

Development of an HPLC Method for the Determination of Capsaicins in Human Plasma



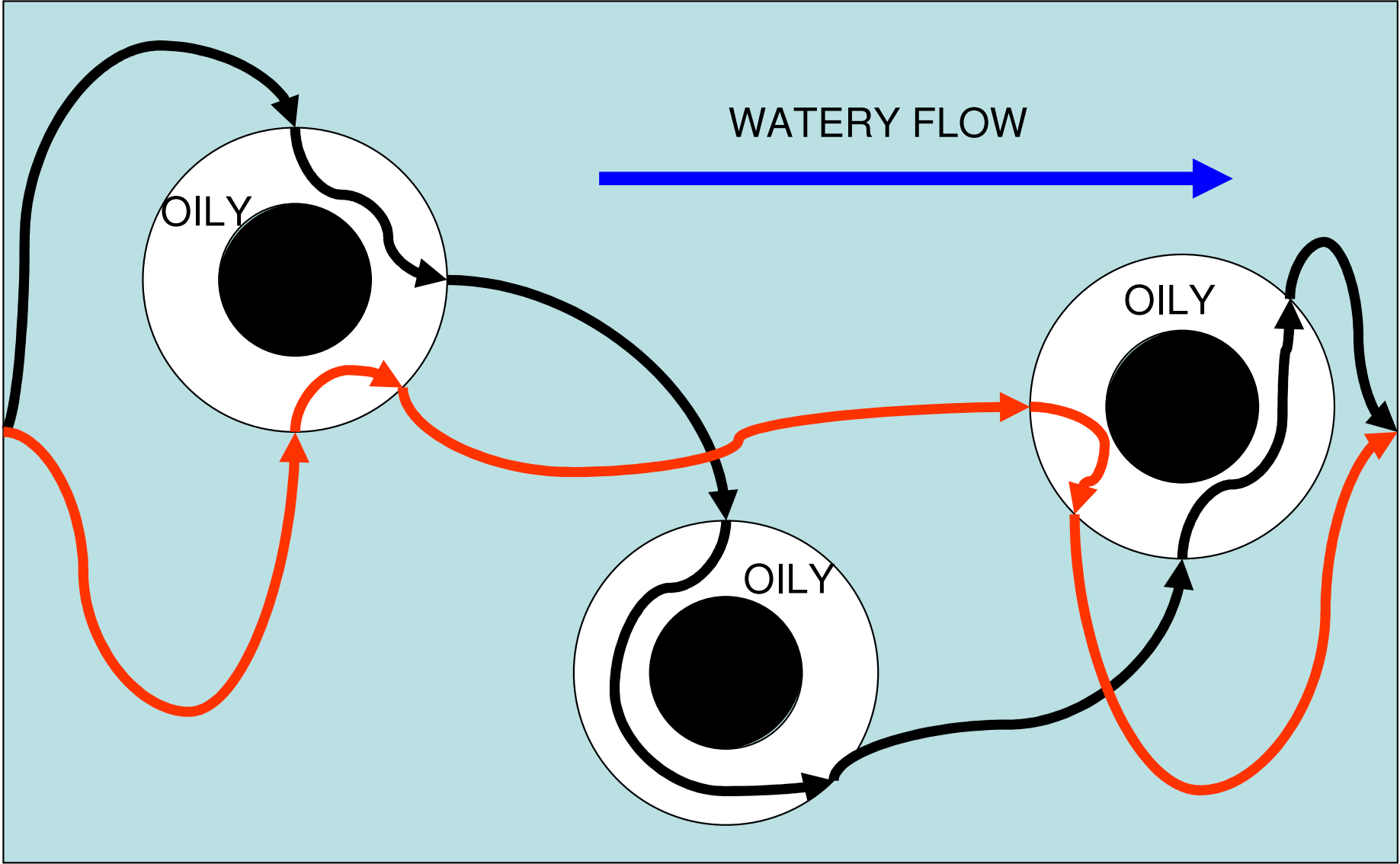
Dr Tom Hartley⁽¹⁾, Dr Brian Stevens⁽²⁾ and Dr Kiran Ahuja⁽¹⁾

