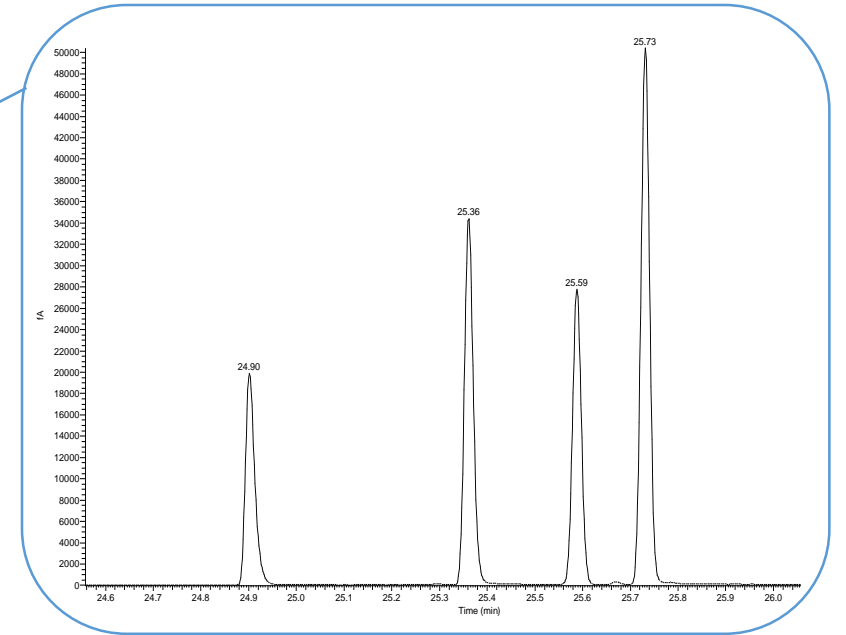
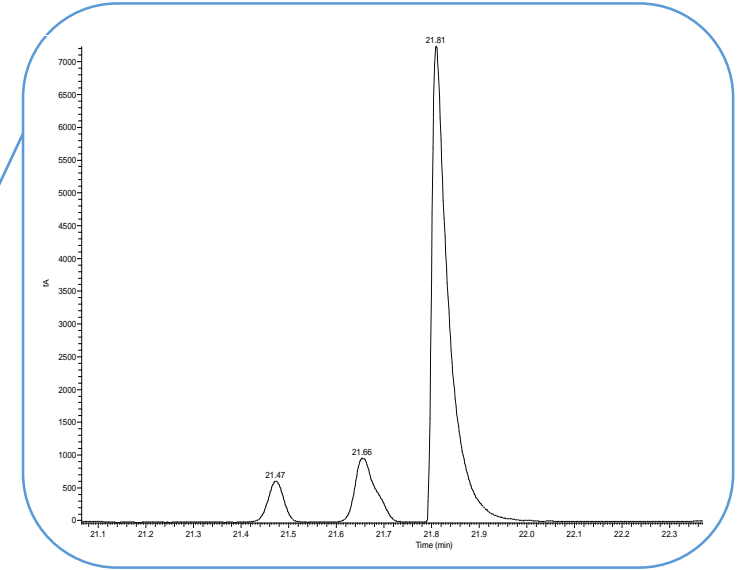
Restek Stabilwax-MS (High polarity column)



方法適用性

- Example 1

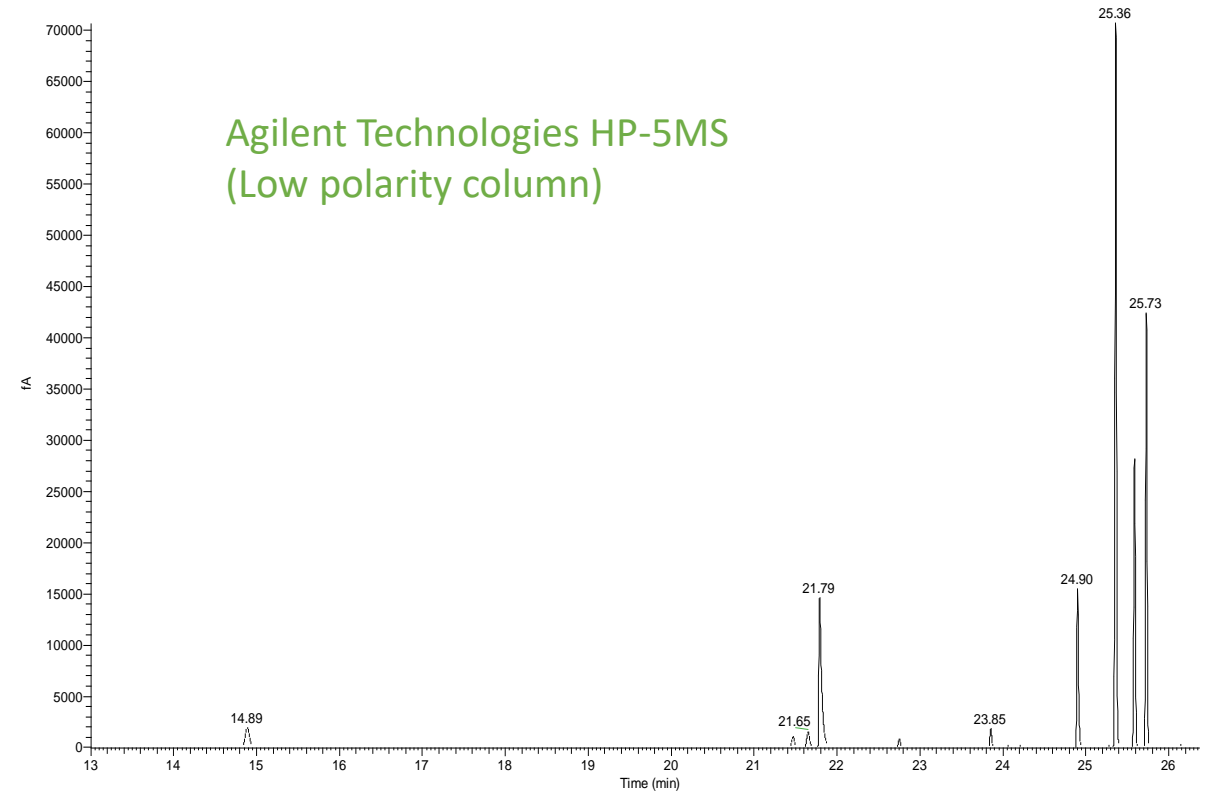
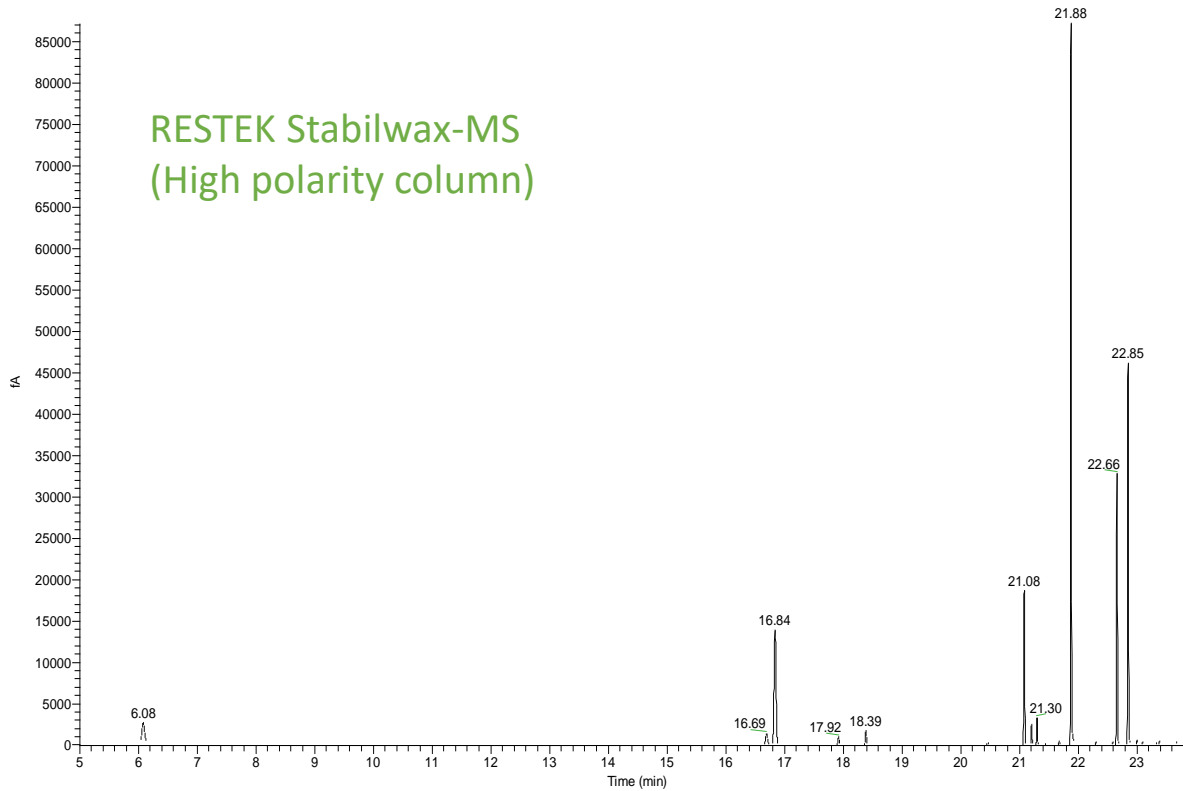
Agilent Technologies HP-5MS
(Low polarity column)



