

利用毛細管電泳線上濃縮技術分析氟硝西泮、 古柯鹼、鴉片類藥物及其相關代謝物

研究生：黃瓊葦 指導教授：謝有容

國立交通大學應用化學系碩士班

摘要

氟硝西泮、古柯鹼與鴉片類藥物為近年來被廣泛濫用之藥物，因此我們選擇了毛細管電泳線上濃縮技術針對氟硝西泮、古柯鹼、鴉片類藥物與其相關代謝物，利用毛細管電泳良好的分離率結合上線上濃縮技術，改善其偵測靈敏度來進行研究。

在氟硝西泮與其代謝物方面，利用掃略式線上濃縮技術來進行分析，其最佳分離條件：緩衝溶液為 pH 9.5, 25 mM 四硼酸鈉緩衝溶液、CTAB 50 mM、甲醇 30% (v/v) ，樣品區帶為 40 mM 四硼酸鈉緩衝溶液，樣品以 0.5 psi 注入 300 sec ，施加 -25 KV 之電壓進行分離。在此條件下氟硝西泮與其代謝物之偵測極限可有效降低至 5.6 ~ 13.4 ppb 之間，堆積效率均在 110 倍以上，在方法的準確度上，對於遷移時間與波峰面積之相對標準偏差(RSD) 小於 4.1% 。經過固相萃取去除基質干擾後，可成功地應用在尿液樣品上。

在古柯鹼及鴉片類藥物方面，利用陽離子選擇性全注入結合掃掠式線上濃縮法來進行分析，其最佳分離條件：高導電度緩衝溶液為 pH 4.0, 100 mM 檸檬酸/磷酸氫二鈉緩衝溶液、乙腈 15% (v/v) ，背景緩衝溶液為 pH 4.0, 50 mM 檸檬酸/磷酸氫二鈉緩衝溶液、SDS 150 mM ，樣品溶液為 1 mM 檸檬酸/磷酸氫二鈉緩衝溶液以 10 KV 電動注入 600 sec ，再施加-25 KV 之電壓進行濃縮與分離，在古柯鹼及鴉片類藥物之偵測極限可有效降低至 0.13 ppb ~ 0.43 ppb 之間，堆積效率均在 2200 倍以上，在方法的準確度上，對於遷移時間與波峰面積之相對標準偏差(RSD) 均小於 4.78% 。經過固相萃取去除基質干擾後，也可以成功地應用在尿液樣品上。

Analyses of flunitrazepam, cocaine, opiates, and their metabolites by on-line sample concentration techniques in capillary electrophoresis

Student : Chiung-Wei Huang Advisor : You-Zung Hsieh*

Department of Applied Chemistry, National Chiao Tung University

Abstract

Flunitrazepam, cocaine, and opiates are usually used abuse drugs recently. We have developed a on-line sample concentration techniques in capillary electrophoresis for determination of flunitrazepam, cocaine, opiates, and their metabolites. Using capillary electrophoresis has good separation efficiency and on-line sample concentration techniques have good sensitivity, to improve detection limit.

In case of flunitrazepam and it's metabolites, we use sweeping technique. The optimal conditions for separation were achieved at pH 9.5 using a 25 mM borate buffer, 30%(v/v) menthol, 50 mM CTAB, a 300 sec injection time and -25 KV separation voltage. Using sweeping technique, the values of LOD that we obtained were in the range of 5.6 ~ 13.4 ppb. The values of the RSD% for the retention time and the peak area were also < 4.1% and the stacking efficiency were up to 110 times. After solid-phase extraction, we succeeded using this method in urine sample.

