ELSEVIER

Journal of Chromatography B, 692 (1997) 445–451

JOURNAL OF CHROMATOGRAPHY B

Determination of thiamphenicol in human plasma by micellar electrokinetic capillary chromatography

Jing-Zheng Song[a,*], Xiu-Juan Wu[a], Zeng-Pei Sun[a], Song-Jiu Tian[a], Man-Li Wang[b], Ru-Long Wang[b]

[a]*Division of Instrumental Analysis, National Institute for the Control of Pharmaceutical and Biological Products of China, Temple of Heaven, Bejing 100050, China*

[b]*Beijing Friendship Hospital, Yongan Road, Beijing 100050, China*

Received 26 June 1996; revised 18 September 1996; accepted 30 September 1996

Abstract

A micellar electrokinetic chromatographic method is described for the determination of thiamphenicol in human plasma. The plasma sample was basified by adding K_2HPO_4 and was then extracted with ethyl acetate. After the solvent was evaporated, the residue was reconstituted in water. Approximately 40 nl of the solution were injected hydrodynamically. The running buffer was 20 m*M* borate (pH 9.2) containing 40 m*M* sodium dodecyl sulfate and 10% acetonitrile. The applied voltage was 18 kV and the detector wavelength was set at 195 nm. On-column sample stacking was achieved during the analysis to enhance the sensitivity; the limit of quantitation was 0.1 μg/ml. Linearity was over the range of 0.2 to 10 μg/ml. Recovery was 93.7±3.3%, the intra-day precision and accuracy was 99.6±2.8%; the inter-day precision and accuracy was 98.4±3.4%. The concentration of thiamphenicol in human plasma from eight volunteers was measured after administering thiamphenicol capsules orally.

Keywords: Thiamphenicol

1. 1. Introduction

Thiamphenicol (TAP), an analog of chloramphenicol in which the nitro group of chloramphenicol is replaced with a methyl-sulphonyl group, is an antibiotic with a similar broad spectrum activity. TAP has a more weakly basic functionality (pK_a = 7.2) than chloramphenicol, is only slightly bound to plasma proteins (approximately 10%) and is not inactivated in the body by metabolic processes [1,2]. TAP is a chiral drug and its L-form was used in humans [21]. A few results have previously been reported concerning the concentration of TAP in serum and cerebrospinal fluid [3], bovine plasma [4], muscles of chicken and beef [5–7] and in human plasma by gas chromatography (GC) after intravenous injection [8], but there are no reports of the determination of TAP in human plasma after oral administration.

Although high-performance liquid chromatography (HPLC) is frequently used in the determination of biological fluids such as plasma, serum, urine, tissue fluids, etc., capillary electrophoresis (CE) has become an important separation technique due to its high resolving power, minimal solvent

*Corresponding author

PII S0378-4347(96)00465-3

consumption, various modes of separation and speed of analysis. Among the different modes of the technique, micellar electrokinetic capillary chromatography (MEKC) is commonly used for the separation of a variety of drug classes including antibiotics, non-steroidal anti-inflammatory agents, steroids and analgesics [9–14]. MEKC has recently gained popularity as a method for the quantitation of drugs and metabolites in biological samples [13,14]. For CE, the small volume of sample introduced into the column makes it difficult to detect low concentrations of the analyte. However, sensitivity can be improved using several approaches, such as by employing short wavelength UV absorbance detection, modifying the optical pathlength of the capillary, or by sample stacking, field-amplified sample injection and other on-column preconcentration procedures [13–16].

Sample stacking in capillary zone electrophoresis (CZE) has been extensively discussed in a series of recent papers [15,16]. When a sample is dissolved in water or a buffer with lower electrical conductivity than that of the electrophoresis running buffer, concentration or solute stacking occurs when the sample is injected. Because the electrical field strength in the sample region is higher than that in the running buffer, the electrophoretic velocity is accelerated and the ions migrate rapidly to the boundary between the lower and higher conductivity zones. At this boundary, the electrophoretic velocity of the ions is reduced and the ions are stacked into a narrow zone.

