Determination of biogenic amines in wine by micellar electrokinetic capillary chromatography

本研究利用微胞電動力毛細管電泳檢測酒類之生物胺含量，可在11分鐘內偵測九種生物胺：色胺(T)、酪胺(TA)、血清素(5-HT)、5 -羥基色胺酸(5-HTP)、多巴胺(DA)、色胺酸(Trp)、酪胺酸(Tyr)、腎上腺素(E)、去甲基腎上腺素(NE)。分別探討背景電解質磷酸濃度、pH值與十二烷基硫酸鈉濃度變化對於生物胺分離情形之影響，從中找出最佳化條件。

最佳分離條件為使用內徑75 μm，外徑365 μm，全長50 cm，有效長度40 cm之毛細管，填充背景電解質為5 mM磷酸緩衝溶液 (pH 3.7)，含20 mM十二烷基硫酸鈉，以上述溶液為背景電解質，外加電壓-15 kV，以紫外光/可見光偵測器進行偵測，偵測波長設定在 200 nm進行毛細管電泳實驗。在此最佳分離條件下，線性範圍介於0.55 ~ 10 μM之間，相關係數皆大於0.99，以訊號/雜訊比(S/N ratio)等於3作為判定標準計算得到偵測極限介於0.11 ~ 0.61 μM，證明本方法具有良好的定量能力。

應用於市售酒類樣品(白酒、米酒、啤酒)，進行目標分析物的鑑定以及定量，發現酒類含有酪胺(TA)、色胺酸(Trp)和酪胺酸(Tyr)，並得到回收率介於96.5 ~ 111.6 %，回收率之相對標準偏差介於1.25 ~ 8.29 %，結果顯示本方法可以有效檢測酒類所含的生物胺。

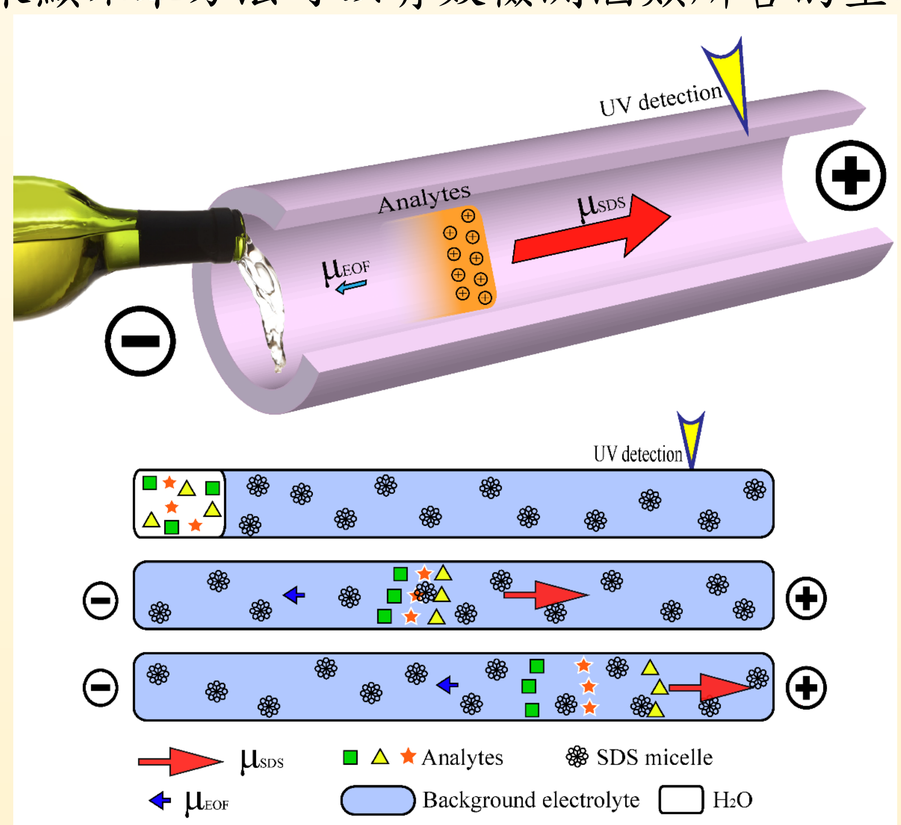

Figure 1 Procedure for the separation of the samples by using MEKC.

