嘉南藥理科技大學專題研究計畫成果報告

市售酒精性飲料中甲醇之快速氣相層析定量分析法

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A Rapid and Simple Gas Chromatographic Method for Direct Determination of Methanol in Alcoholic Beverages

計畫編號：90-FH-10　　執行期間：90年1月1日至90年12月30日

計畫主持人：王美苓

一、中文摘要

本研究建立了直接注入氣相層析分析市售酒精性飲料液體樣品中甲醇及乙醇之快速、簡便定量方法。採用直接注入(direct injection)之方式，以極性之CP-Wax 58 CB管柱 (30 m × 0.53 mm)同時分析定量酒精性飲料中甲醇及乙醇含量，選擇水溶性之乙　(acetonitrile) 爲內標準，甲醇及乙醇最低檢出量分別爲0.1及0.05 μg/mL左右。添加甲醇100及500 μg 於1mL於威士忌及紅酒中，直接注入GC分析，其回收率分別爲102-107%及95-104%，變異係數均在5.5%以下。而添加乙醇50及100 mg 於1mL於威士忌及紅酒中，其回收率分別爲97-104%及98-106%，變異係數均在5.4%以下。以本方法分析市售不同廠牌之酒精性飲料，包括蒸餾酒19件及非蒸餾酒16件，樣品共35件之甲醇及乙醇含量。結果顯示，甲醇及乙醇之含量分別爲：蒸餾酒，10.2-1284.7 μg/mL及154.3-507.1 mg/mL；非蒸餾酒，21.8-294.8 μg/mL及43.2-280.4 mg/mL。35件樣品中有3件甲醇含量在876-1285 μg/mL及154.3-507.1 mg/mL，顯然超出規定值許多。

關鍵詞：酒精性飲料，甲醇，乙醇，直接注入法，氣相層析，定量分析。

ABSTRACT

A simple and rapid method was developed to determine the methanol and ethanol in alcohol beverages using megapore polar column (CP-Wax 58 CB, 30 m × 0.53 mm) with direct injection gas chromatography. Direct quantitative analysis of methanol and ethanol in alcohol beverages was carried out without any sample pretreatment procedure. Water soluble compound acetonitrile was used as internal standard. The detection limit for methanol and ethanol was 0.1 and 0.05 μg/mL, respectively. Recovery studies were performed using 1 mL of whisky and red wine, each spiked with methanol and ethanol at 100, and 1000.0 μg, respectively. The recoveries were found in the range of 94~106% and 90-105%, respectively. The coefficients of variation being less than 5.1%. Fourty-two commercial alcohol beverages were analyzed by the current method. The methanol and ethanol content were found as: 131-246, 24-112, 0-134 and 0-263 μg/mL, respectively. 3 out of 35 methanol of commercial alcohol beverages exceeded the Taiwan ADI level (400 μg/mL).

二、緣由與目的

甲醇和乙醇的味覺是不同的，而且甲醇氧化分解較乙醇慢，有蓄積作用，不易排出體外而有一定毒性，一次攝入4 -10 g 即引起嚴重中毒。甲醇不像乙醇那樣迅速氧化爲二氧化碳和水，甲醇經氧化變爲甲醛和甲酸，都是毒性較強的物質，甲醛和甲酸的毒性分別比甲醇大30倍和6倍。甲醇中毒可導致視網膜受損，甚至失明。酒精的品質或酒精中含甲醇量的多少，除與生產酒精的原料種類有關外，還與生產過程有關。不久的將來，私釀酒將合法化，爲了維護消費者之安全，酒類中甲醇含量之測定，尤其是快速定量方法之建立更顯得有其急迫性。

氣相層析具有高解析度及高靈敏度等優點，仍爲現代最重要分析技術之一。本研究室發現市面販售廣口徑之GC分離毛細管柱（megapore column） 之耐水性相當高。即使直接注入水溶液之樣品於GC中，GC管柱之分離效果與滯留時間之再現性仍與原先之新管柱一樣。基於上述之理由，本研究擬將酒精性飲料樣品，不經任何前處理，加入適當之內標準溶液後，直接注入氣相層

