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NOTEBOOK NO. 51
ISSUED TO Yaxan Didantha-Amrang
ON March 8 **20** 07
DEPARTMENT Chemical Constituents
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Calculation 1 drop of furan (organic cpd) ≈ 0.8 mg ~ so u need around 5-6 drop

W_1 (volumetric flask) = 40.0146 g

W_2 (furan) = 0.0499 g

W_3 (furan + hexane) = 33.3077 g

$0.0499\ g/50\ ml = 0.998\ mg/ml$

100.0 µg → 1000 µl

100 µl → 1 mg

100 µl → 1/1000 × 100

1/10 0.1 mg/ml

1500 µl → 1 mg

100 µl → 0.1 mg = 100 µg/ml → diluted 1/10

10 µg/ml

Project No. ____________

TITLE ____________ Book No. ____________

From Page No. ____

Objective :- To prepare Furan stock solution with a concentration of 1 mg/ml in hexane
4.0 - Assessment of chromatographic peak purity of furan standard solution
~~Procedures~~. Using ~~HP~~-WAX capillary column (30m × 0.25 × μ)
ZB
4.0 4.0

Materials

1. Volumetric flask
2. Balance
3. micropipette
4. graduated cylinder
5. vials
6. GC-MS
7. 1 ml Pipette

Reagents

1. Furan
2. Hexane

Methods

I. Preparing 1mg/ml furan standard solution in hexane

1. Calculating the amount of furan needed in 50ml hexane solution. (1mg/ml → 50mg/50ml)
2. Bring out clean volumetric flask and Weight it on the balance. Tare to 0.000
3. In a fume hood, Use clean micropipette and drop certain amount of furan (~6 drops) and close the flask. Weight on the tarred balance closest to 0.050 grams.
4. Weight 50ml of hexane using graduated cylinder. Pour around half of the hexane into the volumetric flask to thoroughly mix hexane and furan.
5. Pour almost all of the hexane into the flask and use the micropipette to add to the final volume of 50ml.
6. Keep it in a labeled amber glass bottle.

II Assessment of chromatographic peak purity by using ZB-Wax column (30m×0.25m ×1μm)

1. Prepare furan working solution the concentration of ~~100~~ 10 μg/ml
   a. 100 μl of 1mg/ml furan std. solution diluted in labeled vial that contain 900~~ml~~ μl hexane.
   b. Mix by using micropipette. Capped the vial. Load it on the autosampler
2. Set up GC methods. Inlet 200°C. Transfer line 240 °C. (temperature in the transfer line should be a little higher than the oven temperature of GC to prevent condensate of gas before going in the MS-detector.) The oven temperature is set at 40°C for 2.5 mins and increase to 230 °C at 20°C a min and held for 3 mins. Injection mode; Splitless. Split value on every 1 min. Use Helium as mobile phase at the rate of 35cm/sec. (Put in the pressure value of 45 Pa. calculated by the program).
   Mass Spectrometer scan mode range from 20-100. MS detector is on at 5 min and reduced to 2.5 mins (excess time since flow rate is at 35 cm/sec and pass through 30m column will take around 1.30min).

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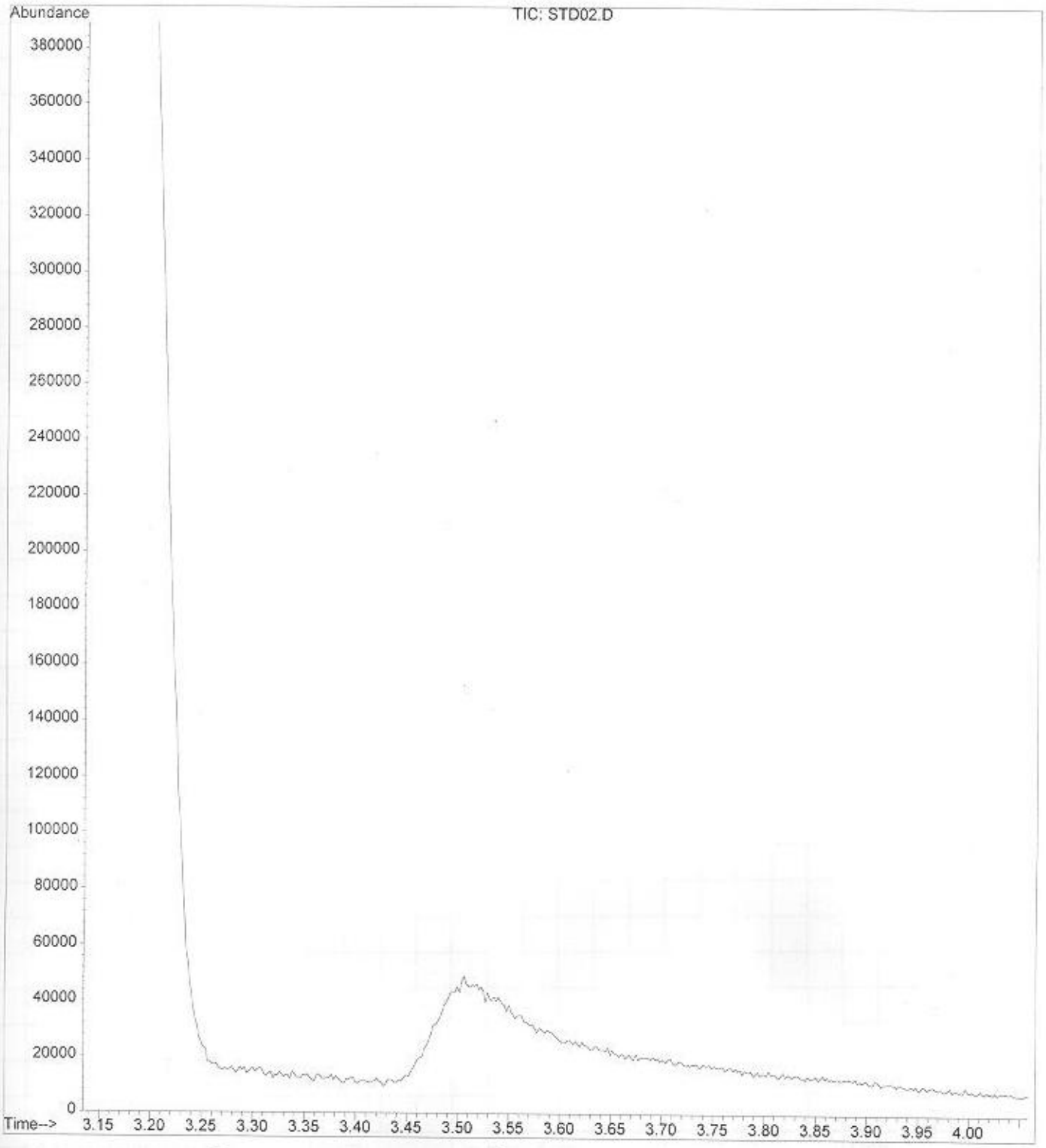
Result

```
File        : C:\HPCHEM\1\DATA\STD02.D
Operator    : yanan
Acquired    :  8 Mar 2007  12:29     using AcqMethod FURAN
Instrument :   GC/MS Ins
Sample Name: furan_solution_in_hexane
Misc Info   : 100ug/ml
Vial Number: 1
```



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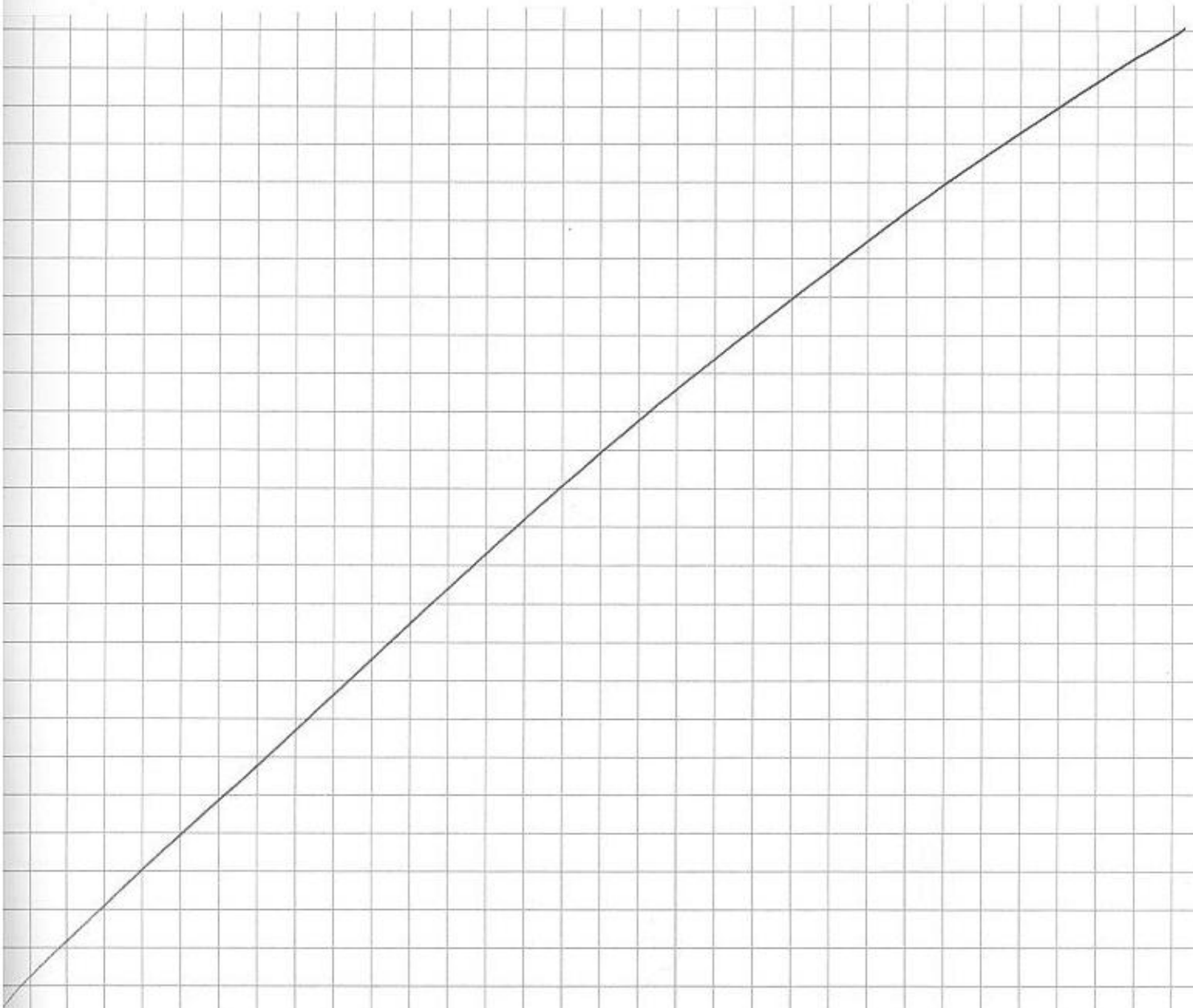
Discussion

- The column, ZB-WAX column (30m×0.25×1μm), is not suitable for furan quantization and detection because furan is a very volatile compound (bp. =31°C) and the compound does not retain in the column. Instead, it quickly volatize and flow out with the solvent as shown in the chromatogram. The furan comes out at 3.5 min which is too quickly to use it to separate furan from food. After assessing the chromatogram, column used should be changed into the one that has the ability to retain very volatile compounds.



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Project No. _____

TLE Assessment of chromatographic peak using CP-Porabond Q column Book No. _____

m Page No. ____

Objective: To determine the suitability of using CP-Para Bond Q column. to quantitize furan.

Procedures

1. Assessment of chromatographic peak by using CP-Porabond Q column(25m × 0.32mm)
   1.1 Set up GC methods. Inlet 200°C. Transfer line 240 °C. (temperature in the transfer line should be a little higher than the oven temperature of GC to prevent condensate of gas before going in the MS-detector.) The oven temperature is set at 40°C for 2.5 mins and increase to 230 °C at 20°C a min and held for 3 mins. Injection mode; Splitless. Split value on every 1 min. Use Helium as mobile phase at the rate of 35cm/sec. (Put in the pressure value of 45 Pa. calculated by the program).
   Mass Spectrometer scan mode range from 20-100. MS detector is on at 5 min and reduced to 2.5 mins (excess time since flow rate is at 35 cm/sec and pass through 30m column will take around 1.30min).

2. Set up GC for SPME analysis
   2.1. Change the injection type from auto sampler to manual.
   2.2 GC methods for SPME analysis is 50°C and increase to150°C and hold for 4 minutes at the rate of 20°C/mins. Run time is 10 mins
   2.2. Turn off the inlet purge valve, splitless mode
   2.3 Change the nut of the injection
   2.4 Prepare furan working solution (100µl furan/ 20ml water) in a vial. Put it in a water bath that has constant temperature of 48°C. Expose SPME fiber to the furan working solution for 15 mins. Run three times to check the retention time.

3. Set up GC methods for SPME conditioning
   3.1. Set the injection type to manual. For first use of SPME fiber, the SPME fiber go through conditioning for 2 hours as suggested by the producer. After that, the SPME fiber needs to go through conditioning for 30 mins before each experiment to clean up the residue left over in the fiber. GC condition is set to have initial temperature at 50 °C and increase to 300°C at 25°C/min.

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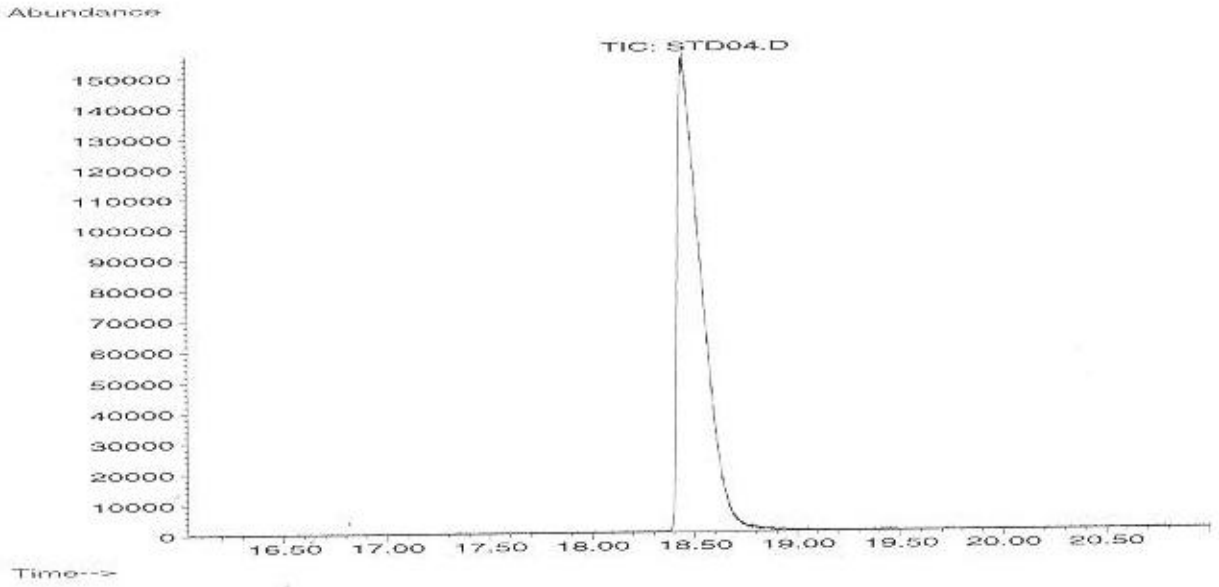

Results and Discussion Y.D.

1. ~~(Chromatogram of standard furan)~~

- After changing the column into Porabond Q porous polymer, sharp and high signal can be obtained with a retention time of 18.45. This shows that volatile furan can retain in the Porabond Q column which makes it suitable to use it in to analysis of furan in food.

- The mass spectrometer gives high respond to standard furan at *m/z* of 68, 39 and 37. As a result, these numbers are being used to detect furan in food

2. SPME analysis

Trials	Rt (mins)
SPME01	6.811
SPME02	6.821
SPME03	6.844

After running SPME analysis of furan three times, the results show stabilize retention time.

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Project No. _____

TLE SPME OPTIMIZATION. Book No. _____

om Page No. ____

04/03/2007

Objective: Optimization of SPME absorption time

Procedures

1. Set up GC methods for SPME analysis.
2. SPME optimization

1.1. Set up a waterbath system which can maintain constant temperature between 45 – 50 °C.

1.2. 0.0011 mg/ml (1.1µg/ml) of furan is prepared from standard (2.2mg of furan in water) by

a.) Dilute to 10^{-1} by pipette 10 ml of water and use micropipette to add 1000µl of 2.2mg/ml furan std. into water

b). Take 100µl of the dilution (0.22mg/ml) into a vial containing 20 ml water.

1.3. Drop a stir bar and put the vial in the waterbath. Let it sits for 10 minutes to let the solution reach the target temperature.

1.4. Spike SPME on top of the vial and vary the absorption of 15, 30, 45, 60 minutes (triplicate trial)

1.5. Manually inject the SPME and determine the amount of furan by using GC (cond^{n} in P.5)

Results and Discussion

After incubate SPME fiber for 10mins and the extraction time of 15 mins the result is as shown below

Trials	Peak area
1	5339740
2	5176759
3	4688291
4	4994457
Mean	5049812
Stdev.	279246.2

The result shows a deviation of 5.5% of the data from the mean. For better repeatability, the same method will be repeated.

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TITLE _____ Book No. _____

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Objective: SPME analysis of furan (Cont.)

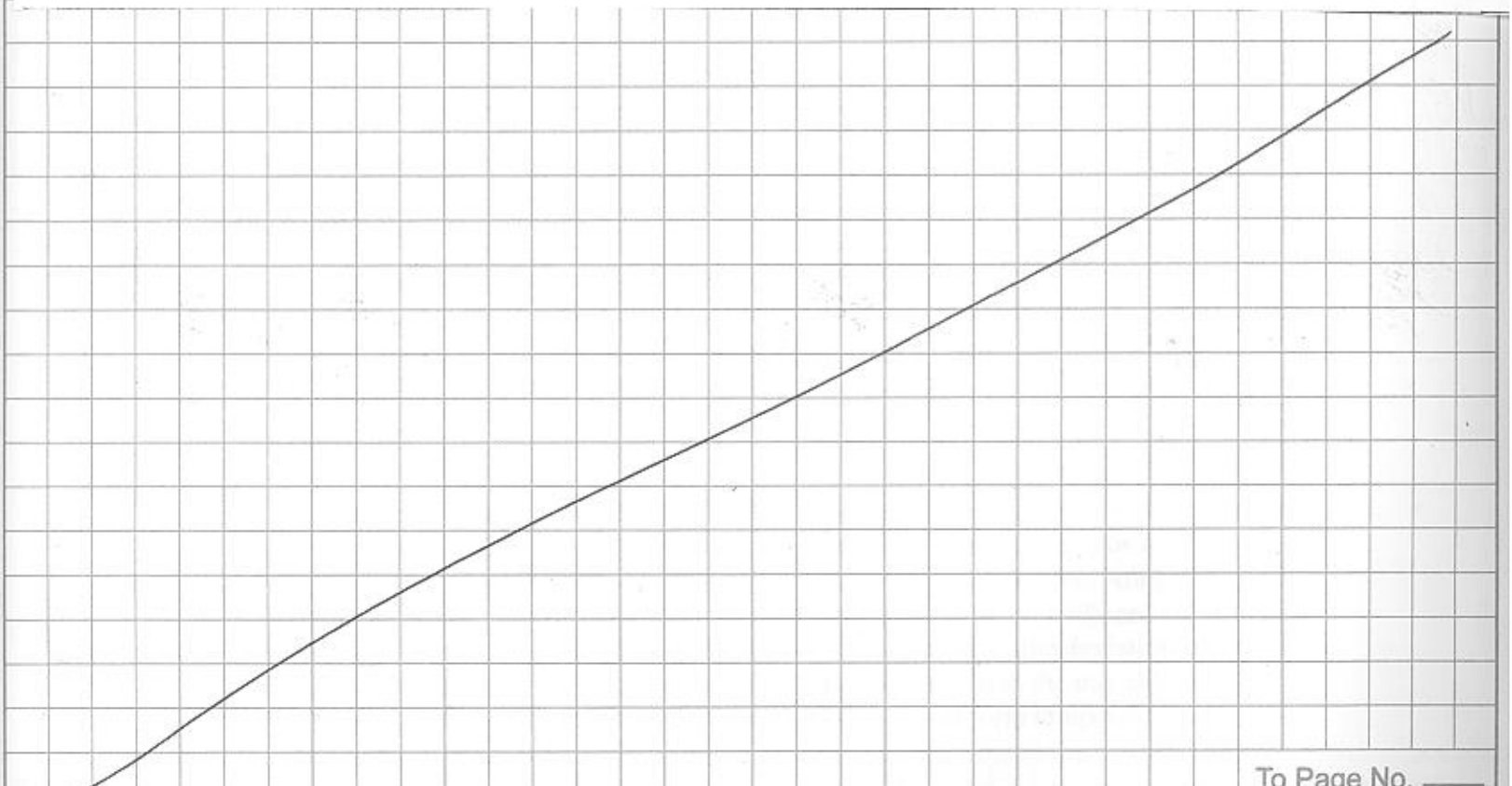
Procedures

Repeating the procedures in page 7 but let the vial sit in the water bath for 10 mins before SPME extraction

Result and Discussion

Trials	Peak Area
1	15625887
2	13889830
3	13444288
4	11588820
Mean	13637206.25
Std.	1658491

The result shows a deviation of 12% of the data from the mean. For better repeatability, the same method will be repeated. Also, 1.1µg/ml of furan gives the chromatogram with wide base, the next SPME analysis of furan will be using smaller amount of furan to give a sharper peak.



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TITLE SPME analysis - Book No. _____

From Page No. ____

Objective : To ~~per~~ TD develop a method in SPME analysis, in order to reduce errors.

i4/11/07 TD

~~Objective: SPME analysis of 0.22µg/ml~~

Procedures

1. Prepare 0.22µg/ml furan working solution by micropipetting 200 µl of 2.2 mg/ml standard furan and dispense into 20ml water. Then do second dilution by adding 1ml of furan into 100ml of water. And distribute to 3 vials containing stir bar (300rpm) by using volumetric pipette to cut out errors from micopipetting

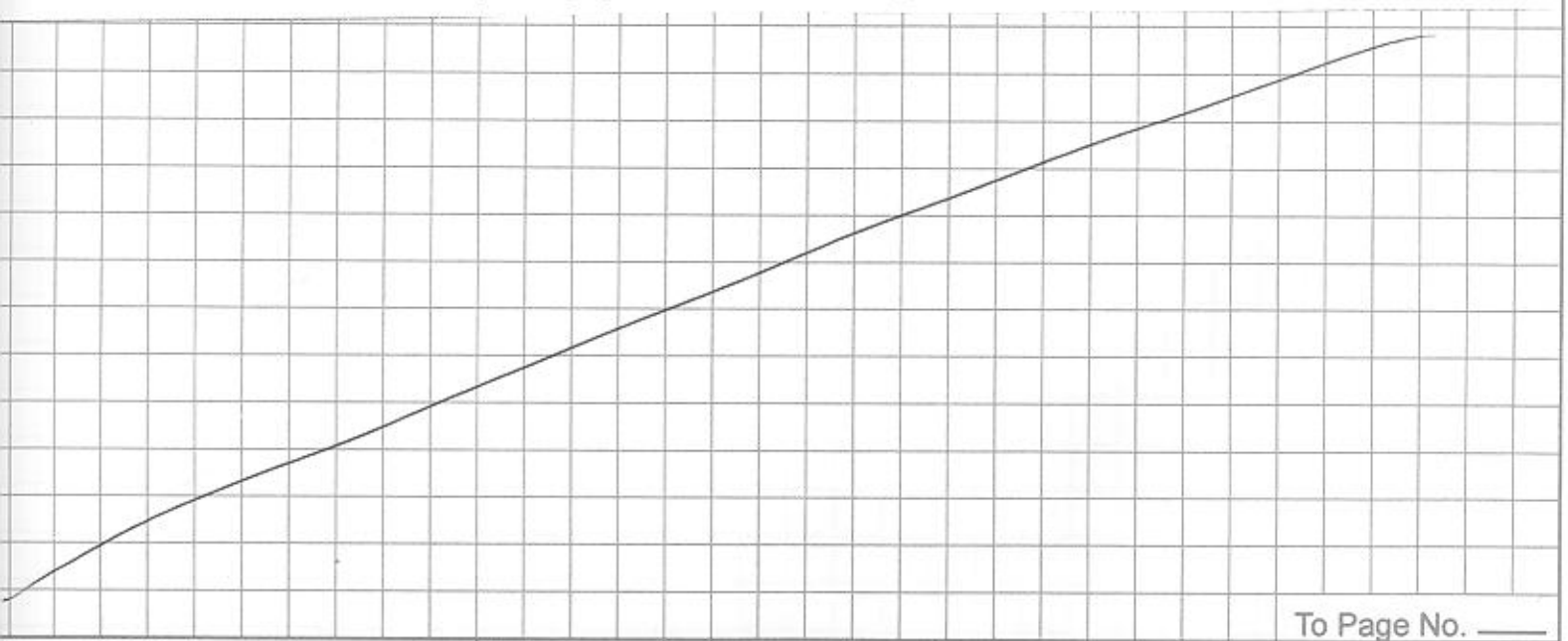
   Std→ 200µl/20ml → 1ml/100ml

2. Set up a waterbath to a constant 0f 48°C
3. SPME extraction for 15 mins
4. GC-MS analysis (Cond^{n} in P.5)

Results and Discussion

Trials	Peak Area
1	1186985
2	1257784
3	1167668
Mean	1204146
Stdev	47445.6

- Without using micropipette, the result is more precise.



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TITLE Detection of d4 furan using GC MS. Project No. _____ Book No. _____

From Page No. ____

4/23/07

Objective: To develop furan quantitation method with d4-furan as internal standard

Procedure

1. Condition SPME fiber
2. Prepare d4 furan standard solution by weigh the water and water+furan and then calculate the exact concentration of d4 furan

   Water weight (3 times): 19.8980 g

   Water + d4 furan : 19.9198 g

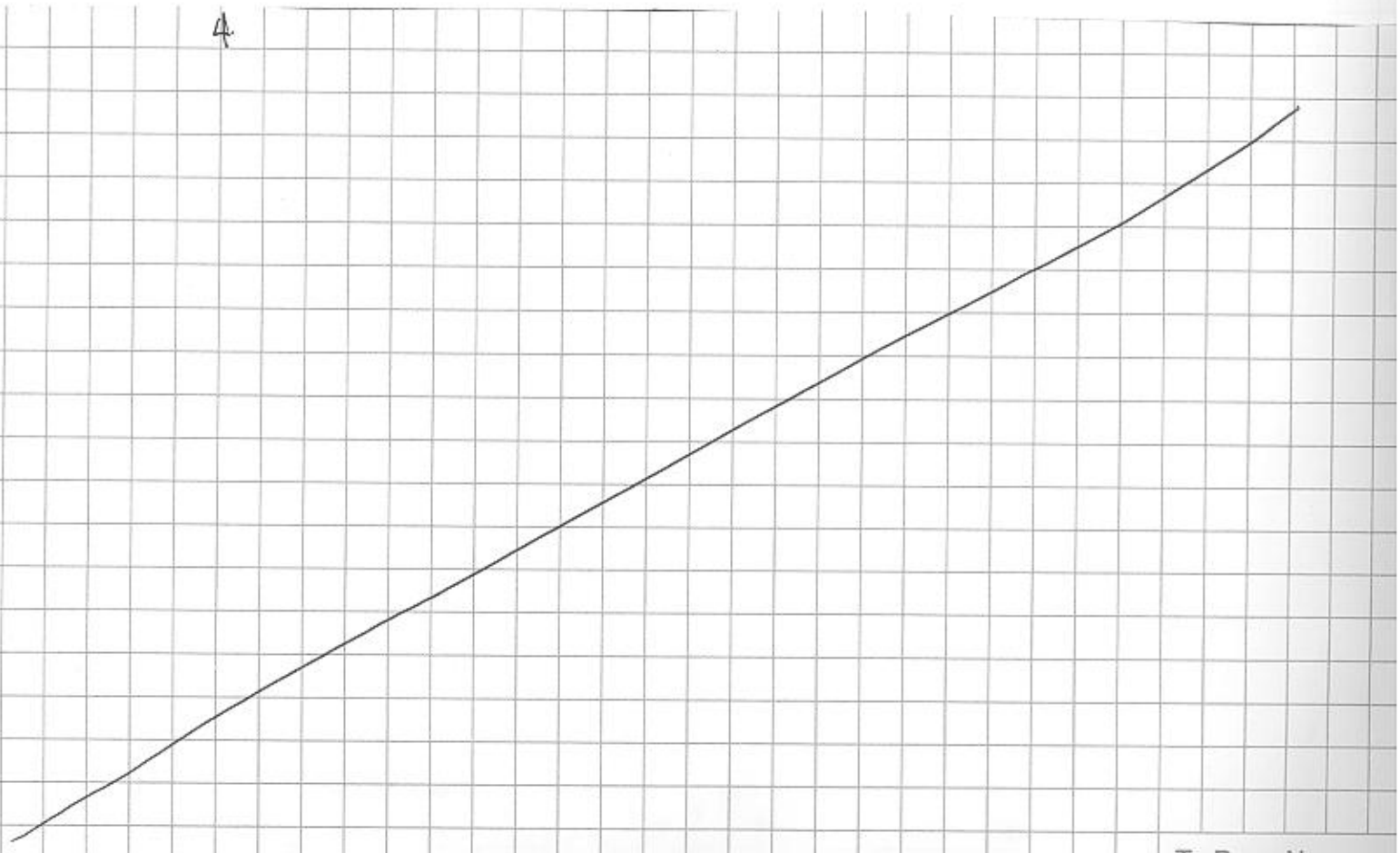
   d_4-Furan : 0.021 g

Thus; the amount of ~~furan~~ d4-furan is 0.021g/19.9198ml = 1.1 mg/ml

Then, Prepare Working sol'n by taking std. furan 200 µl /20 ml DIwater. The working d4-furan sol'n concentration is 11µg/m

3. Analysis of d4 furan using GC-MS with a GC condition of Inlet is 275°C. GC oven is 50°C and increase to 150°C, hold for 2 minutes at the rate of 20°C/min and increase the temperature to 295°C; hold for 6.2 mins at the rate of 25°C/mins. The run time is changed to 20mins to make sure that d4 furan is washed out of the column because d4 furan residues will affect the quantification of furan in further experiment.

4.



