Analysis by gas chromatography

oven temperature on the separation of methyl and ethyl esters. Also the optimum separation condition for the analyte (methyl, ethyl and unknown ester) is to be identified. Finally for this part of the experiment, the effect of ‘ split injection’ and ‘ splitless injection’ is determined. ‘ Split injection’ is when the split valve is kept open when sample is injected while ‘ splitless injection is when the split valve is closed before the sample is introduced1.

Methods

Apparatus

Gas chromatography machine. Agilent technologies 7802A GC system, serial number CN10022002 ITL9002 GC6.

Column: Agilent J&W GC column, Part No 19091J-413 HP-5, Serial No US9035232L, Length 30m, I. D 0. 320mm, film thickness 0. 25 µm

Autosampler: G4513A Serial No: CN95303257

Instrumental condition

Nitrogen carrier gas flow: 2ml/min (split ratio 1: 50).

Oven temperature = 100-150oC (isothermal or temperature gradient programmed)

Injector temperature = 200oC

Detector temperature = 250oC

Air and hydrogen flows preset

Detector: FID

Further Instrumental condition

Condition 1: Initial oven temperature: 100 oC; Final temperature: 100oC run time: 6min.

Condition 2: Initial oven temperature: 150 oC; Final temperature: 150oC; hold time: 6min; ramp rate: 0oC/min; total run time: 6min.

Condition 3: Initial temperature: 100oC; final temperature: 150 oC; hold time: 0min; ramp rate: 10°C/min; total run time: 5min.

Condition 4: Initial temperature: 100oC; final temperature: 150 oC; hold time: 0min; ramp rate: 15°C/min; total run time: 3. 33min.

Condition 5: Initial temperature: 100oC; final temperature: 150 oC; hold time: 0min; ramp rate: 20°C/min; total run time: 2. 5min.

Condition 6: Initial temperature: 55oC; initial time: 1min; optimal ramp rate to 150°C; purge valve: open 0. 7min after the start of the run.

Reagents

Sample A: Methyl ester mixture in hexane: methyl pentanoate 2. 0%; methyl hexanoate 2. 5%; methyl heptanoate 3. 0%; methyl octanoate: 3. 5%

Sample B: Methane.

Sample C: Ethyl ester mixture in hexane: ethyl butanoate 2%; ethyl pentanoate 2. 25%; ethyl hexanoate 2. 75%; ethyl heptanoate 3%

Sample D: Unknown EST 1212 esters.

Sample E: Diluted Ethyl ester mixture.

Steps

From the Lab manual

Step 1: Inject 0. 1Î¼l Sample A using a split injection into GC using Condition 1.

Step 2: Inject 100µl Sample B using a split injection into GC using Condition 1

Step 3: Inject 0. 1Î¼l Sample A using a split injection into GC using Condition 2.

Step 4: Repeat the injection (0. 1Î¼l) of Sample A using Condition 3, 4 and 5.

Step5: Inject Sample C into GC using Condition 5.

Step6: Run Sample D the mixture containing three unknown esters (EST1212) using Condition 5.

Step7: Repeat the analysis of the Sample C using Condition 5 and selecting the splitless option in the injector parameters window (split/splitless)

Step8: Dilute the Sample C five hundred fold by transferring 20 µl into a 10ml volumetric flask and diluting to the with hexane and inject 1Î¼l of the diluted sample into GC using Condition 5.

Step 9: Repeat the analysis, but this time uses the “ sandwich” injection technique to inject 1Î¼l of the diluted Sample C.

Set the injector parameters to the following:

1. 0Î¼l hexane, 0. 2Î¼l air, 1. 0Î¼l sample, 0. 2Î¼l air, 0. 2Î¼l hexane, 1. 0Î¼l air. Inject the whole 3. 6Î¼l sample.

Comment

Condition 5 resulted in optimum separation of Sample A (methyl ester) as shown in page 21. This condition gave a short retention and a good chromatogram compared to other conditions

In the splitless injection (step 7) for the ester peaks are fronting as shown in page 24. This is because the carrier gas continuously mixes with the vapour in the injector, making it more and more dilute but never completely flushing the sample from the injector2. Splitless injection is not well-suited for volatile compounds3.

