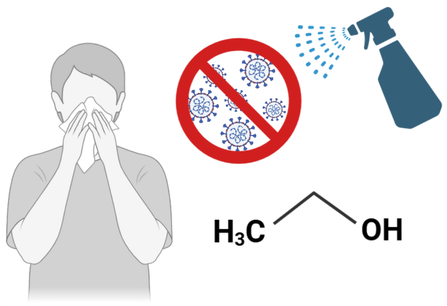
Experimental Production of 70% Alcohol Solution and Validation of Ethanol Analysis Methods

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Abstact

This project aimed to experimentally produce 70% alcohol solution and evaluate an analytical method for assessing the quality of ethanol, focusing on the validation of organic impurity analysis in ethanol using Gas Chromatography (GC), in accordance with the ICH Q2(R2) guideline. The initial phase involved testing the produce 70% alcohol solution. Results showed that the ethyl alcohol content (69.93 - 7032 %v/v), absorbance at 640 nm (0.2 - 0.3), and pH (5.12 - 6.54) all met the established standard criteria. The study evaluated system suitability, specificity, linearity, limits of detection and quantitation (LOD/LOQ), and precision. The method validation results confirmed compliance with all ICH Q2(R2) acceptance criteria. The analytical method demonstrated high specificity, yielding a resolution value between acetaldehyde and methanol of 1.607 (criterion: NLT 1.5). Good linearity was observed with a correlation coefficient (R) of greater than 0.990 for all target compounds. Furthermore, the Precision, reported as percent relative standard deviation (%RSD), ranged from 0.000% to 7.092%, which is lower than the specified acceptance criteria of 7.3%. The signal-to-noise ratio (S/N) for the LOQ solution was confirmed to be greater than 10.0. In conclusion, the developed method is effective, highly reliable, and suitable for the quality control of ethanol, supporting future drug registration processes.

Introduction



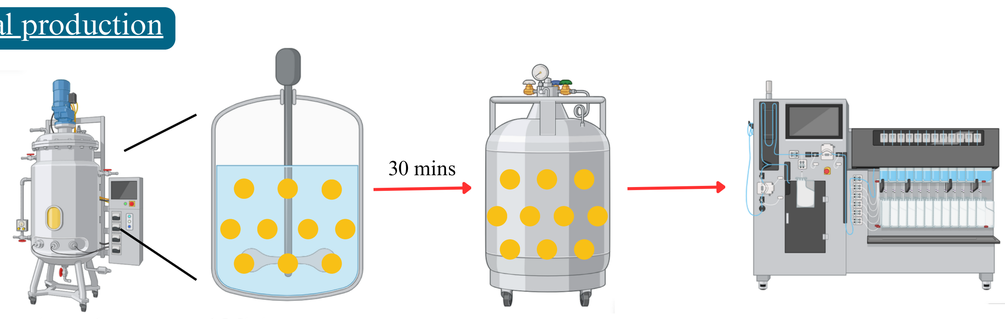
Prior to commercial manufacturing, it is imperative to undergo rigorous Research and Development (R&D), pilot-scale production, and analytical method validation. These processes ensure the effiency and reliability of the analysis of both raw materials and finished products. This report focuses on verifying the most appropriate analytical methods to support the drug registration process, thereby ensuring that the manufactured products comply with regulatory standards and are safe for public consumption.

Objectives

1. To study the manufacturing process of 70% alcohol solution
2. To perform Analytical Method Validation (AMV) to ensure the accuracy of analytical method

Methodology

Trial production



Preparation substances and Mixing process

- Sampling point:
  - Top: 3 points
  - Middle: 4 points
  - Bottom: 3 points

Filtration process

- Sampling point:
  - Top: 3 points
  - Middle: 4 points
  - Bottom: 3 points

Packaging process

Sampling point:
- Begin: 3 points
- Middle: 4 points
- End: 3 points



Conduct assay testing on all collected samples using Density meter, UV-Vis spectrophotometer, and pH meter.

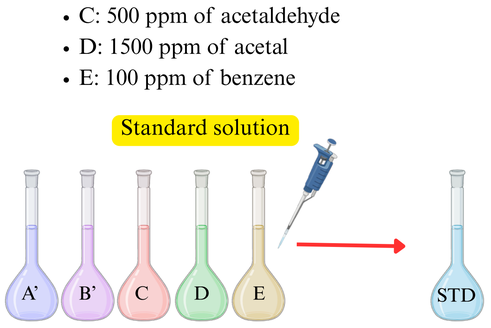
Analytical Method Validation for Organic solvent of GC-FID