⁽¹⁾ University of Tasmania : School of Human Life Sciences : Australia

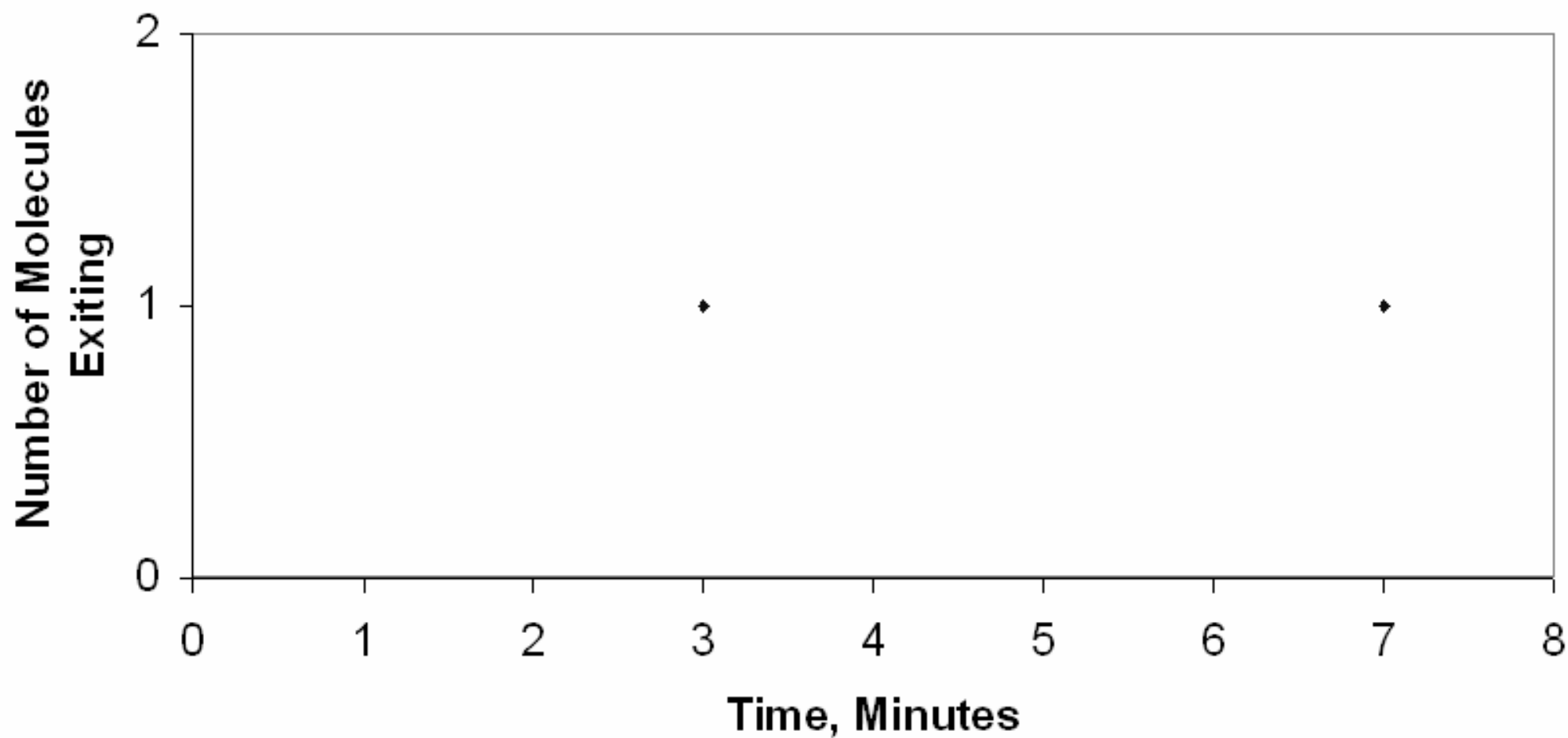
⁽²⁾PathLab : Melbourne : Australia

⁽¹⁾ Sponsor : Nancy Dale Scholarship : AACB

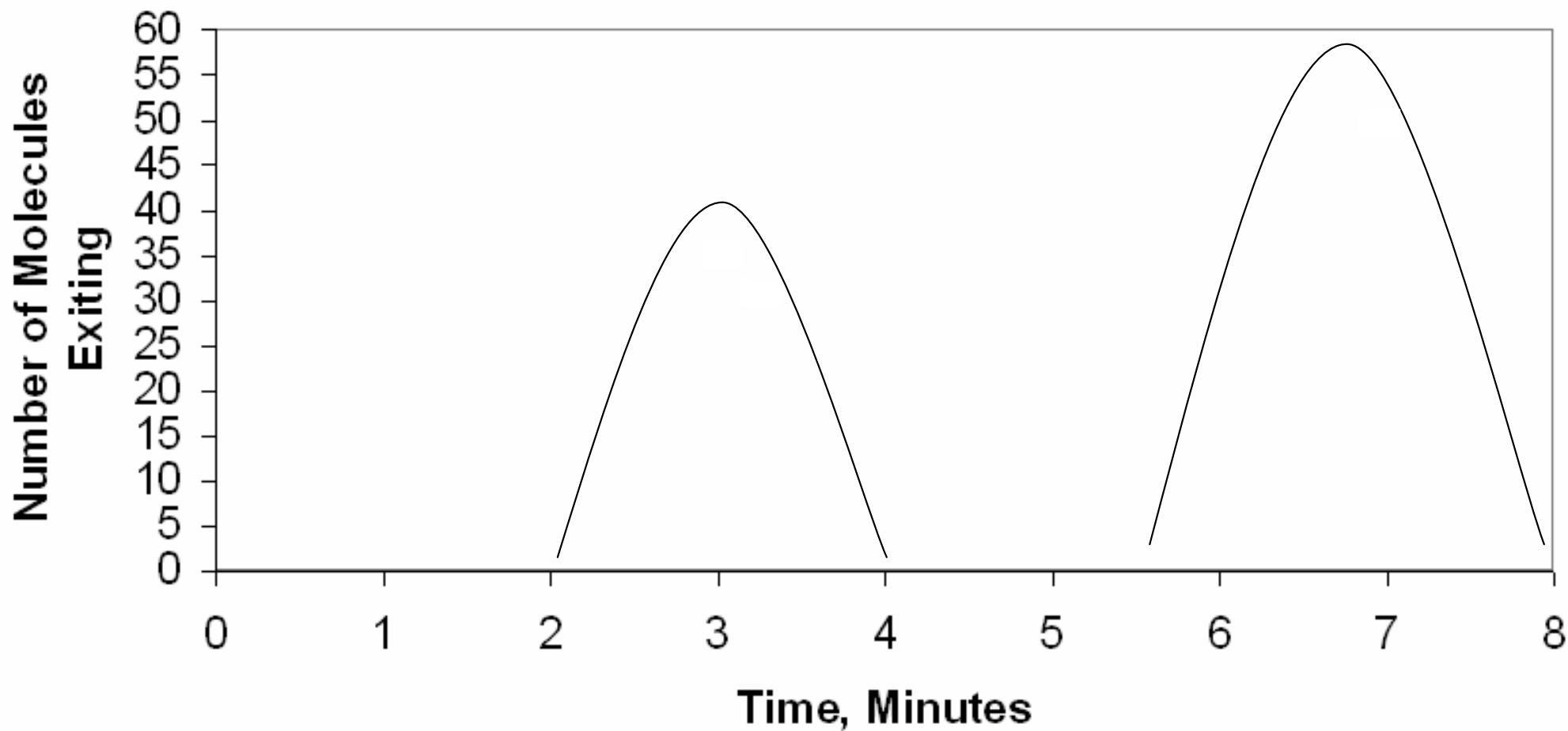
ANALYTICAL PRINCIPLE OF HPLC

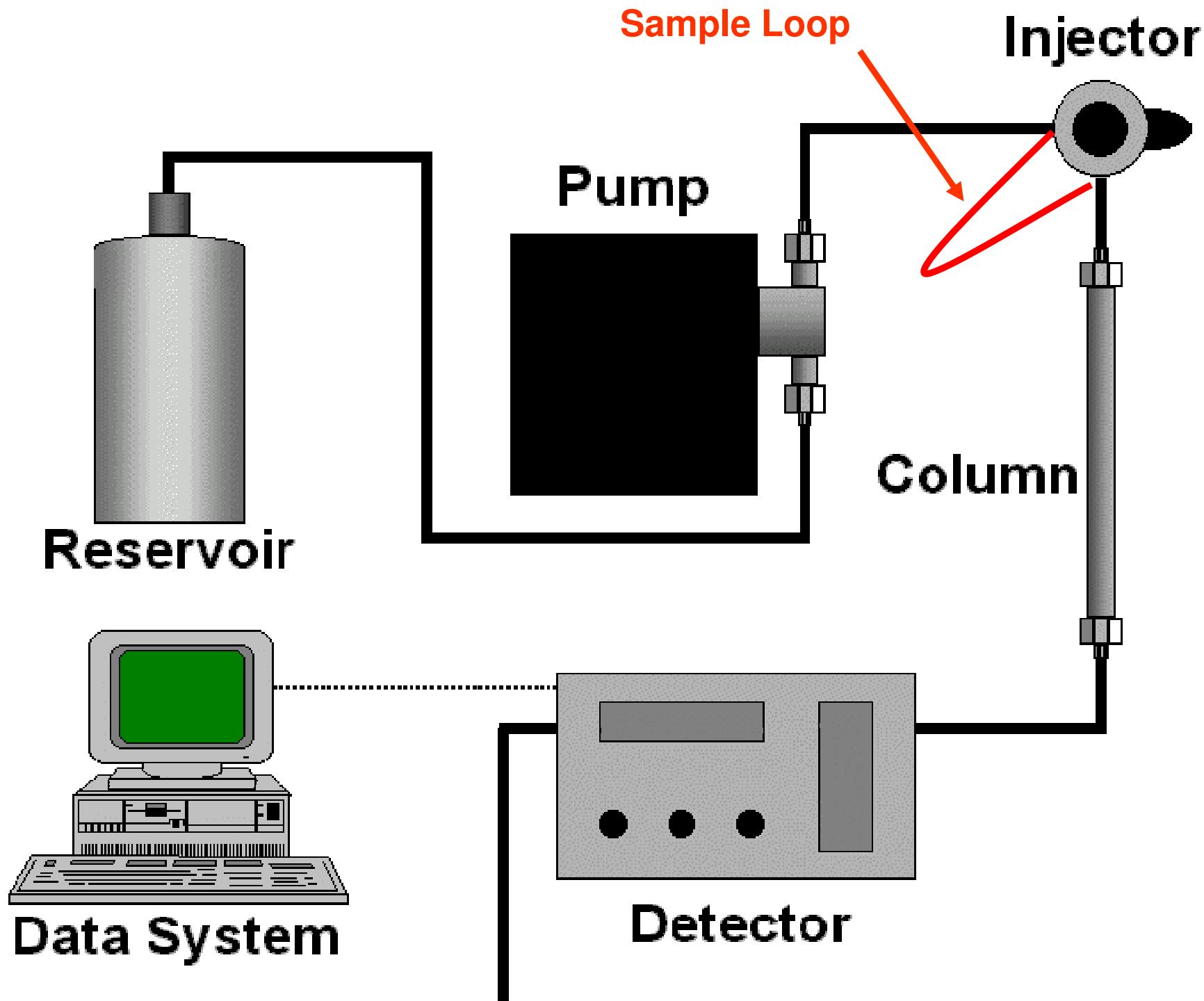


RETENTION TIME OF TWO DIFFERENT MOLECULES



RETENTION TIME OF A MIXTURE OF TWO DIFFERENT MOLECULES





INSTRUMENTATION USED IN THIS WORK



0:00 19
ELECTRONIC
TIMER CLOCK

varian
9010

Meth 40
1 40
Flow Pres Relays
8.78 124
4.88

METHOD STATE

- NOT READY
- READY
- RUNNING
- STOPPED
- INSTRUMENT
- FAULT
- POWER

OPERATION

- START
- STOP
- STATUS
- RESET
- PURGE
- PRIME
- PUMP
- A
- B
- C

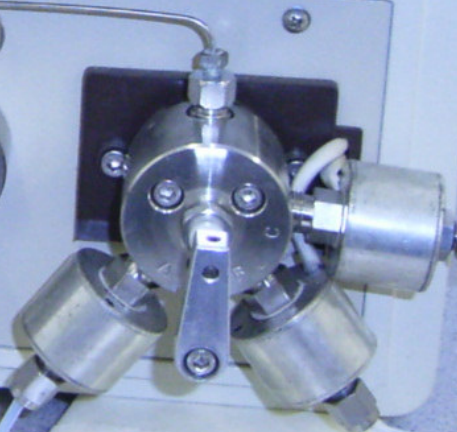
EDIT

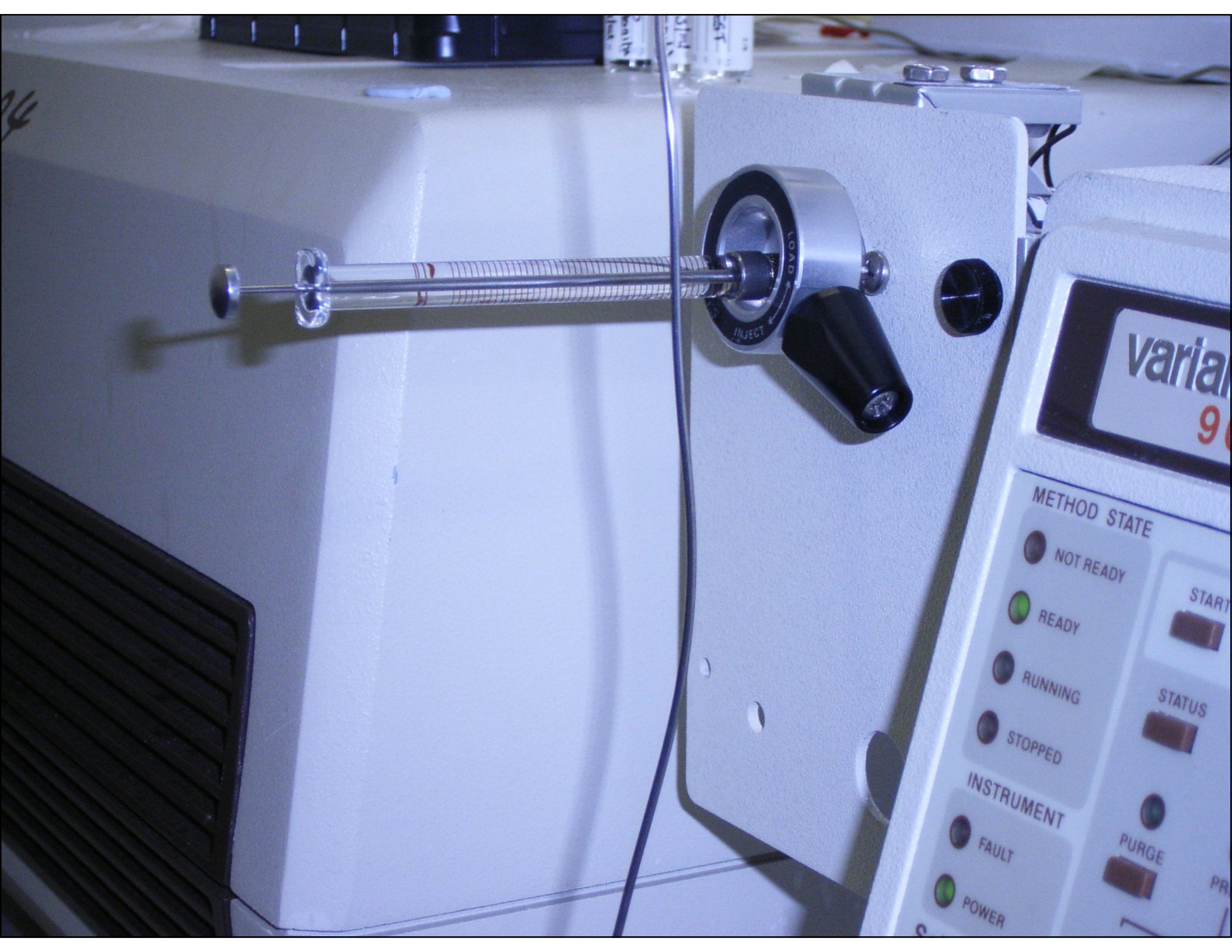
- CURSOR
- PREVIOUS LINE
- NEXT LINE
- SELECT
- CLEAR
- INSERT LINE
- DELETE LINE
- AUTOMATION
- MENU

