

MS解析における各種前処理の自動化

直接イオン化の最新技術 DART
ID-CUBE(New!!)

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エーエムアール株式会社

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- Head Office: Tokyo Meguro
- Employee: 20
- Incorporated in 1986

Application Lab



Solution for LC/MS and GC/MS

- Phamacetical analysis
- Environmental analysis
- Proteomics
- Polymer Analysis

GC/MS用オートサンプラー



LC用オートサンプラー



COMBI PAL はさまざまなGCに装着可能



Agilent 5890 / 6890 / 6850 / 7890

Thermo Trace 2000 / FOCUS / CE 8000top

Shimadzu 14 / 17 / 2010 / 2014

Varian GC 3400 / 3600 / 3800 / 3900

Perkin Elmer Autosystem XL / Clarus 500 / 600

COMBI PAL CombiPALの多機能性



シリンジユニットを交換するだけで
さまざまなインジェクションが可能



液体インジェクション



ヘッドスペース分析



SPME(固相マイクロ抽出)



ITEX(超高感度ヘッドスペース)

基本システムから目的に合わせてアップグレード可能

Upgrade option Liquid ➡ Headspace ➡ SPME ➡ ITEX

LHSO 



1. 液体インジェクションシステムの導入
2. ヘッドスペースオプションの追加
3. SPME/ITEXオプションの追加



Liquid Syringe



HS Option



SPME Option

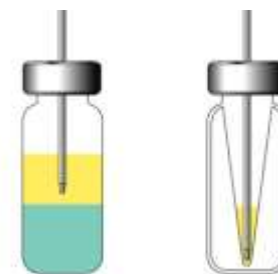


ITEX Option

GC 液体インジェクション



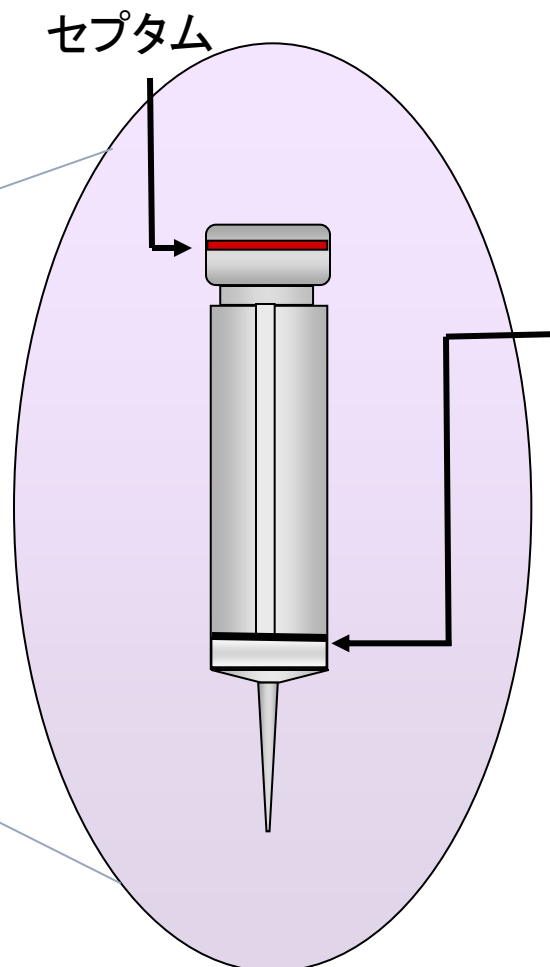
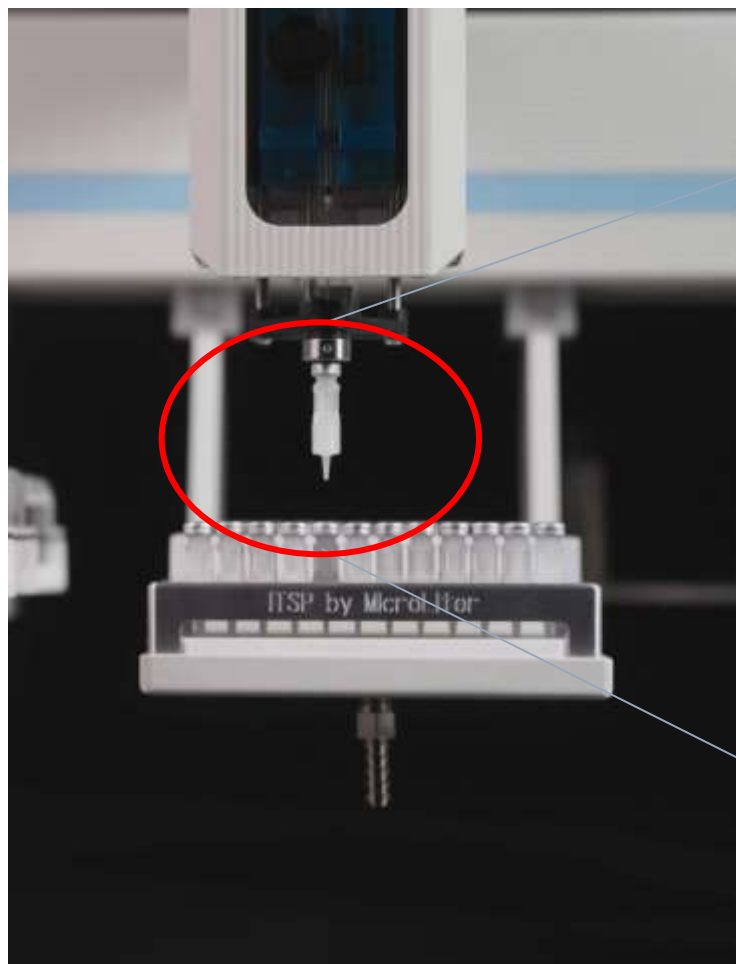
- 液体インジェクションユニットを簡単に装着可能
- 幅広い注入容量: 100nl – 500 μ l
- 注入スピード: 0.01 μ l/s – 250 μ l/s
- 各注入は独自にコントロール可能
- 大量注入にも対応 (LVI)
- 分注、希釈など溶液トランスファーが可能
- オンラインでの誘導體化試薬の添加
- すべての注入モードに対応 (SSL, PTV, On Column)
- バイアル内液/液抽出サンプルの注入も可能 (Liquid – Liquid Extraction in Vial)



Instrument Top Sample Prep(ITSP)オプション



各種固相抽出レジンが充填されたPAL専用のカートリッジ



逆相レジン

C18 Varian SPEC 6mg
 C18 Orochem 10mg
 C18AR Varian End-cap
 C8 Varian SPEC 6mg
 C8 Orochem 10mg
 Phenyl, Varian SPEC
 Phenyl, Orochem

極性レジン

Silica Varian SPEC
 Silica Orochem
 Cyanopropyl
 Amino

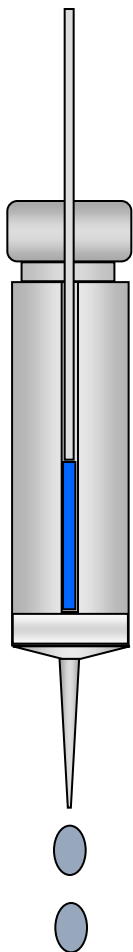
イオン交換レジン

Strong Anion Exchange
 Strong Cation Exchange
 Weak Anion
 Weak Cation

フィルター

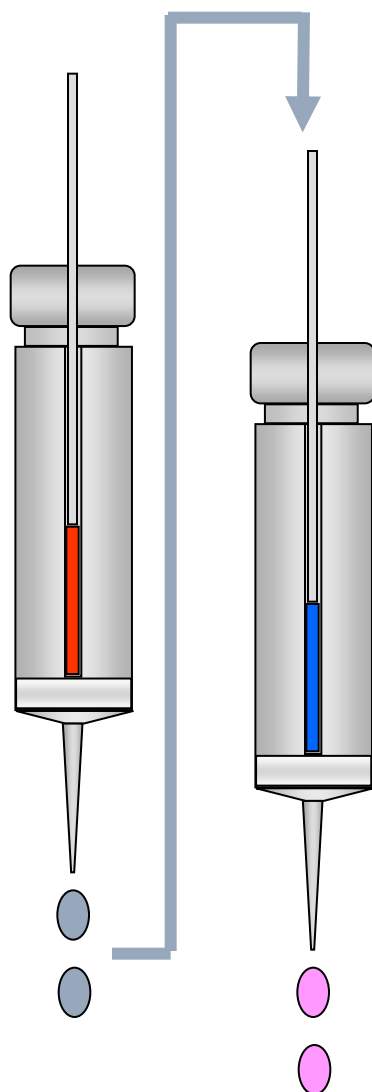
0.45um

溶液のコントロール



オートサンプラーのシリンジがカートリッジ内にタイトに装着されるため各種溶液は押し出しまたは吸引が自由に行うことができるためバキュームシステム(吸引)を使う必要がない。

異なるSPEによるマルチSPE処理



逆相レジン

C18 Varian SPEC 6mg
C18 Orochem 10mg
C18AR Varian End-cap
C8 Varian SPEC 6mg
C8 Orochem 10mg
Phenyl, Varian SPEC
Phenyl, Orochem

極性レジン

Silica Varian SPEC
Silica Orochem
Cyanopropyl
Amino

イオン交換レジン

Strong Anion Exchange
Strong Cation Exchange
Weak Anion
Weak Cation

フィルター

0.45um

ガスパージのメカニズム

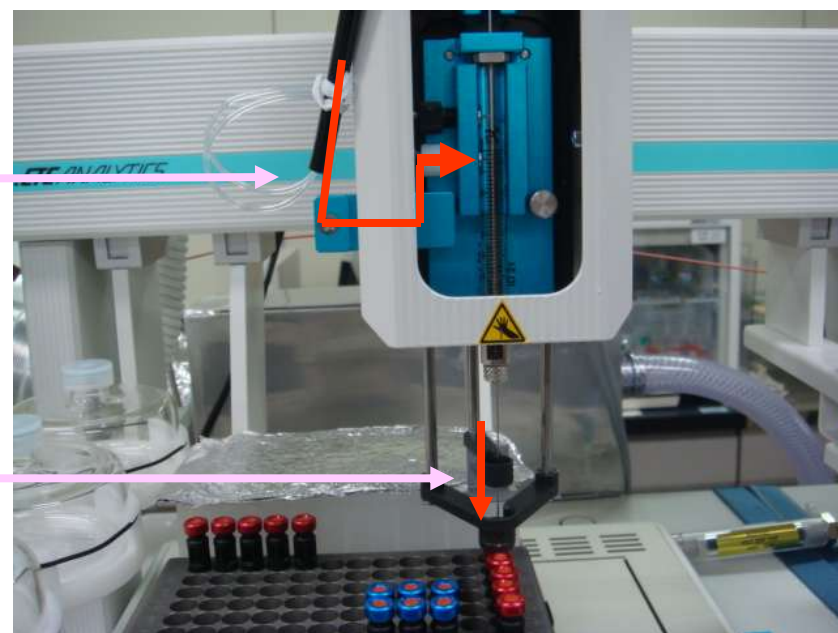
サイドポート搭載インジェクションシリンジ80ul



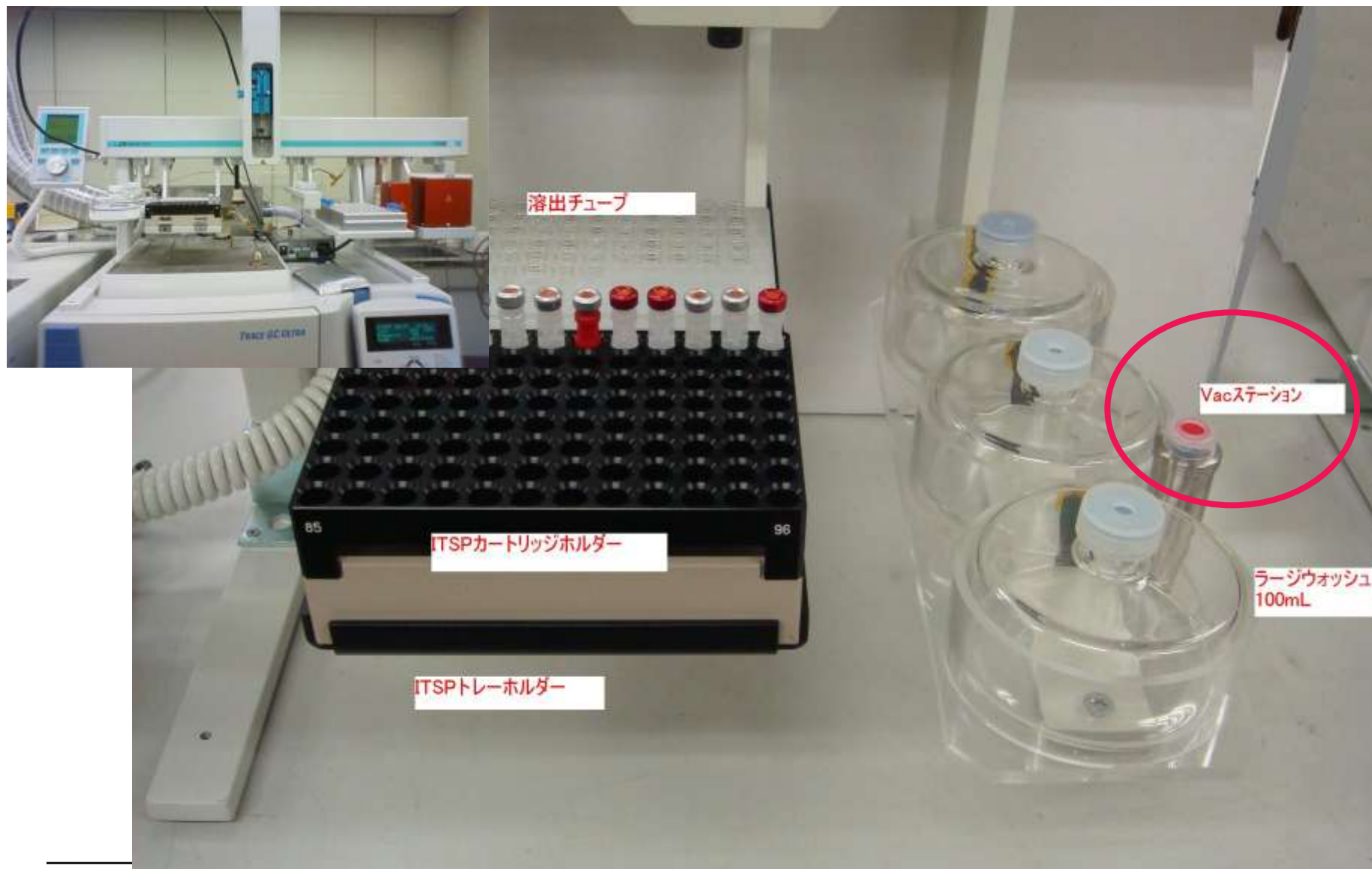
ガスパージ用サイドポート

ガスラインがサイドポートシリンジに接続されクリーンなヘリウムガス又は窒素ガスがシリンジに供給される

ガスはシリンジニードルの先端から固相抽出カートリッジに供給されカートリッジ中の水分を乾燥させて取り除く



ITSP装着CombiPALを搭載したGC/MS



各機能を用いた前処理の自動化に伴う高感度分析の応用例

固相抽出カートリッジと大量注入を用いた
水中の微量農薬の解析

オンラインSPE-GC/MS

サンプル前処理 SPE固相抽出

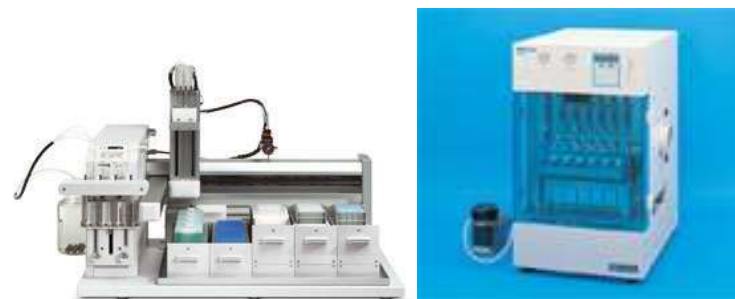
固相抽出カートリッジ



固相抽出プレート

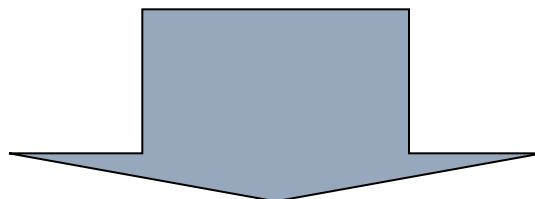


固相抽出前処理自動化装置



マニュアルでの固相抽出の問題点

- 手間がかかり前処理に時間を要する
- 再現性が得られない
- 分析の精度が得られない



**SPE前処理から注入
までの自動化**

サンプル前処理の流れ



オフラインでのSPE前処理の自動化

問題点

溶出後サンプルから水を抜く手動作業が必要
手動工程があるため稼働率が低い



チャレンジ

SPE固相抽出後のサンプルGC/MSにインジェクション(大量注入)



SPE-GC/MSの自動化

CombiPALでのサンプル前処理／GCインジェクションの流れ

- ① カートリッジコンディショニング アセトン/ヘキサン ×1
- ② カートリッジコンディショニング メタノール ×1
- ③ カートリッジコンディショニング H₂O ×1
- ④ Sample Loading 80uL x 25 times (Total 2mL)
- ⑤ シリンジ洗浄 メタノール ×1
- ⑥ シリンジ洗浄 アセトン/ヘキサン ×1
- ⑦ シリンジにてエアーをパージ (80uL Syringe 3 Strokes)
- ⑧ ヘリウムガスパージ 5min
- ⑨ シリンジ洗浄 アセトン/ヘキサン ×1
- ⑩ 溶出 50uL～ 溶出液をロード
- ⑪ シリンジにてエアーをパージ (80uL Syringe 5 Strokes)
- ⑫ サンプルをGCに注入 20uL～
- ⑬ シリンジ洗浄 メタノール ×2
- ⑭ シリンジ洗浄 アセトン/ヘキサン ×2

オフラインSPE前処理自動化とオンラインSPE前処理自動化の比較

オフラインSPE前処理自動化 (300mgSPEレジン)

コンディショニング

試料ロード

500ml / 500ng

洗浄

脱水

溶出

3ml / 500ng

濃縮

3ml → 1ml / 500ng

水の除去1: シリンジで吸い取り **マニュアル操作**

内部標準添加

水の除去2: 吸水剤添加(硫酸ナトリウム)

オフライン

GC インジェクション

2ul / 1ng

オンラインSPE前処理自動化 (12mgSPEレジン)

コンディショニング

試料ロード

2ml / 2ng

洗浄

脱水

溶出

50ul / 2ng

濃縮

必要なし

水の除去1: シリンジに吸い取り **必要なし**

内部標準添加

水の除去2: 吸水剤添加(硫酸ナトリウム) **必要なし**

GC インジェクション

20ul / 0.8ng

大量注入の利点

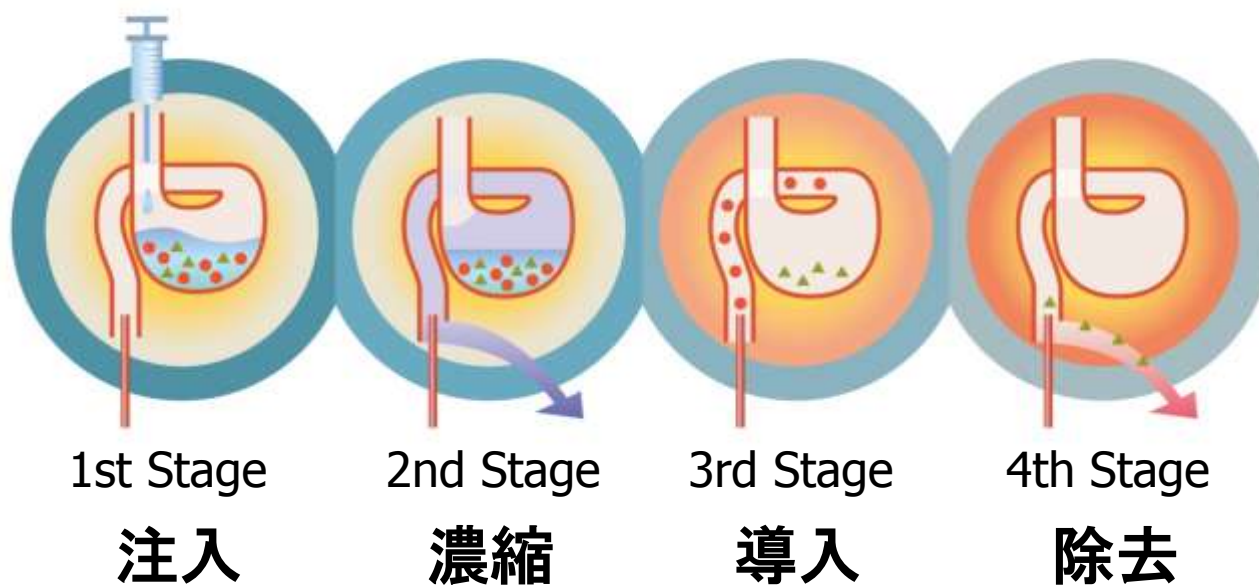
- 高感度分析が可能
 - ・感度向上(10倍から100倍の感度向上が期待できる)
 - ・SCAN分析(一斉分析、データ信頼性の向上)
- 前処理操作の迅速化および簡易化
 - ・試料量の少量化
 - ・濃縮操作の省略
- ハイフネーション技術のインターフェース
 - ・前処理装置との連結、オンラインGC/MS分析システム(SPE-GC、LC-GC、GPC-GC等)

AISTI SCIENCE社 LVI-S200



大量注入法

- 胃袋型インサートがその大量注入を容易にします。



CombiPALを用いることで...