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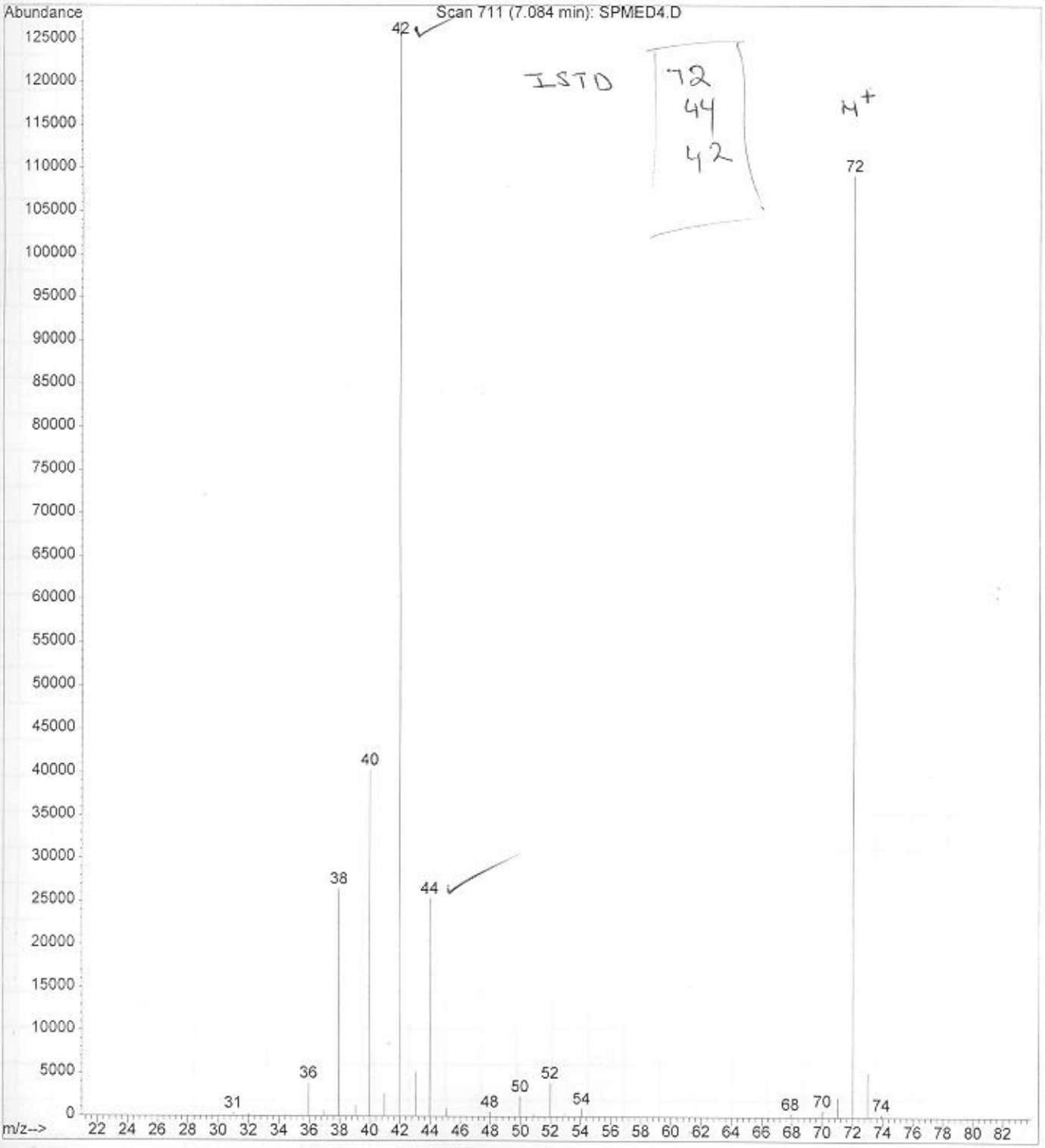
File : C:\HPCHEM\1\DATA\FURAN\SPMED4.D
Operator : Yanan
Acquired : 23 Apr 2007 17:07 using AcqMethod SPME
Instrument : GC/MS Ins
Sample Name: furan0.22ug/ml
Misc Info : 15mins
Vial Number: 1



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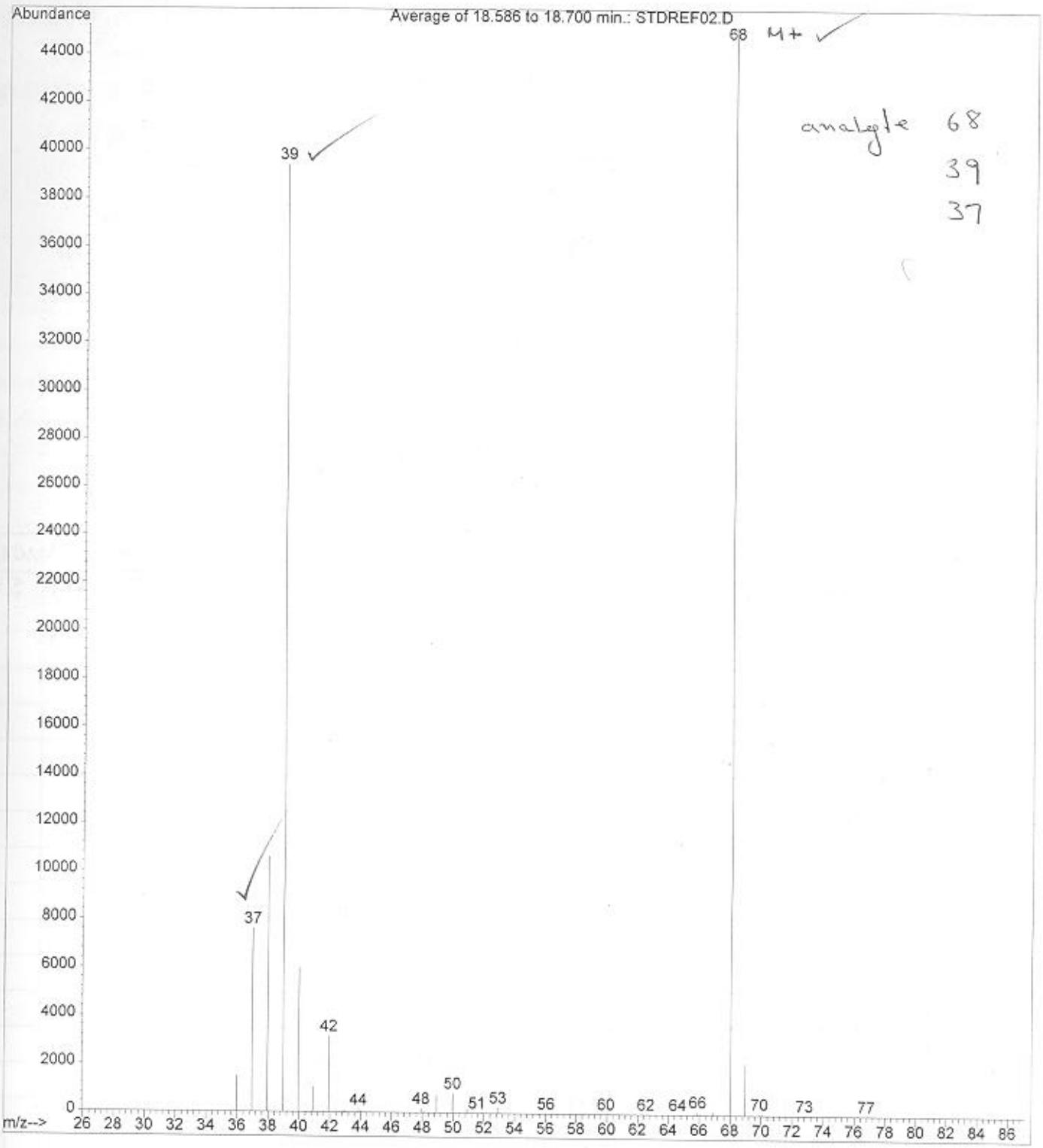

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Project No. _____
Book No. _____

TLE Detection of furan in apple juice using SPME GC-MS.

om Page No. ____

Objective: To see the ability ~~of detecting~~ to detect ppb furan in food sample apple juice using SPME extraction and GC-MS method.

Procedure

1) Volumetric pipette 20 ml of apple juice into a vial that contain a stir bar.
2) Incubate vial in 45°C waterbath. for 10 mins. (agitate 400 rpm)
3) SPME extraction for 15 mins.
4) GC-MS analysis as stated in ~~P. 11~~. P.5 (SPME.M)

Result.

Area : 185,754

Discussion.

SPME extraction shows ability to absorb furan from vial headspace containing ~~low~~ ppb Apple juice. ~~GC~~ SPME GC MS shows good detectability in food.

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Project No. _____

SPME optimization of furan in apple juice using d4-furan as internal standard. Book No. _____

4/24/07

Objective: To optimize SPME extraction time of analyzing furan in apple juice

Procedure

1. Make 10^{-2} dilution of d4furan from working solution (11ug/ml) by suspend 200ul working solution into 20ml DIwater and then transfer 1ml of the first dilution into 10ml DI water
2. Take 100ul of (1) into 20ml of apple juice
3. Incubate in a 48°C waterbath for 10 mins, agitate at 700rpm
4. SPME extraction for 15 mins to see the ratio of d4 furan and detected furan in the sample (ITSD should have peak area 3-5 times of the furan peak area)
5 GC-MS analysis P. 5 SPME. M.
6 Do triplicate trials of 15 mins extraction using the same method

Result and Discussion

Triplicate experiment of 15 mins extraction of SPME fiber

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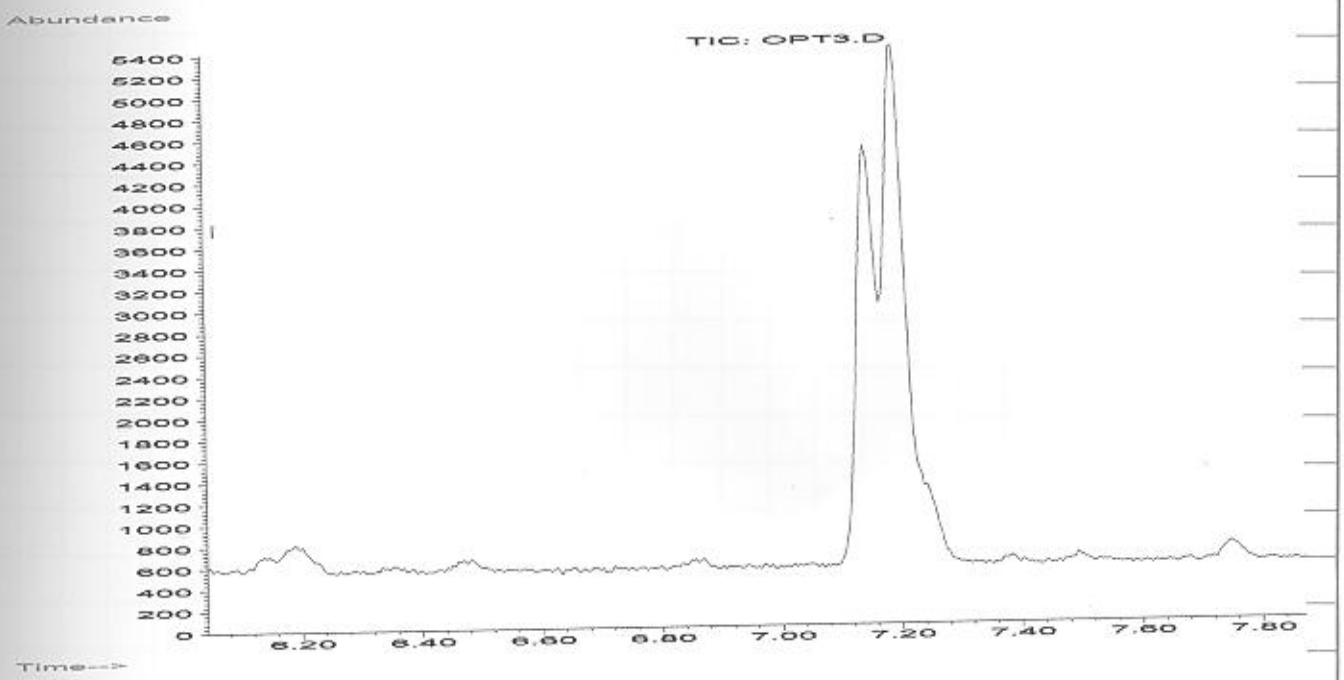

m/z	Area	Rt
68	5562	7.193
72	4510	7.147

Discussion

- There is a reduction of internal standard d4 furan, when compared to furan, from the beginning of the experiment which could have cause from the volatility property of the internal standard which volatile at room temperature while doing suspension. The internal standard, when use, need to be kept cool in ice-water bath or in the fridge when use in any experiment.

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E SPME OPTIMIZATION Book No. _____

Page No. ____

4/30/07, 5/2/07

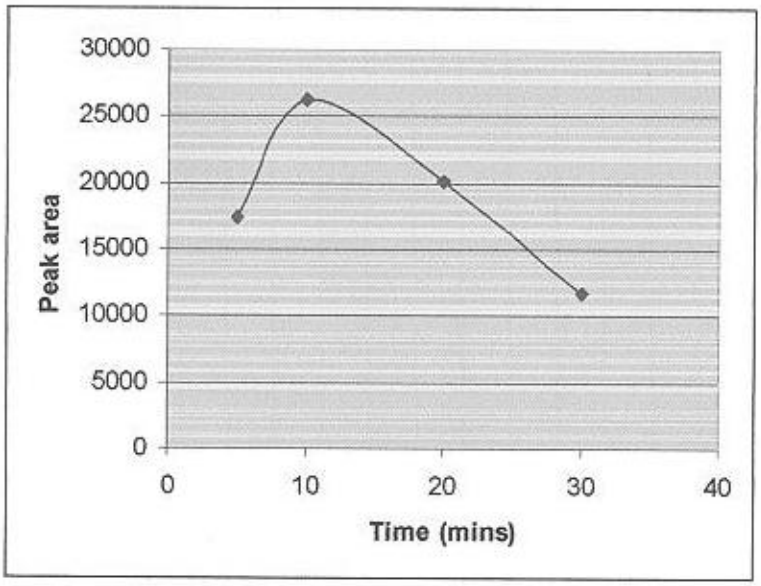
Objective: SPME optimization by using apple juice as food sample

Procedures

1. Let apple juice comes to room temperature
2. Volumetric pipette 20 ml of apple juice into a vial
3. Incubate in 43° waterbath for 10 mins
4. SPME extraction for 5 mins P.5 SPME .M
5. GC-MS analysis follow the condtion in ~~P.11~~
6. Do triplicate trials and then change the SPME extraction time to 10, ~~15~~ 20 ٢٠ and 30 mins respectively.

Result and Discussion

Time	Peak area	Mean	Std
5	16980		
	17398	17399	614.6828
	17819		
10	26523	26179.33	614.6828
	26699		
	25316		
20	17087		
	21368	20064.67	2110.969
	21739		
30	14327		
	11206	11671.67	2005.183
	9482		



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TLE _____ Project No. _____
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6/13/07

Objective: To measure the amount of furan in unknown apple juice using standard addition method

Materials
1. d4 furan working solution 11 ug/ml
2. spiked furan working solution 19.158 ug/ml
3. unknown apple juice kept refrigerated in a laboratory

Procedures
1. Prepare 4 vial and label Xo, X1, X2 and X3
2. Tear vial
3. Volumetric pipette 20ml of DI water into vials and weigh on the balance
4. Micropipette 500 ul of apple juice into vials and weigh amount of water+furan
5. Chill in ice-water bath for 5 minutes
6. Add the internal standard and spiked furan as follow
   Xo : 500ul apple/ 20ml water +200ul ITSD
   X1 :500ul apple/ 20ml water + 200ul ITSD + 25 ul of spiked furan
   X2: 500ul apple/20ml water + 200ul ITSD + 50ul spiked furan
   X3: 500ul apple/20ml water + 200ul ITSD + 100ul spiked furan
   * every procedures are done while sample is rest in ice-water bath
   *while one vial is running the others are kept cold in fridge
7. Incubate in water bath (30-35 °C) for 10 minutes
8. SPME 10 mins
9. GC analysis; the GC condition is

Result and Discussion

Spiked furan	Trials	Sample (g)	Water +sample	*m/z*(68)	*m/z*(72)	ratio	mean
0	1	0.5166	20.0798	386570	236012	1.63793	
	2	0.5109	20.0167	464377	233262	1.9908	1.93949
	3	0.5179	19.9966	485304	221625	2.18975	
25ul(0.47895ug)	1	0.531	20.0549	466410	223577	2.08613	
	2	0.5103	20.0324	537149	227439	2.36173	2.27866
	3	0.5154	20.0243	541029	226549	2.38813	
50ul(0.9579ug)	1	0.5164	20.087	570249	223590	2.55042	
	2	0.509	20.028	602867	223788	2.69392	2.58641
	3	0.5164	20.0955	407996	162233	2.51488	
100ul(1.9158ug)	1	0.5112	20.0473	680637	195984	3.47292	
	2	0.5231	20.0772	709501	190398	3.72641	3.64486
	3	0.5187	20.008	682213	182642	3.73525	

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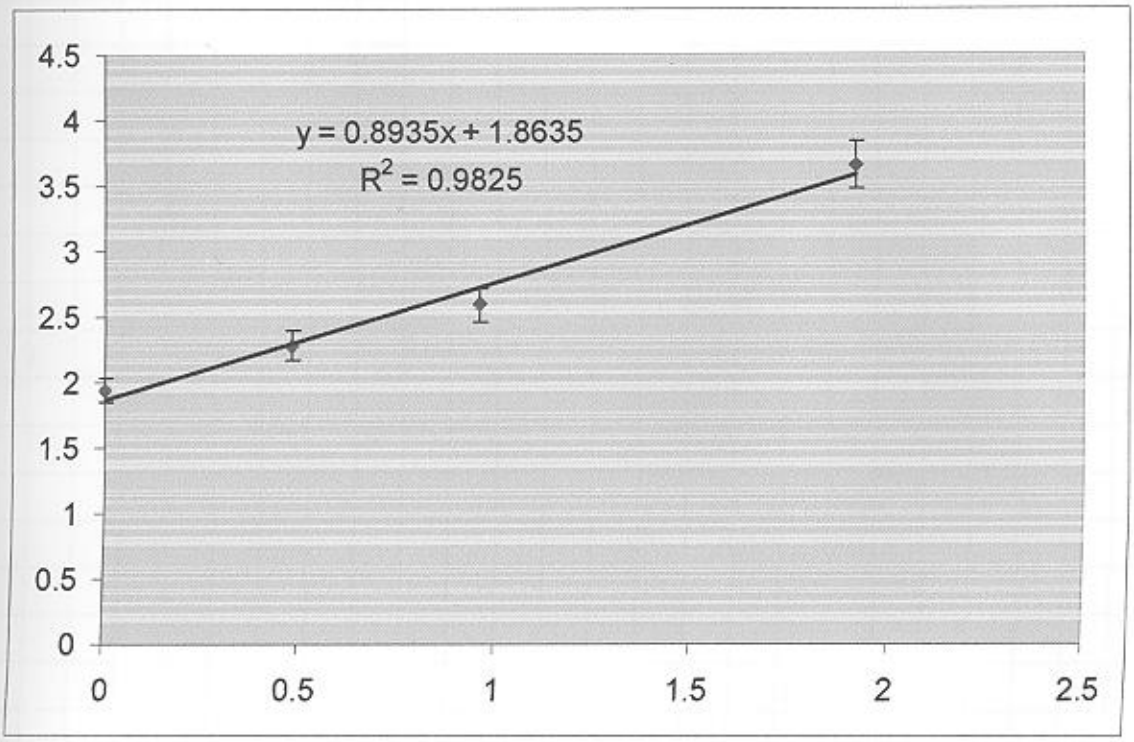

Discussion

The amount of furan in 20.5ml sample = 2.08 ug .The sample is diluted by the factor of 41. The amount of furan per ml sample = 4.16 ug/ml.

However, the correlation is less than 99 % (98.25%). It would be more reliable if the result has better correlation.

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Furan stock std. sol^{n}

W_1 (water) 19.9119 g

W_2 (water + furan) 19.9527 g.

· Conc^{n} = $\frac{W_2 - W_1}{20}$ $^{g}/_{ml}$ $\frac{19.9527 - 19.9119}{20\ ml} = \frac{0.0408\ g}{20\ ml} = 0.00204\ g/ml = 2.04\ mg/ml$

Develop sample preparation protocol and prepare standard solution ~~procedure~~. Y.D.

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6/18/07

Objective: 1. Write a standard procedure of furan standard addition methods for food samples.
2. Write a standard procedure of furan standard stock preparation and working solution preparation.
3. Prepare furan stock standard solution and furan stock solution

Protocol in furan analysis in Food sample

1. Sample preparation :
   a. The canned food is prepared according to the instruction labeled on the container
   b. 100g of prepared food is put in a 225ml bottle and cooled down in a fridge for 15 minutes to Y.D. ~~4~~ 5 °C
   c. Homogenize the sample. Keeps the sample cool by putting the bottle in an ice-water bath
2. Furan quantization
   a. Volumetric pipette 10 ml of DI water into 4 vials. Label X_0, X_1, X_2, X_3, respectively
   b. Weigh 10g of homogenized sample and put into each vial
   c. Equilibrate the sample in ice water bath for 5 minutes
   d. Add 50ul of d4-furan internal standard while the vial is kept chill in ice-water bath
   e. Spiked 0, 25, 50 and 100 ul of _____ ug/ul of furan working solution into X_0, X_1, X_2, X_3, respectively
   f. Incubate X_0 vial in a water bath(35°C, 700rpm) for 10 minutes while the rest of the vials are kept chilled in a fridge.
   g. SPME extraction for 10 minutes.
   h. GC-MS analysis

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Protocol for preparing Furan stock standard and standard solution

All dispensing operation involving furan must be done in fume hood

Furan stock standard solution

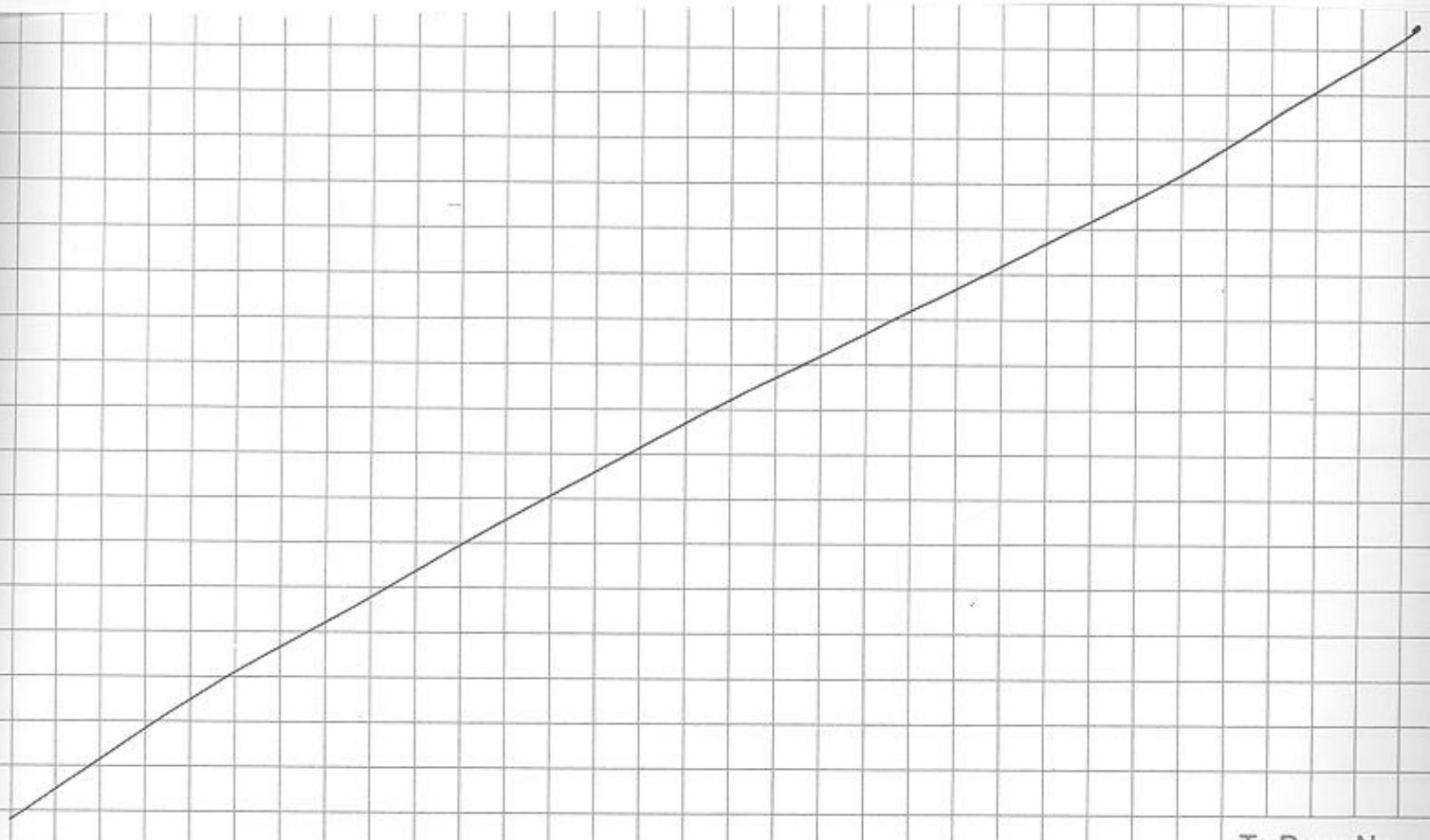
1. Volumetric pipette 20ml of DI water and weigh the water weight---- (1)
2. Let furan comes to room temperature
3. Pasteur Pipette 2 to 3 drops pf furan into the vials.

Weigh the furan solution.- - (2)

4. The concentration of furan standard stock solution is [(2) – (1)]/ 20ml

Furan working standard solution

1. Take 100 ul of Furan stock solution and dispense into 20ml water
2. The concentration of working solution is $\frac{0.1ml \times [stock]}{20.1ml}$



Witnessed & Understood by me, cjanan Date 6/18/07

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TITLE Method development and furan determination in pea food sample. YP Project No. _____

From Page No. ____ using SPME GC MS. Book No. _____

Column length 30.00 m diameter 0.250 mm

6/25/07

Objective: 1. To develop a method in detecting furan in food
2. To detect furan in can pea

Procedures

1. Put a can of Pea in an ice bath for 30 minutes to 1 hour
2. Separate the juice into another bottle and leave the solid pea in a can. Cover the can by aluminum foil and tighten it with rubber band
3. For solid pea
   a. Weigh 50g of can pea into a bottle containing 50ml of DI water
   b. Homogenize while the food sample is kept cool in water bath
   c. Take 20 ml of homogenized sample into 4 vials which contain a magnetic stirrer.
   d. Cool in an icebath for 5 minutes
   e. Take one vial and put it in a 35°C waterbath for 10 mintutes.
   f. SPEM extraction for 10 minutes
   g. GC-MS analysis (Data file: PEA_S.D)

Int. temp 55°C → 115°C (5°C/min) hold 2 min
115°C → 295°C (25°C/min) hold 2.8 min

3. For liquid pea
   a. Take 10ml of pea juice into 10ml of DI water into a vial containing magnetic stirrer
   b. Put in an icebath for 5 minutes
   c. Put a vial into a 35°C waterbath for 10 minutes
   d. SPME extraction for 10 minutes
   e. GC-MS analysis(Data file: PEA.D) : Oven

runtime 25 min
Inlet A 225°C
Det 300°C
Pres ure 47 kPa @ 40°C temp°
Helium flow 1ml/min (35.9 cm/sec)
Split 1 ml/min

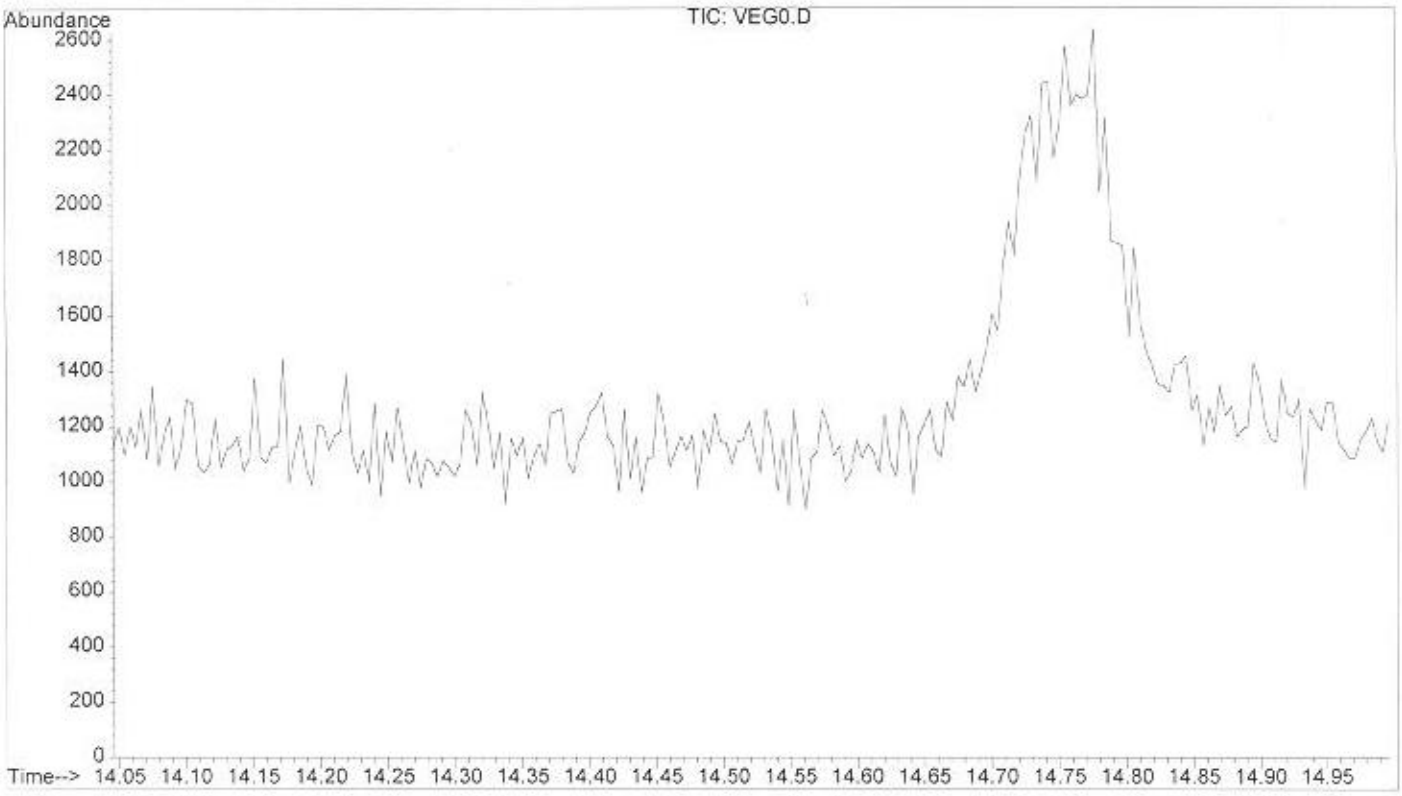
Results

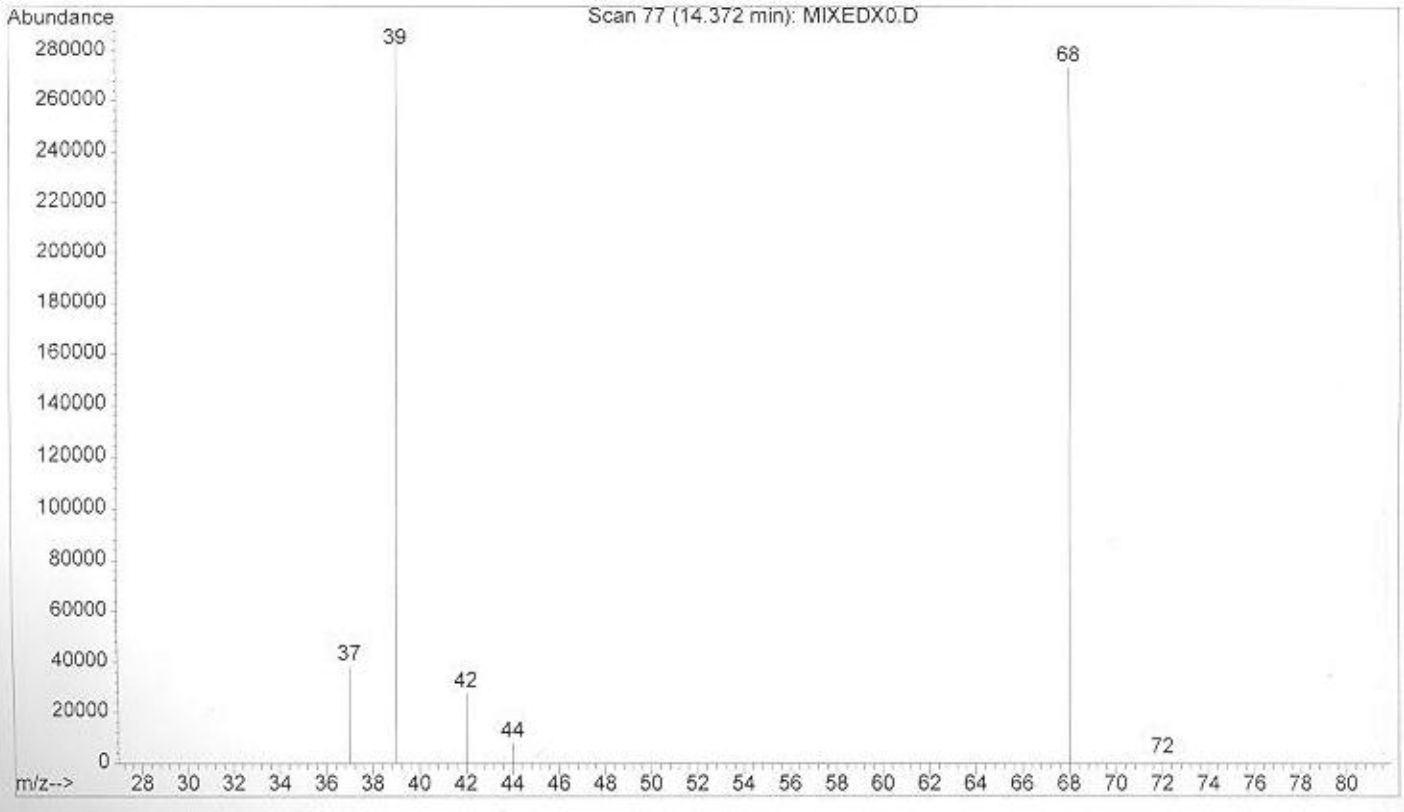
There is no peak detected in both solid part and liquid part of pea sample.