TABLE SELECT

- SELECT LINE
- DELETE LINE
- RECALL
- SAVE
- CONTROL

Solvent Delivery System









Data Logger

File Display Option Help

Auto RS232

DC 099.1 mV

Begin

Digital

Analog

List

Graph

Recording

Close

Continue

Time: 600 s

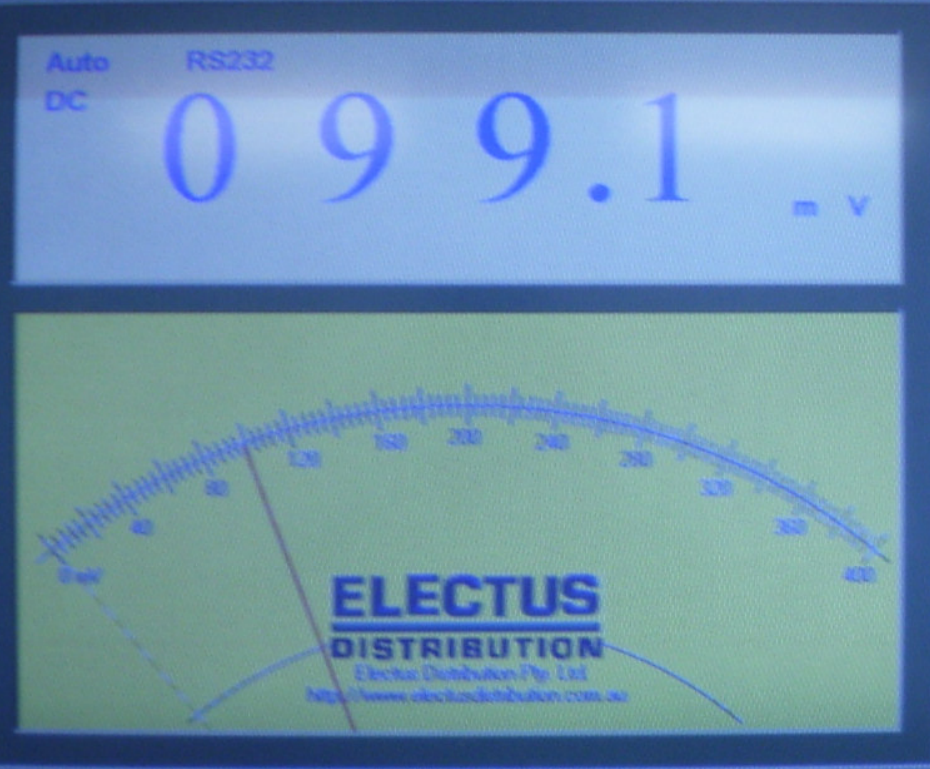
Sample interval: 1 s

Min 0.0mV Max 99.3mV Average 99.1450mV 07/21 13:43:55

1:43 PM

Data Logger

Time
07/21 13:36:06
07/21 13:36:07

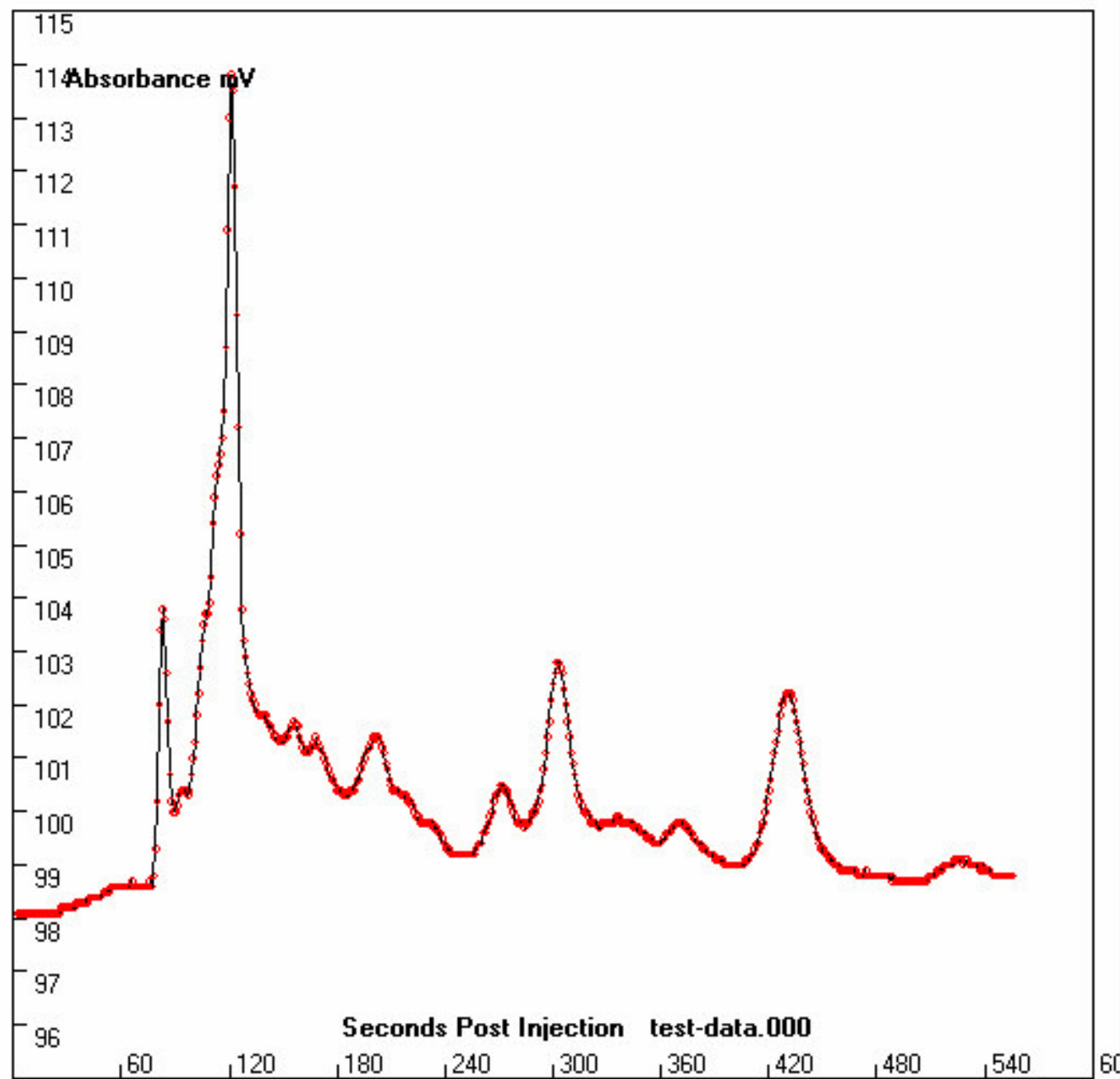


175N 4330

Videocom

PUSH





	MIN	MAX	Tick Marks Every
X	<input type="text" value="0"/>	<input type="text" value="600"/>	<input type="text" value="60"/>
Y	<input type="text" value="95"/>	<input type="text" value="115"/>	<input type="text" value="1"/>

Draw lines between the points ?

HPLC DATAFILE NAME

X is in Column # TOTAL NUMBER OF COLUMNS

Y is in Column #

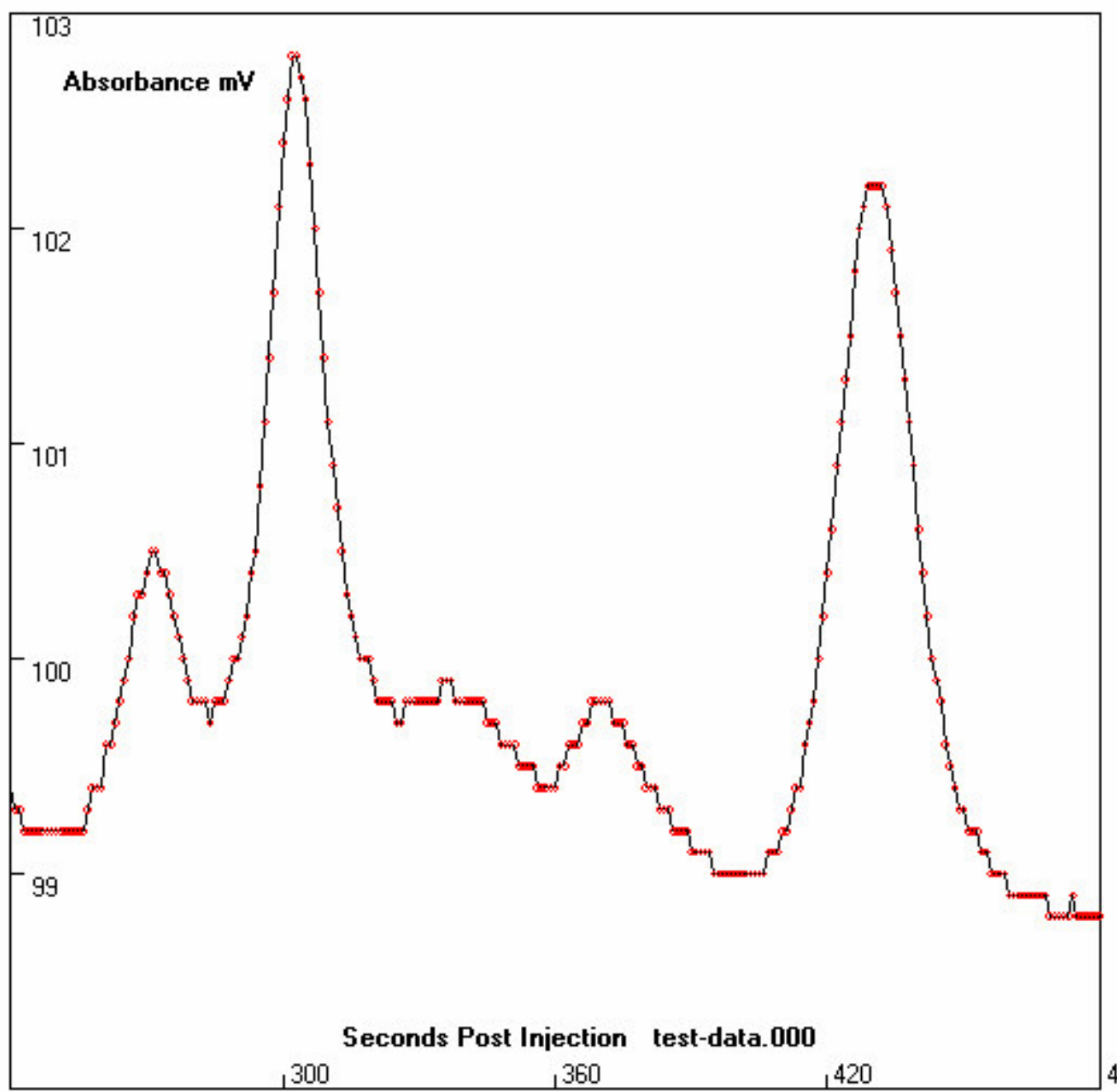
X AXIS LABEL

Y AXIS LABEL

PLOT GRAPH

CLEAR GRAPH

EXIT



	MIN	MAX	Tick Marks Every
X	<input type="text" value="240"/>	<input type="text" value="480"/>	<input type="text" value="60"/>
Y	<input type="text" value="98"/>	<input type="text" value="103"/>	<input type="text" value="1"/>

Draw lines between the points ?

HPLC DATAFILE NAME

X is in Column # TOTAL NUMBER OF COLUMNS

Y is in Column #

X AXIS LABEL

Y AXIS LABEL

LITERATURE REVIEW

A BRIEF REVIEW OF THE LITERATURE

B. SOME KEY PAPERS

1. Early 1970's

First utilisation of GC

Tedious derivatisation procedures

2. 1981 Saria et al

HPLC

Comparison of UV and fluorescence detection

UV-60 ng

Fluorescence – 3 ng

3. 1982 Johnson et al

Toxicological aspects

HPLC

Urine – 50ml extracted 3x into 200ml dichloromethane

4. **1987 Attuquayefio et al**

Use of Sep-pack cartridges

5. **1997 Lu and Cwik**

HPLC with fluorescence detection

Heated column

Complex solvent extraction

Detection limit 0.5 ng/ml

6. **Reilly et al**

High efficiency solvent extraction

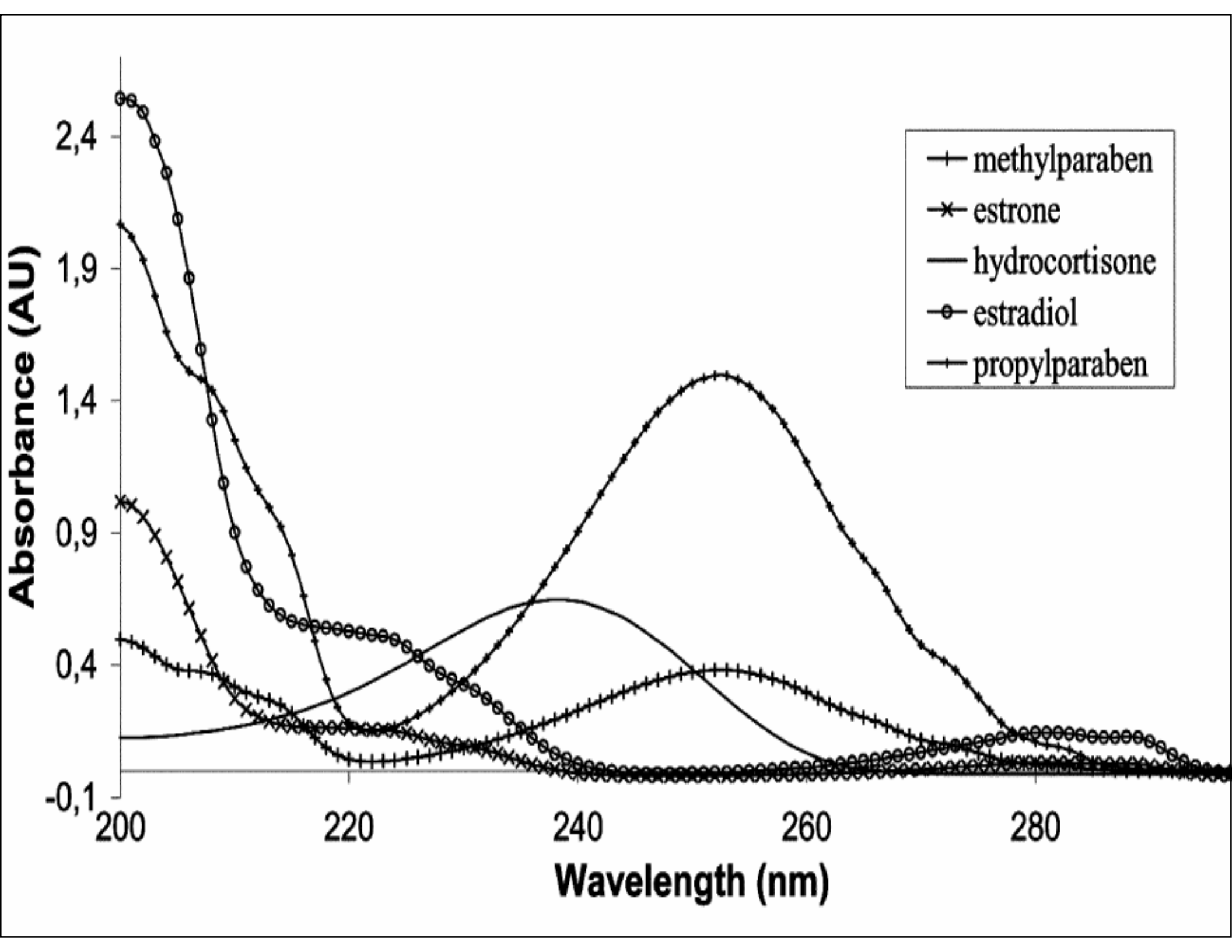
HPLC plus tandem mass spectrometry

Detection limit 0.25 ng/ml

**SELECTION OF THE INTERNAL
STANDARD**

and

PLASMA SAMPLE PREPARATION



OUR APPROACH TO THE ASSAY

1. Oestradiol selected as internal standard.
2. Maybe we needed a protein releasing agent
3. So we tried:
 - 10% trichloroacetic acid
 - 1 to 4 molar acetic acid
 - acid alcohol
 - 10% sulphosalicylic acid
 - isopropanal/HCl
 - acidified acetonitrile
 - 20% PEG 200
 - Saturated sodium carbonate
 - etc. etc. etc.

