

La tecnica SPME e sue applicazioni



7° CONVEGNO NAZIONALE FITOFARMACI E AMBIENTE



Torino

06 – 07 dicembre 2007

Sala Conferenze – ARPA Piemonte – Via Pio VII, 9



*A.R.P.A.F-VG dip. TRIESTE A.R.P.A.
F-VG dip. Trieste*

SPME

Metodiche di screening per la ricerca di Fitofarmaci con la tecnica SPME abbinata alla GC/MS

L. Giorgini – R. Bruno – L. Colugnati – N. Miani – L. Capriglia – J. Falomo
A.R.P.A. FVG dipartimento di Trieste
labts@arpa.fvg.it

*A.R.P.A.F-VG dip. TRIESTE A.R.P.A.
F-VG dip. Trieste*



I perché dei pesticidi?

- L'analisi sugli alimenti è un'eredità passata all'ARPA F-VG con legge regionale
- Il Laboratorio del dipartimento di Trieste ha una collaborazione pluriennale con gli organi di Confine
- I limiti di rilevabilità dei metodi di riferimento e i limiti di legge
- Le difficoltà analitiche dei metodi classici
- I tempi ristretti per dare le risposte

■ Perché usare SPME?

Manuale ?



*A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.
F-VG dip. Trieste*





o Automatica ?

*A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.
F-VG dip. Trieste*



L'equilibrio delle fasi



$$K_1 = C_L / C_G$$
$$K_2 = C_F / C_L$$
$$K_3 = C_F / C_G$$

$$C_0 V_L = C_G V_G + C_L V_L + C_F V_F$$

A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.

F-VG dip. Trieste

Varie matrici alimentari e non...

Analisi per immersione

- **Acquose:** campione con nessuna preparazione
- **Frutta e Verdure:** una estrazione rapida prima dell'analisi in SPME
- **Agrumi, semi e granaglie:** breve macinatura seguita da una estrazione rapida e analisi in SPME



Varie matrici alimentari e non...

Analisi per Spazio di Testa

- **Frutti di mare: sgusciati e omogeneizzati**
- **Pesce e crostacei: freschi e omogeneizzati**
- **Prodotti liofilizzati: tal quale**
- **Latte e latticini**
- **Terreni e sedimenti essiccati e setacciati**

A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.

F-VG dip. Trieste



Matrici acquose e non...

Tal quale

- *Acque minerali*
- *Acque superficiali*
- *Acque potabili*
- *Acqua di mare*
- *Scarichi in uscita*
- *Succhi di frutta 12%*
- *Vino*

Dopo diluizione

- *Succhi di frutta conc.*
- *Miele*
- *Purea di frutta*
- *Purea di vegetali*
- *Ecc ...*





Metodica : ...

- In una Vial da 22 ml
- 1 ml mix THF / isoPOH 2/3
contenente 10 ppb S.I. (TPP e TBP)
- 2 gtt. H₃PO₄ 85%
- 20 ml. di campione
- Tappare e porre nel sistema SPME
- Fibra Grey (PDMS/CX/DVB)

- Analisi per immersione

A.R.P.A.F-VG dip. TRIESTE A.R.P.A.

F.V.G. dip. Trieste





La Strumentazione

Condizioni Strumentali GC

- GC Agilent 6890
- Carrier gas: He ultrapuro 99.9999%
- Pulsed splitless
- In liner Ø int. 0.75 mm (SPME)
- Colonna Supelco tipo SLB 5 ms da 30 m id 0.25 mm film 0.25 µm (o altra colonna equivalente per GC-MS con PDMS al 5%)

A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.

F-VG dip. Trieste



Condizioni strumentali: MS

- **MS 5973 Agilent**
- **Transfer liner MS a 290°C**
- **Sorgente a 240°C**
- **Quadrupolo a 170°C e 70 eV**
- **Range scansione 60 ÷ 490 amu**
- **1 scansione/sec**
- **Threshold 50**

A.R.P.A. F-VG dip. TRIESTE.A.R.P.A.

F-VG dip. Trieste



Condizioni Strumentali: Autocampionatore



Temperatura: 30° C per 3'

Estrazione per immersione: 45'

Agitazione: 500 rpm

Lavaggio: 1'

Iniezione: 210''

A.R.P.A.F-VG dip. TRIESTE.A.R.P.A.

F-VG dip. Trieste

L'esperienza: Acqua Panna

Circuito UNICHIM

Con circa 100 Laboratori

Ricerca dei seguenti pesticidi:

- *Lindano*
- *Eptacloro*
- *Aldrin*
- *Isodrin**
- *Dieldrin*
- *Endrin*

A.R.P.A.F-VG dip. TRIESTE A.R.P.A.

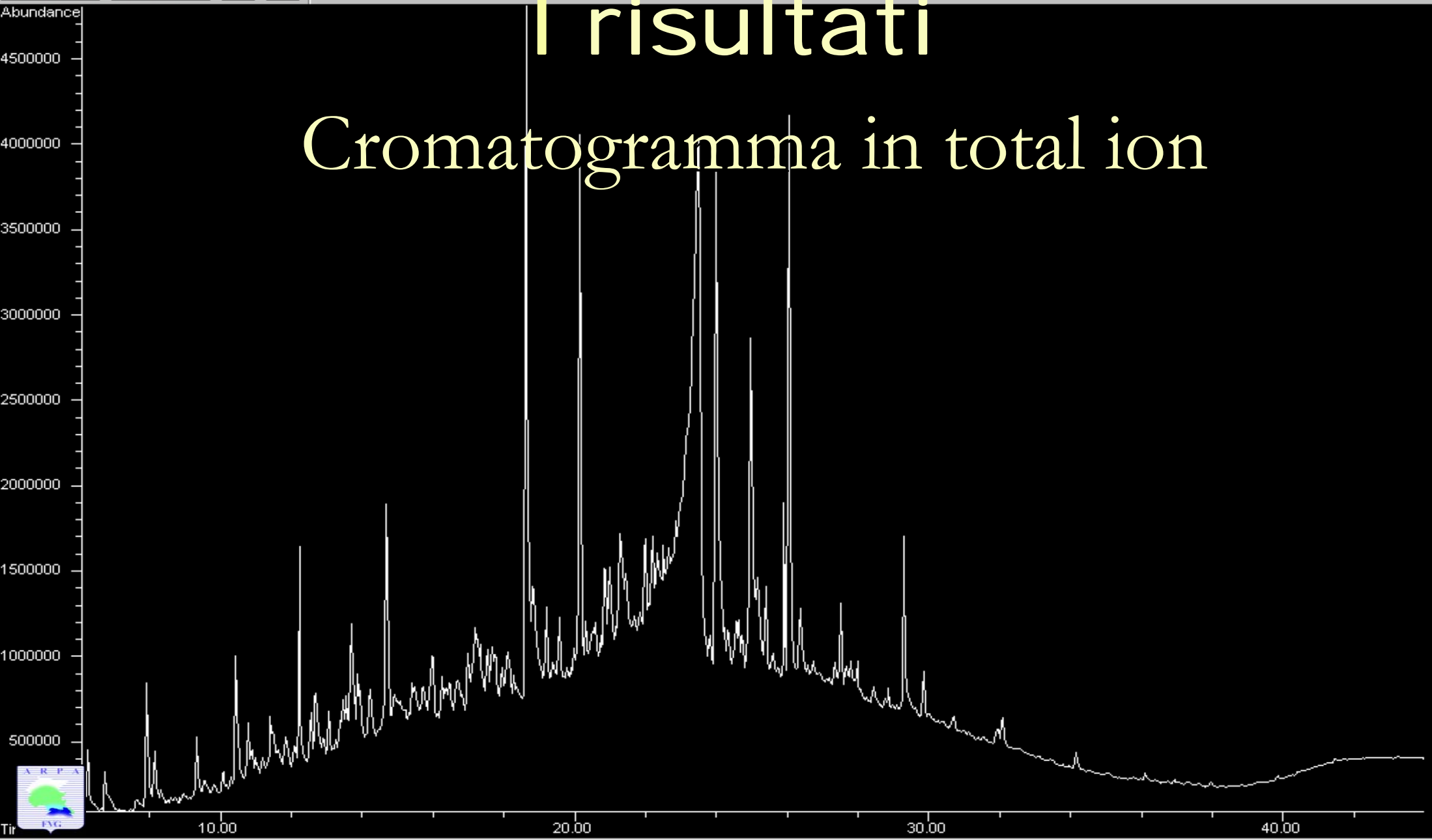
** non determinato per mancanza di standard*





I risultati

Cromatogramma in total ion



Tir

10.00

20.00

30.00

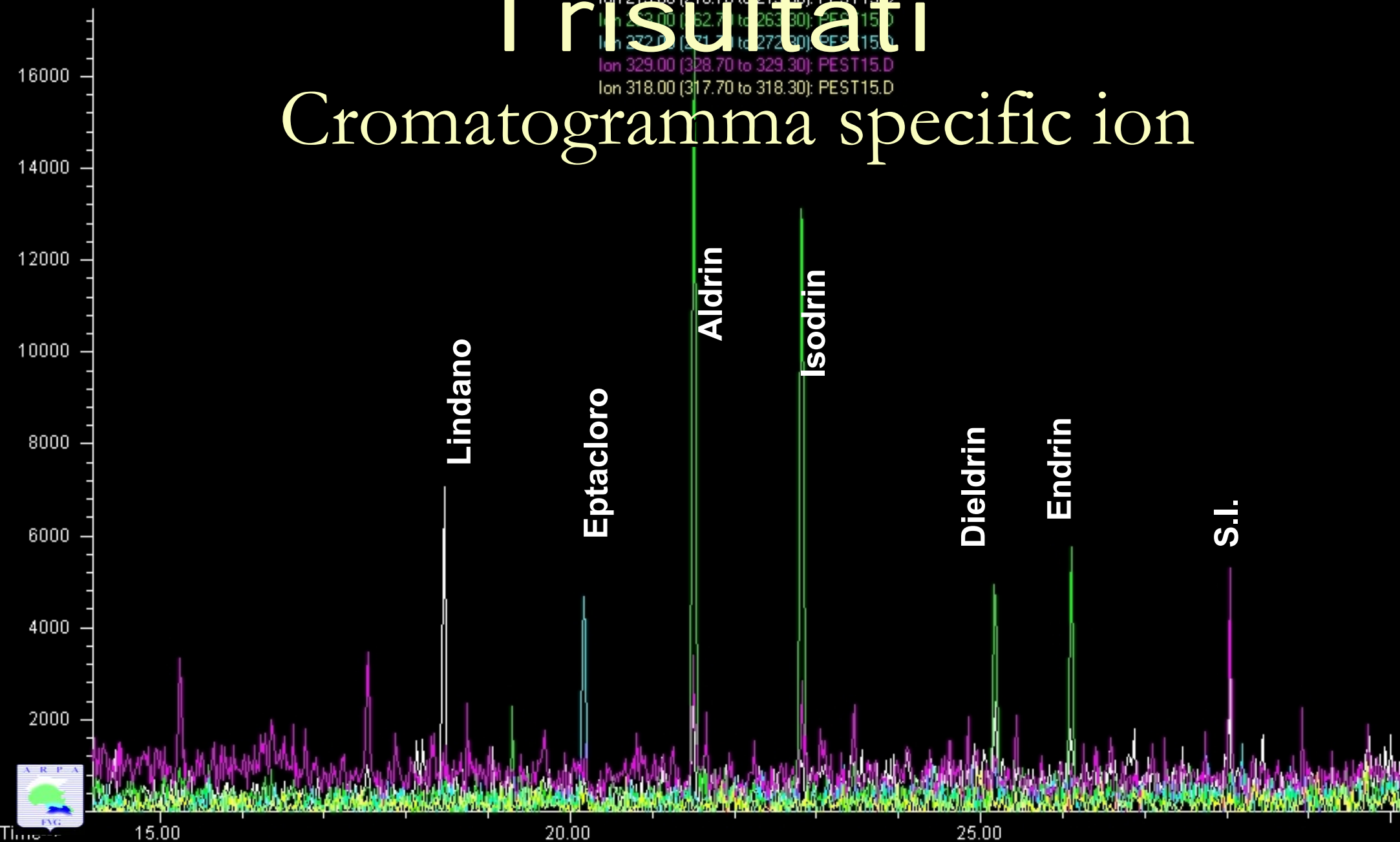
40.00

Abundance

I risultati

Cromatogramma specific ion

Ion 219.00 (218.70 to 219.30): PEST15.D
Ion 263.00 (262.70 to 263.30): PEST15.D
Ion 272.00 (271.70 to 272.30): PEST15.D
Ion 329.00 (328.70 to 329.30): PEST15.D
Ion 318.00 (317.70 to 318.30): PEST15.D