In cocaine and opiates, we use CSEI-sweeping-MEKC technique. The optimal conditions for separation were achieved at HCB pH 4.0, 100 mM citric acid/disodium hydrogen phosphate buffer and 10%(v/v) acetonitrile; BGS 50 mM citric acid/disodium hydrogen phosphate buffer and 150 mM SDS; sample solution 1 mM, pH 2.2 citric acid/disodium hydrogen phosphate buffer; electrokinetic injection 10 KV, 300 s; -20 KV separation voltage. Using CSEI- sweeping-MEKC technique, the values of LOD that we obtained were in the range of 0.13 ppb ~ 0.43 ppb. The values of the RSD% for the retention time and the peak area were also < 4.78% and the stacking efficiency were up to 2200 times. After solid-phase extraction, we succeeded using this method in urine sample.

誌 謝

在研究所二年求學生涯中，感謝吾師 謝有容教授在學術上與生活上的關心與指導，使學生能夠順利地完成碩士的學業與訓練，在將進入人生下一個旅程之時，心中會牢記著老師的諄諄教誨。亦非常感謝二位口試委員 余艇教授與 李耀坤教授在百忙之中撥空指導學生的論文，賦予寶貴之建議，使學生的論文更加流暢與完善。

感謝實驗室修平學長在實驗與處世上的幫助與指導，使我能順利地進行研究，並且在待人處世上有更進一步的認知與學習，在二年相處的過程中，特別感謝學長對我的笨拙予以包容。再者感謝秀麗同學一路的陪伴與扶持，還有瑜婷學妹、小愷學弟、滄浩同學與冠文學長，因為有了你們豐富了我研究所的生活。

感謝一直在我身邊支持我的家人與朋友們，有了你們的鼓勵與諒解使我在面對挫折時，都能再次昇起元氣與勇氣，對於研究所二年幫助我的朋友們，在此深表感謝之意。

最後感謝國科會在實驗經費上的支助 (NSC 92-2113-M-009-031, NSC 93-2113-M-009-022)



目錄

頁次

中文摘要.....	I
英文摘要.....	II
誌謝.....	III
目錄.....	IV
表目錄.....	VIII
圖目錄.....	IX
一、毛細管電泳.....	1
1.1 分離原理.....	2
1.1.1 帶電粒子的電泳遷移行為.....	2
1.1.2 電滲流.....	3
1.1.3 分離效率.....	5
二、線上濃縮技術介紹.....	6
2.1 線上濃縮技術之模式.....	6
2.1.1 線上堆積.....	7
2.1.1.1 電場放大堆積.....	8
2.1.1.2 大體積樣品堆積.....	8
2.1.1.3 緩衝溶液酸鹼值調整修飾濃縮.....	9
2.1.2 掃掠式線上濃縮.....	10
2.1.2.1 陰離子界面活性劑掃掠式線上濃縮.....	10
2.1.2.2 陽離子界面活性劑掃掠式線上濃縮.....	10
2.1.2.3 中性界面活性劑掃掠式線上濃縮.....	11
2.1.2.4 掃掠式線上濃縮模式之濃縮公式.....	11
2.1.2.5 陽離子選擇性全注入結合掃掠式線上濃縮法.....	13
2.1.2.6 陰離子選擇性全注入結合掃掠式線上濃縮法.....	13
2.1.3 不同酸鹼值緩衝溶液接合濃縮.....	14
2.1.4 不同酸鹼值緩衝溶液接合結合掃掠式線上濃縮.....	15
2.1.5 大體積樣品堆積結合掃掠式線上濃縮.....	15