方法適用性

- Example 2

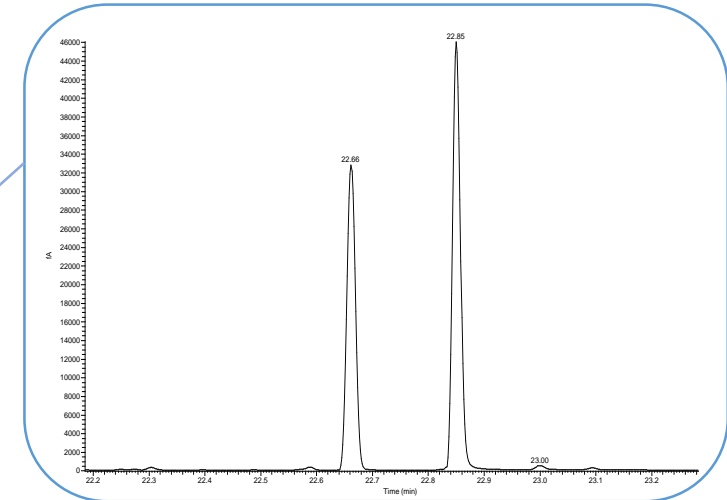
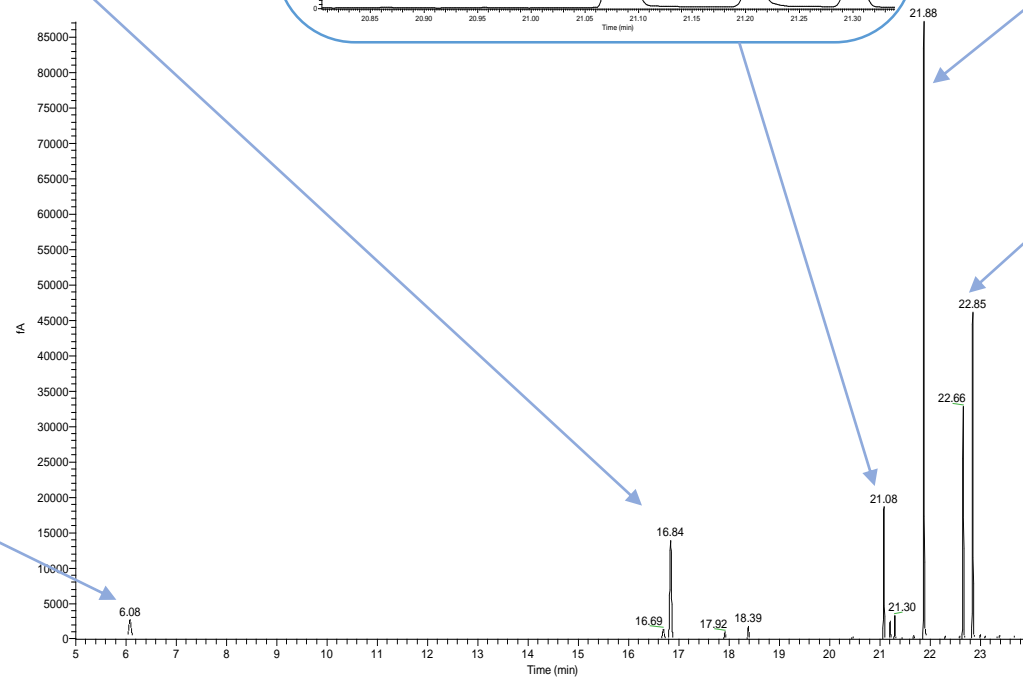
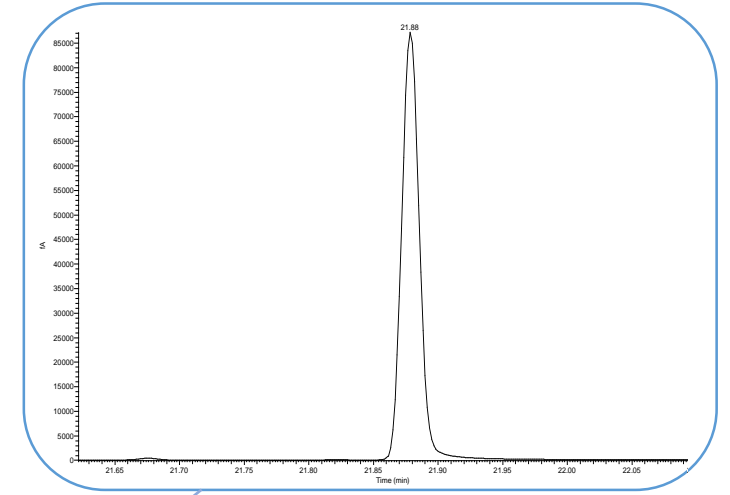
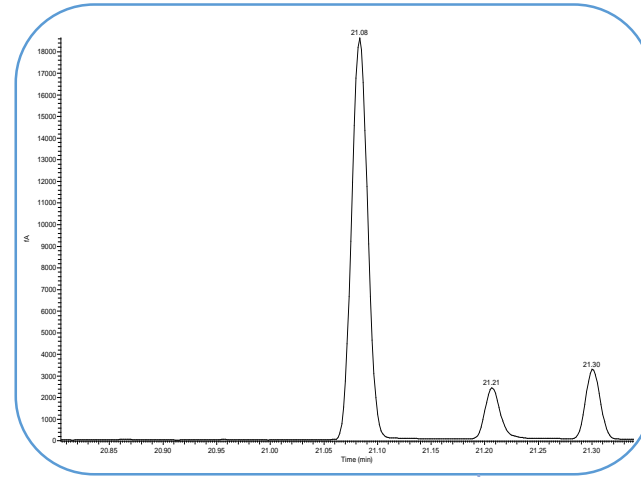
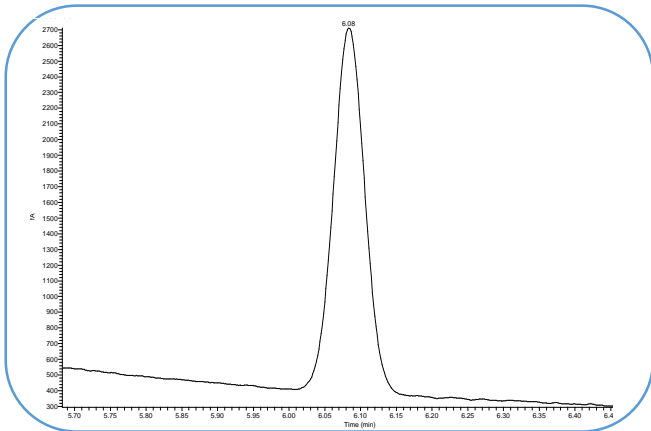
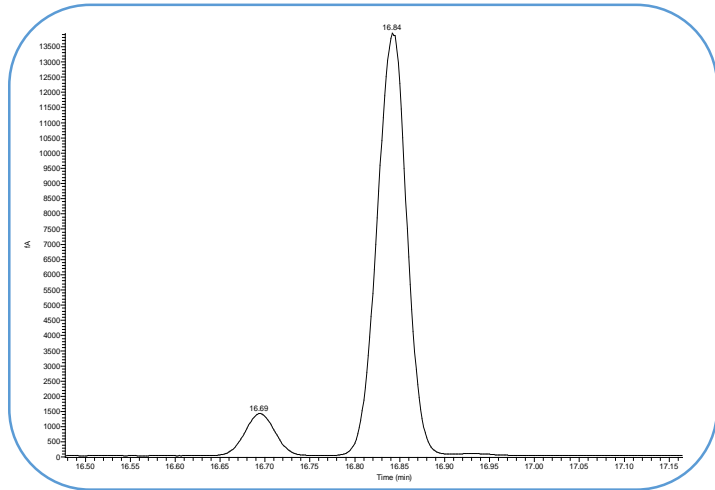
➤ 有效成分：三七，冬青油，薰衣草油，樟腦，生川烏，生天南星，生附子，生半夏，薄荷腦，松節油，桉葉油等