I dati di tre analisi comparati con il circuito

Espressi in ppt	1	2	3	X
lindano	29,3	30,6	32,7	40
eptacloro	42,2	42,0	39,3	40
aldrin	52,4	51,2	49,8	50
dieldrin	40,2	37,9	39,2	40
endrin	47,3	46,1	47,2	50

Triazine su acqua potabile



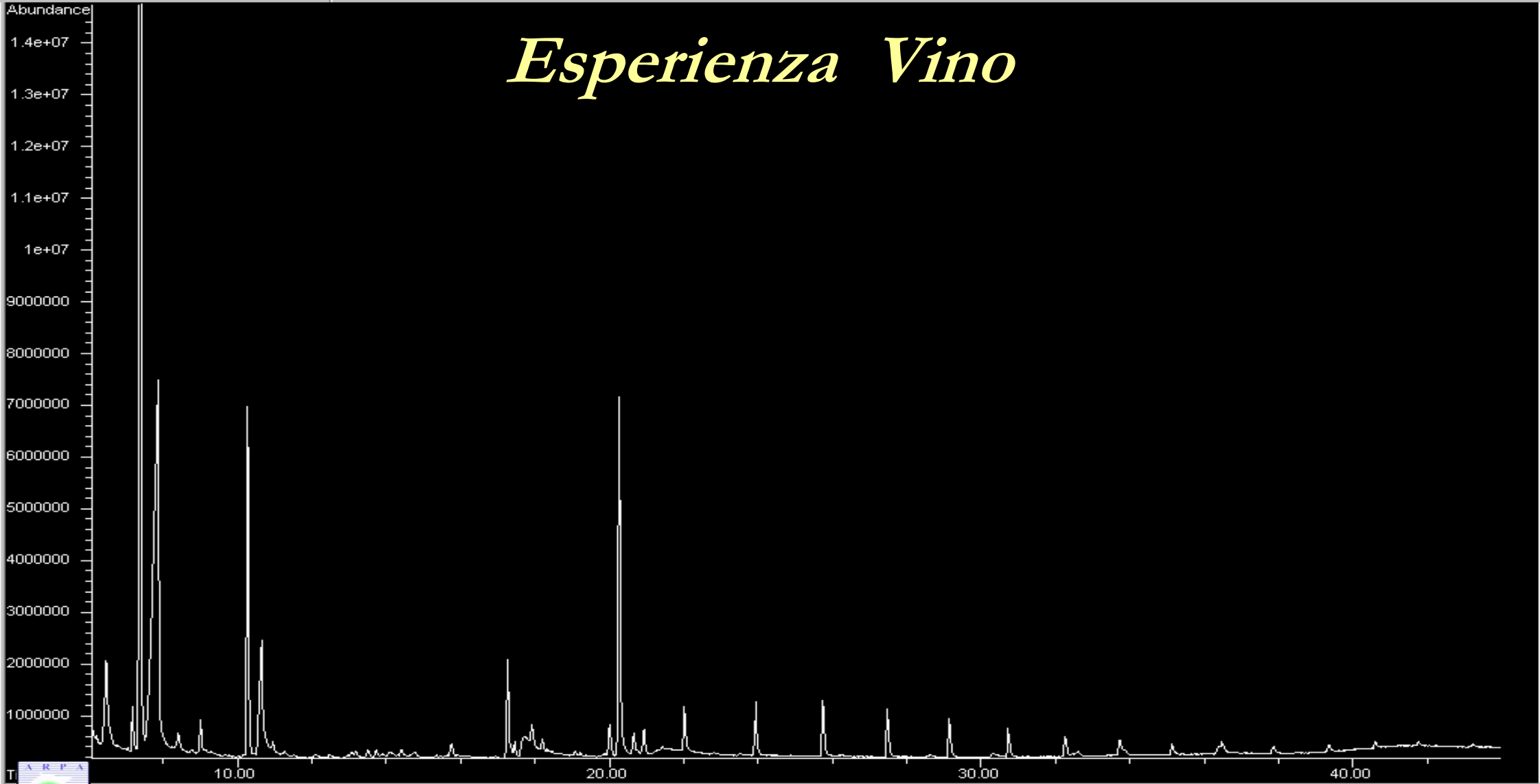
Triazine su acqua potabile

Target Compound	0.00	284	0	N.D.	Value
1) HCB	0.00	284	0	N.D.	
2) desetylatriazina*	15.86	172	190	45.14 ppt	# 41
3) trifluralin	0.00	306	0	N.D.	
4) desetylterbutylatriazina	0.00	186	0	N.D.	
5) simazina*	18.68	201	665	50.31 ppt	# 31
6) atrazina*	18.88	200	1543	54.42 ppt	# 64
7) desetylseebutylazina	18.73	172	6912	53.73 ppt	# 47
8) Atraton	0.00	196	0	N.D.	
9) propazina*	19.02	214	2235	52.35 ppt	# 62
10) g HCH	0.00	181	0	N.D.	
11) terbutylazina*	19.52	214	8128	57.80 ppt	# 73
12) Simetrina	0.00	213	0	N.D.	
13) desmetryne	0.00	213	0	N.D.	
14) alaclor*	23.35	160	2126	54.34 ppt	# 78
15) prometryna	0.00	241	0	N.D.	
16) terbutryne	0.00	226	0	N.D.	
17) dipropetryne	0.00	255	0	N.D.	
18) a	0.00	208	0	N.D.	
19) metolachor*	25.05	162	3878	50.12 ppt	# 62
20) cyanazina	25.77	173	302	3.84 ppt	# 6
21) bentazone	25.33	198	150	No Calib	#
22) metazaclor	0.00	132	0	N.D.	
23) pendimenthalin	0.00	252	0	N.D.	
24) pp'DDE	0.00	246	0	N.D.	
25) pp'DDD	0.00	235	0	N.D.	
26) pp'DDT	0.00	235	0	N.D.	
27) chloridazon	0.00	221	0	N.D.	

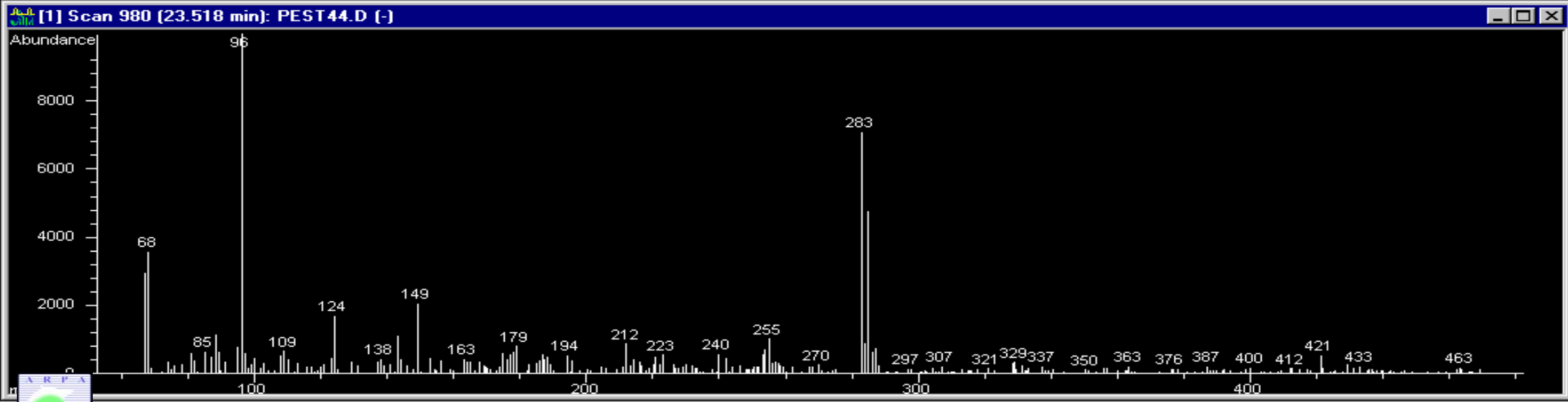
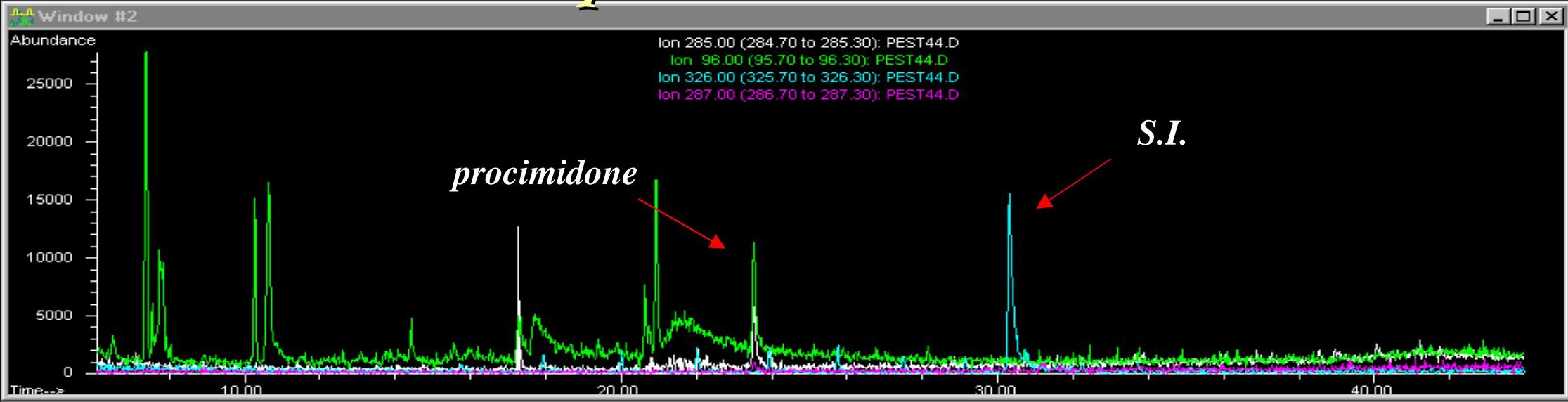
(#) = qualifier out of range (m) = manual integration (+) = signals summed



LINE.M Fri Nov 16 13:05:08 2007



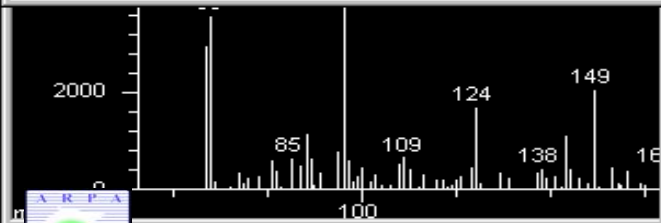
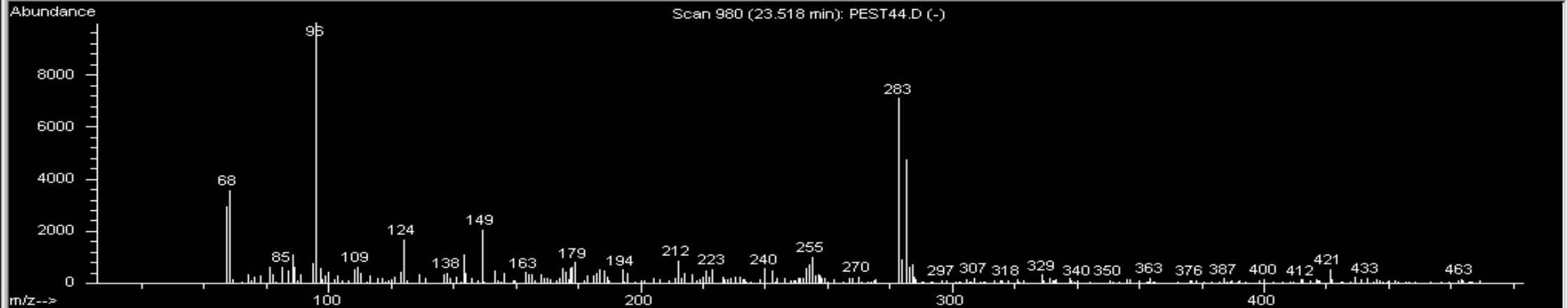
Esperienza Vino



Esperienza Vino



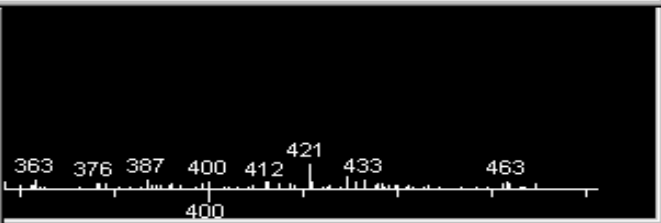
Window #24 Scan 980 (23.518 min): PEST44.D (-)



PBM Search Results: C:\Database\Wiley275.L

Rank	Name	Ref No.	MW	Qual
1	3-Azabicyclo[3.1.0]hexane-2,4-dione, 3-(3,5-dichlorophenyl)-	164094	283	81
2	Procymidone \$\$ Sumiscles \$\$ Sumilex	164093	283	74
3	1-(2,6-Dichlorophenoxy)-4-nitrobenzene \$\$ B...	164045	283	72

Difference



Metodica: ...