析儀中，配合適當的分離管柱及氣相層析條件，以期建立簡便、快速又精確之酒精性飲料之甲醇及乙醇定量分析方法。

另外，目前有關界面活性劑合成之研究報告均以游離脂肪酸爲疏水基原料和胺基酸或有機酸（如乳酸）親水基原料進行反應合成界面活性劑，甚少以脂肪醇（原料亦相當便宜）和胺基酸或有機酸合成界面活性劑之文獻及專利報告，實值得研究探討。乳化劑及界面活性劑經濟價值高，若能以低價的脂肪醇爲疏水基原料配合國產大量又便宜之蛋白質（黃豆蛋白、乳清蛋白、卵蛋白等…或）、胺基酸（如麩胺酸等）等作爲親水性原料合成高價且廣效性食品乳化劑或界面活性劑，則不但可提高原料的附加價值，同時對於國內產業技術的提升助益很大，開發此類源自再生性資源的新界面活性劑，實值得我們深入探討。此爲本研究計畫之動機。

本研究室在先前主持之研究計畫（NSC 87-2314-B-127-003及NSC 88-2313-B-127-001）以針對以脂肪醇爲疏水性材料和親水性材料麩胺酸(glutamic acid)進行縮合，並探討其產物之基本特性及界面活性。目前研究計畫之進行亦相當順利，已獲得相當不錯之成果，相關之研究乃在進行中。

本計畫則擬針對另一項主題作進一步深入探討、開發。本研究擬以脂肪醇爲疏水性材料和親水性材料蛋白質及其水解物進行縮合，並探討其產物（PFAD）之基本特性及界面活性。經本研究

三、結果與討論：

一、　氣相層析條件之探討：

在考慮管柱之選擇方面，乃採用極性之 CP-Wax 58CB 管柱來分析甲醇及乙醇，樣品不需前處理，直接取樣注入氣相層析儀中進行分析，管柱分析之昇溫程序如方法（六）所示。依上述條件直接注入甲醇及乙醇標準品溶液，其氣相層析儀滯留時間分別爲 3.04 min 及 3.79 min，如圖一所示。

在內標準品之選擇方面，於蒸餾酒（如whisky）及非蒸餾酒（如紅葡萄酒）樣品中逐一加入微量之 1-propanol、2-propanol、acetonitrile及 acetone 四種水溶液之標準品，然後依上述氣相層析條件分析之。結果顯示，上述四種標準品之氣相層析滯留時間分別爲 6.43、6.37、5.15 及 6.06 min，如表一所示。經與蒸餾酒及非蒸餾酒空白樣品之 GC 圖比對，乙　（acetonitrile）及 2-propanol 之 GC 波峰完全不會與樣品中之成分波峰重疊，其中乙　與甲醇、乙醇之波峰較接近，故最適合作爲本研究中甲醇及乙醇定量之內標準品。

本研究之氣相層析分析條件，首先採用較低溫之程序 38℃（4 min）→4℃/min→50℃，讓甲醇、乙醇及乙　（內標準品）都先後分離出來後（約 7 min 後，）即採取快速升溫（40℃/min）至 245℃，將樣品中滯留管柱內之成分快速帶出，如此可以在 16 min 內完成一個樣品之分析，以達到建立快速分析定量。紅葡萄酒及 whisky 之氣相層析圖分別如圖二及圖三所示

二、甲醇及乙醇對內標準品之相對感應係數：

本研究選擇水溶性之acetonitrile 作爲直接定量市售酒精性飲料中甲醇及乙醇之內標準，要有準確之定量，必需先定出甲醇及乙醇對內標準之相對感應係數(RRF)，則樣品中甲醇及乙醇之含量可依公式(2)計算之。由甲醇及乙醇對內標準品之GC波峰面積比(Y軸)對其濃度比(X軸)作圖，其線性迴歸係數均在R^2=0.98以上。並計算求得甲醇及乙醇對內標準品之相對感應係數分別爲0.62、0.91。