Witnessed & Understood by me, yaran Date 6/25/07 Invented by: Recorded by: Date

To Page No. ____

File : C:\HPCHEM\1\DATA\2007_F~1\FURAN\VEG0.D
Operator : yanan
Acquired : 27 Jun 2007 13:01 using AcqMethod FURAN
Instrument : GC/MS Ins
Sample Name: mix_veg_solid
Misc Info :
Vial Number: 1





TITLE Method development and furan determination in food sample using SPME-GC-MS. Project No. _____ Book No. _____

From Page No. ____

6_26_07, 6_27_07

Objective: To measure furan in mixed vegetable

Procedures

1. Cool the can in the fridge overnight
2. Condition SPME fiber at 295 for 30 mins
3. Chill the bottle in icebath
4. Separate the liquid part of can mixed vegetable into another bottle and keep in a fridge

- For mixed vegetable solid

1. Take 50g of mixed vegetable solid into a bottle containing 50 ml of HPLC grade water.
2. Homogenize until completely mixed together
3. take 20g of sample into a vial containing magnetic stirrer
4. Chill the vial in an icebath for 5 minutes
5. Determine the amount of ISTD and spiked furan need to be added
6. Incubate in 35°C waterbath fo 10 minutes
7. SPME extraction for 10mins
8. GC-MS analysis

- For mixed vegetable juice

1. Pipette 20 ml of mixed vegetable juice into a vial
2. Incubate in an icebath for 5 minutes
3. Determine ISTD and amount of spike furan need to be added
4. Incubate in the 35°C waterbath for 10 minutes
5. SPME extraction for 10 minutes
6. GC-MS analysis

Result and Discussion

Sample	Filename	m/z 68
Mixed vegetable solid	VEG0.D	No peak detected
Mixed vegetable juice		

To Page No. ____

Witnessed & Understood by me, Date 6/27/07 Invented by: Date Recorded by:

Project No. _____

TITLE Measure furan in food sample (baby food) Book No. _____

From Page No. ____

7/02/07

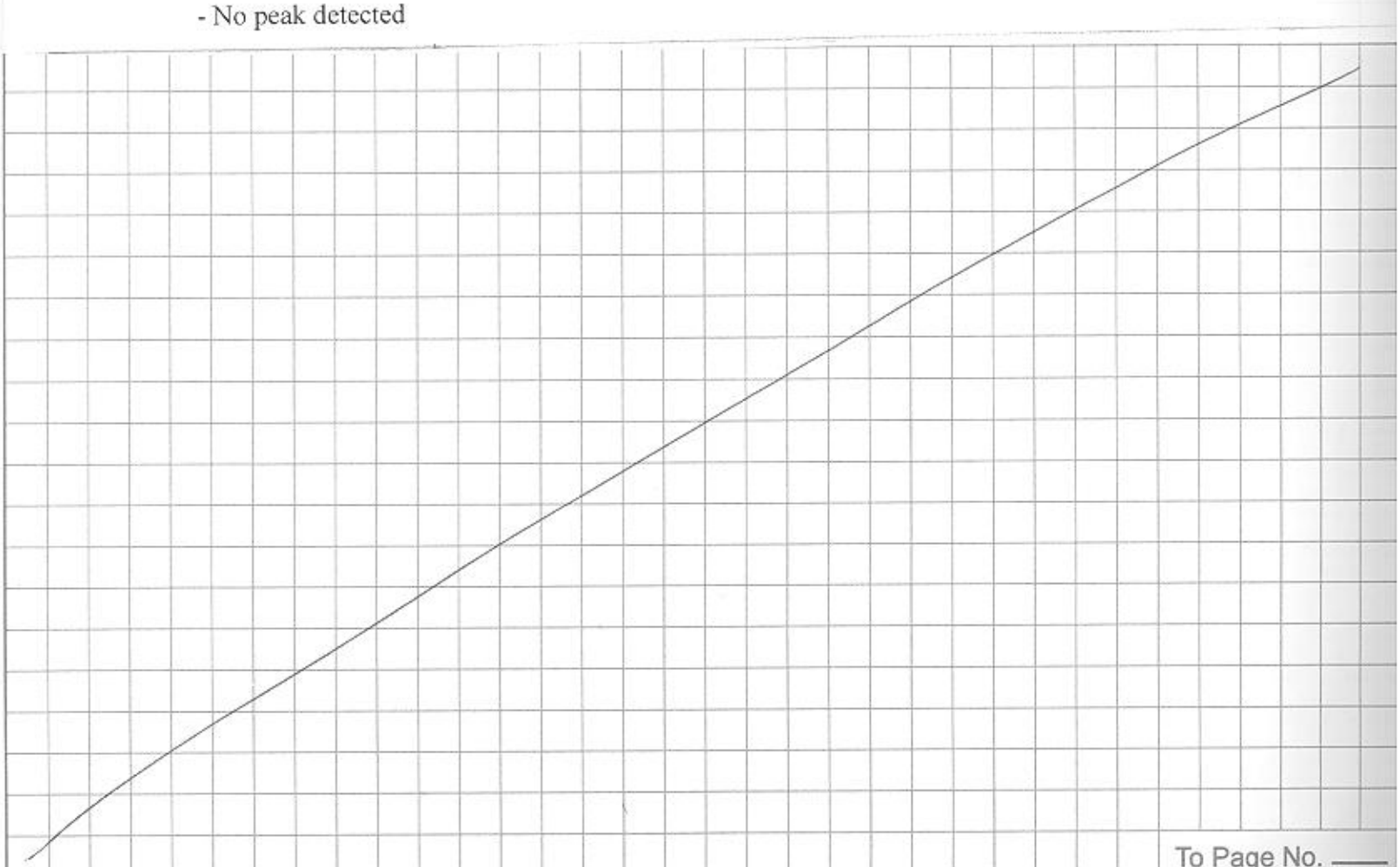
Objective: To use SPME GC-MS to measure furan in baby food sample

Procedures

1. Cool a jar of baby food overnight
2. Take 10g into 10 ml of cold HPLC grade water
3. Incubate in an ice bath for 5 min
4. Incubate in35°C waterbath for 10 mins
5. SPME extraction for 10 mins
6. Analysis amount of furan using GC-MS

Result

- No peak detected



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Witnessed & Understood by me,	Date	Invented by:	Date
Yuran Ding	7/02/07	Recorded by:	

Project No. ____________

TITLE Method development and furan determination in food Book No. ____________

From Page No. ____

Objective: To check the chromatographic peak

7/03/07 (check the peak in GC)

Procedure

* -Change the sampling of asolid food to 50g of food in 50 ml of HPLC grade water
- Take 20 g of dilute mash sample into 4 vials
- Incubate in an icebath for 5 minutes
- Incubate in 35°C waterbath for 10 mins
- SPME extraction for 10 mins
- GC-MS analysis

Result

- The peaks obtained have a very high baseline

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
[illegible signature]	7/3/07	Recorded by:	

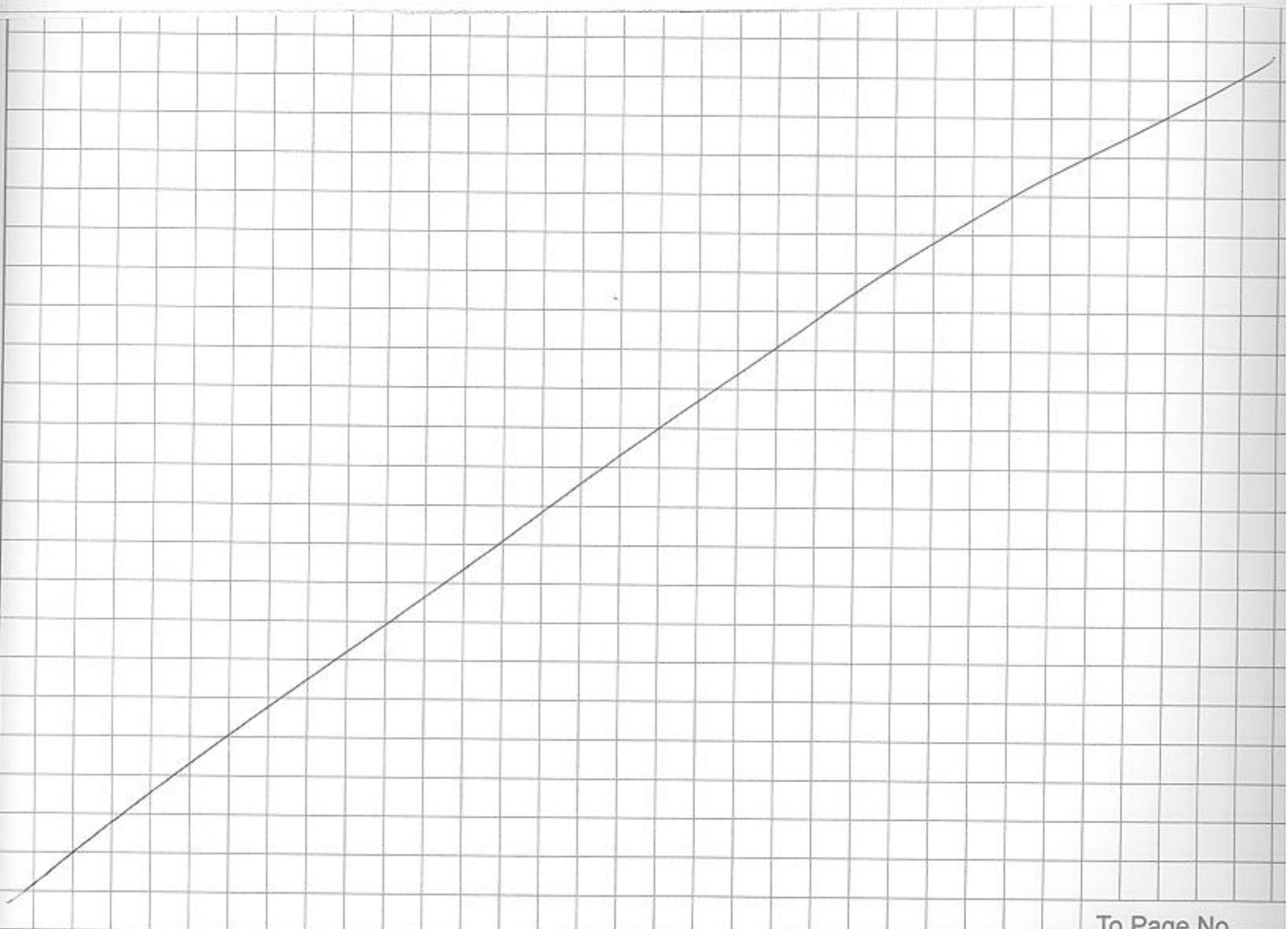
7/09/07 (check the peak in GC)
- Prepare mixed vegetable sample by separate the solid part of mixed vegetable and liquid part.

Solid mixed vegetable
- Take 50g of cold mix vegetable from a can into 50 ml of water.

Mixed vegetable liquor
- Take 10 ml of the juice into 10 ml of cold HPLC grade water

Result
- no peak detected

Discussion
- Since it is expected to find some furan in mixed vegetable, there should be some factors that cause problems in furan determination. After checking the fiber, it is found that the fiber is not intact. The fiber should be changed in the next experiment.



Witnessed & Understood by me,	Date	Invented by:	Date
yaman Dahmj	7/9/07	Recorded by:	

TITLE Method development and furan determination in Foods. Project No. _____ Book No. _____

From Page No. ____

Objective: To Find the best S:D ratio of solid and water that gives the best mixing

7/13/07

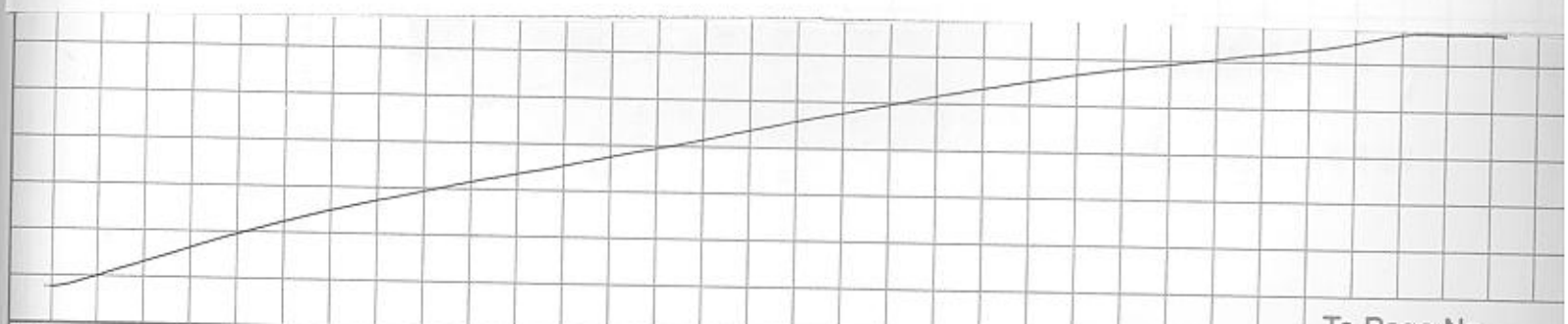
Objective: Test the dilution factor that gives the best mixing

Procedure

1. Prepare solid food sample by taking 50g into 100 ml of HPLC water and compare with 50g and 150 ml of HPLC water (dilution factor =3)

Result

- The one with 50g and 150 ml HPLC grade water gives much better mixing than the one that has 100 ml water. The magnetic stirrer gives good mixing into the bottom. Therefore, we are using 150 ml of HPLC grade water dilute 50g of sample before homogenizing.
- The procedure in sample preparation is optimized into-
    1. The container is chilled overnight
    2. The can is opened and the juice is separated into 225 ml plastic bottle. Keep refrigerated until needed to be analyzed for furan
    3. 50g of solid part of the food sample were taken from the can and place into 225 ml plastic bottle containing 150 ml of HPLC grade water
    4. Homogenize while kept the bottle cool in an ice bath
    5. 20 g sample test portion were taken from the bottle into 4 headspace glass vial for 4 levels of standard calibration curve plot- 0, 0.5 X°, 1X° and 2 X°
    6. Incubate in an ice bath for 5 minute
    7. Place internal standard which makes the area of the internal standard equal to 1X° and add spike furan in 3 headspace vials in increasing concentration of 0.5 X°, 1 X°, and 2 X°
    8. Incubate in a 35 X° water bath for 10 minutes
    9. SPME extraction for 10 minutes
    10. GC/MS analysis (FURAN .M)



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Witnessed & Understood by me,	Date	Invented by:	Date
Yaman Dalmaj	7/13/07	Recorded by:	

TITLE _____ Project No. _____

From Page No. ____ Book No. _____

7_16_07

Objective: To measure the furan amount in baby apple

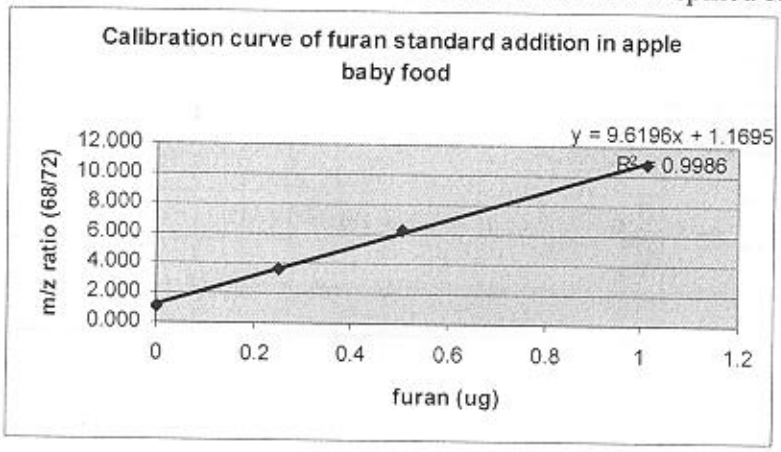
Procedures

1. Take out 50 g of baby apple food and put into a bottle containing 150 ml of HPLC grade water
2. Mixed thoroughly
3. Put 20 g of the sample into 4 vials that contain magnetic stirrer
4. Incubate in an icebath for 5 minutes
5. Determine the amount of ISTD and spiked furan. Add appropriate amount into each vial- X0 20g + 50ul of 11ug/ml d4 furan ISTD
   - X1 20g + 50ul of 11ug/ml d4 furan ISTD +25 ul of 10.1 ug/ml furan
   - X2 20g + 50ul of 11ug/ml d4 furan ISTD + 50ul of 10.1ug/ml furan
   - X3 20g + 50ul of 11ug/ml d4 furan ISTD + 100ul of 10.1 ug/ml furan
6. Incubate a vial in 35°C waterbath for 10 minutes
7. SPME extraction for 10 minutes
8. GC-MS :

Results

Sample	Date file	Level	amt added ug	Abundance Analyte m/z =68	Abundance ISTD m/z =72	ratio area
Gerber Apple Baby food	BBAPPLE.D	0	0	7,815,260	7,437,826	1.051
	BBAPPLE1.D	1	0.2525	29,914,421	8,270,107	3.617
	BBAPPLE2.D	2	0.505	45,392,428	7,278,315	6.237
	BBAPPLE3.D	3	1.01	79,204,550	7,350,164	10.776
					Amount	furan = 0.12 ug
					Amount (ng/g food)	24ng/g

- Calibration curve between m/z 68 and 72 ratio and spiked furan (ug)



- The amount of furan detected in baby food is 24 ng/g. The result shows good linearity with R^2 of 0.9986.

Witnessed & Understood by me,	Date	Invented by:	Date
yana	7/16/07	Recorded by:	

7/18/07

Objective: To collect data of furan and qualifier ions of furan in water and use it in QC data when determine furan in food

Procedure

1. Pipette 20 ml of HPLC water into 3 glass vials
2. Take 200 ul of 10.1 ug/ml furan into each vial containing water
3. Put the vial in an ice bath for 5 minutes
4. Put the vial in 35°C water bath for 10 minutes
5. SPME extraction for 10 minutes
6. GC-MS analysis

Result

File name	m/z	Area	Rt
STDINH2O.D	68	121284322	14.434
	39	131913824	14.433
	37	17093529	14.432
	72	24147766	14.310
	42	22845603	14.308
	44	5976720	14.309
STDINH21.D	68	122716029	14.425
	39	131857633	14.423
	37	16842619	14.423
	72	25154669	14.300
	42	23495923	14.297
	44	5572063	14.298
STDINH22.D	68	126094339	14.432
	39	134824594	14.430
	37	17082393	14.429
	72	26037556	14.309
	42	24344832	14.307
	44	5577978	14.307

Result and Discussion

The average area of m/z qualifier ions used in QC data is

For m/z = 68; the average area is 123364897

m/z = 39; the average area is 132865350

m/z =37 ; the average area is 17006180

m/z = 72; the average area is 25113330

m/z = 42; the average area is 23562119

m/z = 44; the average area is 5708920

The m/z 39 and 37 will be the qualifier ions for furan analyte (m/z= 68) , and m/z 44 will be the qualifier for internal standard– d4 furan (m/z =72).

These data will be using for quality control when analyze furan content in food sample.

Witnessed & Understood by me,	Date	Invented by:	Date
jason Dang	7/18/07	Recorded by:	

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

Microsoft Excel - Pea juice

File Edit View Insert Format Tools Data Window Help

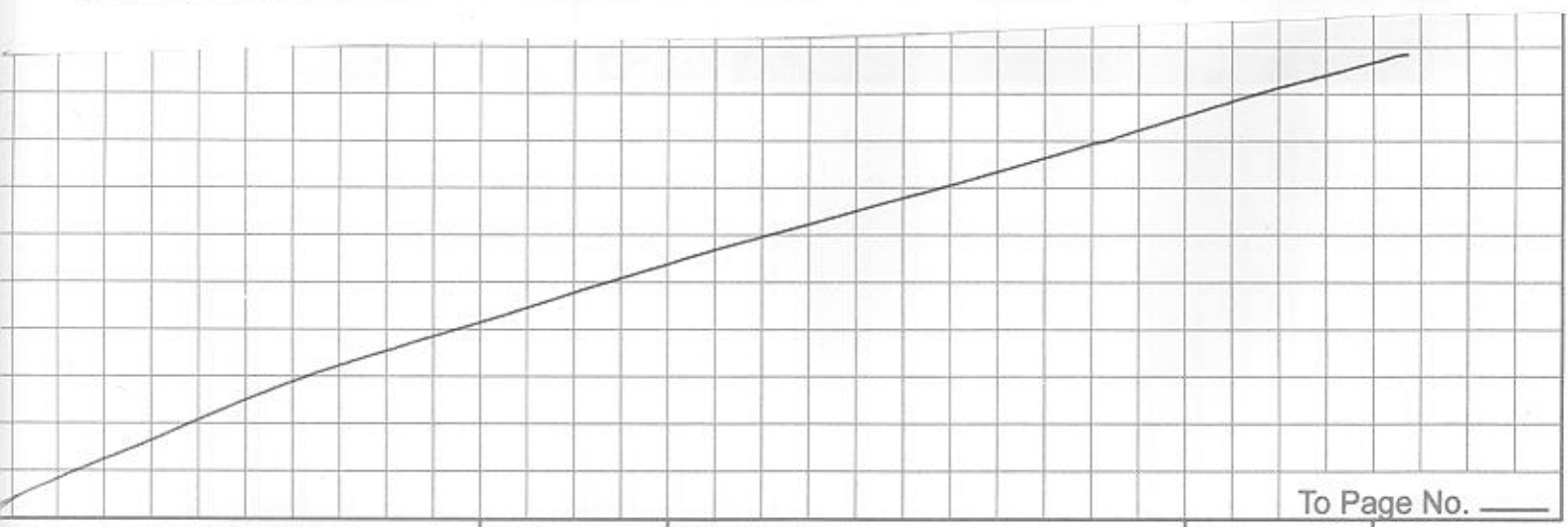
G57 =ABS(F54-E57)

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Date	9/2/2007											
2	Sample	Pea Juice											
3	Dilut. Factor	10											
4		Con of		ug/ml in water									
5		ISTD	d4 furan	11.00									
6		Spiking Solutio	Furan	10.10									
7													
8													
9	Furan												
10	Target Ion	Data File	Spike amount	Rt	Area	Area Ratio(68/72)							
11	m/z = 68		ug										
12	Lv1	PEALIQX0.D	0	14.431	4,319,979	1.15							
13	Lv2	PEALIQX1.D	0.2525	14.436	7,942,631	1.97							
14	Lv3	PEALIQX2.D	0.505	14.443	10,226,682	3.02							
15	Lv4	PEALIQX3.D	1.01	14.443	17,553,581	4.91							
16													
17	Qualifier			Rt	Area	Qualifier Ratio (%)	Expected ratio (%)	Differences					
18	Q1 = m/z 39												
19	Lv1	PEALIQX0.D		14.429	4,497,914	104.12	107.7	3.58					
20	Lv2	PEALIQX1.D		14.435	8,197,671	103.21		4.49					
21	Lv3	PEALIQX2.D		14.441	10,518,594	102.93		4.87					
22	Lv4	PEALIQX3.D		14.441	17,943,085	102.22		5.48					
23													
24													
25													
26	Q2 = m/z 37			Rt	Area	Qualifier Ratio (%)	Expected ratio (%)	Differences					
27													
28	Lv1	PEALIQX0.D		14.288	519,875	12.03	13.79	1.75					
29	Lv2	PEALIQX1.D		14.435	961,216	12.10		1.68					
30	Lv3	PEALIQX2.D		14.439	1,221,862	11.95		1.83					
31	Lv4	PEALIQX3.D		14.439	2,110,564	12.02		1.76					
32													
33													
34			d4-furan ISTD										
35													
36	Target Ion			Rt	Area								
37	m/z = 72												
38	Lv1	PEALIQX0.D		14.288	3,757,596								
39	Lv2	PEALIQX1.D		14.296	4,041,868								
40	Lv3	PEALIQX2.D		14.303	3,392,324								
41	Lv4	PEALIQX3.D		14.306	3,573,082								
42													
43													
44	Qualifier			Rt	Area	Qualifier Ratio (%)	Expected ratio (%)	Differences					
45	Q1 = m/z 44												
46	Lv1	PEALIQX0.D		14.285	786,484	21.20	22.07	0.87					
47	Lv2	PEALIQX1.D		14.284	926,264	20.44		1.63					
48	Lv3	PEALIQX2.D		14.300	721,792	21.28		0.79					
49	Lv4	PEALIQX3.D		14.306	775,435	21.70		0.37					

Data / STD Addition Curve / Calculation /

Ready NUM

start



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Witnessed & Understood by me,

Date 7/18/07

Invented by:

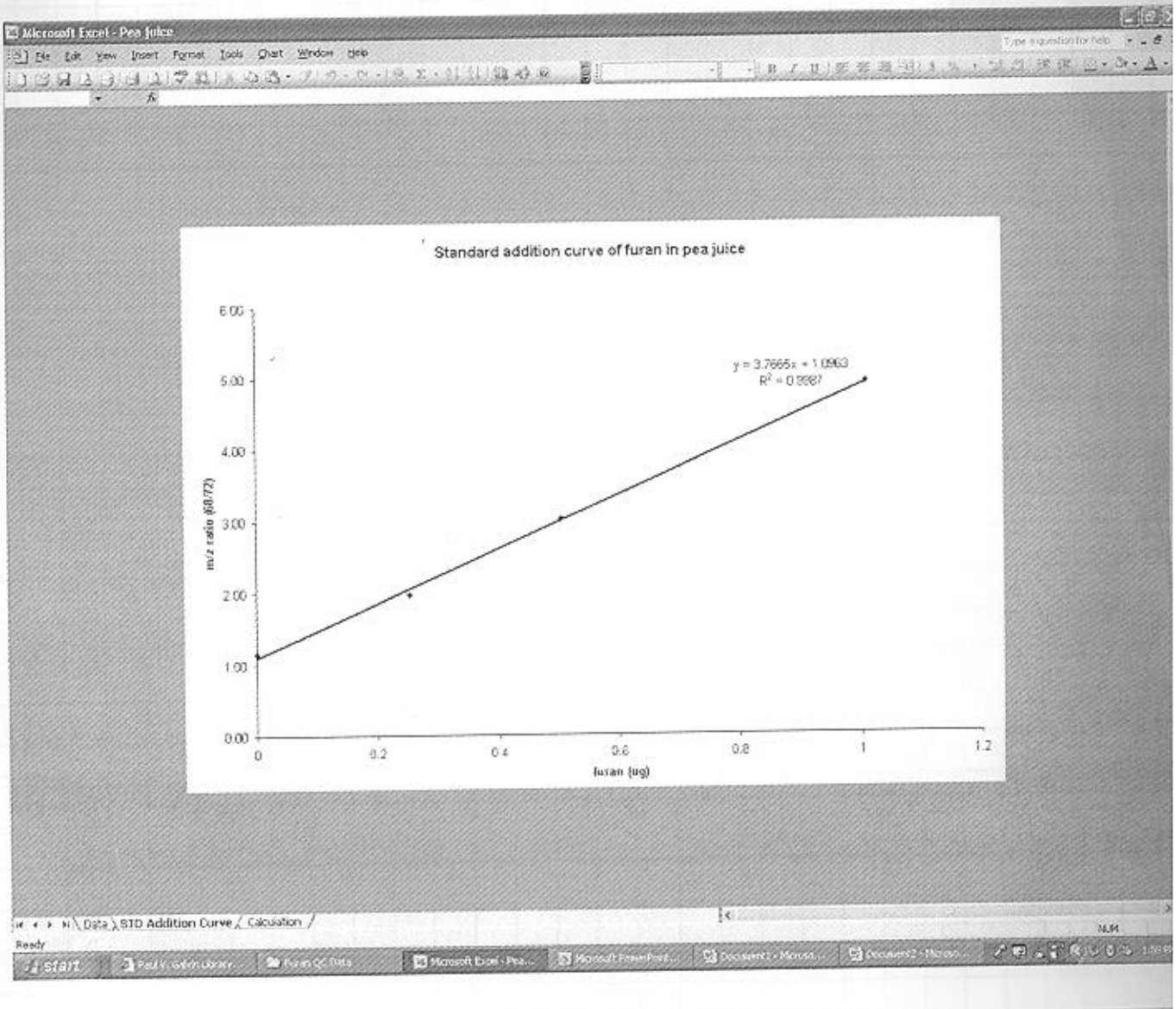
Recorded by:

Date

Project No. _____

TITLE _____ Book No. _____

From Page No. ____



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garan	7/18/07	Recorded by:	

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

Date 7/31/07

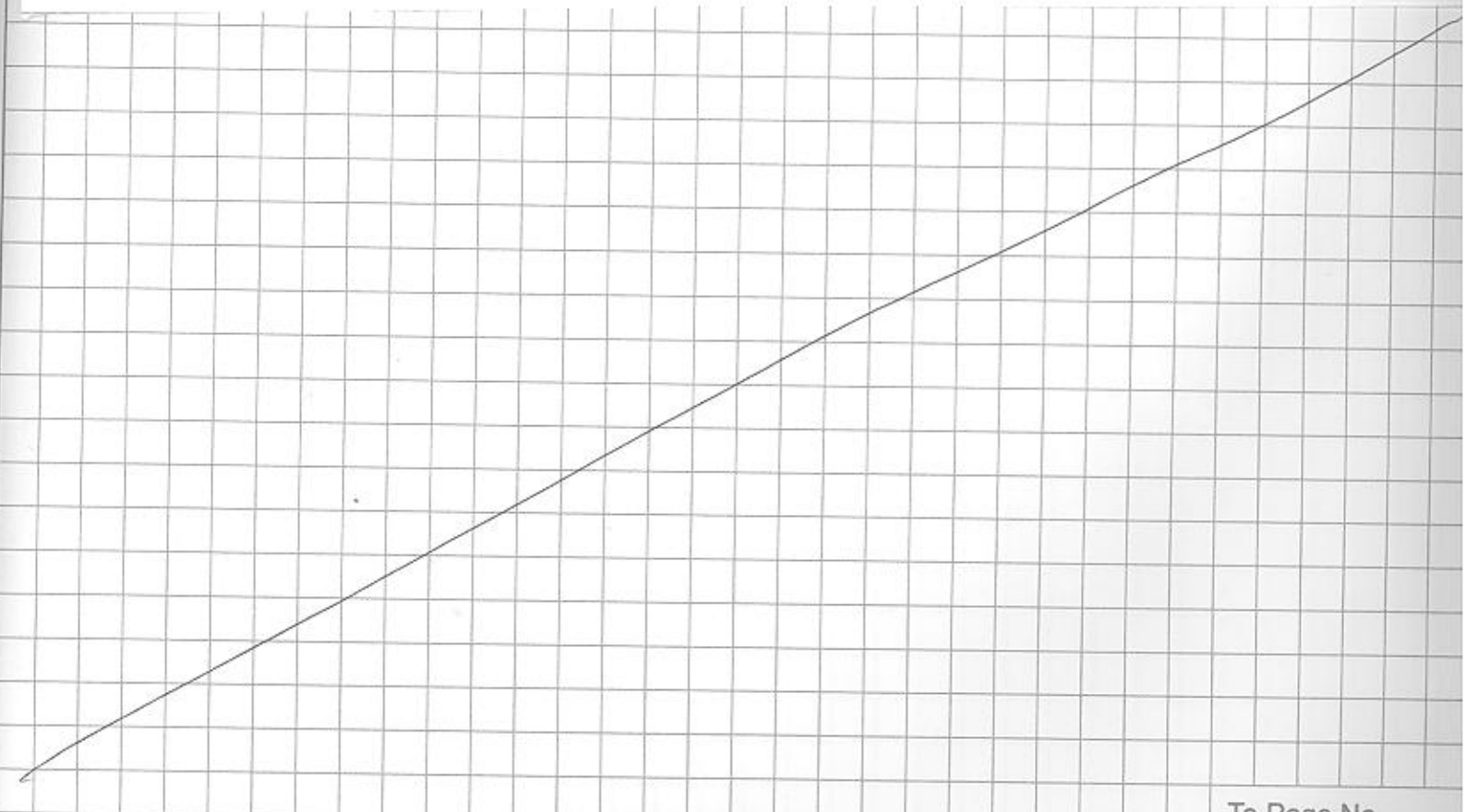
Objective: To measure the amount of furan in organic whole kennel corn

Procedures

1. Drain the liquid of refrigerated corn into 225 bottle and keep the solid part into an ice bath covered with foil and rubber band.
2. Take 50 grams of solid corn into 225 plastic bottle containing 150 ml of water.
3. Homogenize while keeping it cool in an ice bath
4. Transfer 20 ml of sample into 40ml glass vial
5. Incubate in an ice bath for 5 minutes. Then added 250 ul of d_4 –furan ISTD.
6. Incubate in 35°C for 10 mins
7. SPME extraction for 10 mins
8. GC MS analysis using FURAN.M method (Injection port 275°C, Oven 55°C→ 115°C(2) @ 5°C/min then increase to 295°C (2.8) @ 25°C/min)

Results and Discussions

The d_4 –furan added was in very high concentration (0.275 mg/ml; 250ul from 1.1mg/ml of d_4 –furan). This is inappropriate to use the *m/z* ration (68/72) to calculate amount of furan in ~~sold~~ solid corn. However, There is very little furan observed and we believed that furan should be dissolve in the liquid part of canned corn and we decided not to do further analysis on solid corn.



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Witnessed & Understood by me,	Date	Invented by:	Date
Yaman Deng	7/13/07	Recorded by:	

Project No. ____________

TITLE ______________________________ Book No. ____________

From Page No. ____

Date 8/1/07

Objective: To measure the amount of furan in organic whole kernel corn (continue from page 33)

Product details: Organic Kernel Corn. Net WT. 425 g. Company ID: H6PU231A. Lot No.: 5994 CORN. Exp.: Jan 31 2008. Brand: O organics.