- In una Vial da 22 ml
- 1 ml mix THF / isoPOH 2/3
contenente 10 ppb S.I. (TPP e TBP)
- 2 gtt. H₃PO₄ 85%
- 1 g di campione portare a volume
- Tappare e porre nel sistema SPME
- Fibra Grey (PDMS/CX/DVB)

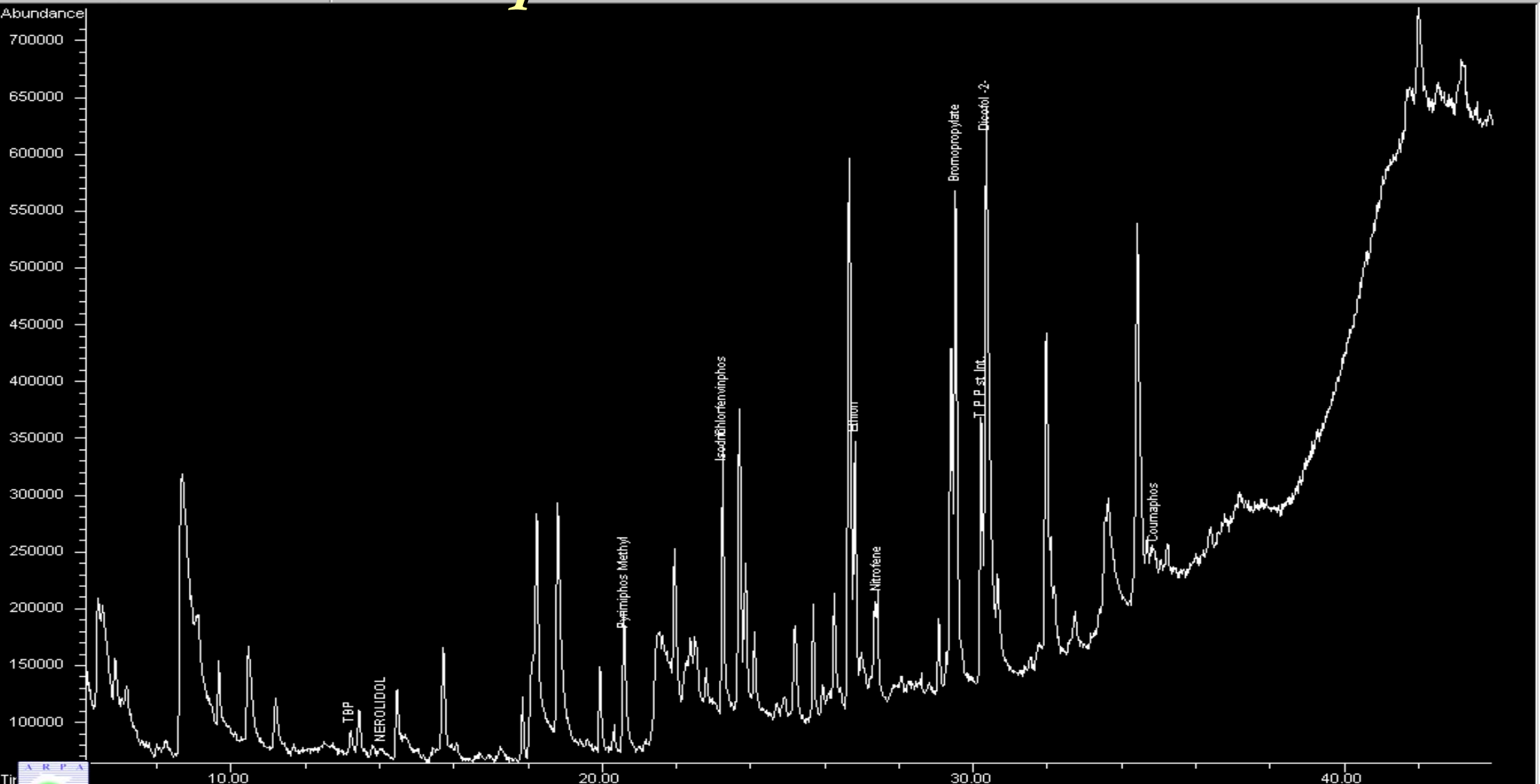
A.R.P.A.F-VG dip. TRIESTE A.R.P.A.

F-VG dip. Trieste

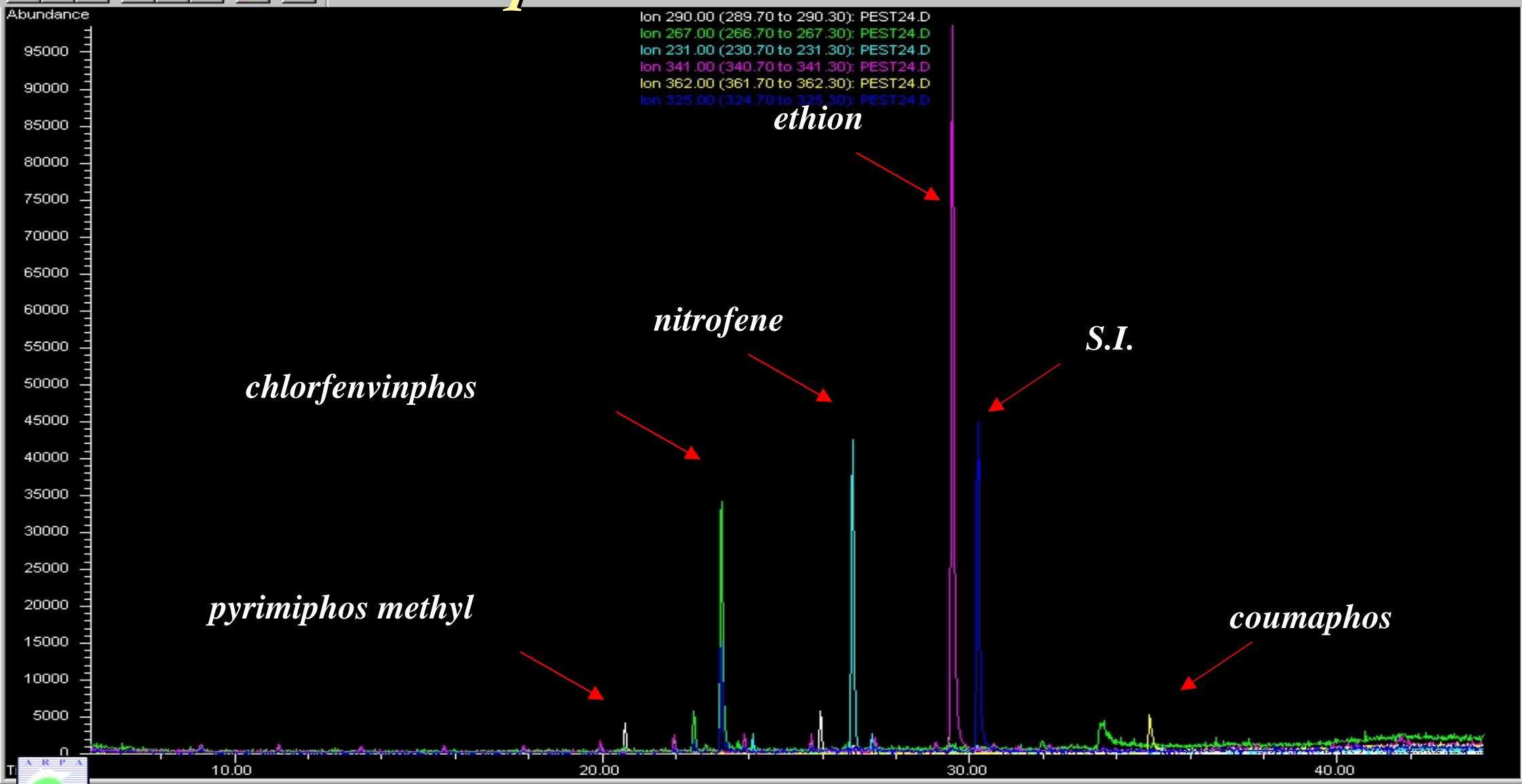
- Analisi per immersione



Esperienza Miele dil.



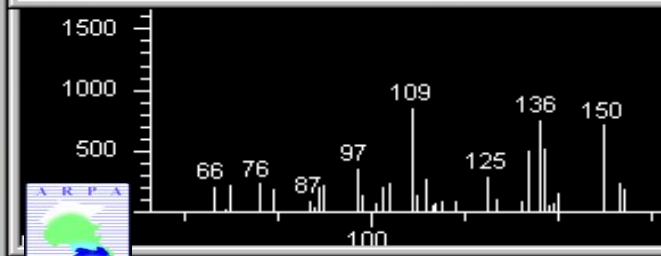
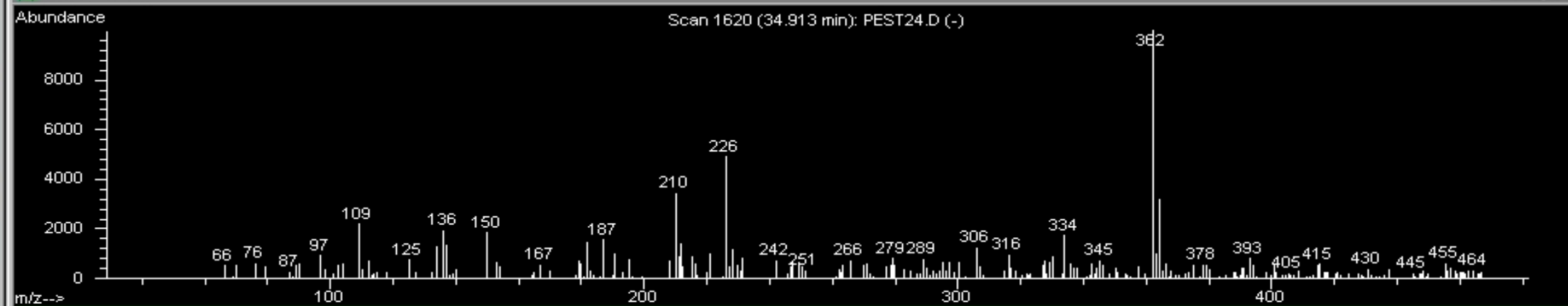
Esperienza Miele dil.



Esperienza Miele dil.



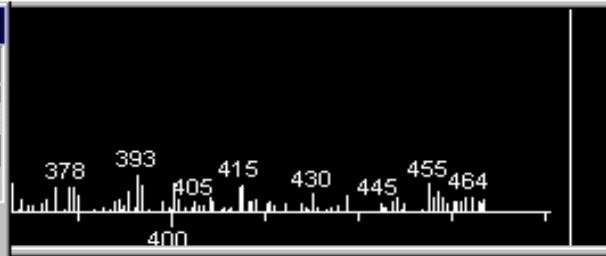
Window #26



PBM Search Results: C:\Database\Wiley275.L

Rank	Name	Ref No.	MW	Qual
3	Coumaphos \$\$ Asuntol \$\$ Muscatox \$\$ Resitox	217601	362	56
4	Meldone \$\$ Phosphorothioic acid, O-(3-chlor...	217597	362	42
5	[2,2'-Binaphthalene]-1,1':4,4'-tetrone, 3,8,8'-tri...	217839	362	25

Difference



Esperienza Miele dil.