4. Choice of **solvent** for extraction:

hexane

chloroform

diethyl ether

* 70-80% efficiency

* volatile

n-chlorobutane (Reilly et al)

* 100% efficiency (to be confirmed)

PRESENT STATUS OF THE METHOD : Part 1

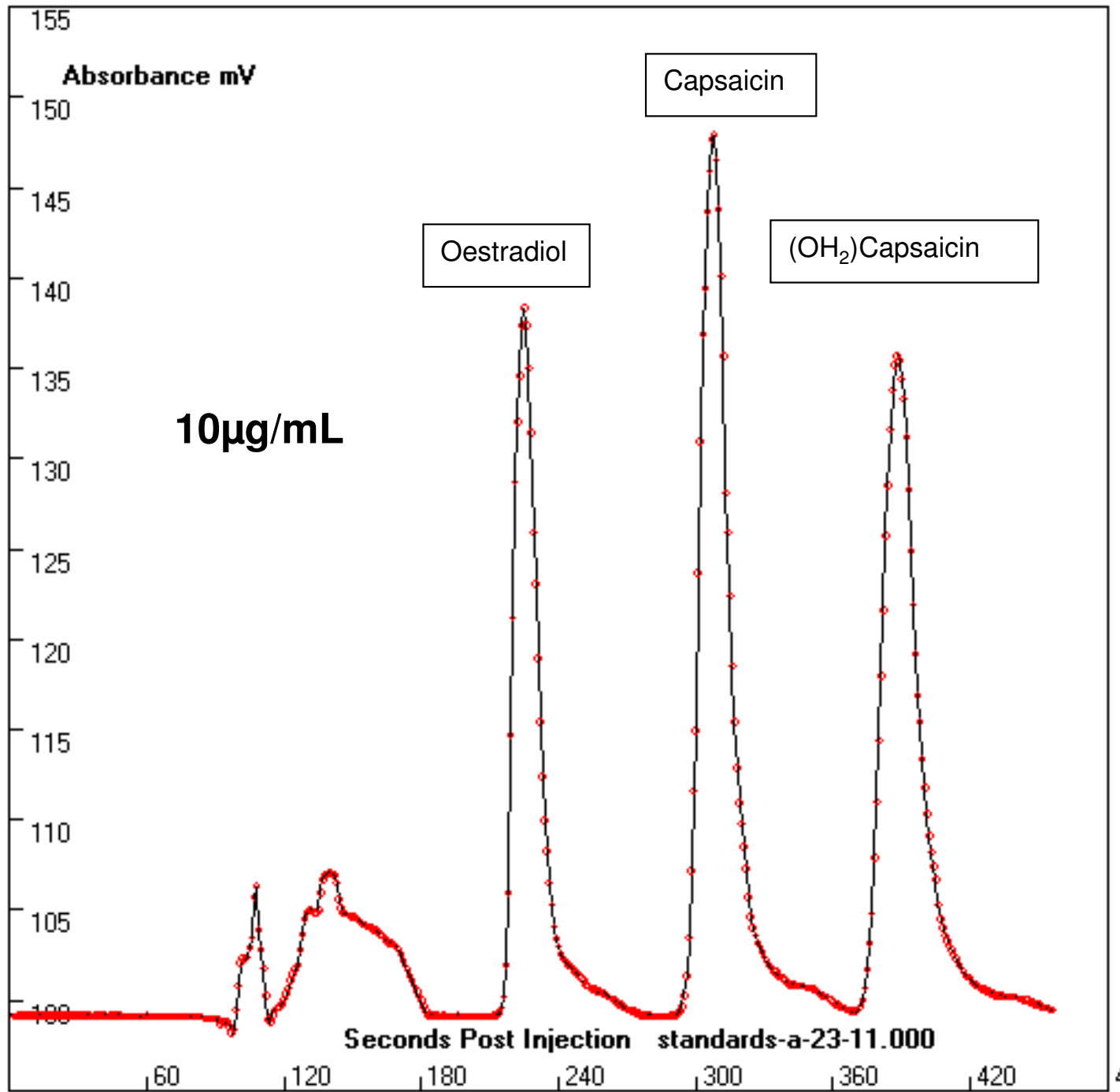
- **Spiking of the sample tubes with internal standard:**
- Spike the 10 mL “Hach” tubes with 200 μ L of the 200 ng/mL oestradiol in methanol solution and bring to dryness under nitrogen at 40 deg C.
- **Sample preparation:**
- Place a 2 mL sample of heparinised plasma in a spiked 10 mL “Hach” tube.
- Add 1.0 mL of phosphate buffer and mix.
- Add 5.0 mL of 1-chlorobutane. (Use a 5 mL measuring cylinder).
- Stopper the tube and place on a rotary mixer for 20 mins.
- Centrifuge at 3000 rpm for 10 mins at 4 deg C.

PRESENT STATUS OF THE METHOD : Part 2

- Quantitatively transfer 4.5 mL of the solvent layer to a 5 mL measuring cylinder. Then using the same transfer pipette, place 2.0 mL of this sample in a 5 mL glass bottle and evaporate to dryness under nitrogen (flow rate 5 L/min). Then add the second 2.0 mL of solvent and again bring to dryness.
- **N.B.** After centrifugation, if the solvent separation is not distinct, gently mix the contents and centrifuge again.
- Take up the dry residue to 150 μ L of acetonitrile. – **giving us a concentrating effect of 26.7 times** - Cap the tube and vortex for one minute and briefly again immediately before injecting into the HPLC.

PRESENT STATUS OF THE METHOD : Part 3

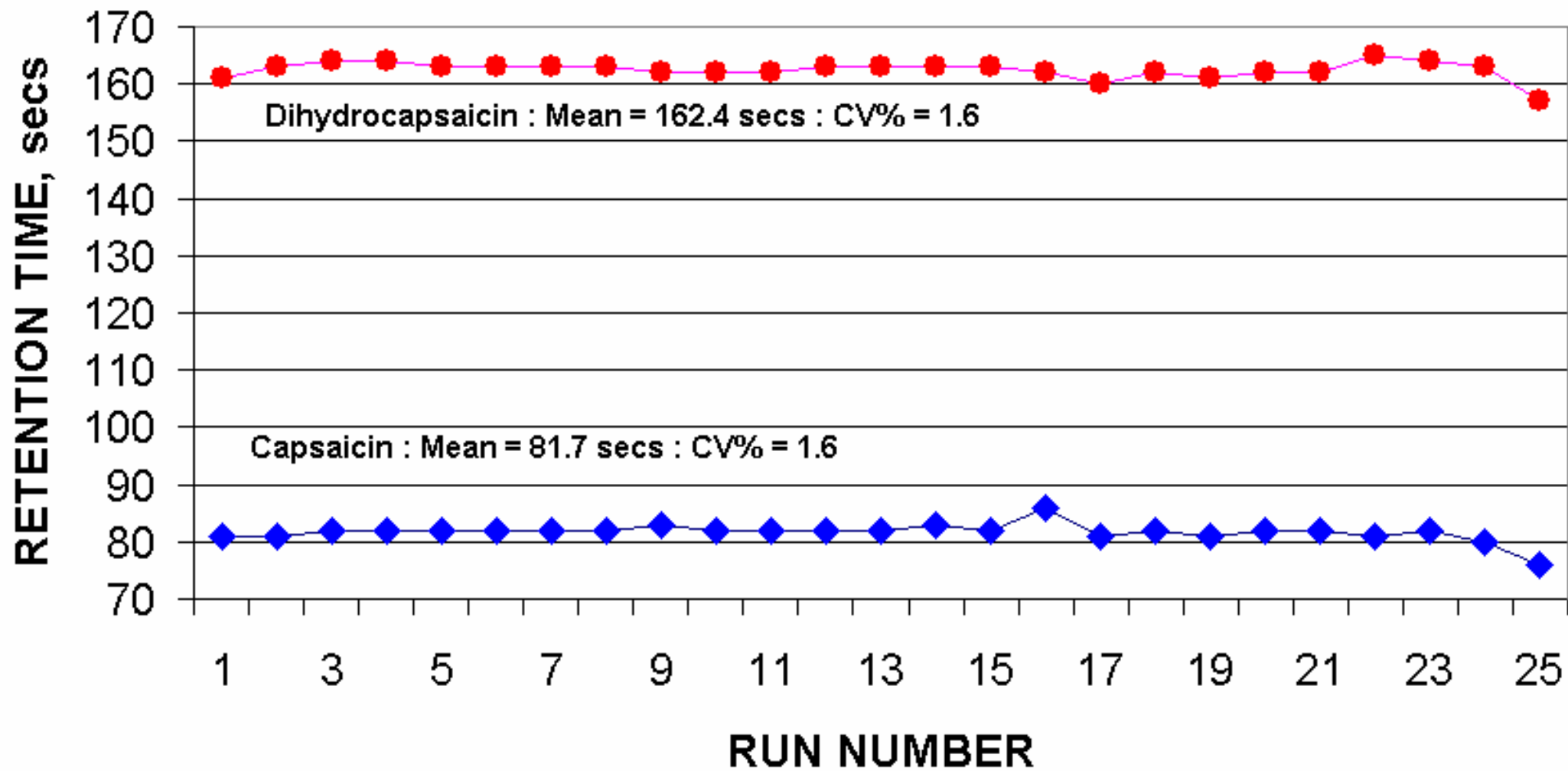
- **Injection Procedure:**
- Rinse the Hamilton syringe with acetonitrile
- Draw up approx 60 uL of sample without bubbles.
- Inject into sample loop
- Observe the peaks:
 - Injection peak – approx 1 min. 28 sec.
 - Oestradiol – 3 min. 30 sec.
 - Capsaicin – 4 min. 55 sec.
 - Dihydrocapsaicin – 6 min. 30 sec.



	OESTRADIOL	CAPSAICIN	(OH₂)CAPSAICIN
	Peak Heights for 10ug/mL in mV		
	39.2	48.6	35.7
Derived Correction Factors		39.2/48.6 = 0.8066	39.2/35.7 = 1.098

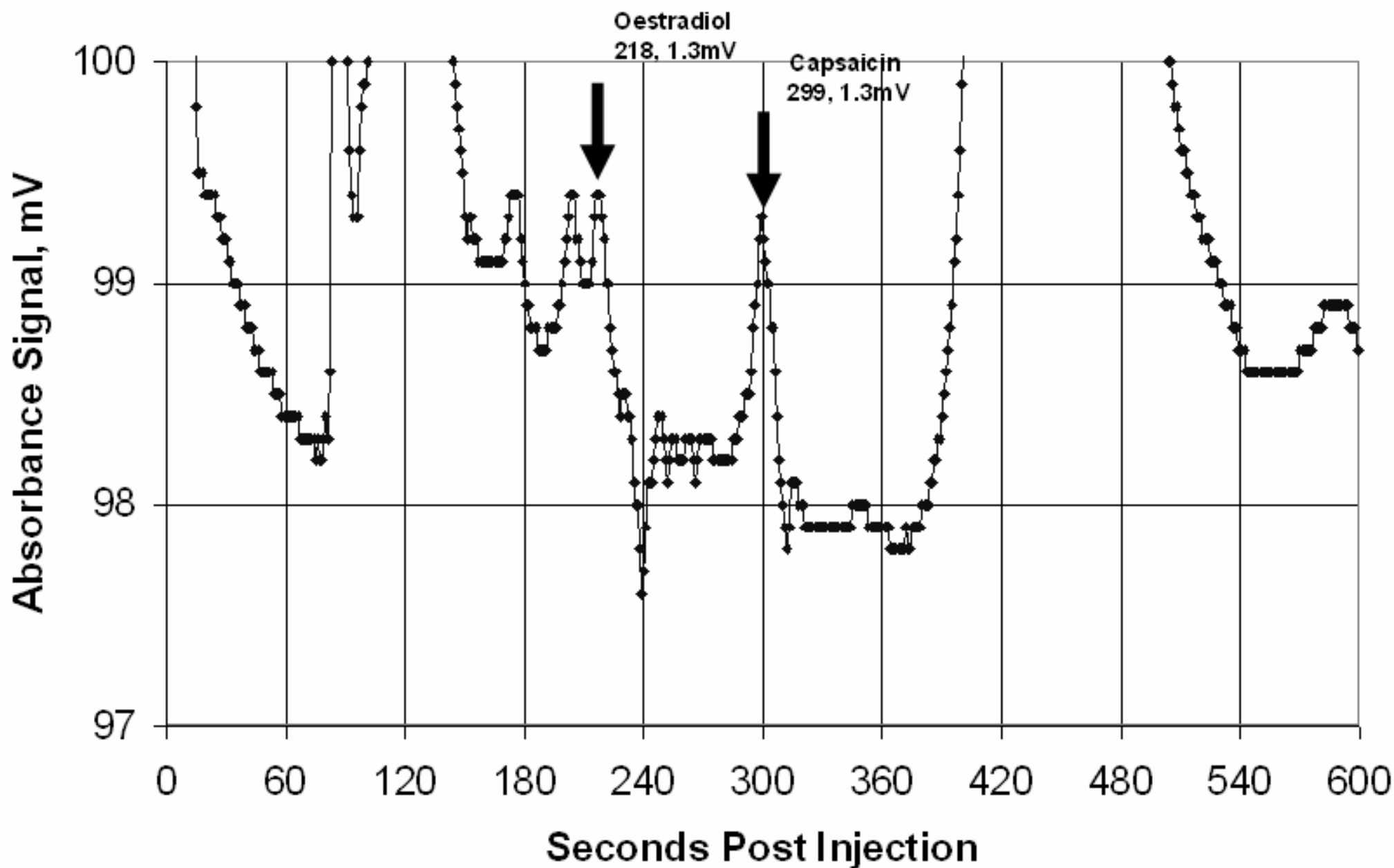
				RETENTION TIME RELATIVE TO OESTRADIOL PEAK	
Run #	OESTRADIOL	CAPSAICIN	DIHYDROCAPSAICIN	CAP RT Secs	(OH)2 CAP RT Secs
1	225	306	386	81	161
2	226	307	389	81	163
3	225	307	389	82	164
4	224	306	388	82	164
5	226	308	389	82	163
6	224	306	387	82	163
7	206	288	369	82	163
8	225	307	388	82	163
9	224	307	386	83	162
10	225	307	387	82	162
11	224	306	386	82	162
12	225	307	388	82	163
13	225	307	388	82	163
14	214	297	377	83	163
15	214	296	377	82	163
16	183	269	345	86	162
17	222	303	382	81	160
18	222	304	384	82	162
19	224	305	385	81	161
20	223	305	385	82	162
21	216	298	378	82	162
22	220	301	385	81	165
23	223	305	387	82	164
24	222	302	385	80	163
25	217	293	374	76	157
Mean	220.2	301.9	382.6	81.7	162.4
SD	9.11	8.54	9.39	1.59	1.55
CV%	4.1	2.8	2.5	2.0	1.0