Standard stock solution



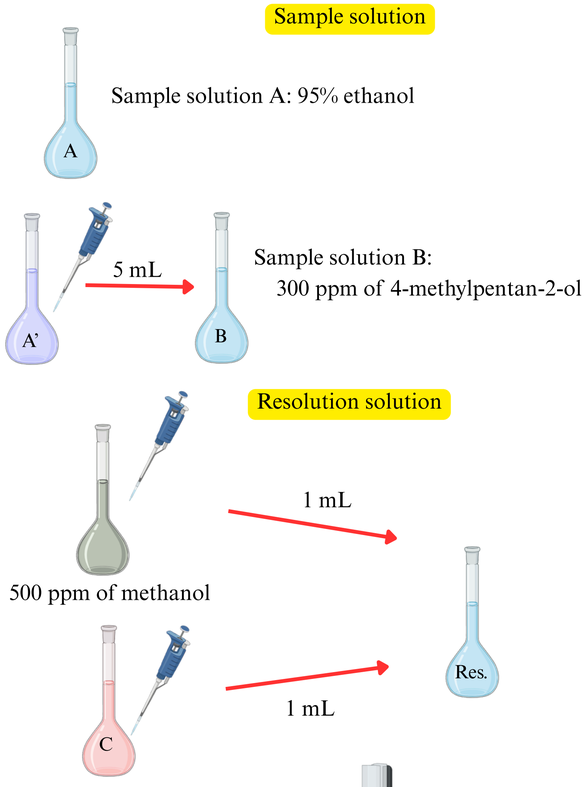
- A': 3000 ppm of 4-methylpentan-2-ol
- B': 2000 ppm of methanol
- C: 500 ppm of acetaldehyde
- D: 1500 ppm of acetal
- E: 100 ppm of benzene

Standard solution



The final concentration of 4-methylpentan-2-ol, methanol, acetaldehyde, acetal, and benzene are 300, 200, 10, 30, and 2 ppm, respectively

Sample solution



Results & Discussion

Trial production

Density meter: Acceptance criteria 69.6 - 70.5 %v/v

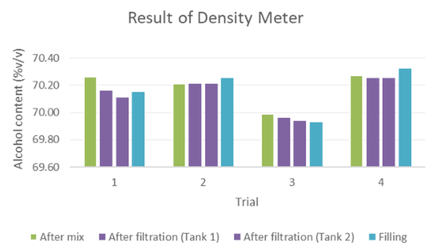


Fig. 1: The results from the density meter indicate that the alcohol content is within the specified acceptance criteria.

pH meter: Acceptance criteria 5 - 8

Trial	pH			
	After mix	After filtration		Filling
		Tank 1	Tank 2	
2	5.29	5.12	5.15	5.13
3	6.54	6.45	6.53	6.48
4	6.07	5.98	5.72	6.07

Fig. 2: The pH values measured by the pH meter ranged from 5.12 to 6.53.

UV-Vis spectrophotometer: Acceptance criteria 0.2 - 0.3

Trial	Absorbance			
	After mix	After filtration		Filling
		Tank 1	Tank 2	
2	0.2323	0.2322	0.2329	0.2323
3	0.2222	0.2225	0.2228	0.2233
4	0.2422	0.2403	0.2407	0.2393

Fig. 3: The absorbance at 640 nm, measured by the UV-Vis spectrophotometer, was within the specified acceptance criteria.

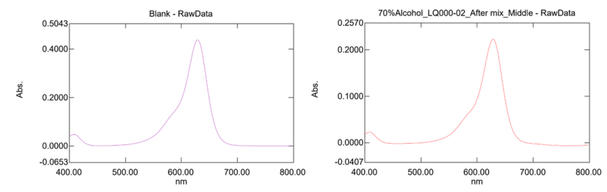
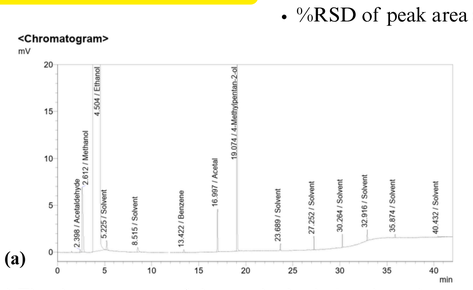


Fig. 4: Absorption Spectrum of the product samples at 640 nm in Trial Batch 2

Analytical Method Validation for Organic solvent of GC-FID

System suitability & Specificity:

- The resolution between acetaldehyde and MeOH >1.5
- %RSD of peak area (n=6) < 7.3% and %Interference < 10%



(a)

Resolution solution	
Solution	Resolution
Acetaldehyde	-
Methanol	1.607

(b)

Standard solution			
Solution	Ret. Time (mins)	%RSD of Area response	%Interference
Acetaldehyde	2.393	0.988	0.22
Methanol	2.610	0.349	0.02
Benzene	13.416	1.255	0.00
Acetal	16.993	0.273	0.25
4-Methylpentan-2-ol	19.071	0.155	0.00

Fig. 5: (a) The chromatogram of the standard solution showed a resolution between acetaldehyde and methanol of 1.601. **(b)** The %RSD for the peak areas of acetaldehyde, methanol, benzene, acetal, and 4-methylpentan-2-ol were 1.836, 0.324, 1.335, 0.251, and 0.168, respectively. Additionally, the %interference was less than 10%.