Quantitation Report (Not Reviewed)

Data File : D:\ANALISI\PEST\PEST13\PEST24.D
 Acq On : 1 Sep 2006 16:26
 Sample : miele 4283+mix miele
 Misc :

Vial: 23
 Operator:
 Inst : Gas_Massa
 Multiplr: 1.00
 Sample Amount: 0.00

MS Integration Params: Pest.p
 Quant Time: Sep 12 17:12:23 2006

Quant Results File: 50PEST.RES

Quant Method : D:\METODI\50PEST.M (RTE Integrator)
 Title : System Performance Check - CH4/PCI - BZP
 Last Update : Mon Sep 11 10:16:56 2006
 Response via : Initial Calibration
 DataAcq Meth : 50PEST

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)	
Target Compounds							Qvalue
8) TBP	13.21	155	18116	6.52	ppb		92
11) NEROLIDOL	14.06	69	9112	0.13	@@	#	22
21) Dimethoate	18.80	93	13982	Below	Cal	#	59
40) Pyrimiphos Methyl	20.61	290	17929	50.35	ppb	#	81
46) Chlorfenvinphos	23.25	267	167817	50.13	ppb	#	82
48) Isodrin	23.23	195	11903	11.34	ppb	#	42
66) Nitrofene	27.40	283	9695	1.14	ppb	#	1
70) Ethion	26.81	231	203253	50.02	ppb	#	63
78) T P P st Int.	30.23	326	298017	29.80	ppb	#	83
84) Dicofol -2-	30.34	251	11297	4.39	ppb	#	1
87) Bromopropylate	29.52	341	567963	47.09	ppb	#	88
90) Coumaphos	34.90	362	35208	50.00	ppb	#	82

(#) = qualifier out of range (m) = manual integration
 PEST24.D 50PEST.M Tue Sep 12 17:12:25 2006

GAS_MASSA



Frutta e Verdure: una estrazione rapida prima dell'analisi in SPME

- 
- pomacee
 - drupacee
 - bacche
 - uve
 - fragole
 - agrumi
 - ecc ...
 - cavoli
 - ortaggi a tubero
 - ortaggi a bulbo
 - ortaggi a foglia
 - cucurbitacee
 - solanacee
 - ecc...

Metodica: ...

10 grammi di frutta fresca o verdura tritурata + 50 ml di ACN + 15 g. di NaCl; agitare per 30' e filtrare. Andare quasi a secchezza su rotovapor e rievaporare con 5 ml di acetone. Riprendere con 5ml mix THF / isoPOH 2 : 3 contenente 10 ppb S.I. (TPP e TBP)

Nella vial

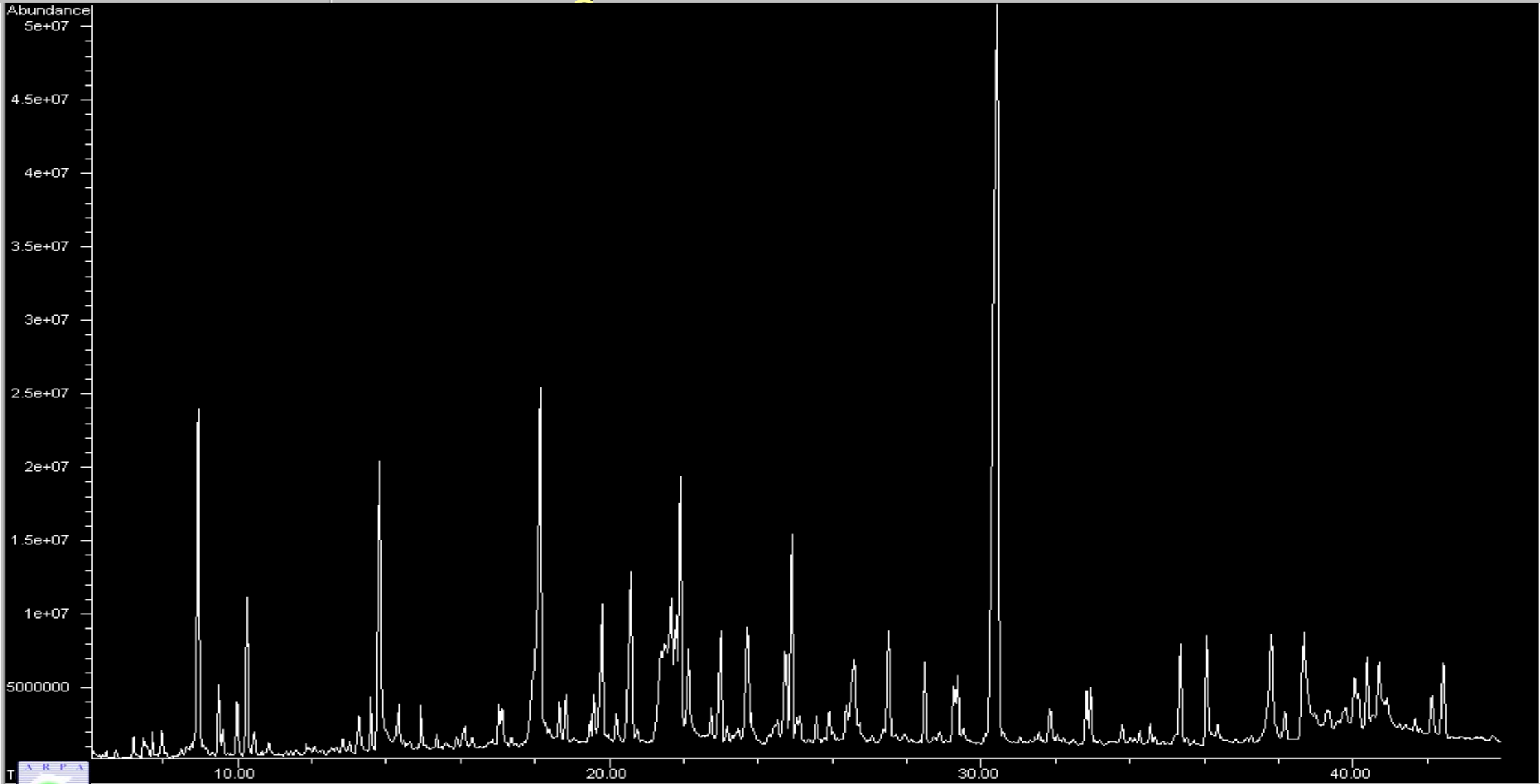
- 1 ml mix THF / isoPOH 2 : 3
- 2 gtt. H₃PO₄ 85%
- 20 ml. di acqua dal rubinetto
- Tappare e porre nel sistema SPME
- Fibra Grey (PDMS/CX/DVB)
- Analisi per immersione

ARPA FVG dip. TRIESTE A.R.P.A.

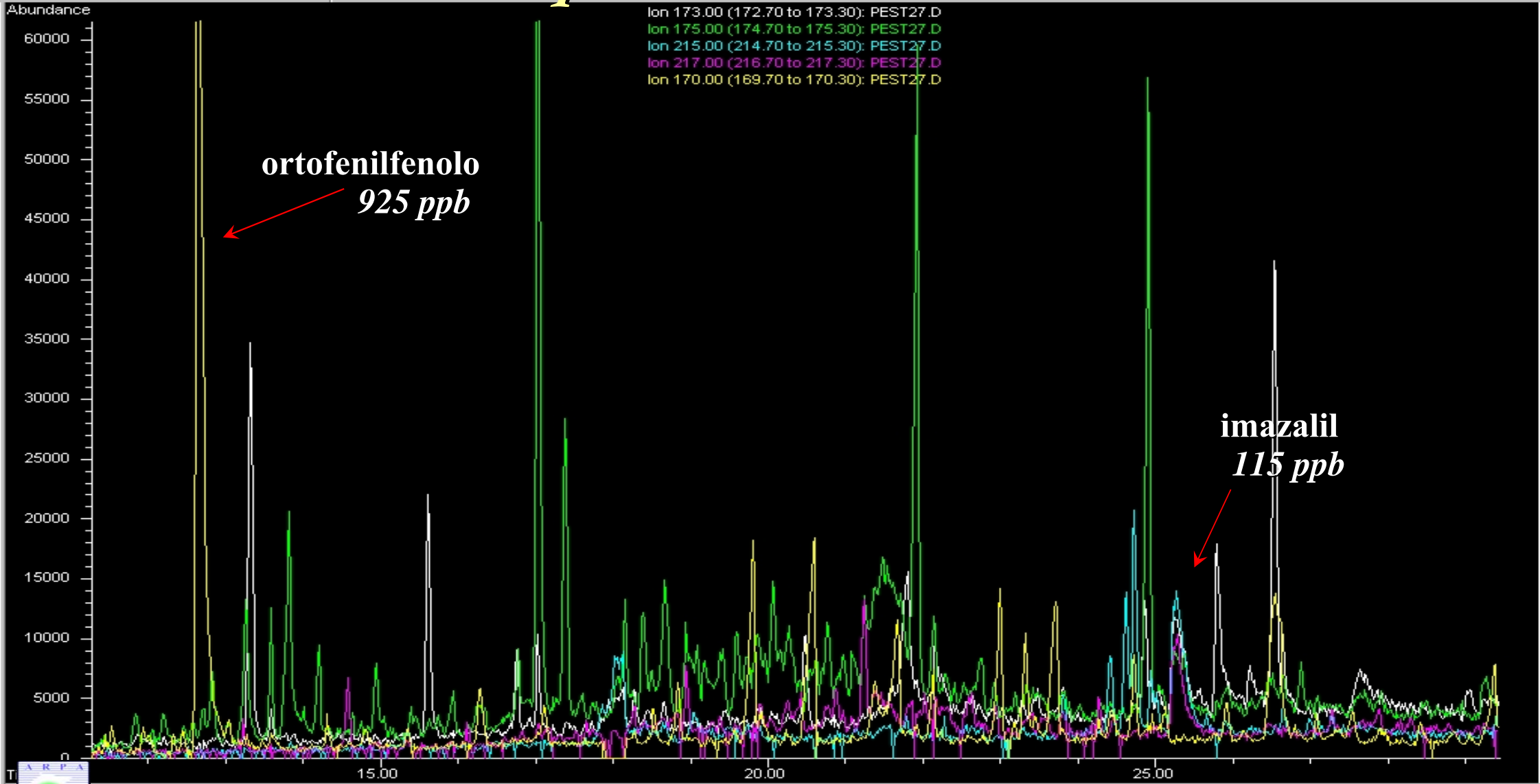
F-VG dip. Trieste



Esperienza arance



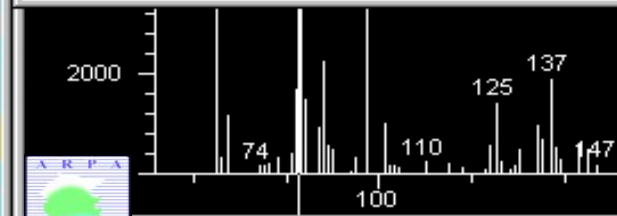
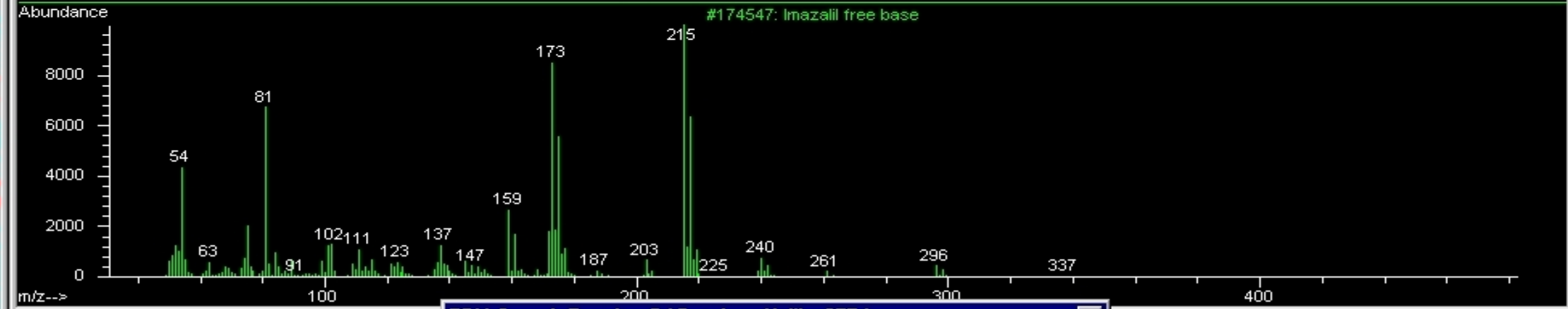
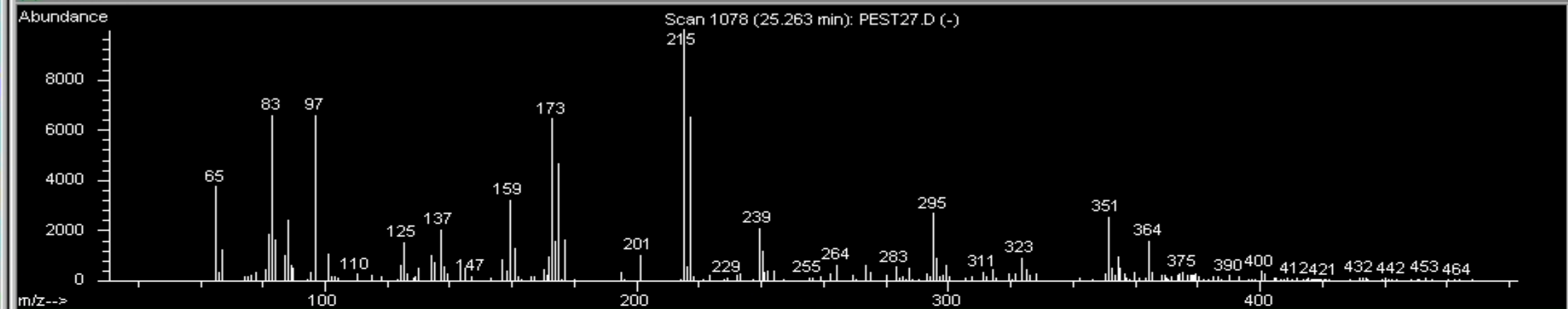
Esperienza arance