Procedures

1. Use pipette to take out 10 ml of refrigerated liquor of kernel corn and put into 40 ml headspace vial containing 10 ml of water. Prepare 4 test portions and label X_0-X_3
2. Incubate in an ice bath for 5 minutes
3. Added 250 µl of 11ug/ml d_4 –furan in each test portions
4. Added 25ul, 50ul and 100 µl of 10.1ug/ml of furan working solution into vial X_1, X_2 and X_3 consecutively.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
Yanan Ding	8/1/07	Recorded by:	

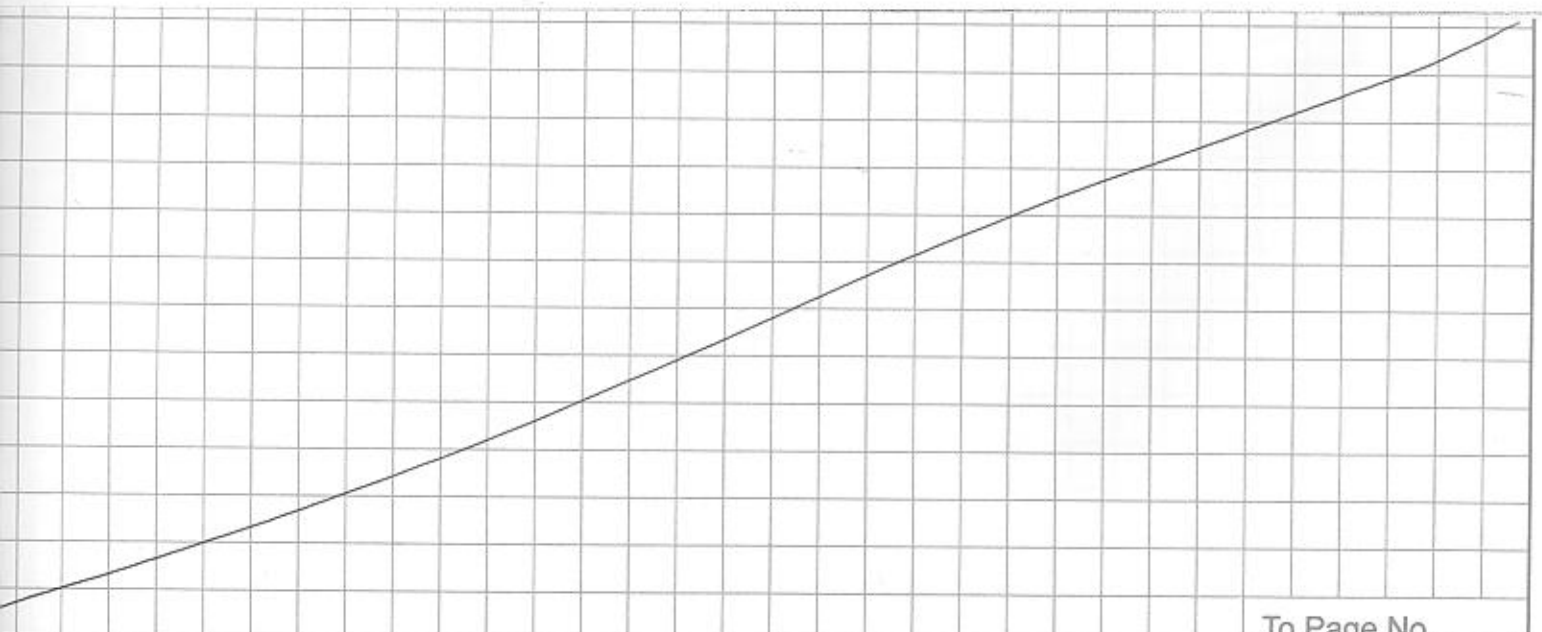
Project No. _____

TLE _____ Book No. _____

m Page No. 34

Results and Discussions

	File name	*m/z*	Peak Area	Rt
X0	LCORN0.D	68	1598515	14.397
		39	1692969	14.396
		37	200101	14.394
		72	2504759	14.257
		42	2732995	14.254
		44	508925	14.257
X1	LCORNX1.D	68	3384685	14.376
		39	3542831	14.375
		37	407373	14.375
		72	2646040	14.239
		42	2682508	14.236
		44	581480	14.237
X2	LCORNX2.D	68	5353642	14.359
		39	5599209	14.357
		37	645504	14.355
		72	2611207	14.222
		42	2622527	14.219
		44	575801	14.220
X3	LCORNX3.D	68	7482824	14.391
		39	7820354	14.389
		37	925094	14.389
		72	2147554	14.255
		42	2149813	14.253
		44	459732	14.251



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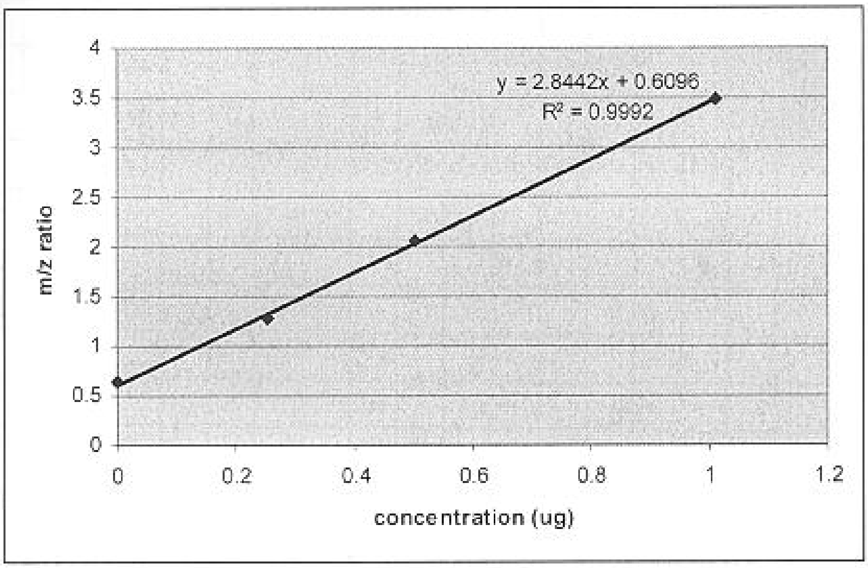
Recorded by:

- Data analysis

conc (ug)	68	72	m/z ratio(68/72)
0	1598515	2504759	0.638191
0.2525	3384685	2646040	1.279151
0.501	5353642	2611207	2.050256
1.01	7487824	2147554	3.486676



- The concentration of furan in corn liquor is 0.2143μg in diluted 20ml sample (dilution factor =2). Thus, the amount of furan is (0.2143*2)/20 = 0.02143μg/ml or 21.43ng/ml.

Witnessed & Understood by me, [signature] | Date 8/1/07 | Invented by: | Date
Recorded by:

Project No. _____

TLE _____ Book No. _____

om Page No. ____

8/2/07

Objective: To analysis furan in Pea Juice

Procedures

1. Pipette 10 ml of pea juice into a glass vial containing 10 ml of water **

** The peak area of pea juice is much higher than the adding 250 ul of ISTD. Therefore, the dilution factor is changed

2. 1 ml of sample was transferred into 9 ml of water (dil. factor = 10)
3. 250ul of 11 ug/ml of d4-furan was added after 5 min incubation in an ice bath
4. 4 concentrations of standard additions which are by adding 0, 25ul, 50, and 100ul of 10.1 µg/ml furan working standard solution.
5. Incubate at 35°C for 10 mins
6. SPME extraction for 10 mins
7. GC/MS analysis using FURAN.M

Results and Discussions

	File name	*m/z*	Peak Area	Rt
X0	PEALIQX0.D	68	4319979	14.431
		39	4497914	14.429
		37	519875	14.429
		72	3757596	14.288
		42	4209935	14.287
		44	796484	14.285
X1	PEALIQX1.D	68	7942631	14.436
		39	8197671	14.435
		37	961218	14.435
		72	4041869	14.296
		42	3984578	14.294
		44	826264	14.294
X2	PEALIQX2.D	68	10228628	14.443
		39	10518599	14.441
		37	1221862	14.439
		72	3392324	14.303
		42	3316180	14.301
		44	721792	14.300
X3	PEALIQX3.D	68	17553581	14.443
		39	17945722	14.441
		37	2110564	14.439
		72	3573082	14.308
		42	3452869	14.306
		44	756223	14.306

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Recorded by:

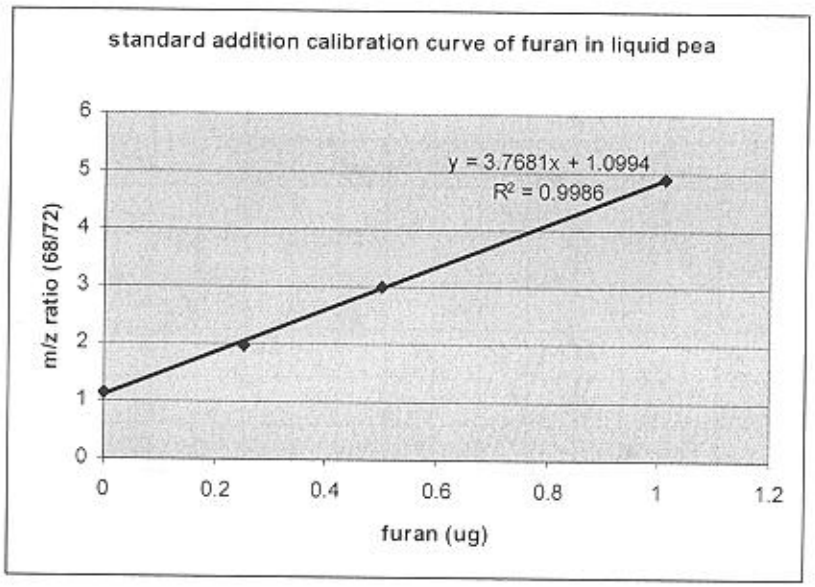
TITLE _____ Project No. _____

Book No. _____

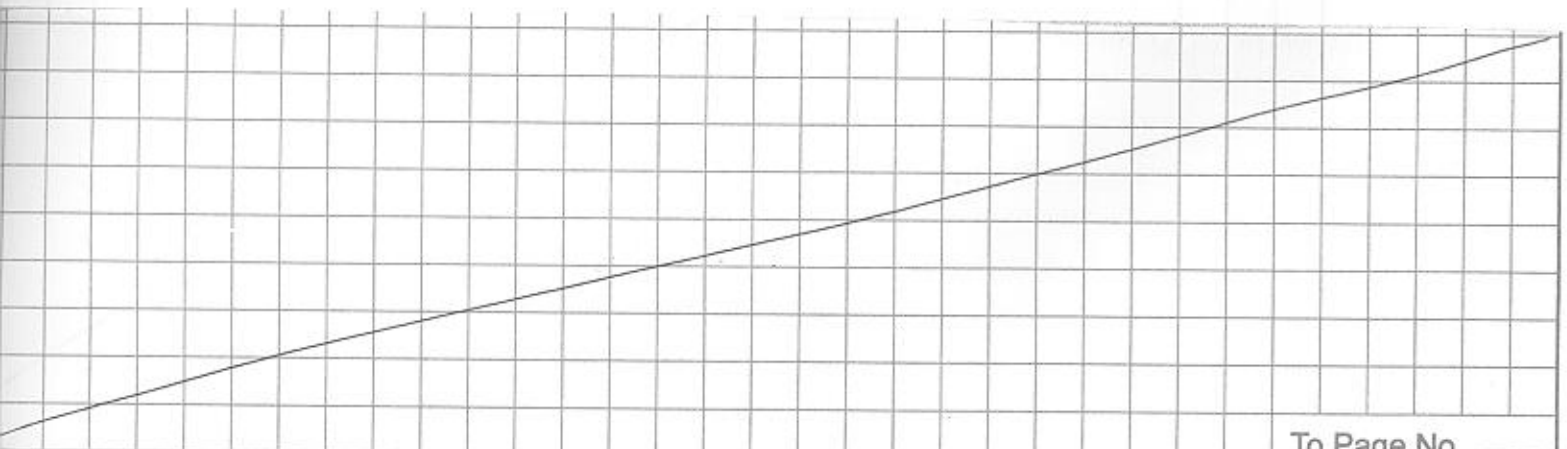
From Page No. 37

- Data analysis

conc (ug)	68	72	*m/z ratio(68/72)*
0	4319979	3757596	1.149666
0.2525	7942631	4041869	1.965089
0.501	10228682	3392324	3.015243
1.01	17553581	3573082	4.912728



- The concentration of furan in corn liquor is 0.2918 µg in diluted 10ml sample (dilution factor =10). Thus, the amount of furan is (0.2143*10)/10 = 0.2918 µg/ml or 291.8 ng/ml.



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Yaren Dicky	8/2/07	Recorded by:	

TITLE _____ Project No. _____

Book No. _____

From Page No. ____

8/6/07

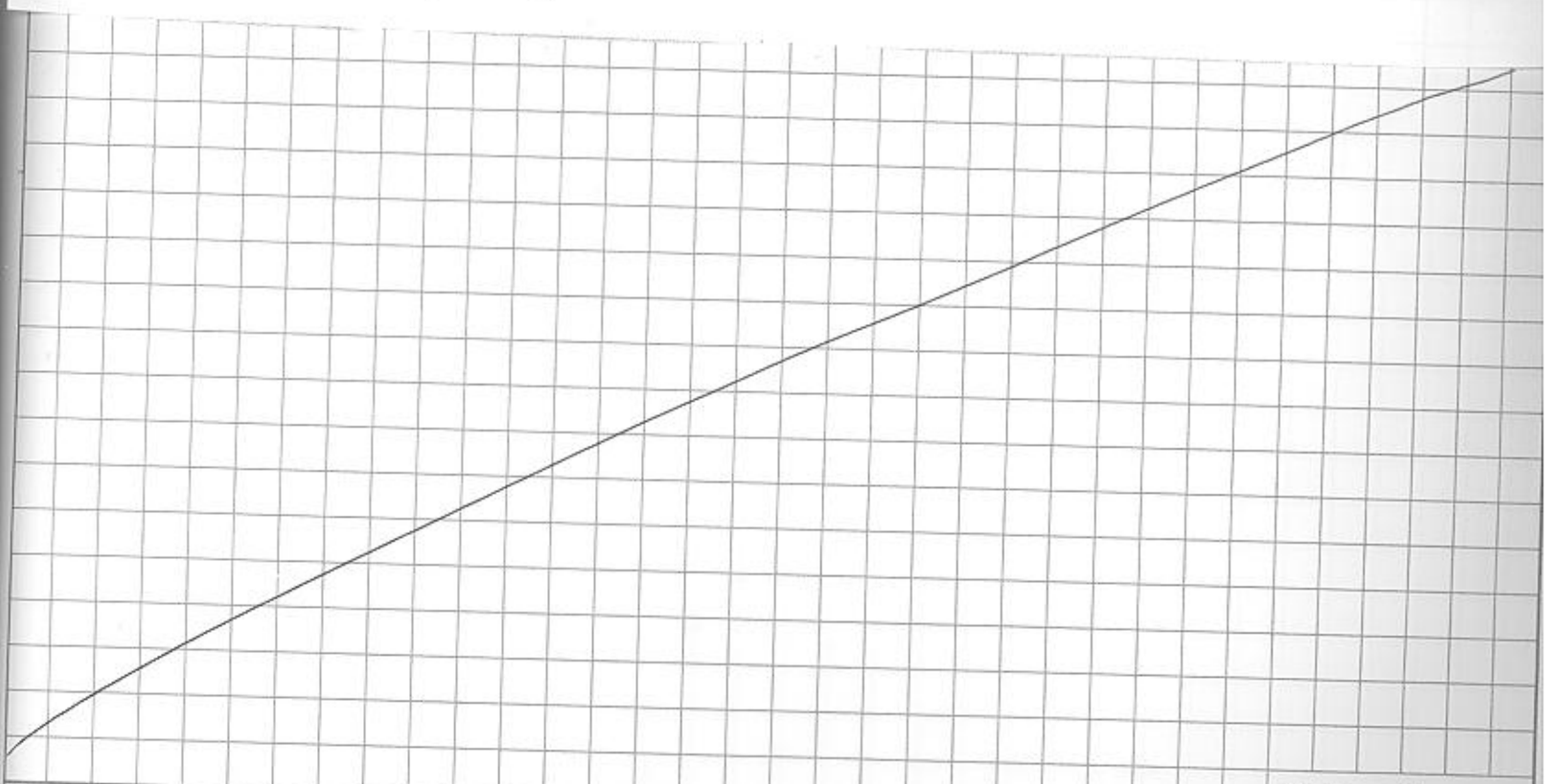
Objective: To measure furan in solid pea sample

Procedures

1. Take 50 g of refrigerated pea into 150 ml of water
2. Homogenize until it becomes homogeneous
3. Transfer 20 g into 4 headspace vials
4. Incubate in an ice bath for 5 min
5. Furan and d4-furan is fortified as below
   X0 : 20g + 250 ISTD
   X1 : 20g + 250 ISTD +25µl furan
   X2 : 20g + 250 ISTD +50µl furan
   X3 : 20g + 250 ISTD + 100µl furan
6. Incubate in a 35°C water bath for 10 min
7. SPME extraction for 10 mins
8. GC/MS analysis (FURAN.M)

Results and Discussion

The working solution needs to be prepared fresh. The furan working solution is now will be prepared every 2 weeks.



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Yanan Diao	8/6/07	Recorded by:	

TITLE _____ Project No. _____
Book No. _____

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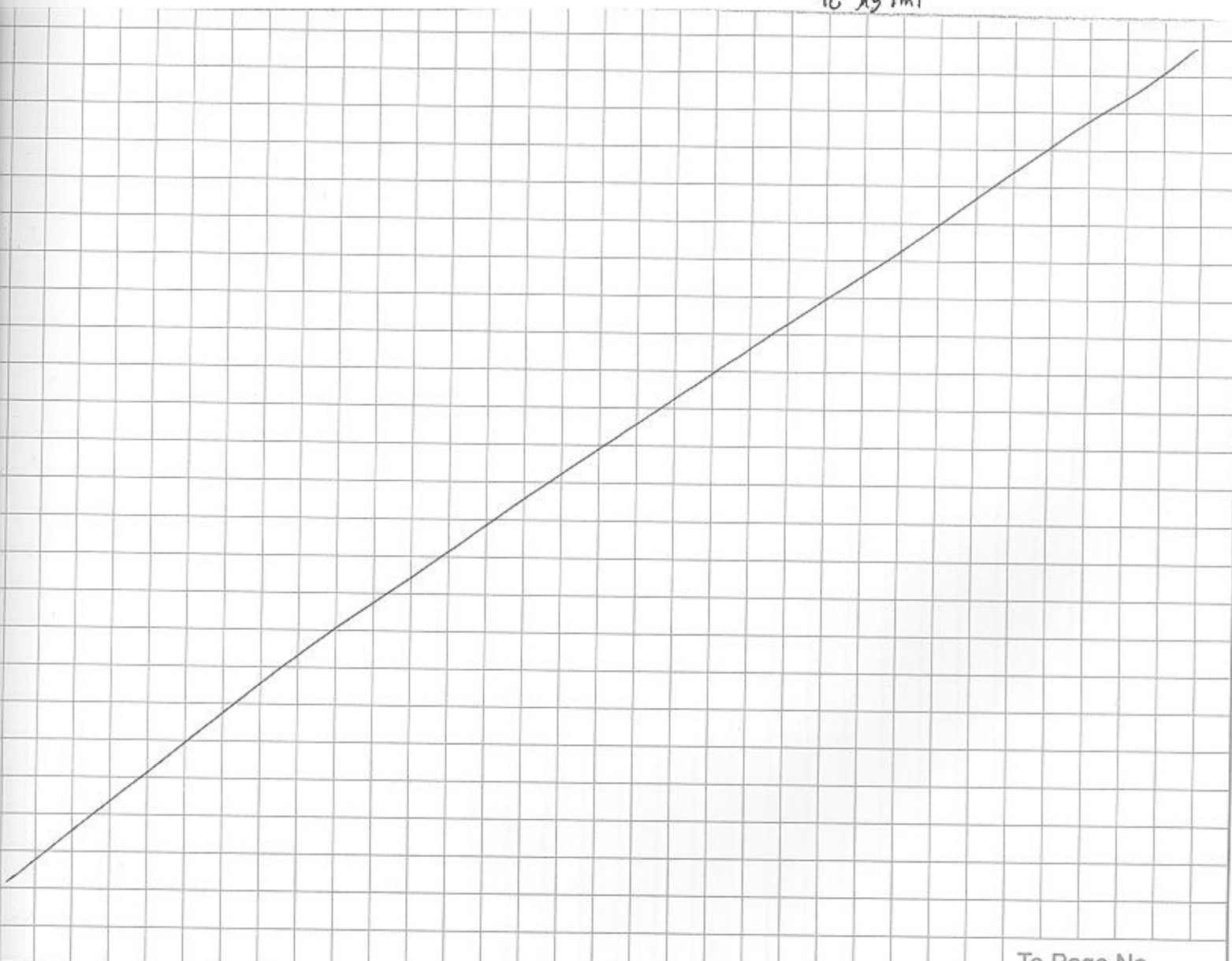
8/7/07

Objective: To prepare furan and d4-furan working solution

Procedures

1. Prepare 10.9 µg/ml of d4-furan working solution by using micropipette to transfer 200 µl of 1.1 mg/ml d4-furan into 20 ml of water. The concentration of d4-furan working solution is (0.2*1.1)/20 = 10.9 µg/ml
2. Prepare 10.1 µg/ml of furan working solution by using micropipette to transfer 100 µl of 2.04 mg/ml furan standard solution into 20 ml of water. The concentration of furan working solution is (0.1*2.04)/20 = ~~10.9~~ 10.2 µg/ml
   10 µg/ml



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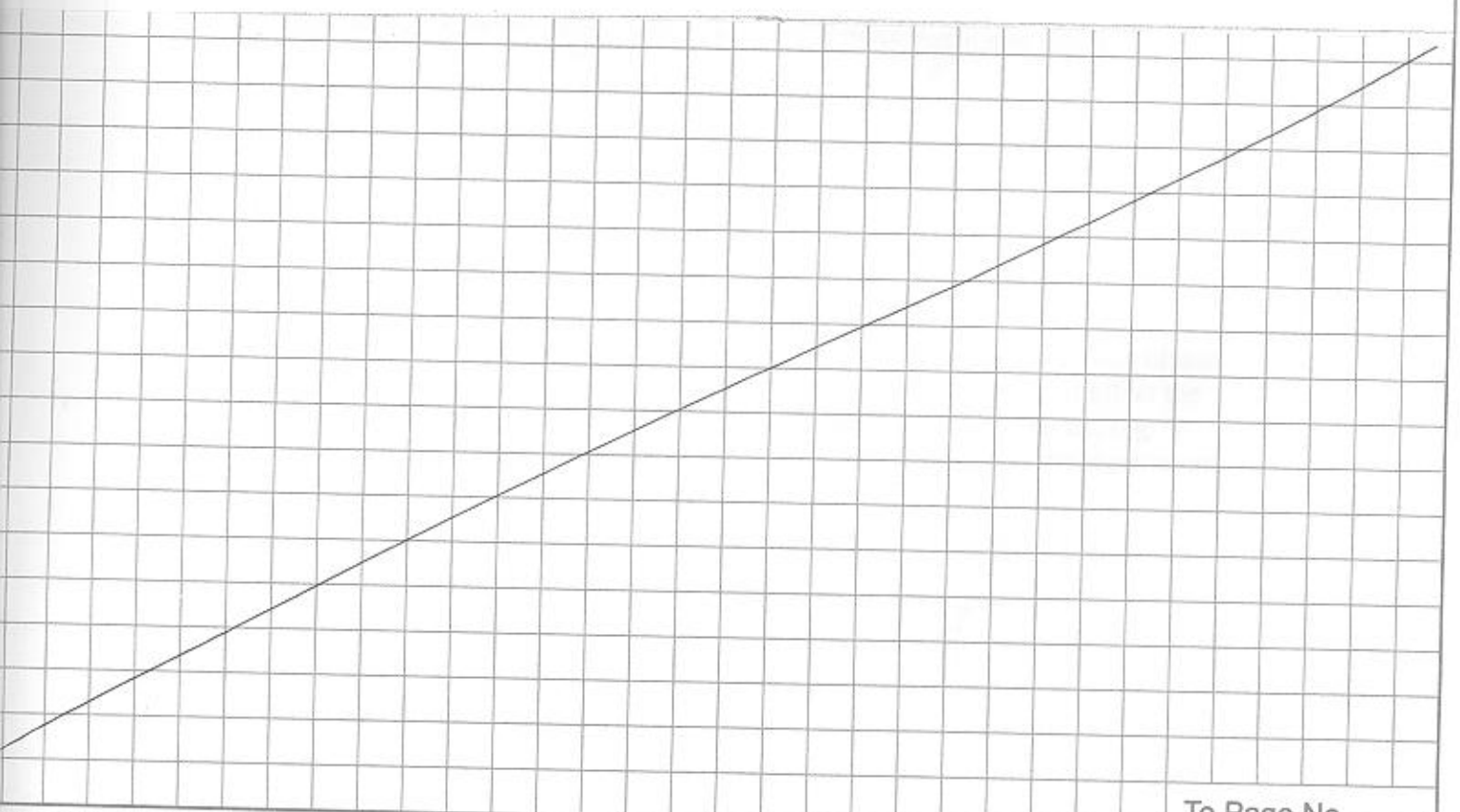
tnessed & Understood by me,	Date	Invented by:	Date
8/7/07 Yuan Dilong	8/7/07	Recorded by:	

8/9/07

Objective: To measure the amount of furan in pea sample

Procedures
Spiked furan concentration: 10.1 µg/ml, ISTD concentration is 10.9 µg/ml

1. Measure 50 g of solid pea and place into 225 plastic bottle containing 150 ml of water
2. Homogenize until the matrix is unify
3. Transfer 20 ml into 4 vials
4. Incubate in an ice bath for 5 minutes
5. Add ISTD and spiked furan as follow
    a. X_0: 20 g sample + 10 µl ISTD
    b. X_1: 20 g sample + 10 µl ISTD + 25 µl of spiked furan
    c. X_2: 20 g sample + 10 µl ISTD + 50 µl of spkied furan
    d. X_3: 20 g sample + 10 µl ISTD + 100 µl of sapiked furan
6. Incubate in the 35°C water bath for 10 minutes
7. SPME extraction for 10 minutes
8. GC-MS analysis (FURAN.M)



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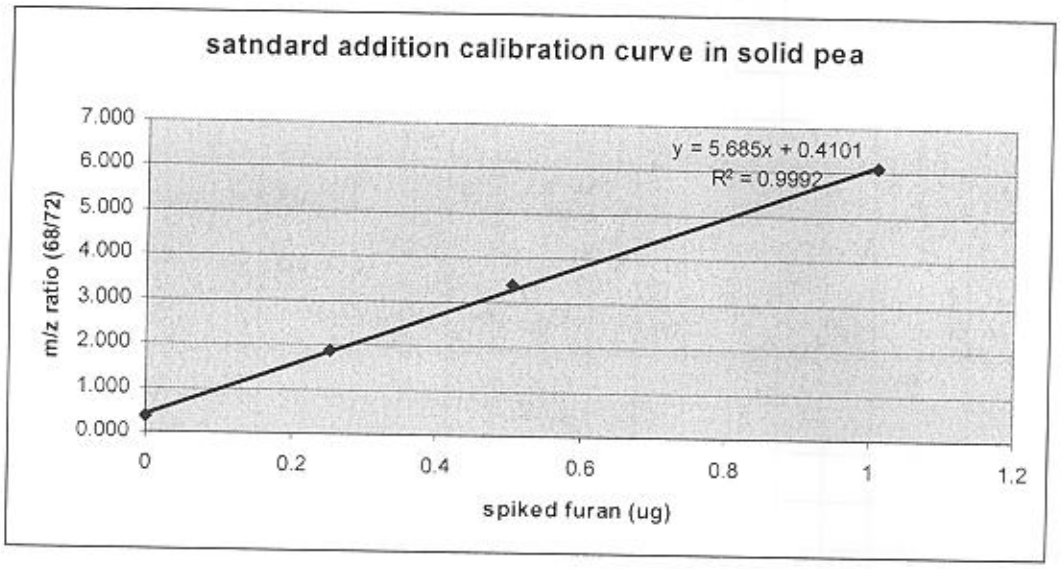
Results and Discussions

Data file	Level	Concentration	Area #68	Area # 72	*m/z* ratio (68/72)
SPEAX0.D	0	0	3,442,118	9,595,181	0.359
SPEAX1.D	1	0.2525	13,853,258	7,482,876	1.851
SPEAX2.D	2	0.505	29,221,764	8,658,045	3.375
SPEAX3.D	3	1.01	42,431,270	6,952,041	6.103



- The difference between level 0 and level 1 is too high therefore the concentration obtain from the graph may overpass co-ordinates that make a considerably change or effect the slope of the standard calibration curve which alter the concentration (0.07ug in 20 g sample diluted by the factor of 4; the concentration= 14ng/ml). Therefore, the experiment should be repeated.

essed & Understood by me,

Date 8/9/07

Invented by:

Recorded by:

Date

Project No. ______

TITLE ______ Book No. ______

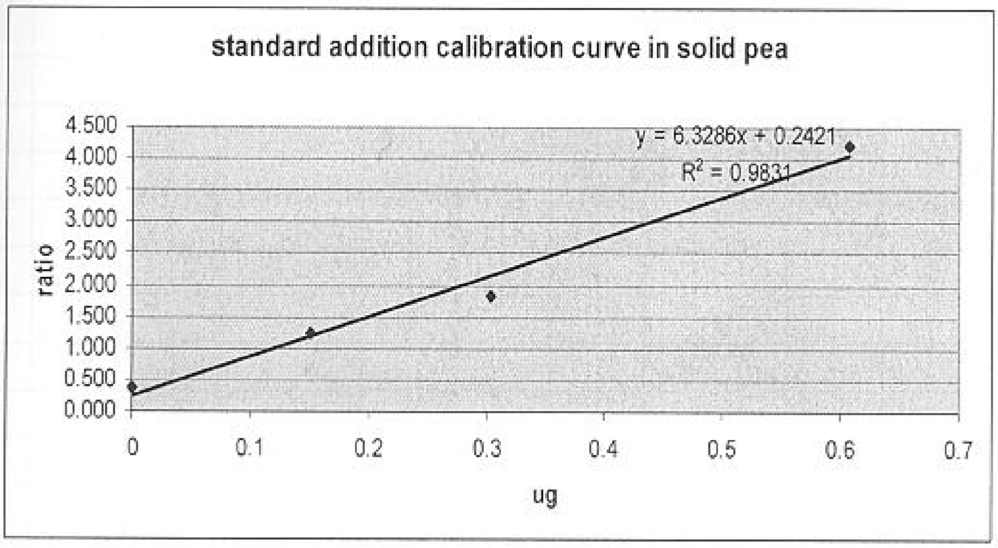
From Page No. ____

- Repeat the same experiment as above but reduce the volume of spiked furan into –

a. X_0: 20 g sample + 10 µl ISTD
b. X_1: 20 g sample + 10 µl ISTD + 15 µl of spiked furan
c. X_2: 20 g sample + 10 µl ISTD + 30 µl of spkied furan
d. X_3: 20 g sample + 10 µl ISTD + 60 µl of sapiked furan

Results

SPEAX0.D	0	0	2,741,130	7,603,660	0.361
SPEAX1.D	1	0.1515	10,534,198	8,398,797	1.254
SPEAX2.D	2	0.303	12,325,461	6,688,534	1.843
SPEAX3.D	3	0.606	25,233,432	5,976,215	4.222
				Amount	furan=0.038



- The results still jumps too high from level 0. The spiked solution may need to be more diluted.