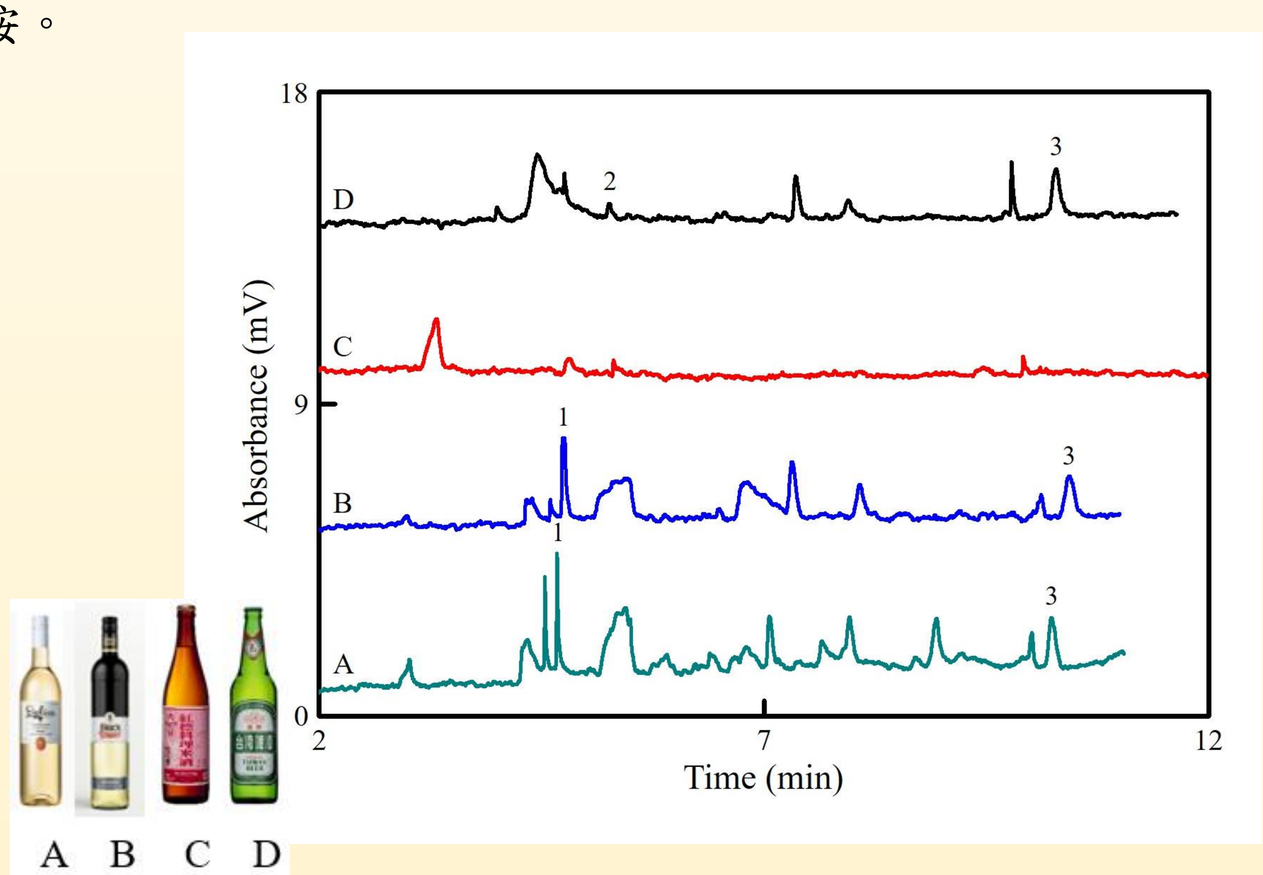

Figure 3 Electropherograms of (A) 10-fold diluted white wine A, (B) 16-fold diluted white wine B, (C) rice wine, (D) 50-fold diluted beer. Peaks 3: TA; 5: Trp; 9: Tyr

Table 1 Optimum conditions.

Wavelength	200 nm
Concentration of phosphate buffer	5 mM
pH values of phosphate buffer	3.7
Concentration of SDS	20 mM
Applied voltage	-15 kV
Injection time for sample	10 s
Capillary length	50 cm (40 cm effective length)
Capillary diameter	75μm i.d. and 375μm o.d.

Table 2 Recovery results of analytes in real samples (n = 3).

Analyte	White wine A			White wine B			Beer			Rice wine		
	Original (μM)	Recovery (%)	RSD (%)	Original (μM)	Recovery (%)	RSD (%)	Original (μM)	Recovery (%)	RSD (%)	Original (μM)	Recovery (%)	RSD (%)
T	N.D.			N.D.			N.D.			N.D.	111.6%	2.89%
5-HT	N.D.			N.D.			N.D.			N.D.	101.5%	2.32%
TA	3.52	105.7%	4.66%	3.57	96.8%	4.60%	N.D.			N.D.	115.6%	5.54%
DA	N.D.			N.D.			N.D.			N.D.	111.1%	3.17%
Trp	N.D.			N.D.			1.52	96.5%	7.01%	N.D.	98.8%	1.25%
E	N.D.			N.D.			N.D.			N.D.	107.4%	2.65%
5-HTP	N.D.			N.D.			N.D.			N.D.	104.2%	3.67%
NE	N.D.			N.D.			N.D.			N.D.	104.2%	3.67%
Tyr	2.23	99.6%	2.65%	3.59	104.0%	5.02%	3.95	104.8%	8.29%	N.D.	104.4%	4.81%

The recovery results were calculated by spiking 4.0 μM standards to each sample.