Esperienza arance



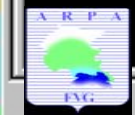
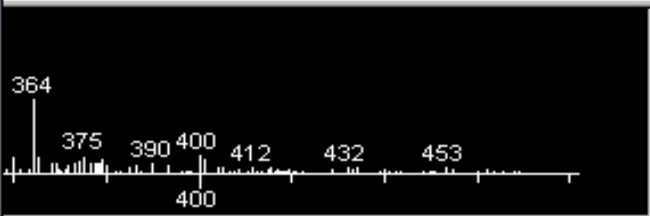
Window #24



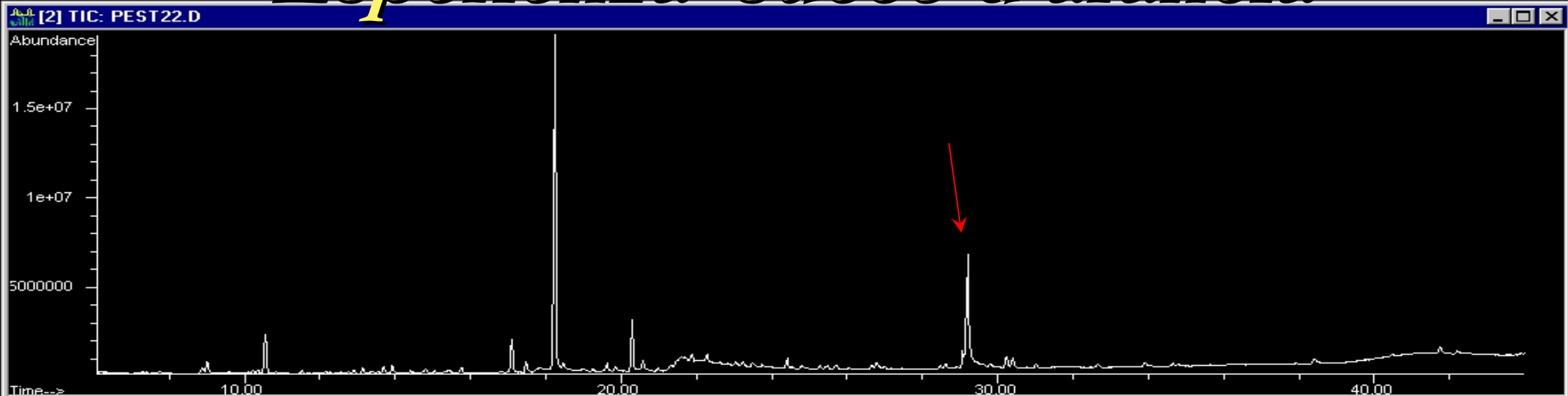
PBM Search Results: C:\Database\Wiley275.L

Rank	Name	Ref No.	MW	Qual
1	Imazalil free base	174547	296	50
2	Imazalil \$\$ Enilconazole	174545	296	38
3	5,6-DIHYDRO-7-METHYL-12-HYDROXYME...	183021	306	25