To Page No. ____

Witnessed & Understood by me, Yaran Dai

Date 8/9/07

Invented by:

Recorded by:

Date

8/10/07

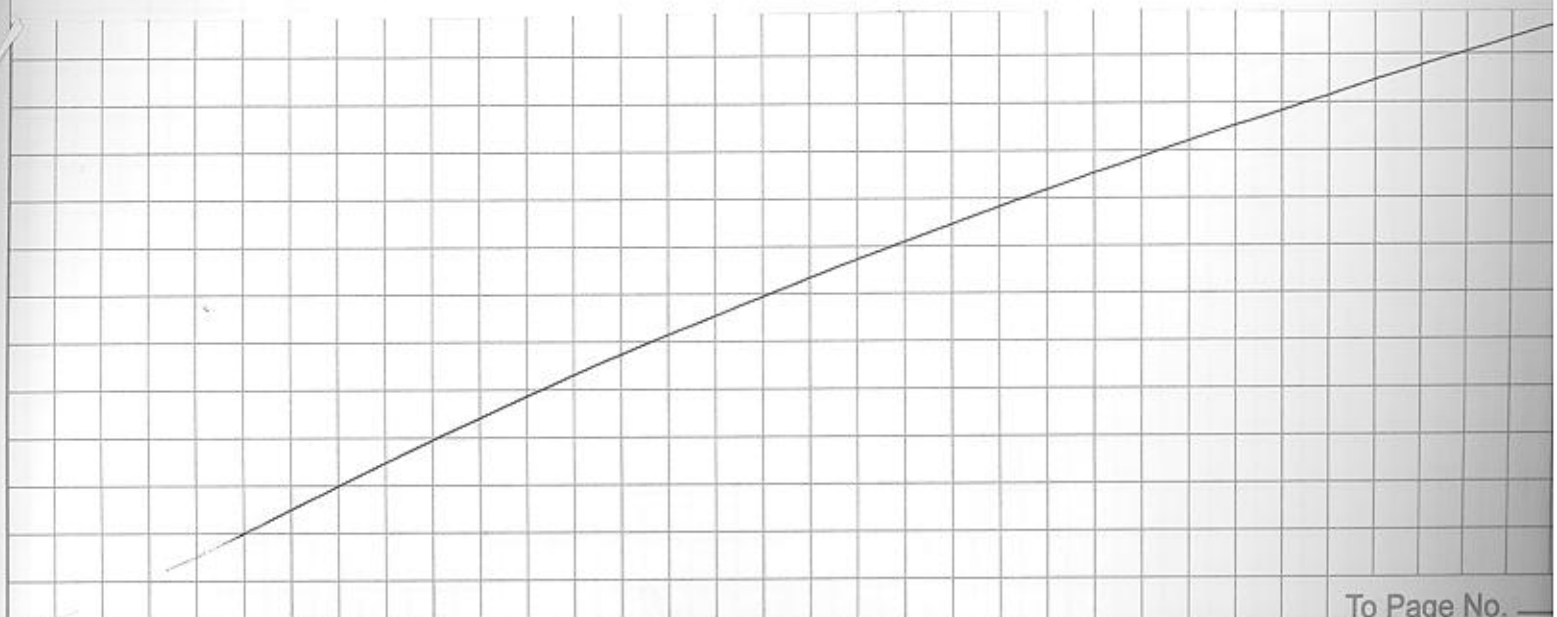
Objective: To measure furan in solid pea samples

Procedures

Procedures
Spiked furan concentration: 2.1 µg/ml , ISTD concentration is 10.9 µg/ml

1. Measure 50 g of solid pea and place into 225 plastic bottle containing 150 ml of water
2. Homogenize until the matrix is unify
3. Transfer 20 ml into 4 vials
4. Incubate in an ice bath for 5 minutes
5. Add ISTD and spiked furan as follow
    e. X_0: 20 g sample + 10 µl ISTD
    f. X_1: 20 g sample + 10 µl ISTD + 25 µl of spiked furan
    g. X_2: 20 g sample + 10 µl ISTD + 50 µl of spkied furan
    h. X_3: 20 g sample + 10 µl ISTD + 100 µl of sapiked furan
6. Incubate in the 35°C water bath for 10 minutes
7. SPME extraction for 10 minutes
8. GC-MS analysis (FURAN.M)

Results
- The results show X1 ratio is less than X0, which should be by the diluted furan concentration added. After trying several dilutions, 5.05 µg/ml shows to be the most suitable concentrations.



Witnessed & Understood by me,	Date	Invented by:	Date
Yanan Dai	8/10/07	Recorded by:	

Product Information.

Product. : Peas, early june medium

Brand : Jewel

Net Wt : 15 OZ (425g)

Bar code : 41280 07134

Company code : ATC 7086

Batch : 001 012 J

TITLE _____ Project No. _____

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8/15/07

Objectives: To measure furan in solid pea sample

Procedures

Spiked furan concentration: 5.05 µg/ml, ISTD concentration is 10.9 µg/ml

1. Measure 50 g of solid pea and place into 225 plastic bottle containing 150 ml of water
2. Homogenize until the matrix is unify
3. Transfer 20 ml into 4 vials
4. Incubate in an ice bath for 5 minutes
5. Add ISTD and spiked furan as follow
    i. X_0: 20 g sample + 20 µl ISTD
    j. X_1: 20 g sample + 20 µl ISTD + 50 µl of spiked furan
    k. X_2: 20 g sample + 20 µl ISTD + 100 µl of spkied furan
    l. X_3: 20 g sample + 20 µl ISTD + 300 µl of sapiked furan
6. Incubate in the 35°C water bath for 10 minutes
7. SPME extraction for 10 minutes
8. GC-MS analysis (FURAN.M)

Witnessed & Understood by me, | Date 8/15/07 | Invented by: | Recorded by: | Date

Results

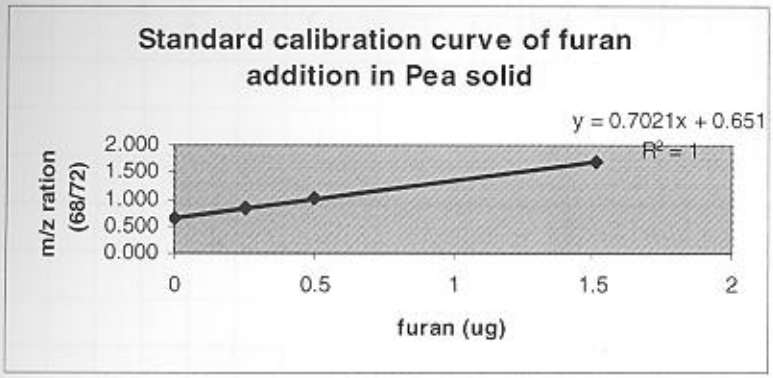
	File name	*m/z*	Peak Area	Rt
X_0	PEAS9.D	68	3479884	14.480
		39	3667680	14.478
		37	434484	14.477
		72	5333851	14.333
		42	5777161	14.331
		44	1093530	14.330
X_1	PEAS10.D	68	4925720	14.485
		39	5150019	14.483
		37	607537	14.485
		72	5969459	14.339
		42	6511197	14.336
		44	1238301	14.336
X_2	PEAS11.D	68	5748970	14.443
		39	5947762	14.487
		37	698338	14.484
		72	5706366	14.342
		42	6258146	14.340
		44	1183403	14.340
X_3	PEAS12.D	68	9066316	14.491
		39	9317184	14.489
		37	1109298	14.486
		72	5287629	14.349
		42	5216737	14.437
		44	1087600	14.349

- Data analysis

File name	Level	Conc.	Area #68	Area #72	ratio
PEAS09.D	0	0	3,479,884	5,333,851	0.652
PEAS10.D	1	0.2525	4,925,720	5,969,459	0.825
PEAS11.D	2	0.505	5,748,970	5,706,366	1.007
PEAS12.D	3	1.5151	9,066,316	5,287,629	1.715
				Amount	furan=0.927ug

tnessed & Understood by me, | Date 8/15/09 | Invented by: | Date

Recorded by:



- The amount of furan in 20 g pea test portion is 0.927 µg. Thus, the concentration of solid pea is (0.927*4)/20 = 0.185 µg/g or 185 ng/g.

Witnessed & Understood by me,	Date	Invented by:	Date
	8/15/07	Recorded by:	

8/16/07

Objective: To measure the furan concentration of pea sample after centrifuge to remove the solid matrix (pellet), compare with the control (homogenize sample that has not been centrifuge)

Procedures

1. Thaw frozen canned pea
2. Take 50 g of solid pea into 225 ml plastic bottle containing 150 ml of water.
3. Homogenize until the matrix becomes smooth
4. Transfer 20 g of sample into 4 headspace vials containing a stir bar
5. Incubate the 4 vials in ice for 5 minutes
6. Add 20 µl ISTD of 11 µg/ml of d4-furan into each vial and spiked furan (10.1 µg/ml) into 3 of the vials- 50µl into X_1, 100 ul into X_2, and 300 µl into X_3.
7. Analyze the test portion for furan using the same method in the page before (P.)
8. Bring the rest of the prepared sample out and transfer 50g of homogenized sample into 2 centrifuge tubes
9. Centrifuge at 5000 rpm, 5°C for 15 mins
10. Transfer 10 ml of the supernatant into 4 headspace vial
11. Incubate in ice for 5 minutes
12. Put 20 µl of ISTD into each vial and 50µl, 100 µl and 300 µl into X1, X2 and X3 consecutively
13. Analyze for furan using the same method. GC/MS analysis using FURAN.M

Witnessed & Understood by me,	Date	Invented by:	Date
yanmei Ding	8/16/07	Recorded by:	

TITLE _____ Project No. _____

Book No. _____

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8/16/07

Objective: To measure the furan concentration of pea sample after centrifuge to remove the solid matrix (pellet), compare with the control (homogenize sample that has not been centrifuge)

Procedures

1. Thaw frozen canned pea
2. Take 50 g of solid pea into 225 ml plastic bottle containing 150 ml of water.
3. Homogenize until the matrix becomes smooth
4. Transfer 20 g of sample into 4 headspace vials containing a stir bar
5. Incubate the 4 vials in ice for 5 minutes
6. Add 20 µl ISTD of 11 µg/ml of d4-furan into each vial and spiked furan (10.1 µg/ml) into 3 of the vials- 50µl into X_1, 100 ul into X_2, and 300 µl into X_3.
7. Analyze the test portion for furan using the same method in the page before (P.)
8. Bring the rest of the prepared sample out and transfer 50g of homogenized sample into 2 centrifuge tubes
9. Centrifuge at 5000 rpm, 5°C for 15 mins
10. Transfer 10 ml of the supernatant into 4 headspace vial
11. Incubate in ice for 5 minutes
12. Put 20 µl of ISTD into each vial and 50µl, 100 µl and 300 µl into X1, X2 and X3 consecutively
13. Analyze for furan using the same method. GC/MS analysis using FURAN.M

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	8/16/07	Recorded by:	

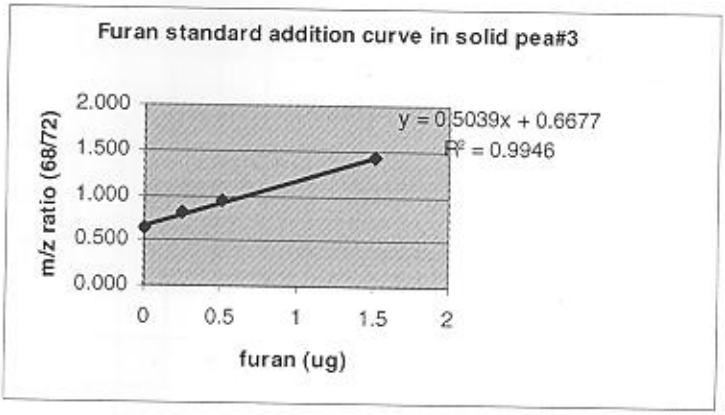
Results and Discussions.

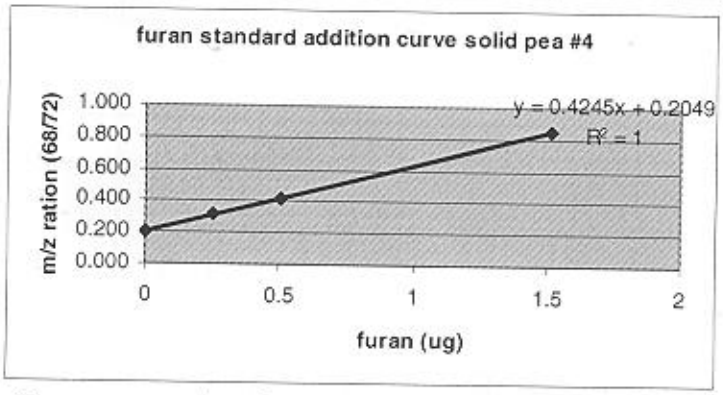
Before Centrifuge

File name	Level	Concentration	Area # 68	Area #72	Ratio (68/72)
PEAS17.D	0	0	3,354,388	5,241,895	0.640
PEAS18.D	1	0.2525	4,231,378	5,254,466	0.805
PEAS19.D	2	0.505	4,295,811	4,517,542	0.951
PEAS20.D	3	1.5151	7,297,798	5,139,713	1.420
				Amount	furan=1.32ug

After Centrifuge

File name	Level	Concentration	Area # 68	Area # 72	Ratio (68/72)
PEAS21.D	0	0	1,248,161	6,130,857	0.204
PEAS22.D	1	0.2525	2,089,175	6,692,453	0.312
PEAS23.D	2	0.505	2,849,097	6,765,806	0.421
PEAS24.D	3	1.5151	5,381,510	6,350,853	0.847
				Amount	furan=0.48ug





- The concentrations between before and after centrifuge are 0.263 µg/ml and 0.193 µg/ml.

Witnessed & Understood by me, | Date 8/16/07 | Invented by: | Date

Recorded by:

8/28/07

Objective: To measure furan in solid corn sample

Procedures

1. Thaw the refrigerated corn sample
2. Take 50 g of corn sample and put into 225 ml plastic bottle containing 150 ml of water
3. Homogenize until the sample is unify
4. Transfer 20 g of sample into 4 headspace vials
5. Incubate in an ice bath for 5 minutes
6. Put 125 µl of ISTD into each vial
7. Use micropipette to transfer 25, 50 and 100 µl of 10.1 µg/ml into X_1, X_2 and X_3
8. Incubate in the 35°C water bath for 10 minutes
9. SPME extraction for 10 minutes
10. GC/MS analysis (FURAN.M): 55°C → 115°C(2) at5°C/min, increase to 115°C to 295°C (2.8) at 25°C/minute

Results and Discussions

- The ratio of X1 ≈ X2. A fresh furan working solution should be prepared.

Prepare furan and d4-furan working solution

- Use micropipette to transfer 200 µl of 1.1 mg/ml of d_4- furan into 20 ml of water. The concentration of d4-furan is (0.2/20.2) * 1.1 mg/ml = 10.9 µg/ml
- Use micropipette to transfer 100 µl of 2.04 mg/ml of furan standard solution into 20 ml of water. The concentration of furan is (0.1/20.1)*2.04 = 10.1 µg/ml

Witnessed & Understood by me,	Date	Invented by:	Date
	8/28/07	Recorded by:	

TITLE ______ Project No. ______ Book No. ______

From Page No. ____

9/4/07

Objective: To repeat the furan determination in corn solid sample

Procedures

1. Take 50g of sample into a 225 ml plastic bottle containing 150 ml of water
2. Homogenize until the texture is unified
3. Transfer 20 g of homogenized sample into 4 glass vials which contains a magnetic stirrer
4. Incubate in an ice bath for 5 minutes
5. Dilute spiked furan by using micropipette to take 1 ml of 10.1 ug/ml furan into a vial containing 2 ml of water (the concentration is 10.1/3 = 3.367 μg/ ml)
6. The 3.367μg/ml furan and 10.9 μg/ml d-4 furan are spiked as follow:
   - X0 : 20 g sample + 30μl of d4-furan
   - X1 : 20 g sample + 30μl of d4-furan + 50 μl of furan
   - X2 : 20 g sample + 30μl of d4-furan + 100 μl of furan
   - X3 : 20 g sample + 30 μl of d4-furan+ 200 μl of furan
7. Incubate in 35°C water bath for 10 minutes
8. SPME extraction for 10 minutes
9. GC/MS analysis (FURAN.M)

Results

	File name	*m/z*	Peak Area	Rt
X_0	CORN08.D	68	6090205	14.558
		39	6048310	14.556
		37	742763	14.555
		72	26158533	14.407
		42	26361475	14.405
		44	5451623	14.405
X_1	CORN09.D	68	11018873	14.569
		39	10851548	14.567
		37	1315326	14.566
		72	25548462	14.418
		42	26054180	14.416
		44	5211380	14.417
X_2	CORN10.D	68	14283037	14.568
		39	14097717	14.566
		37	1711769	14.566
		72	26542262	14.417
		42	27218543	14.415
		44	55142134	14.416
X_3	CORN11.D	68	18528923	14.567
		39	18086603	14.565
		37	2167122	14.567
		72	24094165	14.420
		42	25084209	14.418
		44	4886414	14.418

Witnessed & Understood by me, Yanan Dong

Date 9/4/07

Invented by:

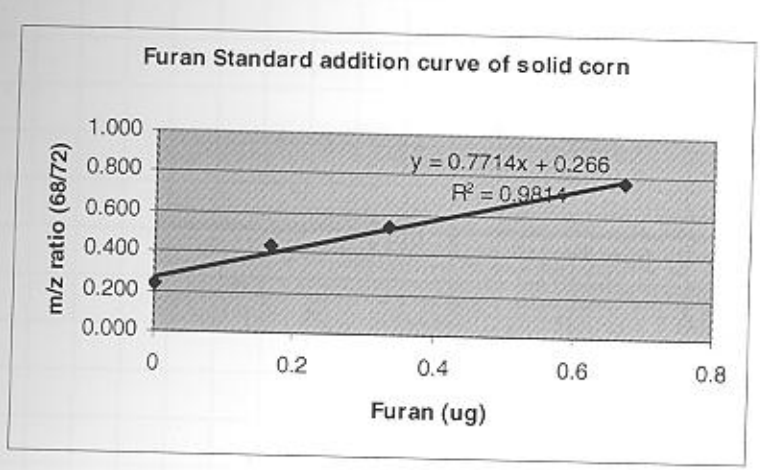
Recorded by:

Date

To Page No. ______

-Data Analysis

Sample	Date file	Level	amt added	Abundance Analyte	Abundance ISTD	ratio area
Corn Solid	CORN08.D	0	0	6090205	26158533	0.233
	CORN09.D	1	0.168	11018873	25548462	0.431
	CORN010.D	2	0.336	14283037	26542262	0.538
	CORN011.D	3	0.672	18528923	24094165	0.769
					Amount=0.34ug	



-The furan concentration is 0.34 µg in 20 g sample; Thus, The furan in solid corn is (0.34/20) * 4 = 0.068 ug/ g or 68 ng/g sample.

Witnessed & Understood by me,	Date	Invented by:	Date
Yanan Ding	9/4/07	Recorded by:	

9/5/07

Objective: -To use the developed method to measure furan concentration in Vitamin water Glaceau

- To use the developed method to measure furan in sliced carrot (brand Jewel)

Procedure

1. Use pipette to take out 20 ml of vitamin water into 4 headspace vials
2. Incubate in an ice bath for 5 minutes
3. Add 5 µl of 10.9 µg/ml into each vial
4. Make a 1 in 10 dilution of 10.1 µg/ ml furan working solution and add 50 µl, 100µl and 300µl into X_1, X_2 and X_3
5. Incubate in 35°C for 10 minutes
6. SPME extraction for 10 minutes
7. GC/MS analysis (FURAN.M)

Results

	File name	*m/z*	Peak Area	Rt
X_0	VITWAT4.D	68	418627	14.624
		39	420125	14.621
		37	ND	
		72	2110785	14.469
		42	2137504	14.464
		44	429166	14.462
X_1	VITWAT5.D	68	1338048	14.619
		39	1377674	14.614
		37	128897	14.620
		72	2471005	14.468
		42	2565817	14.468
		44	531606	14.468
X_2	VITWAT6.D	68	1810375	14.638
		39	1827406	14.635
		37	188464	14.629
		72	2169414	14.488
		42	2273111	14.485
		44	532251	14.480
X_3	VITWAT7.D	68	4034736	14.468
		39	4014747	14.645
		37	454083	14.649
		72	2058807	14.498
		42	1984072	14.497
		44	548853	14.497

Witnessed & Understood by me, Yanan Ren

Date 9/5/07

Invented by:

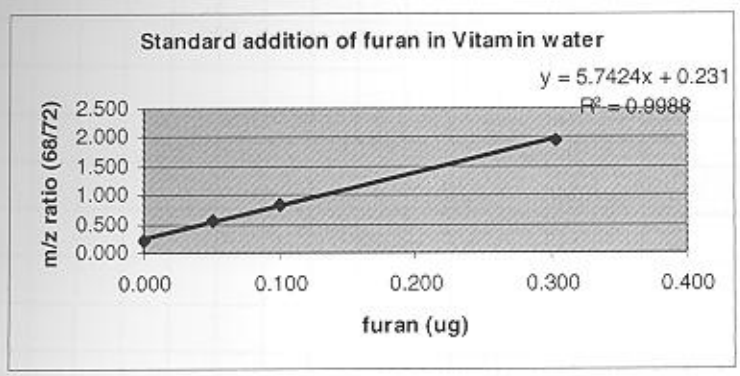
Recorded by:

Date

-Data Analysis

Sample	Date file	Level	amt added	Abundance Analyte	Abundance ISTD	ratio area
Vitamin water	Vitwat4.D	0	0.000	418627	2110785	0.198
	Vitwat5.D	1	0.051	1338048	2471005	0.541
	Vitwat6.D	2	0.101	1810375	2169414	0.834
	Vitwat7.D	3	0.303	4034736	2058807	1.960

Amount = 0.04ug



- The amount of furan in Vitamin enhance water is 2 ng/ml

Witnessed & Understood by me,	Date	Invented by:	Date
Yaran Dang	9/5/07	Recorded by:	

II.) Measure furan in sliced carrot (Jewel sliced carrot. Net wt. 14.502,411g. Company code : NSCA206. Lot number: 004026)

Procedures

1. Bring out refrigerated can carrot
2. Open the can and quickly drained the carrot juice into 225 ml plastic bottle. Keep the bottle refrigerated until it is to be analyzed for furan.
3. Take 50 g of solid carrot into a plastic bottle containing 150 ml of HPLC grade water
4. Homogenize while the bottle is kept cool in the ice bath
5. Transfer 20 g of homogenized sample into 4 headspace vials
6. Incubate the vials in an ice bath for 5 minutes
7. Add 30 µl of 10.9 µg/ml d_4-furan and 25, 50 and 100 µl of 5.05 µg/ml working furan solution (diluted working solution by taking 1 ml of furan into 1 ml of water)
8. Incubate the vial in a 35 °C water bath for 10 minutes
9. SPME extraction for 10 minutes
10. GC/MS analysis (FURAN.M)

Results and Discussions

	File name	*m/z*	Peak Area	Rt
X_0	CARROTS.D	68	6914638	14.616
		39	6977290	14.615
		37	797059	14.615
		72	19289239	14.467
		42	19384665	14.464
		44	3908828	14.467
X_1	CARROTS1.D	68	8285322	14.635
		39	8233876	14.633
		37	957925	14.633
		72	17522118	14.486
		42	17866433	14.483
		44	3497098	14.483
X_2	CARROTS2.D	68	8855796	14.649
		39	8814797	14.647
		37	1014305	14.649
		72	15932904	14.499
		42	16393870	14.497
		44	3109459	14.497
X_3	CARROTS3.D	68	13750520	14.652
		39	13635111	14.650
		37	1574234	14.648
		72	17225264	14.503
		42	17961201	14.501
		44	3411017	14.501

Witnessed & Understood by me,	Date	Invented by:	Date
Yuran Dang.	9/5/07	Recorded by:	

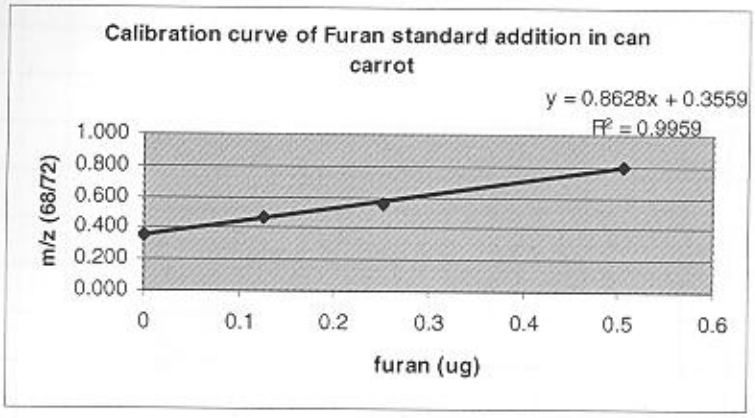
TITLE _____ Project No. _____

From Page No. ____ Book No. _____

- Data analysis

Sample	Date file	Level	amt added	Abundance Analyte	Abundance ISTD	ratio area
Solid carrot	CARROTS.D		0	6914638	19289239	0.358
	CARROTS1.D	1	0.126	8285322	17522118	0.473
	CARROTS2.D	2	0.252	8855796	15932904	0.556
	CARROTS3.D	3	0.505	13750520	17225264	0.798

Amount= 0.413ug



- The amount of furan in solid carrot is (0.413/20 g) * 4 (dilution factor) = 0.0826 µg/g or 82.6 ng/g

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
	9/5/07	Recorded by:	

9/6/07

Objective: To use the developed method to measure furan in carrot juice

Procedures

1. 20 ml of carrot juice was taken and transferred to 4 headspace vials
2. Incubate in an ice bath for 5 minutes
3. Put 75 µl of 10.9 µg/ml of ISTD into each headspace vial
4. Add 25, 50 and 100 µl of 10.1 µg/ml into X_1, X_2 and X_3
5. Incubate in a 35°C water bath for 10 minutes
6. SPME extraction for 10 minutes
7. GC/MS analysis (FURAN.M)

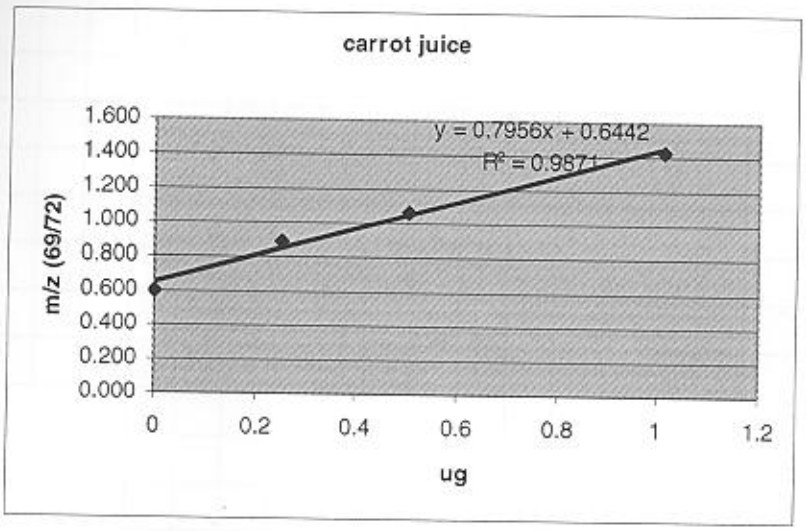
Results

	File name	*m/z*	Peak Area	Rt
X_0	CARROTJ1.D	68	23408006	14.628
		39	23142400	14.625
		37	2732290	14.624
		72	38937861	14.473
		42	19384665	14.471
		44	3908828	14.472
X_1	CARROTJ2.D	68	33708383	14.642
		39	33234983	14.639
		37	3966376	14.639
		72	37897855	14.492
		42	39768066	14.489
		44	7803521	14.490
X_2	CARROTJ3.D	68	36228215	14.650
		39	35725126	14.648
		37	4302910	14.647
		72	34007283	14.501
		42	36179380	14.499
		44	6929279	14.499
X_3	CARROTJ4.D	68	51671956	14.643
		39	50905797	14.641
		37	6828138	14.641
		72	36212565	14.497
		42	39658490	14.495
		44	7338044	14.495

Witnessed & Understood by me,	Date	Invented by:	Date
	9/6/07	Recorded by:	

- Data analysis

Sample	Date file	Level	amt added	Abundance Analyte	Abundance ISTD	ratio area
Carrot juice	CARROTJ1.D	0	0	23408006	38937861	0.601
	CARROTJ2.D	1	0.2525	33708383	37897855	0.889
	CARROTJ3.D	2	0.505	36228215	34007283	1.065
	CARROTJ4.D	3	1.01	51671956	36212565	1.427



- The amount of furan in 20 ml sample is 0.8097. Thus, the amount of furan per ml is 40 ng/ ml.

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

9/13/07

Objective: To use the developed method to measure furan in vitamin enhance water propel

Procedure

1. Thaw the bottle of propel that has been frozen overnight
2. Use pipette to place 20 ml of vitamin enhanced water into 4 headspace vials
3. Incubate in an ice bath for 5 minutes
4. Apply 2.18 µg/ml d_4-furan (Prepare by dilute d_4-furan by taking 1 ml of 10.9 µg/ml of d_4-furan into a vial containing 4 ml of water) and 1.01 µg/ml spiked furan (prepare by making 1/10 dilution by take 1 ml of 10.9 µg/ml into 9 ml of water)
    a. X0 : 20 ml + 25 µl ISTD
    b. X1 : 20 ml + 25 µl ISTD + 25 µl spiked furan
    c. X2 : 20 ml + 25 µl ISTD + 50 µl spiked furan
    d. X3 : 20 ml + 25 µl ISTD + 100 µl spiked furan

Results

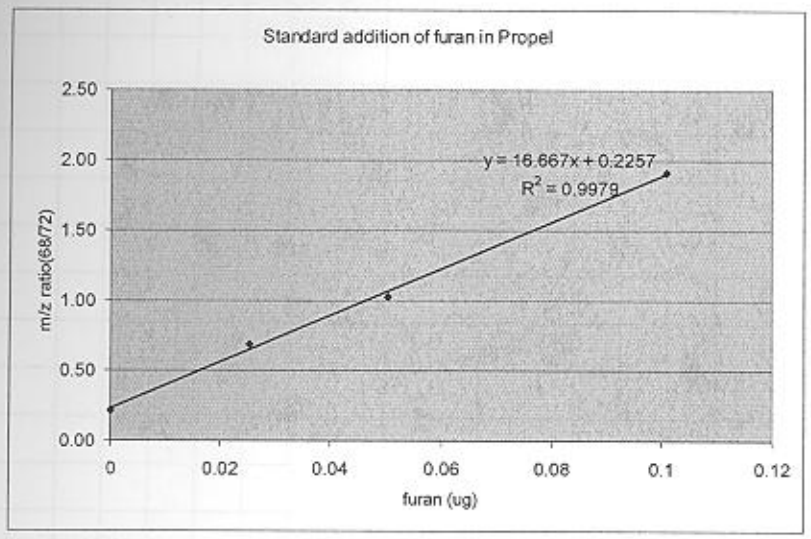
	File name	*m/z*	Peak Area	Rt
X_0	PROPEL01.D	68	267114	14.639
		39	298144	14.629
		37	22125	14.634
		72	1236349	14.482
		42	1258053	14.478
		44	236565	14.490
X_1	PROPEL04.D	68	668677	14.659
		39	690769	14.668
		37	68456	14.667
		72	974867	14.517
		42	1003754	14.516
		44	223200	14.517
X_2	PROPEL02.D	68	1044142	14.657
		39	1075817	14.657
		37	122905	14.657
		72	1015969	14.508
		42	1098390	14.506
		44	210423	14.502
X_3	PROPEL03.D	68	1711332	14.676
		39	1743420	14.675
		37	194512	14.672
		72	891721	14.526
		42	1009284	14.522
		44	201335	14.531

Witnessed & Understood by me,	Date	Invented by:	Date
yaran Dang	9/13/07	Recorded by:	

-Data analysis

Sample	Date file	Level	amt added	Abundance Analyte	Abundance ISTD	ratio area
Propel	Propel01.D	1	0	267144	1236349	0.22
	Propel04.D	2	0.025	668677	974867	0.69
	Propel02.D	3	0.0505	1044142	1015969	1.03
	Propel03.D	4	0.101	1711332	891721	1.92

Amount= 0.0135 µg



- The concentration calculated from graph is the x-intercept of the graph, which equals $|-0.2257/16.667| = 0.0135$ µg. The dilution factor is 1 (The sample wasn't diluted). Thus, the amount of furan in Propel is (0.00846/20 ml) * 1 (dilution factor) = 0.000677 or 0.677 ng/ml

Witnessed & Understood by me,	Date	Invented by:	Date
Yaran Ding	9/13/07	Recorded by:	