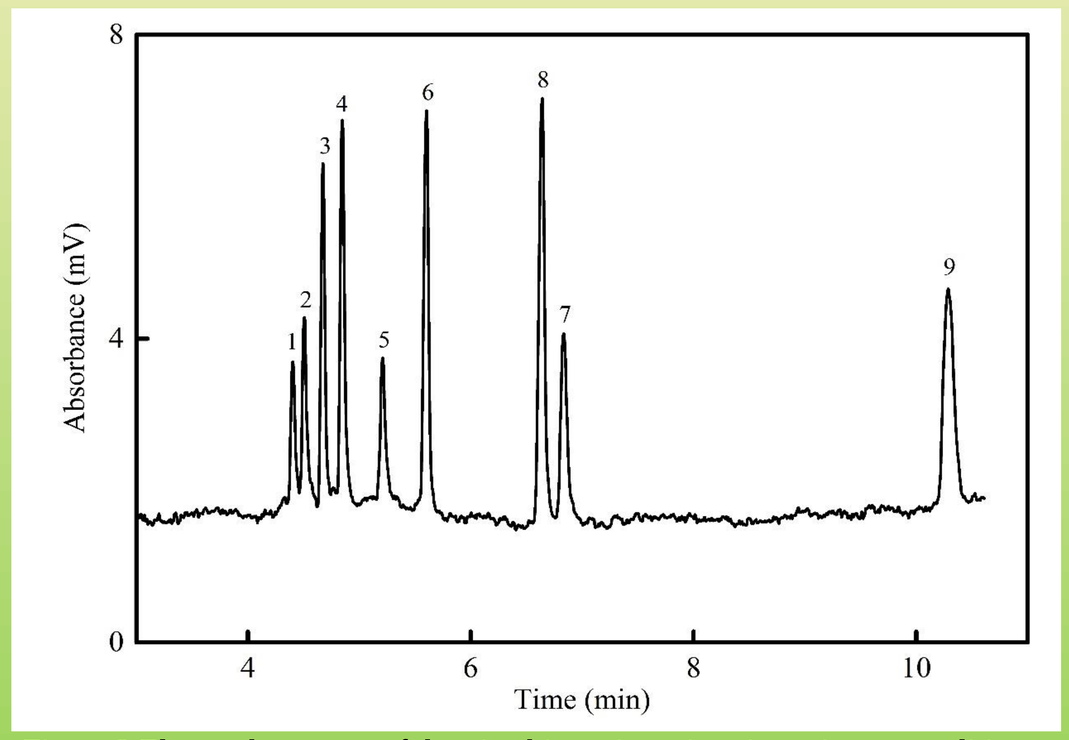

Figure 2 Electropherogram of the nine biogenic amines in optimum conditions. Peaks 1: T; 2: 5-HT; 3: TA; 4: DA; 5: Trp; 6: E; 7: 5-HTP; 8: NE; 9: Tyr.

本研究成功開發一種簡單且快速的分析方法來檢測酒類中的生物胺含量，真實樣品僅需要離心、過膜再稀釋後即可上機檢測，並於11分鐘內完成分析。未來可望將此方法推展至更多酒類的應用，甚至其他真實樣品檢測上。

1. Bacaloni, A.; Insogna, S.; Sancini, A.; Ciarrocca, M.; Sinibaldi, F., Sensitive profiling of biogenic amines in human urine by capillary electrophoresis with field amplified sample injection. *Biomedical Chromatography* **2013,** *27* (8), 987-993.
2. Wang, W.-f.; Ju, F.-r.; Ran, Y.-l.; Zhang, H.-g.; Chen, X.-g., Detection of biogenic amines in C57BL/6 mice brain by capillary electrophoresis electrokinetic supercharging. *Analyst* **2016,** *141* (3), 956-962.
3. Ginterova, P.; Marak, J.; Stanova, A.; Maier, V.; Sevcik, J.; Kaniansky, D., Determination of selected biogenic amines in red wines by automated on-line combination of capillary isotachophoresis-capillary zone electrophoresis. *J Chromatogr B Analyt Technol Biomed Life Sci* **2012,** *904*, 135-9.
4. Kvasnicka, F.; Voldrich, M., Determination of biogenic amines by capillary zone electrophoresis with conductometric detection. *J Chromatogr A* **2006,** *1103* (1), 145-9.