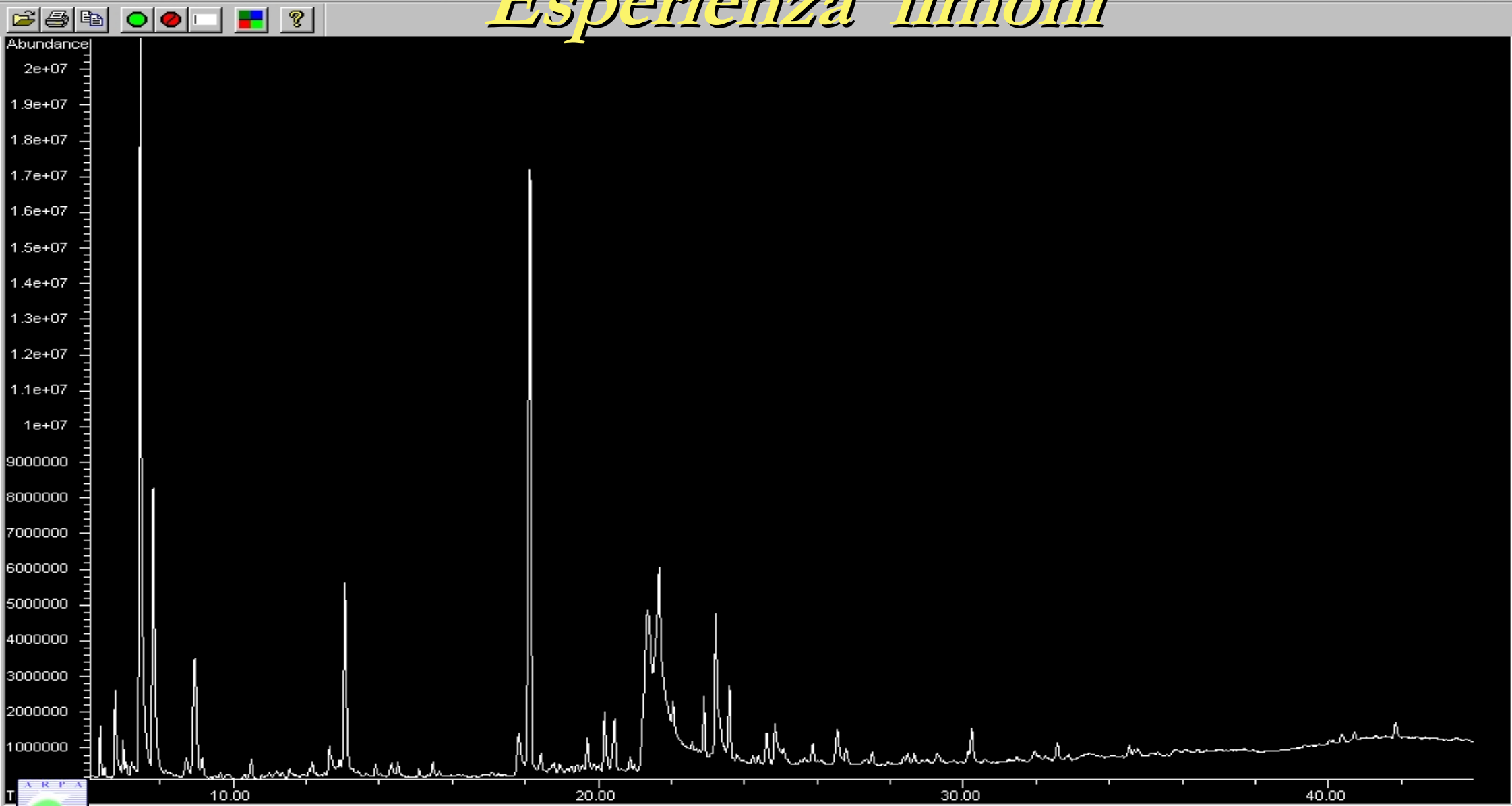
Difference



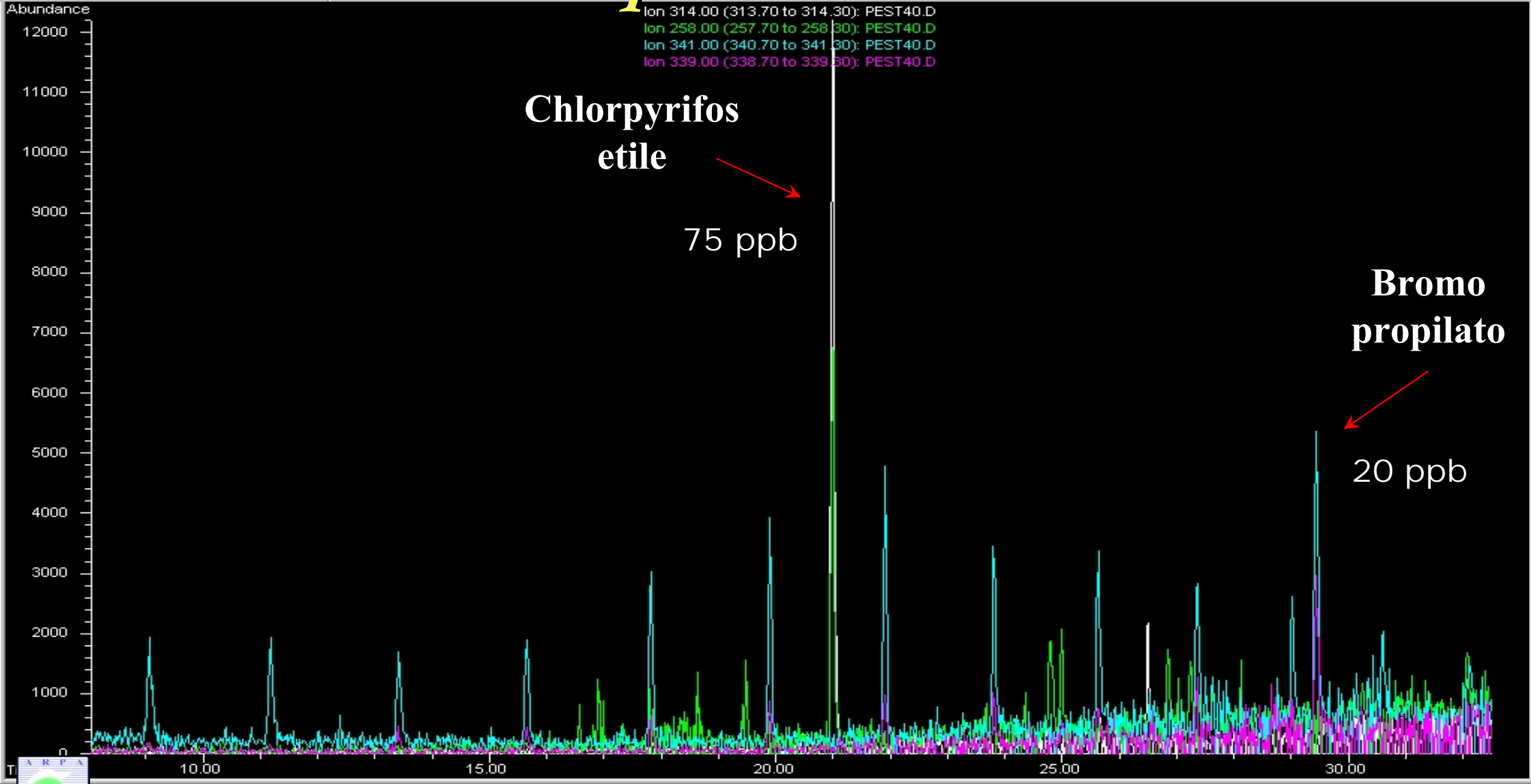
Esperienza succo d'arancia



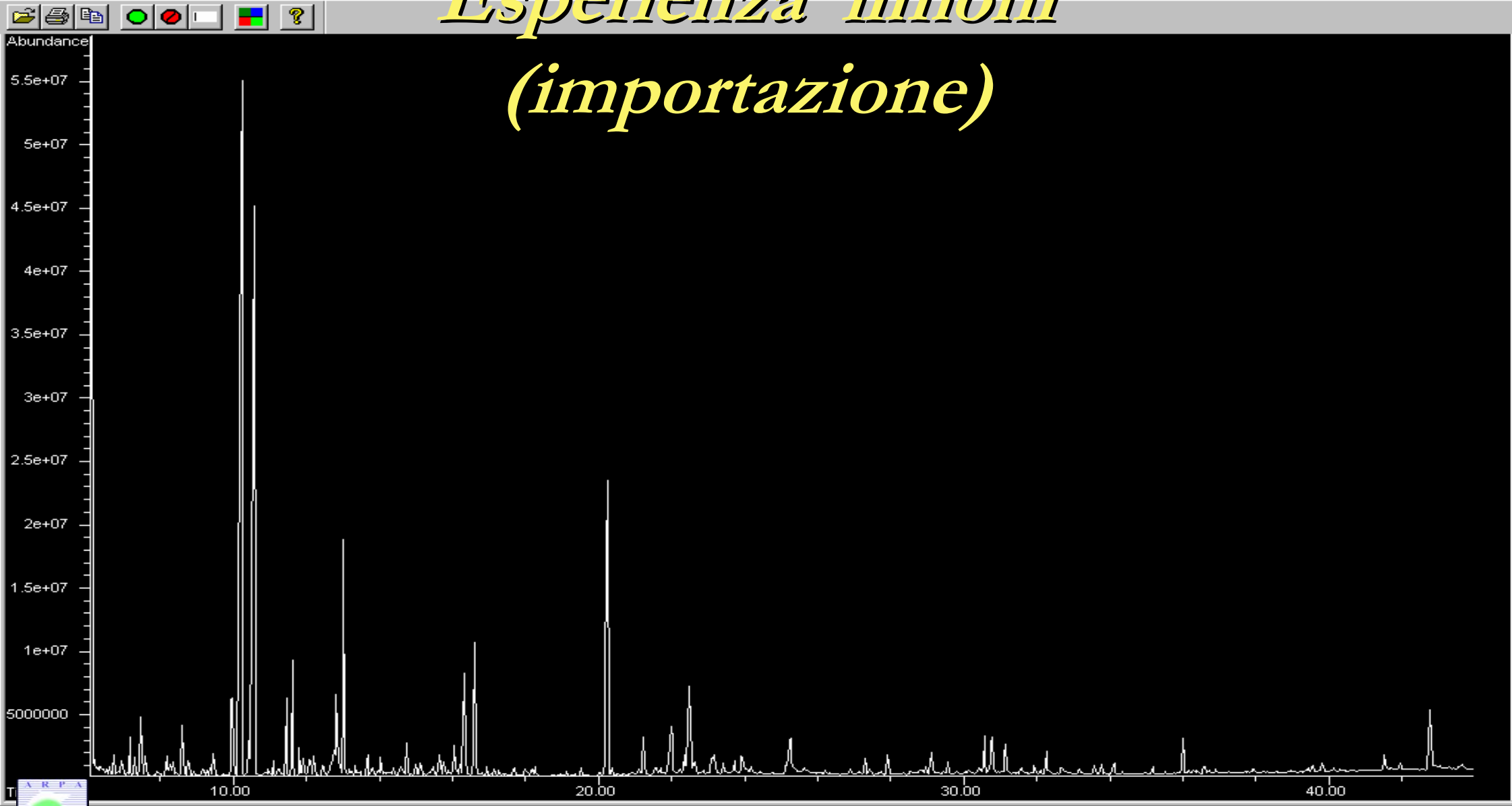
Esperienza limoni



Esperienza limoni

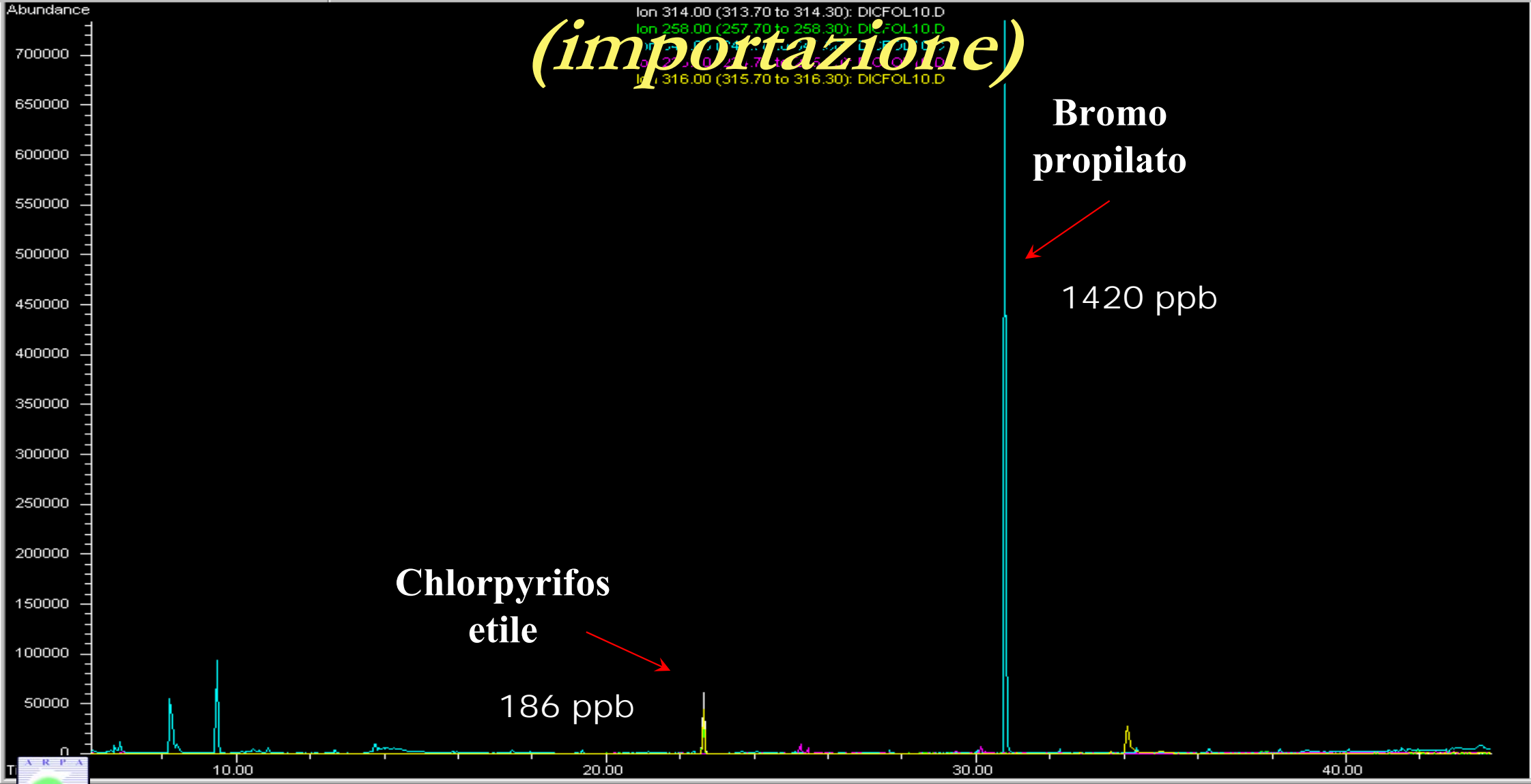


Esperienza limoni *(importazione)*



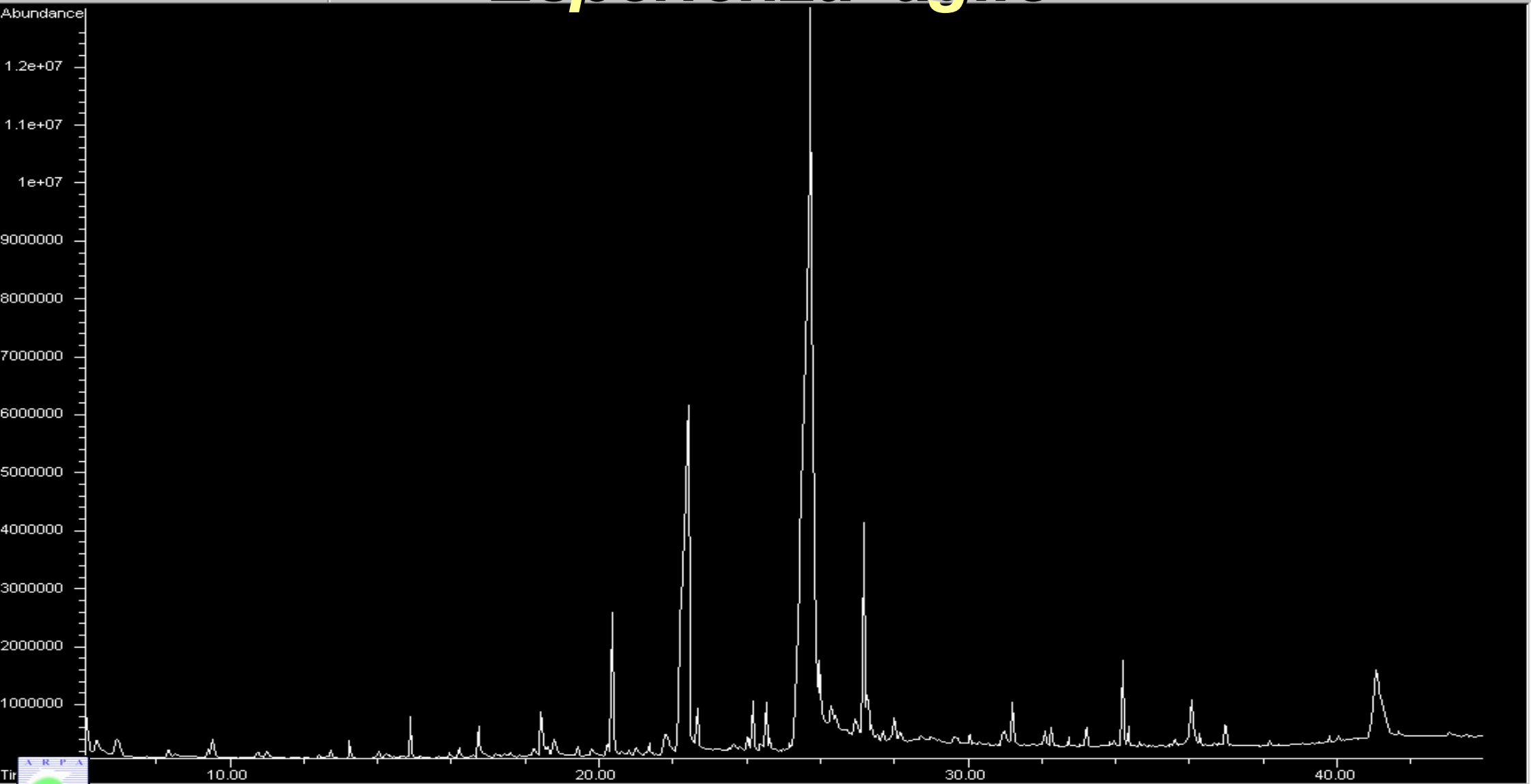
Esperienza limoni

(importazione)