- **固相抽出から大量注入までを自動化することにより再現性の優れた超高感度GC/MS解析が可能。**
- 大量注入で大きなシリンジを使用する場合、洗浄溶媒が直になくなってしまふという欠点があるが、CombiPALでは**十分な洗浄溶媒量が確保できる**ため、安心して分析できる。
- いろいろな大きさのシリンジサイズに簡単に換えられ、またそのシリンジサイズの最大まで注入することが可能である。
- 新しく設置したLVI-S200の注入口と既存の注入口の両者をそのまま使用することが可能である。
- LVI-S200を用いることで注入口の中で誘導体化を行うことが可能。

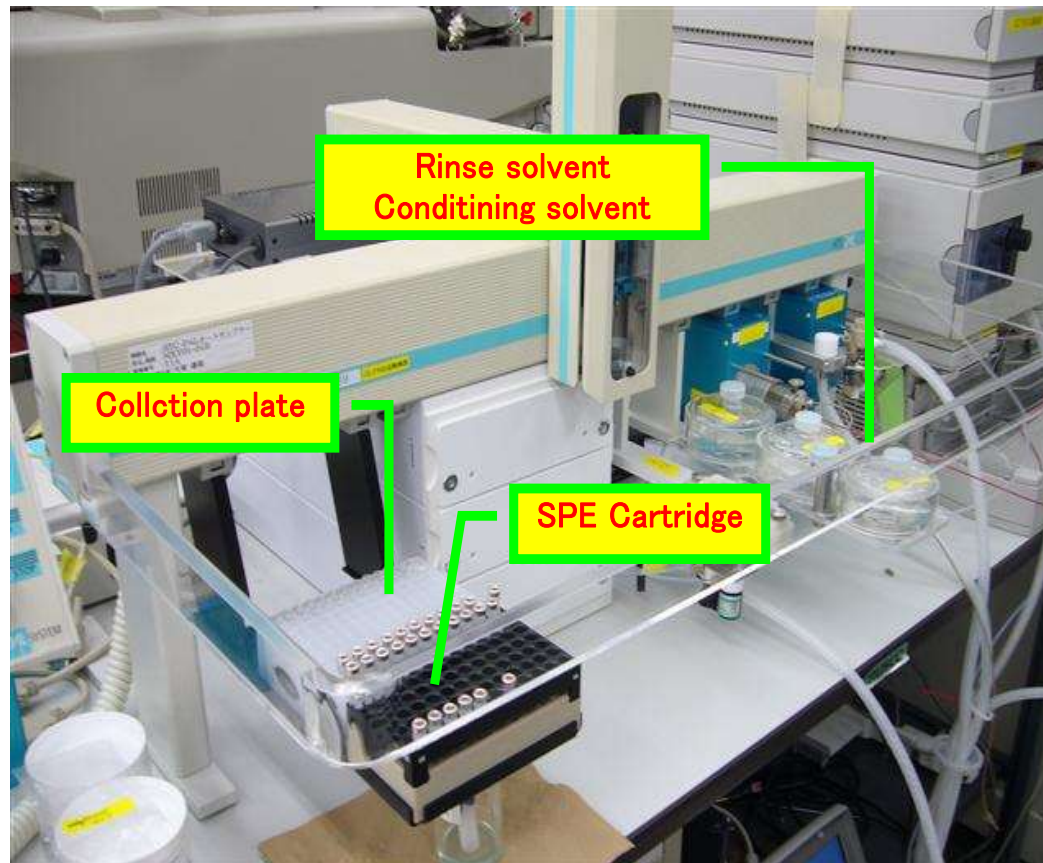
Method Development for Determination of Atorvastatin and Its Metabolites in Human Plasma Using ITSP System

A. Sakurai ¹, Y. Bandoh ²
(Toray Research Center, Inc. ¹, AMR, Inc. ²)



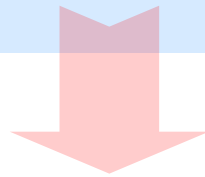
ITSP System

ITSP can automatize the process of conditioning, sample loading, elution and injection using PAL.



Expected Benefit

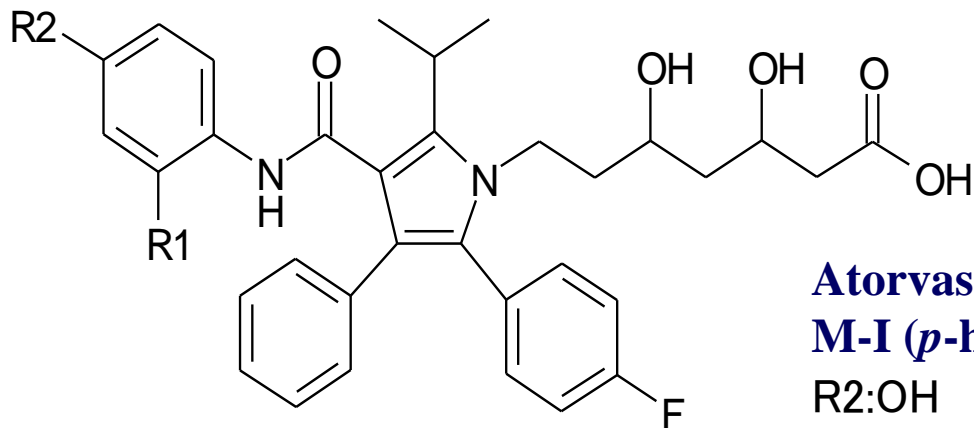
1. Efficient sample processing
2. Reduction of re-analysis caused by human error
3. Saving reagent consumption
4. Improvement of reproducibility in SPE and analysis
etc



ITSP system could have a high degree of availability for processing of huge number of samples.
(e.g. clinical examination, clinical research . . .)



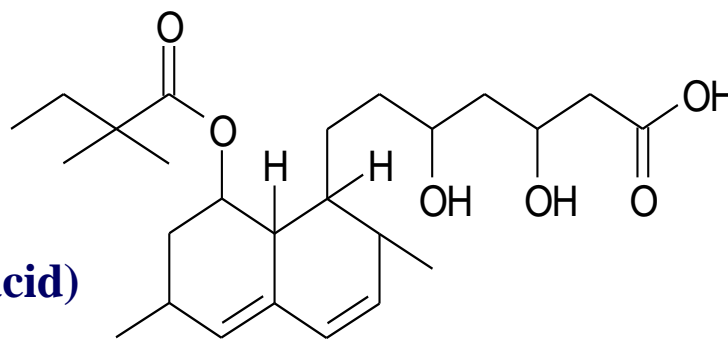
Structures of Atorvastatin and Its Metabolites



Atorvastatin / R1:H, R2:H

M-I (*p*-hydroxy atorvastatin) / R1:H,
R2:OH

M-II (*o*-hydroxy atorvastatin) / R1:OH,
R2:H



IS (Simvastatin hydroxy acid)

Quantitative Analysis

- ◆ **Human plasma: 20 μ L**
- ◆ **Calibration curve: 0.25 to 100 ng/mL**
- ◆ **Assay reproducibility**
QC samples; LLQC, LQC, MQC and HQC (4 levels)

Acceptance criteria

Calibration curve

%Nominal: 85.0 to 115.0% (80.0 to 120.0% at the LLOQ)
At least three-quarters of the calibration standards including the LLOQ and the ULOQ of the calibration standards should meet the above criteria.

Assay reproducibility

%Nominal: 85.0 to 115.0% (80.0 to 120.0% for LLQC)



Pretreatment Procedure

Plasma
sample

ITSP



STEP 1 SPE cartridge conditioning

STEP 2 SPE cartridge conditioning

STEP 3 Load onto SPE cartridge

STEP 4 Wash

STEP 5 Elute

STEP 6 Dilution

STEP 7 Injection



Calibration Curve

(1/x weighting)

Atorvastatin concentration (ng/mL)						
0.250	0.500	1.00	5.00	25.0	50.0	100
0.215	0.538	0.984	5.36	24.9	51.6	98.1
<i>86.0</i>	<i>107.6</i>	<i>98.4</i>	<i>107.2</i>	<i>99.6</i>	<i>103.2</i>	<i>98.1</i>

Upper value: Observed concentration (ng/mL)

Lower value: %Nominal

(1/x weighting)

M-I concentration (ng/mL)						
0.250	0.500	1.00	5.00	25.0	50.0	100
0.236	0.505	0.983	5.38	23.8	52.8	98.1
<i>94.4</i>	<i>101.0</i>	<i>98.3</i>	<i>107.6</i>	<i>95.2</i>	<i>105.6</i>	<i>98.1</i>

Upper value: Observed concentration (ng/mL)

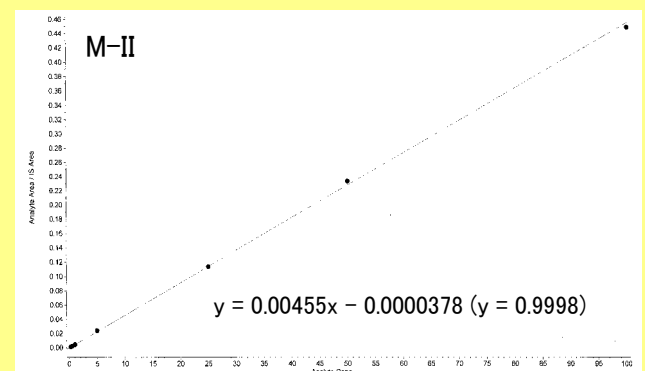
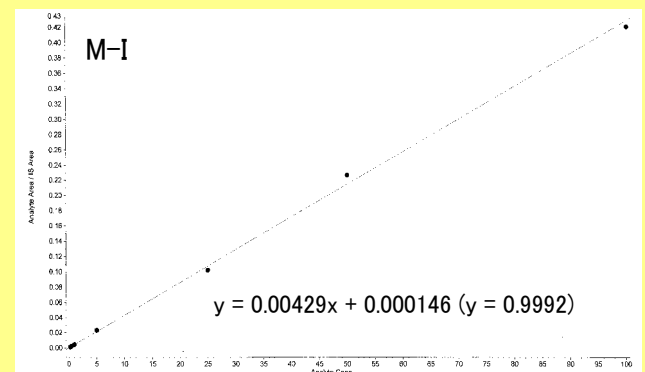
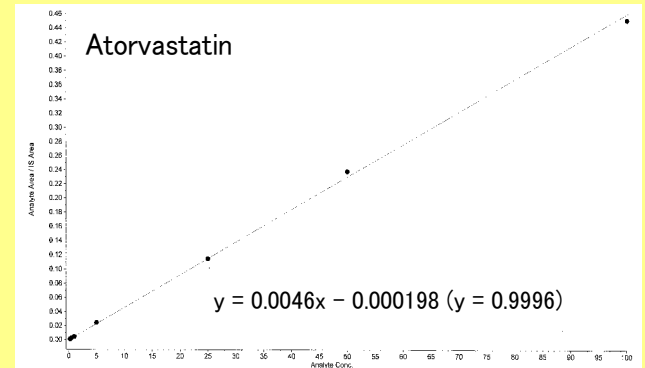
Lower value: %Nominal

(1/x weighting)

M-II concentration (ng/mL)						
0.250	0.500	1.00	5.00	25.0	50.0	100
0.223	0.539	0.964	5.30	25.0	51.2	98.6
<i>89.2</i>	<i>107.8</i>	<i>96.4</i>	<i>106.0</i>	<i>100.0</i>	<i>102.4</i>	<i>98.6</i>

Upper value: Observed concentration (ng/mL)

Lower value: %Nominal



Assay Reproducibility

Quantitative results

Compound name	Atorvastatin				M-I				M-II			
	LLQC	LQC	MQC	ULQC	LLQC	LQC	MQC	ULQC	LLQC	LQC	MQC	ULQC
QC sample	0.250	0.500	5.00	80.0	0.250	0.500	5.00	80.0	0.250	0.500	5.00	80.0
Found	0.301	0.524	4.68	71.9	0.215	0.468	4.47	69.5	0.235	0.561	4.90	72.5
Concentration (ng/mL)	0.263	0.384	5.61	77.7	0.211	0.520	5.24	69.9	0.213	0.464	5.34	77.1
	0.287	0.495	4.66	70.7	0.247	0.448	4.29	68.2	0.202	0.466	4.54	72.1
Mean	0.284	0.468	4.98	73.4	0.224	0.479	4.67	69.2	0.217	0.497	4.93	73.9
%Nominal	113.5	93.5	99.7	91.8	89.7	95.7	93.3	86.5	86.7	99.4	98.5	92.4

$$\%Nominal = \frac{X}{C_{nom}} \times 100$$

C_{nom} : Nominal concentration (ng/mL)

X: Mean found concentrations (n=3)

%Nominal:

LLQC 86.7to

113.5%

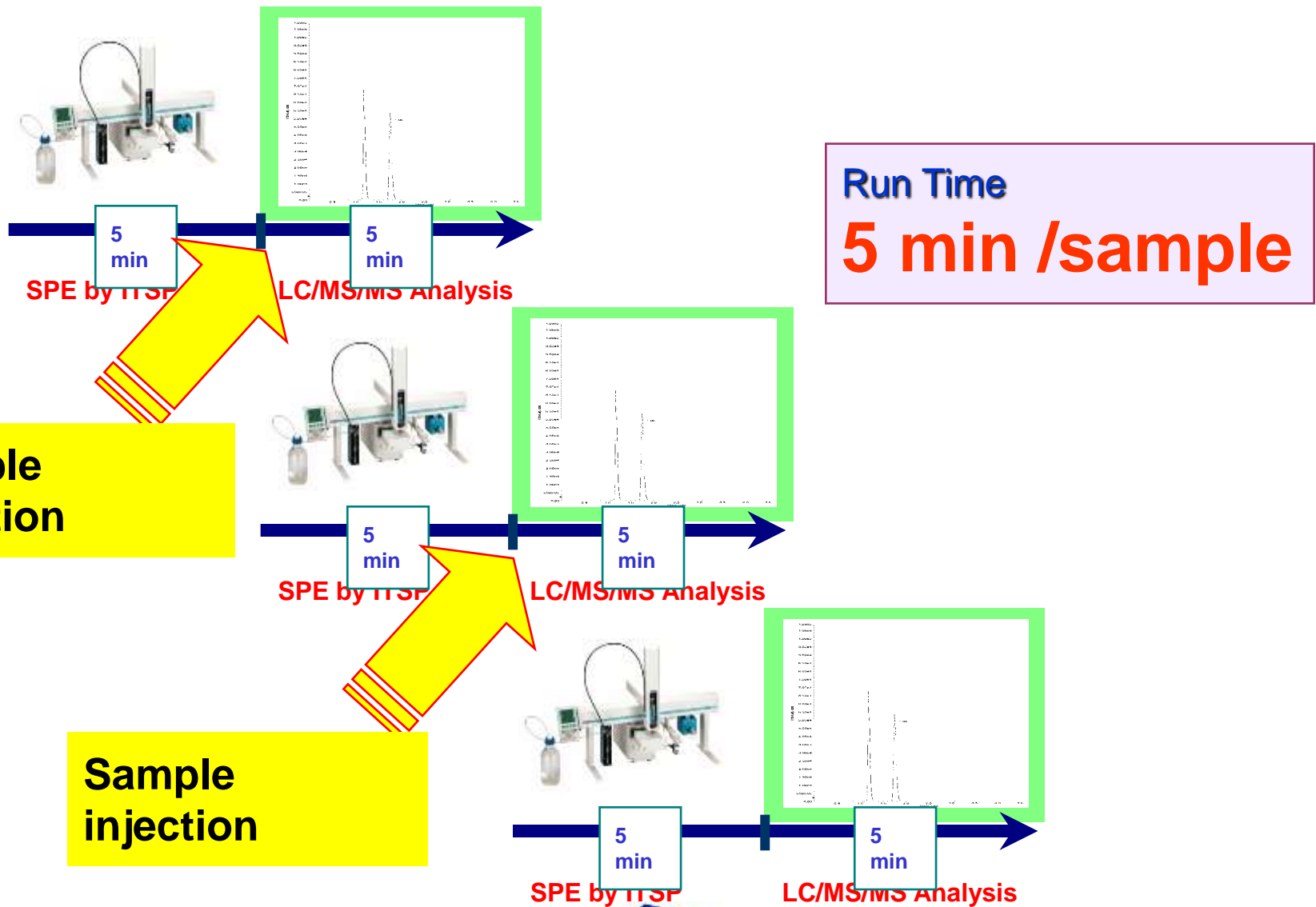
LQC, MQC and HQC 86.5 to

99.7%

The accuracy (%Nominal) values of analytes met the acceptance criteria.



Run Cycle



Sample injection

Sample injection

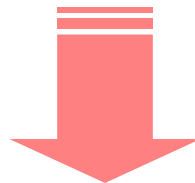
Run Time
5 min /sample



Conclusion

Other evaluation items

Item	Conditions	Result
Recovery test	Atorvastatin	72.7 to 75.6%
	M-I	78.5 to 98.6%
	M-II	71.6 to 89.3%
	IS	67.8%
System reproducibility	IS peak area (n = 20)	8.9% (%CV)
Cotamination	—	Not detected

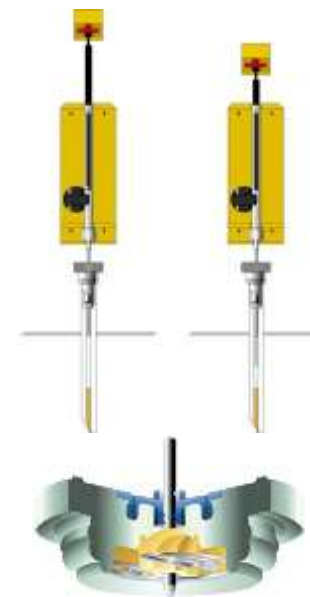
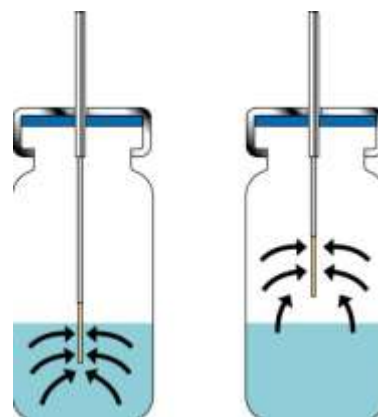
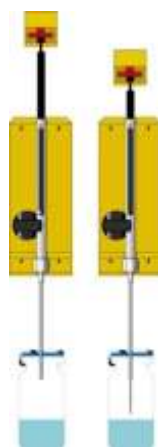


This method can be useful for quantitative assay on Atorvastatin and its metabolites in human plasma.



SPME（固相マイクロ抽出）インジェクション

- SPMEシリンジユニットを簡単に装着
- SPMEシリンジファイバーを装着したまま攪拌が可能：250rpm – 750 rpm
- ファイバーコンディショニングステーションによりファイバーのコンディショニングが自動化
- 溶媒を用いないサンプル濃縮

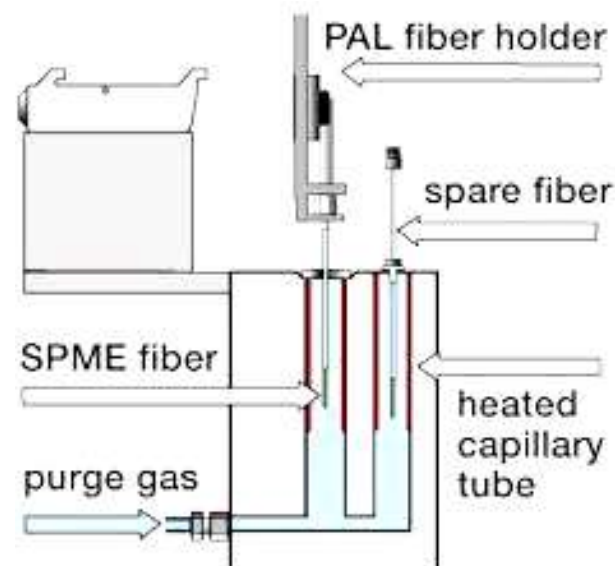


Merlin Septumless Injector

SPME Fiber Conditioning Station



- SPMEファイバーのコンディショニングの自動化
- 350°Cまで温調可能
- イナートなガスでパージ (He or N2)
- セカンドコンディショニングポートも用意



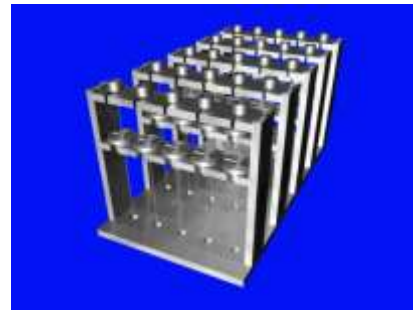
SPME Multi-fiber System オプション

環境分野などでフィールドサンプリングが可能になるといろいろな場所または定期的な採取によるサンプル数が増加し多くのSPMEファイバーの分析が必要になる。

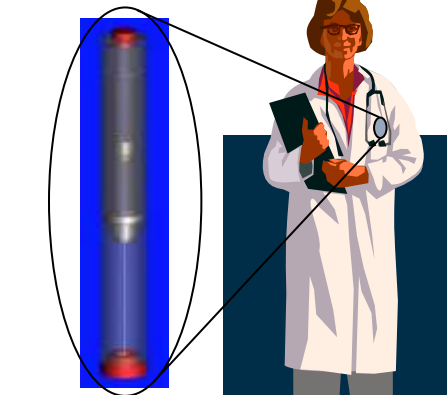
エアースAMPLING

さまざまな環境(野外フィールド、病院、研究所、工場、
火事現場、室内)での揮発物質/VOCのモニタリング

ペンサイズPassiveSPME サンプラー



サンプリング

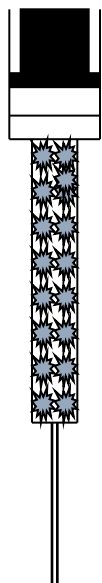


PALファイバーホルダーに装着(25本)