三、利用掃略式線上濃縮毛細管電泳法分析氟硝西泮及其代謝物.....	30
3.1 氟硝西泮.....	30
3.2 線上濃縮方法.....	33
3.3 儀器裝置.....	34
3.4 試藥.....	35
3.5 固相萃取.....	35
3.6 實驗方法.....	36
3.6.1 新毛細管的調態.....	36
3.6.2 實驗前、後毛細管的處理.....	36
3.6.3 氟硝西泮標準溶液之配製.....	36
3.6.4 不同濃縮分離條件之測試.....	37
3.6.4.1 緩衝溶液不同 pH 值之影響.....	37
3.6.4.2 緩衝溶液不同離子濃度之影響.....	38
3.6.4.3 緩衝溶液含量不同濃度 CTAB 之影響.....	38
3.6.4.4 緩衝溶液含量不同甲醇之影響.....	38
3.6.4.5 樣品區帶不同離子濃度緩衝溶液之影響.....	38
3.6.4.6 樣品注射時間.....	39
3.6.4.7 樣品掃集濃縮效果及放大倍率.....	39
3.6.4.8 再現性分析及定量校正曲線.....	39
3.6.5 藥錠之偵測.....	40
3.6.6 尿液樣品之固相萃取步驟.....	40
3.6.6.1 固相萃取之回收率.....	40
3.7 結果與討論.....	41
3.7.1 緩衝溶液不同 pH 值之影響.....	41
3.7.2 緩衝溶液不同離子濃度之影.....	41
3.7.3 緩衝溶液含量不同濃度 CTAB 之影響.....	42
3.7.4 緩衝溶液含量不同甲醇之影響.....	43
3.7.5 樣品區帶不同離子濃度緩衝溶液之影響.....	43
3.7.6 樣品注射時間.....	44

3.7.7 樣品掃集濃縮效果及放大倍率.....	44
3.7.8 再現性分析及定量校正曲線.....	45
3.7.9 藥錠之偵測.....	45
3.7.10 尿液樣品之偵測與回收率.....	46
3.8 結論.....	46
四、利用陽離子選擇性全注入結合掃掠式線上濃縮法分析古柯鹼及鴉片類藥物.....	63
4.1 古柯鹼.....	63
4.2 喀啡.....	64
4.3 海洛因.....	64
4.4 6-乙醯嗎啡.....	65
4.5 可待因.....	65
4.6 線上濃縮方法.....	66
4.7 儀器裝置.....	66
4.8 試藥.....	67
4.9 固相萃取.....	67
4.10 實驗方法.....	67
4.10.1 新毛細管的調態.....	67
4.10.2 實驗前、後毛細管的處理.....	67
4.10.3 古柯鹼及鴉片類藥物標準溶液之配製.....	68
4.10.4 不同濃縮分離條件之測試.....	68
4.10.4.1 高導電度緩衝溶液不同 pH 值之影響.....	68
4.10.4.2 背景緩衝溶液含量不同濃度 SDS 之影響.....	69
4.10.4.3 高導電度緩衝溶液含量不同乙晴之影響.....	69
4.10.4.4 背景緩衝溶液不同離子濃度之影響.....	69
4.10.4.5 樣品注射時間.....	69
4.10.4.6 再現性分析及定量校正曲線.....	70
4.10.5 粉末之偵測.....	70
4.10.6 尿液樣品之固相萃取步驟.....	70
4.10.6.1 固相萃取之回收率.....	71
4.11 結果與討論.....	71
4.11.1 緩衝溶液不同 pH 值之影響.....	71

4.11.2 背景緩衝溶液含量不同濃度 SDS 之影響.....	72
4.11.3 高導電度緩衝溶液含量不同乙晴之影響.....	72
4.11.4 背景緩衝溶液不同離子濃度之影響.....	73
4.11.5 樣品注射時間.....	74
4.11.6 樣品濃縮效果及放大倍率.....	74
4.11.7 再現性分析及定量校正曲線.....	75
4.11.8 粉末之偵測.....	76
4.11.9 尿液樣品之偵測與回收率.....	76
4.12 結論.....	77
五、參考資料.....	92



表 目 錄

頁 次

Table 3-1 Regression equations, r^2 , LOQ, LOD, migration times, and values of RSD for flunitrazepam and its major metabolite during separation using the normal MEKC and sweeping-MEKC techniques.....	36
Table 3-2 Recoveries for 7-aminoflunitrazepam, flunitrazepam and N-desmethylflunitrazepam.....	37
Table 4-1 Stacking efficiency in term of peak height.....	78
Table 4-2 Regression equations, r^2 , LOQ, LOD, migration times, and values of RSD for cocaine and opiates during separation using CSEI-sweeping-MEKC technique.....	79
Table 4-3 Identified compound and purity of three suspect powders.....	80
Table 4-4 Recoveries for cocaine and opiates.....	81