方法適用性

- Example 2

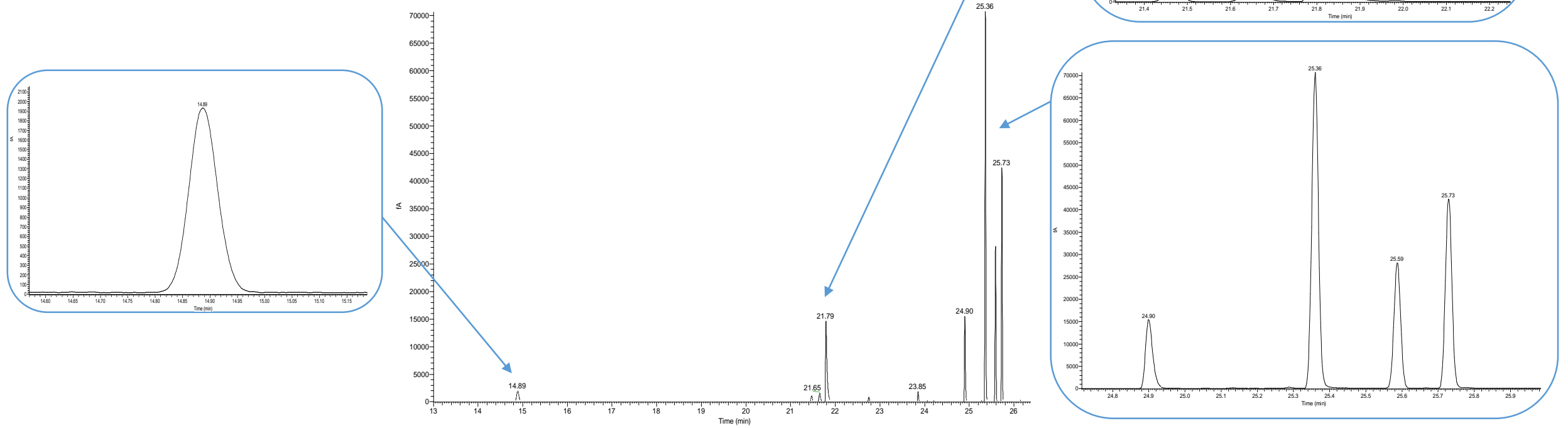
RESTEK Stabilwax-MS
(High polarity column)



方法適用性

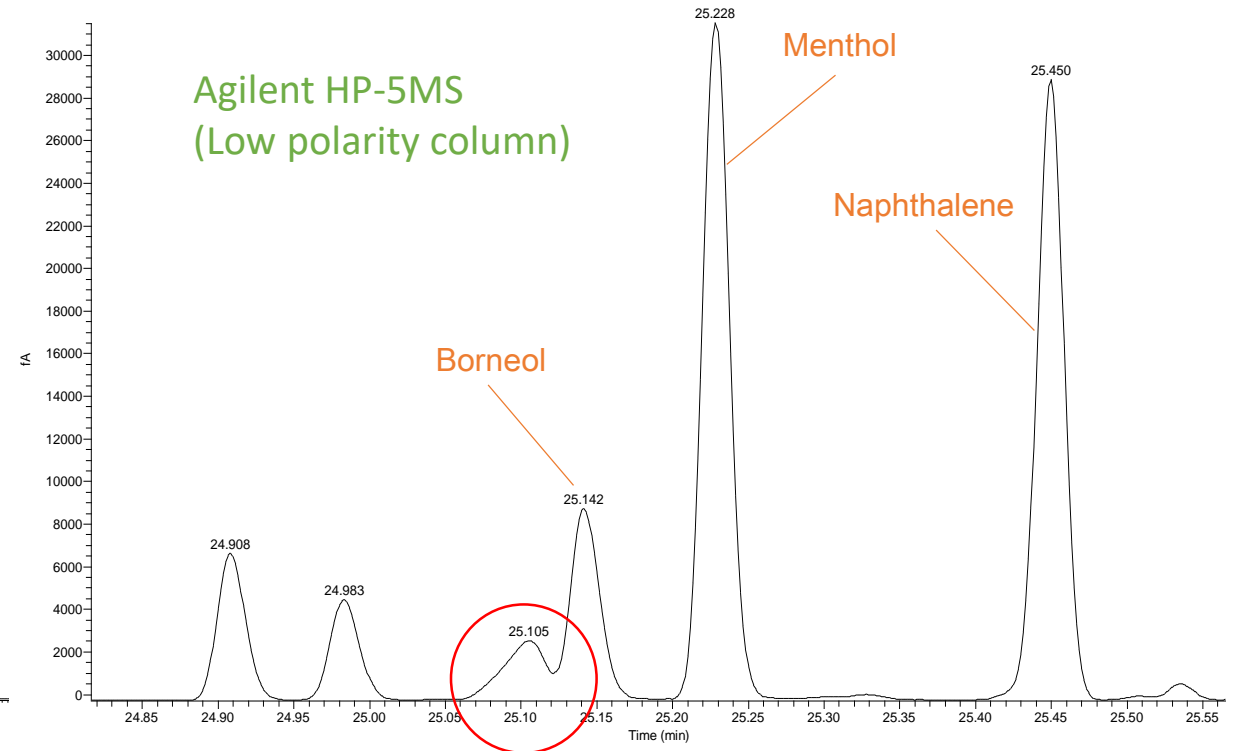
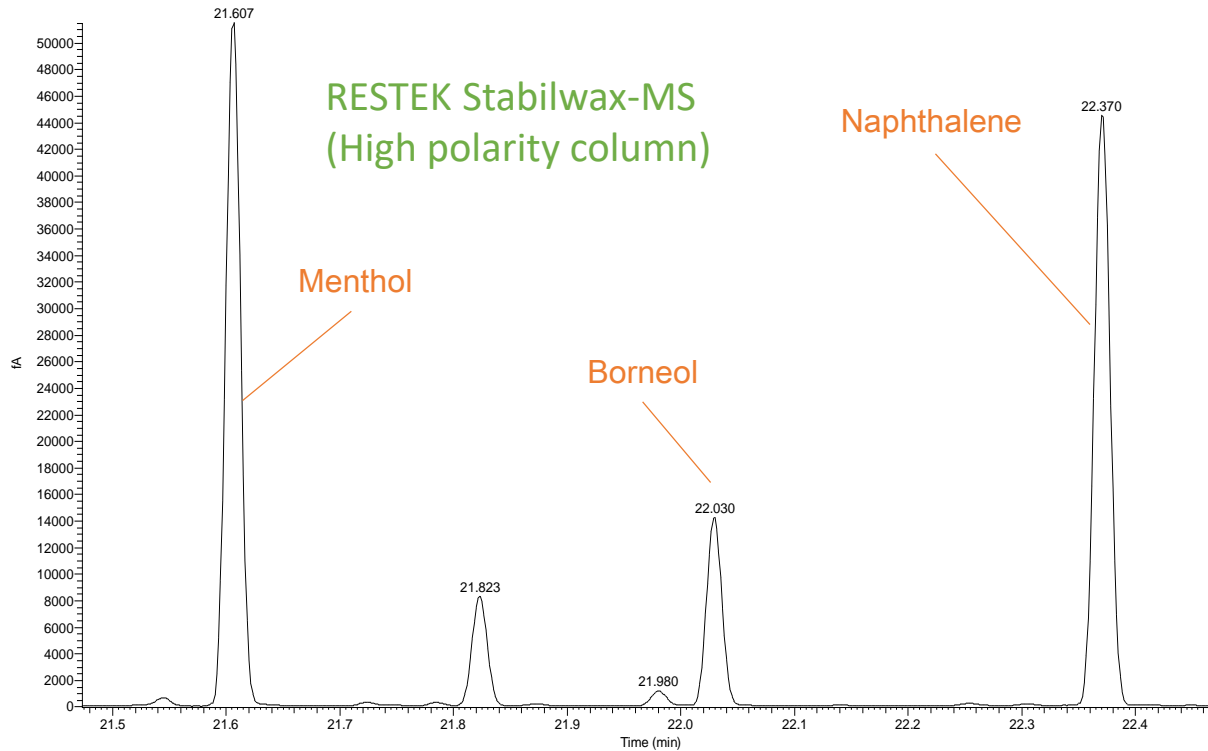
- Example 2

Agilent Technologies HP-5MS
(Low polarity column)