Linearity:

- The correlation coefficient (R) > 0.990

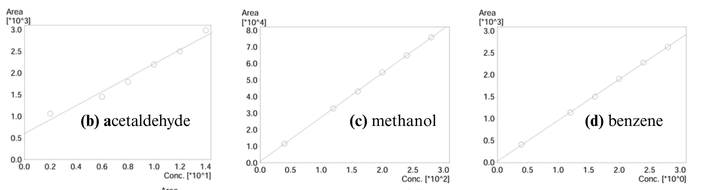


Fig. 6: (a) The correlation coefficients (R) for acetaldehyde, methanol, benzene, acetal, and 4-methylpentan-2-ol were greater than 0.990 for all five analytes. **(b), (c), (d), (e),** and **(f)** display the calibration curves for acetaldehyde, methanol, benzene, acetal, and 4-methylpentan-2-ol, respectively.

(a)

Solution	Slope	%Y-intercept (%)	Correlation coefficient (R)	Coefficient of determination (R^2)
Acetaldehyde	55.6120	64.7336	0.9937901	0.9876188
Methanol	258.008	-0.16340	0.9996470	0.9992941
Benzene	903.041	-8.02570	09995401	0.9990804
Acetal	428.107	26.5587	0.9997874	0.9995748
4-Methylpentan-2-ol	665.582	-4.55890	0.9997616	0.9995233

LOD and LOQ precision:

- The S/N of LOD solution > 3.0 and The S/N of LOQ solution > 10.0
- %RSD of peak area (n=6) in LOQ solution < 7.3%

(a)

LOD solution			
Solution	S/N in each injection No.		
	LOD-1	LOD-2	LOD-3
Acetaldehyde	16.57	6.50	7.08
Methanol	7.90	8.13	8.02
Benzene	4.16	3.03	5.77
Acetal	150.22	187.17	218.02
4-Methylpentan-2-ol	403.80	360.05	656.77

(b)

LOQ solution							
Solution	S/N in each injection No.						%RSD of Area response
	LOQ-1	LOQ-2	LOQ-3	LOQ-4	LOQ-5	LOQ-6	
Acetaldehyde	17.45	15.63	17.73	21.48	20.32	22.48	5.476
Methanol	24.29	18.66	18.45	21.24	21.59	21.04	0.621
Benzene	13.02	11.14	13.37	13.00	11.91	13.56	4.802
Acetal	237.7	313.2	258.4	191.5	206.2	185.7	0.510
4-Methylpentan-2-ol	1925	2127	1564	2093	1886	2044	0.317

Fig. 7: (a) The Signal-to-Noise (S/N) ratios for acetaldehyde, methanol, benzene, acetal, and 4-methylpentan-2-ol in the LOD solution were greater than 3.0. **(b)** For the LOQ solution, the S/N ratios exceeded 10.0, indicating high sensitivity and reliable signal detection for the analytical method. Furthermore, the %RSD of peak areas at the LOQ level for all analytes—recorded at 5.476, 0.621, 4.802, 0.510, and 0.317, respectively

Precision:

- %RSD of sample solution for Repeatability precision and Intermediat precision < 7.3%
- The overall statistical %RSD (repeatability + intermediat) < 13%

(a)

Solution	%RSD of Area response		
	Repeatability	Intermediate	Overall
Acetaldehyde	7.092	5.320	6.321
Methanol	2.516	1.628	2.038
Benzene	0.000	0.000	0.000
Acetal	3.897	6.178	6.259
4-Methylpentan-2-ol	0.000	0.000	0.000

(b)

Solution	Acceptance criteria	Repeatability	Intermediate
Methanol	NMT 0.5, corresponding to 200 µL/L	0.04405	0.04403
Acetaldehyde and acetal	NMT 10 µL/L, expressed as acetaldehyde	4.716	2.410
Benzene	NMT 2 µL/L	0	0
Other impurities	NMT 300 µL/L	0	0

Fig. 8: (a) The %RSD for Repeatability of acetaldehyde, methanol, benzene, acetal, and 4-methylpentan-2-ol were 7.092, 2.516, 0.000, 3.897, and 0.000, respectively. For Intermediate Precision, the %RSD values were 5.320, 1.628, 0.000, 6.178, and 0.000, respectively. **(b)** The overall Method Precision showed total %RSD values were less than 13%. These results demonstrate that both Repeatability and Intermediate Precision meet the specified acceptance criteria, indicating that the analytical method is highly precise and provides consistent results when performed under the same or varied conditions.

Conclusion

Based on the pilot production of 70% Alcohol, samples were analyzed using a Density Meter, UV-Vis Spectrophotometer, and pH Meter. All three analytical parameters met the specified acceptance criteria, confirming that the manufacturing process is appropriate and viable for the production of 70% alcohol.

Furthermore, the Analytical Method Validation for Organic Impurities of Ethanol by GC-FID demonstrated that all validation parameters—including System Suitability, Specificity, Linearity, LOQ/LOD Precision, and Precision—successfully passed the acceptance criteria. These results confirm that the equipment, reagents, and analytical instruments at the Chulabhorn Royal Pharmaceutical Manufacturing Facility are suitable for this analytical method. Consequently, this method can be officially transferred to the Quality Control (QC) Department for routine production analysis and used as supporting data for future drug registration.

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