ITEX II インジェクション 超高感度ヘッドスペース分析

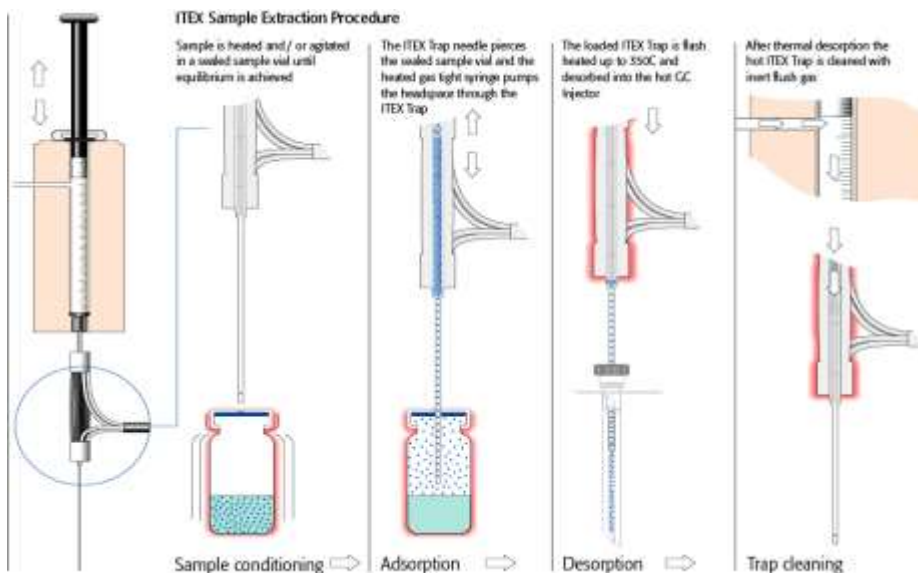


- Tenax等の吸着剤をシリンジ本体に装備して吸着/熱脱離を行う。
- 簡単に超高感度ヘッドスペース分析が可能
- PALシステムのみ装着可能な技術
- GCの改良や変更、クライオフォーカシングも必要ない。
- 新タイプのITEXIIオプションは現行のPALのインジェクターに簡単に装着可能(Z軸交換は不要)
- シリンジニードルはSiltek処理(不活性化)



吸着レジン

Tenax TA 80/100
 Tenax GR 80/100
 Carbosieve S3
 80/100
 Carboxen 1000 60/80
 Carbopack X 60/80
 Carbopack X 20/40
 Carbopack C 80/100
 Hayesep D 100/120
 Porapak Q 50/80
 Porapak Q 120/150
 Porapak R 120/150
 Tenax GC 60/80



GC Conditions			MS Parameters			ITEX-2 Conditions			Headspace Conditions		
Injector: Varian 1177 – 250 °C			Trap Temp.: 180 °C			Trap: Tenax TA 80/100 mesh			Syringe Temp: 80 °C		
Split: 1:10			Transfer Line Temp.: 200 °C			Agitation Time: 5 min			Agitator Temp: 80 °C		
Flow: Helium – 1.0 mL/min			Manifold Temp.: 50 °C			Agitation Temp.: 80 °C			Incubation Time: 5 min		
Liner: Siltek split liner with glass frit (Varian Part Number RT21045214)			Mass Range: 41-200 Da			Extraction Strokes: 20			Plunger Fill Speed: 100 µL/s		
Column: FactorFour™ VF-5 ms, 30 m x 0.25 mm x 0.25 µm, (Varian Part Number CP8944)			Target TIC: 20,000			Extraction Volume: 1.0 mL			Injection Speed: 250 µL/s		
Oven Program:			Emission Current: 10 µA			Desorption Rate: 50 µL/s			Injection Volume: 1 mL		
	Temp	Rate	Hold		µScans: 3						
	(°C)	(°C/min)	(min)								
	60	0	1								
	150	10	0								
	275	25	0								

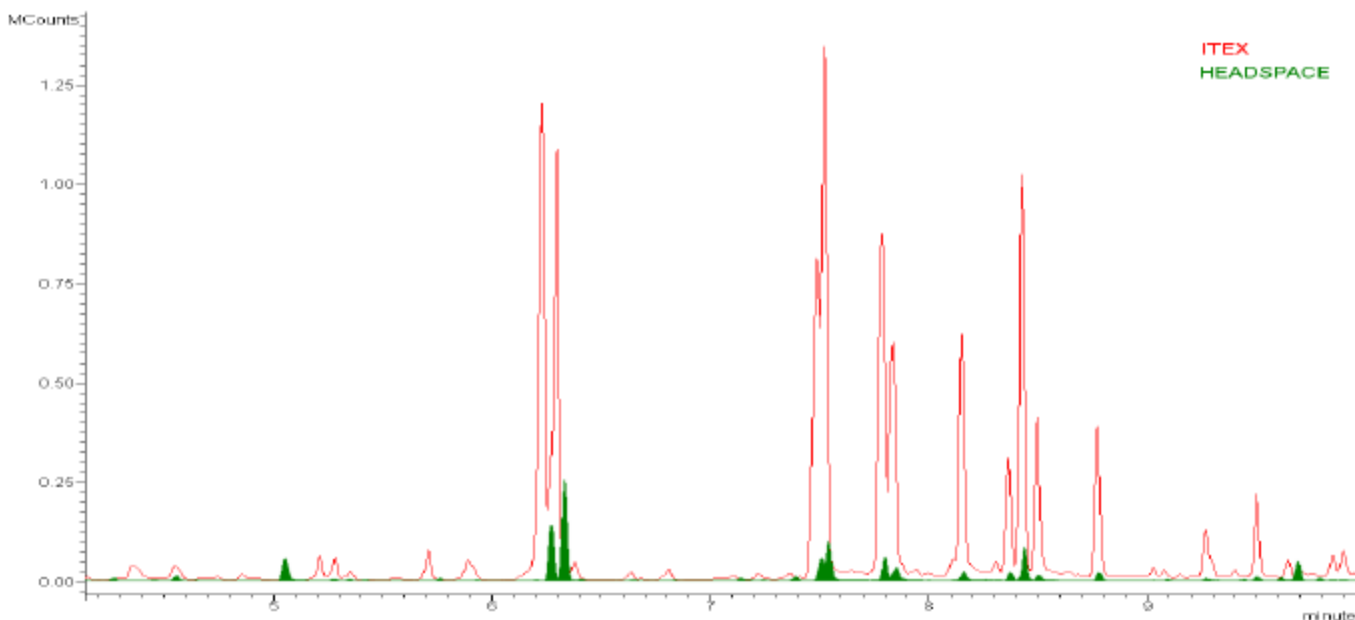
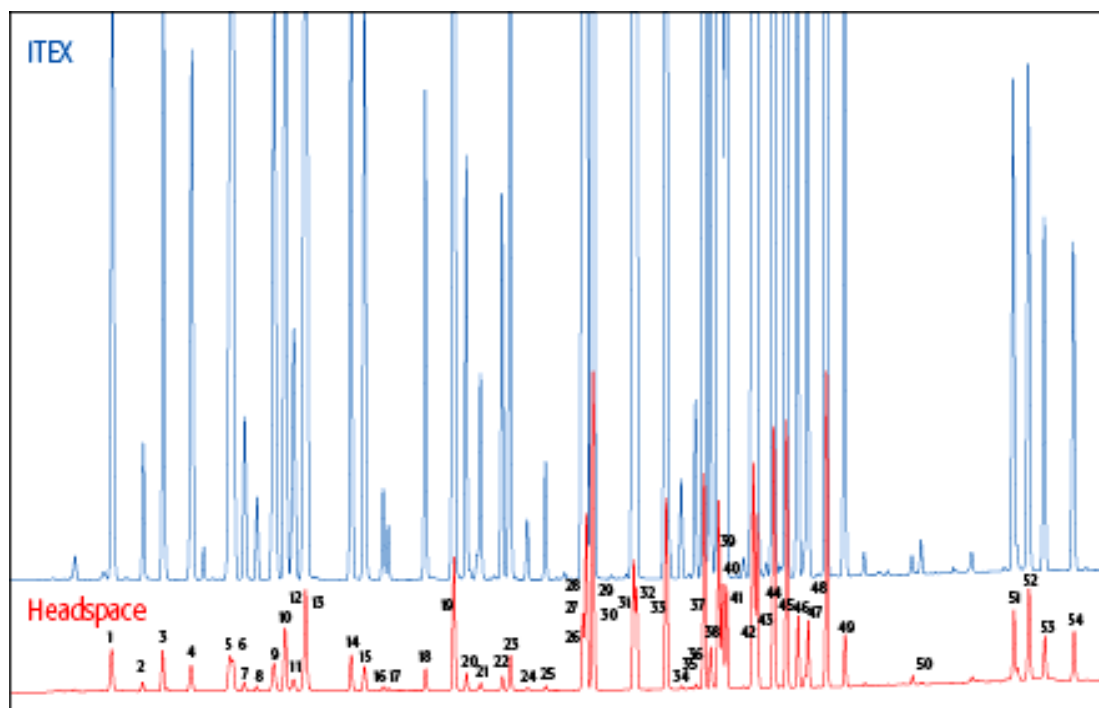


Figure 2. Overlay of TICs for standards run with static headspace (green) and ITEX-2 (red). In this example, ITEX-2 is 10-30 times more sensitive than static headspace.

ITEXと通常のヘッドスペース分析との比較

ITEX Extraction versus Static Headspace Analysis



Static Headspace Parameter

60C / 10min / 1ml sample volume

ITEX Parameter:

Extraction Speed: 100l / sec.

Total Pumping Strokes: 50

Temperature Pumping Syringe / Sample Incubation: 60C / 10min.

Desorption at 200C, 15sec. splitless

Chromatography:

Injection: Splitless 15sec. at 250C / Carrier gas: 0.2bar hydrogen

Column: Rtx-502.2 60m x 0.32mm ID, 1.8m film

Temperature Program: 40C - 1min. - 10C / min to 220C

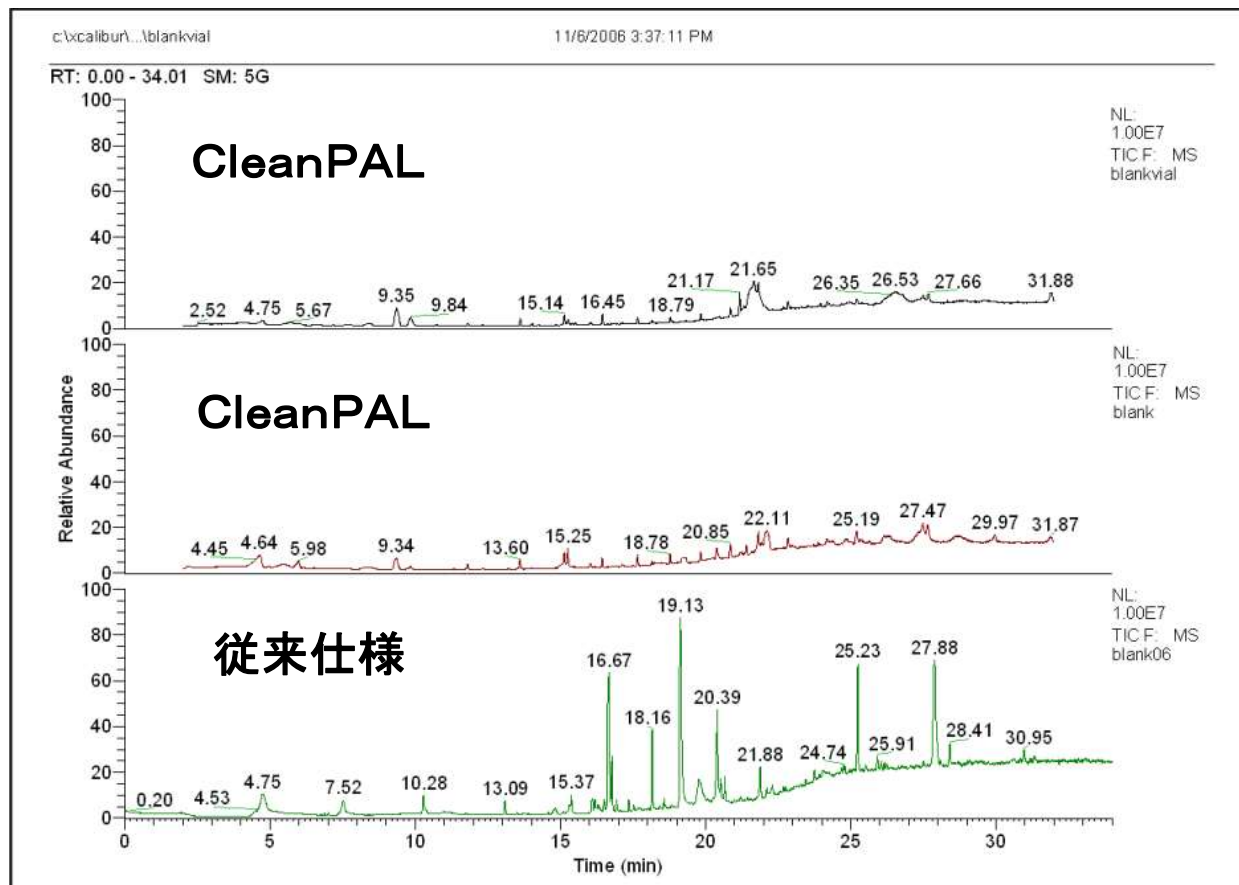
Detection: FID 250C

Comparison of ITEX analysis versus Static Headspace

Sample: Purge and Trap calibration mix (Restek Cat.No. 30431 502.2 CAL2000 Mega-Mix)

独自開発されたClean PAL仕様 (AMR)でのITEX分析

パーズラインを溶出によるコンタミネーションがないような材質に改良されたAMR独自開発のCleanPAL仕様を併用することで優れたS/Nを達成



DARTはサンプルかざすだけ

Introducing the latest in DART technology



■ DARTとは?

DARTイオン化法

による新しいタイプの質量分析用イオン源

EI (電子イオン化)法

CI (化学イオン化)法

MALDI (マトリックス支援レーザー脱離イオン化)法

ESI (エレクトロスプレーイオン化)法

APCI (大気圧化学イオン化)法

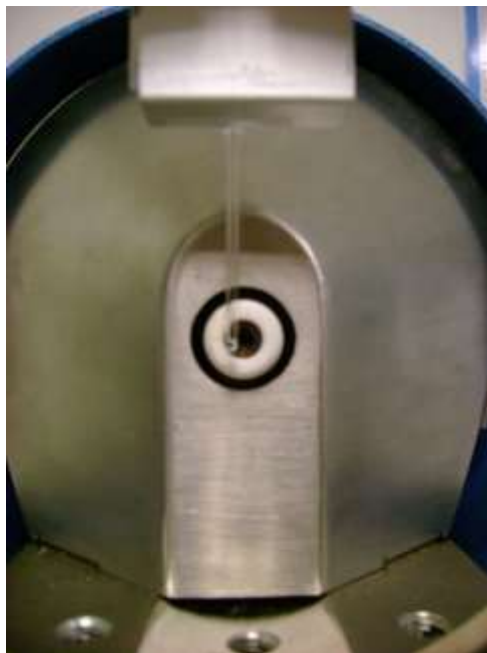
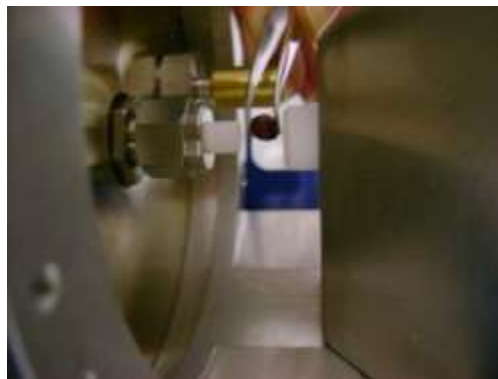
APPI (大気圧光イオン化)法

DART

(リアルタイムで直接イオン化(?))法

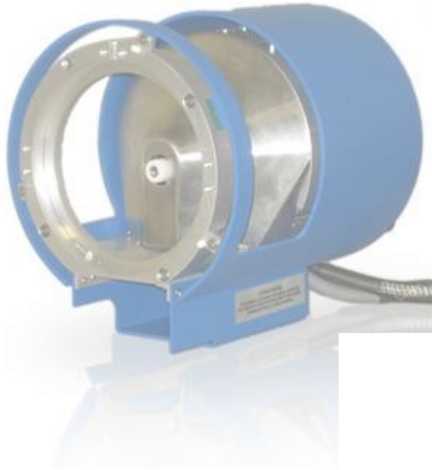


かざすだけでイオン化



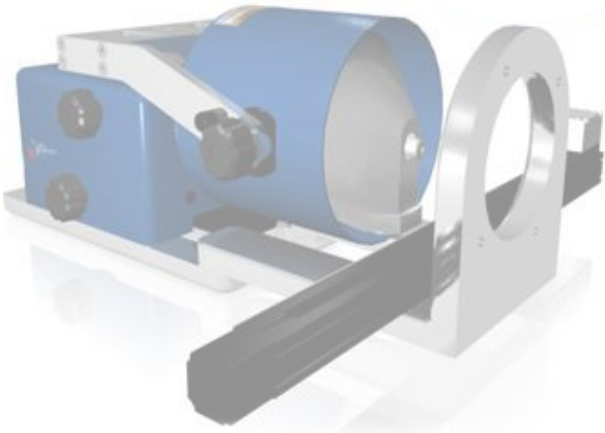
DART-SVP

DART 100 CE



NEW

DART-ET



互換性



AB SCIEX



Agilent Tech



BRUKER

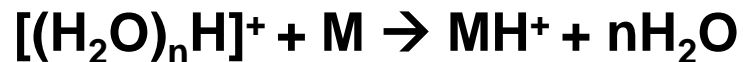
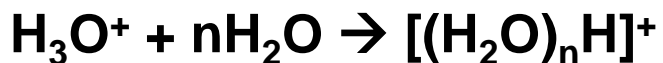
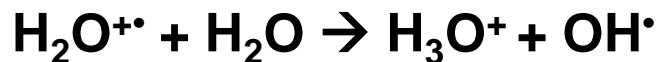
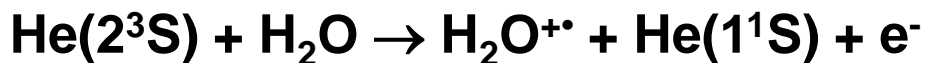