MEKC differs from CZE in that a surfactant is present as a pseudostationary phase in the background electrolyte. The sample-stacking effect was also used in MEKC to enhance the sensitivity of the measurements. Ions dissolved in water [17,18] and neutral compounds dissolved in a low concentration of surfactant solution [19,20] were reported. In this paper, a simple MEKC assay was developed for the quantitation of TAP in human plasma. By use of on-column sample stacking, combined with low UV wavelength detection, the required sensitivity could be obtained and the method was used for the determination of the bioequivalence of TAP following oral administration of thiamphenicol capsules from two factories.

2. Experimental

2.1. Materials

L-Thiamphenicol and chloramphenicol (internal standard, I.S.) were obtained from the Division of Reference Substances of our Institute. The thiamphenicol capsules used were Thiamcol, from Union Chemical and Pharmaceutical (Taipei, China), and Urfamycin, from Zambon Group (Vicenza, Italy). Sodium dodecyl sulfate (SDS) was of research grade and was obtained from Serva (Heidelberg, Germany). Acetonitrile was of HPLC grade and other reagents were of analytical grade. Water was redistilled before use.

2.2. CE instrumentation

All separations were conducted on a SpectraPhoresis 1000 system with FOCUS detection (Thermo Separation Products, San Jose, CA, USA) and an IBM 350-450DX2 PC running with PC1000 version 2.61 software. The electrophoretic separation was performed on a fused-silica capillary of 70 cm×75 μm I.D. (62.5 cm effective length, Yongnian Optical Fiber Factory, Hebei Province, China). All buffers were filtered through a 0.25-μm filter. Sample injection was performed hydrodynamically for 10 s from the positive side of the capillary (approximately 40 nl). The applied voltage was 18 kV and the current was about 58 μA before the electroosmotic flow and then raised to 69 μA. The average current was 64 μA. The capillary temperature was controlled at 30°C. The detection wavelength was 195 nm. The running buffer was composed of 40 m*M* SDS, 20 m*M* di-sodium tetraborate (pH 9.2) and 10% acetonitrile.

The capillary was conditioned with 1 *M* NaOH for 20 min at 60°C, followed by 0.1 *M* NaOH for 5 min at 60°C and water for 5 min at 30°C, prior for use. Before each run, the capillary was "pre-washed" with 0.1 *M* NaOH and water for 1 min, then rinsed with running buffer for 2 min.

The electroosmotic flow was detected with methanol, and the micellar migration time t_{mc} was detected with Sudan III.

2.3. Preparation of a standard solution and a plasma standard

Thiamphenicol standard and I.S. stock solutions were prepared by dissolving 12.5 mg in 25 ml of water. All solutions were stable when stored at 4°C for one month. The I.S. stock solution was diluted five-fold to form a working solution before use.

2.4. Extraction procedure

A 0.5-ml plasma sample was spiked with 20 μl of I.S. working solution and 3–5 mg of K_2HPO_4 were added. The solution was thoroughly mixed. A 5-ml volume of ethyl acetate was accurately added and then rotary mixed for 1–2 min, followed by centrifugation at 3500 *g* for 5 min. Exactly 4 ml of the ethyl acetate layer were removed and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 100 μl of deionized water prior to being assayed.

3. Results and discussion

3.1. Method development

3.1.1. Extraction and preconcentration

In sample stacking, the resistivity of the sample solution must be higher than that of the bulk buffer. However, with given samples such as plasma, this is not a controllable factor, because there are a lot of ions, proteins, etc., in the samples. When similar injection and analytical conditions were applied to analysis, ineffective stacking and unsatisfactory chromatograms were obtained. Therefore, it was determined that the plasma should be deionized. lonic substances, proteins and most of other interfering components can be removed simultaneously. Organic solvent extraction is a simple and effective method. Ethyl acetate was often used for extraction from meat, but the recovery was low [7]. A method for extraction with ethyl acetate, with the residue being dissolved in a 10% NaCl solution and partitioned with hexane, followed by reextraction of the aqueous phase with ethyl acetate was reported [6]. Nagata [5] developed a solid-phase extraction procedure with a Sep-Pak Florisil cartridge to determine the residues in muscles of animals and cultured fish by LC. These methods were troublesome and time consuming. Although an ethyl acetate one-step extraction procedure was described [4], the recovery was low in our test. Since TAP is a weak base (pK_a=7.2) and is slightly soluble in water [21], approximately 3 to 5 mg of K_2HPO_4 was added to basify the plasma, thus enhancing partitioning into the ethyl acetate phase. The recovery of TAP was increased from 80 to 93% after K_2HPO_4 was added.

