Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 795 (2003) 329–335
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# Simultaneous determination of theophylline and dyphylline by micellar electrokinetic chromatography and application in drug formulations

Wei-Shan Huang, Shun-Jin Lin, Hsin Lung Wu, Su-Hwei Chen*

*Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Received 4 November 2002; received in revised form 22 July 2003; accepted 22 July 2003

**Abstract**

A simple micellar electrokinetic chromatography is described for well resolution of theophylline, dyphylline and caffeine. The separation was performed at 25 °C using a background electrolyte consisting of 10 mM borate buffer at pH 9 and 40 mM sodium dodecyl sulfate (SDS) as running buffer. Under this condition, good separation with high efficiency and short analyses time required is achieved. Several parameters affecting the separation of the drugs were studied, including the pH and concentrations of the borate buffer and sodium dodecyl sulfate. Using caffeine as an internal standard (I.S.), the linear range of the method for the determination of theophylline and dyphylline was over 0.03–1 μmol ml$^{-1}$; the detection limit (signal-to-noise ratio 3; injection 0.3 psi, 3 s) was 0.01 and 0.02 μmol ml$^{-1}$, respectively.

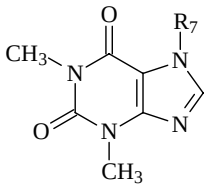

*Keywords:* Theophylline; Dyphylline

## 1. Introduction

Theophylline and caffeine occur naturally in plants [1]. Dyphylline (dihydroxypropyl theophylline) is a derivative of theophylline and not metabolized to theophylline in vivo. They are methylxanthines and have highly similar chemical structure (Fig. 1). Methylxanthines directly relax the smooth muscle of bronchi and pulmonary blood vessels. Therefore, theophylline and dyphylline are usually employed for symptomatic relief or prevention of bronchi asthma and chronic obstructive pulmonary disease clinically. The theophylline therapeutic range is 10–20 μg ml$^{-1}$. The minimal effective therapeutic concentration of dyphylline is 12 μg ml$^{-1}$. Methylxanthines have CNS stimulant properties; its CNS effects are more often encountered as side effects, including nausea, vomiting, anxiety, insomnia and tremors [2]. Generalized convulsions are produced at still high doses and such seizures sometimes occurr in patients in which the blood concentrations of theophylline is only about 50% above the top of the accepted therapeutic range [3]. Dyphylline has a shorter half-life properties causing lower incidence of side effects than does theophylline in clinical use. There are some evidence indicating that dyphylline combined with theophylline may exhibit less adverse

* Corresponding author. Tel.: +886-7312-1101; fax: +886-7321-0683.
*E-mail address:* suhwch@kmu.edu.tw (S.-H. Chen).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1570-0232(03)00601-9



| | $R_7$ | pKa | MW |
|---|---|---|---|
| Dyphylline | $-CH_2CHOHCH_2OH$ | 11.6 | 254.2 |
| Theophylline | -H | 8.8 | 180.2 |
| Caffeine | $-CH_3$ | 14 | 194.2 |

Fig. 1. Chemical structures of theophylline, dyphylline and caffeine.

effects than an equivalent dose of theophylline alone [4,5]. For the safety in medicines, it is quite essential to assure the potency and content uniformity of drugs, especial need concentration monitoring of the narrow effective therapeutic drug, such as dyphylline and theophylline.

Several methods including immunoassay [6–8], high-performance liquid chromatography (HPLC) [9–13] and capillary electrophoresis (CE) [14–19] have been proposed for the determination of these methylxanthines in a biological sample. However, a few chromatographic [20,21] and CE methods [22,23] were developed for determination of methylxanthine in commercial preparations such as simultaneous determination of caffeine and paracetamol in tablets [22]. Many formulations contain components, which are strongly retained and may unduly affect the chromatographic performance of HPLC columns. Therefore, it is often necessary to pretreat sample solutions prior to HPLC analysis. Typical procedures include liquid–liquid extraction and solid phase extraction. However, in the CE analysis of a formulation containing drugs, after the separation, the majority of excipients will be removed during a rinse step. On the other hand, the advantage of a little amount of sample size and running buffer needed CE method which became a major analysis tool to determine chemicals in complicated matrix.