Results and Discussion

Summary of result

Table 1: Result of the separation of methyl esters using condition 1 (as shown in the chromatogram in page 16 and some properties of the mixture of methyl esters

Compound

tm (min)

tR (min)

t’R (min)

log t’R (min)

Boiling point (oC)

Carbon number

Methyl pentanoate

1. 500

1. 974

0. 474

-0. 324

126. 0

6

Methyl hexanoate

2. 447

0. 947

-0. 024

151. 0

7

Methyl heptanoate

3. 356

1. 856

0. 269

172. 5

8

Methyl octanoate

5. 100

3. 600

0. 556

193. 0

9

Where

tm is the retention time of methane.

tR is the retention time of methyl esters.

t’R is the adjusted retention time gotten by using t’R = tR – tm

Boiling points2, 3

Example for the calculation of adjusted retention time.

Using methyl pentanoate values

From the formula2 (t’R = tR – tm)

t’R = 1. 974 – 1. 500 = 0. 474

Figure 1: The plot of log t’R (adjusted retention time) against number of carbon atoms

The relationship shows a linear dependence between the log t’R and the number of carbon atom

Figure2: The plot of log t’R (adjusted retention time) against boiling point

The graph shows a linear dependence of the log t’R (min) on the boiling point (oC).

Table 2: Result of the separation of methyl esters using condition 1 (as shown in the chromatogram on page 16a and the efficiency

Compound

tm (min)

tR (min)

t’R (min)

W1/2 (min)

N

Methyl pentanoate

1. 500

1. 974

0. 474

0. 017

74698

Methyl hexanoate

1. 500

2. 447

0. 947

0. 033

30461

Methyl heptanoate

1. 500

3. 356

1. 856

0. 046

29488

Methyl octanoate

1. 500

5. 100

3. 600

0. 083

20917

Where

tm (min) is the retention time for methane (chromatogram on page 17)

W1/2 (min) is the width at half height and was measured directly from peaks on page 16b

N (dimensionless) is the efficiency, calculated using the formula4

Comment

The efficiency of a column is determined by two factors2:

The difference in the elution times between peaks: the farther apart, the better their separation.

The other factor is how broad the peaks are: the wider the peaks, the poorer their separation.

Therefore, the efficiency of the column for each of the methyl ester peaks using 100oC isothermal analysis is fair because the elution time is not farther apart and some of the peaks are too broad.

Table 3: Result of the separation of the Methyl esters, ethyl esters and the unknown ester sample (EST1212) using Condition 5

Compound

tm (min)

tR (min)

t’R (min)

Page

Methyl pentanoate

1. 500

1. 756

0. 256

16a

Methyl

Hexanoate

1. 500

1. 974

0. 474

16a

Methyl heptanoate

1. 500

2. 299

0. 799

16a

Methyl

Octanoate

1. 500

2. 738

1. 238

16a

Ethyl

Butanoate

1. 500

1. 715

0. 215

22

Ethyl

Pentanoate

1. 500

1. 908

0. 408

22

Ethyl

Hexanoate

1. 500

2. 199

0. 699

22

Ethyl

Heptanoate

1. 500

2. 600

1. 100

22

Unknown ester 1

1. 500

1. 908

0. 408

23

Unknown ester 2

1. 500

1. 974

0. 474

23

Unknown ester 3

1. 500

2. 602

1. 102

23

Where

tm is the retention time of methane. (chromatogram on page 17)

tR is the retention time of methyl esters, ethyl esters and unknown esters.

t’R is the adjusted retention time gotten by using t’R = tR – tm

From the table above

t’R (min) for ethyl pentanoate = 0. 408= t’R (min) for unknown ester 1

t’R (min) for methyl hexanoate = 0. 474 = t’R (min) for unknown ester 2

t’R (min) for ethyl heptanoate (1. 100) is equivalent to t’R (min) for unknown ester 3 (1. 102).

Therefore, the unknown esters in the unknown ester sample (EST1212) are

Ethyl pentanoate

Methyl hexanoate

Ethyl heptanoate

Answers to questions

Comparison of split and splitless chromatograms

The initial oven temperature is lowered to 50oC for the splitless injection because the sample solvent hexane has a boiling point of 69oC and any initial oven temperature that is above the temperature of hexane will lead to the solvent peak tailing , and the early eluting of compounds have broad peak shapes and are poorly resolved from one another2. Therefore, the initial oven temperature is lowered to enable the components condense,

forming a narrow “ slug” of mixture to be injected onto the column, thus minimize peak broadening4.