Thermo
SCIENTIFIC

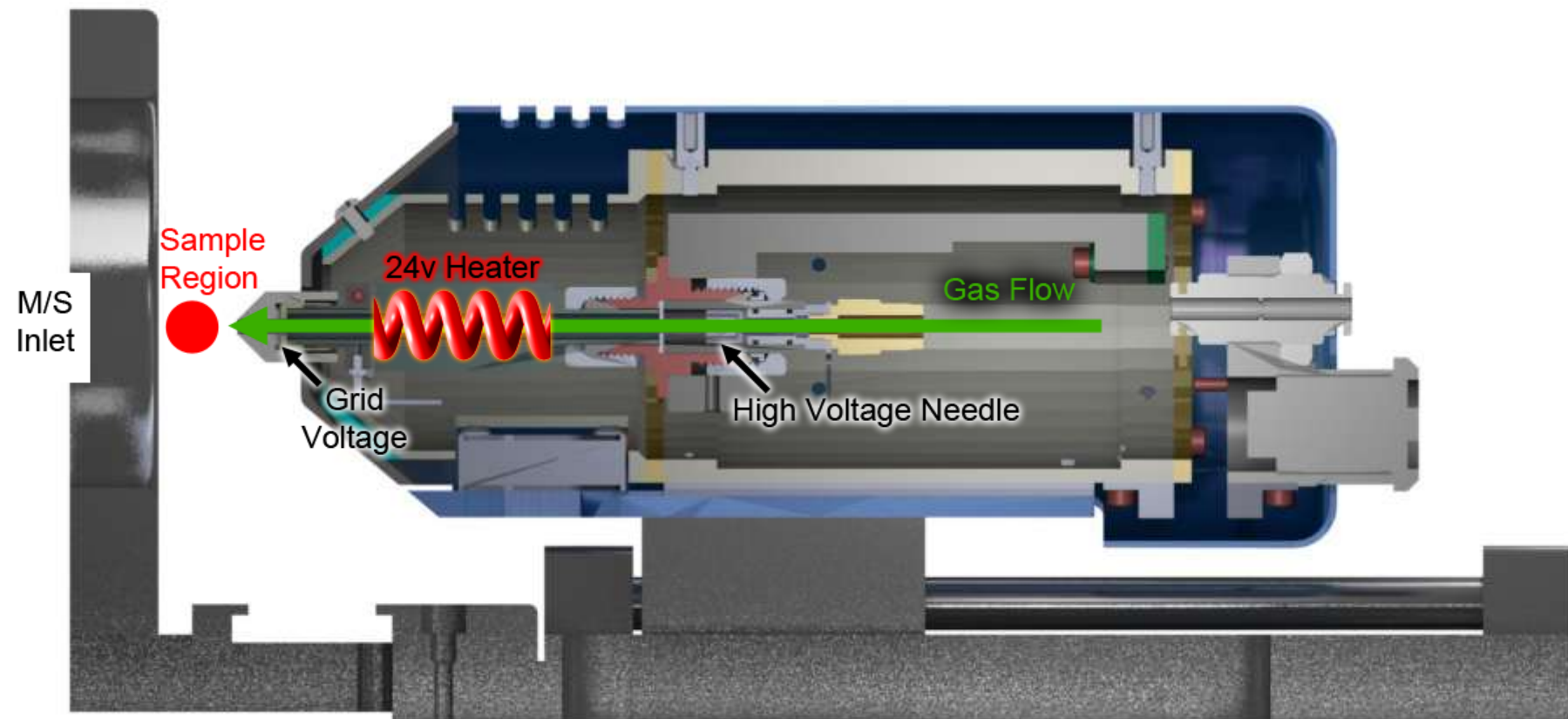


Waters

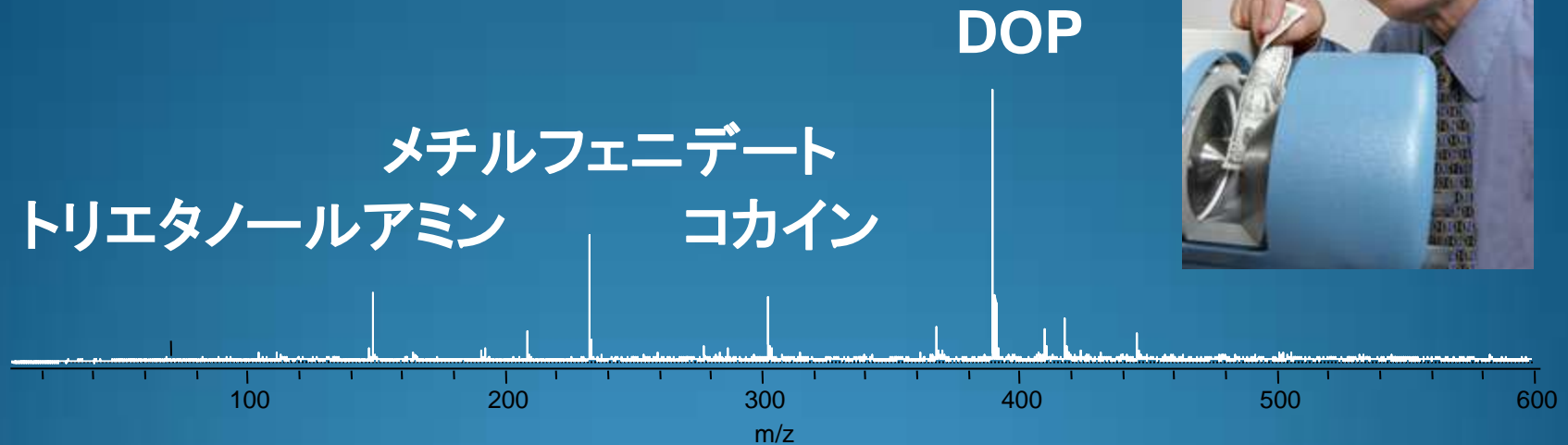
■ DART Ion Source 仕組み



・グロー放電によって生じたHeのメタステーブルが大気中の水分子をイオン化
→クラスターになった水イオンがサンプルにプロトンが付加（ポジティブモード）



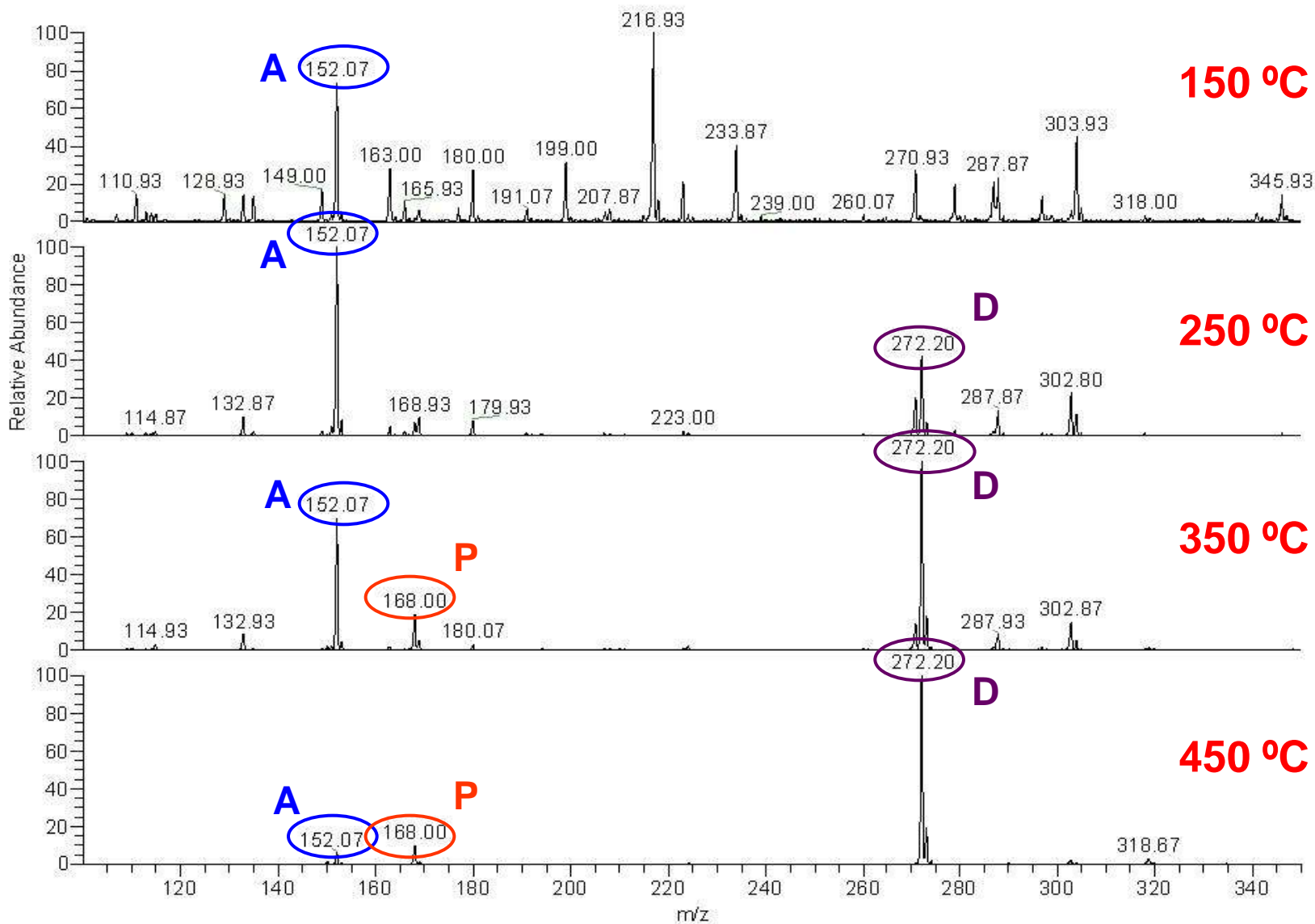
■米流通のドル紙幣、 約90%にコカイン付着！？



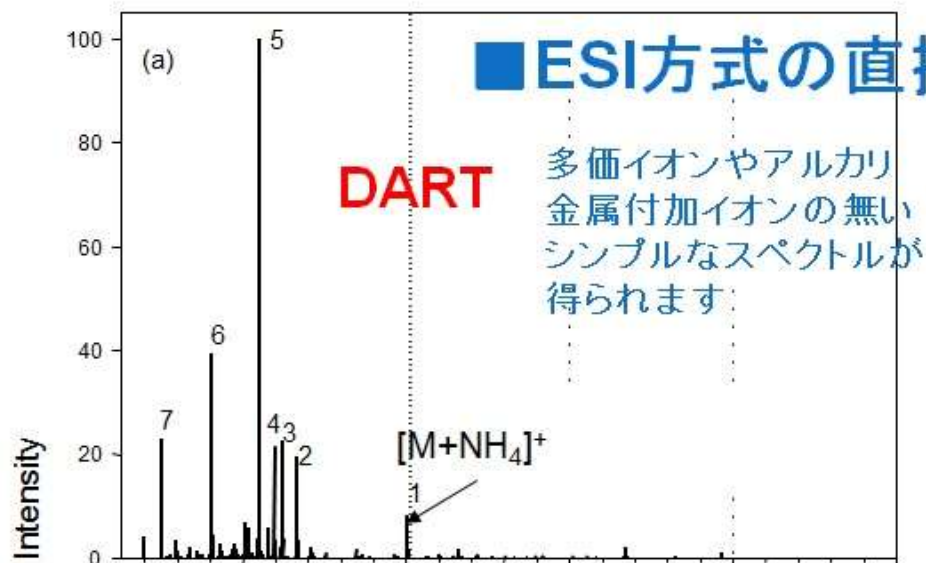
1. トリエタノールアミンは、化粧品で用いられるpH調整用成分です
2. 化合物はMH⁺として検出されます
3. 紙幣上の一般的な物質：日焼け止め、虫よけ (DEET)、ニコチン、グリセロール、ポリエチレングリコール (印刷工程から?)

Temperature Profile Phase of Method Development

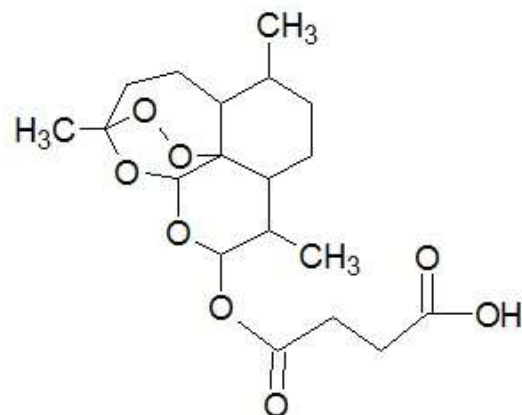
Daytime Caplet – 4 DART-ET Temperatures - Search for 3 Active Ingredients



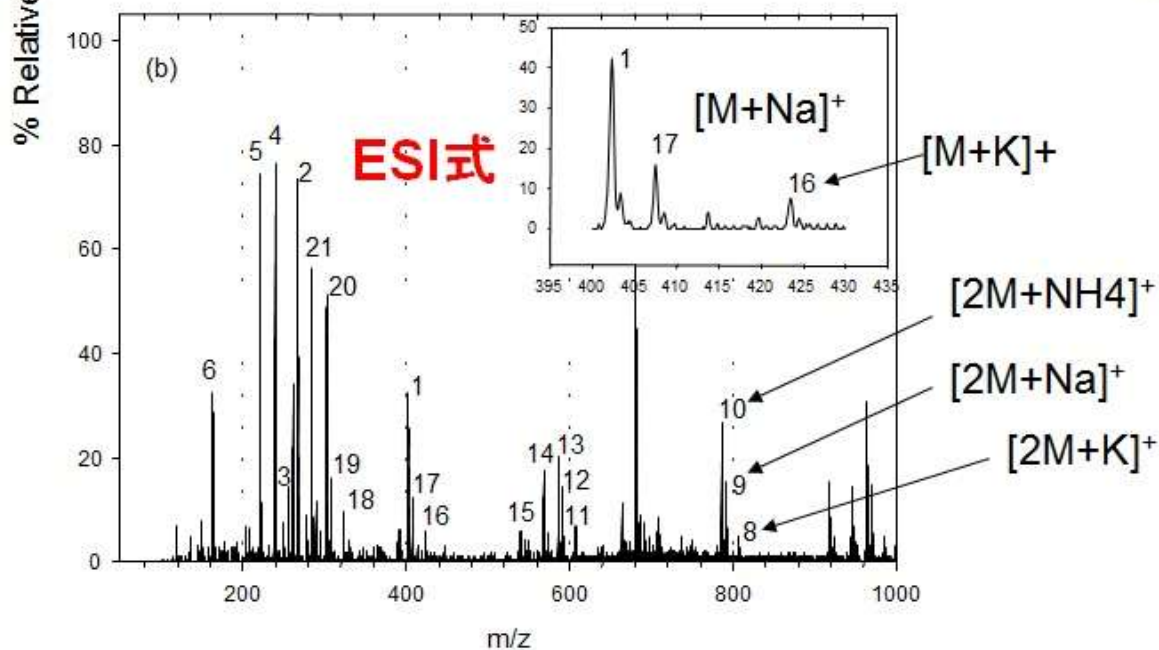
ESI方式の直接分析との違い



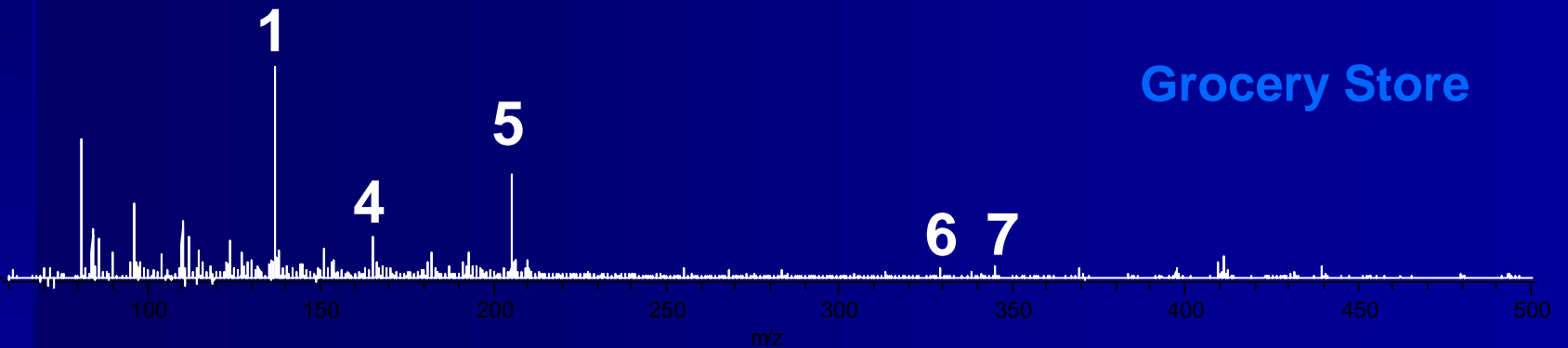
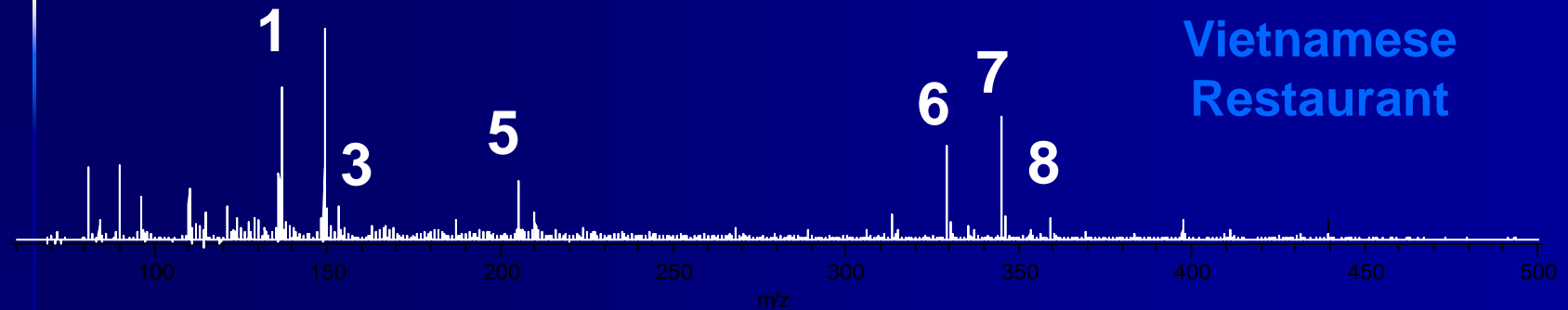
抗マラリア製剤の直接分析



アーテスネート (Artesunate)



Two Basil-Leaf Chemotypes



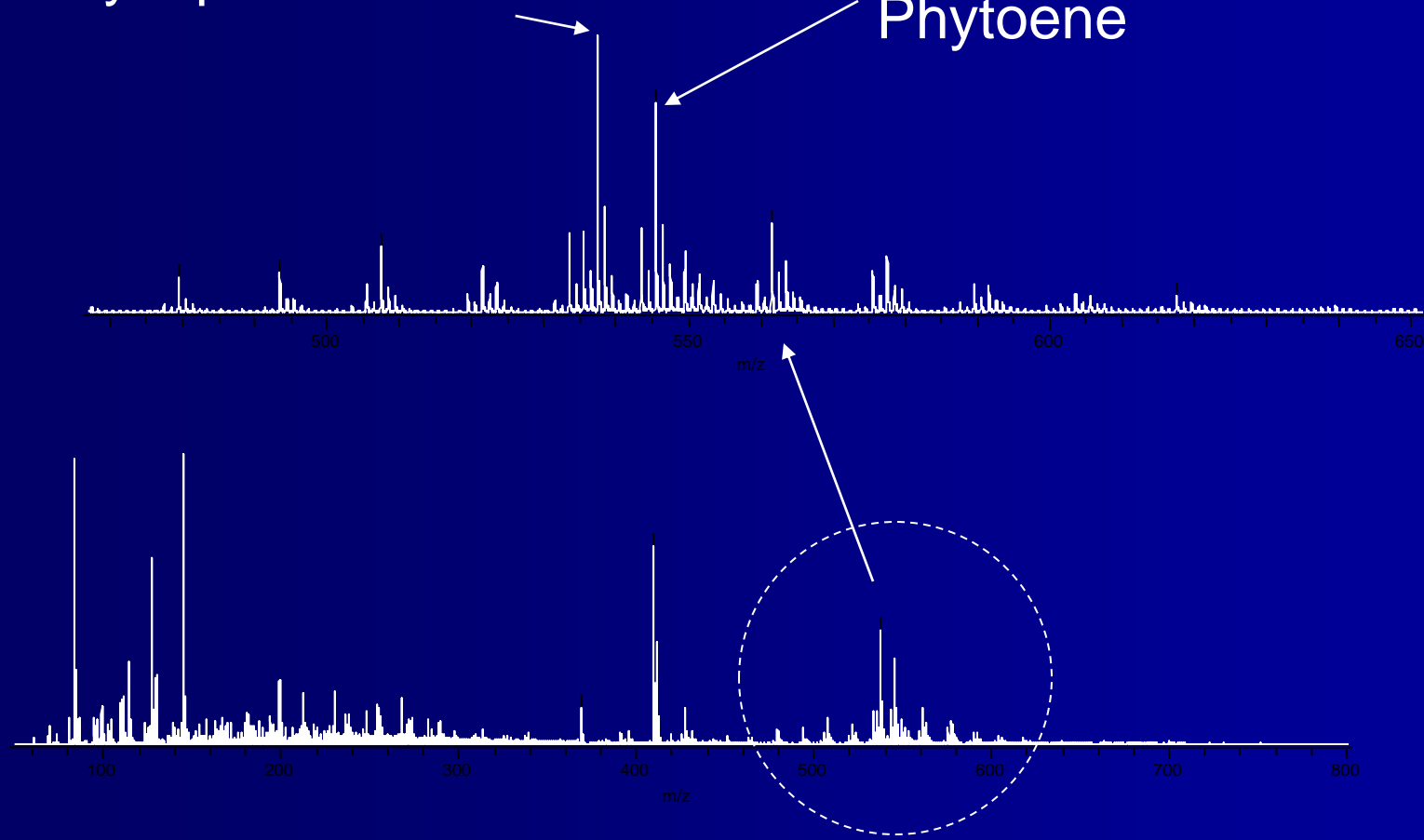
1. $C_{10}H_{16}$ (pinene, terpinine)
2. $C_{10}H_{12}O$ (methylchavicol)
3. $C_{10}H_{16}O$ (citral)
4. $C_{10}H_{12}O_2$ (eugenol)

5. $C_{15}H_{24}$ (sesquiterpenes)
6. Hydroxytrimethoxyflavone
7. Dihydroxytrimethoxyflavone
8. Hydroxytetramethoxyflavone

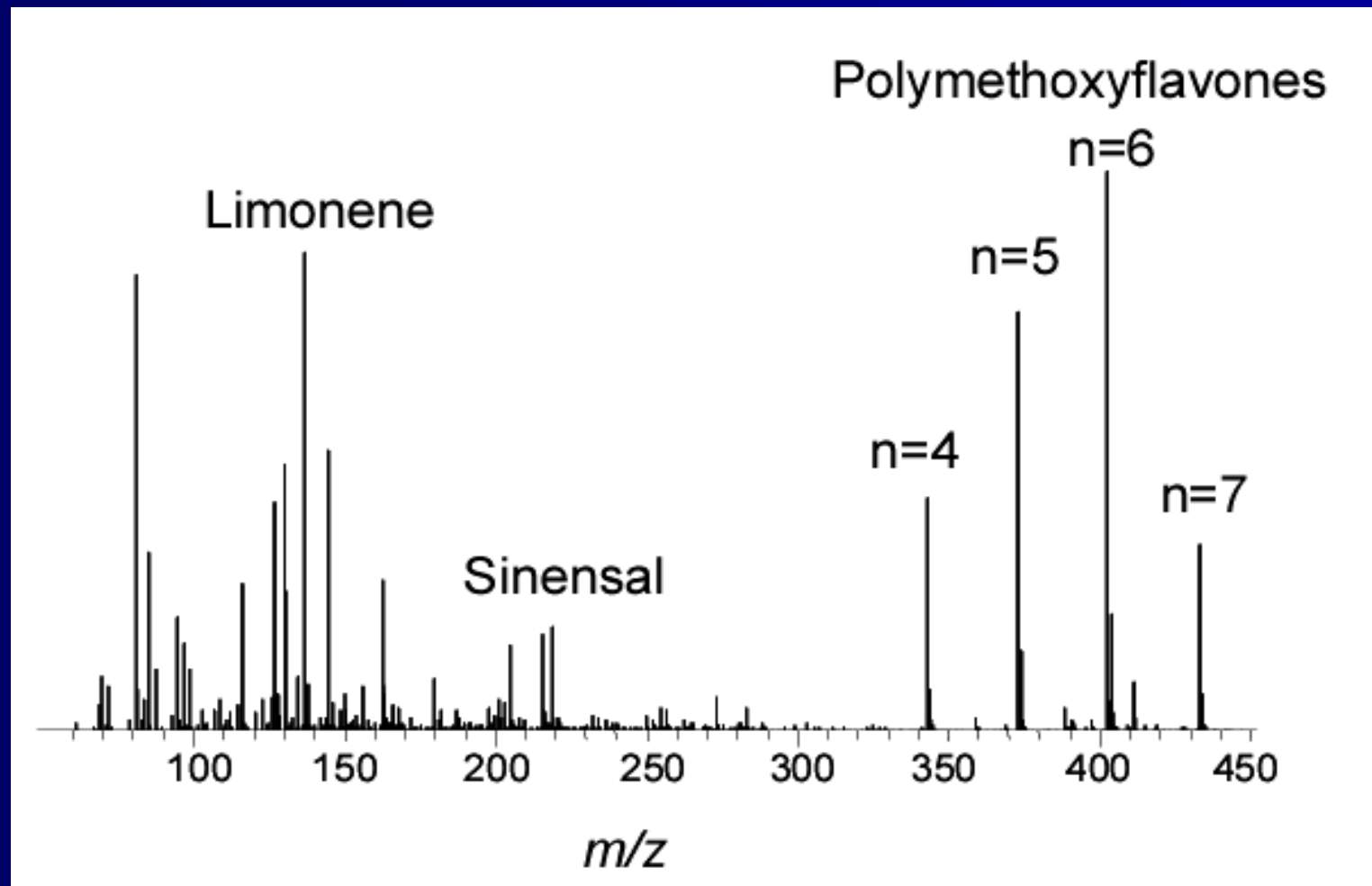
Tomato Skin (Positive ions)