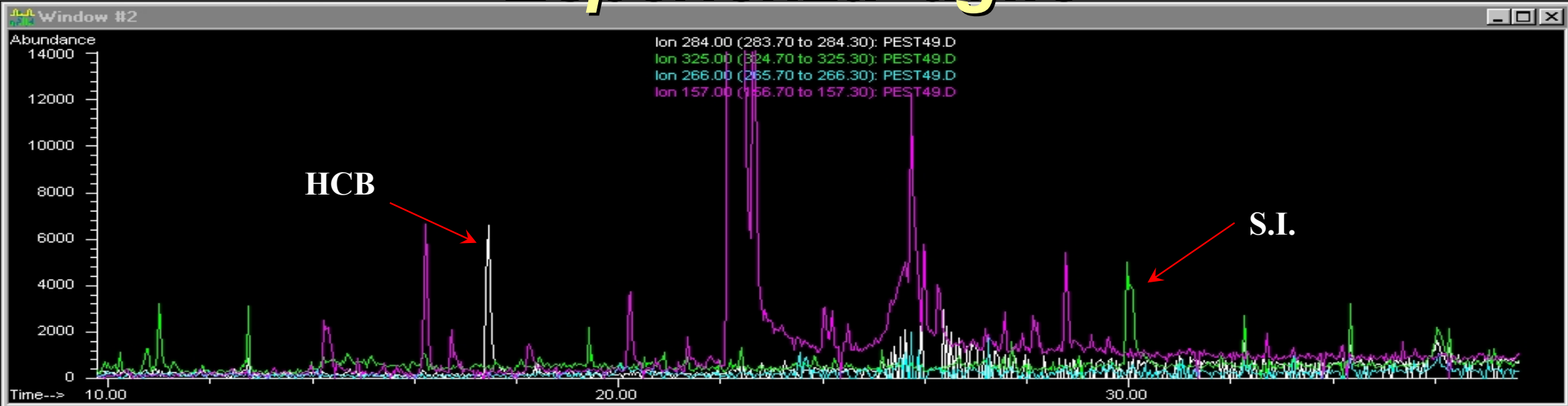




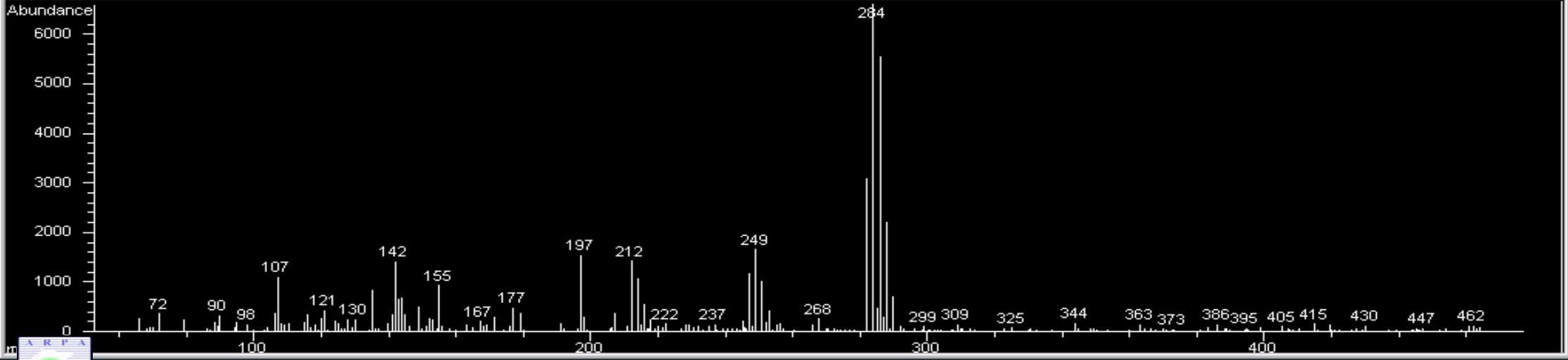
Esperienza aglio



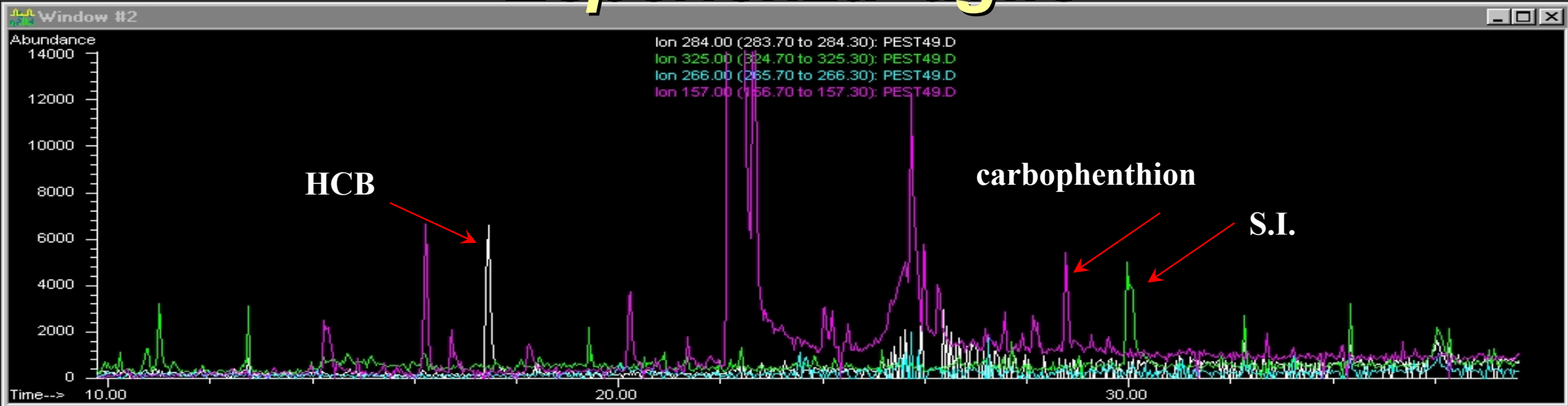
Esperienza aglio



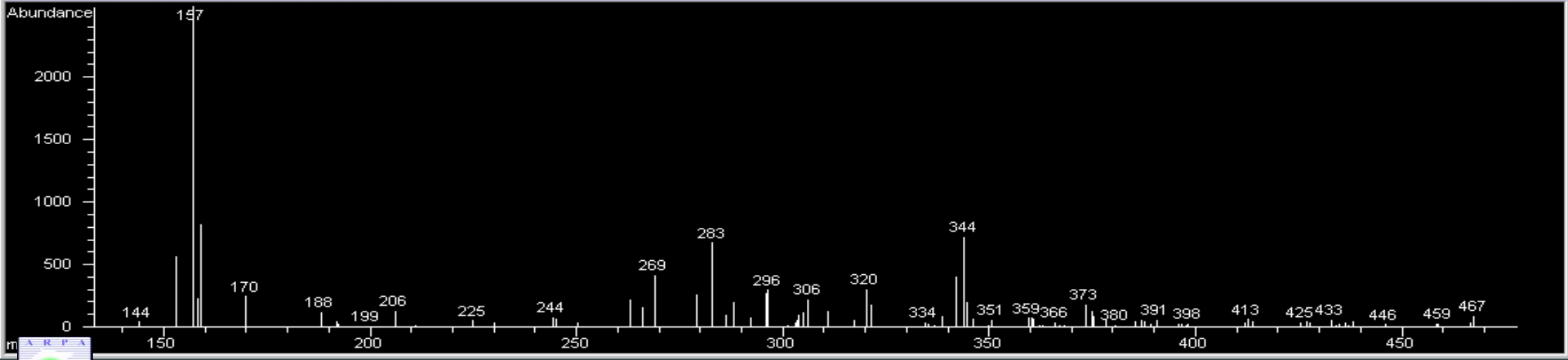
[1] Scan 323 (17.445 min): PEST49.D (-)



Esperienza aglio

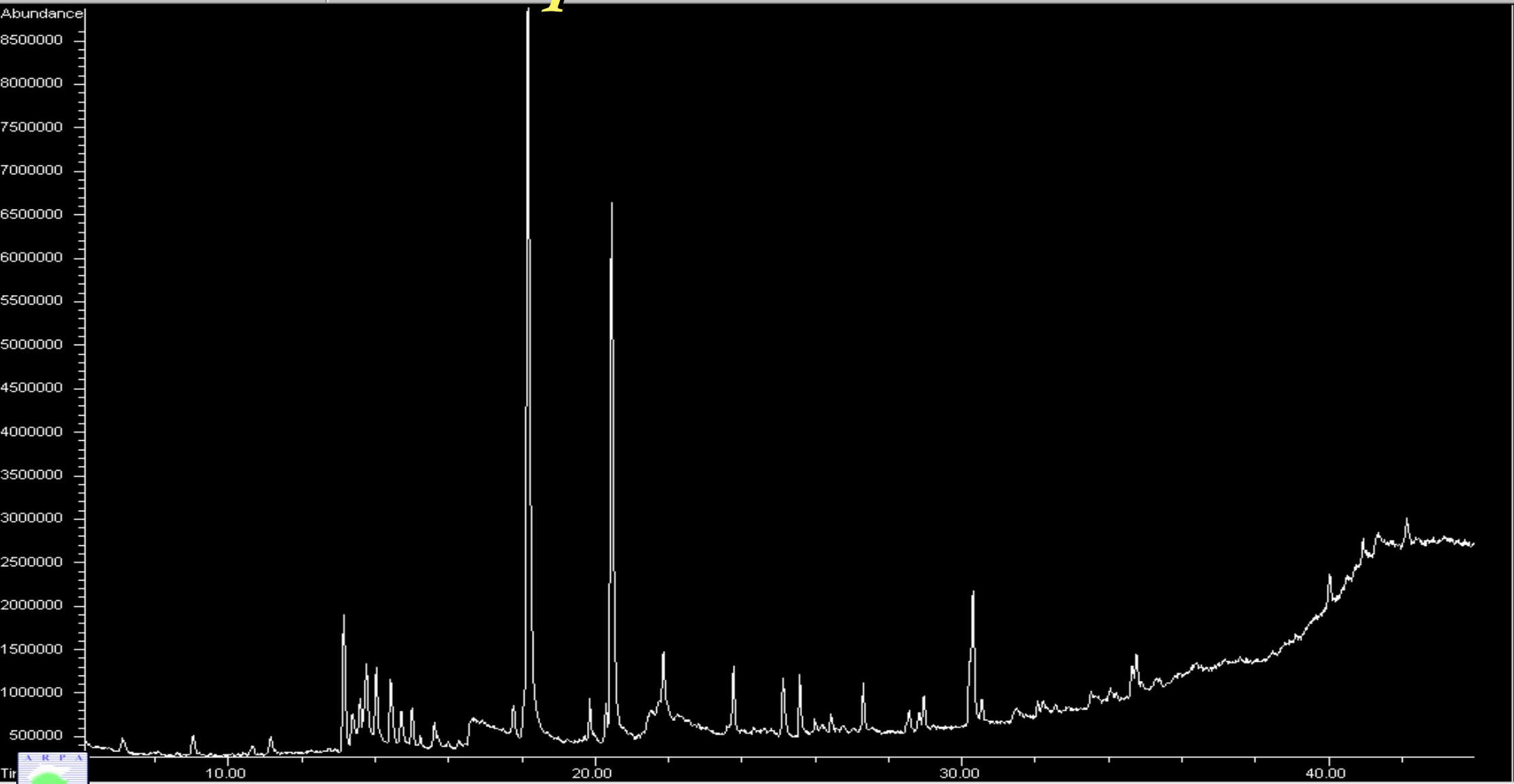


[1] Scan 644 (28.769 min): PEST49.D (-)

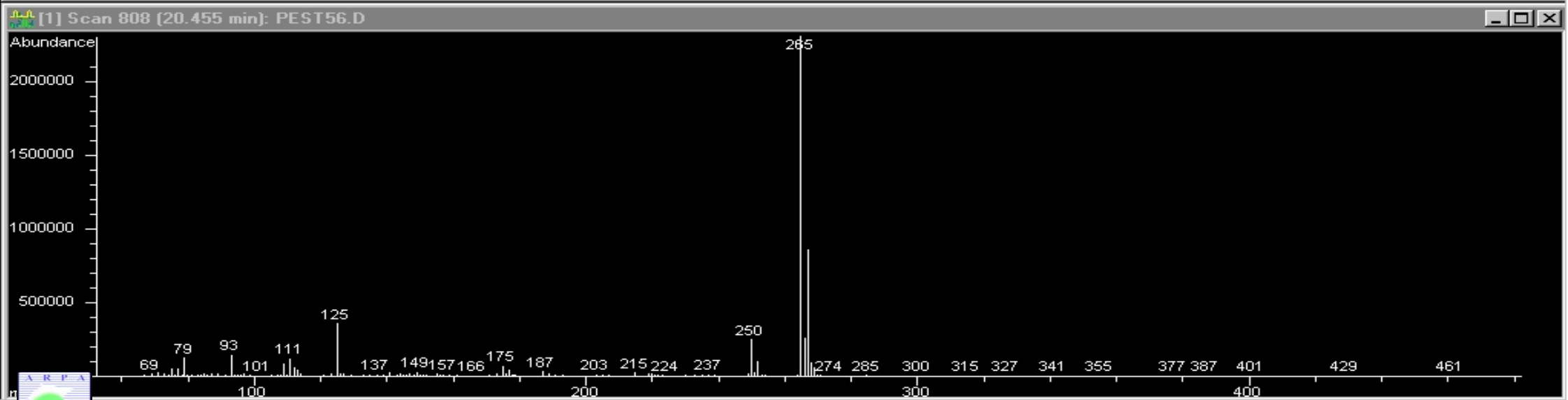
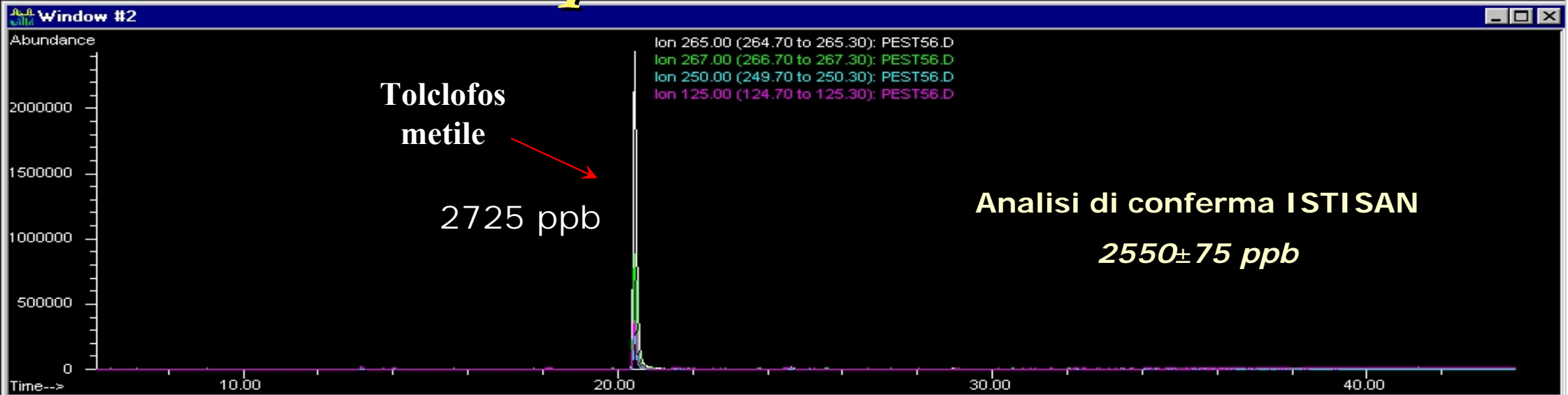