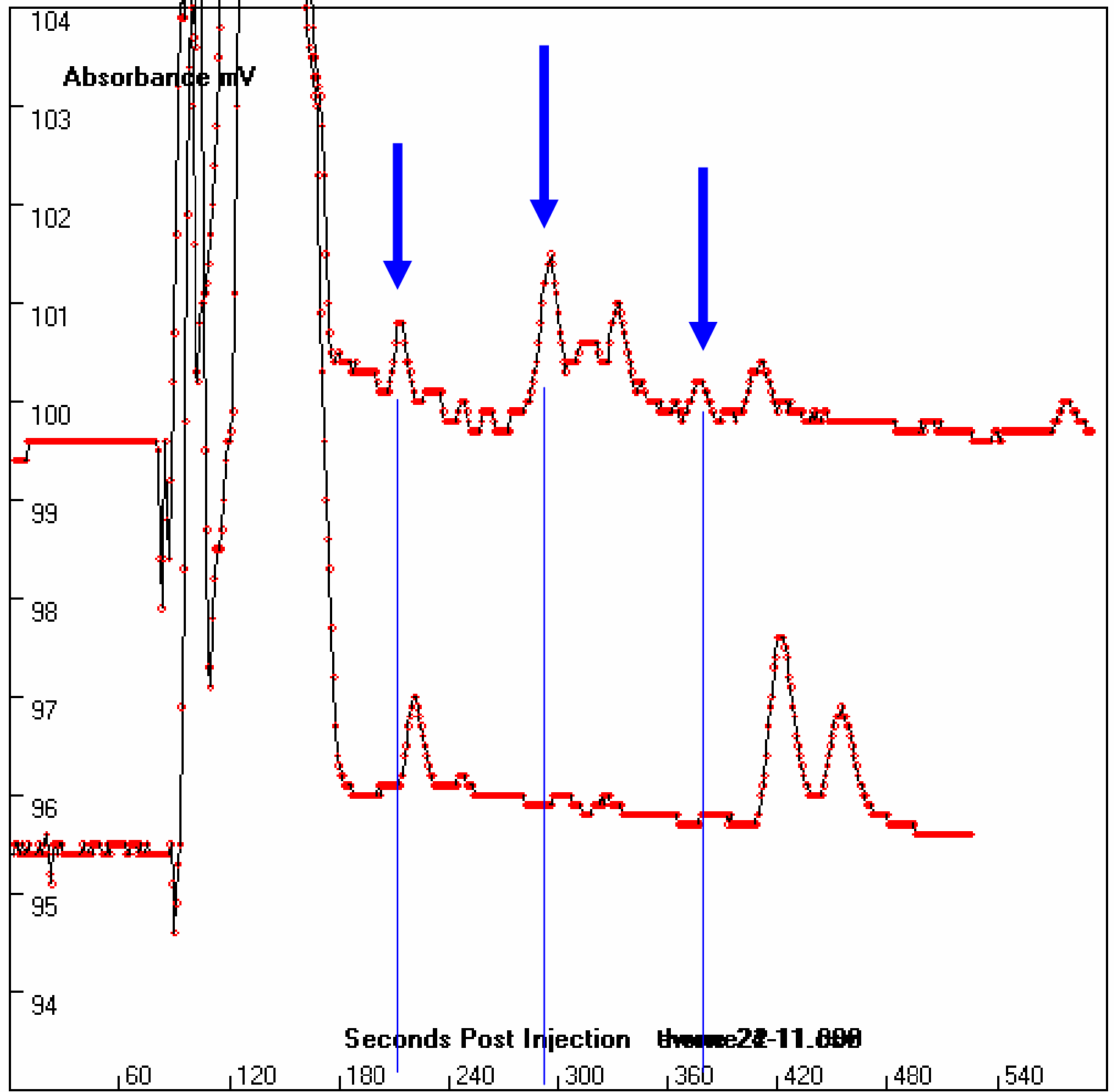
RETENTION TIMES RELATIVE TO OESTRADIOL : 25 Runs



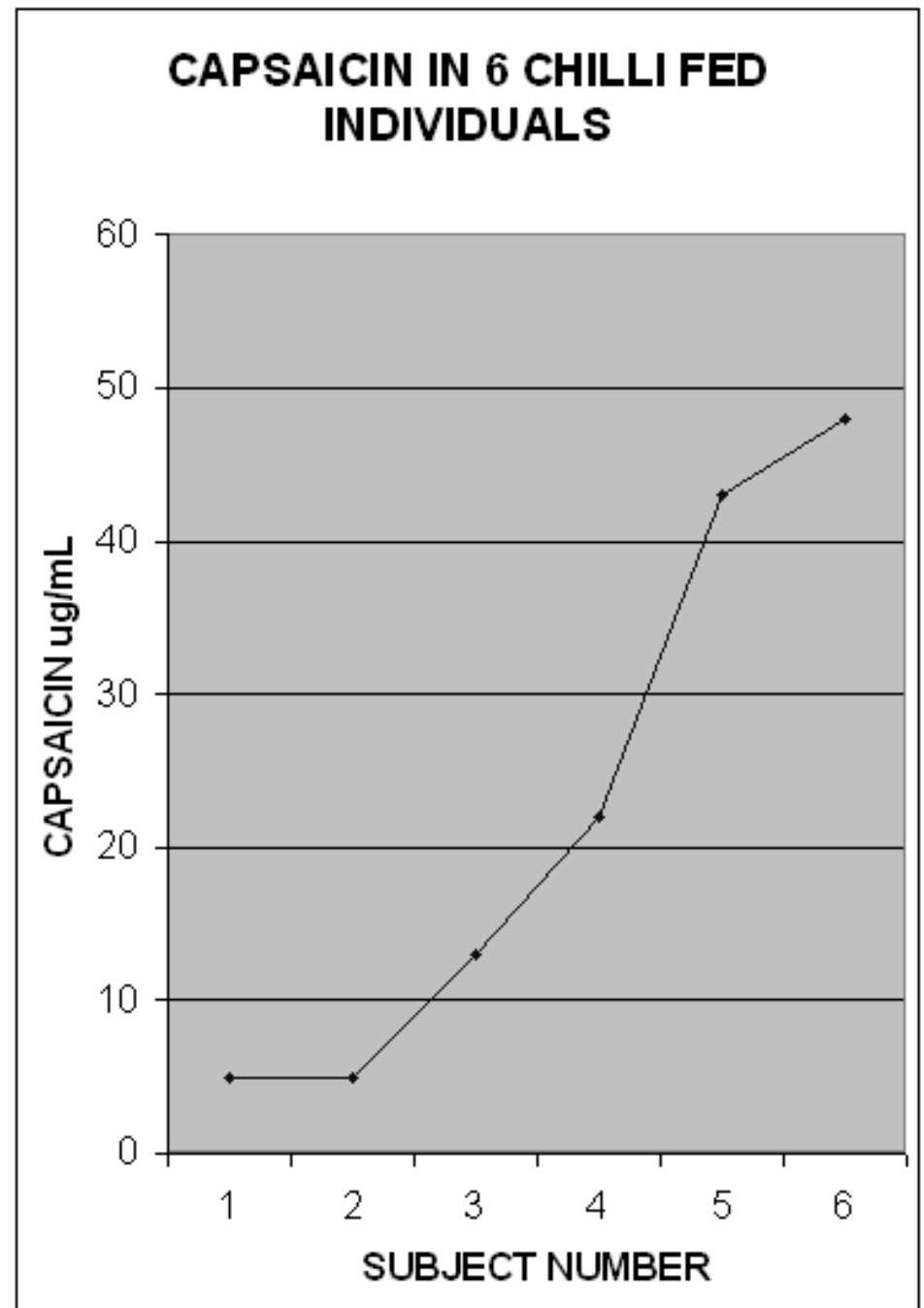
20 ng/mL Capsaicin and Oestradiol Added to Whole Blood