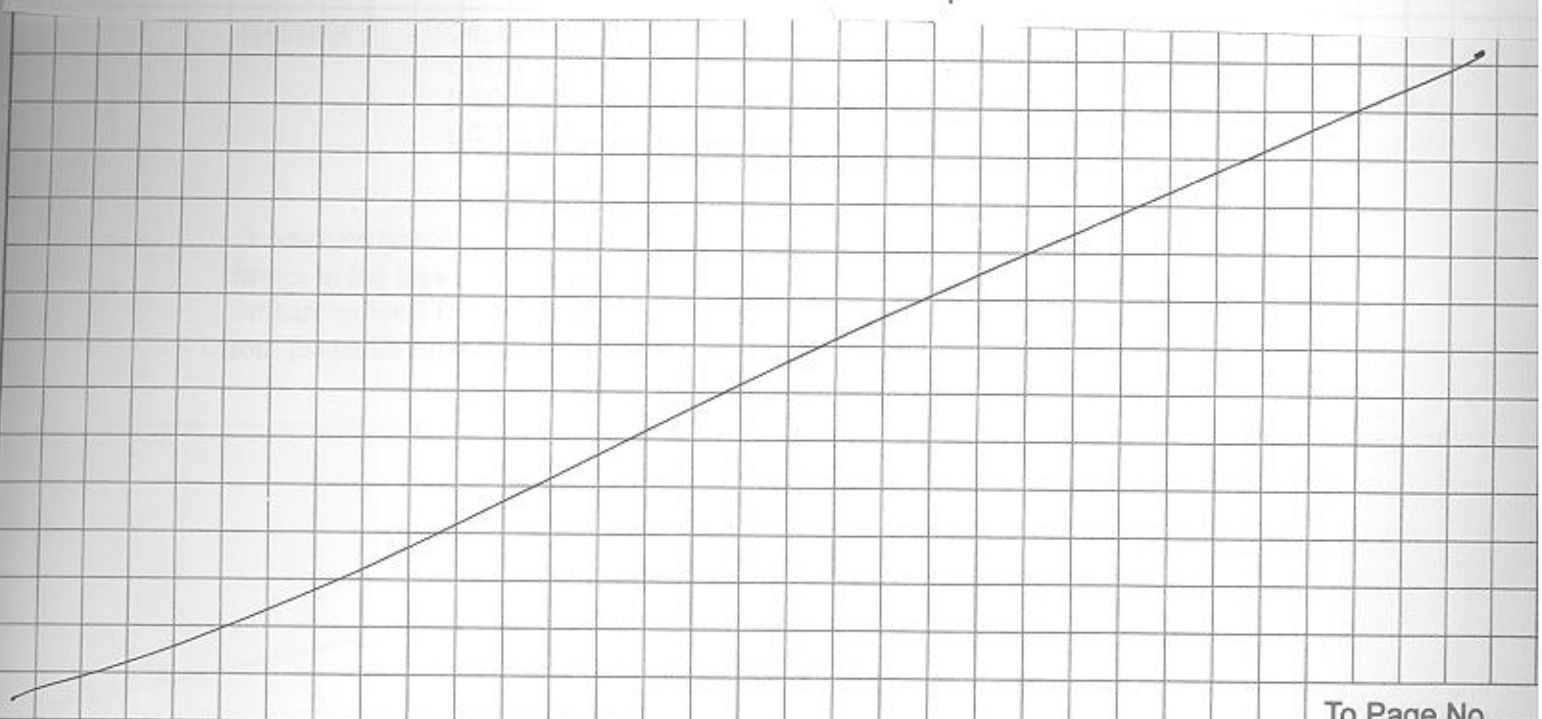
9/13/07

Objective: To use the developed method to measure can corn

Sample: Del Monte Whole kennel corn
Net Wt. 8 ¾ Oz (238 g)
Exp date Jan 31 08
Lot no. 5247P4

Procedures

1. Take out refrigerated can corn and separate corn liquor into a plastic bottle
2. Take out 50 g of corn solid into 225 ml plastic bottle containing 150 g of water
3. Homogenize the sample until it becomes smooth paste
4. Transfer 20 g of sample into 4 vials
5. Incubate in an ice bath for 5 minutes
6. Furan was diluted into 5.05 µg/ml by taking 1 ml of 10.1 µg/ml into a vial containing 1 ml of water. 10.9 µg/ml of d_4-furan was used as ISTD.
   X0 : 20 g sample + 30 µl ISTD
   X1 : 20 g sample + 30 µl ISTD + 25 µl of 5.05 µg/ml furan
   X2 : 20 g sample + 30 µl ISTD + 50 µl of 5.05 µg/ml furan
   X3 : 20g sample + 30 µl ISTD + 100 µl of 5.05 µg/ml furan
7. Vial was incubated in a 35 °C water bath for 10 minutes
8. SPME extraction for 10 minutes
9. GC/MS analysis (FURAN.M)



Witnessed & Understood by me,	Date	Invented by:	Date
yaman Dedy	9/13/07	Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

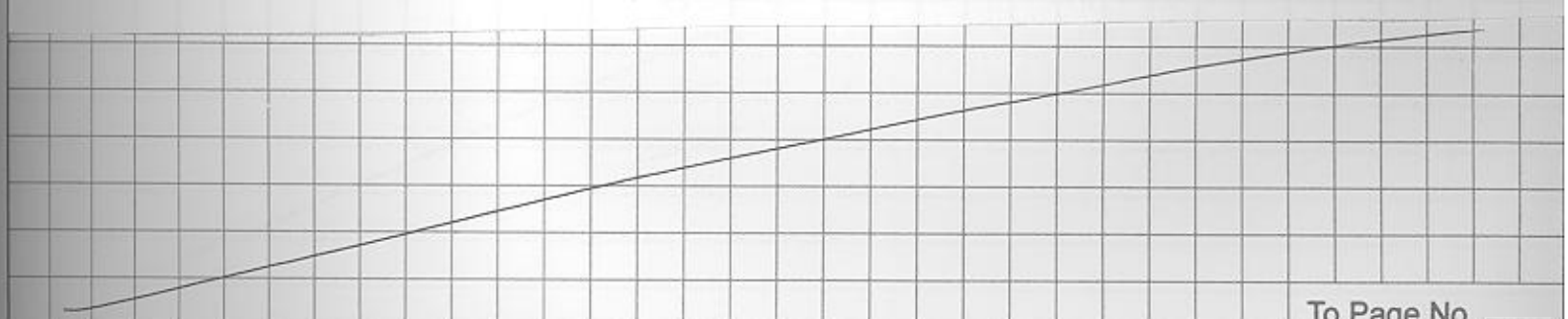
Results and Discussions

	File name	*m/z*	Peak Area	Rt
X_0	DELCORNS.D	68	4199590	14.682
		39	4124401	14.629
		37	476279	14.615
		72	5945273	14.486
		42	6152170	14.484
		44	1272220	14.484
X_1	DELCORNS1.D	68	4157886	14.648
		39	412007	14.645
		37	465796	14.646
		72	5492830	14.500
		42	5693272	14.498
		44	1143207	14.499
X_2	DELCORNS2.D	68	5710581	14.650
		39	641161	14.649
		37	5635927	14.649
		72	6465622	14.505
		42	6746733	14.504
		44	1365845	14.503
X_3	DELCORNS3.D	68	7012504	14.645
		39	789198	14.644
		37	6907973	14.644
		72	6701126	14.499
		42	711790	14.497
		44	1344327	14.498

- Data analysis

corn solid	DELCORNS.D	0	0	4199590	5945273	*0.706*
	DCORNS1.D	1	0.126	4157886	5492830	*0.757*
	DCORNS2.D	2	0.252	5710581	6465622	0.883
	DCORNS3.D	3	0.505	7012504	6701126	1.046

- It could be possible that there is a loss of furan during dilution of furan because the change in the area is very little (from 0.706 to 0.757). Moreover, the area read in level 1, compare to level 0 is about the same, which may cause a bias in the concentration derived from the calibration curve.



To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
Yaran Dong	9/13/07	Recorded by:	

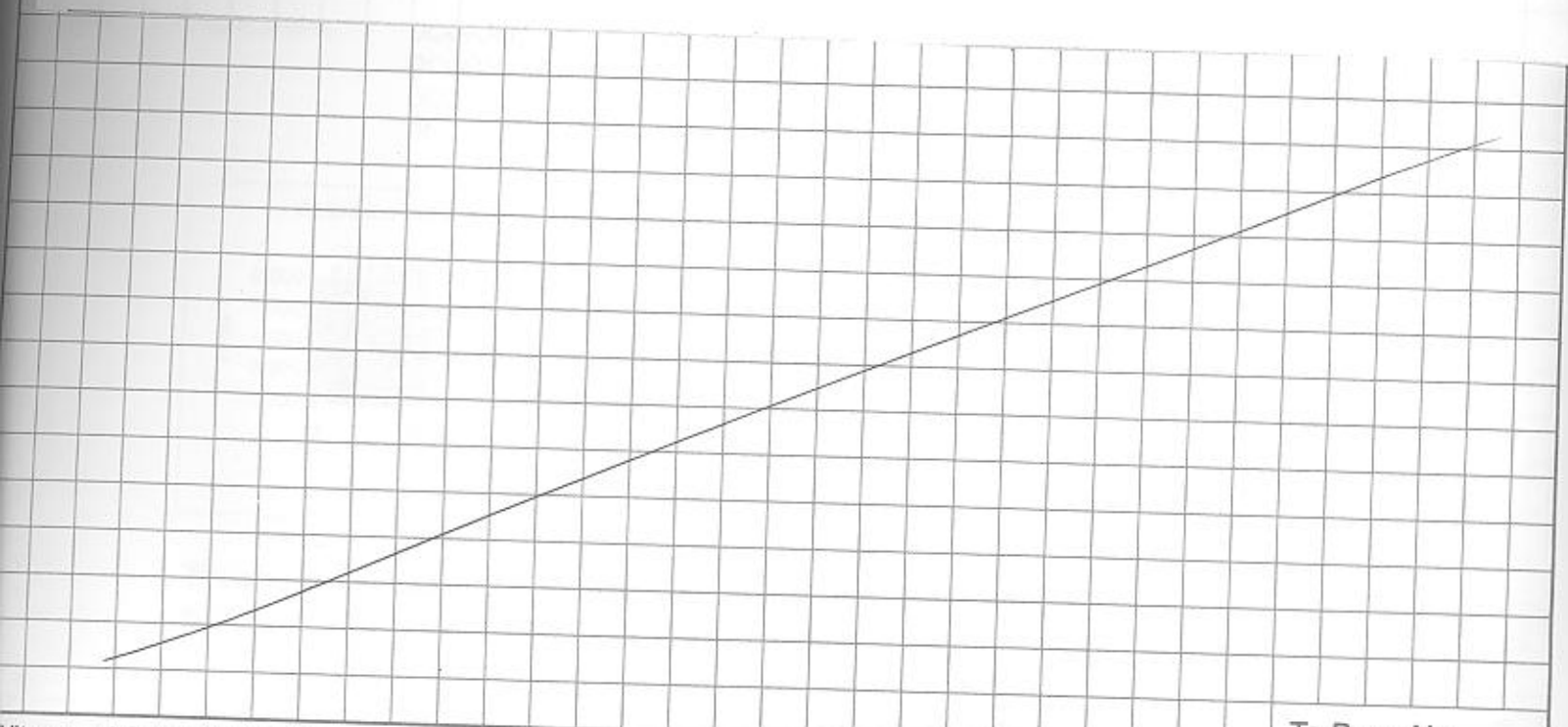
9/17/07

Objective: To measure furan in Del Monte corn Juice

Std. d_4-furan concentration: 10.9 µg/ml
Std furan working solution : 10.1 µg/ml

Procedures

1. Take 10 ml of corn juice into a headspace vial containing 10 ml of water. Prepare 4 test portions
2. Incubate in ice for 5 minutes
3. Each vial were fortified with d_4-furan and furan –
   X0 : 20 ml + 50 µl ISTD
   X1 : 20 µl + 50 µl ISTD + 25 µl spiked furan
   X2 : 20 µl + 50 µl ISTD + 50 µl spiked furan
   X3 : 20 µl + 50 µl ISTD + 100 µl spiked furan
4. Incubate the vials in 35 °C water bath for 10 minutes
5. SPME extraction for 10 minutes
6. GC/MS analysis (FURAN.M)



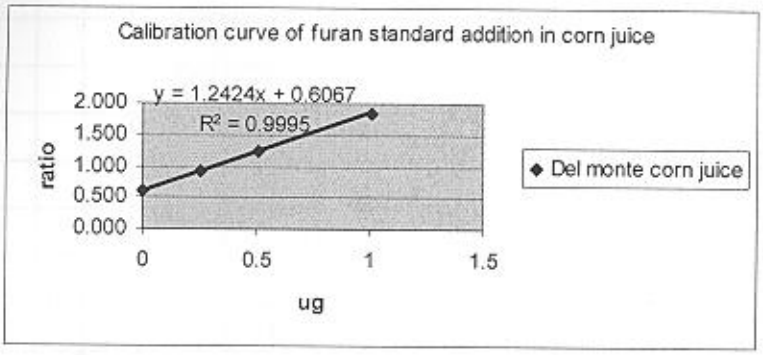
Witnessed & Understood by me, Yuan Ding | Date 9/17/07 | Invented by: | Recorded by: | Date

Results

	File name	*m/z*	Peak Area	Rt
X_0	DELCORNJ.D	68	4313690	14.583
		39	4327532	14.582
		37	500224	14.582
		72	7245706	14.438
		42	7537528	14.435
		44	1481722	14.435
X_1	DELCORNJ1.D	68	6752697	14.597
		39	6658863	14.595
		37	780675	14.594
		72	7286452	14.452
		42	7713874	14.450
		44	1492050	14.451
X_2	DELCORNJ2.D	68	9252422	14.605
		39	9069263	14.604
		37	1071174	14.603
		72	7417069	14.461
		42	7144354	14.459
		44	1538919	14.461
X_3	DELCORNJ3.D	68	1366494	14.625
		39	13407852	14.624
		37	1071174	14.623
		72	7417069	14.483
		42	7144354	14.481
		44	1538919	14.484

- Data analysis

Del monte corn corn JUICE	DCORNJ.D	0	0	4313690	7245706	0.595
	DCORNJ1.D	1	0.2525	6752697	7286452	0.927
	DCORNJ2.D	2	0.505	9252422	7417069	1.247
	DCORNJ3.D	3	1.01	13666494	7374053	1.853



- The amount of furan in corn juice is 0.488 µg in 20 ml sample that has been diluted by the factor of 2. Thus the amount of furan in corn juice is (0.488/20) * 2 = 48.8 ng/ml.

Witnessed & Understood by me,	Date	Invented by:	Date
Yaron Deng	9/17/07	Recorded by:	

TLE Headspace autosampler setup. Project No. _____ Book No. _____

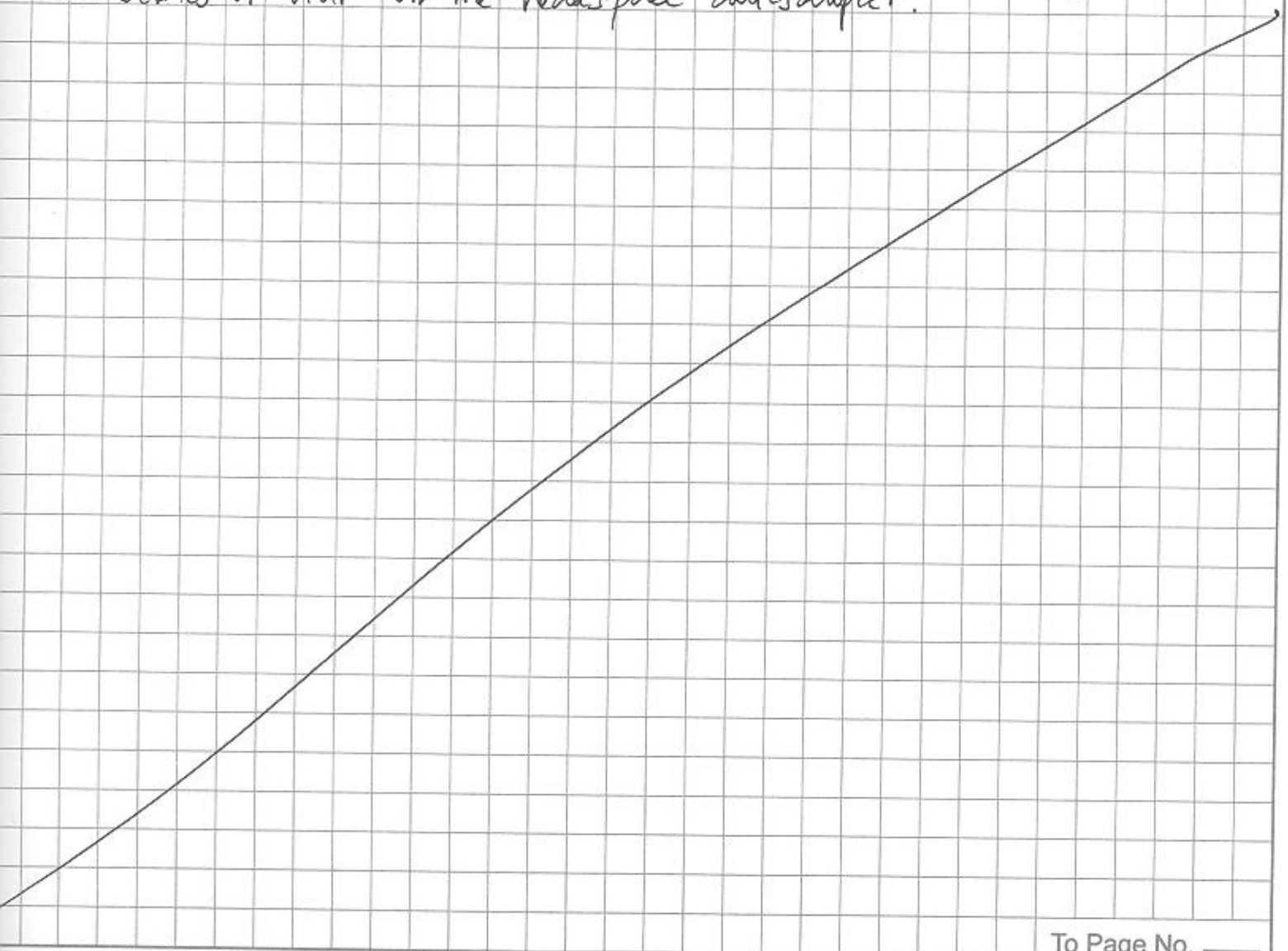
om Page No. ____

Objective : To set up a sequence when using headspace autosampler machine

Procedures

— For Chemstation program —

1. Load method (Furan.M)
2. Go to GC/MS top
   a) Click sequence. Load sequence (eg. test.s)
   b) Edit sample log table, such as name of sample, vial, give file name
3. Save the sequence (In my data pathway (File/set data path)
4. Load and Run sequence
5. Use the set up sequence when run autosampler to automatically run series of vial on the headspace autosampler.



To Page No. ____

essed & Understood by me,	Date	Invented by:	Date
mm Dai	9/24/07	Recorded by:	

9_27_07

Objective: - Prepare d4-furan ISTD and furan working solution
- To measure the repeatability of analytical method using milk sample and bean sample

Procedures

I. Prepare 10.9 ug/ml of d4furan by using micropipette to take out 200 ul of 1.1 mg/ml into 20ml of HPLC grade water

II. Prepare 10.1 ug/ml spiked furan working solution by adding 100ul of 2.04 mg/ml into 20 ml of water

III. Prepare milk sample by pipette 20 ml of milk into a vial and determine the ISTD and spiked furan need to be added

IV. Prepare bean sample by adding 50g of bean sample (cold) into 150 ml of HPLC grade water (no separation of bean liquor due to thickness of sample). Homogenize. Then, distribute 20ml of sample into four vials.
- Incubate in ice for 5 mins
- Add 75ul ISTD and 0, 25,50 and 100 to X0, X1,X2 and X3 vial respectively
-Incubate at 35°C for 10mins
- SPME extraction for 10mins
- GC/MS analysis

Results

I.) Milk sample

(show chromatogram)

Sample	Linear regression equation	Amount of furan
Dean's 2% reduced fat milk Best by 10/01/07 Manu. ID 1738 19:22 113 Size: ½ Gal (1.89L)	-	ND

II.) Bean sample.
Sample: Bean. (Brand: Joan of Arc; Greatest northern bean)
Exp. Date. May 05, 2011
Net Wt. 15.502 (439 g)
Manufacturure ID. F226A2 0733

	File name	*m/z*	Peak area	Rt.
X_0	BEAN02.D	68	4699075	14.700
		39	4600790	14.696
		37	753820	14.700
		72	6387752	14.545
		42	7059237	14.542
		44	1383713	15.544
X_1	BEAN03.D	68	6957891	14.716
		39	6865107	14.714
		37	1331404	14.714
		72	6945159	14.566
		42	7350756	14.563
		44	1409471	14.560
X_2	BEAN04.D	68	9604200	14.710
		39	9449038	14.709
		37	1567517	14.708
		72	6712224	14.503
		42	7353074	14.560
		44	1362320	14.560
X_3	BEAN05.D	68	14145388	14.703
		39	13921464	14.701
		37	2338241	14.702
		72	7118741	14.563
		42	6725028	14.561
		44	1446761	14.560

essed & Understood by me, Date 9/27/07 Invented by: Date
Recorded by:

Project No. _____

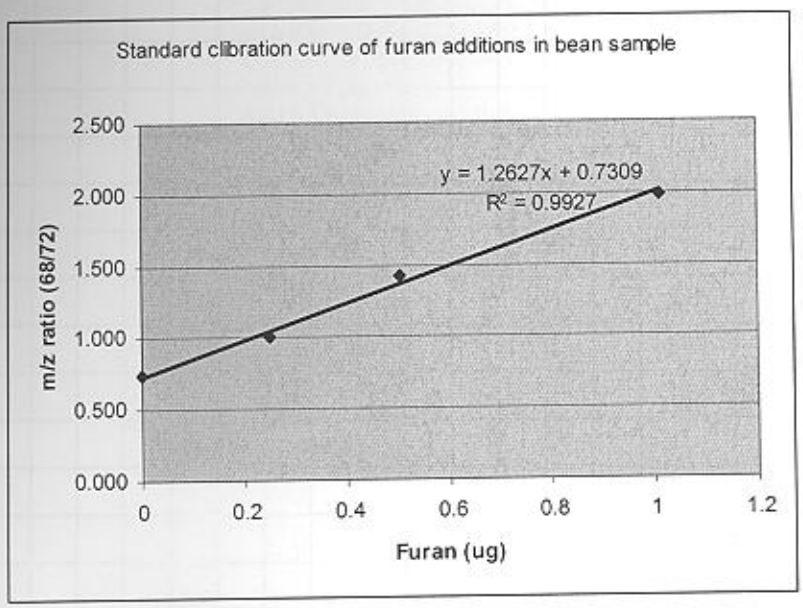
TITLE _____ Book No. _____

From Page No. ____

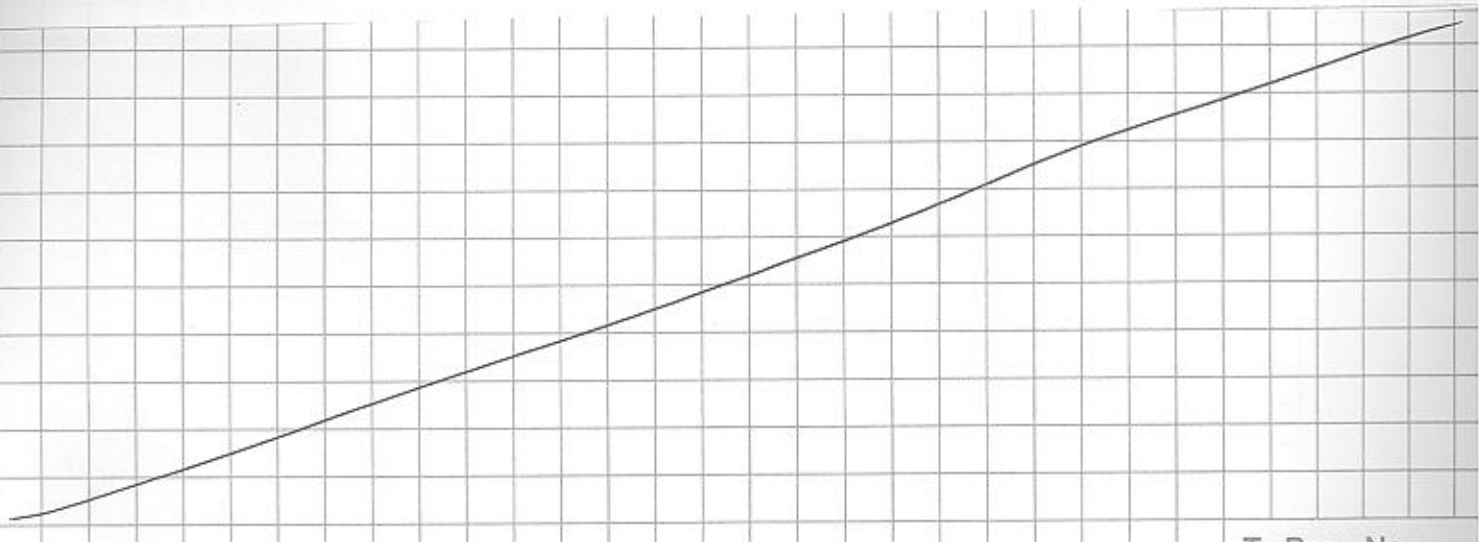
- Data analysis

9/27/2007

Sample	Date file	Level	amt added	Abundance Analyte #68	Abundance ISTD #72	ratio area
Bean	BEAN02.D	0	0	4699075	6387752	0.736
	BEAN03.D	1	0.2525	6957891	6945159	1.002
	BEAN04.D	2	0.505	9604200	6712224	1.431
	BEAN05.D	3	1.01	14145388	7118741	1.987



-The furan content in bean sample is 115.8 ng/g



To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
Yuan Ding	9/27/07	Recorded by:	

Project No. _____

TITLE To use the developed method to measure furan in food Book No. _____

From Page No. ____

Objective. To repeat the measurement of furan in bean sample

Procedure.

1. Take out the refrigerated canned bean.
2. ~~Sim~~ 40. The bean sample is thick. The liquid and solid portion were not separated, 50 g of ~~water~~ 7D. sample was put into a plastic bottle containing 150 ml of water.
3. Homogenize.
4. Transfer 20 ml g of sample into 4 vials containing magnetic stirer. Incubate in ice for 5 minutes and spike 125 µl of 10.9 ~~µg~~/ml d_4-furan ISTD. Label $X_0 - X_3$
5. Add 0, 25, 50, and 100 into X_0, X_1, X_2 X_3 respectively of spiked furan 10.1 µg/ml
6. Incubate in 35°C waterbath for 10 mins. SPME extraction for 10 minutes
7. GC/MS analysis (Furan. M)

Results and Discussion

	File name	m/z	Peak area	Rt.
X_0	BEAN06.D	68	3338637	14.644
		39	3316483	14.643
		37	554960	14.642
		72	10872296	14.482
		42	11053573	14.479
		44	2244912	14.481
X_1	BEAN07.D	68	5935257	14.664
		39		
		37	986352	14.660
		72	12080992	14.503
		42	12410072	14.501
		44	2482974	14.502
X_2	BEAN08.D	68	8463708	14.668
		39	8311740	14.667
		37	1399957	14.668
		72	12390086	14.512
		42	12880001	14.501
		44	2542689	2542689
X_3	BEAN09.D	68	13764315	14.651
		39	13528691	14.649
		37	2290889	14.646
		72	13624325	14.500
		42	14499672	14.497
		44	2793474	14.499

To Page No. ____

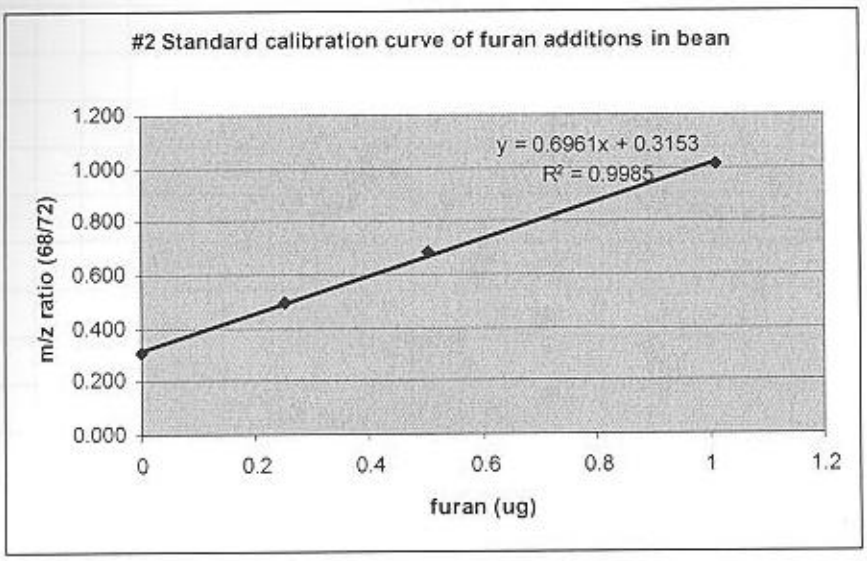
Witnessed & Understood by me,	Date	Invented by:	Date
Yanan	9/28/07	Recorded by:	

- Data analysis

9/28/2007

Sample	Date file	Level	amt added	Abundance Analyte #68	Abundance ISTD #72	ratio area
Bean	BEAN06.D	0	0	3338637	10872296	0.307
	BEAN07.D	1	0.2525	5935257	12080992	0.491
	BEAN08.D	2	0.505	8463708	12390086	0.683
	BEAN09.D	3	1.01	13764315	13624325	1.010

0.4529ug



- The amount of furan in bean sample is 90.6 ng/g. Thus, the average mean of 2 time analysis is(115.8 +90.6)/2 = 103.2 ng/g. The standard deviation is 12.6.