The reproducibility of the split and splitless analysis of ethyl esters can be affected by polarity and the induced vapour pressure volume1.

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Name: Ime Cletus Usen Registration Number: B212859 Partner’s Name: Lufeng Zhao Date: 26-10-2012

Part 2: Quantitative Analysis of Ethanol in Alcoholic Beverages by Internal Standards

Abstract

A method is given for the quantitative analysis of ethanol in alcoholic beverages by gas chromatography. This method uses an internal standard

and flame ionization detector for the accurate and precise determination of ethanol in alcoholic beverages (Quantitative analysis) compared to other methods of analysis commonly used. For this experiment, propan-1-ol is used as an internal standard to determine the relative responds factor for ethanol, which is then use to ascertain the concentration of ethanol in the alcoholic beverage. This experiment has explored and seen the effectiveness of using an internal standard (propan-1-ol) for the determination of the concentration of ethanol in alcoholic beverages.

Introduction

The aim of this experiment is to observe the effect of the internal standard (propan-1-ol) for the determination of ethanol in alcoholic beverages by gas chromatography (with flame ionization detector). The highest precision for quantitative GC is obtained using internal standards because the uncertainties introduced by sample injection, flow rate, and variation in column condition are minimised. 1, 4 An internal standard is a known amount of a compound, different from the analyte, that is added to the unknown2. The internal standard should have the following characteristics

It should elute near the peaks of interest but must be well resolved from them3.

It should be chemically similar to the analytes of interest and not react with any sample components3.

Like any standard, it must be available in high purity3.

Be similar in functional group type to the component(s) of interest. If such a compound is not readily available, an appropriate hydrocarbon should be substituted5.

Be sufficiently non-volatile to allow for storage of standard solutions for significant periods of time5.

A calibration curve is then plotted for the ratio of the analyte peak area to the internal standard peak area as a function of the analyte concentration of the standard1, 4.

Safety

I ensured that the injection syringe was carefully injected into the injection valve.

Method

Summary of instrument and instrumental condition

Instrument

Gas Chromatography machine. Varian CP-3380, Serial Number 05469 ITL1724, GC 2.

Pipette 20-200µl and 500-5000 µl. eppendorf research, US Patent No 5531131.

Unknown Sample: Andrew Peace Chardonnay, South Eastern Australia 75CLe 12. 5% vol. Bottled by W1507 at NR104BG, UK for Bottle Green Ltd LS184BH South Eastern Australia, Andrew Peace wines, Murray valley highway, Piangil, Victoria 3597. www. apwines. com

Column: Agilent J&W GC column, Part No 19091J-413 HP-5, Serial No US9035232L, Length 30m, I. D 0. 320mm, film thickness 0. 25 µm.

SGE Syringe IBR-7 Cat #2477L

Instrumental condition

Nitrogen 2ml/min

Oven temperature = 45oC

Injector temperature = 150oC

Detector temperature = 200oC

Air and hydrogen flows preset

Flame ignited – allow to stabilise for 10mins

Checked detector signal is less than 20 and stable.

Preparation of standard

100µl 10% v/v aqueous ethanol was pipetted into a sample vial.

700µl of distilled water and 200µl 15% v/v aqueous propan-1-ol was added to sample vial containing aqueous ethanol and was cap immediately to prevent lost of volatiles.

These was repeated with varying amount of ethanol and distilled water but with 200µl 15% v/v aqueous propan-1-ol in separate vials as shown in Table a below

Table a. Description of the preparation of ethanol/propan-1-ol standards.

Vial No

Volume 10% aq Ethanol (µl)

Volume 15% aq Propanol (µl)

Volume

Water (µl)

1

100

200

700

2

200

200

600

3

300

200

500

4

400

200

400

5

500

200

300

Each of the standards in the sample vial was injected into the GC using the GC syringe. (0. 1 µl of the solution in each sample vial were injected at approximately 2 minutes between injections).

Also triplicate solution of the unknown sample (beverage sample) was prepared by pipetting 200 µl of the unknown sample into a sample vial and adding 600 µl of distilled water and 200 µl of 15% v/v aqueous propan-1-ol as shown in table b below.

Table b. Description of the preparation of the unknown sample (beverage sample)

Vial No

Volume Unknown sample (µl)

Volume 15% aq Propanol (µl)

Volume

Water (µl)

6

200

200

600

7

200

200

600

8

200

200

600

0. 1µl of the solution in each sample vial (6, 7, 8) were injected into the GC at approximately 2 minutes between injection.