In this work, a simple micellar electrokinetic chromatographic (MEKC) method is developed for the simultaneous separation of theophylline, dyphylline and caffeine. Application of the proposed method to analyse the theophylline or dyphylline in two different commercial preparations were demonstrated and proved to be satisfactory.

## 2. Experimental

### 2.1. Chemicals and reagents

Theophylline and caffeine were from Sigma. Sodium hydroxide (NaOH), di-sodium tetraborate, tris(hydroxymethyl)-aminomethane (Tris), sodium dodecyl sulfate (SDS) and phosphoric acid ($H_3PO_4$, 85%) were supplied by Merck (Darmstadt, Germany). Dyphylline was from Acrós (Geel West Zone, Belgium). Acetonitrile and other reagents were of analytical-reagent grade. Milli-Q (Millipore, Bedford, MA, USA) treated water was used for the preparation of buffer and related drugs.

### 2.2. Preparation of electrolytes and other solutions

Tris, di-potassium hydrogen phosphate and borate buffers at pH 9 were used for preliminary test to assess the suitable electroyte. The 100 mM stock solution of Tris or phosphate buffers were prepared by suitable amount of Tris or di-potassium hydrogen phosphate in 50 ml volumetric flask and adjusted at pH 9, then diluted to 10 mM. From the results, the borate buffer was selected for further study. The stock solution of 100 mM borate buffer was prepared by dissolving 1.006 g of di-sodium tetraborate in 50 ml volumetric flask with 30 ml deionized water and diluted to volume. Solutions of various borate buffers at different pH were prepared by neutralizing the borate solution with $H_3PO_4$. Solutions of SDS borate buffer at various levels of SDS were obtained by dissolving different amounts of SDS in water, then diluted with stock solution of borate buffer as a running buffer. The optimization of separations were performed at about 25 °C in borate buffer (10 mM; pH 9.0) with SDS (40 mM) using a voltage of 12 kV and 20 μA of the current is produced. Stock solutions of three methylxanthines at 2.0 mM were prepared in water and suitably diluted as reference solutions.

### 2.3. CE conditions

A Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a diode-array detector and a liquid-cooling device were used. MEKC was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm (effective length 30 cm) × 75 μm i.d. Samples were injected by pressure (0.3 psi) for 3 s. Before analysis, we scanned theophylline, dyphylline and caffeine by a Beckman spectrophotometer. The wavelengths of maximal absorbance of these drugs were 203–206 nm in running buffer. Detection was carried out by the on-column measurement of UV absorption at 203 nm (cathode at the detection side). Capillary conditioning before startup is: methanol (10 min), 1N HCl aqueous solution (10 min), deionized water (2 min), 1N NaOH aqueous solution (10 min) and deionized water (2 min). The conditioning between runs was effected by rinsing with 0.1N NaOH (3 min), deionized water (2 min), and running buffer (3 min), under positive pressure applied at the injection end. A Beckman P/ACE MDQ Microsoft software system was used for data processing. Acetone was used as EOF marker.

### 2.4. Sample preparations

For the assay of theophylline or dyphylline in tablet formulations, sample solutions were prepared as follows: 10 tablets of theophylline (uniphylline®, labeled amount 300 mg) or 10 tablets of dyphylline (prophylline®, labeled amount 100 mg) were each weighed and finely powdered. We weighed an accurate portion of each of the tablet powder, equivalent to each tablet of about 7.2 mg of theophylline or 10 mg of dyphylline, by dissolving in 25 ml volumetric flask with the aid of water and sonicate for 5 min. A suitable amount of the resulting water extract was centrifuged at 1000 × $g$ for 10 min. 0.5 ml of the supernatant was pipeted into a 1.5 ml Eppendorf vial and 0.5 ml 2 mM of caffeine as internal standard (I.S.) was added. A 0.2 ml of mixed solution was transferred to a 0.2 ml mini-vial that could be placed into the autosampler for CE analyses.