Witnessed & Understood by me,	Date	Invented by:	Date
Yaren Deng	9/28/07	Recorded by:	

Project No. _____

TITLE _____ Book No. _____

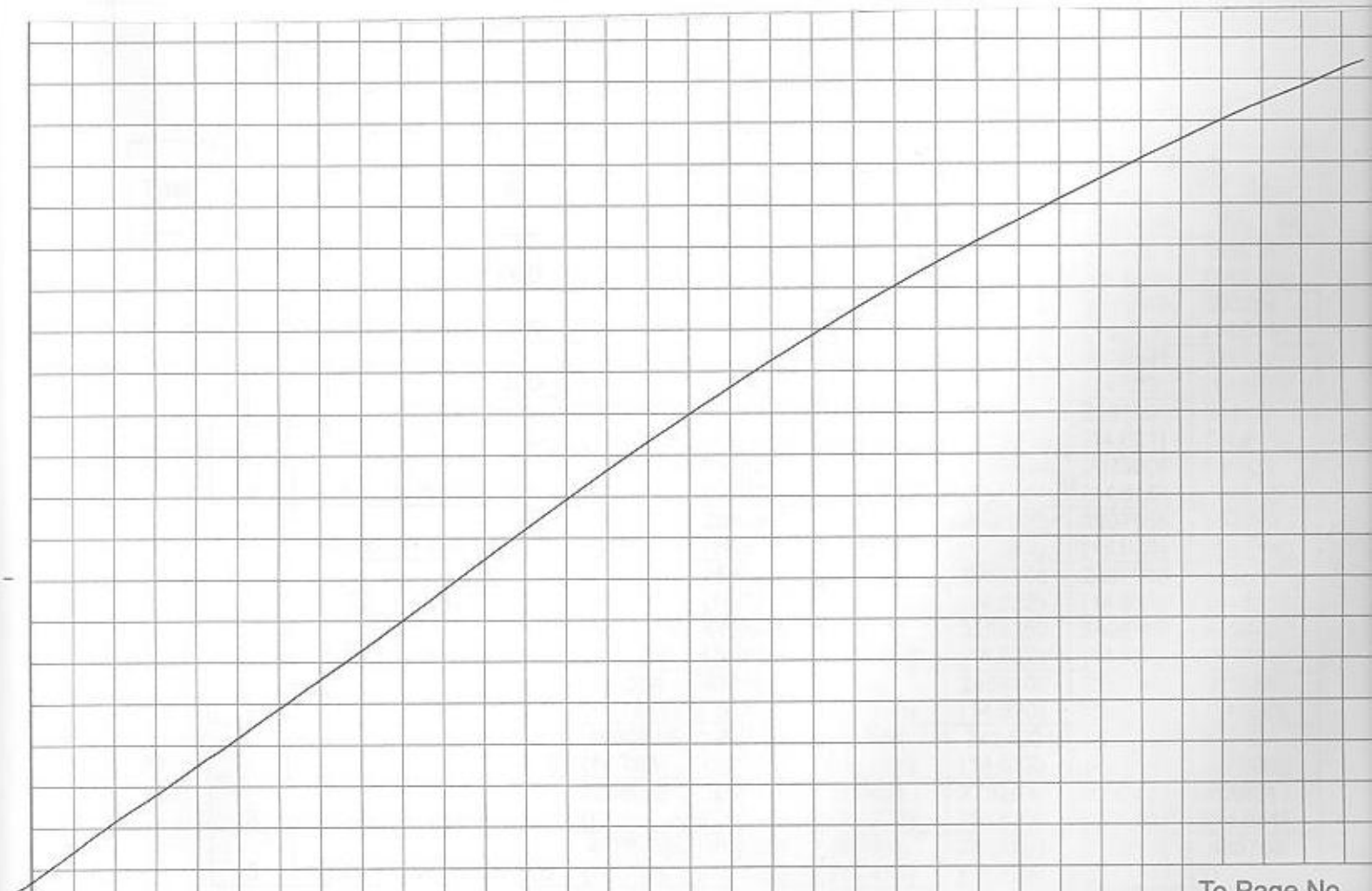
From Page No. ____

10/1/2007

Objective: To set up a Pal system of the auto sampler to measure furan according to the method developed using manual injection

Procedure

1. Parameters for SPME analysis are set as follow
    a. Choose method (a. SPME b. GC HS)
    b. Choose SPME method
    c. Set agitation speed : 400 rpm
    d. Agitation on time: 5 sec
    e. Agitation off time: 2 sec
    f. Injector Penetration 45mm (standard)
    g. Desorption
    h. Where: GC injector port
    i. GC run time: 25 mins



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yaman	10/1/07	Recorded by:	

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10/12/07

Objective: Test the repeatability of sample preparation and furan detection method
Product : UHT gossner whole milk

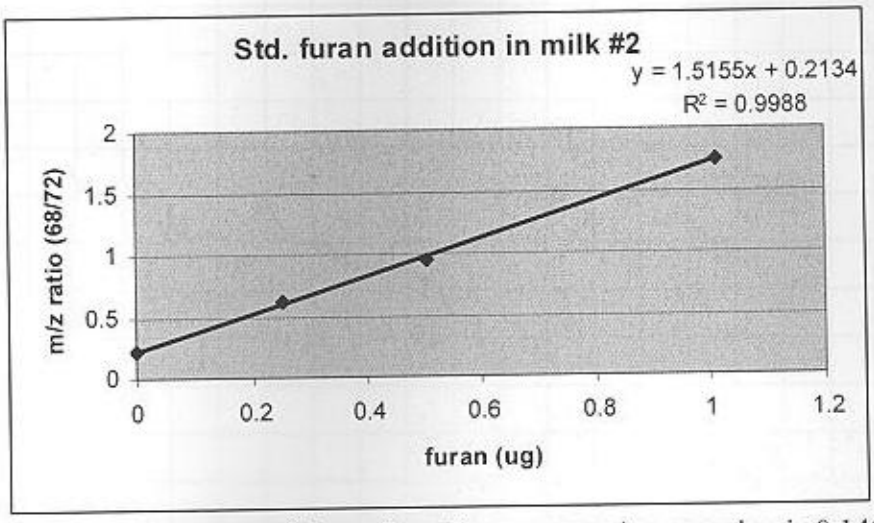
Procedures

1. Refrigerate the milk in the fridge for 1 hour
2. Keep the vial and glass bottle in ice
3. Condition the fiber at 295°C for 30 mins
4. Pipette 20 ml of milk into four 40ml headspace vials containing stir bar
5. Put in icebath for 5 mins
6. Add 50ul of d4 furan internal standard ~~5~~ 10 10-1
7. Add 0 ul, 25ul, 50ul and 100ul of ~~10.9~~ ul furan working solution into each vial
8. Incubate at 35°C for 10 mins
9. SPME extraction for 10 mins
10. GC/MS using method Furan.M

Results

Trial	level	conc. (ug)	Data file	Analyte furan (work std conc.= 10.9ug/ml)			Analyte *d4*-furan (conc. added = 10.9ug/ml)		
				m/z: 68	m/z : 39	m/z : 37	m/z : 72	m/z : 42	m/z : 44
#1	0	0	WHMILK4.D	723696 (14.746)	549629 (14.769)	86370 (14.749)	3509947 (14.593)	2697999 (14.593)	598718 (14.588)
	1	0.2525	WHMILK5.D	2236329 (14.768)	1647385 (14.762)	277232 (14.675)	3541572 (14.615)	2840949 (14.613)	555854 (14.615)
	2	0.505	WHMILK6.D	2404676 (14.776)	1782541 (14.773)	301557 (14.767)	2411966 (14.626)	2006541 (14.623)	375942 (14.627)
	3	1.01	WHMILK7.D	6114340 (14.768)	4514478 (14.766)	793103 (14.764)	3256591 (14.623)	238199 (14.621)	514176 (14.618)
#2	0	0	WHMILK8.D	735337 (14.771)	537624 (14.773)	83154 (14.768)	~~3464804~~ (14.618)	2673008 (14.616)	542438 (14.615)
	1	0.2525	WHMILK9.D	2180756 (14.784)	2546904 (14.773)	264740 (14.773)	~~3669185~~ (14.623)	2997916 (14.620)	588951 (14.619)
	2	0.505	WHMILK10.D	3478955 (14.774)	2546904 (14.773)	438921 (14.773)	3669185 (14.623)	2997916 (14.620)	588951 (14.619)
	3	1.01	WHMILK11.D	5705616 (14.760)	4168936 (14.765)	707682 (14.760)	3253359 (14.622)	2426589 (14.620)	509040 (14.628)
#3	0	0	WHMILK12.D	594384 (14.784)	433348 (14.778)	70801 (14.774)	2958100 (14.630)	2283300 (14.628)	467051 (14.630)
	1	0.2525	WHMILK13.D	1990945 (14.783)	1457150 (14.781)	246885 (14.778)	3424004 (14.632)	2718180 (14.632)	532633 (14.630)
	2	0.505	WHMILK14.D	2588092 ()	1846348 (14.777)	313402 (14.773)	2752647 (14.629)	2230262 (14.628)	458861 (14.624)
	3	1.01	WHMILK15.D	4776665 (14.758)	3449551 (14.752)	595416 (14.755)	2552667 (14.609)	1962167 ()	405758 ()

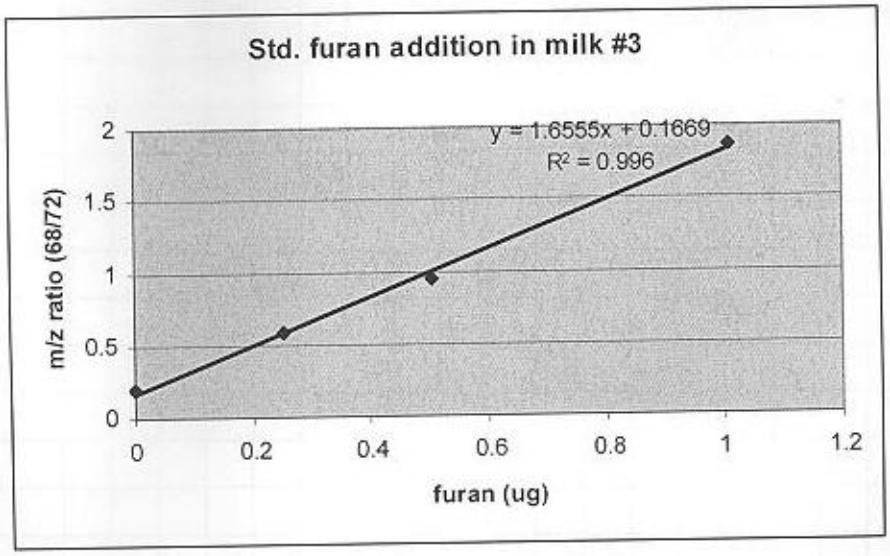
yuan ning | 10/12/07 | Recorded by:



From graph, amount of furan from linear regression equation is 0.1408 ug per 20 ml milk sample. Thus, amount of furan is 0.00704 ug/ml or 7.04ng/ml

#Trail 3

File name	level	Conc. (ug)	m/z=68	m/z=72	m/z ratio (68/72)
WHMILK12.D	X0	0	594384	2958100	0.200934384
WHMILK13.D	X1	0.2525	1990945	3424004	0.581466902
WHMILK14.D	X2	0.505	2588092	2752647	0.94021936
WHMILK15.D	X3	1.01	4776665	2552667	1.871244859



From graph, amount of furan from linear regression equation is 0.100 ug per 20 ml milk sample. Thus, amount of furan is 0.005 ug/ml or 5.ng/ml.

Witnessed & Understood by me,	Date	Invented by:	Date
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10/15/07 (Continue from 10/12/07)

Objective: To measure the repeatability of furan analysis method

Procedure

1. Condition the fiber at 295°C for 30 minutes
2. Take the milk sample out of the fridge. Let it sit in water until the milk defrosts.
3. Take four glass vials and let them sit in an ice bath
4. Put magnetic stirrer into the glass vial and use pipette to take out 20 ml into each glass vial.
5. Incubate in ice for 5 minutes
6. Put in 50 ul of internal standard (d4-furan)
7. Label X0, X1, X2 and X3 and add 0ul, 25ul, 50ul and 100 ul of 10.1 ug/ml furan working solution respectively.
8. Incubate 35°C for 10 minutes
9. SPME extraction for 10 minutes
10. GC/MS analysis using Furan.M

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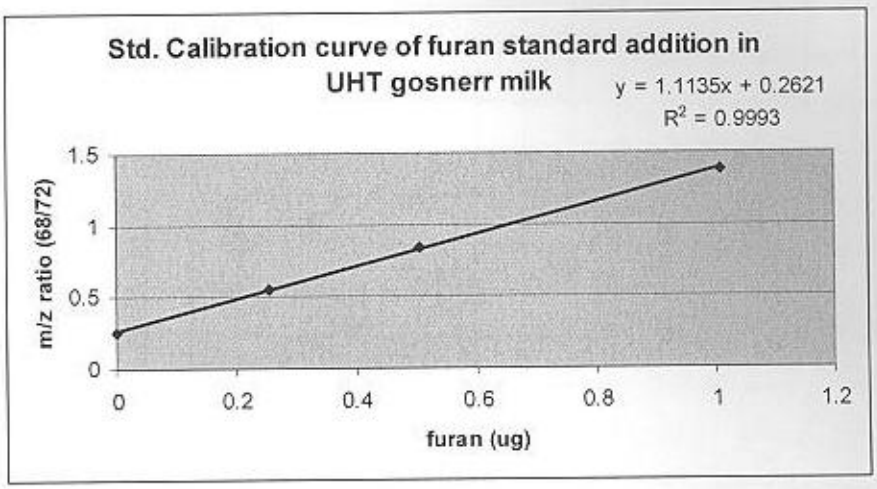
Trial #4

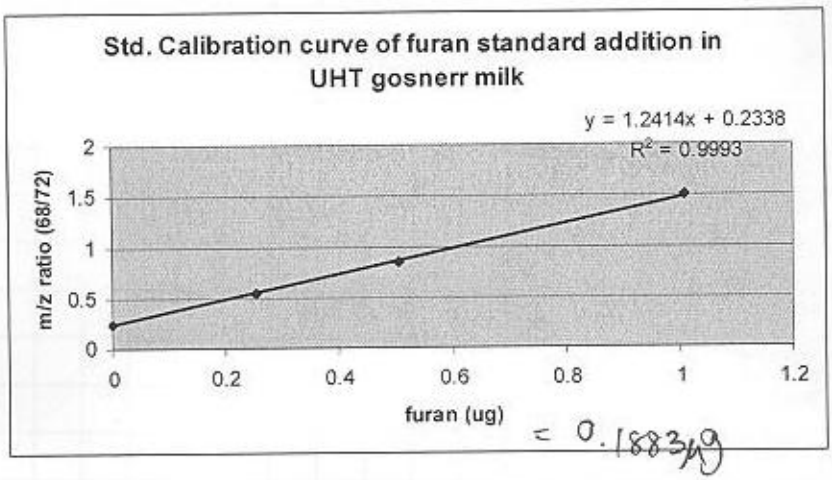
File name	Level	Conc.	m/z=68	m/z=72	ratio (68/72)
WHMILK16.D	X0	0	709099	2839673	0.249711
WHMILK18.D	X1	0.2525	1665084	3029145	0.549688
WHMILK17.D	X2	0.505	2193862	2613291	0.839502
WHMILK19.D	X3	1.01	3803982	2761424	1.377544



From the calibration curve, the amount of furan in milk is 0.2352 ug in 20 ml sample. Thus, the amount of furan in UHT milk sample is 11.769 ng/ml

Trial #5

File name	Level	Conc.	m/z=68	m/z=72	Ratio (68/72)
WHMILK20.D	X0	0	545578	2205162	0.247409
WHMILK21.D	X1	0.2525	1632522	3019445	0.54067
WHMILK22.D	X2	0.505	2376560	2818484	0.843205
WHMILK23.D	X3	1.01	3940112	2630387	1.497921



= 0.188349

= 9.417 ng/ml

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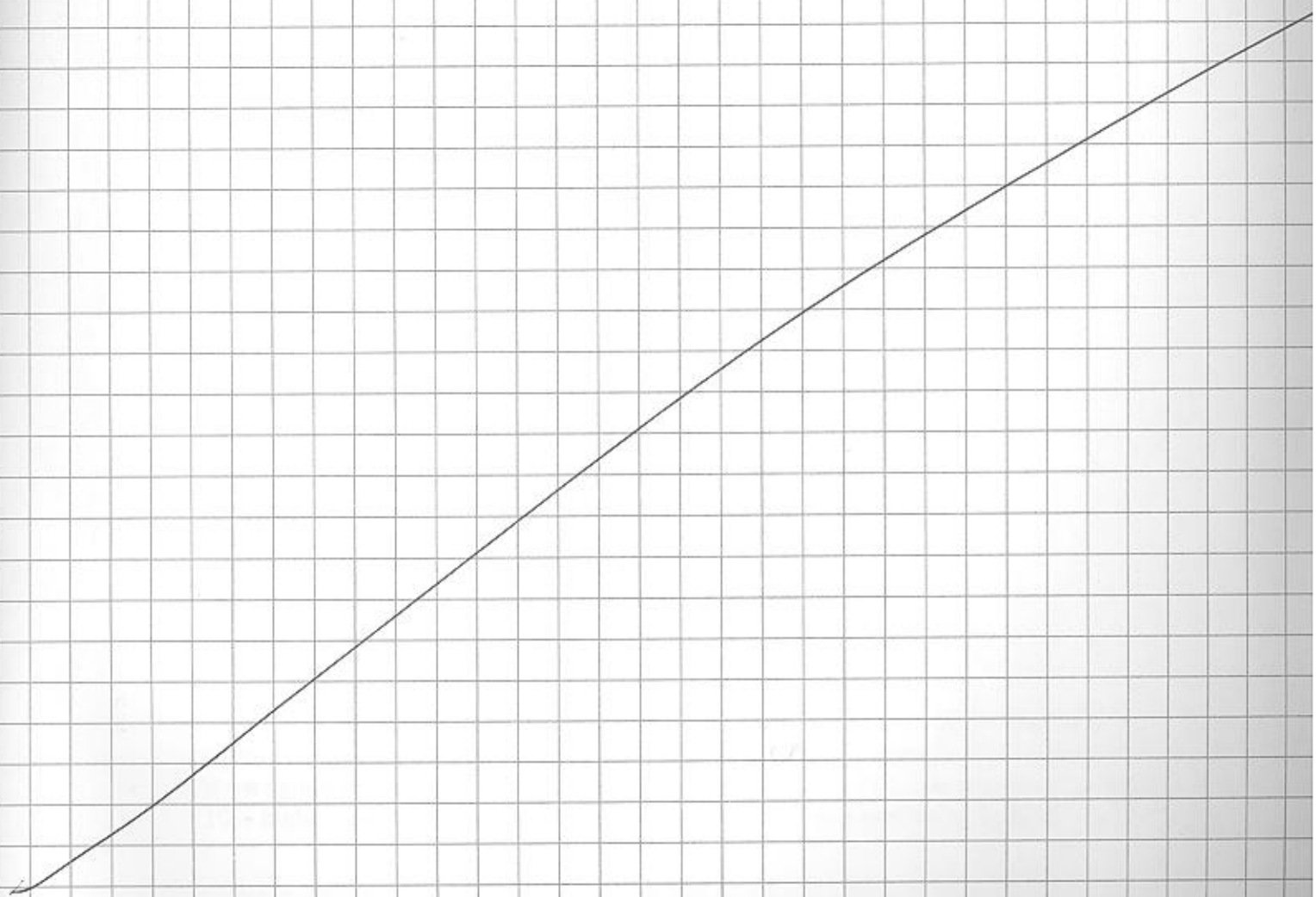
Discussion

From the results, the amount of furan detected in UHT milk in 5 time measurements are 6.07, 7.04, 11.47, 9.417 ng/m respectively. The results show considerably variation but should be because of ~~the~~ 4D. it has low level of furan. Moreover, ~~the~~ milk has distribution of lipid over ~~the homogenous~~ per carton. Therefore, the variation might be due to the unequal distribution of lipi ~~the~~ D. during different day of analysis.



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yeran Ding	10/15/07	Recorded by:	

10/17/07

Objective: To check the repeatability in prepared 10 ng/ml furan in water

Procedure:

1. Take 500 µl of 10.1 µg/ml into 500 ml of HPLC water to make 10ng/ml of furan solution
2. Transfer 20 ml of 10.1ng/ml into 4 vials
3. Analysis for furan by incubate in ice for 5 minutes and spiked 10.1 µg/ml furan standard working solution and 10.9 µg/ml d4-furan working solution as followed:
   X0 20 ml + 25 µl ISTD
   X1 20 ml + 25µl ISTD + 25µl spiked furan
   X2 20 ml + 25 µl ISTD + 50 µl spiked furan
   X3 20 ml + 25 µl ISTD + 100µl spiked furan
4. Incubate in 35°C water bath for 10 minutes
5. SPME extraction for 10 minutes
6. GC/MS analysis
7. Do a duplicate analysis of furan solution by repeating step 2 to step 6

Results

#1

File name	Level	Conc.	m/z=68	m/z=72	ratio (68/72)
KNOWNH20.D	X0	0	1293459	6190010	0.208959
KNH201.D	X1	0.2525	2743453	6141111	0.446736
KNH202.D	X2	0.505	4754082	6284503	0.756477
KNH203.D	X3	1.01	7741822	6678493	1.159217

#2

File name	Level	Conc.	m/z=68	m/z=72	ratio (68/72)
KNH204.D	X0	0	1233825	6099123	0.202295
KNH205.D	X1	0.2525	2628839	6189766	0.424707
KNH206.D	X2	0.505	4081700	6222357	0.655973
KNH207.D	X3	1.01	4646565	4199378	1.106489

Discussion

The amount of furan detected is 11.776 ng/ml. The amount of furan is 11.22 ng/ml respectively for trial 1 and 2. The mean of the measurement is 11.49. The result shows good repeatability with the standard deviation of 11.498. The amount detected was 10% higher than the prepare standard may result from the pipetting technique.

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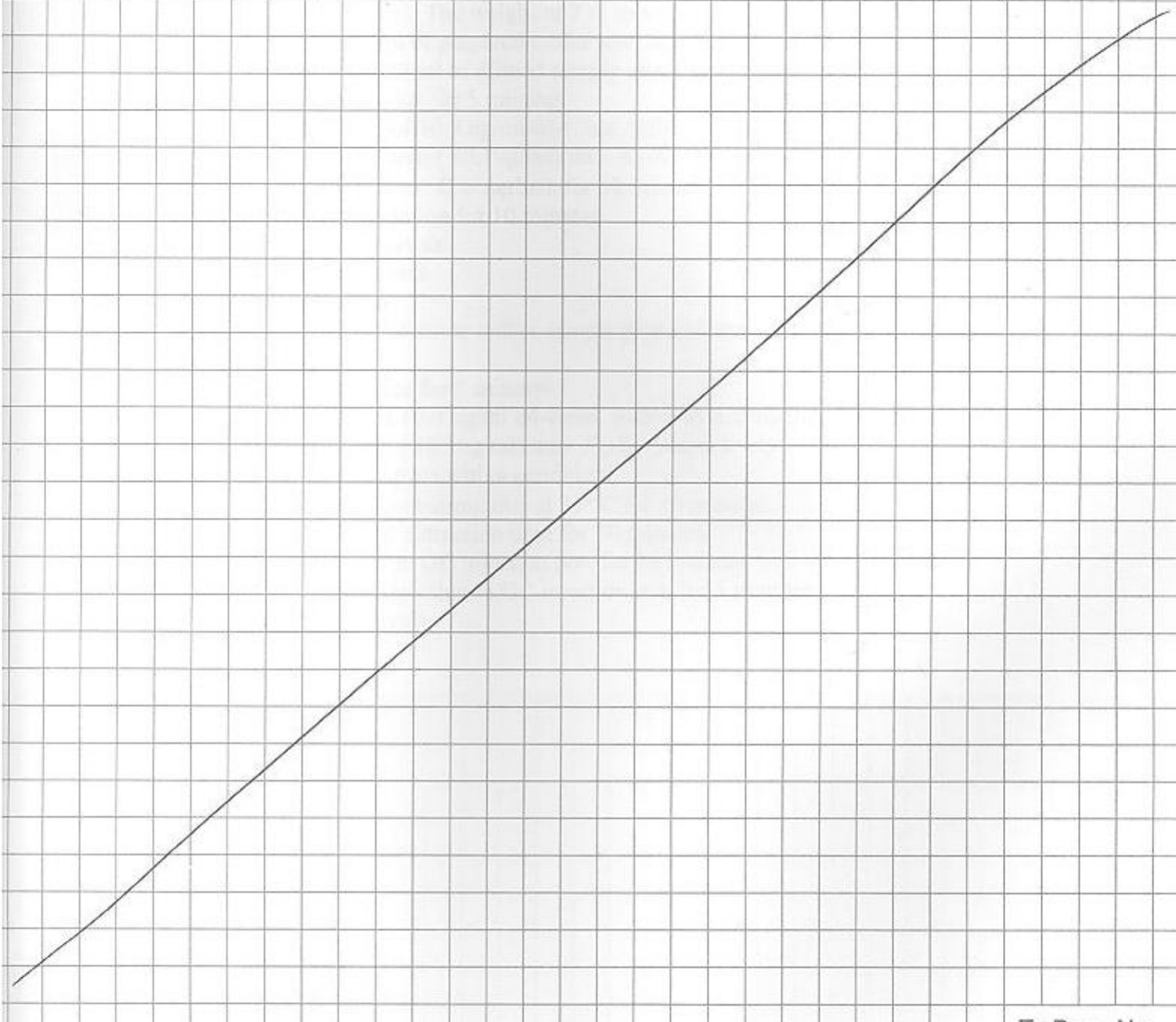
10/23/07

Objective: To prepare furan standard working solution and d4-furan standard working solution.

Procedures:

1. Prepare 11 µg/ml d4-furan by taking 200 µl of 1.1 mg/ml into 20 ml water
2. Prepare 10.1 µg/ml of furan by taking 100 µl of 2.04 mg/ml into 20 ml water
3. Seal both of the vials and keep Refrigerated.



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yaun Ding	10/23/07	Recorded by:	

10/25/07

Objective: To test the repeatability of the developed method in analyzing furan using freshly brewed coffee sample

Procedure

- Manual injection

1. Freshly brew coffee according to instruction on the package (2 tbspoon per 180ml water). The weight of 2 tb spoon coffee is 10.04 g in 180 ml of water.
2. Pipette 15ml of prepared coffee into 285 ml HPLC grade water (cold)
3. Distribute 20 ml of diluted sample into 4 glass vials. Label X_0-X_3 .
4. Incubate in ice for 5 minutes
5. Add 100ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
6. Incubate in 35° C waterbath for 10 minutes
7. SPME extraction for 10 minutes
8. GC/MS analysis
9. Repeat 2 times

- Headspace injection

1. Take 10 ml of dilute coffee sample prepared in manual injection into each headspace vial.
2. Incubate in ice for 5 minutes
3. Add 100ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
4. Set up headspace with a condition:
   - Pre-incubation time at 35° C for 10 minutes
   - SPME extraction time for 10 minutes
   - Inject in GC injection port for 15 minutes
   - Post injection in GC injection port for 5 minutes
5. GC/MS analysis

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Results

	Date file	Level	Amt added	Abundance Analyte	Abundance ISTD	ratio area	Amount (ug)	Amount (ug/ml)
coffee (manual #1)								
	coffee16.D	0	0	4531836	7616137	0.595	0.586	0.586
	coffee17.D	1	0.2525	6412171	7546677	0.850		
	coffee18.D	2	0.505	8150962	7643457	1.066		
	coffee19.D	3	1.01	12217951	7580572	1.612		
coffee (manual#2)								
	coffee20.D	0	0	4143130	7360785	0.563	0.548	0.548
	coffee21.D	1	0.2525	5852817	7304287	0.801		
	coffee22.D	2	0.505	7605608	7200355	1.056		
	coffee24.D	3	1.01	10098232	6392466	1.580		
coffee (headspace#1)								
	hcoff5.D	0	0	1622740	6193305	0.262	0.253	0.506
	hcoff6.D	1	0.2525	4897166	9134266	0.536		
	hcoff7.D	2	0.505	9002392	11317139	0.795		
	hcoff8.D	3	1.01	15678691	11831738	1.325		
coffee(headspace#2)								
	hcoff9.D	0	0	371440	1553479	0.239	0.239	0.478
	hcoff10.D	1	0.2525	4825821	9281411	0.520		
	hcoff11.D	2	0.505	9258150	11884916	0.779		
	hcoff12.D	3	1.01	12438402	9619964	1.293		

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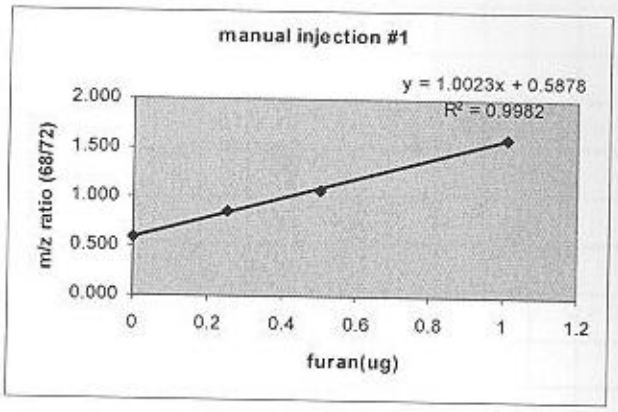

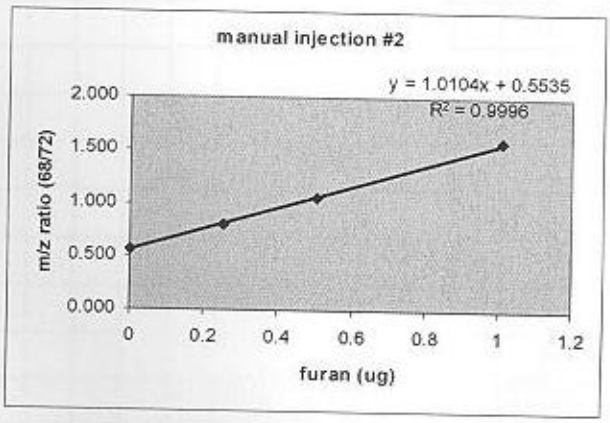

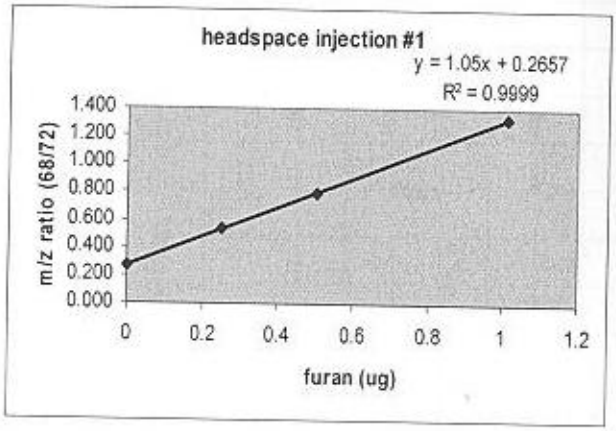

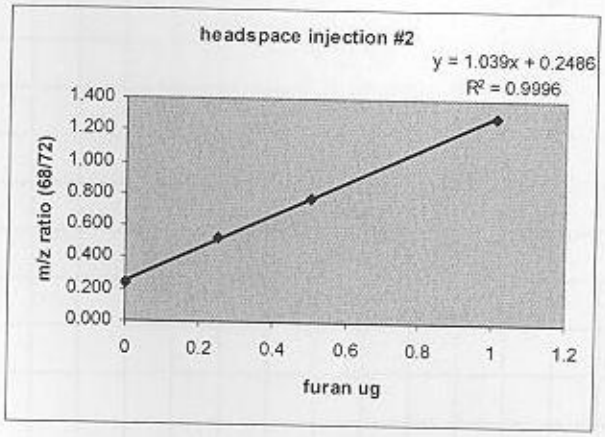

Discussion

The headspace autosampler crash and was unable to perform 5 repeatition of furan quantitation. The method will be repeated.

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10/31/07

Objective: To test the repeatability of developed method in analyzing furan using coffee sample.

Procedure

- Sample Preparation

- Prepare enough sample for 5 times repetition of furan analysis by manual injection and 5 times repetition of furan analysis by headspace injection.

1. Freshly brew coffee according to instruction on the package (2 tbspoon per 180ml water). The weight of 2 tb spoon coffee is 9.5 g in 180 ml of water.
2. Dilute prepared coffee by adding 40 ml of fresh brewed coffee into 760 ml of HPLC water. (40 ml / 800 ml tot. volume)

** dilution factor = 20 **

- Manual injection

1. Distribute 20 ml of diluted sample into 4 glass vials. Label X_0-X_3 .
2. Incubate in ice for 5 minutes
3. Add 200ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
4. Incubate in 35° C waterbath for 10 minutes
5. SPME extraction for 10 minutes
6. GC/MS analysis
7. Repeat 2 times

- Headspace injection

1. Take 10 ml of dilute coffee sample prepared in manual injection into each headspace vial.
2. Incubate in ice for 5 minutes
3. Add 200 ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
4. Set up headspace with a condition:
   - Pre-incubation time at 35° C for 10 minutes
   - SPME extraction time for 10 minutes
   - Inject in GC injection port for 15 minutes
   - Post injection in GC injection port for 5 minutes
5. GC/MS analysis

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11/1/07 (Continue from 10/31/07)

Objective: To continue the repeatability test of furan quantification in coffee sample.

Procedure.

1. The sample prepared from 10/31/07 were refrigerated and brought out to thaw

- Manual injection

1. Distribute 20 ml of diluted sample into 4 glass vials. Label X_0-X_3.
2. Incubate in ice for 5 minutes
3. Add 200ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
4. Incubate in 35° C waterbath for 10 minutes
5. SPME extraction for 10 minutes
6. GC/MS analysis
7. Repeat 2 times
8.