When the ethyl acetate extractive residue was dissolved in separation buffer, the signal obtained with a regular, short injection time (2 s) is unsuitable for quantitation. In contrast, increasing the injection time, which is equivalent to lengthening the injected sample plug, leads only to broadened peaks but not to an increase in peak height, with a limit of detection of only 1.5 μg/ml (S/N=3). However, when the residue was dissolved in water (the examined pH was about 6.2) and the injection time was increased, both the peak height and peak area increased. When the time exceeded 15 s (approximate 60 nl), peak height was not increased and the peak broadened significantly. Fig. 1 shows the peak height and peak area of TAP vs. injection time (approximately 4 nl/s). With an injection time of 10 s, a fifteen-fold gain can be obtained without a significant loss of efficiency. This was satisfactory for the quantitation.

3.1.2. Effect of pH, content of SDS and acetonitrile on the separation

The effect of pH was studied in the range of pH values from 7 to 11 in phosphate buffer and in borate buffer at a concentration of 20 m*M*, in a solution containing 40 m*M* SDS and 10% acetonitrile. It was found that at the lower pH value, the migration times of the TAP peak and the I.S. peak were shorter and then co-migrated with the broad negative water peak. There were also interferences from plasma components. When the pH value was between 9.5 and 11, the migration times of endogenous peaks were longer than those of TAP and I.S., but there were baseline perturbations at the most sensitive detection wavelength of 195 nm. Optimal separation was achieved

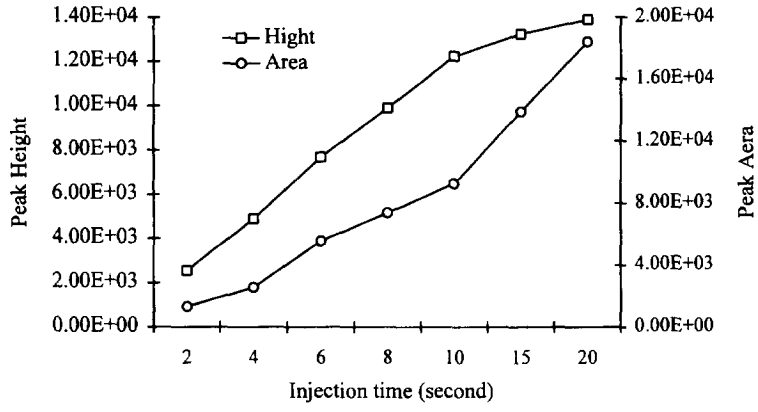

Fig. 1. Effect of injection time (hydrodynamic mode) on peak height and peak area. Sample: thiamphenicol (20 μg/ml) dissolved in water. Column: 70 cm×75 μm I.D. (62.5 cm effective length) fused-silica capillary. Buffer: 20 m*M* borate buffer (pH 9.2) containing 40 m*M* SDS and 10% acetonitrile. Conditions: 18 kV, 30°C, detection at 195 nm.

by using borate buffer (pH 9.2). A typical electropherogram is shown in Fig. 2.

Fig. 3 shows that the effective migration velocity (μ_{eff}) was influenced by the concentrations of SDS (Fig. 3a) and acetonitrile (Fig. 3b). As the concentration of SDS was increased from 20 to 40 m*M*, the μ_{eff} of peak 1 and of the TAP peak decreased, but the influence on the resolution was small. Other peaks were changed very little. When the concentrations of SDS used were 20 and 30 m*M*, the TAP peak was not completely resolved from the tailing interfering peak (peak 1) from endogenous plasma components. At 50 m*M* SDS, TAP and I.S. were baseline-separated from other peaks (Fig. 2), but