三、甲醇及乙醇之GC-FID最低檢出量：

本研究將0.1%(w/v)之甲醇及乙醇標準溶液，經一系列之稀釋後，分別加入適量之內標準液，以直接注入法注入GC中，採用FID爲檢測器，訊號設定FID range=1，且attenuation=1，在上述分析條件下，設定甲醇及乙醇回收率之cv%＝15%，即爲最低檢出量或最低定量濃度(Lowest quantitatively determinable concentration)。結果顯示甲醇及乙醇之最低檢出量分別爲0.1及0.05 μg/mL左右，分別如表二及表三所示。

四、添加回收試驗：

表四及五爲甲醇及乙醇添加至蒸餾酒（whisky）及非蒸餾酒（red wine）之添加回收試驗。結果顯示，甲醇分別添加 100 及 500 μg 於 1mL 之威士忌及紅酒中，其回收率分別爲 102-107%及 95-104%，變異係數均在 8.5%以下，如表四所示。而乙醇分別添加 50 及 100 mg 於 1mL 之威士忌及紅酒中，其回收率分別爲 97-104%及 98-106%，變異係數均在 9.4%以下，如表五所示。由上述結果顯示，本研究採用之直接注入 GC 法，樣品不需任何前處理即可取樣分析，每分析一個樣品僅需 16min，GC 層析圖如圖二及圖三所示。是目前研究報告中，最簡單快速之甲醇及乙醇同時定量分析之方法，故此法可建議作爲一般例行分析用之參考。

五、市售酒精性飲料中甲醇及乙醇之含量：

市售不同廠牌之酒精性飲料，包括蒸餾酒19件及非蒸餾酒16件，直接注入GC分析其甲醇及乙醇含量。結果如表六所示，蒸餾酒中甲醇及乙醇含量分別爲：10.2-1284.7 μg/mL及 154.3-507.1 mg/mL；非蒸餾酒，21.8-294.8 μg/mL及43.2-280.4 mg/mL。35件樣品中有3件甲醇含量在876-1285 μg/mL及154.3-507.1 mg/mL，顯然超出規定值許多，有可能是假酒。

四、計畫成果自評：

本研究將酒精性飲料樣品，加入一定量水溶性之內標準品(acetonitrile)後，直接取0.1 μL注入GC分析，可以簡單、快速又精確的同時定量酒精性飲料之甲醇及乙醇含量，分析每一樣品全程僅需16分鐘左右。以本研究發展出來的直接注入氣相層析法，檢驗市售酒精性飲料35件樣品。結果顯示酒精性飲料中甲醇及乙醇之含量分別在10.2-1284.7及43.2-507.1 mg/mL之間。

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六、附表：

Table 1. Gas chromatographic retention time of methanol and the internal standards

Compound	Retention time (min)[a]	RRF
Methanol	3.04	0.62
Ethanol	3.79	0.91
1-propanol	6.43	–
2-propanol	6.37	–
Acetonitrile	5.15	1.00
Acetone	6.06	–

[a] CP-Wax 58CB column (30m × 0.53mm, 1.5μm) was used. Oven condition = 38℃(4min)→ 5℃/min→60℃(1min)→ 40℃/min→245℃.

Table 2. Recoveries of the methanol in spiked commercial alcoholic beverage by direct injection gas chromatographic method

Sample	Blank[a] (μg) (A)	Amount added (μg)(B)	Amount found[b] (μg) (C)	Recovery (%)[c]	CV (%)[d]
Red wine	294.6	100	401.3	106.7	6.8
		500	803.2	101.7	5.3
Wishky	1048.7	100	1152.5	103.8	8.5
		500	1521.8	94.6	4.6

[a] Methanol in 1 mL alcoholic beverage.
[b] Average of triplicate analyses.
[c] Recovery (%) = (C−A) / B × 100%.
[d] Coefficient of variation (cv %).

Table 3. Recoveries of the ethanol in spiked commercial alcoholic beverage by direct injection gas chromatographic method

Sample	Blank[a] (mg) (A)	Amount added (mg)(B)	Amount found[b] (mg) (C)	Recovery (%)[c]	CV (%)[d]
Red wine	106.5	50	154.9	96.8	6.9
		100	210.1	103.6	8.5
Wishky	351.4	50	404.3	105.8	9.4
		100	449.7	98.3	7.5

[a] Ethanol in 1 mL alcoholic beverage.
[b]Average of triplicate analyses.
[c]Recovery (%) = (C−A) / B × 100%.
[d]Coefficient of variation (cv %).