TFHwholeblood20ngmlCapandOest.xls



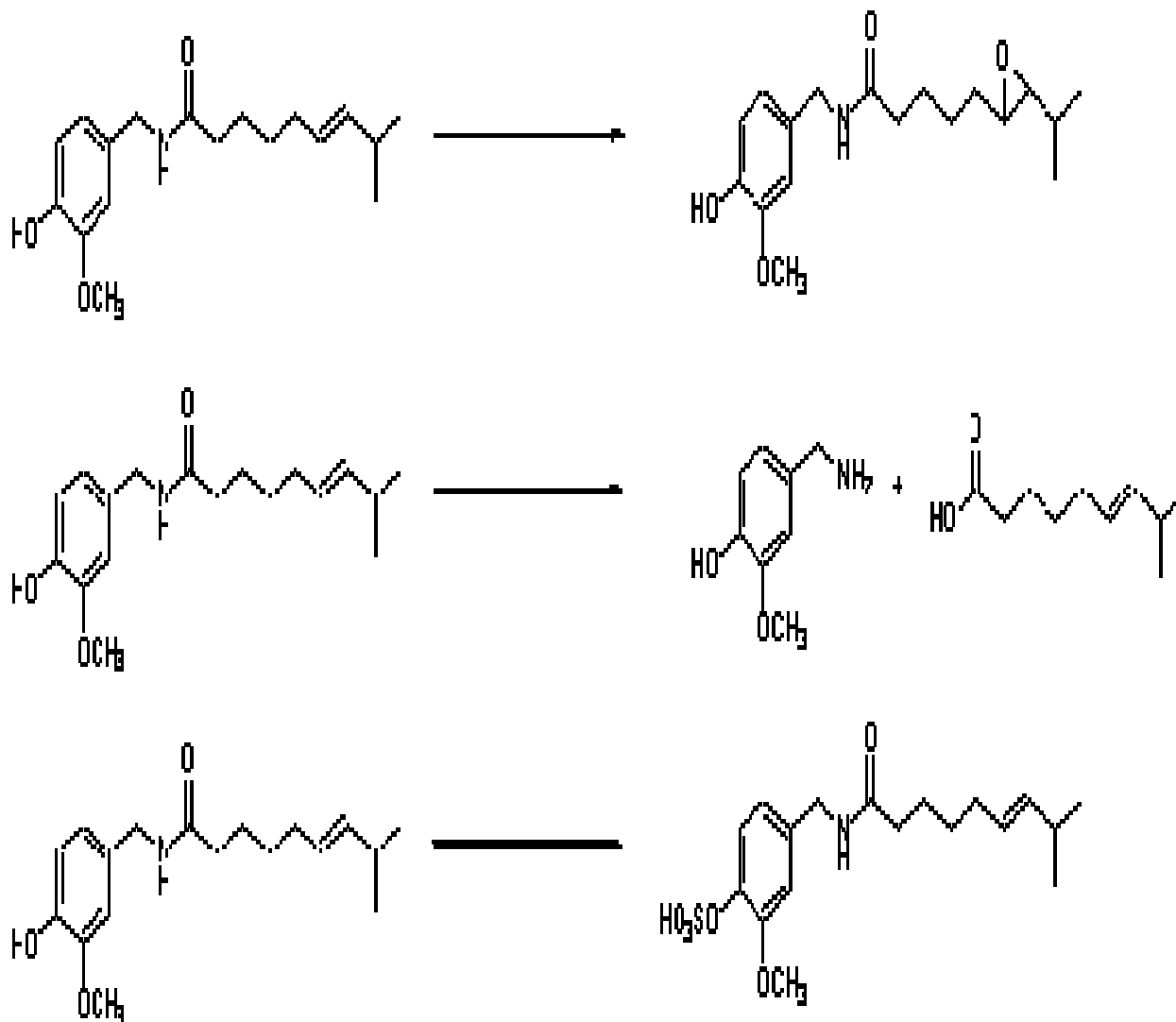


ug / mL Plasma	
CAPSAICIN	Di(OH)CAP.
5	6
5	
13	7
22	
43	8
48	





(a) Draw three different metabolic products expected for capsaicin. Do not truncate the structure - draw the entire molecule each time. Note that only one possible route of benzene ring oxidation is possible. (9 pts)



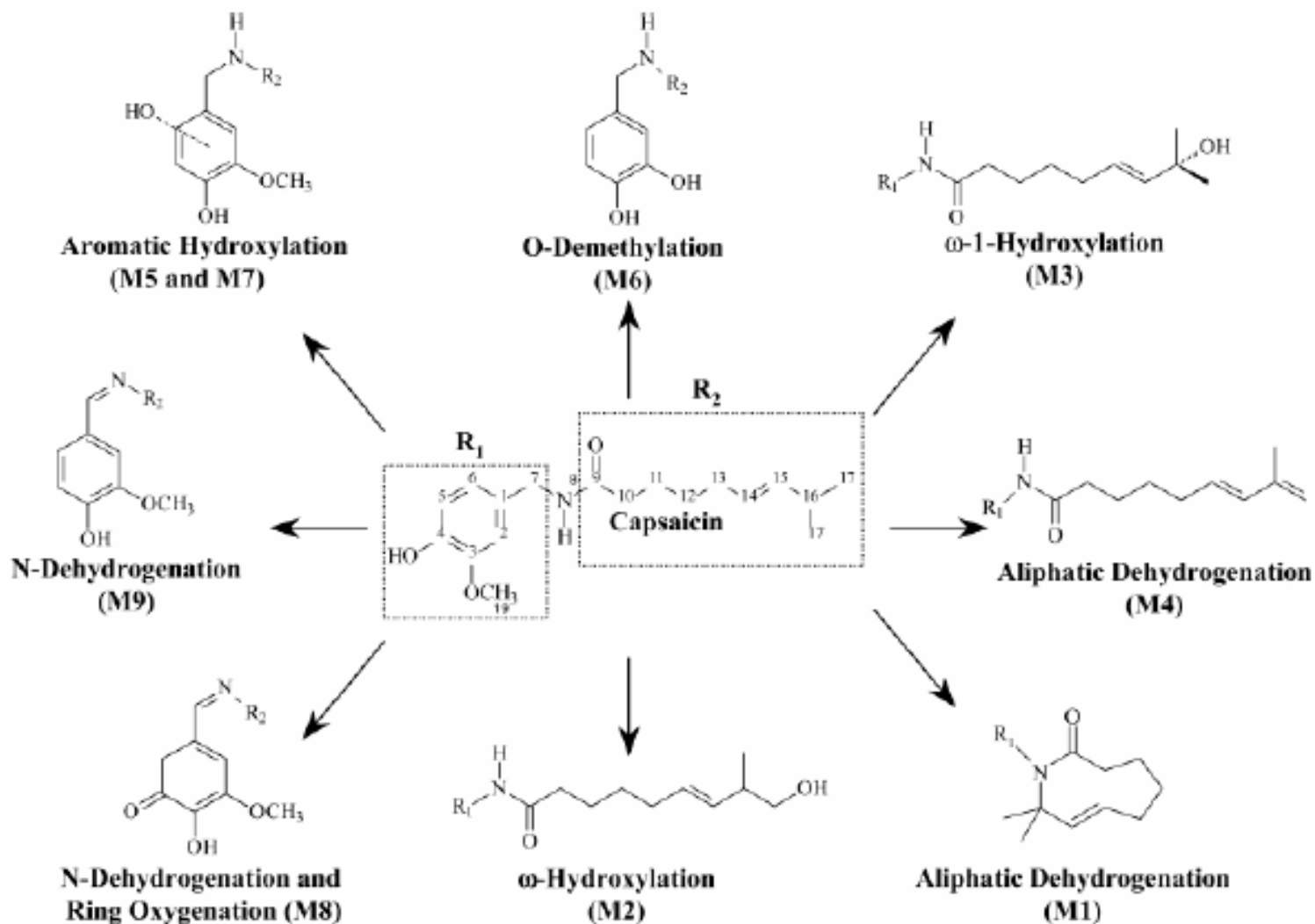
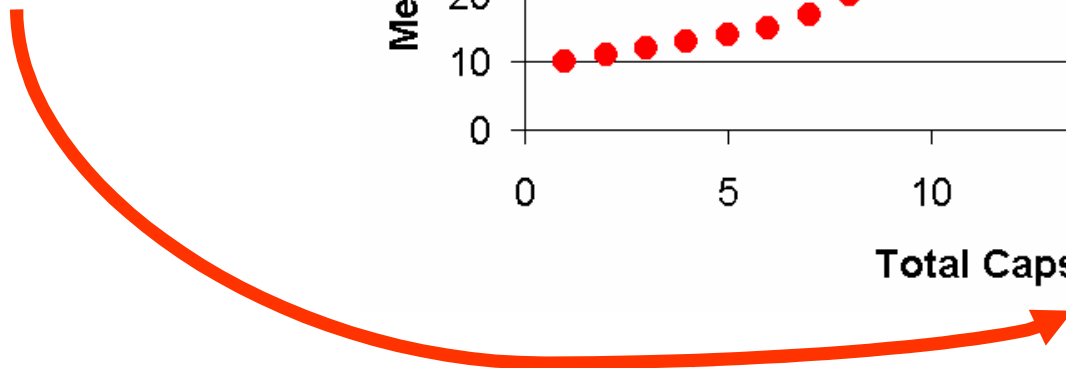
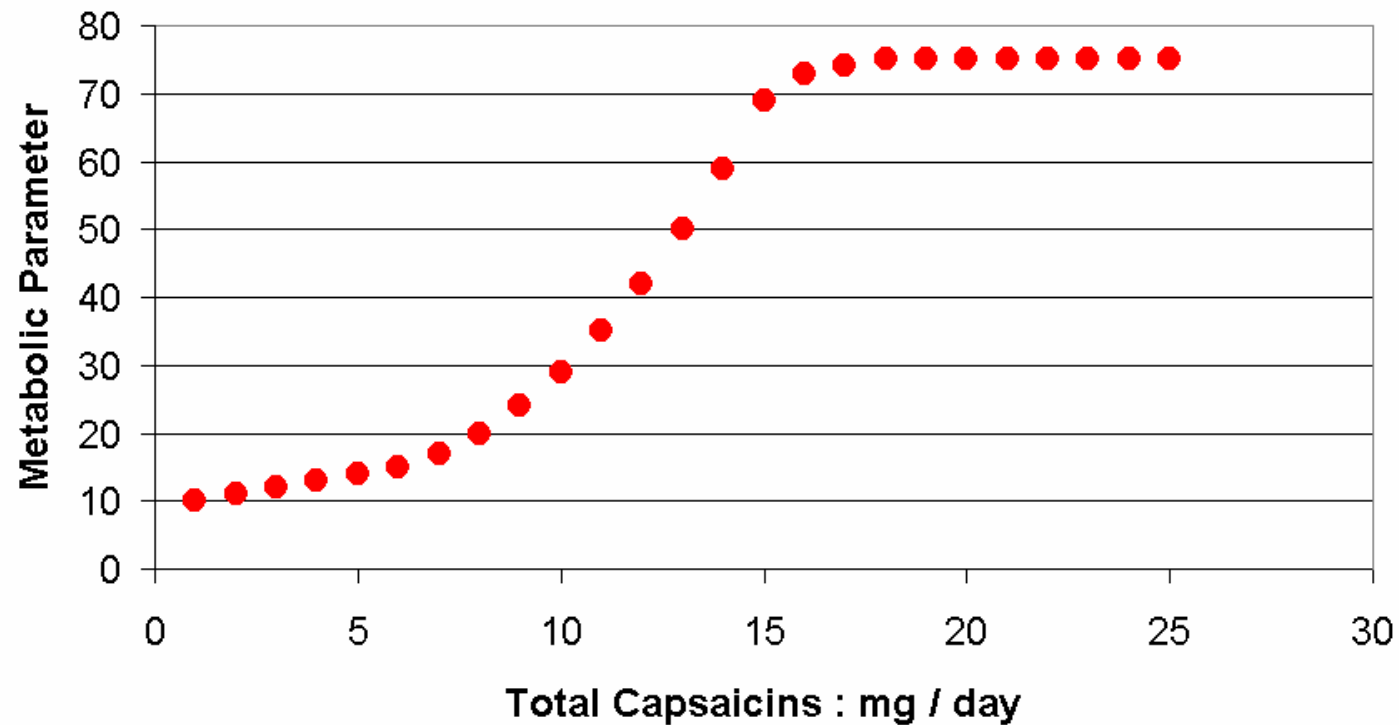


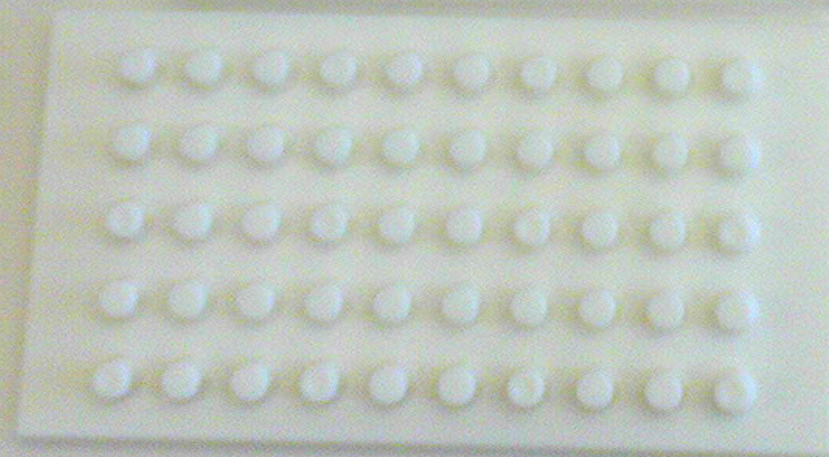
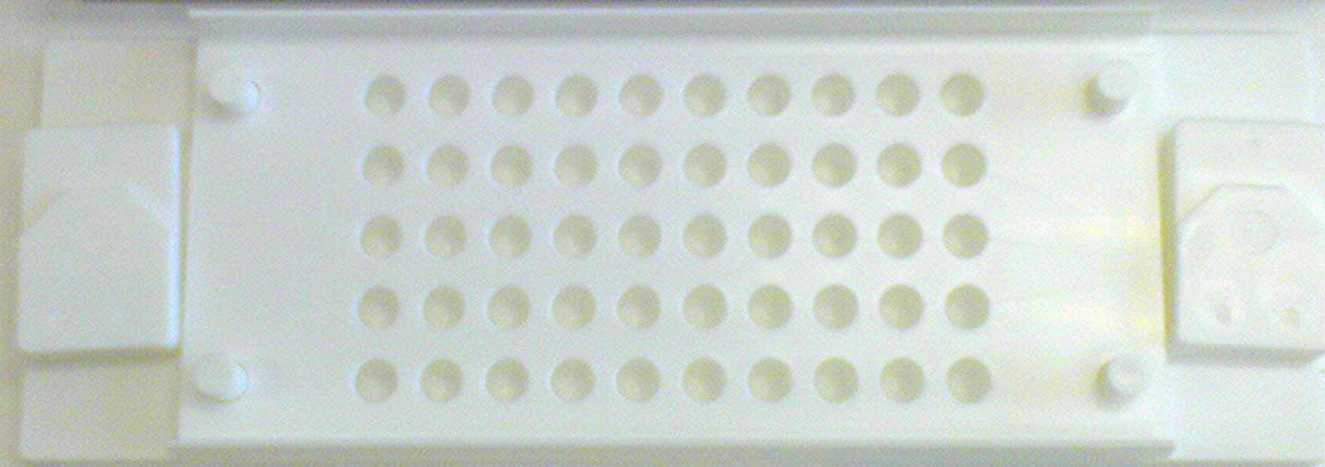
Figure 1. Schematic representation for the metabolism of capsaicin by P450. The structure for capsaicin has been numbered according to Figure 6 to aid in the discussion of the results presented throughout the study.