Lycopene and carotenes

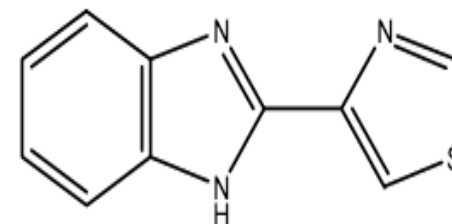
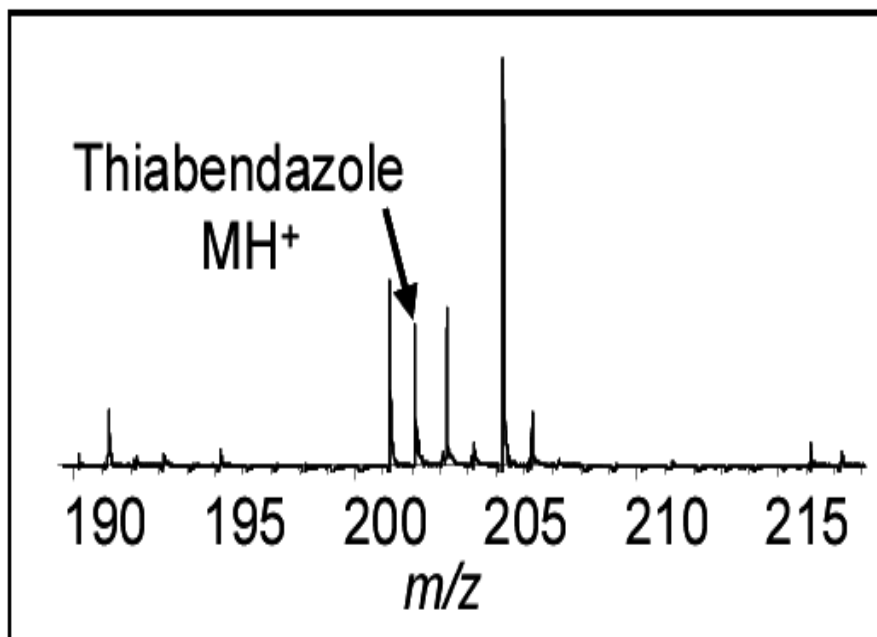
Phytoene



Orange Peel (Positive ions)



Pesticide Residue on Orange Peel (Positive ions)



Thiabendazole

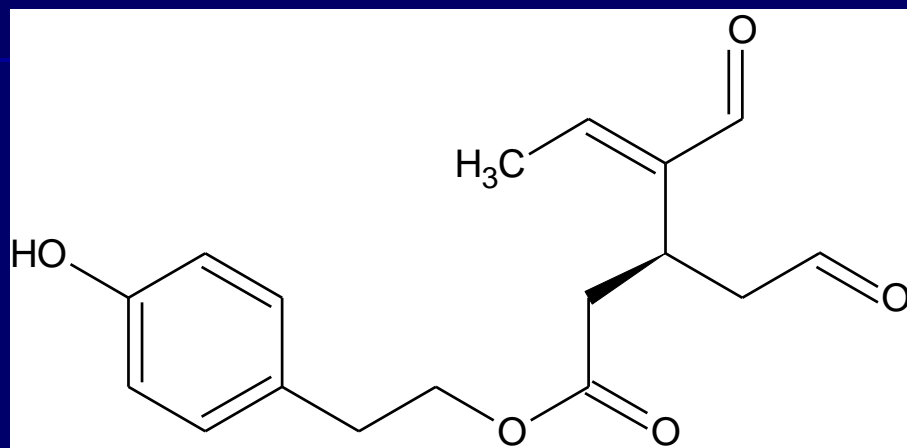
$C_{10}H_7N_3S$

Measured: 202.0444 Da

Calculated: 202.0439 Da

Difference: 0.0005 Da

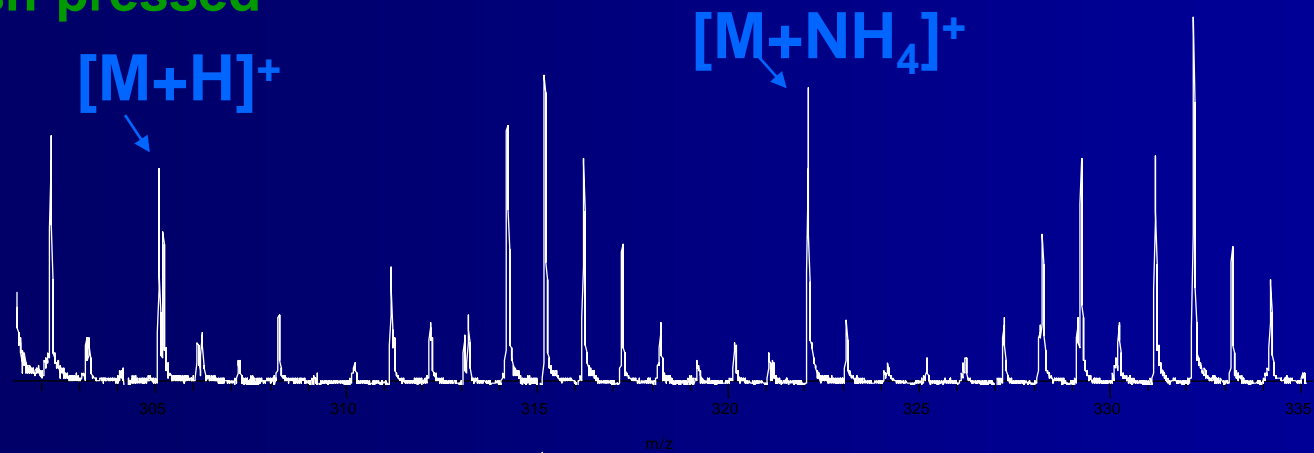
Oleocanthal



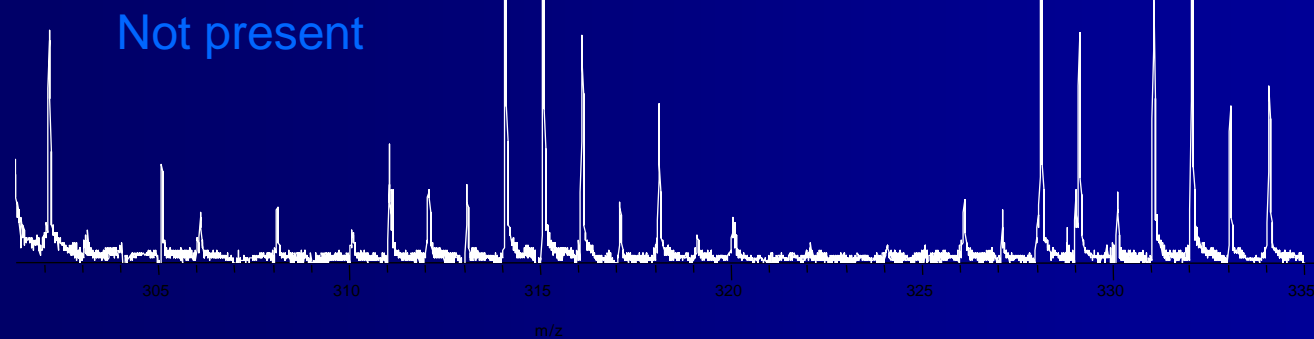
- Present in fresh extra-virgin olive oil
- Reported to have ibuprofen-like activity
- Beauchamp, G. K. et. al. *Nature* **2005**, *437*, 45-46.
"Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil"

Oleocanthal in Olive Oil

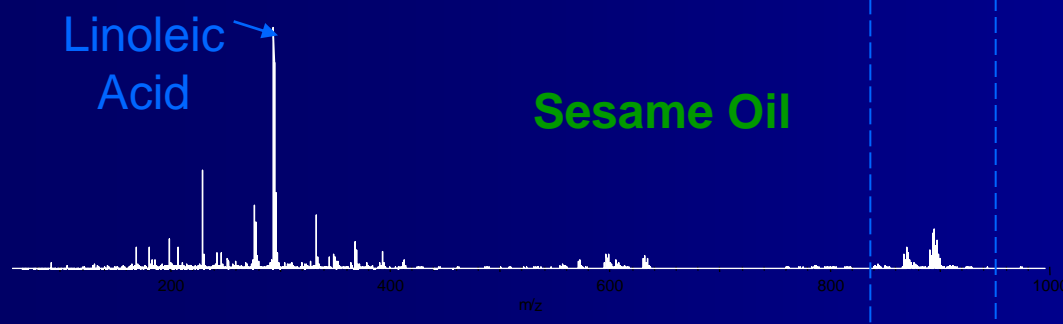
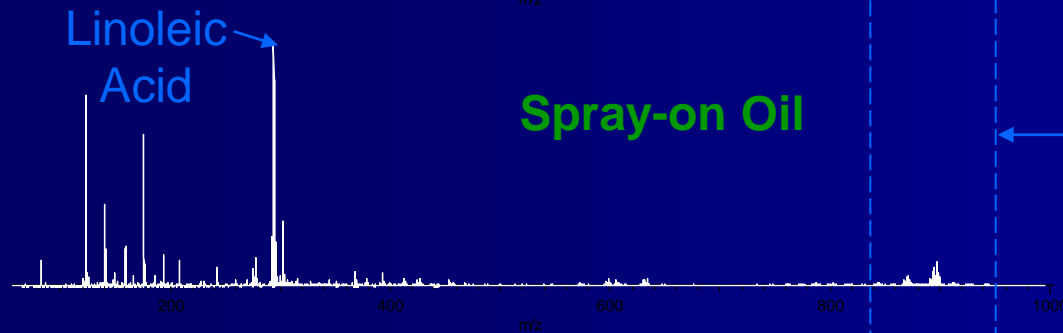
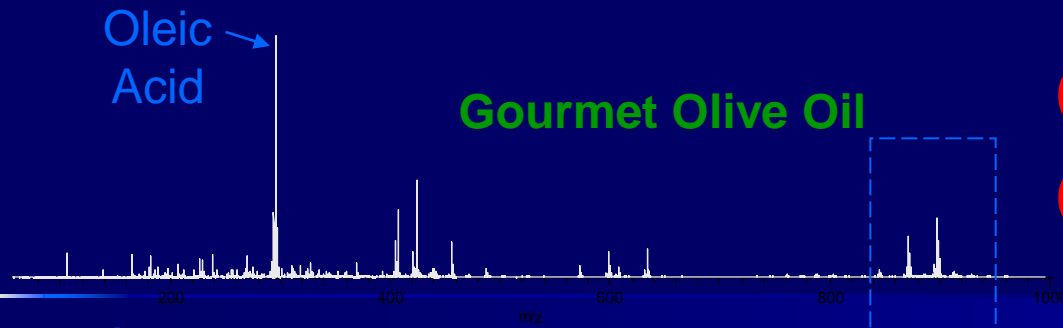
Fresh-pressed



Grocery brand



Cooking Oil Comparison



← Triglycerides

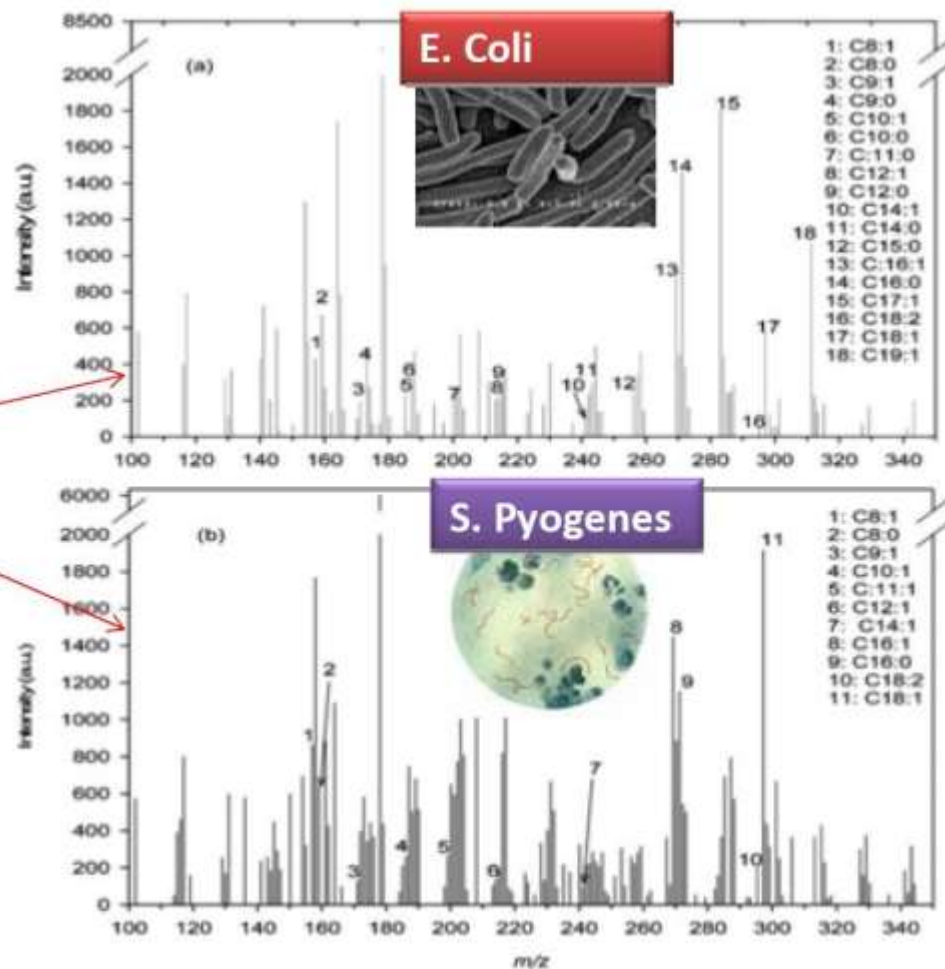
DART/MS—生化学分野への応用

バクテリアの細胞から
脂肪酸メチルエステル (FAME)
イオンがDARTによって測定されました

(a)大腸菌(グラム陰性)

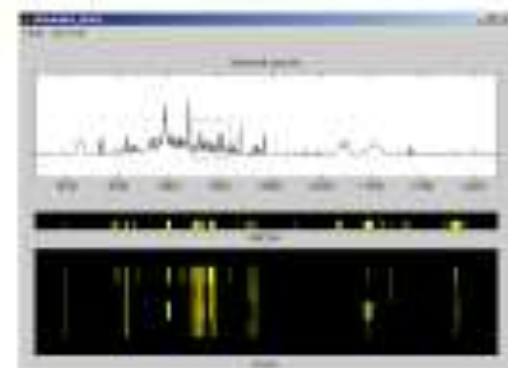
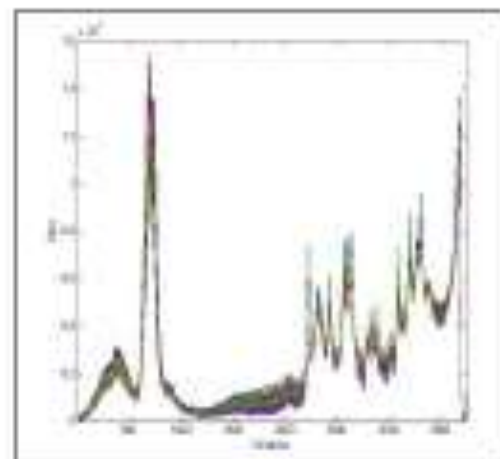
(b)化膿レンサ球菌 (グラム陽性)

バクテリアの脂肪酸の熱加水分解/メチル化
の後、DART-TOF MSで測定



→ Fingerprint methods employing advanced instrumental techniques

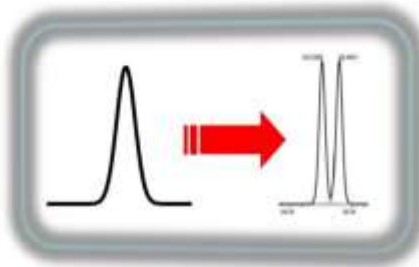
- Near infrared spectroscopy
- Fourier-transform mid-infrared spectroscopy
- Fourier-transform Raman spectroscopy
- Nuclear magnetic resonance spectroscopy
- Direct infusion mass spectrometry
- Gas / liquid chromatography-mass spectrometry



高分解能の威力



DART-TOF MS
medium resolution (1~ 6000 fwhm)

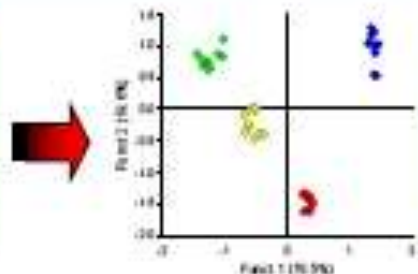


DART-Orbitrap MS
high resolution (10k-100k fwhm)

CHEMOMETRIC TOOLS

Investigation of data structure, statistical model formation

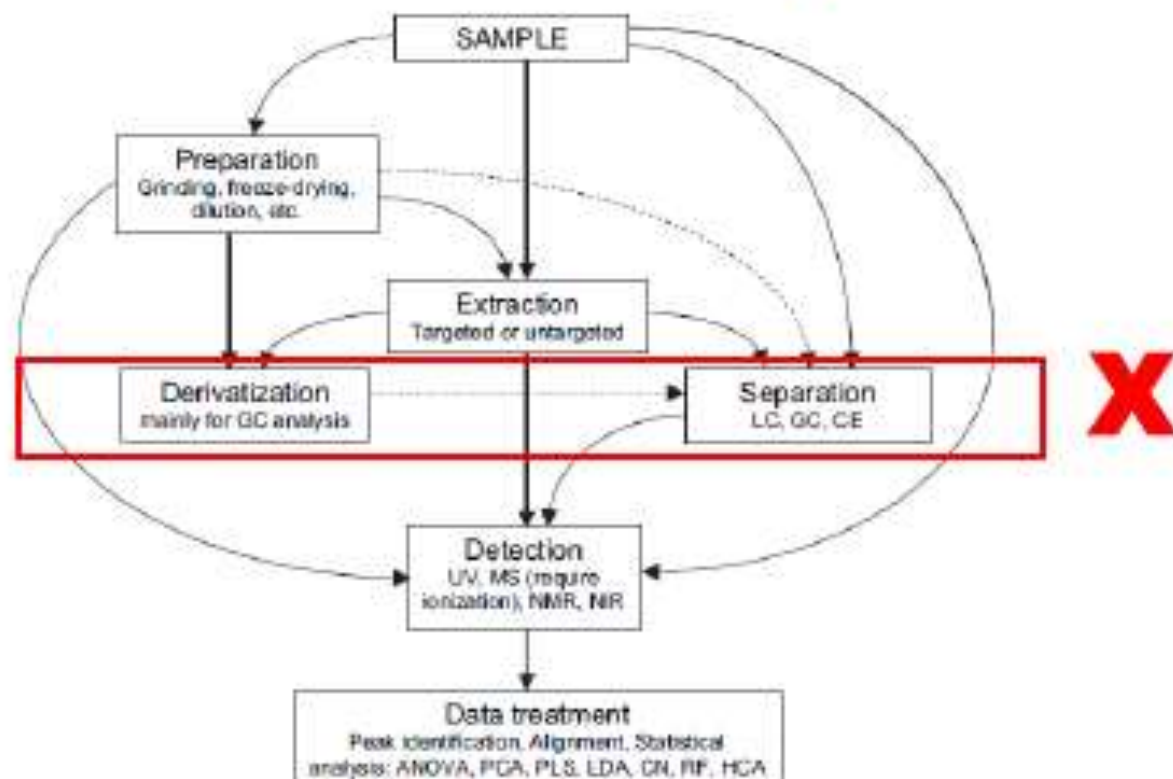
Sample	ExVOO	HO	OO	OPD	MIX	Correctly Classified
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
21	0.00	0.00	0.00	0.00	0.00	0.00
22	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00	0.00	0.00
29	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00
31	0.00	0.00	0.00	0.00	0.00	0.00
32	0.00	0.00	0.00	0.00	0.00	0.00
33	0.00	0.00	0.00	0.00	0.00	0.00
34	0.00	0.00	0.00	0.00	0.00	0.00
35	0.00	0.00	0.00	0.00	0.00	0.00
36	0.00	0.00	0.00	0.00	0.00	0.00
37	0.00	0.00	0.00	0.00	0.00	0.00
38	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00	0.00	0.00
41	0.00	0.00	0.00	0.00	0.00	0.00
42	0.00	0.00	0.00	0.00	0.00	0.00
43	0.00	0.00	0.00	0.00	0.00	0.00
44	0.00	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00	0.00
46	0.00	0.00	0.00	0.00	0.00	0.00
47	0.00	0.00	0.00	0.00	0.00	0.00
48	0.00	0.00	0.00	0.00	0.00	0.00
49	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	0.00	0.00



	Pred. Group (Holdout)					Correctly Classified
	ExVOO	HO	OO	OPD	MIX	
ExVOO	2	0	1	0	0	0.200
HO	0	10	0	0	0	1.000
OO	1	0	9	0	0	0.900
OPD	0	0	0	10	0	1.000
MIX	0	1	1	0	14	0.875
						0.929

- Principal Component Analysis (PCA)
- Partial Least Squares Discriminant Analysis (PLS-DA)
- Linear Discriminant Analysis (LDA)
- Artificial Neural Networks (ANN)

Metabolomic based analysis



J.M. Cevallos-Cevallos et al. / Trends in Food Science & Technology 20 (2009) 557–566

Case study # 1

OLIVE OIL, ANIMAL FATS



OLIVE OIL

Examined samples

various quality grade and botanical origin:

- 🌿 Extra virgin olive oil (EVOO)
- 🌿 Olive oil (OO)
- 🌿 Olive pomace oil (OPO)
- 🌿 Hazelnut oil (HO)



EXTRA VIRGIN OLIVE OIL

The oil obtained from the fruit of the olive tree solely by mechanical or other physical means under the conditions, particularly thermal conditions, that do not lead to alternations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation and filtration.