### 2.5. Method validation

Calibration curves were prepared by simultaneously adding theophylline and dyphylline at five different concentrations and fixed concentration of caffeine (I.S.) in running buffer to make the final concentrations of theophylline, dyphylline as 0.03, 0.05, 0.1, 0.5 and 1 μmol ml$^{-1}$ and caffeine 1 μmol ml$^{-1}$. The calibration graphs were established with the peak-area ratio of theophylline and diphylline to caffeine (I.S.) as ordinate ($y$) versus the concentration of these drugs in μmol ml$^{-1}$ as abscissa ($x$). Intra-day precision and accuracy were tested by analyzing six identical samples for three concentration levels at 0.05, 0.5 and 1 μmol ml$^{-1}$ for theophylline and dyphylline. Inter-day precision and accuracy were calculated from repeated analysis of identical samples on six consecutive days for these concentrations of theophylline and dyphylline.

## 3. Results and discussion

Preliminary test of theophylline, dyphylline and caffeine standards by capillary zone electrophoresis (CZE) was briefly studied at 12 kV with 10 mM of Tris, borate or phosphate buffers at pH 9.0 in the absence of SDS. A broader peak of caffeine and un-symmetric dyphylline peak was observed. Incomplete separation with resolution of about 0.9 between dyphylline and caffeine were obtained in these background electrolytes. To compare different separation systems with respect to the separation efficiency, the plate number is often referred to a capillary length of 1 m. The calculation of separation efficiency was based on $N = 16(t_R/w)^2$, where $N$ is the number of theoretical plates, $t_R$ the migration time of the compound and $w$ the peak width [24]. The theoretical plates of about $1.83 \times 10^4$ and $2.99 \times 10^4$ cm$^{-1}$ for caffeine and dyphylline, respectively, were obtained in these background electrolytes. This indicates that based on the differences in the electrophoretic mobilities resulting in different velocities of migration is not suitable to determine the neutral methylxanthine, dyphylline and caffeine. Therefore, MEKC with SDS as a micellar source was tried to separate the analytes. The SDS in 10 mM of Tris, borate, or phosphate buffer was studied; containing more than 30 mM of SDS in Tris or in borate buffers can get efficiency separation between theophylline, dyphylline and caffeine standard. But higher response to detection of the tested drugs was obtained in borate buffer. The SDS

micelle may provide the chromatographic pseudostationary phase to differentiate the methylxanthines. In order to determine the optimal separation conditions, parameters affecting the theophylline, dyphylline and caffeine separations were studied. Different voltage were tested, 12 kV can provide suitable separation and better efficiency. The detection of theophylline, dyphylline and caffeine in borate buffer (10 mM; pH 9.0) with SDS (40 mM), were monitored at 203 nm in this study.

### 3.1. Optimization of the experimental conditions

The retention behavior of theophylline, dyphylline and caffeine in borate buffer (pH 9.0) at concentration range of 5–25 mM with 40 mM SDS was studied. MEKC of the methylxanthines in borate buffer (pH 9.0) in the concentration range of 5–25 mM can give good separation efficiency (results not shown). To prevent the generation of too much Joule heating, 10 mM of borate buffer was chosen. Because theophylline ($pK_a = 8.8$) with a hydrogen atom at position 7 is ionizable, its migration time was found to increase with increasing pH. The 10 mM borate buffers with SDS (40 mM) at different pH (7.0, 7.5, 8.0, 8.5 and 9.0) are studied. The peak shape of the tested drugs has no significant change at various pH values, but partial overlap between theophylline and dyphylline is observed at pH 7.0–8.0 and incomplete separation between caffeine and theophylline occured at pH 8.5. While increasing pH of the buffer at pH 9, the migration velocity of theophylline decrease and well resolution of the tested drugs are obtained (Fig. 2). The migration velocity of the analyte in MEKC mode depends on the distribution coefficient between the micellar and the non-micellar phase. Dyphylline is theophylline derivative containing a polar functional group, 2,3-dihydroxypropyl at position 7. These two hydroxy groups interacted with borate ions in running electrolyte forming negatively charged complexes and gave it more solubility than theophylline and caffeine in non-micelle [25]. This phenomenon makes dyphylline to have higher migration velocity. So, dyphylline has a shortest migration time in this study. Based on the $pK_a$ values of theophylline and caffeine at position 7 are 8.8 and 14, respectively [13,25]. In theophylline, a proton can be donated from position 7 and its migration velocity is affected by pH of running buffer. Although at high pH such as pH 9, EOF is large, but theophylline does not remain positively charged at position 7 under this condition. Therefore, the migration velocity of theophylline slows down and well resolution of three methylxanthines can be obtained at pH 9.