Current Work – Selection of ‘Strong’ Chilli Powders to Put Into Capsules for Use in **Dose Response Studies** in Human Volunteers

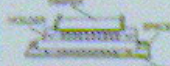


DOSE RESPONSE





Cap-M-Quik
 Home Product
 TAMPRESS

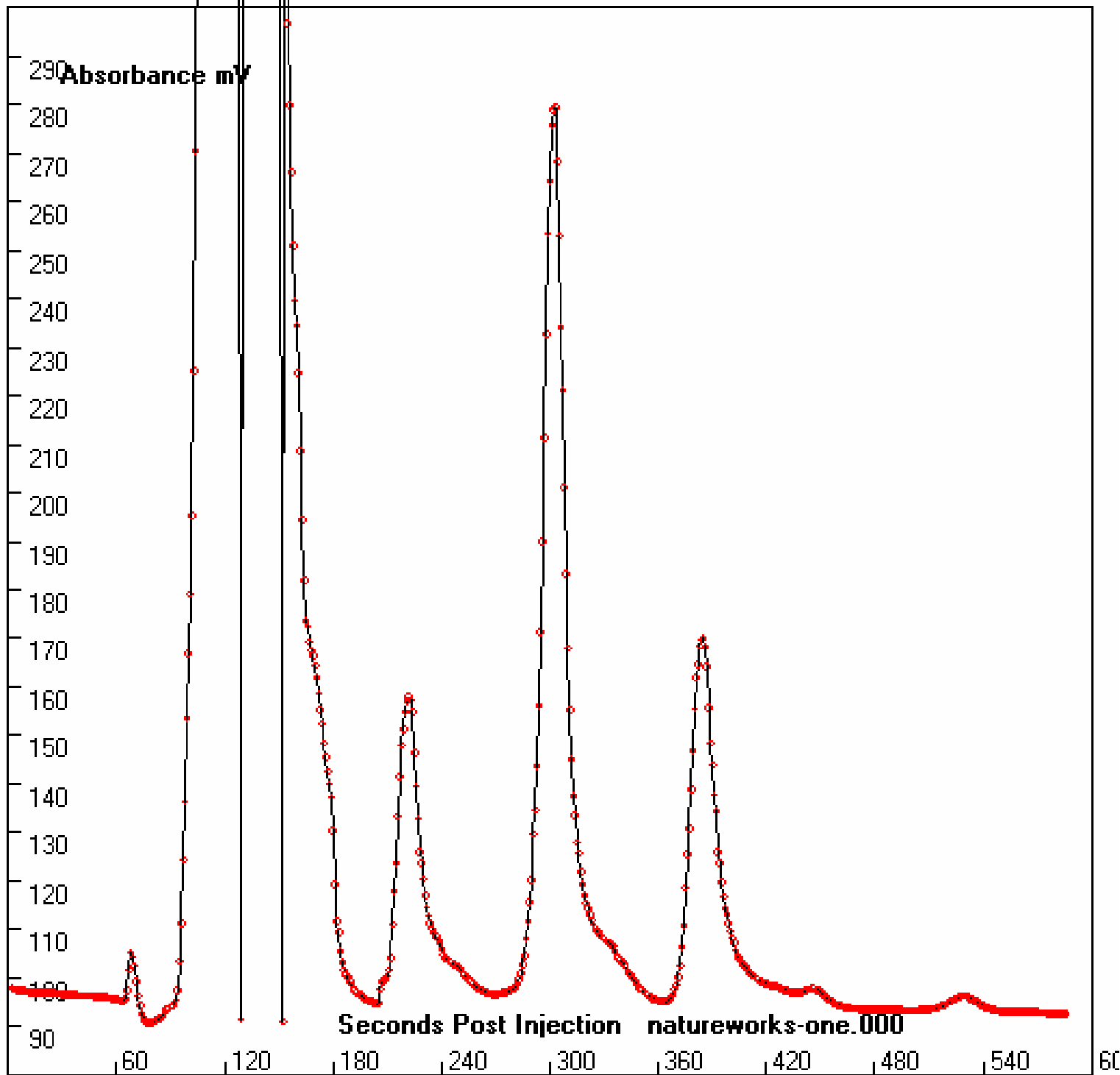


Simply load 50 pills to press down the contents of 50 00 size capsules. Being able to press Cap-M-Quik Capsule Filler.

Cap-M-Quik Filler consists of base, holder and cap. It is manually operated and loads 50 capsules in upright position after each turn has been turned. Push substance into holder and spread over open end of upright capsules with lid. Turn cap over 90° holder slides down spreading opening over full of filled capsules. Naturally replace for complete.


1-800-783-QUICK(7243)

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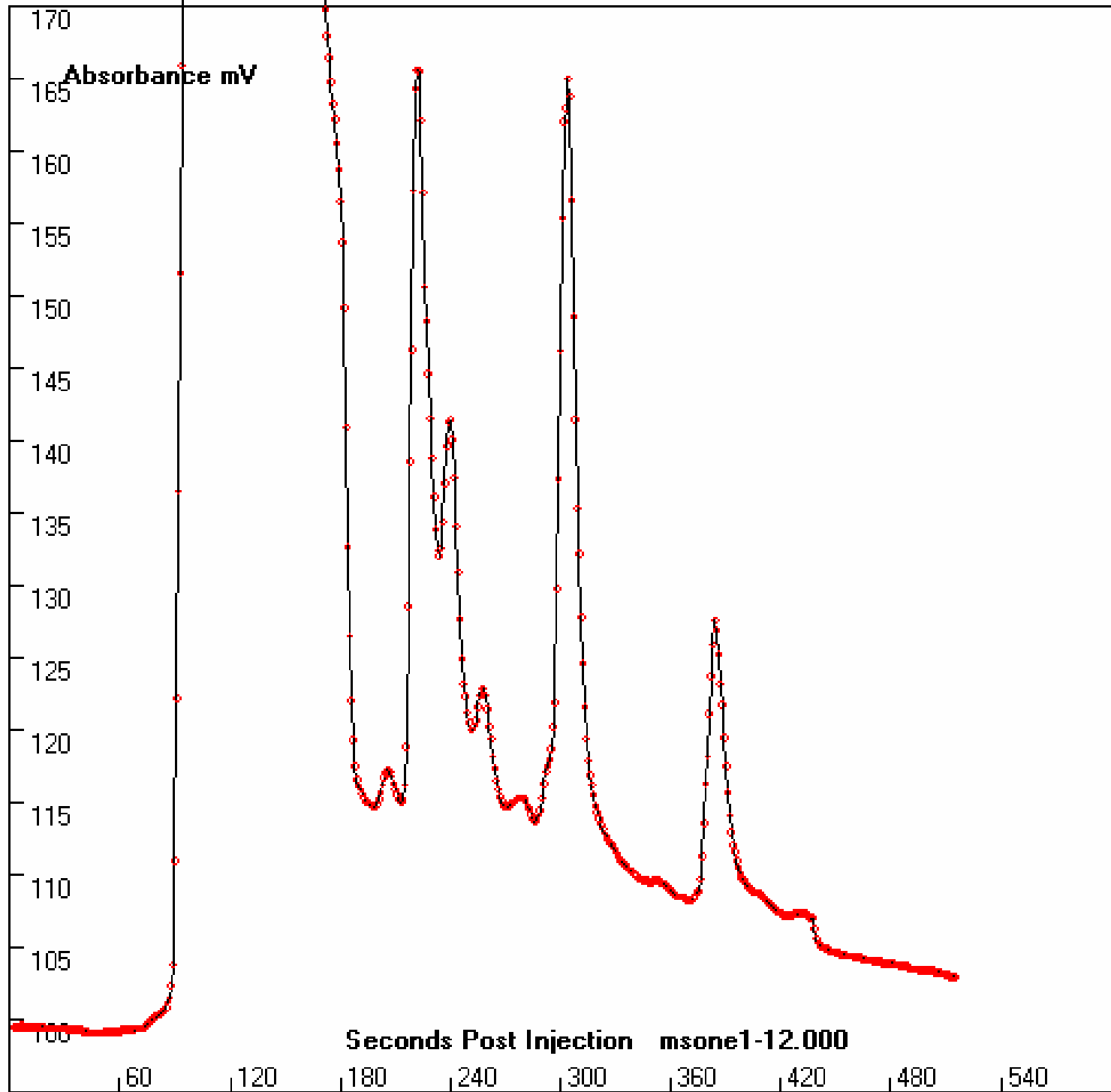
			TOTAL CAPSAICINS ug/g
	CAP	(OH)2CAP	
Menora Powder	624	410	
	541	353	
	565	357	
Average	577	373	950
%	61	39	
Hoyt	689	417	
	950	655	
	994	672	
Average	878	581	1459
%	60	40	
SpiceWorld	540	571	
	702	865	
	722	906	
Average	655	781	1435
	46	54	
NaturesWorks	1548	865	
	1727	935	
	1737	968	
	1671	946	
Average	1671	929	2599
%	64	36	

Development of an HPLC Method for the Determination of Capsaicins in Human Plasma

Thank You  *ANY QUESTIONS*

Results - Sauces





**TABLE 1 : Capsaicin Concentrations in Chilli Containing Food Products
(Datafile = three-sauces.xls)**

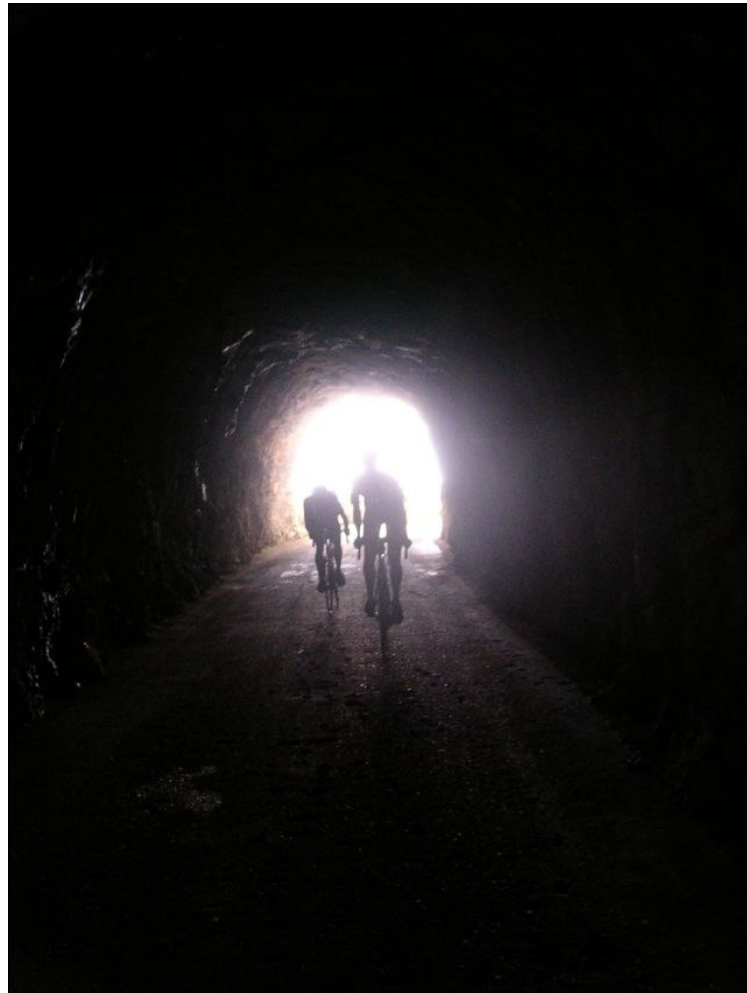
	Capsaicin, ug/g	Mean Capsaicin, ug/g	(OH ₂)Capsaicin	Mean (OH ₂)Capsaicin	Total Capsaicins, ug/g
Ayam Sweet Chilli Sauce	12		7		
ditto	23	18	9	8	26
Mama Sita Pepper Sauce	75		40		
ditto	84	80	47	44	124
Tabasco Sauce	97		45		
Ditto	103	100	45	45	145
Master Foods Chilli Paste		183		139	322
Cayenne Pepper Capsules		535 ug/capsule		495 ug/capsule	1030 ug/capsule

Results – Human Beings !