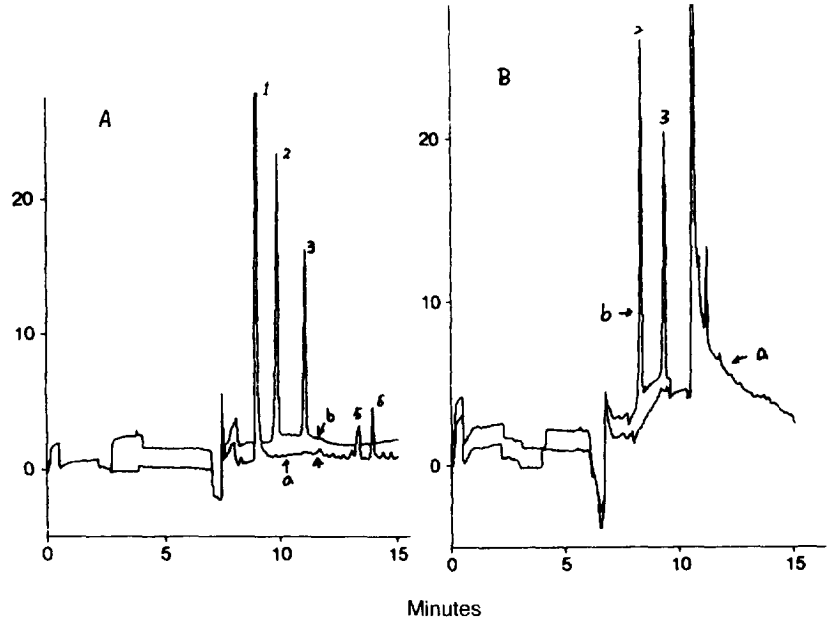

Fig. 2. Effect of pH on the separation of TAP and blank plasma. (A) Borate buffer (pH 9.2). (B) Borate buffer (pH adjusted to 11 with NaOH). (a) Blank plasma. (b) TAP standard solution with I.S. (20 g/ml each). Peaks: 2=TAP, 3=chloramphenicol (I.S.). Peaks 1, 4, 5 and 6 are due to substances in the plasma. Other conditions as in Fig. 1.

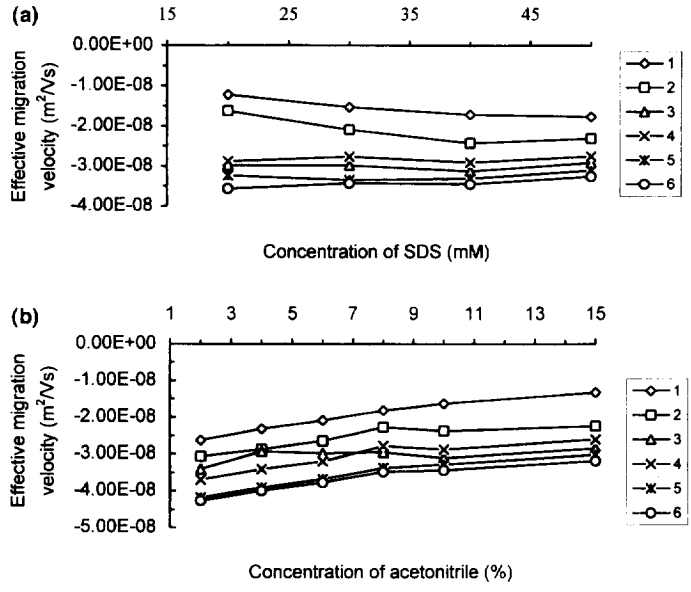

Fig. 3. Effect of varying the concentration of SDS (20–50 m*M*) (a) and of acetonitrile (2–10%, v/v) (b) on peak resolution. Peaks and other conditions as in Fig. 2.

there was a small new peak (not shown in figure) that overlapped with the TAP peak. The area of the new peak was equivalent to that of TAP at a concentration of 1 μg/ml. It is obvious that the deviation of quantitation would be increased especially at the time points of beginning and tailing in Fig. 4. When the concentration of SDS was 40 m*M*, there was no interfering peak in the migration time window where TAP and I.S. appeared.

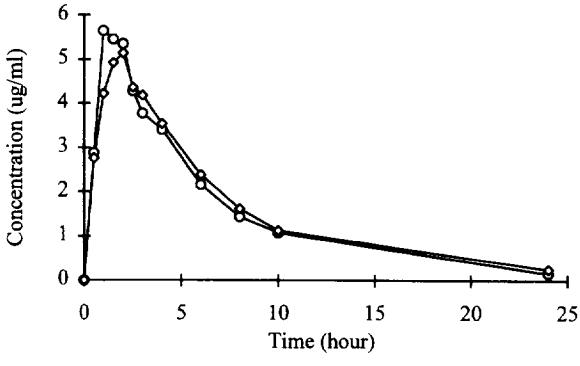

Fig. 4. Profile of the concentration of thiamphenicol in human plasma vs. time after oral administration of a 500-mg thiamphenicol capsule to eight volunteers. (○) Thiamcol capsule; (□) Urfamycin capsule.