- Headspace injection

1. Take 10 ml of dilute coffee sample prepared in manual injection into each headspace vial.
2. Incubate in ice for 5 minutes
3. Add 200 ul of 10.9 ug/ml d4-furan, follow by adding 0ul, 25ul, 50ul and 100 ul of spiked furan (10.1ug/ml) into X_0-X_3 respectively.
4. Set up headspace with a condition:
   - Pre-incubation time at 35° C for 10 minutes
   - SPME extraction time for 10 minutes
   - Inject in GC injection port for 15 minutes
   - Post injection in GC injection port for 5 minutes
5. GC/MS analysis

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Witnessed & Understood by me,	Date	Invented by:	Date
Yan Ding	11/1/07	Recorded by:	

- Data analysis

1. Manual injection repeatability

Date file	Level	Amt added	Abundance	Abundance	ratio area	Amount (ug)	Amount (ug/ml)
10/31/07			Analyte	ISTD			
coffee (manual #1)							
coffee30.D	0	0	3615568	4892873	0.73894581	0.572	0.572
coffee31.D	1	0.2525	5038075	4617531	1.091075512		
coffee32.D	2	0.505	7470947	5242537	1.425063285		
coffee33.D	3	1.01	10225350	4939526	2.070107537		
coffee (manual#2)							
coffee34.D	0	0	3375032	4581497	0.736665767	0.542	0.542
coffee35.D	1	0.2525	4395978	3956862	1.110975819		
coffee36.D	2	0.505	6869986	4686887	1.465788699		
coffee37.D	3	1.01	9733968	4543821	2.142242839		
11/1/07							
coffee(manula#3)							
coffee38.D	0	0	3136960	3302548	0.949860532	0.634	0.634
coffee39.D	1	0.2525	4677964	3496882	1.33775289		
coffee40.D	2	0.505	6209373	3694698	1.680617198		
coffee41.D	3	1.01	8773845	3558448	2.46563811		
coffee(manula#4)							
coffee42.D	0	0	3486325	3494634	0.997622355	0.61	0.61
coffee43.D	1	0.2525	4774601	3496609	1.365494684		
coffee44.D	2	0.505	6115513	3416981	1.789741588		
coffee45.D	3	1.01	8721695	3341924	2.609782568		
coffee(manual#5)							
coffee46.D	0	0	3309712	3229752	1.024757319		
coffee47.D	1	0.2525	4460950	3113241	1.432895815	0.657	0.657
coffee48.D	2	0.505	5999706	3233935	1.855233949		
coffee49.D	3	1.01	8523917	3256978	2.617124525		

Witnessed & Understood by me, [signature]

Date 10/31/07

Invented by:

Recorded by:

Date

2. Headspace repeatability

	Date file	Level	Amt added	Abundance	Abundance	ratio area	Amount (ug)	Amount (ug/ml)
10/31/07				Analyte	ISTD			
coffee (headspace#1)								
	hcoff13.D	0	0	4618436	10093107	0.45758318	0.295	0.589
	hcoff14.D	1	0.2525	8170391	9676852	0.844323237		
	hcoff15.D	2	0.505	12030496	9813744	1.225882395		
	hcoff16.D	3	1.01	18039098	8949252	2.01571014		
coffee(headspace#2)								
	hcoff17.D	0	0	4606797	9720580	0.473922029	0.289	0.578
	hcoff18.D	1	0.2525	8239112	9299293	0.885993376		
	hcoff19.D	2	0.505	11484586	8976740	1.279371576		
	hcoff20.D	3	1.01	17708484	8352884	2.120044287		
11/1/07								
coffee (headspace#3)								
	hcoff21.D	0	0	2116096	4049997	0.522493227	0.326	0.653
	hcoff22.D	1	0.2525	3309177	3630837	0.911408857		
	hcoff23.D	2	0.505	7365308	5475423	1.345157808		
	hcoff24.D	3	1.01	9338294	4385529	2.129342663		
coffee(headspace#4)								
	hcoff25.D	0	0	2282036	4266448	0.5348796	0.335	0.67
	hcoff26.D	1	0.2525	4124997	4371353	0.943643078		
	hcoff27.D	2	0.505	5314503	3955421	1.343599834		
	hcoff28.D	3	1.01	203762	74507	2.734803441		
coffee(headspace#5)								
	hcoff29.D	0	0	3392285	5943154	0.570788676	0.325	0.65
	hcoff30.D	1	0.2525	5607968	5696173	0.984515042		
	hcoff31.D	2	0.505	7854223	5758256	1.363993369		
	hcoff32.D	3	1.01	12381834	5415896	2.28620232		

- Discussion

- The amounts of furan found in coffee sample by manual injection are 0.572, 0.542, 0.634, 0.61, 0.657 ug/ml. The mean of 5 repetitions is 0.603 ± 0.04 ug/ml (%RSD = 6.63%)

- The amounts of furan found in coffee sample by headspace injection are 0.589, 0.578, 0.653, 0.67, and 0.65 µg/ml. The mean of 5 repetitions is 0.628 ± 0.037 µg/ml (% RSD = 5.89%)

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11/15/07- 11/23

Objective: To test the repeatability of developed method using pea sample
Product : Pea June Medium Brand : Cantrella

Procedure

I. Prepare standard working solution

a) 10.9 µg/ml d_4-furan: Take 200 µl of 1.1 mg/ ml of d_4-furan stock standard solution into 20 ml of water.

b) 5.087 µg/ml furan : Take 50 µl of 2.04 mg/ml of furan stock standard solution into 20 ml of water

II. Sample preparation

Prepare 750 g of pea sample for 10 repetition (5 for manual and 5 for autosampler) by

a). Take 75 g of pea sample into 225 ml plastic bottle containing 150 ml of water. Then homogenize and put into a large 1 L glass container. Rinse with 150 ml of water × (2)

- The total amount is 150 g sample in 600 ml of water or 150 g /750 tot. in water
** Dilution factor = 5 **

III. Repeatability test

- Manual -

1. Tare 40 ml glass vial on the balance and transfer the sample into the glass vial until it reaches amount needed.
2. Transfer 20 g sample into 4 glass vials containing magnetic stirer
3. Incubate in ice for 5 minutes
4. Add 5.087 µg/ml furan and and 10.9 µg/ml d_4-furan as follow:

X_0 : 20 g sample + 150 µl ISTD
X_1 : 20 g sample + 150 µl ISTD + 25 ul spiked
X_2: 20 g sample + 150 µl ISTD + 50 µl spiked
X_3 : 20 g sample + 150 µl ISTD + 100 µl spiked

5. Incubate each vial in 35°C water bath
6. SPME extraction at 35°C for 10 minutes
7. GC/ MS analysis
8. Repeat 5 times

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- Headspace –

1. Tare 20 ml headspace vial
2. Transfer 10 g of pea sample into 4 vials
3. Incubate in an ice bath for 5 minutes
4. Add 5.087 µg/ml furan and 10.9 µg/ml d_4-furan as follow:
   For headspace #1 and #2
   X0 : 10 g sample + 100 µl ISTD
   X1 : 10g sample + 100 µl ISTD +15 µl spiked
   X2: 10 g sample + 100 µl ISTD + 25 µl spiked , (#3 to #5 use 30 µl spiked)
   X3: 10 g sample + 100 µl ISTD + 75 µl spiked

5. Put it on the headspace autosampler which was programmed as below
   - Autosampler 32 vials tray
   - Preincubation in an agitation box : 10 mins
   - Incubation temperature: 35 °C
   - Agitation on time 5 sec
   - Agitation off time 2 sec
   - Injection penetration 45.0 mm
   - Vial penetration 22.0 mm
   - Extraction time 10 mins
   - Desorption : GC Injector Port for 15 minutes
   - Injection penetration : 45 mm.

Results and Discussions

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Date file	Level	Amt added	Abundance Analyte	Abundance ISTD	ratio area	Amount (ug)	Amount (ug/g)
11/15/07							
Pea (manual #1)							
RPEA21.D	0	0	3379252	6412615	0.526969419	0.239	0.05975
RPEA22.D	1	0.127	5935070	7050373	0.841809362		
RPEA24.D	2	0.508	11567672	6888941	1.679165491		
11/22/07							
Pea (manual#2)							
RPEA25.D	0	0	2243468	4634358	0.484094669	0.264	0.066
RPEA26.D	1	0.127	3433132	4382954	0.783291816		
RPEA27.D	2	0.254	3657637	3369775	1.085424695		
RPEA28.D	3	0.508	8026450	5370406	1.494570429		
Pea (manula#3)							
RPEA29.D	0	0	1890798	3275761	0.577208777	0.264	0.066
RPEA30.D	1	0.127	3125076	3532000	0.884789354		
RPEA31.D	2	0.254	4135702	3744803	1.104384396		
RPEA32.D	3	0.508	6358662	3725976	1.70657621		
11/23/07							
Pea							
(manula#4)							
RPEA33.D	0	0	1579721	2630024	0.600648891	0.322	0.0805
RPEA34.D	1	0.127	2376315	2731797	0.869872469		
RPEA35.D	2	0.254	2907124	2678500	1.085355236		
RPEA36.D	3	0.508	3918010	2489518	1.57380264		
Pea(manual#5)							
RPEA37.D	0	0	1599689	2510183	0.637279832		
RPEA38.D	1	0.127	2331307	2620260	0.889723539	0.319	0.07975
RPEA39.D	2	0.254	2736299	2571816	1.063955975		
RPEA40.D	3	0.508	3464870	2114597	1.638548622		

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Date file	Level	Amt added	Abundance Analyte	Abundance ISTD	ratio area	Amount (ug)	Amount (ug/ml)
11/20/07							
Pea (headspace#1)							
HSPEA18.D	0	0	3831228	9172722	0.417676236	0.119	0.0595
HSPEA19.D	1	0.076	5524310	9393601	0.588092894		
HSPEA20.D	2	0.127	6442258	9137508	0.705034458		
HSPEA21.D	3	0.382	16064811	10212312	1.573082667		
Pea(headspace#2)							
HSPEA22.D	0	0	3786615	8611384	0.439722001	0.117	0.0585
HSPEA23.D	1	0.076	5296113	8344558	0.634678673		
HSPEA24.D	2	0.127	7258758	9517370			
HSPEA25.D	3	0.382	12775147	7304371	1.74897291		
Pea (headspace#3)							
HSPEA26.D	0	0	3713343	8715173	0.426077945	0.109	0.0545
HSPEA27.D	1	0.076	5513981	8161704	0.675591886		
HSPEA28.D	2	0.153	7398100	8433361			
HSPEA29	3	0.382	14386970	7779678	1.849301475		
11/21/07							
Pea(headspace#4)							
HSPEA30.D	0	0	3750934	8136880	0.460979393	0.134	0.067
HSPEA31.D	1	0.076	5260467	7227598	0.727830602		
HSPEA32.D	2	0.153	7447784	7745709	0.961536768		
HSPEA33.D	3	0.382	12921334	7297303	1.770699942		
Pea(headspace#5)							
HSPEA34.D	0	0	2871174	6097322	0.470890991	0.153	0.0765
HSPEA35.D	1	0.076	4306099	6236001	0.6905225		
HSPEA36,D	2	0.153	6043492	6287863	0.961136081		
HSPEA37.D	3	0.382	10131769	6170304	1.642021041		

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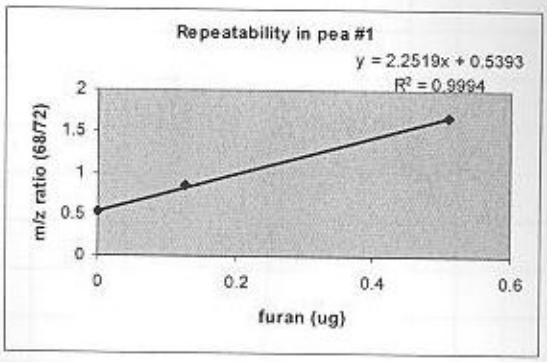
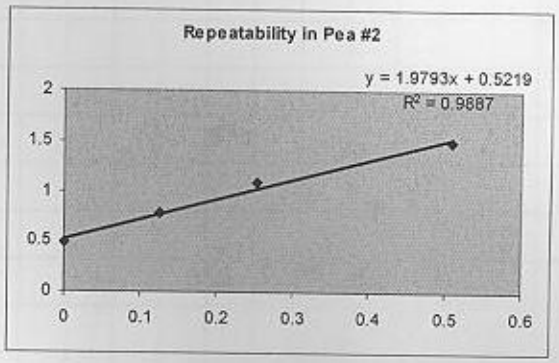
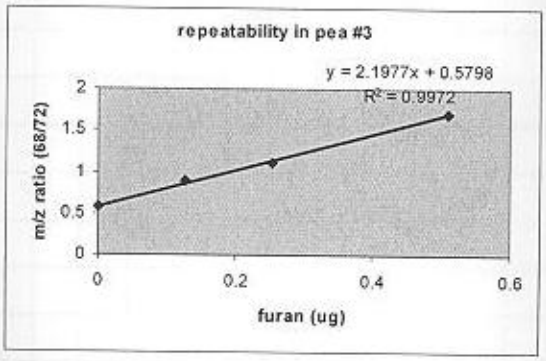
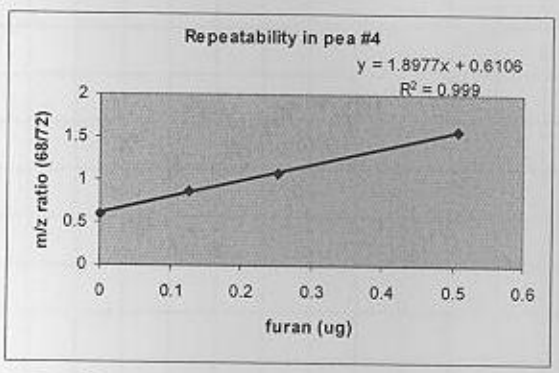
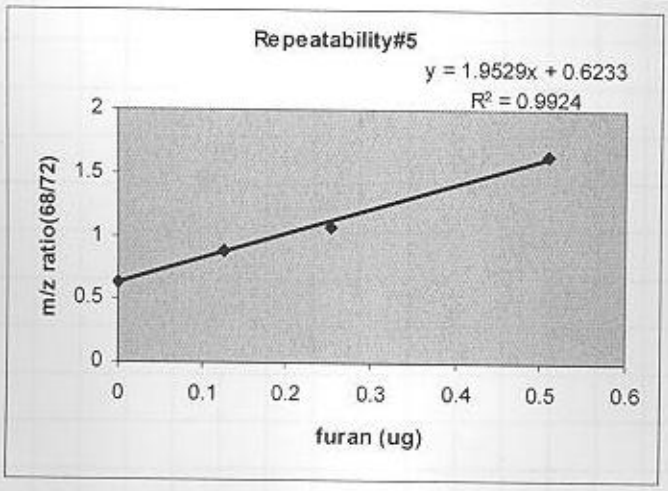
- Repeatability using manual injection- Standard addition calibration curve











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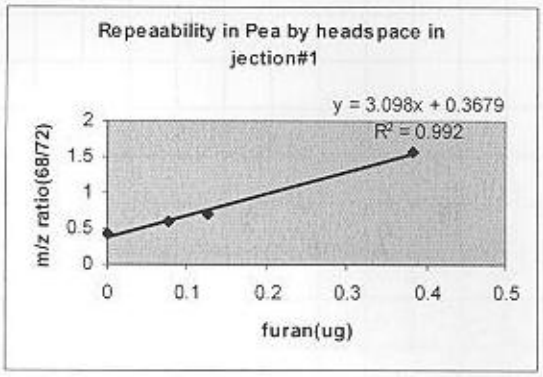
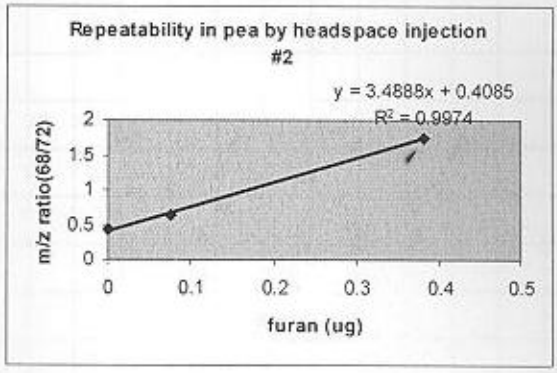
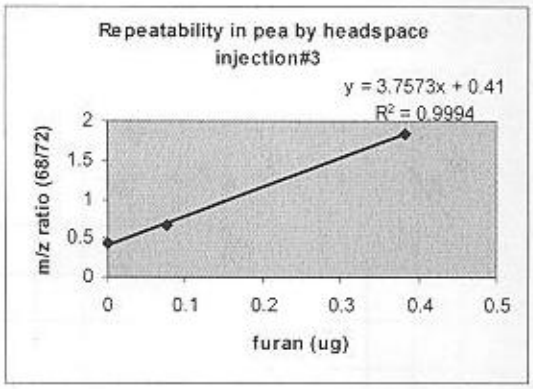
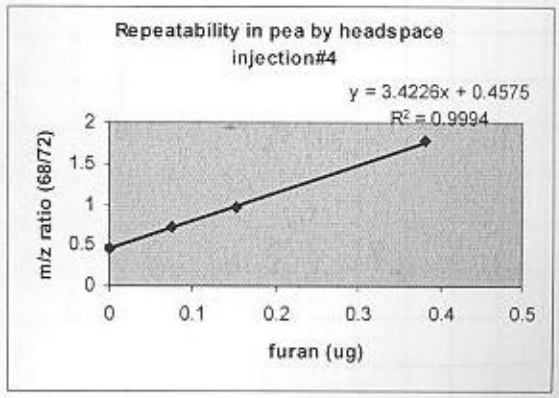
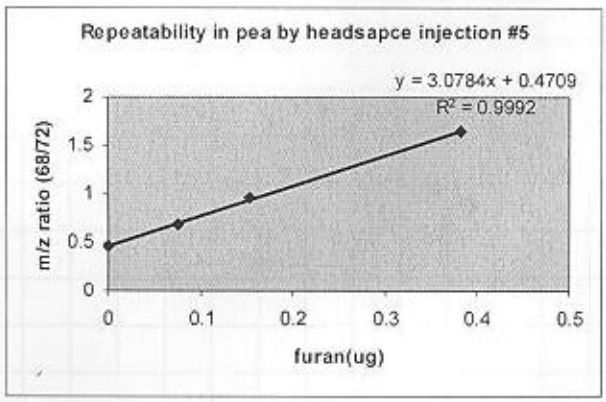
- Repeatability standard addition calibration curve; using autosampler











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Furan

Cal^n

- 8.5 µg/ml Furan working sol^n → From [1.7 mg/ml stock]

1000 µl → 1.7 mg

100 µl → $\frac{1.7}{1000} \times 100 = 0.17$ mg / 0.1 ml = 8.5 µg/ml

- 4.2 µg/ml workin sol (add 50 µl)

100 c — 1.7 mg

50 µl — $\frac{1.7}{1000} \times 50 = 0.085$ mg / 0.05 ml = 4.2 µg/ml

D4 furan. from 0.847 mg/ml stock

1000 — 0.847 mg

200 — $\frac{0.847}{1000} \times 200$ = ~~0.847~~ 0.169 mg / 0.2 ml

= 0.00839 mg/ml = 8.39 µg/ml

PEA : JENEL : EARLY June medium

lot AYB6217

Furan Stock (water) W_1 = 19.8972g

water + Furan μl W_2 = 19.9265 g

Furan Working 10cc — 1.27 mg

100 μl $\frac{1.27}{10cc} \times 100 / 20.1 =$ 0.0063 mg/ml or 6.3 μg/ml

Furan

1000 — 1.27

50 μl — $\frac{1.27}{1000} \times 50 = 0.063\,mg / 20.05\,ml = 0.00316\,mg/ml$

$= 3.2\,\mu g/ml$

liquor 8ml / water 8ml → 2/10 = dilfact = (5)

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I Prepare new stock furan

1) Volumetric pipette 20 ml of water into 40 ml vial Weight W_1 = ~~19.91217 g~~ 19.87729 Y.D.
2) Add 3 ~~-6~~ drops of furan 99%.
3) Weight W_2 = ~~19.91705 g~~ 19.90265 g = W_2 Y.D.
4) Conc^{tr} = ~~19.91217 - 19.9~~ ~~19.91217 g~~ = ~~0.0488 g~~ / 20 ml Y.D.
= 19.90265 - 19.87729 = 0.02536 g ~~20 ml~~
= 0.02536 g / 20ml = 1.268 mg/ml ≈ 1.27 mg/ml
= ~~0.0033449~~
= ~~3.3449 mg/ml~~

II Prepare new Furan working solution

1) Volumetric pipette 20 ml of water into 40 ml vial
2) Add 100 µl into the vial using micropipette
3) Conc^{tr} = $\left[\frac{1.27}{1000} \times 100\right] \times \frac{1}{20.1\,ml}$ = 6.3 µg/ml

III Prepare d_4-furan from 1.0154 77 mg/ml

1) Volumetric pipette 20 ml of water into 40ml vial
2) Add ~~250~~ $\frac{50}{200}$ µl into ~~20~~ the vial Y.D.
3) Conc^{tr} = $\left[\frac{1.0154}{1000} \times \frac{50}{200}\right] \times \frac{1}{20.05}$ = ~~4 µg/ml~~ 2.53 µg/ml

IV Sample preparation

1) Open a pea can and drain liquor into a glass bottle
2) ~~Wei~~ Tare another glass bottle and weigh 50 g into 150 ml water (Dil. factor = 4)
3) Put it in ice ~~for~~ Y.D.
4) Homegenize using Torrex
5) Weigh 10 g into 20 ml headspace vial label x_0, x_1, x_2, x_3
6) After testing the sample the spiked furan should be lower to generate curve that is not too drift. As a result, prepare ~ 3 µg/ml furan for spiked
7) Prepare Working furan by adding 50 µl of 1.27 mg/ml into 20 ml water
(the concentration = $\frac{1.27\,mg}{1000\,\mu l} \times 50$ = 0.063 mg / 20.05ml = 3.2 µg/ml)

SPME Extraction & GC-MS

8) Continue incubate in ice 5 min and fortified furan nd d_4-furan.
(3.2 µg/ml furan, 2.53 µg/ml d_4-furan.
9) ~~After test, the spike furan is still too high, so dilute 5 times (dil factor = 5)~~
~~by using micropipette~~ Y.D.

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9) -Make 1 in 10 dilution of ~~6.3 µg/ml~~ Y.D. 3.2 µg/ml by using micropipette to transfer 1000 µl into 9 ml of water to obtain 0.32 µg/ml furan
- Make 1 in 10 dilution of 2.53 by using micropipette to transfer 1000µl into 9 ml of water to obtain 0.25 µg/ml d_4-furan.

X_0	10 ml sample +	~~50~~ 100 µl IS (Y.D. 0.25 µg/ml d_4-furan)
X_1	10 ml sample +	~~50~~ 100 µl IS + 25 spiked (0.32 µg/ml furan)
X_2	10 ml sample +	~~50~~ 100 IS + 50 spiked "
X_3	10 ml sample +	~~50~~ 100 IS + 100 spiked

10) Also, prepare liquid pea by putting 2 ml of liquid pea into 8 ml of water ($2/10_{(f)}$ = dilu factor = (5))

X_0	10 ml sample +	~~75~~ 100 µl IS Y.D. (0.25 µg/ml d_4-furan)
X_1	10 ml sample +	~~75~~ 100 µl IS + 25 µl spiked (0.32 µg/ml furan)
X_2	10 ml sample +	~~75~~ 100 IS + 50 µl spiked
X_3	10 ml sample +	~~75~~ 100 IS + 100 µl spiked

Results

- The machine crash, filament burnt - no result retrieved.

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7/9/07 Measurement : Jewel Pea lot AYB6217 can b solid and liquid

1) Prepare a 32 µg/ml as stated in P. 95

2) ~~test d4 furan YD con~~ Prepare Pea sample

2.1) Bring out refrigerated can of pea

2.2) Open a can and drain liquor into a glass bottle

2.3) Cover a can with an aluminum foil and tighten it with rubber band

2.4) ~~Pip~~ YD. Use cylinder to measure 300 ml of water and place into a glass bottle (600 ml bottle)

2.5) Weight 100 g of pea into a bottle.

2.6) Chill in ice

2.7) Homogenize until getting a smooth paste

2.8) transfer 10 g into headspace vial

2.9) incubate in ice for 5 mins.

~~2.10~~ 2.10) Prepare blank : 10 ml of water in headspace vial

3) Fortified with 2.5 µg/ml d_4-furan (IS) and 0.32 µg/ml furan.

X_0 : 10 ml sample + 25 µl IS

X_1 : 10 ml sample + 25 µl IS + 50 µl spiked furan.

X_2 : 10 ml sample + 25 µl IS + 100 µl spike furan.

X_3 : 10 ml sample + 25 µl IS + 200 µl spiked furan.

4) Autosampler SPME GC-MS analysis : method Furan_M.

~~Result~~ YD

~~5~~ YD) 5) Liquid sample measurement

~~Results~~ YD

5.1) Pipette 8 ml of water into a headspace vial

5.2) Pipette 2 ml of liquor into the vial (2ml/10 tot ; dil. factor = 5)

5.3) Incubate in ice for 5 minutes.

5.4) Fortified 0.32 µg/ml furan and 2.5 µl d_4-furan.

X_0 10 ml + 25 µl IS

X_1 10 ml + 25 µl IS + 50 µl spiked furan

X_2 10 ml + 25 µl IS + 100 µl spiked furan

X_3 10 ml + 25 IS + 200 µl spiked furan.

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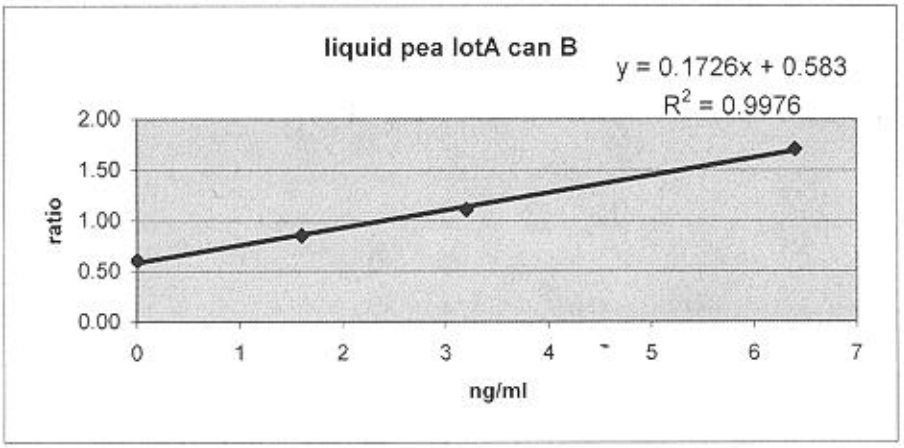
	7/9/07	Recorded by:	

	ul added	ng/ml	68	72	ratio	39	37	42	44
Lv1 LPEA09.D	0	0	201,388	333,752	0.60	274,254	ND	471,821	88,155
Lv2 LPEA10.D	50	1.6	276,851	325,265	0.85	380,827	ND	465,599	88,874
Lv3 LPEA11.D	100	3.2	343,790	310,831	1.11	474,008	ND	453,772	
Lv4 LPEA12.D	200	6.4	471,052	276,472	1.70	648,108	105,670	415,076	



Amount 16.8 ng/ml

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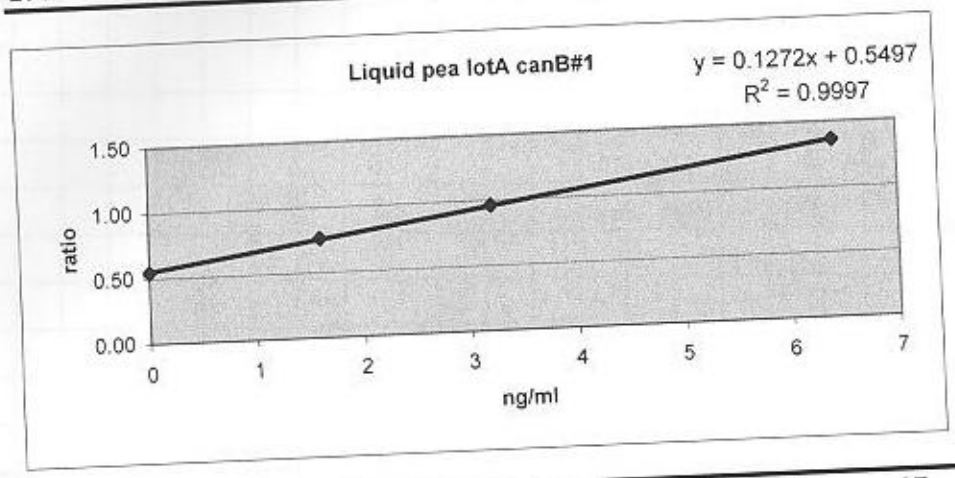
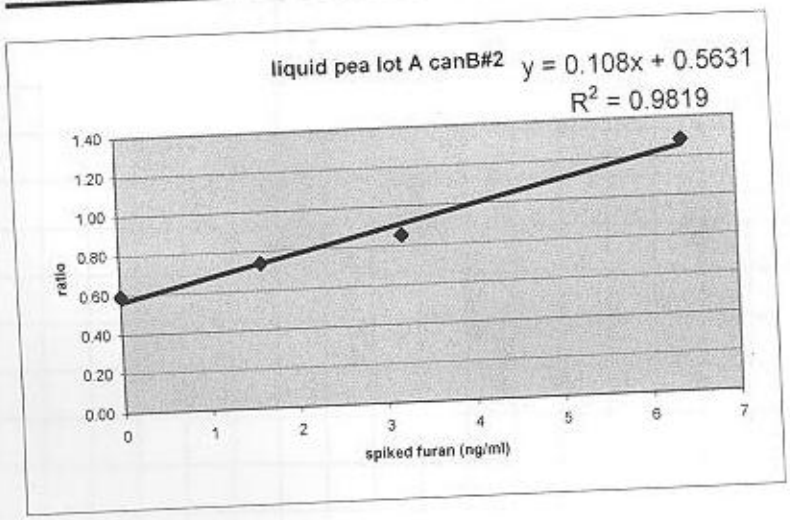
Results

	ul added	ng/ml	68	72	ratio	39	37	42	44
Lv1 LPEA01.D	0	0	205,672	378,634	0.54	290,504	ND	542,479	104,154
Lv2 LPEA02.D	50	1.6	289,128	380,433	0.76	402,908	ND	545,089	104,346
Lv3 LPEA03.D	100	3.2	359,913	375,036	0.96	508,101	ND	546,599	103,156
Lv4 LPEA04.D	200	6.4	504,155	370,509	1.36	696,837	115,668	554,687	101,589



Amount 21.6 ng/ml

	ul added	ng/ml	68	72	ratio	39	37	42	44
Lv1 LPEA05.D	0	0	223,828	378,634	0.59	353,536	ND	505,424	97,920
Lv2 LPEA06.D	50	1.6	280,122	380,433	0.74	345,727	ND	497,115	96,092
Lv3 LPEA07.D	100	3.2	319,492	375,036	0.85	305,779	ND	446,517	
Lv4 LPEA08.D	200	6.4	475,206	370,509	1.28	109,237	109,237	331,394	90,573



Amount 26 ng/ml

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	7/9/08	Recorded by:	

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1/10/08

Procedure

1) Prepare new 2.5 µg/ml of d_4 furom by adding 50 µl of 1.015477 mg/ml into 20ml of water (Conc = $\frac{1.0154}{1000} \times 50] \times \frac{1}{20.05}$ = 2.53 µg/ml

2) Keep YP - Measure liquid pea (third trial of can B from day 7/9/08 Use procedure in P. 96)

3) Do standard addition of furom in water to use in QC data (Use 2.5 µg/ml furom (25 µl) and 6.3 µg/ml furom (25^{X1} µl, 50^{X2} µl and 100^{X3} µl)

4) Prepare Pea sample (different can (can C, same lot - AYB6217 (100 g pea into 300 ml water; dilution = 4)

5) Measure furom in solid pea (triplicates)

id pea – X_0 10 ml + 25 µl of 2.5 µg/ml d_4-furom (IS)

X_1 10 ml + 25 µl IS + 50 µl of 0.32 µg/ml furom

X_2 10 ml + 25 µl IS + 100 µl of 0.32 µg/ml furom

X_3 10 ml + 25 µl IS + 200 µl of 0.32 µg/ml furom.

uid pea – (Pipette 8 ml of water into the vial follow by 2 ml of sample ($\frac{2\text{ml}}{10\text{fold}}$) ; dil factor = 5)

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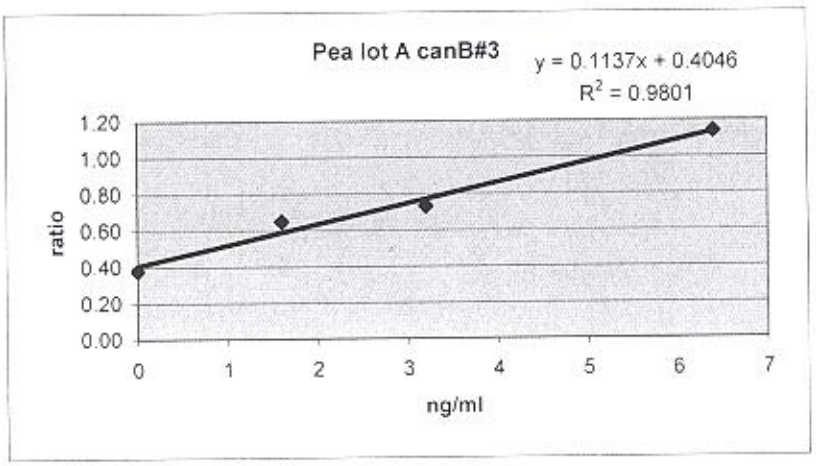
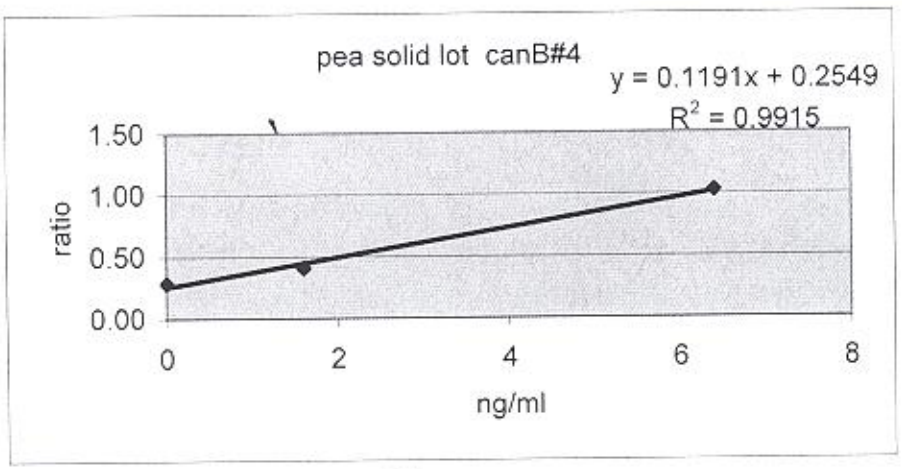
tnessed & Understood by me,	Date	Invented by:	Date
	7/10/08	Recorded by:	

	ul added	ng/ml	68	72	ratio	39	37	42	44
Lv1 PEAB09.D	0	0	160,271	424,092	0.38	220,879		598,053	172,217
Lv2 PEAB10.D	50	1.6	253,987	392,302	0.65	355,777		559,399	159,029
Lv3 PEAB11.D	100	3.2	273,916	375,046	0.73	382,558		537,243	132,097
Lv4 PEAB12.D	200	6.4	420,495	370,114	1.14	581,650	96,753	550,197	126,495



Amount 14 ng/ml

	ul added	ng/ml	68	72	ratio	39	37	42	44
Lv1 SOLB01.D	0	0	122,176	427,846	0.29	236,848	ND	592,485	113,142
Lv2 SOLB02.D	50	1.6	178,379	440,774	0.40	251,559	ND	614,900	111,847
Lv3 SOLB03.D	100		232,549	493,082		336,193	ND	660,423	134,815
Lv4 SOLB04.D	200	6.4	331,959	323,042	1.03	483,496	96,753	442,913	ND



Amount 8.6 ng/ml