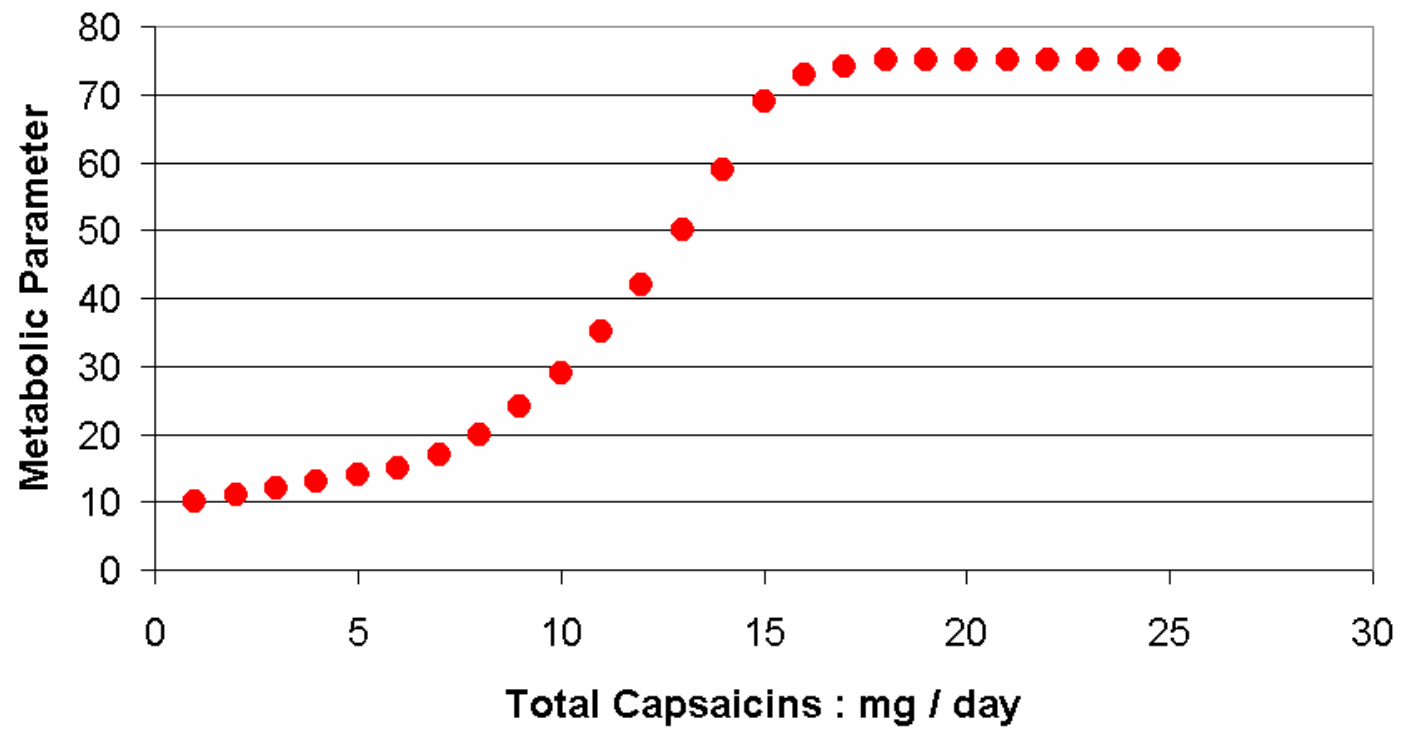
I Know This Much is True

..... *Wally Lamb*





DOSE RESPONSE





Mama Sita

Mama Sita's[®]

**PURE
LABUYO**

**Red Hot
Pepper Sauce**



**FROM NATIVE
PHILIPPINE BIRD'S EYE
RED HOT PEPPERS**

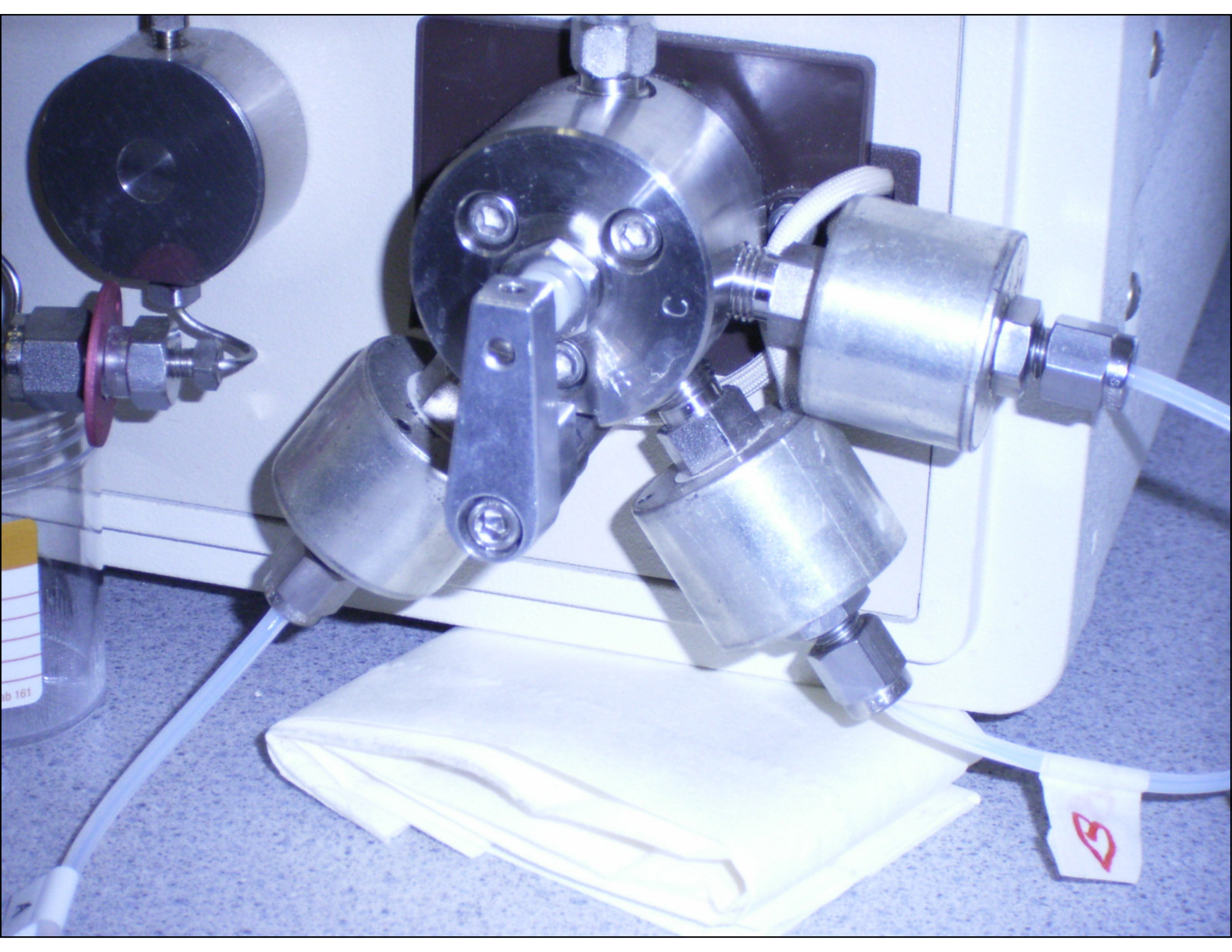
NET 2 FL. OZ.

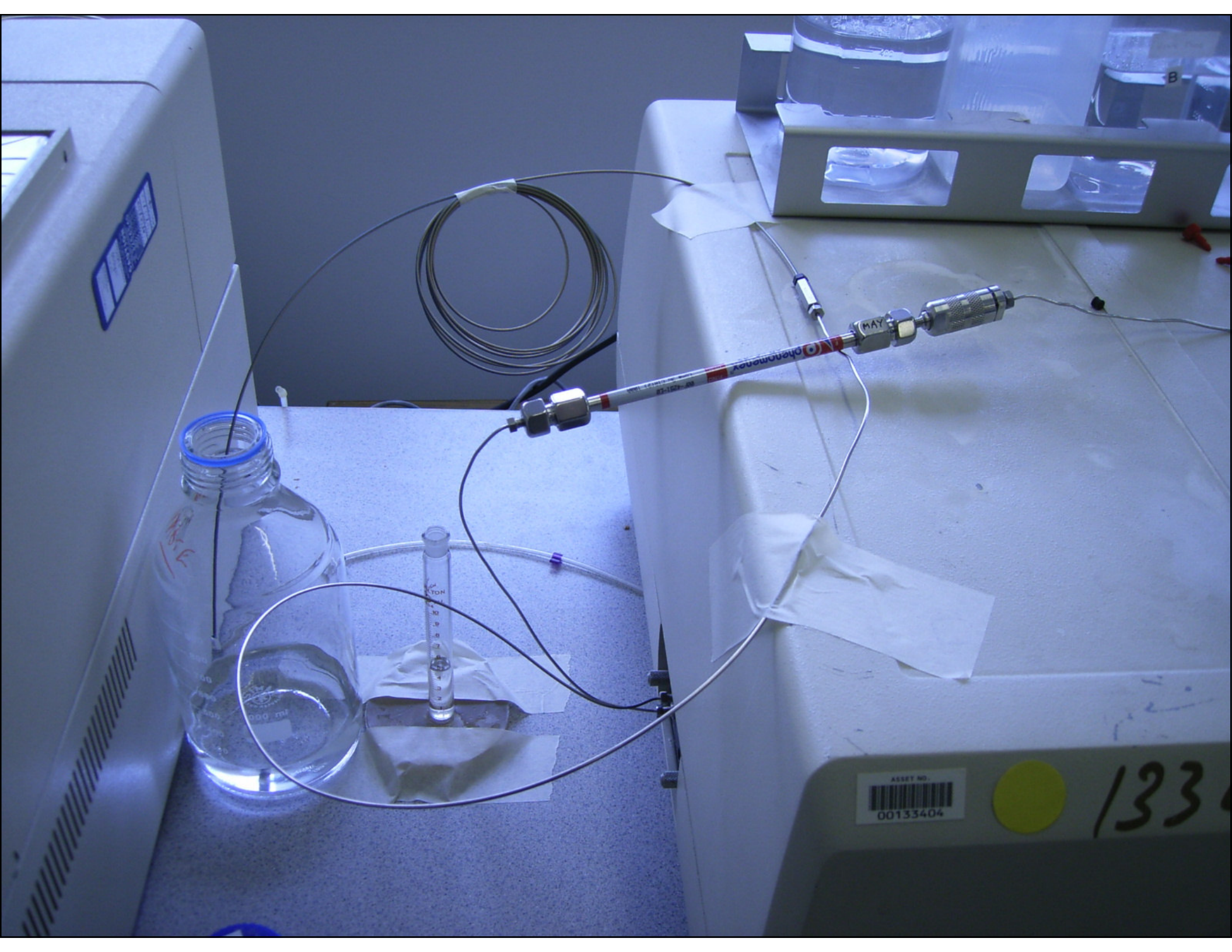
**POIDS NET
PESO NETTO
NETTOGEWICHT
60 mL**











ASSET NO.
00133404



ASSET NO.
00133404



133

THE METHOD – IN SUMMARY

- Excellent for sauces and powders
- Lengthy – long cleanup period
- Low sensitivity for plasma
(D.L. 20 ng/ml)