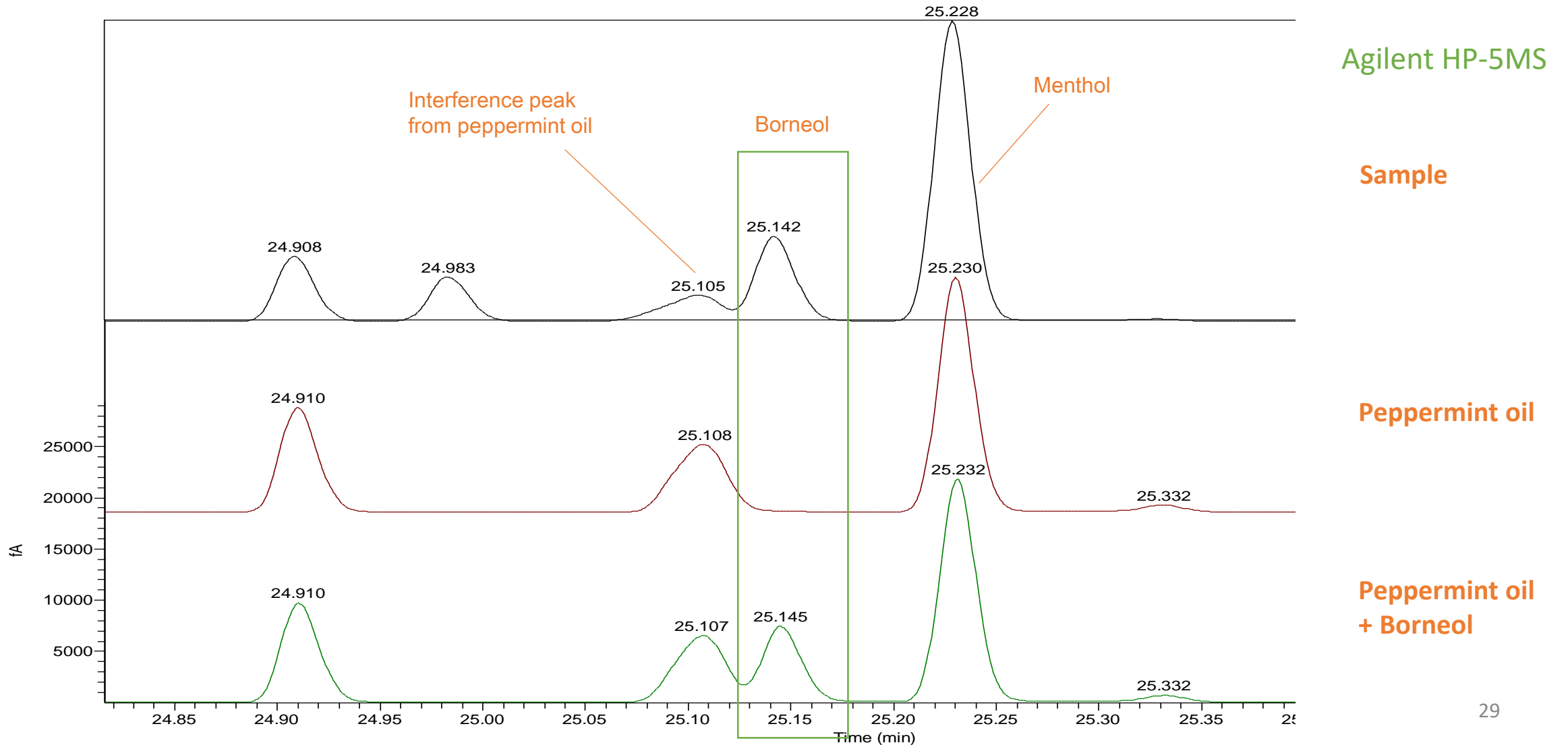


方法適用性

Interference peak from was observed for analysis of borneol using Agilent column in some samples



方法適用性



外用藥油中中藥材指標成分的分析

GCMTI RD-1:2019 & RD-2:2020

- 儀器 : GC-FID
- 指標成分: 6種 (Camphor, Eucalyptol, Menthol, Methyl Salicylate, α -Pinene, Borneol)
- 特點
 - ✓ 經驗證的一測多檢方法
 - ✓ 方法方便，簡單易用和普及
 - ✓ 提供雙色譜柱方法作選擇

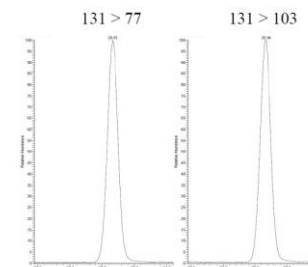
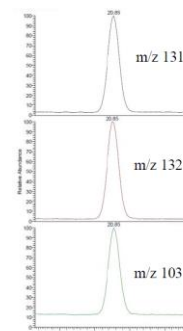
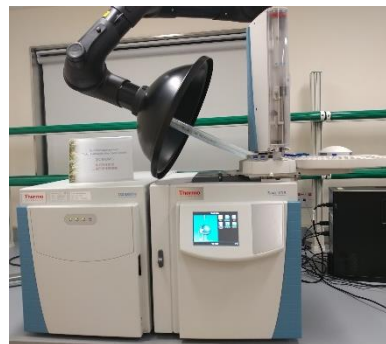
有效成分 Active ingredient	指標成分 Chemical markers
樟腦 Camphor	樟腦 Camphor
桉油 Eucalyptus oil	桉油精 Eucalyptol
薄荷腦 Menthol	薄荷腦 Menthol
冬青油 Methyl Salicylate	水楊酸甲酯 Methyl Salicylate
松節油 Turpentine oil	α -蒎烯 α -Pinene
冰片 Borneolum	龍腦 Borneol

外用藥油中中藥材指標成分的分析

GCMTI RD-3:2020

- 儀器 : GC-MS or GC-MS/MS
- 建立及確認定性分析6種指標成分的方法

有效成分 Active ingredient	指標成分 Chemical markers
肉桂 Cinnamon / 肉桂油 Cinnamon oil	桂皮醛 Cinnamaldehyde
香茅 Citronella / 香茅油 Citronella oil	香茅醛 Citronellal
丁香 Clove / 丁香油 Clove oil	丁香酚 Eugenol
薰衣草油 Lavender oil	芳樟醇 Linalool、 乙酸芳樟酯 Linalyl acetate
百里香油 Thyme oil	百里香酚 Thymol



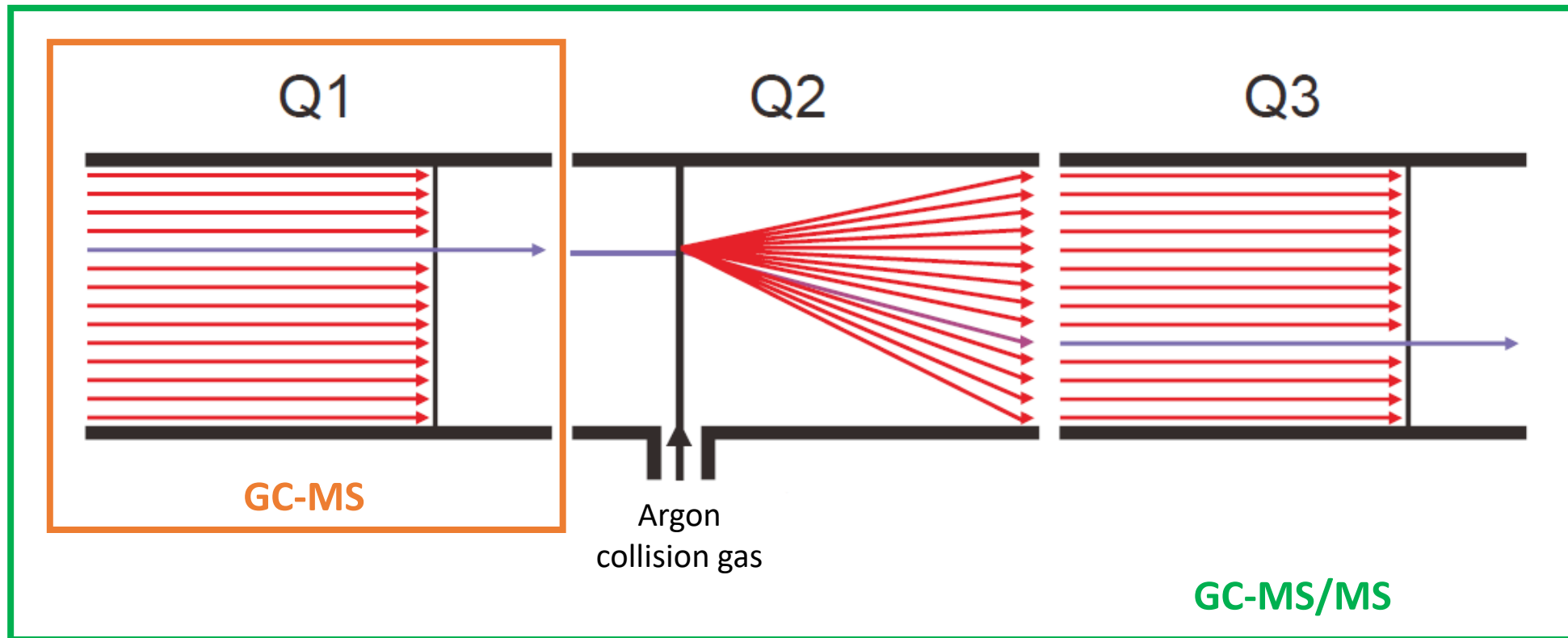
【中成藥註冊】

品質性資料

技術指引

GC-MS 及 GC-MS/MS 原理

- 選擇離子監測 vs 選擇反應監測
- Selected ion monitoring (SIM) vs Selected Reaction Monitoring (SRM)