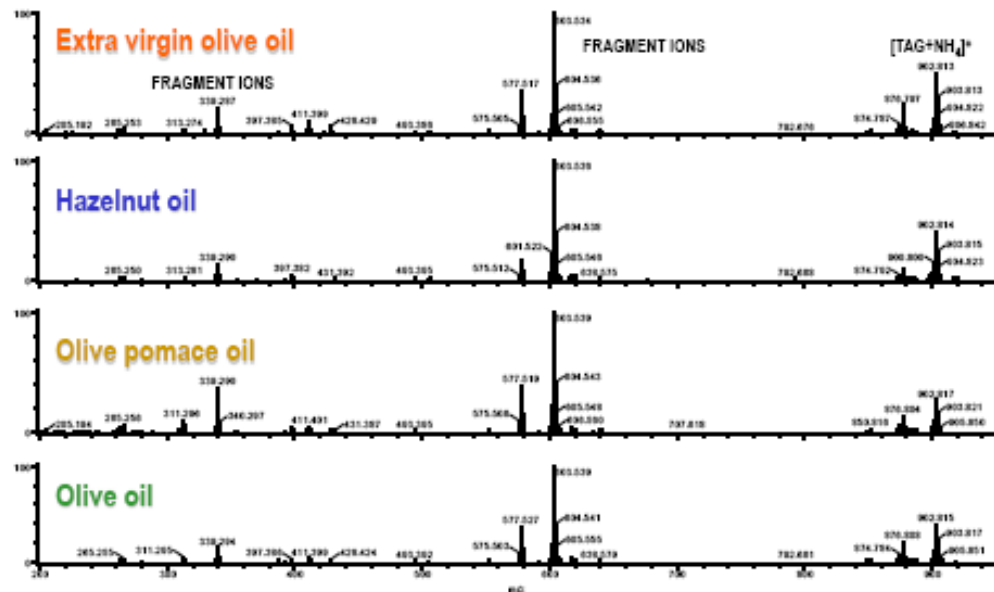
DART-TOFMS [+] mass spectra of DILUTED OILS

SAMPLE PREPARATION

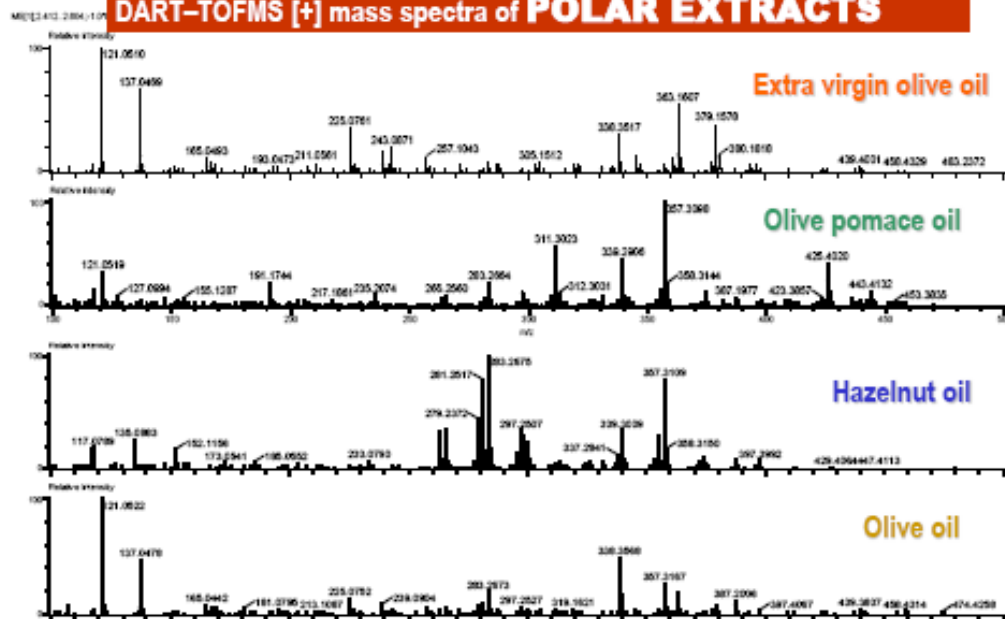
- **TAGs analysis:** oil dilution with toluene (1:50, v/v)
- **Polar compounds analysis:** 2 min shaking of oil with MeOH-H₂O mixture (80:20, v/v)

DART-TOFMS method

- IONIZATION MODE: positive
- ANALYSIS TIME: **1 min**
- GAS BEAM TEMPERATURE
 - 350°C,
 - 220°C
- For TAGs analysis ammonia solution was employed as dopant



DART-TOFMS [+] mass spectra of POLAR EXTRACTS



GROUPING ANALYSIS USING LINEAR DISCRIMINANT ANALYSIS (LDA)

SAMPLE PREPARATION

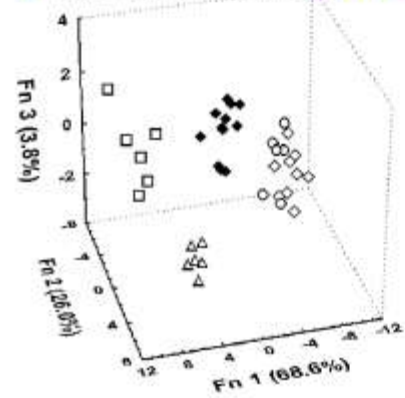
- **TAGs analysis:** oil dilution with toluene (1:50, v/v)
- **Polar compounds analysis:** 2 min shaking of oil with MeOH-H₂O mixture (80:20, v/v)

DART-TOFMS method

- IONIZATION MODE: positive
- ANALYSIS TIME: **1 min**
- GAS BEAM TEMPERATURE
 - (i) 350°C,
 - (ii) 220°C
- For TAGs analysis ammonia solution was employed as dopant



Based on profiles of **TAGs**



- △ EVOO
- HO
- ◇ OPO
- OO
- ◆ MIX

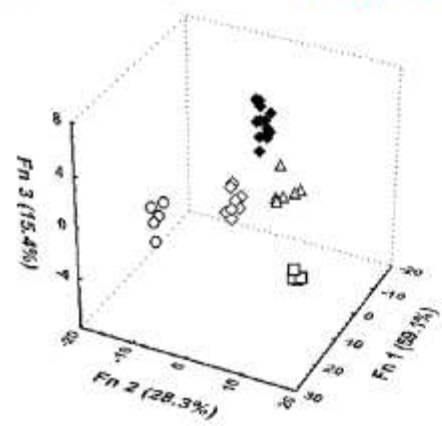
- EVOO, HO, OO, OPO, MIX → **objects**
- TAGs: markers → 11 masses → **variables**

Prediction ability 100% for the EVOO/HO mixtures in the range **50:50 – 85:15 (v/v)**

Software statistiXL 1.8

GROUPING ANALYSIS USING LDA

Based on profiles of **POLAR COMPOUNDS**



- △ EVOO
- HO
- ◇ OPO
- OO
- ◆ MIX

- EVOO, HO, OO, OPO, MIX → **objects**
- Polar compounds: markers → 12 masses → **variables**

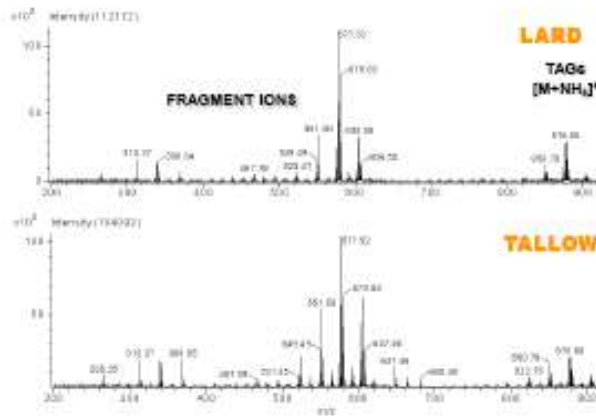
Prediction ability 100% for the EVOO/HO mixtures in the range **50:50 – 94:6 (v/v)**

Software statistiXL 1.8

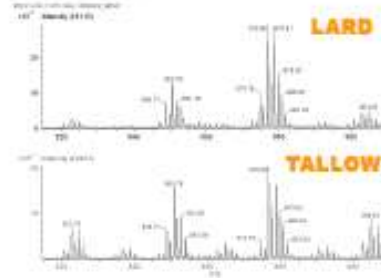
Note: Prediction ability was obtained on the basis of leave-one-out cross validation (LOOCV)

TRIACYLGLYCEROLS PROFILES OF LARD AND TALLOW

Dissolved fat (toluene, 1:50, v/v)



DART [+]
350°C

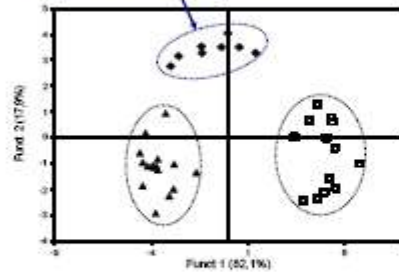


CHEMOMETRIC ANALYSIS

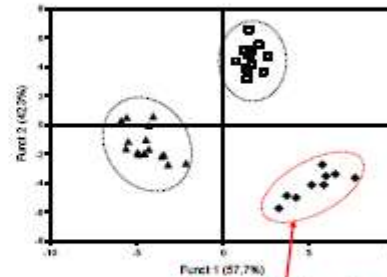
LINEAR DISCRIMINANT ANALYSIS

TRIACYLGLYCEROLS DART [+]
(34 ions used as variables)

Mixes of lard and tallow
from 5:95 to 90:10 (w/w)



▲ Lard
□ Beef tallow



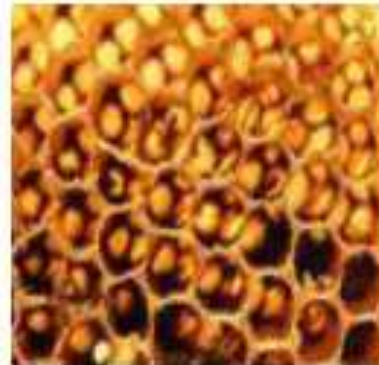
Mixes of pork and beef
from 10:90 to 90:10 (w/w).
corresponds to mixtures of
lard and tallow from 7:93 to
80:20

- both recognition and prediction ability of the LDA model was 100%.
- detection of 5% of tallow in lard and 10% of lard in tallow.
- using mass spectra of fat isolated from meat, model enables detection of 10% pork added to beef and vice versa.

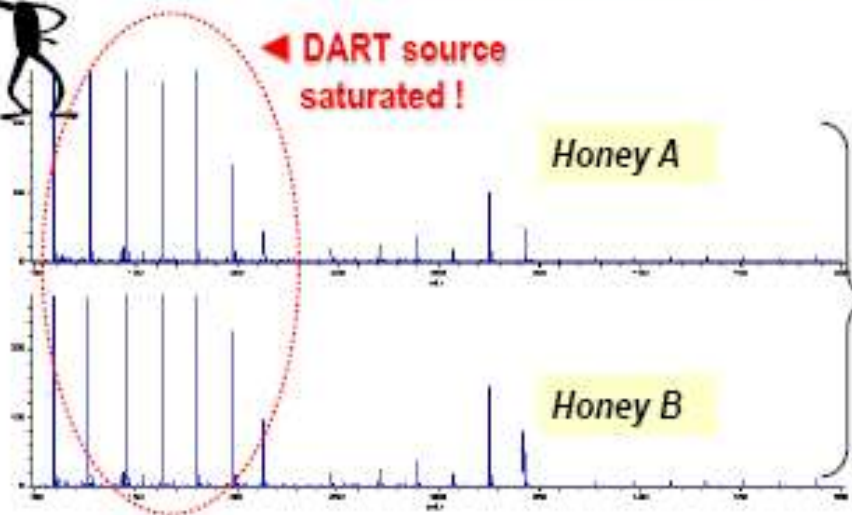
Case study # 2

HONEY

🍯 *Floral origin?*



Can direct be honey analysed directly?



NO!

High intensity of non-characteristic ions - mostly degradation products of sugars (caramelization)

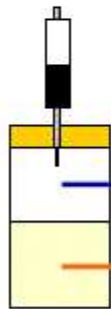
DART: positive ion mode, DART temperature: 250°C

Honey + water (2 g + 2 mL)



PRE-CONCENTRATION USING SPME

- ▶ isolation of volatile compounds in the presence of (abundant) non-target compounds (e.g. sugars)
- or
- ▶ absorption also less volatile (more polar) fraction by immersion of a fibre into sample solution?



HEADSPACE: honey + water (2 g + 2 mL)

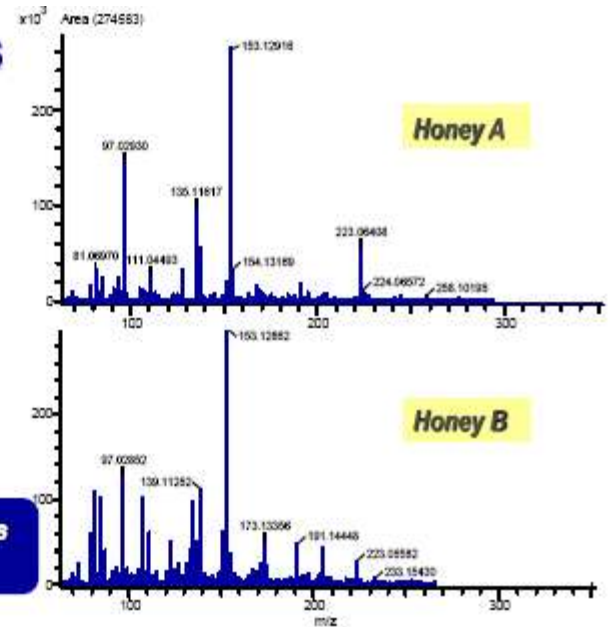
DIRECT IMMERSION: honey + water (2 g + 6 mL)

DART TOF MS Head-space SPME profile

Fiber: DVB/CAR/PDMS 50/30 μm
(divinylbenzene/Carboxen/
polydimethylsiloxane)

DART: positive ion mode
DART temperature: 250°C

lower MW compounds
extracted

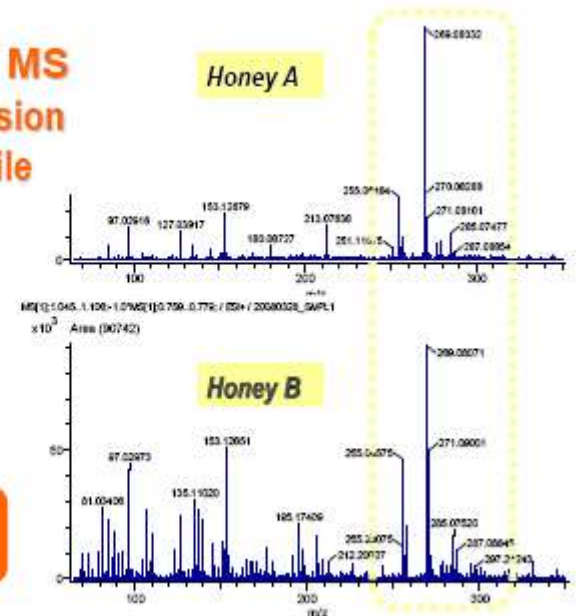


DART TOF MS direct immersion SPME profile

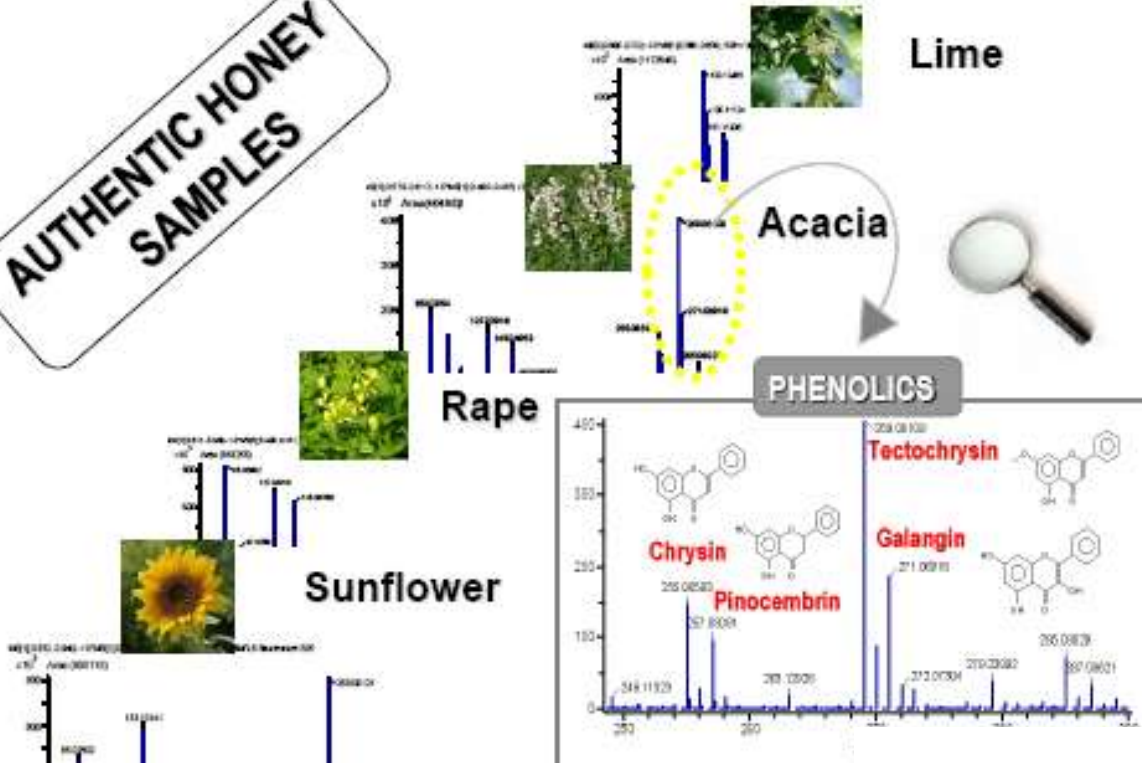
Fiber: DVB/CAR/PDMS 50/30 μm
(divinylbenzene/Carboxen/
polydimethylsiloxane)

DART: positive ion mode
DART temperature: 250°C

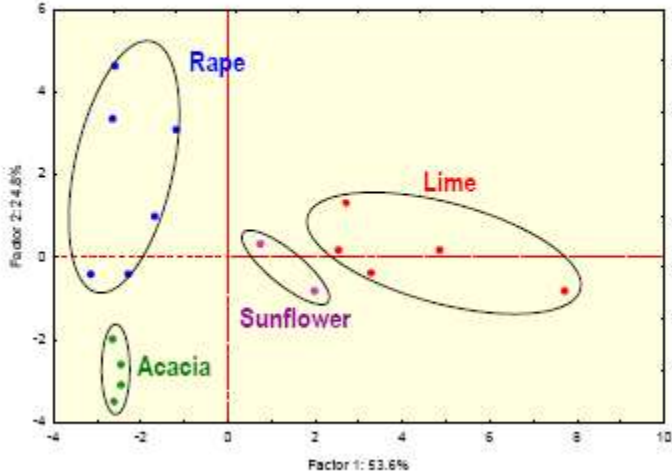
Larger range of
compounds extracted



**AUTHENTIC HONEY
SAMPLES**



**CLASSIFICATION USING PCA
(20 marker masses selected)**



Case study # 3

MEAT

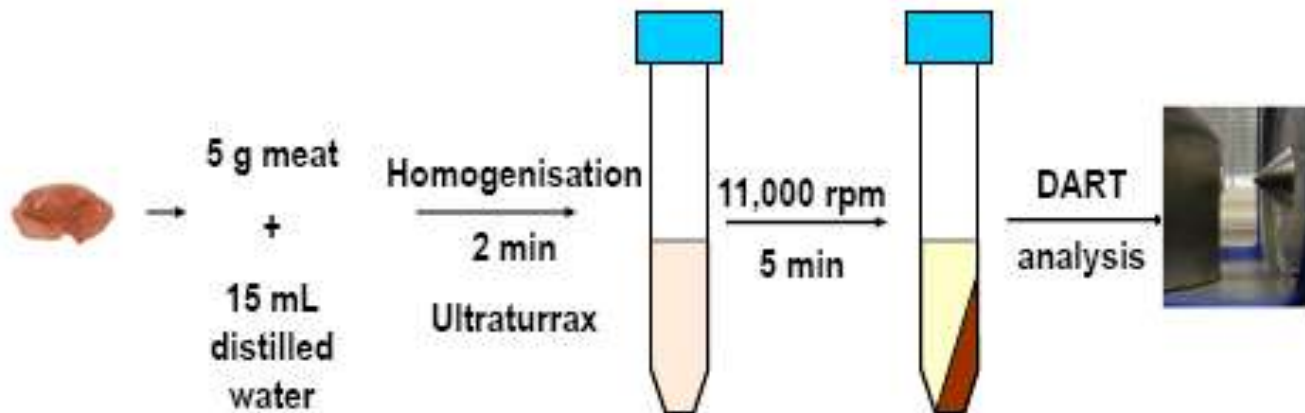


● *Meat freshness*

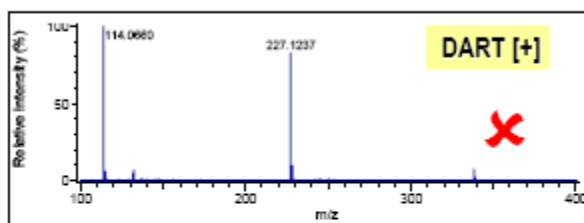


POLAR COMPOUNDS ANALYSIS

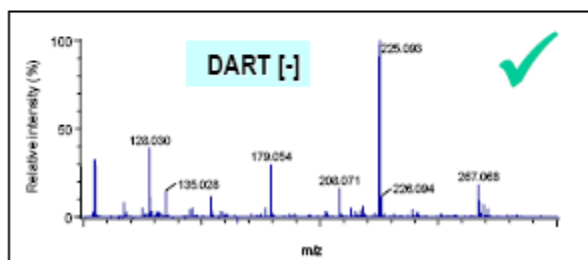
Sample preparation



DART MS PROFILES: PORK



Not too many diagnostic ions present...