The effects of SDS at a concentration range of 0–60 mM in borate buffer (10 mM; pH 9.0) on the separation were studied. The results indicate that electrophoresis of the drugs at SDS <30 mM result in poor resolution of dyphylline and caffeine. Well resolution of the methylxanthines standard is obtained at 30–50 mM SDS (results not shown). Optimization of the MEKC conditions was at 10 mM borate buffer (pH 9.0) with SDS (40 mM) using 12 kV and monitored at 203 nm. Peaks 1–3 represent dyphylline, caffeine, and theophylline, respectively. In this CE condition, the apparent mobility of EOF and the tested drugs are $5.86 \times 10^{-4}$, $5.03 \times 10^{-4}$, $4.64 \times 10^{-4}$ and $4.24 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ for EOF, dyphylline, caffeine, and theophylline, respectively [26]. The naturally methylxanthines, caffeine, theophylline, and theobromine, have highly similar chemical structure. Especially, theophylline and theobromine have same molecular weight. Therefore, we add theobromine to test the selectivity of the proposed method. Structural related methylxanthine, theobromine, did not appear in the interference in the separation of dyphylline, caffeine and theophylline in this MEKC condition.

### 3.2. Analytical calibration

For evaluating the quantitative applicability of the method, five different concentrations of theophylline and dyphylline in the range 0.03–1.0 $\mu mol\,ml^{-1}$ were analyzed using caffeine (1 $\mu mol\,ml^{-1}$) as an I.S. The linearity between the peak-area ratios ($y$) of the related analyte to I.S. and the concentration ($x$, $\mu mol\,ml^{-1}$) of the analyte was investigated. The linear regression equations were obtained as follows: for theophylline assay: $y = (1.1128 \pm 0.0060)x - (0.0009 \pm 0.0010)$ for intra-day ($n = 6$, $r = 0.999$) and $y = (1.1450 \pm 0.0186)x - (0.0049 \pm 0.0022)$ for inter-day ($n = 6$, $r = 0.999$); for dyphylline assay: $y = (0.9312 \pm 0.0081)x - (0.0006 \pm 0.0026)$ for intra-day ($n = 6$, $r = 0.999$) and $y = (0.9406 \pm 0.0094)x + (0.0005 \pm 0.0042)$ for inter-day ($n = 6$, $r = 0.999$). The data indicate good linearity of the proposed method over