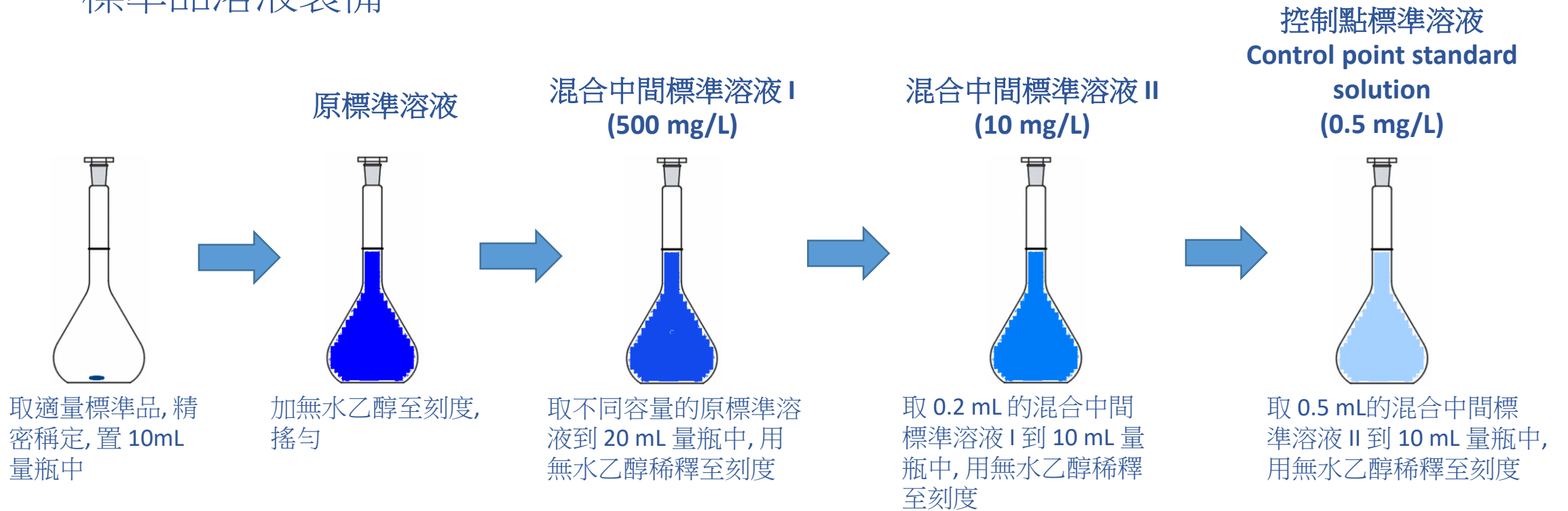
儀器 Apparatus

- 分析天平 Analytical balance
- 容量瓶 Volumetric flasks
- 移液管 Pipettes
- PTFE過濾膜 PTFE membrane filters
- 石英毛細管柱 Fused silica capillary column
- 氣相色譜質譜儀 GC-MS /
氣相色譜串聯三重四極桿質譜儀 GC-MS/MS



標準品制備

- 標準品溶液製備

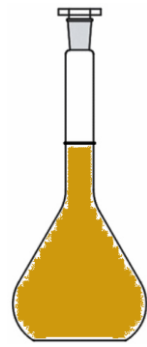
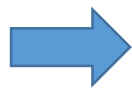


樣品制備

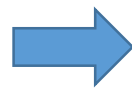
- 樣品溶液製備



取樣本100 mg, 精密稱定, 置10 mL量瓶中



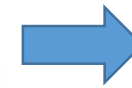
加無水乙醇至刻度, 搖勻



取0.1 mL 樣品溶液到10 mL 量瓶中, 無水乙醇稀釋至刻度



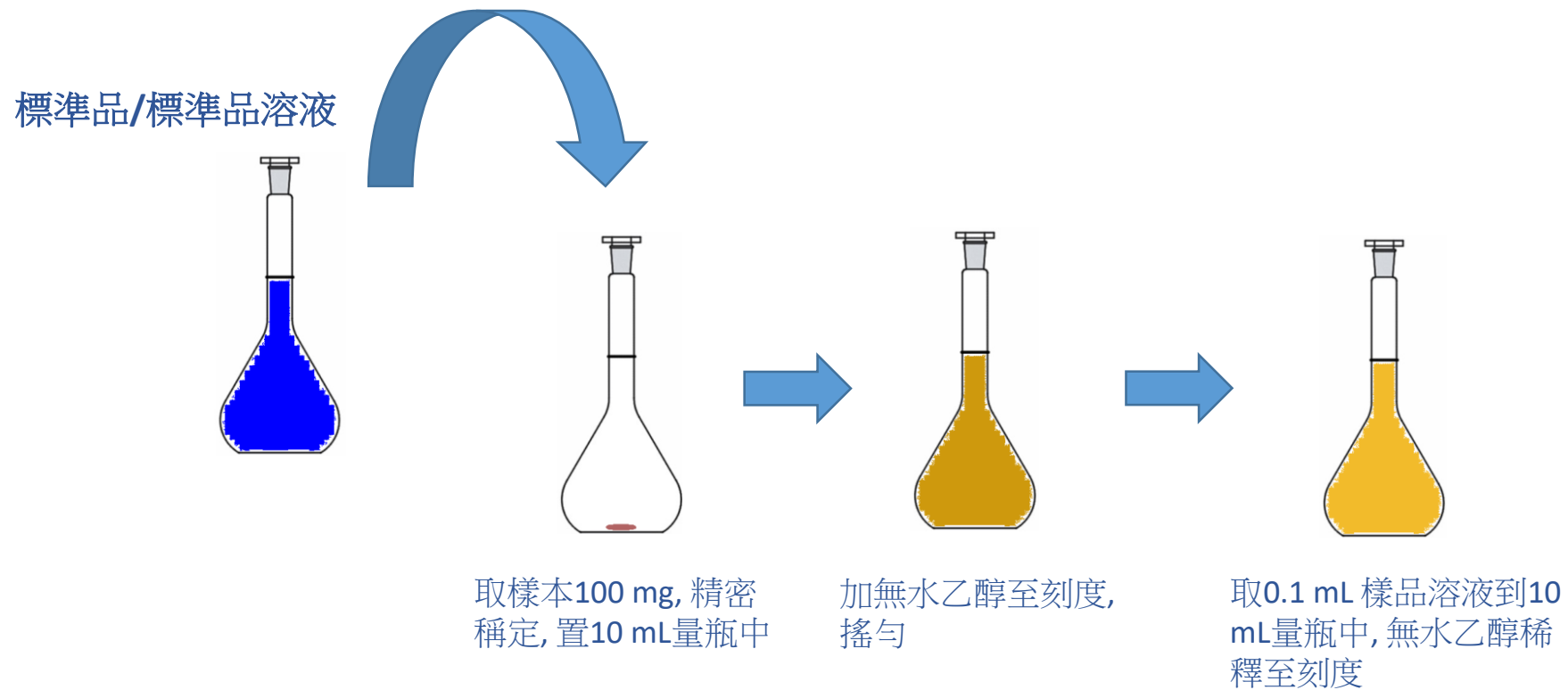
經0.45 μm 微膜過濾至 GC vial



GC-MS 或 GC-MS/MS 分析

樣品制備

- 樣品加標溶液製備



儀器設置



- 色譜柱

Column	Dimension	Stationary Phase
Restek Stabilwax-MS	0.25 mm ID x 30 m x 0.25 μ m	Polyethylene glycol (PEG)

- GC-MS, GC-MS/MS設置

Injection volume	1 μ L
Injection mode	Split mode, split ratio 50:1
Column flow rate	1.0 mL/min
Temperature programme	60°C for 10 min, 15°C/min to 180°C for 6 min, 40°C/min to 220°C for 3 min (Total run time 28 min)

儀器設置

- GC-MS

Marker	SIM ions (m/z)		
	Qualifier ion 1	Qualifier ion 2	Qualifier ion 3
Cinnamaldehyde	131	132	103
Citronellal	69	41	95
Eugenol	164	149	103
Linalool	71	93	80
Linalyl acetate	93	80	121
Thymol	135	150	91