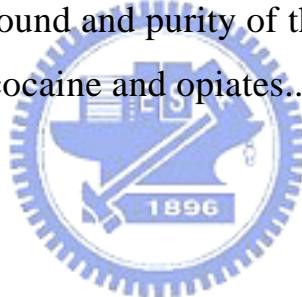


Figure 1-1 The profiles of EOF and double-layer charge distribution at a negatively charged capillary wall.....	7
Figure 2-1 Schematic diagrams of the FASS model.....	18
Figure 2-2 Schematic diagrams of the LVSS mode.....	19
Figure 2-3 Schematic diagrams of the pH-Mediate stacking model.....	20
Figure 2-4 Schematic diagram of a stacking mechanism by sweeping with anionic micelles mode.....	21
Figure 2-5 Schematic diagram of a stacking mechanism by sweeping with cationic micelles model.....	22
Figure 2-6 Evolution of micelles and neutral analyte molecules during sweeping.....	23
Figure 2-7 Schematic diagram of the CSEI-sweep-MEKC mode.....	24
Figure 2-8 Schematic diagram of the ASEI-sweep-MEKC mode.....	25
Figure 2-9 Schematic diagram of the dynamic pH junction model.....	26
Figure 2-10 Schematic diagrams of the dynamic pH junction-sweeping model.....	27
Figure 2-11 Evolution of analyte zones in LVSS-sweeping-MEKC.....	28
Figure 2-12 The choice of on-line concentration techniques.....	29
Figure 3-1 The major mechanisms of flunitrazepam metabolism in humans	50
Figure 3-2 Schematic diagram of sweeping using a CTAB micellar phase..	51
Figure 3-3 Effect that the pH of the buffer solution has on CE separation...	52
Figure 3-4 Variation of the mobility of flunitrazepam and its major metabolite as a function of the buffer electrolyte pH.....	53
Figure 3-5 Effect that ion strength of buffer solution has on CE separation..	54
Figure 3-6 Variation of the mobility of flunitrazepam and its major metabolite as a function of the buffer electrolyte concentration.....	55

Figure 3-7 Effect that CTAB concentration in buffer solution has on CE separation.....	56
Figure 3-8 Effect that the percentage of methanol in the buffer solution has on CE separation.....	57
Figure 3-9 Effect that the sample matrix (borate buffer) concentration has on CE separation.	58
Figure 3-10 Effect that the injection time has on CE separation.....	59
Figure 3-11 A comparison between the sweeping-MEKC method and that of normal hydrodynamic sample injection.....	60
Figure 3-12 Sweep-MEKC electropherogram of suspect's powders.....	61
Figure 3-13 Sweep-MEKC electropherogram of extraction urine.....	62
Figure 4-1 The structure of cocaine and opioid drugs.....	82
Figure 4-2 Effect that the pH of the buffer solution has on CE separation... ..	83
Figure 4-3 Effect that SDS concentration in background buffer solution....	84
Figure 4-4 Effect that the percentage of acetonitrile in the high conductivity buffer solution.....	85
Figure 4-5 Effect that ion strength of background buffer solution.....	86
Figure 4-6 Effect that the injection time.....	87
Figure 4-7 A comparison between the CSEI-sweeping-MEKC, sweeping-MEKC method and normal MEKC.....	88
Figure 4-8 CSEI-sweeping-MEKC electropherogram of suspect's powders.....	89
Figure 4-9 On-line UV spectrum of morphine, herion, cocaine and three suspect's powders.....	90
Figure 4-10CSEI-sweeping-MEKC electropherogram of extraction urine	91