Table 4. Methanol and ethanol levels found in some alcoholic beverage

Sample	Content	
	Mthanol (μg/mL)	Ethanol (mg/mL)
Distilled spirit		
Whisky 1	1056.9	351.6
Whisky 2	1284.7	431.2
Kirin whisky 3	867.3	407.4
White liquor 1	213.5	404.1
Kao liang 1	321.7	402.3
Kao liang 2	285.6	385.7
XO-1	293.9	384.5
XO-2	189.6	376.8
VSOP 1	159.7	289.7
VSOP 2	291.6	305.6
95% alcohol	219.3	951.1
Rice wine 1	18.7	165.2
Rine wine 2	10.2	154.3
Selfmade-Rine wine	83.6	301.1
Plum wine 1	93.1	189.5
Plum wine 2	80.1	208.3
Plum wine 3	77.9	200.9
Medecine wine 1	321.6	197.7
Medecine wine 2	310.1	507.
Non-distilled spirit		
Small rice wine 1	123.1	77.2
Small rice wine 1	120.2	89.9
Red wine 1	76.3	90.1
Red wine 2	294.8	106.5
Red rose 1	195.6	128.4
Red rose 2	202.3	117.1
Red rose 3	163.6	140.3
Fruit wine 1	62.8	91.2
White wine 1	127.6	100.4
White wine 2	167.1	103.9
Yellow wine 1	265.4	213.3
Yellow wine 2	218.8	280.4
Shao-Hsing wine 1	76.8	141.2
Shao-Hsing wine 2	84.4	138.6
Beer 1	21.8	43.2
Beer 2	34.1	45.8

[a] Average of two analyses.

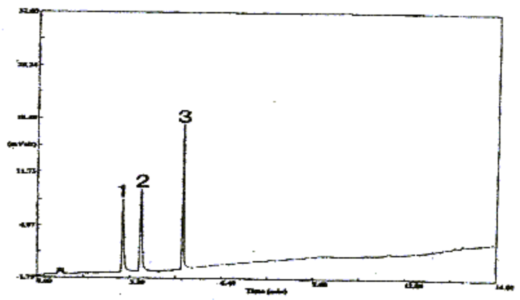

Figure 1. Gas chromatogram of methanol, ethanol and acetonitrile authentic compound by direct injection method.

Peaks: 1 = methanol, 2 = ethanol, and 3 = acetonitrile (IS)

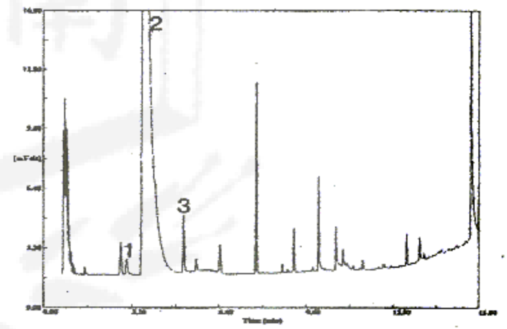

Figure 2. Gas chromatograms of the methanol and ethanol in non-distilled spirit (red grape wine) by direct injection method. Peak identification is the same as in Figure 1.

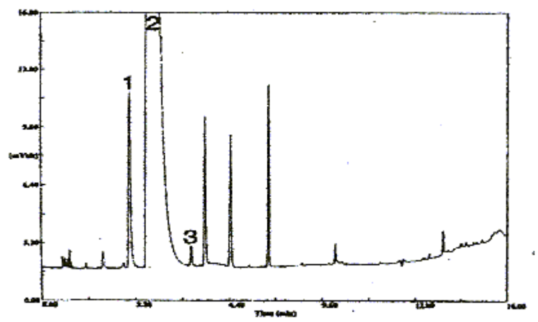

Figure 3. Gas chromatograms of the methanol and ethanol in distilled spirit (whisky) by direct injection method. Peak identification is the same as in Figure 1.