A lot of markers present in MS spectrum

**DART
negative
ion mode**

Major ions

**DART
positive ion
mode**

Major ions

Identified compounds

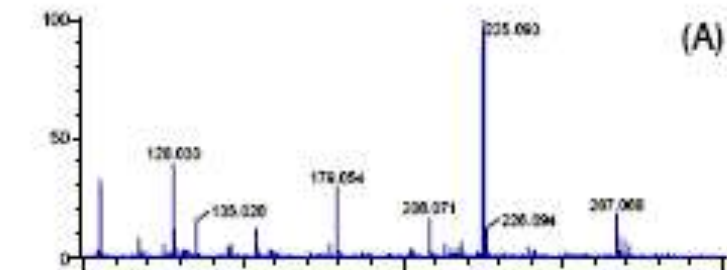
Analyte	El. composition	[M-H] ⁻
Lactic acid	C3H6O3	89.024
Glyceric acid	C3H6O4	105.019
Creatinine	C4H7N3O	112.051
Succinic acid	C4H6O4	117.019
5-Oxoproline	C5H7NO3	128.035
Hypoxanthine	C5H4N4O	135.031
Glutamine	C5H10N2O3	145.061
Glutamic acid	C5H9NO4	146.045
Xanthine	C5H4N4O2	151.026
Glucose	C6H12O6	179.056
Carnosine	C9H14N4O3	225.099
Anserine	C10H16N4O3	239.114
6-Hydroxyl-1,6-dihydroxy	C10H14N4O5	269.089

Identified compounds

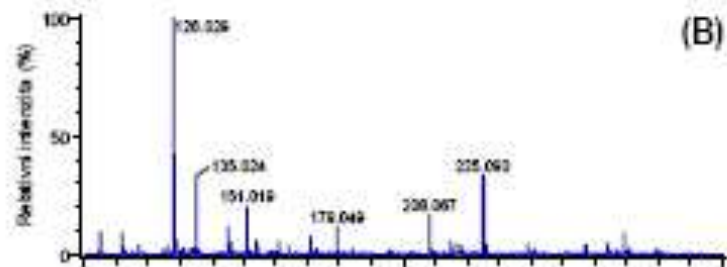
Analyte	El. composition	[M+H] ⁺
Glycine	C2H5NO2	76.040
Cadaverine	C5H14N2	103.124
GABA	C4H9NO2	104.071
Histamine	C5H9N3	112.087
Creatinine	C4H7N3O	114.067
Proline	C5H9NO2	116.071
Threonine	C4H9NO3	120.066
Nicotinamide	C6H6N2O	123.056
Scatole	C9H9N	132.081
Hypoxanthine	C5H4N4O	137.046
Spermidine	C7H19N3	146.166
Methionine	C5H11NO2S	150.059
Histidine	C6H9N3O2	156.077
Carnosine	C9H14N4O3	227.114
Anserine	C10H16N4O3	241.130

MEAT PROFILES

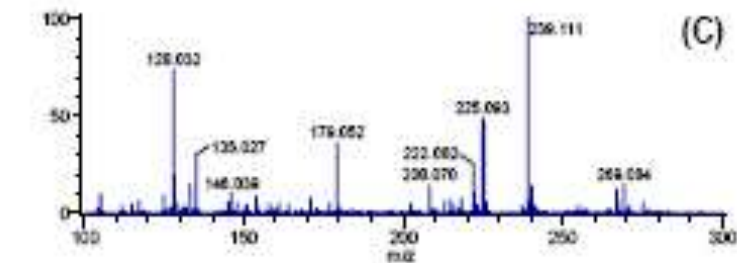
DART
negative ion
mode



Pork

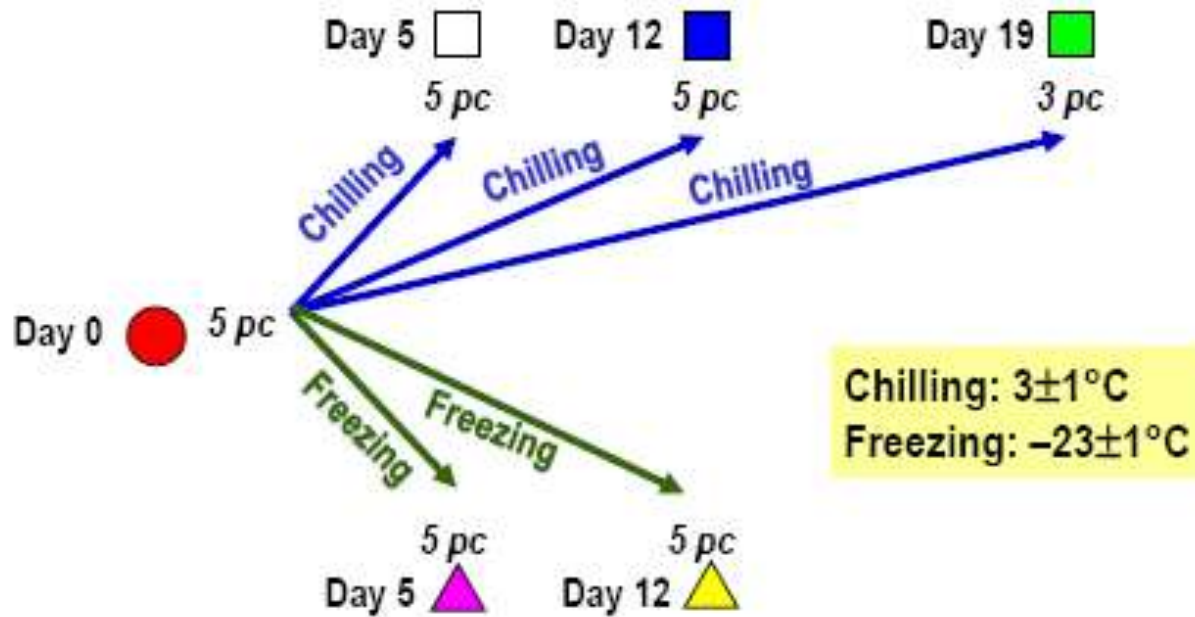


Beef



Rabbit

PORK FRESHNESS



☒ CHEMOMETRIC ANALYSIS

Step 1: 30 markers selected (DART negative ion mode) →

Step 2: Normalisation to a maximum variable in the spectrum of each sample

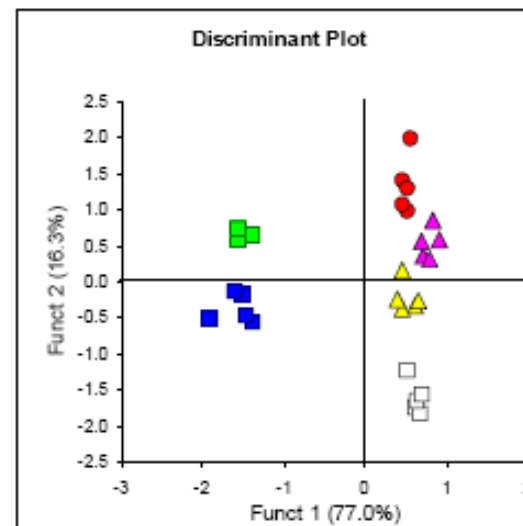
Step 3: Principal Component Analysis

Step 4: Linear Discriminant Analysis
→ model formation using the most important principal components

m/z

Marker	Marker
105.01	226.10
117.01	237.06
128.03	239.11
135.02	241.09
145.05	251.11
146.04	267.07
147.03	269.09
171.00	271.10
177.03	275.10
179.05	293.16
202.08	315.13
208.07	333.09
218.07	356.12
223.08	434.18
225.10	451.20

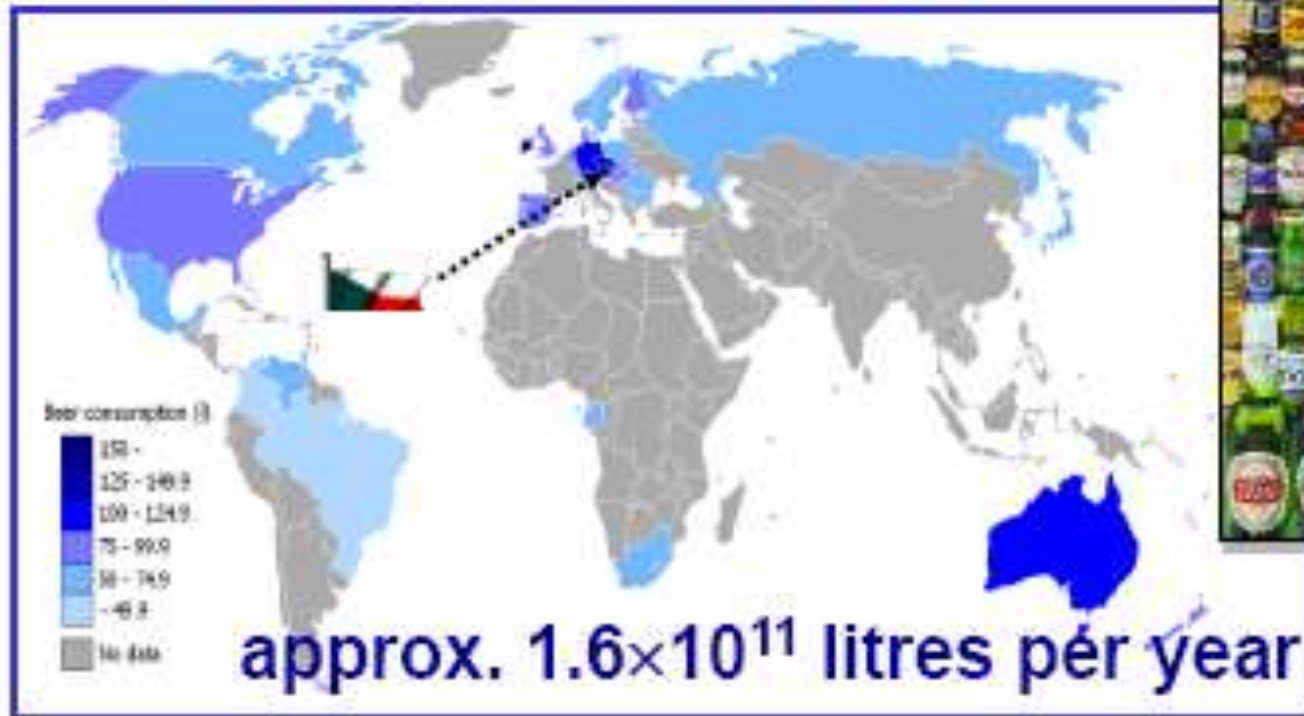
☒ CHEMOMETRIC ANALYSIS



- Day 0
- Day 5 (chilling)
- Day 12 (chilling)
- Day 19 (chilling)
- ▲ Day 5 (freezing)
- ▲ Day 12 (freezing)

BEER CONSUMPTION IN THE WORLD

Question: Where is the darkest spot...?



Source: en.wikipedia.org

BACKGROUND OF THE STUDY

- Aim of study



Tracing the origin of food



DISTINGUISH ROCHEFORT BRAND FROM OTHER TRAPPIST AND NON-TRAPPIST BEERS

- 400 different beers in Belgium
- Trappist beer is local speciality
- Beer samples involved in study:
 - Rochefort
 - other Trappist
 - non-Trappist



OVERVIEW OF SAMPLES

- Trappist beer ($n = 137$)
 - Rochefort 6 ($n = 6$)
 - Rochefort 8 ($n = 48$)
 - Rochefort 10 ($n = 26$)
 - Other Trappist beers ($n = 57$)
- Non-Trappist beers ($n = 128$)

265 samples



Samples were collected continuously over one year to cover possible seasonal variability of the products.

A. HS-SPME-GC-TOFMS

Step 1:
SPME extraction
of volatiles



Step 2:
GC/TOF-MS

Leco TruTOF HT system



25 min per
sample
(including data
processing)

HS-SPME-GC-TOFMS VOLATILES PROFILE

Compound	t_r (N)	m/z
1 Ethyl acetate	382	81
2 Octanol	511	84
3 Ethyl heptanoate	552	85
4 Hexan-1-ol	564	87
5 Undecane (n: 45, 55, 111, 145)	577	95
6 Ethyl acetate	587	81
7 Nonanol	604	90
8 Heptan-1-ol	600	90
9 Acetic acid	607	60
10 Hexan-2-carboxylate	605	96
11 Octyl acetate	671	96
12 2-Ethylhexan-1-ol	676	93
13 Decan-2-one	685	95
14 1,2-Pyridinediol	682	96
15 Nonan-3-ol	712	95
16 (E)-1,7-Dimethylcyclohexan-2-one	712	95
17 Decyl acetate	725	98
18 1,7-Dimethylcyclohexan-2-one (isomer)	724	91
19 Octan-1-ol	730	86
20 Octan-2-one (polyketone)	735	85
21 Malonic acid	791	60
22 Ethyl benzoate	795	91
23 2-Phenylacetate	797	83



45
markers

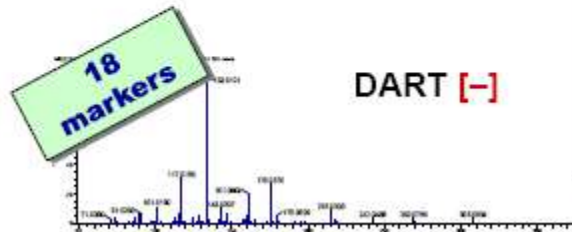
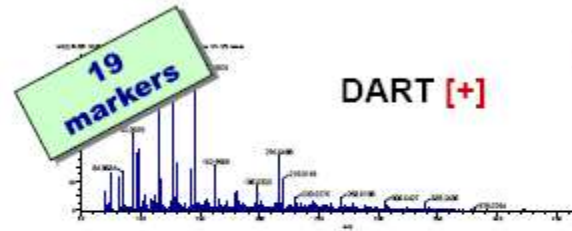
Compound	t_r (N)	m/z
24 2-Furylfuranol	824	98
25 Nonan-1-ol	825	98
26 2-Methylbutyl acetate	810	127
27 Ethyl benzoate	812	126
28 Ethyl benzoate	819	131
29 Ethyl benzoate	822	130
30 3-Methylbutyl acetate	844	126
31 Undecan-2-ol	848	95
32 Decan-1-ol	871	84
33 (E)-3,7-Dimethylcyclohexan-2-one (isomer)	876	123
34 Dec-2-yn-1-ol	912	95
35 Phenethyl acetate	916	91
36 (E)-2,6,6-Triethylcyclohexan-2-one	921	98
37 (E)-3,7-Dimethylcyclohexan-2-one (isomer)	922	98
38 Ethyl benzoate	926	131
39 Phenolic acid	929	92
40 2-Phenylacetol	934	92
41 Octanoic Acid	1033	88
42 Undecane (n: 45, 55, 67, 83, 91, 107, 123, 145)	1082	92
43 Nonanoic acid	1085	88
44 Decanoic acid	1126	88
45 Undecane (n: 41, 55, 67, 79, 91, 103, 119, 145)	1129	92

B. DART-TOFMS: direct measurement

<3 min
per sample
(including data
processing!)



DART-TOFMS SPECTRA



No.	m/z (Positive)	No.	m/z (Negative)
1	81.95	1	75.02
2	84.95	2	80.02
3	93.01	3	101.02
4	95.98	4	115.00
5	99.00	5	117.02
6	116.02	6	128.03
7	125.99	7	129.01
8	130.00	8	133.01
9	145.00	9	143.03
10	163.01	10	161.04
11	160.04	11	175.06
12	156.03	12	179.05
13	216.06	13	197.02
14	219.02	14	215.03
15	256.03	15	217.03
16	278.05	16	289.06
17	289.02	17	328.09
18	296.03	18	317.00
19	305.02		

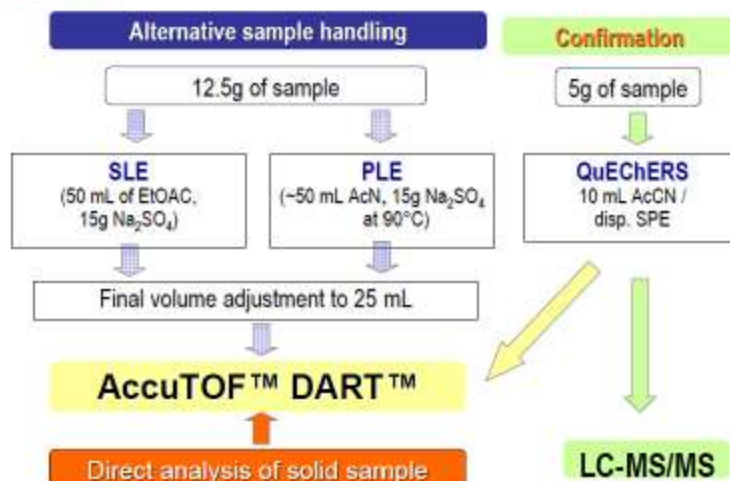
Overall aim of the Work package



Development of rapid screening methods for a specific class of fungicides (strobilurins) in cereals

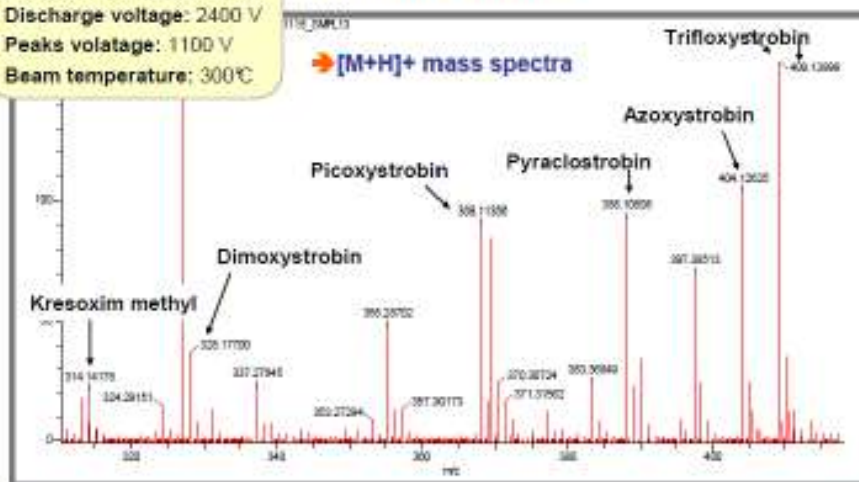
Work to be completed at ICT Prague...

- (iv) Testing of a new generation of DART ion source "Baby DART" → improvement of performance characteristics expected...



Polarity: positive (DART+)
Helium flow rate: 2.6 L/min
Discharge voltage: 2400 V
Peaks voltage: 1100 V
Beam temperature: 300°C

wheat extract



Crude ethyl acetate extract spiked with strobilurins at 120 µg/kg

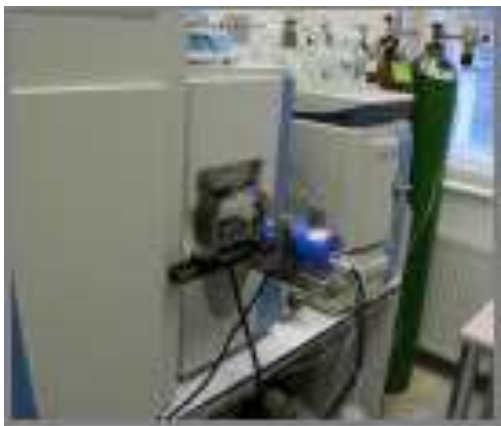
Institute of Chemical Technology, Prague, CZ



Leading Czech research institution in chemistry and related fields with a long history of relationships with industry both in the Czech Republic and abroad.



Direct successor to the Chemistry Department of the Prague Polytechnic, officially called the Royal Czech Polytechnical Institute, founded by Emperor Francis II. in 1803.