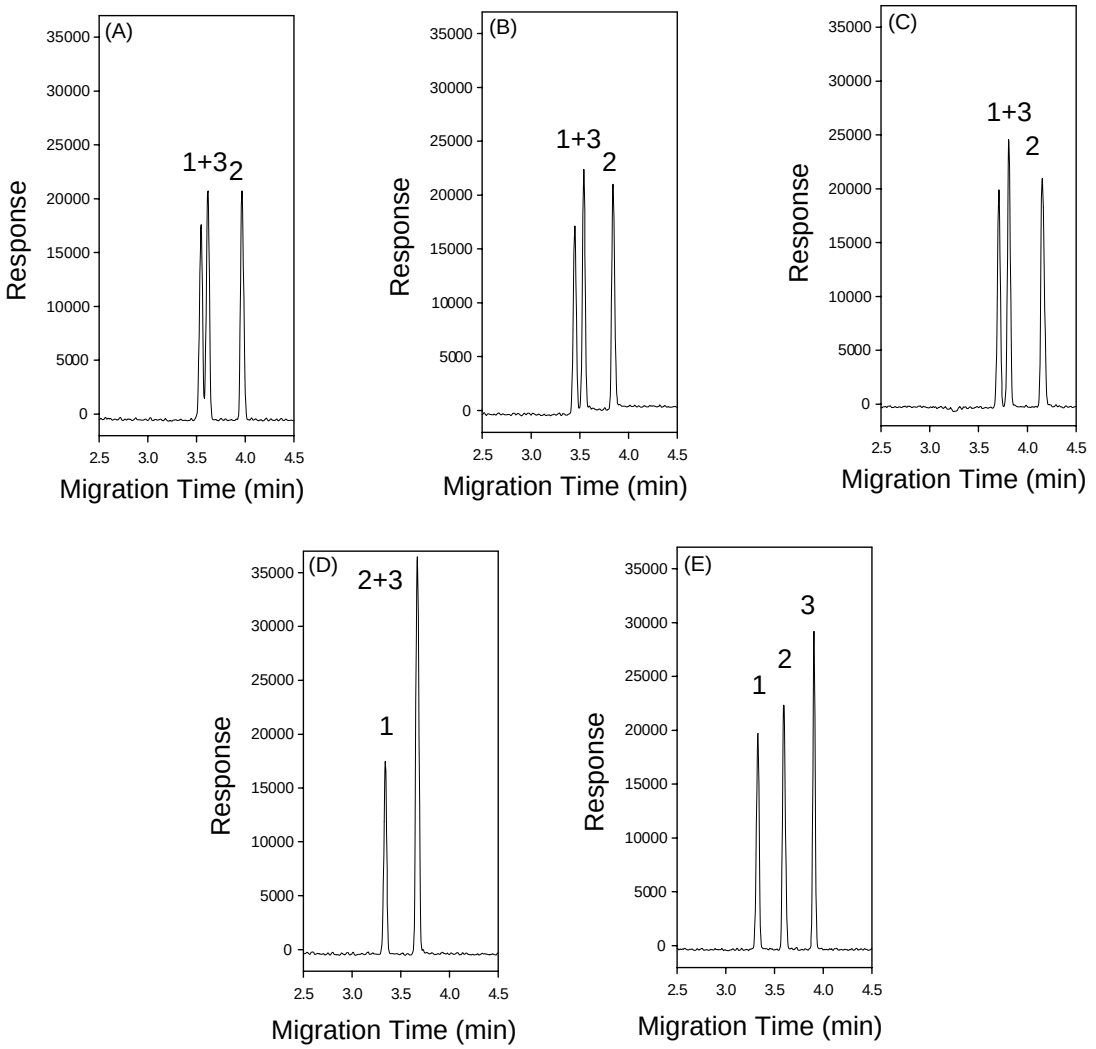



Fig. 2. Effect of pHs of 10 mM borate buffer with 40 mM SDS on the migration of theophylline, dyphylline, and caffeine. Electropherograms: (A) pH 7.0; (B) pH 7.5; (C) pH 8.0; (D) pH 8.5; (E) pH 9.0. Peaks: 1–3 for dyphylline, caffeine, and theophylline, respectively. CE conditions: applied voltage, 12 kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length) × 75 μm i.d.; sample size, 0.3 psi, 3 s; wavelength, 203 nm.

the range studied. The precision (relative standard deviation, R.S.D.) and accuracy (relative error, R.E.) of the proposed method was studied. The results (Table 1) show that the R.S.D. ($n = 6$) and R.E. of intra-day and inter-day of the analytes at three concentrations were all below 2.04 and 4.6%, respectively. The limit of quantitation (LOQ) of theophylline and dyphylline from linear regression was 30 nmol ml$^{-1}$. Limit of detection (LOD) were calculated on the basis of the baseline noise, which was defined as the sample concentration generating a peak of height three times the level of the baseline noise. The LOD of the proposed method for theophylline and dyphylline was 10 and 20 nmol ml$^{-1}$, respectively.