Result and Discussion

Table c. Summary of data

Vial No

Page

Ce (µl)

Cp (µl)

Ae (mV. s)

Ap (mV. s)

Ce/Cp

Ae/Ap

1

10

30

377. 497

1593. 899

0. 333

0. 237

2

20

30

752. 516

1507. 398

0. 667

0. 499

3

30

30

1229. 607

1567. 497

1. 000

0. 784

4

40

30

1878. 929

1880. 070

1. 333

0. 999

5

50

30

474. 427

495. 571

1. 667

0. 957

6

Unknown

30

729. 010

1294. 222

n/a

0. 563

7

Unknown

30

813. 806

1286. 530

n/a

0. 633

8

Unknown

30

1127. 405

1749. 031

n/a

0. 645

Where

Ce is the concentration of ethanol

Cp is the concentration of propan-1-ol

Ae is the peak area of ethanol

Ap is the peak area of propan-1-ol.

Example for the calculation of Ae/Ap, Ce, Cp, and Ce/Cp.

Using Vial 1 of table c, where Ae= 377. 497mV. s and Ap= 1593. 899mV. s

Ae/Ap= 377. 497mV. s / 1593. 899mV. s = 0. 236839

Using Vial 1 of table a,

Ce= 10%v/v *100 µl

Ce= 10/100* 100 µl

Ce= 10 µl.

Cp= 15%v/v*200 µl

Cp= 15/100*200 µl

Cp= 30 µl

Using the result of Ce and Cp above

Ce/Cp= 10 µl / 30 µl

Ce/Cp= 0. 333333

Figure . A plot of Ae/Ap versus Ce/Cp

y = 0. 5824x + 0. 1131

R^2 = 0. 9021

Slope of the graph= 0. 5824

The variation in the last point is due to some experimental error.

Answers to Questions

The relative response factor for ethanol is the slope of the graph(). Therefore RF= 0. 5824 from figure 1. This is significant because it has to be use to determine the concentration of ethanol in the unknown sample.

Table d: Showing peak area of the unknown sample

Vial

Ae (mV. s)

Ap (mV. s)

Ae/Ap

6

729. 010

1294. 222

0. 563

7

813. 806

1286. 530

0. 633

8

1127. 405

1749. 031

0. 645

Mean of Ae/Ap = 0. 563+0. 633+0. 645 = 1. 841 = 0. 614

3 3

The relationship between Ce/Cp= x and Ae/Ap= y is y= 0. 5824x+0. 1131 from the graph (Figure 1)

Therefore, the mean = y= Ae/Ap= 0. 614

And x = y-0. 1131 = 0. 614 – 0. 1131 = 0. 8600

0. 5824 0. 5824

Thus x= Ce/Cp= 0. 8600

And Cp is known as 15% v/v

So Ce= CpÃ-0. 8600= 15% v/vÃ-0. 8600= 12. 9%v/v

The associated uncertainty is the standard deviation of the mean6, 7.

http://standard-deviation. appspot. com/images/standard-deviation-1. png

Where

Ïƒ is the standard deviation

x is each value of Ae/Ap

x is the mean of the values of Ae/Ap

N is the number of values.

Table e: Values for the calculation of standard deviation

x

x

x – x

(x -x)2

0. 563

0. 614

– 0. 051

2. 601 x 10-3

0. 633

0. 614

0. 019

3. 610 x 10-4

0. 645

0. 614

0. 031

9. 610 x 10-4

âˆ‘(x – x)2 = 2. 601 x10-3 + 3. 610 x 10-4 + 9. 610 x 10-4 = 3. 923 x 10-3

Therefore

Ïƒ = âˆš 3. 923 x 10-3 = 0. 036 = 3. 6%

3

Thus, the concentration of ethanol in the unknown beverage and the associated uncertainty is

12. 9%v/v ±3. 6% = 25. 8µl ± 7. 2 is the concentration of ethanol in the unknown sample.

A good internal standard must have a close peak to the analyte and must be well from the analyte and must be chemically similar to the analytes3, 4. Therefore propan-1-ol is a suitable internal standard for the determination of ethanol because it gives a close peak to ethanol and it is well separated and also has similar chemical properties.