Esperienza insalata



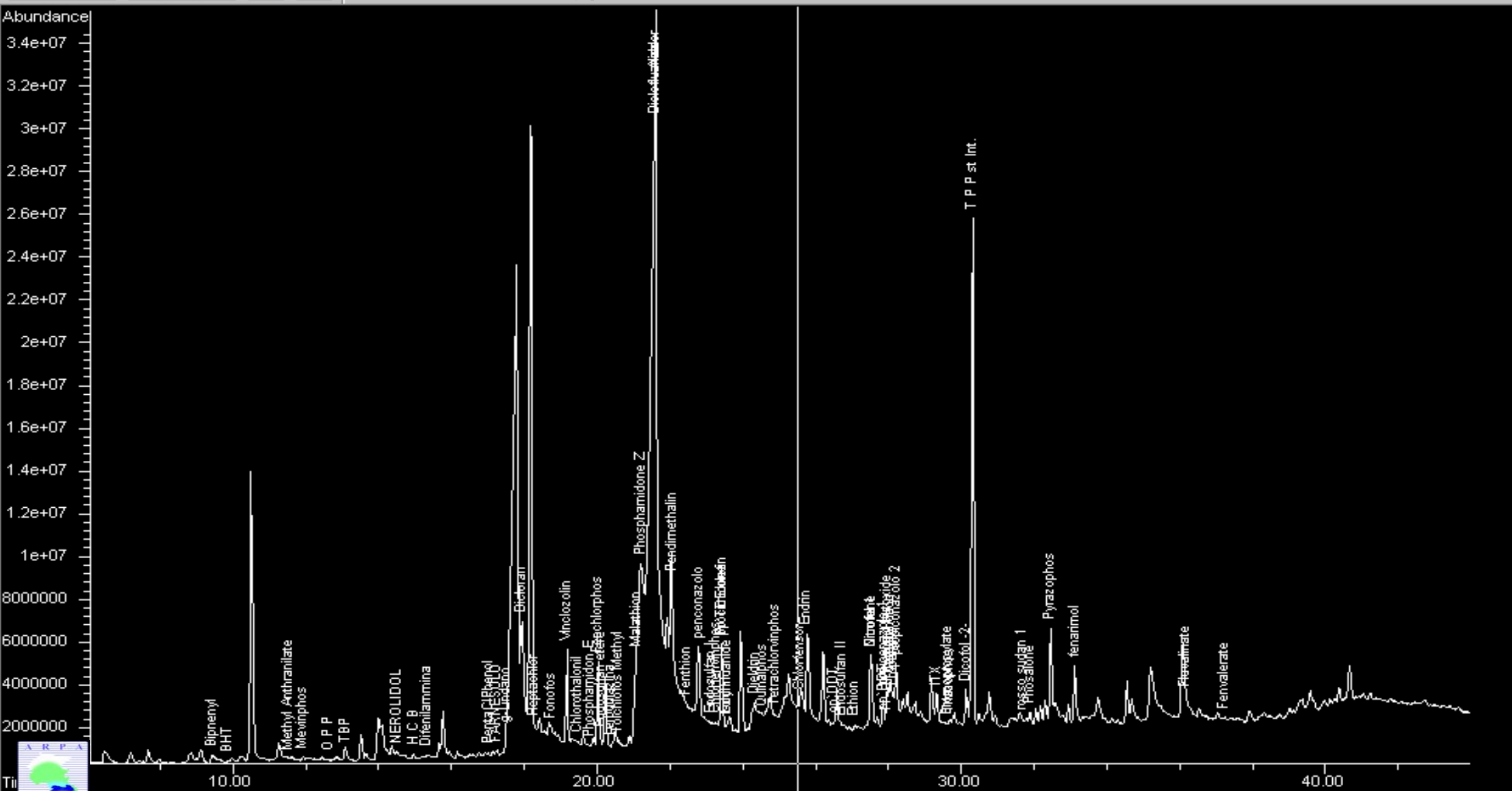
Esperienza insalata



Semi e granaglie

- Legumi secchi
- Semi (papavero, anice, miglio, sesamo, ecc)
- Spezie
- Tè
- Mais
- Frutta secca
- ...

Esperienza mais



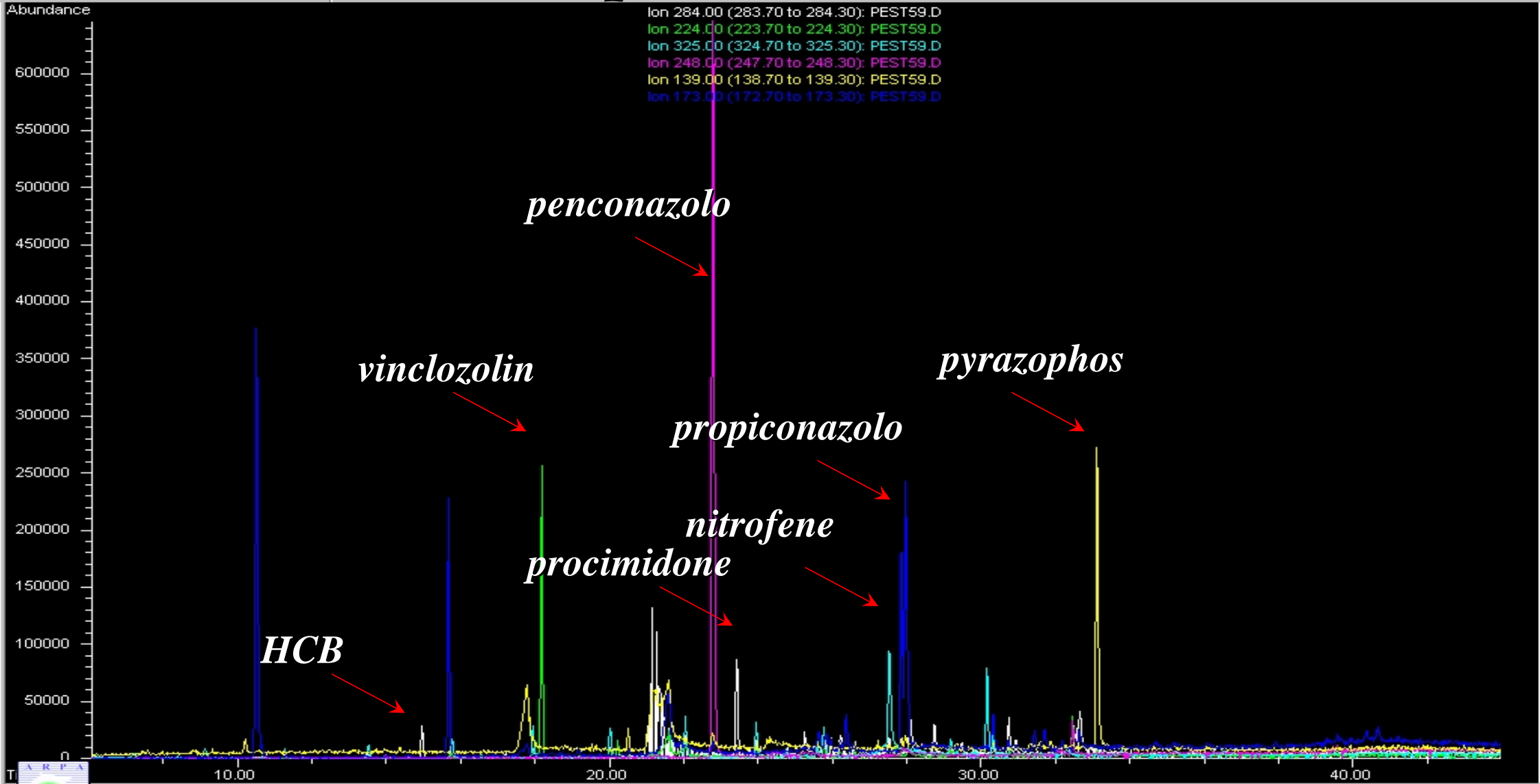
Esperienza mais

Sample : 886 mais
 Misc :
 MS Integration Params: Pest.p
 Quant Time: Sep 14 12:43:40 2006
 Quant Method : D:\METODI\50PEST.M (RTE Integrator)
 DataAcq Meth : 50PEST
 Multiplr: 1.00

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)	Qvalue
Target Compounds							
4) Bipnenyl	9.43	154	43469	2.87	ppb		87
5) Methyl Anthranilate	11.52	119	9787	0.47	ppb	#	18
6) Mevinphos	11.93	127	43982	31.65	ppb	#	60
8) TBP	13.12	155	160228	57.23	ppb		83
9) BHT	9.88	205	58893	5.89	ppb	#	76
16) H C B	14.97	284	120801	365.41	ppb		87
19) PentaClPhenol	17.02	266	33604	3.36	@@	#	62
20) Fonofos	18.75	246	8952	1.04	ppb	#	1
21) Dimethoate	19.05	93	14699	Below	Cal	#	21
22) g-Lindano	17.50	219	9418	5.21	ppb	#	25
23) Chlorothalonil	19.46	266	15957	9.91	ppb	#	53
25) Terbutilazina	20.35	173	10875	8.21	ppb	#	11
31) Tolchlofos Methyl	20.60	265	13070	1.17	ppb	#	41
32) Vinclozolin	19.17	285	942991	313.59	ppb	#	58
33) Heptachlor	18.28	272	15391	5.88	ppb	#	11
34) Alaclor	21.59	160	87024	5.68	ppb	#	46
36) Fenclorphos	20.03	285	16303	5.06	ppb	#	16
39) penconazolo	22.79	248	2939186	973.53	ppb	#	85
42) Malathion	21.08	173	9066	1.73	ppb	#	4
43) Fenthion	22.47	278	24441	7.08	ppb	#	44
46) Chlorfenvinphos	23.27	267	115277	34.77	ppb	#	24
53) Chlorfenson	25.56	175	27366	2.60	ppb	#	94
54) Procimidone	23.43	283	2179763	242.44	ppb	#	70
55) TolyFluanide	23.59	238	9115	0.44	ppb	#	14
59) Dieldrin	24.32	263	17703	35.06	ppb	#	17
61) Tetrachlorvinphos	24.89	329	13201	3.78	ppb	#	14
62) Endosulfan I	23.16	237	19760	26.57	ppb	#	33
66) Nitrofone	27.52	283	233484	32.41	ppb	#	41
67) op`DDT	26.53	235	26388	11.54	ppb	#	51
68) Endosulfan II	26.74	195	29454	44.88	ppb	#	32
70) Ethion	27.08	231	18164	4.52	ppb	#	59
71) Triazophos	29.68	162	8817	5.36	ppb	#	31
72) Carbofenotion	28.04	157	49608	24.43	ppb		95
74) Propiconazole 1	27.86	173	537677	592.59	ppb		85
75) propiconazolo 2	27.96	173	14831	614.25	ppb	#	86
76) Propargite	28.13	135	39634	16.02	ppb	#	62
77) Piperonyl butoxide	27.97	176	43617	16.93	ppb	#	1
81) fenarimol	33.11	139	1167154	736.60	ppb	#	91
87) Bromopropylate	29.62	341	47522	4.18	ppb	#	77
88) Phosalone	31.90	182	19212	10.55	ppb	#	57
89) Pyrazophos	32.46	221	4035467	725.18	ppb	#	82
92) Fenvalerate	37.21	167	10449	10.45	ppb	#	22



Esperienza mais



Varie matrici alimentari e non...

Analisi per Spazio di Testa

- Frutti di mare: freschi e omogeneizzati
- Pesce e crostacei: sgusciati e omogeneizzati
- Prodotti liofilizzati: tal quale
- Latte e latticini
- Terreni: campioni essiccati e setacciati

A.R.P.A.F-VG dip. TRIESTE A.R.P.A.

F-VG dip. Trieste



L'esperienza: ...

Preparazione



- 1 g di materiale in vial da 20 mL
- 3 g di $(\text{NH}_4)_2\text{SO}_4$ (da contenitori in vetro!!!)
- 1 mL di acqua
- 5 mL H_2SO_4 al 1% goccia a goccia

L'esperienza: ...

Estrazione ed analisi

- Tappare e sottoporre a riscaldamento 105°C per 30' a 500 giri/min
- Estrazione con SPME – Fibra $65\mu\text{m}$ PDMS/DVB (pink) per 20' a 105°C (HS)
- Iniezione in GC-MS (iniettore a 263°C ; programma di temperatura $70^{\circ}\div 310^{\circ}\text{C}$ a varie rampe; flusso 1.1 mL/min)