### 3.3. Application

The application of the proposed method in quality control of theophylline or dyphylline in tablets was studied. The uniformity test (a test to evaluate the

Table 1
Precision and accuracy for the analysis of dyphylline and theophylline

| Concentration known ($\mu$mol ml$^{-1}$) | Concentration found ($\mu$mol ml$^{-1}$) | R.S.D. (%) | R.E. (%) |
|---|---|---|---|
| Dyphylline | | | |
| Intra-day[a] ($n = 6$) | | | |
| 0.05 | 0.051 ± 0.001 | 1.96 | 2.0 |
| 0.5 | 0.493 ± 0.007 | 1.42 | −1.4 |
| 1 | 1.003 ± 0.011 | 1.09 | 0.3 |
| Inter-day[a] ($n = 6$) | | | |
| 0.05 | 0.050 ± 0.001 | 2.00 | 0 |
| 0.5 | 0.487 ± 0.004 | 0.82 | −2.6 |
| 1 | 1.006 ± 0.011 | 1.09 | 0.6 |
| Theophylline | | | |
| Intra-day[a] ($n = 6$) | | | |
| 0.05 | 0.049 ± 0.001 | 2.04 | 2.0 |
| 0.5 | 0.497 ± 0.003 | 0.60 | −0.6 |
| 1 | 1.001 ± 0.012 | 1.19 | 0.1 |
| Inter-day[a] ($n = 6$) | | | |
| 0.05 | 0.051 ± 0.001 | 1.96 | 2.0 |
| 0.5 | 0.556 ± 0.004 | 0.72 | 1.2 |
| 1 | 1.046 ± 0.011 | 1.05 | 4.6 |

[a] Intra-day data were based on six replicate analyses and inter-day were from six consecutive days.

content variation of the drug in tablets) and the assay (an analysis for evaluating the average content of the drug in 20 tablets) is usually required by an official pharmacopeia for quality control of the tablet

Table 2
Analytical results for content uniformity of dyphylline tablet obtained from a commercial source

| Tablet[a] | Amount found[b] (mg) | Claimed content[c] (%) |
|---|---|---|
| 1 | 98.1 ± 3.7 | 98 |
| 2 | 99.4 ± 0.6 | 99 |
| 3 | 98.5 ± 3.5 | 99 |
| 4 | 99.1 ± 2.9 | 99 |
| 5 | 99.9 ± 4.1 | 100 |
| 6 | 98.4 ± 1.7 | 98 |
| 7 | 98.5 ± 1.9 | 99 |
| 8 | 98.3 ± 2.0 | 98 |
| 9 | 98.6 ± 3.1 | 99 |
| 10 | 98.2 ± 1.2 | 98 |
| Mean (%) | | 99 |
| S.D. | | 0.6 |

[a] Labeled amount of dyphylline in each tablet is 100 mg.
[b] Mean ± S.D. ($n = 4$).
[c] Content uniformity test is used to check the variation of dyphylline in each tablet.

Table 3
Analytical results for content uniformity of theophylline tablet obtained from a commercial source

| Tablet[a] | Amount found[b] (mg) | Claimed concent[c] (%) |
|---|---|---|
| 1 | 305.0 ± 11.4 | 101 |
| 2 | 294.3 ± 8.4 | 98 |
| 3 | 300.8 ± 10.4 | 100 |
| 4 | 297.3 ± 11.8 | 99 |
| 5 | 299.5 ± 9.7 | 100 |
| 6 | 305.2 ± 10.2 | 102 |
| 7 | 303.9 ± 9.0 | 101 |
| 8 | 301.3 ± 7.4 | 100 |
| 9 | 305.3 ± 10.9 | 102 |
| 10 | 302.6 ± 10.6 | 101 |
| Mean (%) | | 101 |
| S.D. | | 1.2 |

[a] Labeled amount of theophylline in each tablet is 300 mg.
[b] Mean ± S.D. ($n = 4$).
[c] Content uniformity test is used to check the variation of theophylline in each tablet.

formulation. The content uniformity is more important in control of pharmaceutical products. The analytical results of percentage of claimed content (%) are 98.1–101.8% for theophylline and 98.1–99.9% for dyphylline (Tables 2 and 3). All the analytical values fell within labeled amount of 94.0–106.0% for theophylline and 90–110% for dyphylline required by the USP 25 [20]. The analytical results indicated that the water used as the solvent is suitable for the extraction of the drug from pulverized tablet. The small amount of sample and short analysis time needed (4.5 min) in this study allowed content uniformity in individual tablets to be easily assessed. And there is no interference with theophylline and dyphylline analysis in tablets under this MEKC conditions.