儀器設置

- GC-MS/MS

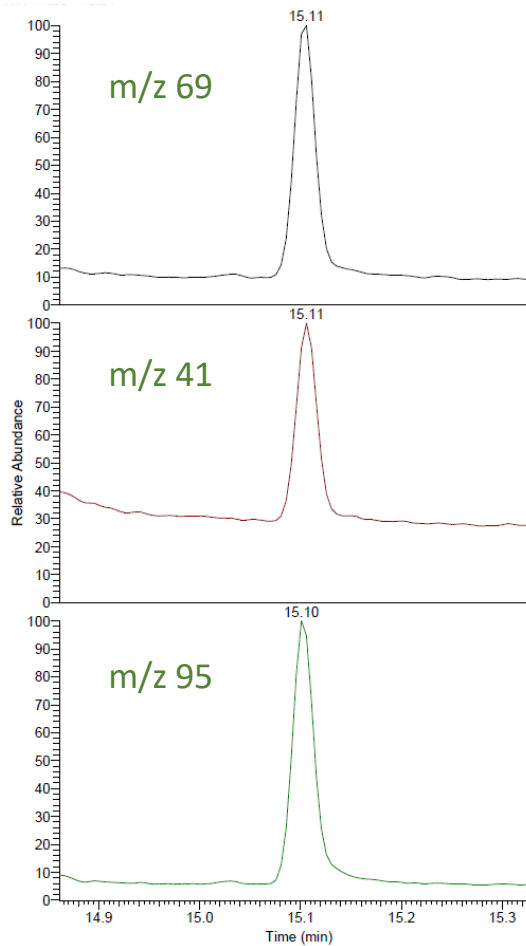
Marker	SRM transition		CE (eV)
	Precursor ion (m/z)	Daughter ion (m/z)	
Cinnamaldehyde	131	77	25
	131	103	10
Citronellal	95	55	15
	109	67	5
Eugenol	164	149	10
	164	131	10
Linalool	121	93	5
	121	77	20
Linalyl acetate	136	93	10
	136	121	10
Thymol	135	91	15
	150	135	10

數據分析

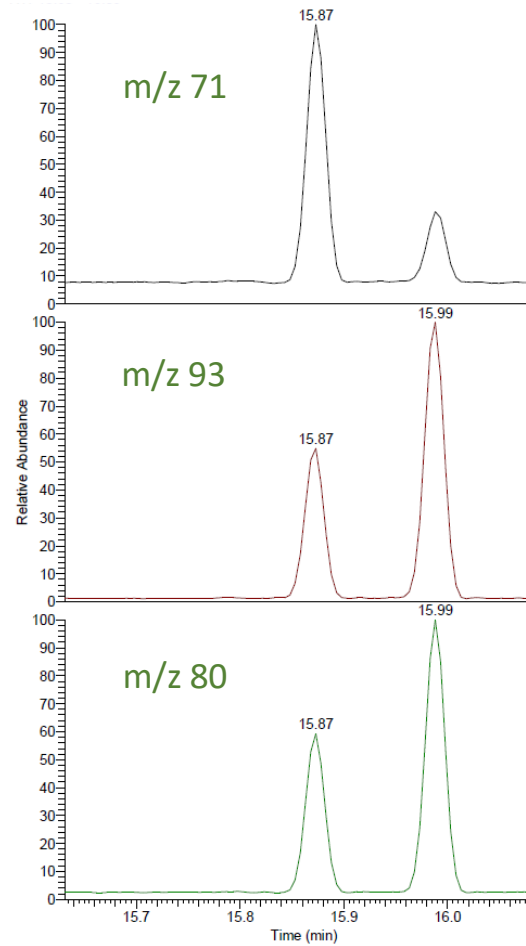
- GC-MS

0.5 mg/L standard

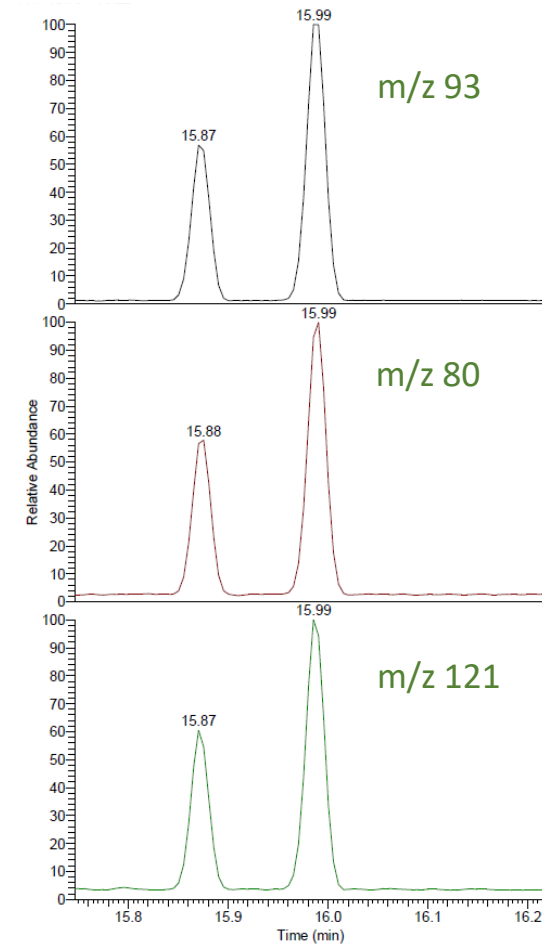
Citronellal



Linalool



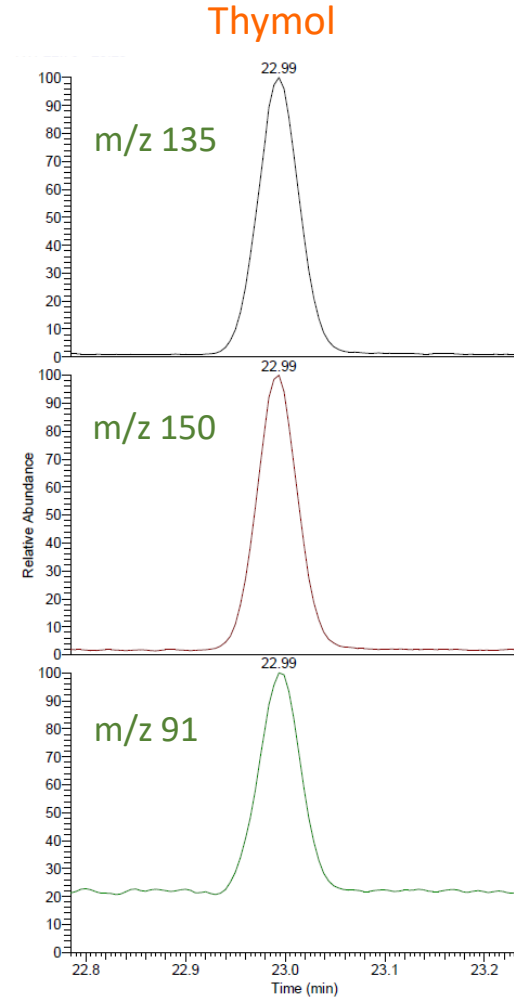
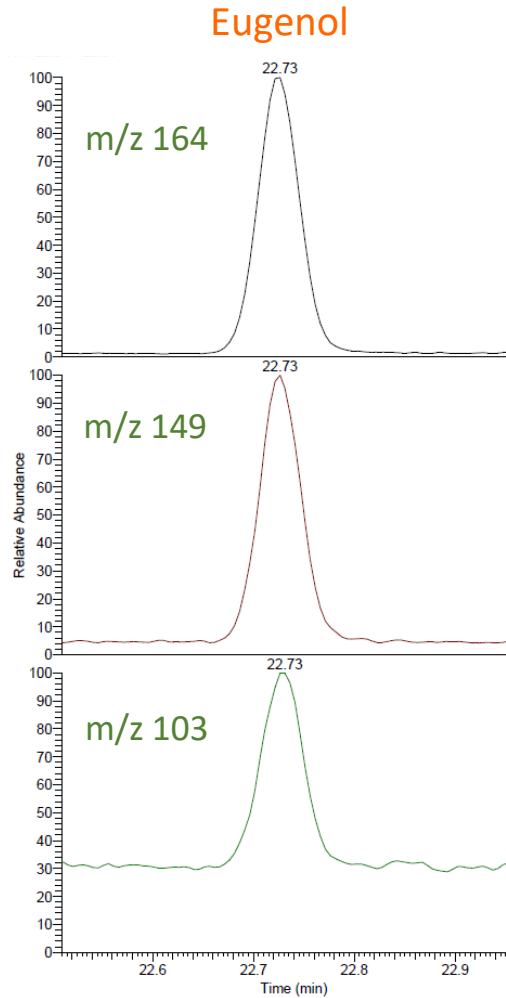
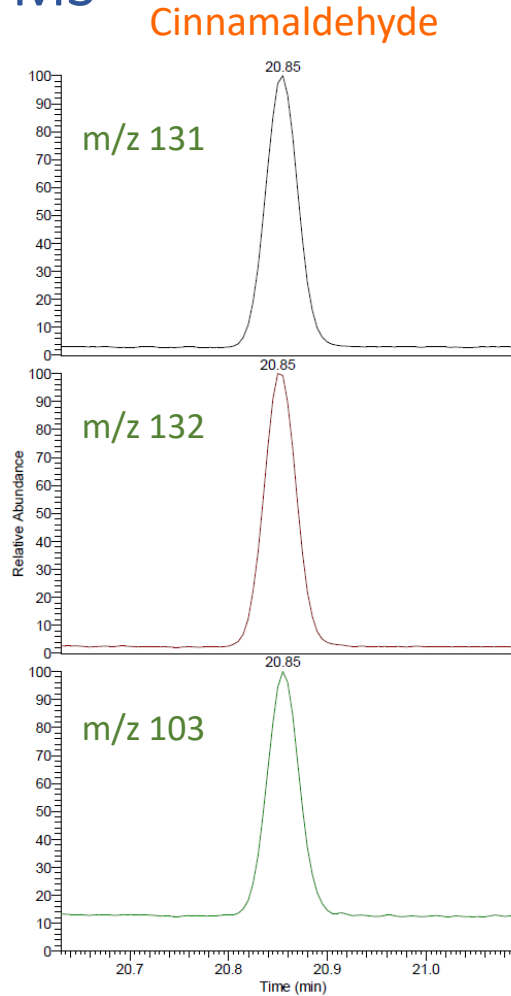
Linalyl acetate