5th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

1 - 4 November, 2011 • Prague, Czech Republic

Symposium sessions introducing RECENT & EMERGING (BIO)ANALYTICAL APPROACHES in areas:

- Allergens
- Flavours and odours
- Genetically modified organisms (GMO's)
- Industrial contaminants
- Mycotoxins, marine and plant toxins
- Nanoparticles
- Packaging contaminants
- Pesticide residues
- Processing contaminants
- Veterinary drugs



▪ Authenticity, Traceability, Fraud

- Novel foods & supplements
- Organic crops & foodstuffs

▪ QA/QC and chemometrics in food analysis, Accreditation





互換性



AB SCIEX



Agilent Tech



BRUKER



Thermo
SCIENTIFIC



Waters

■まとめ

- DARTは物質の表面を大気圧下・非破壊でサンプリングできる新しいタイプの質量分析用イオン源
- 気体、液体、固体の分析
- ESIやAPCIなどでカバーしきれない極性範囲もイオン化が可能
- 一価イオンのみ生成し、アルカリ金属付加イオンなども生じないため、シンプルなスペクトルが得られる
- Thermo, Waters, Bruker, ABI, Agilentなど各社MSに対応
- 食品中の成分、フレグランス、脂質、紙上のインク、尿中の代謝物、錠剤中の賦形剤・有効成分、ポリマー・ポリマー添加剤などの分析、リアクションモニタリングなど様々なアプリケーションが可能



ID CUBE™

Enabling Near Instantaneous Characterization of
Chemicals using DART Technology



DART-SVP Ion Source



ID-CUBE: Built on DART Technology



Fundamentals of the ID CUBE

- Stand alone source
- High current power supply
- Enables consumable: OpenSpot™ sample consumable card (OSSC)
- Designed for rapid vaporization of sample into the DART ionizing gas
- No method development required for different samples

MS Instrumentation

Thermo Exactive

- Accurate mass measurements within 3 ppm for all work presented (external)
- Scan Parameters:
 - Positive & Negative Ion Modes
 - Resolution: "High" - 50,000
 - Fragmentation: None, HCD Gas Off
 - Scan Settings: 1 μ -scan by 250 ms max inject time
 - AGC Target: Balanced ($1e^6$)
- Exactive Inlet Parameters:
 - Capillary Temp: 200° C 200° C
 - Capillary Voltage: 25 V -50 V
 - Tube Lens Voltage: 120 V -120 V
 - Skimmer Voltage: 26 V -25 V



- For all ID-CUBE experiments the Exactive Spray Voltage was set to 4 kV and the following parameters set to zero:
 - Sheath Gas Flow, Aux Gas Flow, Sweep Gas Flow

The OpenSpot Sample Card (OSSC)

- Consumable for ID Cube
- Business Card shape and size
- Sample applied to narrow cutout in metal screen
- Sample information can be written on the card
- Integral part of the thermal desorption process



Spotting the card

- Spot solution with pipettor, or place droplet of liquid containing analyte on center spot

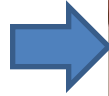


- Card is ready to analyze

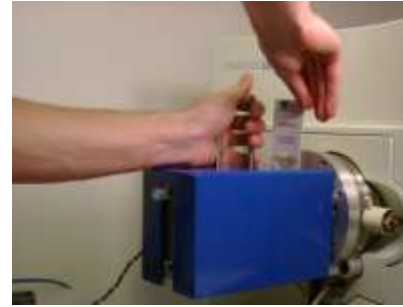


Actions

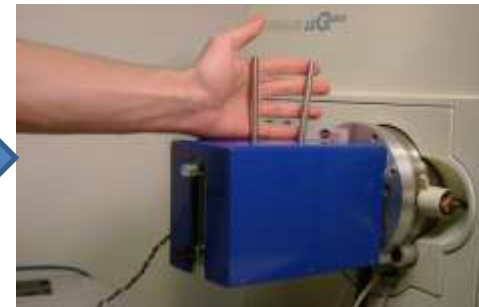
- Finger clamps are squeezed



- Sample card is inserted into the guide slot



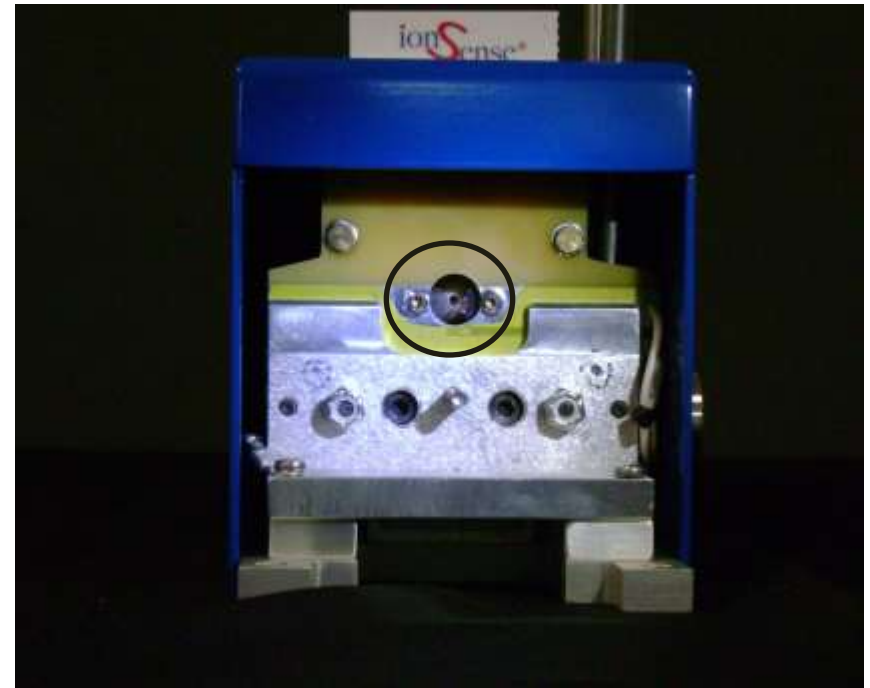
- User releases the clamps to complete the electrical circuit



Positioning

- The card carrying the sample positioned in the circuit
- Without current no desorption occurs unless the sample vapor pressure is high

ID CUBE™ with sample in place

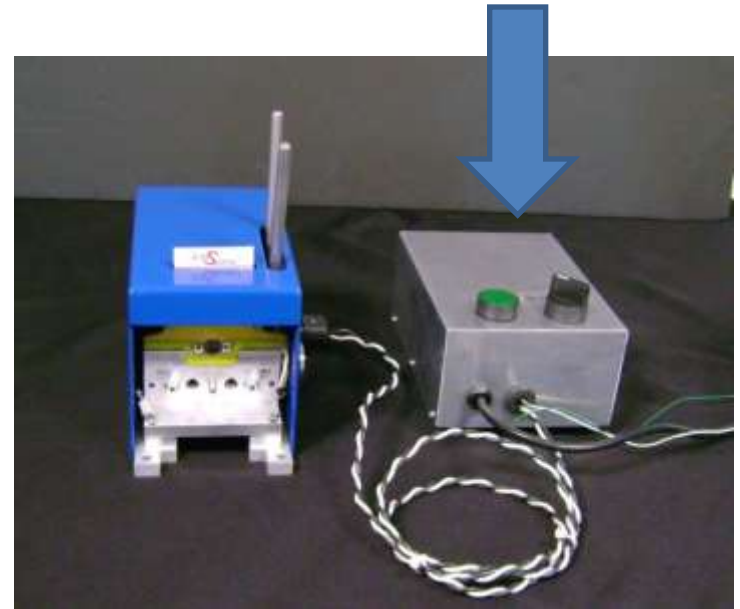


Vapur® interface removed for picture

The Power Supply

- A novel power supply provides high current through the circuit
- User selects heating rate (High-Med-Low)
- Push button to start and release to stop
- Resistance in the wire screen generates a localized heat

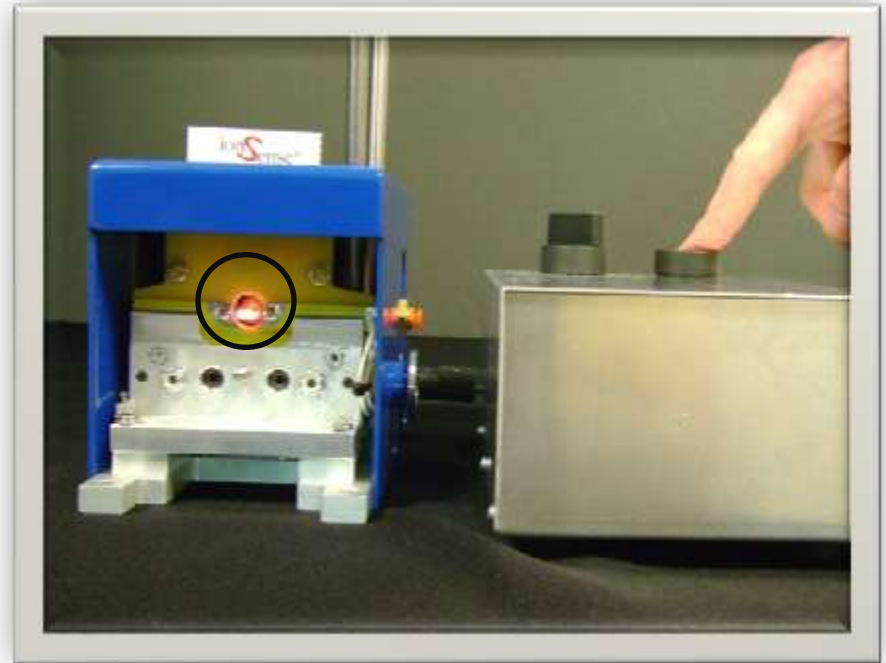
Variable current power supply



Simple and easy to use design!

Rapid Heating of the Sample

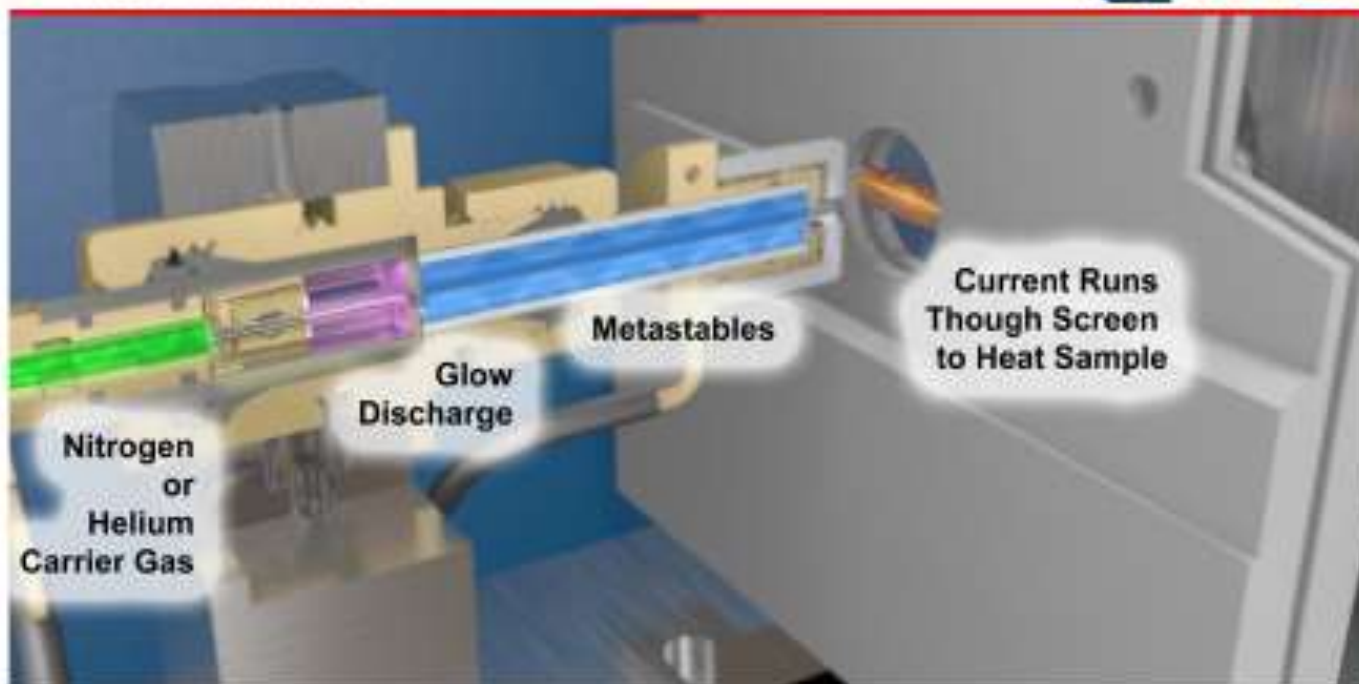
- Depressing the power supply switch drives several amps through the mesh screen
- Vaporization is rapid as the screen becomes red hot in seconds
- Releasing the power supply switch stops the current and ion production as well



Sample Vaporized in 5-10 seconds!

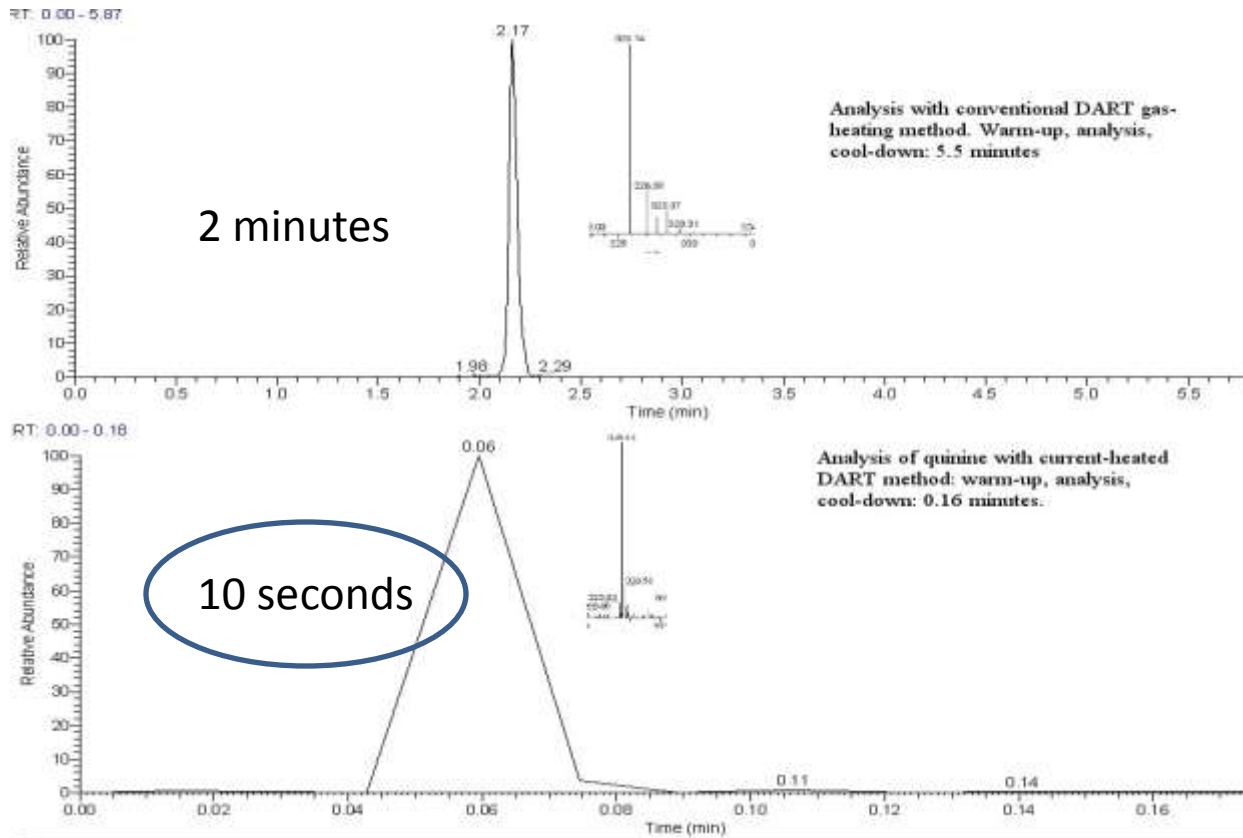
ID-CUBE™ Ionization Source

How it Works



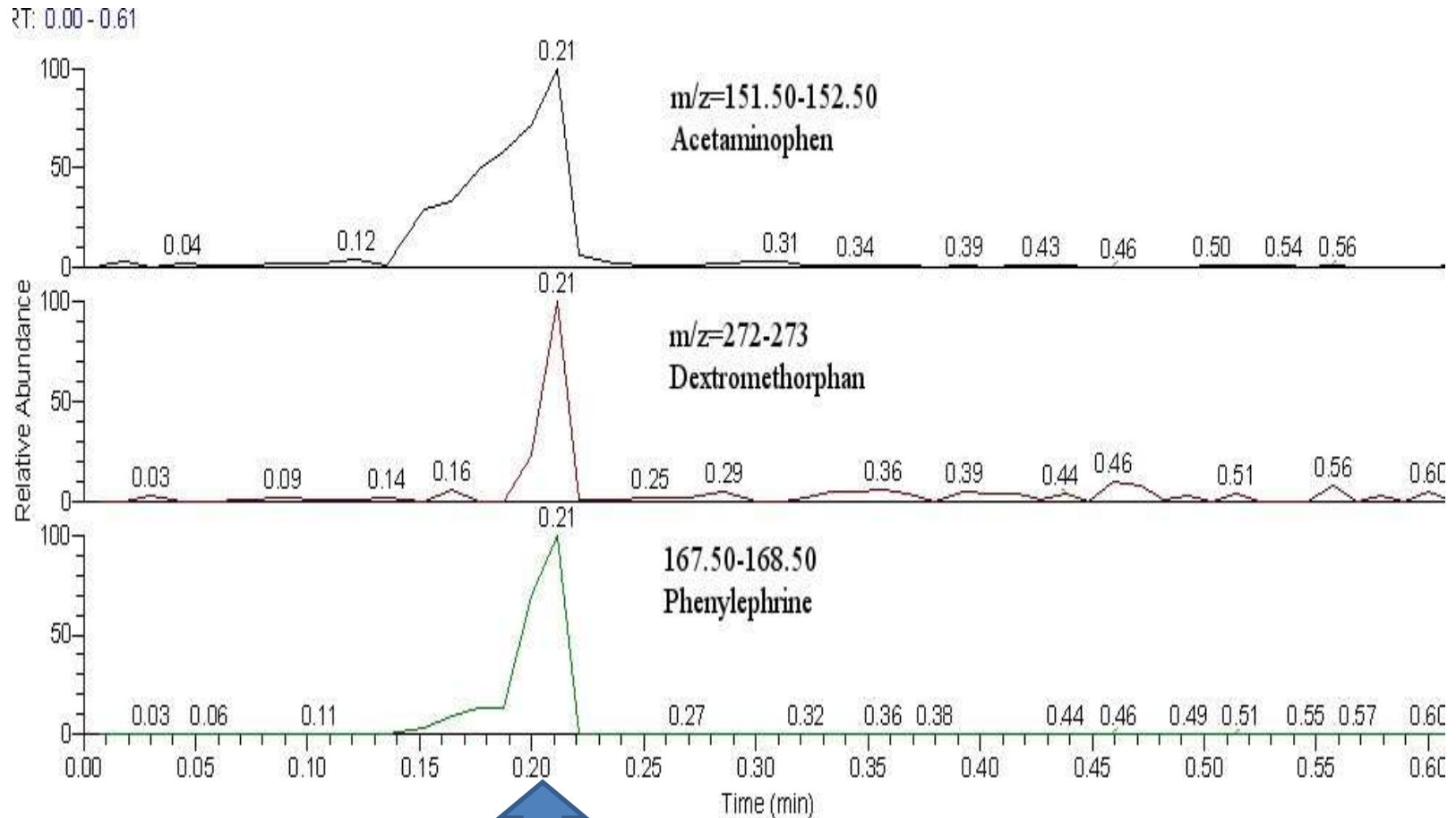
Thermal Assisted DART

Next Generation Open Access



Instantaneous Up and Run time!

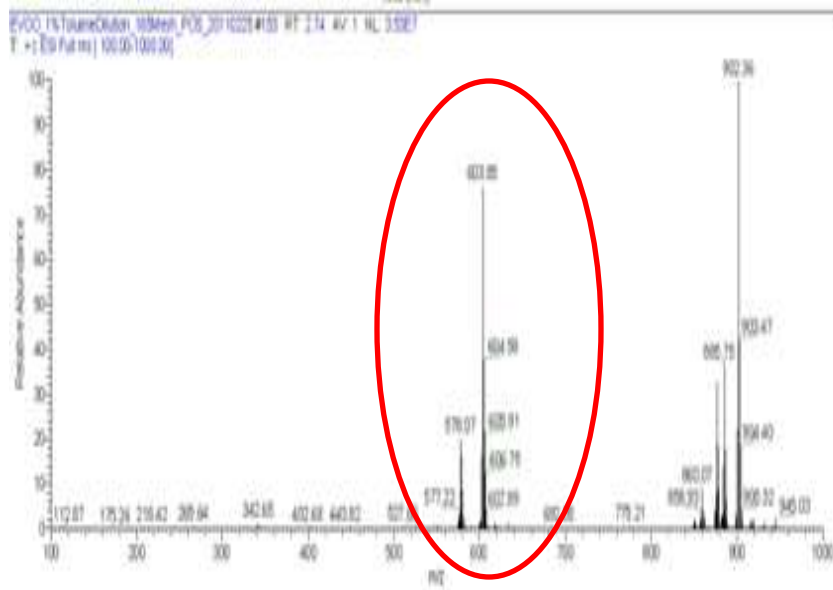
Analysis of Tylenol Tablet



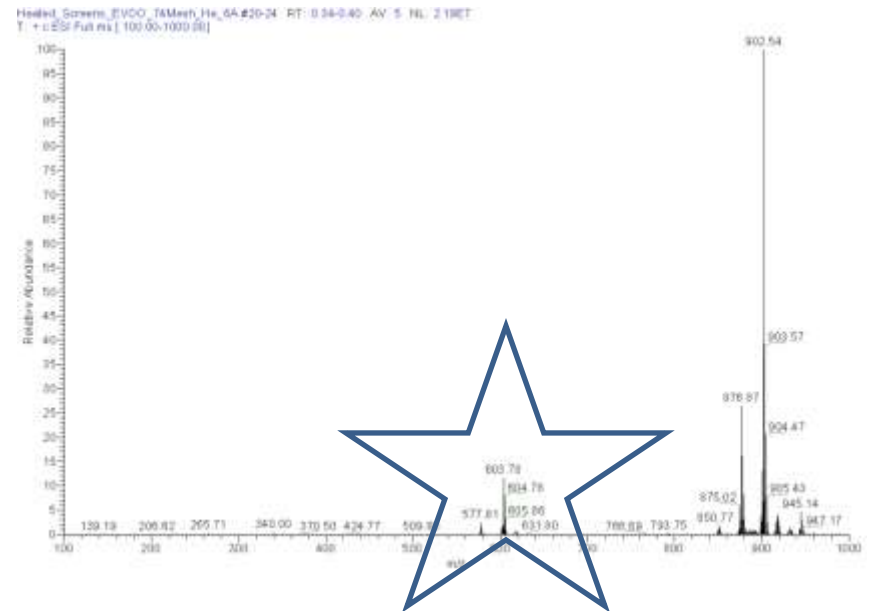
0.2 minutes

Effect on Thermal Degradation

Conventional DART 450° C



ID-CUBE Technology



Reduced fragmentation!