## 4. Conclusion

The MEKC method is developed for determination of theophylline, dyphylline and caffeine. The proposed method has been successfully demonstrated for the assay of theophylline and dyphylline in two commercial tablets. The method is also considered as a useful and time effective tool for analysis of caffeine and theophylline in tea. In conclusion, the simple, speedy and specific MEKC method offers a completely different selectivity method and is a completementary and

alternative technique for HPLC in pharmaceutical assay for reliable quality control.

## Acknowledgements

The authors would like to thank Prof. Dr. I.M. Wang, School of Medicinal and Applied Chemistry, for determination of p$K_a$ of dyphylline and the National Science Council, ROC, for financial support of the work (NSC 90-2745-p-037-001).

## References

[1] G.K. McEvoy (Ed.), AHFS Drug Information, The American Society of Health-System Pharmacist Inc., MD, 2001, p. 2294.
[2] G.K. McEvoy (Ed.), AHFS Drug Information, The American Society of Health-System Pharmacist Inc., MD, 2001, pp. 2296 and 3486.
[3] A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, Pergamon Press, New York, 1991, p. 618.
[4] M.B. Kester, C.L. Saccar, H.C. Mansmann Jr., J. Chromatogr. 416 (1987) 91.
[5] M. Wenk, B. Eggs, F. Follath, J. Chromatogr. 276 (1983) 341.
[6] R.M. Garcinuño, P. Fernández, C. Pérez-Conde, A.M. Gutiérrez, C. Cámara, Talanta 52 (2000) 825–832.
[7] A. Ius, M.A. Bacigalupo, R. Longhi, G. Meroni, Fresenius J. Anal. Chem. 366 (2000) 869.
[8] C.M. Rico, M. del P. Fernandez, A.M. Gutierrez, M. Concepcion, P. Conde, C. Camara, Analyst 120 (1995) 2589.
[9] K.A. Georga, V.F. Samanidou, I.N. Papadoyannis, J. Chromatogr. B 759 (2001) 209.
[10] R.D. Smet, P. Vogeleere, J.V. Kaer, N. Lameire, R. Vanholder, J. Chromatogr. A 847 (1999) 141.
[11] T. Umemura, R. Kitaguchi, K. Inagaki, H. Haraguchi, Analyst 123 (1998) 1767.
[12] W.M. Mullett, E.P.C. Lai, Anal. Chem. 70 (1998) 3636.
[13] M.S. Bispo, M.C.C. Veloso, H.L.C. Pinheiro, R.F.S. De Oliveira, J.O.N. Reis, J.B. De Andrade, J. Chromatogr. Sci. 40 (2002) 45.
[14] N. Chiem, D.J. Harrison, Anal. Chem. 69 (1997) 373.
[15] Z.Y. Zhang, M.J. Fasco, L.S. Kaminsky, J. Chromatogr. B 665 (1995) 201.
[16] L. Steinmann, J. Caslavska, W. Thormann, Electrophoresis 16 (1995) 1912.
[17] M. Johansson, M.B.G. Rydberg, B. Schmekel, J. Chromatogr. A 652 (1993) 487.
[18] L.L. Garcia, Z.K. Shihabi, J. Chromatogr. A 652 (1993) 465.
[19] Z.K. Shihabi, J. Chromatogr. A 652 (1993) 471.
[20] US Pharmacopeia 25-National Formulary 20, Asian, United States Pharmacopeial Convention Inc., MD, 2001, pp. 629 and 1686.
[21] C. Barbas, A. Garcia, L. Saavedra, M. Castro, J. Chromatogr. A 870 (2000) 97.
[22] A. Haque, X. Xu, J.T. Stewart, J. Pharm. Biomed. Anal. 21 (1999) 1063.
[23] M. Blanco, I. Valverde, Electrophoresis 23 (2002) 578.
[24] R. Kuhn, S. Hoffstetter-Kuhn (Eds.), Capillary Electrophoresis: Principles and Practice, Springer, Berlin, 1993, p. 30.
[25] M. Blanco, I. Valverde, J. Chromatogr. A 950 (2002) 293.
[26] K.D. Altria, Capillary Electrophoresis Guidebook: Principle, Operation, and Application, Humana Press Inc., New Jersey, 1996, p. 7.