數據分析

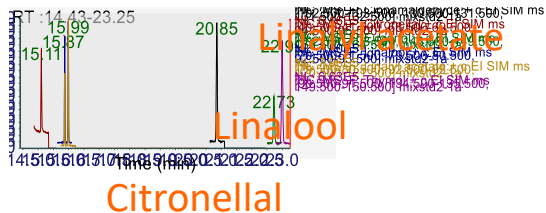
- GC-MS

0.5 mg/L standard



數據分析

- GC-MS



0.5 mg/L standard

Cinnamaldehyde

Thymol

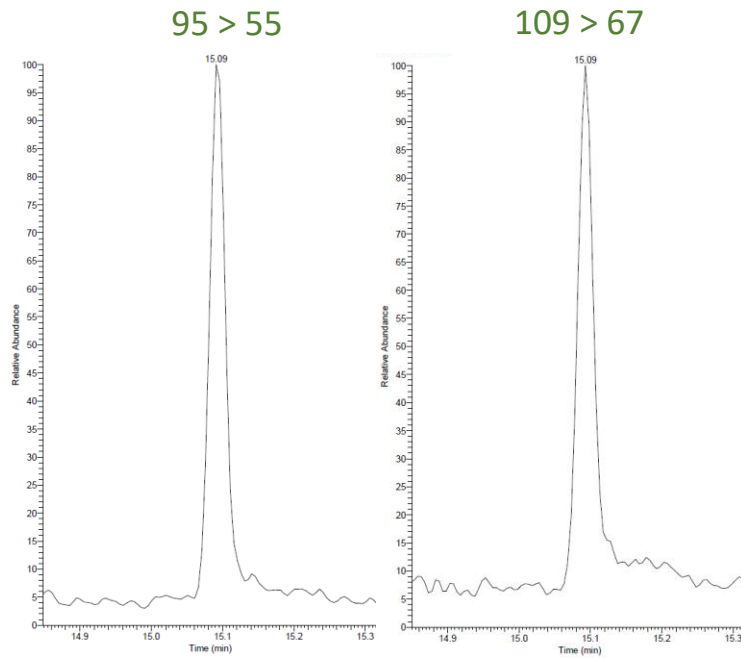
Eugenol

數據分析

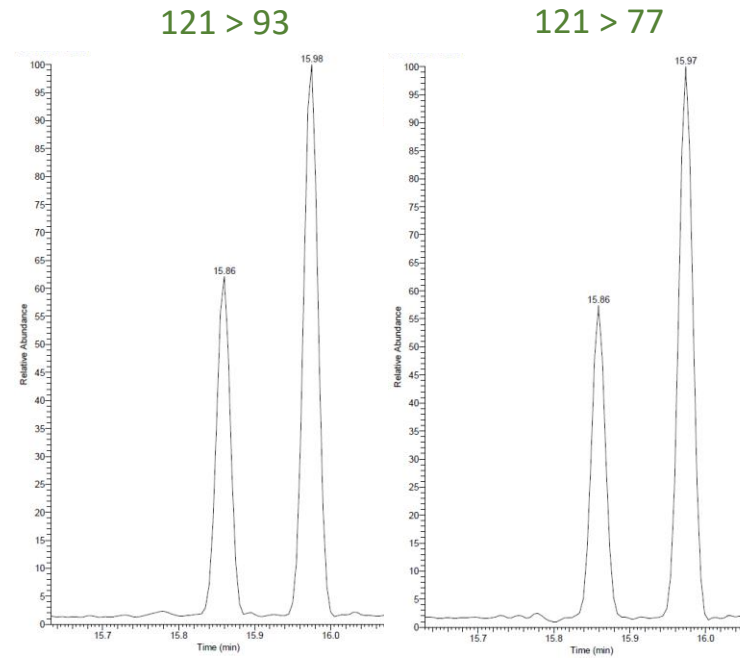
- GC-MS/MS

0.5 mg/L standard

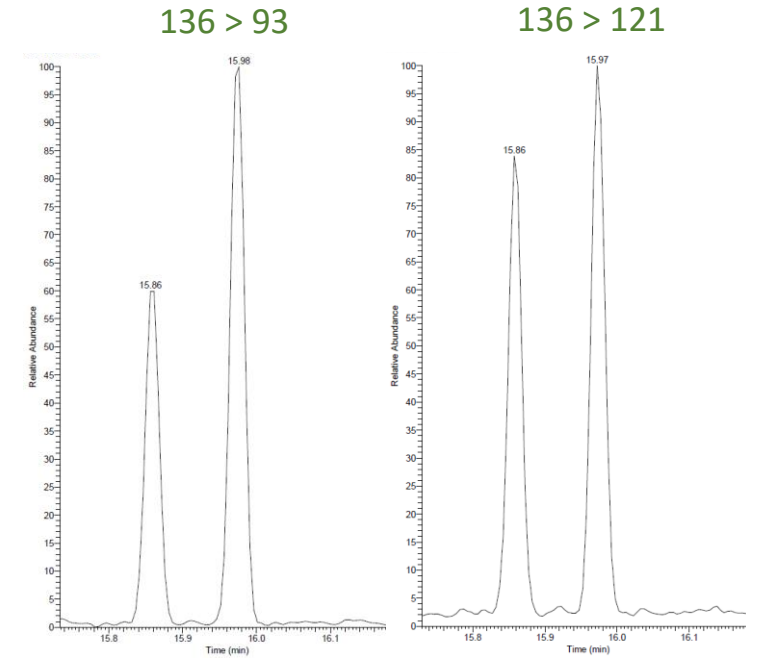
Citronellal



Linalool



Linalyl acetate



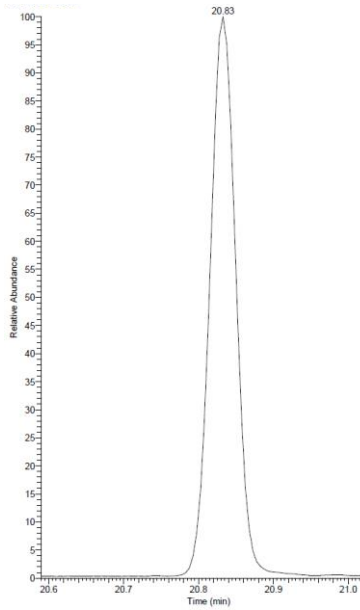
數據分析

- GC-MS/MS

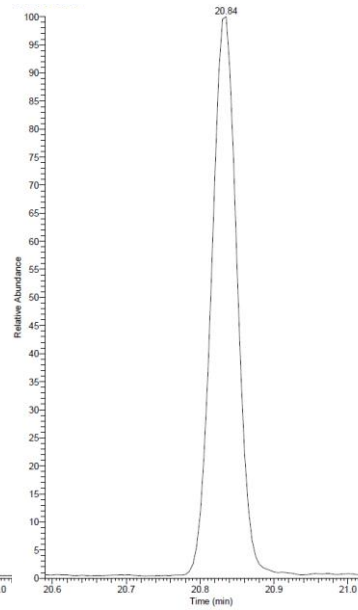
0.5 mg/L standard

Cinnamaldehyde

131 > 77

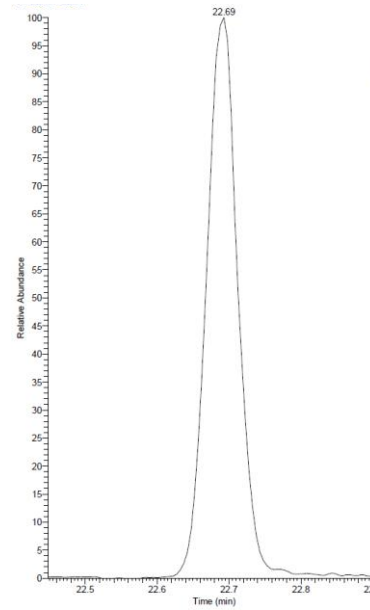


131 > 103

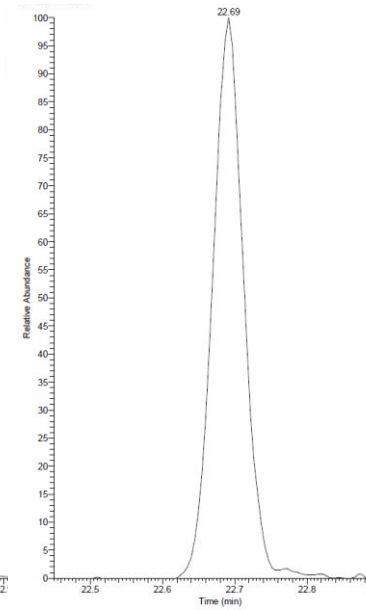


Eugenol

164 > 149

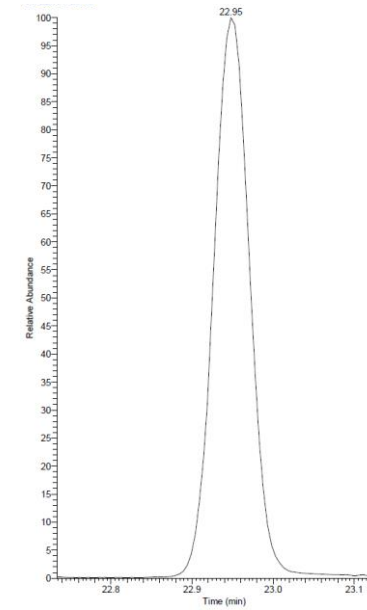


164 > 131

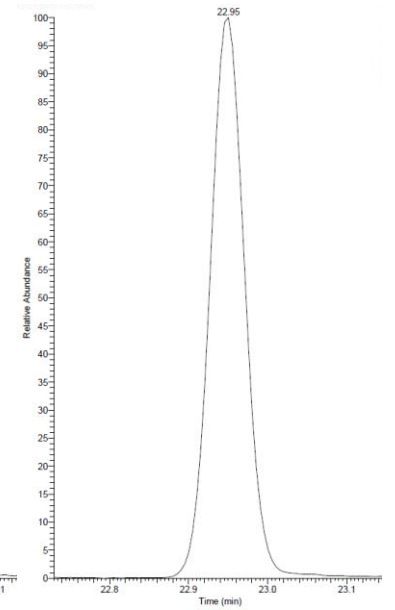


Thymol

135 > 91



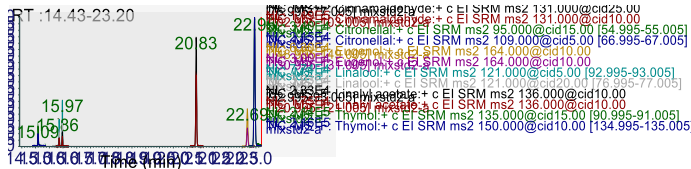
150 > 135



數據分析

- GC-MS/MS

0.5 mg/L standard



Thymol

Cinnamaldehyde

Linalyl acetate

Linalool

Citronellal

Eugenol

數據分析

- Positive identification

Parameters for target analyte	Criteria																	
Deviation of RT (%)	<ul style="list-style-type: none"> < 2 % compared with control point standard 																	
Signal response	<ul style="list-style-type: none"> Signal response of base peak for sample solution ≥ 70 % of that of control point standard Signal-to-noise ratio of detected peak > 5:1 																	
Relative abundances of diagnostic ions	<ul style="list-style-type: none"> Meet the tolerances according to 2002/657/EC <table border="1"> <thead> <tr> <th rowspan="2">Relative intensity (% of base peak)</th> <th colspan="2">Maximum permitted tolerance (%)</th> </tr> <tr> <th>GC-MS</th> <th>GC-MS/MS</th> </tr> </thead> <tbody> <tr> <td>> 50%</td> <td>± 10</td> <td>± 20</td> </tr> <tr> <td>> 20 to 50%</td> <td>± 15</td> <td>± 25</td> </tr> <tr> <td>> 10 to 20%</td> <td>± 20</td> <td>± 30</td> </tr> <tr> <td>$\leq 10\%$</td> <td>± 50</td> <td>± 50</td> </tr> </tbody> </table>	Relative intensity (% of base peak)	Maximum permitted tolerance (%)		GC-MS	GC-MS/MS	> 50%	± 10	± 20	> 20 to 50%	± 15	± 25	> 10 to 20%	± 20	± 30	$\leq 10\%$	± 50	± 50
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Linalyl acetate	136	93	10
	136	121	10
Thymol	135	91	15
	150	135	10

假陰性 False Negative Rate

- False negative rate
 - Proportion of false negative results obtained to the number of blank samples spiked at control point level studied

Marker	False negative rate (%)	
	GC-MS	GC-MS/MS
Cinnamaldehydye	0 (12)	0 (12)
Citronellal	0 (13)	0 (13)
Eugenol	0 (10)	0 (10)
Linalool	0 (10)	0 (10)
Linalyl acetate	0 (12)	0 (12)
Thymol	0 (15)	0 (15)

Number in parentheses indicates the total number of samples

方法適用性

- 目的

- ✓ 購買市面上已獲中成藥註冊的藥油，用建立的方法進行分析，測試方法對不同樣本的適用性

- 結果

- ✓ GC-MS及GC-MS/MS的質譜圖皆沒有明顯的干擾
- ✓ 2個方法的結果一致

Proficiency Testing Programme

- The main objective of the proficiency testing programme is to provide a platform to evaluate the testing capability of participating laboratories in analyzing chemical markers in Chinese medicinal oil.
- A total of 12 laboratories enrolled in the programme and 10 of them returned the analytical results to the organiser on or before the deadline of result submission.
- The z-scores of the participants are summarized as follows:

Chemical Marker	Number of participants (Percentage)			Total participants
	$ z \leq 2.0$	$2.0 < z < 3.0$	$ z \geq 3.0$	
α -Pinene	8 (89%)	--	1 (11%)	9
Eucalyptol	8 (89%)	--	1 (11%)	9
Camphor	8 (89%)	--	1 (11%)	9
Menthol	9 (90%)	--	1 (10%)	10
Methyl Salicylate	8 (89%)	--	1 (11%)	9

CHEMICAL MARKERS IN CHINESE MEDICINAL OIL

PROFICIENCY TESTING PROGRAMME

GLHK PT 19-03

FINAL REPORT

28 April 2020



GCMTI RD-1:2019

GCMTI method publications



Determination of α -Pinene, Eucalyptol, Camphor,
Menthol and Methyl Salicylate in Chinese Medicinal
Oil for External Use by Gas Chromatography

方法適用性

- 此方法旨在提供一種可靠的測試方法，作為含有**12種化學標記物**中的一種或多種的有效成分，如**松節油、桉葉油、樟腦、薄荷醇、水楊酸甲酯、冰片、肉桂油、香茅油、丁香油、薰衣草油和百里香油**的外用藥油的質量控制方法
- 在採用此方法時，使用者有責任評估其外用藥油產品是否適用於此測試方法，特別是其產品中的**其他成分或輔料**是否含有選定的**12種化學標記物**的任何一種或多種，以及**其他中藥材或中藥材提取物**的影響



總結

- 此項目是為中藥業界和檢測業界提供經驗證的分析方法作參考
- 中藥商可採用本項目所建立的一測多檢方法，作為質量控制方法供中成藥註冊使用
- 有助減少中藥商在方法建立及確認方面的投資
- 中成藥製造商亦可採用此方法作為內部生產的質量控制手段，加強業界對其產品的品質控制能力
- 藉此建立香港中藥品牌形象

Thank You