When the concentration of acetonitrile was changed from 2 to 15%, the μ_{eff} of all peaks was increased. The separation of peak 1 and the TAP peak was improved by adding some acetonitrile to the running buffer. The optimal concentration of acetonitrile in the running buffer was 10%. The symmetry of all peaks was also improved.

3.2. Linearity, limit of quantitation and recovery

3.2.1. Linearity and limit of quantitation

Linearity of the calibration curve was assessed over a concentration range of 0.2 to 10 μg/ml of plasma. Control plasma samples were spiked to give concentration of 0.2, 0.4, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 μg/ml of TAP and 20 μl of I.S. solution were added to samples prior to extraction. The linearity of the standard curve was confirmed by plotting the ratio of the TAP and I.S. peak areas versus the concentration of TAP. A straight line obtained in the 0.2 to 10 μg/ml range was $y=3.37\cdot10^{-3}+0.3992x$, with a correlation coefficient of 0.9998. Where y is the ratio of TAP to the I.S. peak area and x is the concentration of TAP (in μg/ml). The standard errors in the slope and intercept of the calibration curve were $1.29\cdot10^{-3}$ and 0.0167, respectively.

Table 1
Assay recovery, precision and accuracy for determination of thiamphenicol in human plasma samples

Spiked concentration (μg/ml)	Recovery (mean±R.S.D.) (%)	Intra-day precision and accuracy (%)	Inter-day precision and accuracy (%)
0.40	91.55±4.0	94.92±3.6	95.55±3.4
4.01	92.56±2.6	103.5±2.6	99.90±3.3
8.02	97.11±3.3	100.3±2.3	99.72±3.6

The limit of quantitation (LOQ) is defined as the lowest concentration on the standard curve that can be measured with acceptable accuracy and precision. At the LOQ, an acceptable precision was a value of ≤20% R.S.D., obtained from a set of measured concentrations used for the analysis [22]. In this paper, the measured LOQ was 0.1 μg/ml (R.S.D.= 17.9%, $n=5$).

3.2.2. Recovery

The recovery of thiamphenicol from human plasma was evaluated by comparing the peak areas of TAP extracted from spiked plasma with that of an untreated standard TAP solution of the same concentration as that of the final injected plasma sample. Both samples had TAP concentrations of 0.4, 4 and 8 μg/ml, with $n=5$ for each concentration. Samples (injected for 10 s) of each of these were analyzed. The results are presented in Table 1.

3.3. Precision and accuracy of the method

The intra-day and inter-day precision and accuracy study was carried out by analyzing quality control samples prepared by spiking control human plasma samples with a standard solution of TAP at three concentrations, i.e., 0.4, 4 and 8 μg/ml. The samples were stored at −20°C. Intra-day precision and accuracy was carried out by analyzing a set of samples ($n=5$) at each of the three concentrations. An inter-day precision and accuracy study was carried out by analyzing these samples at each concentration on five separate days. These results are presented in Table 1. From these data, it was found that the TAP was stable in plasma for at least five days.

3.4. Application

Thiamphenicol capsules from two companies were administered orally to eight volunteers. Blood samples were taken at time points up to 24 h and plasma samples were separated and stored at −20°C, before being assayed as described above. The plasma species were completely determined within 48 h. Results are shown in Fig. 4.

References

[1] F. Azzolini, A. Gazzaniga and E. Lodola, Int. Z. klin. Pharm. Ther., 4 (1970) 303.
[2] F. Azzollini, A. Gazzaniga, E. Lodola and R. Natangelo Int. J. Clin. Pharm., 62 (1972) 130.
[3] A. Meulemans, C. Manuel, J. Mohler, A. Roncoroni and M. Vulpillat, J. Liq. Chromatogr., 4 (1981) 145.
[4] F. Lawrence, A.E. Hassane and A. Muhammed, J. Assoc. Off. Anal. Chem., 71 (1988) 1156.
[5] T. Nagata, J. Liq. Chromatogr., 15 (1992) 2045.
[6] T. Nagata and M. Sakei, J. Chromatogr., 565 (1991) 471.
[7] J.E. Psomas and E.G. Iosifidog, J. Liq. Chromatogr., 16 (1993) 2653.
[8] T.A. Plomp and R.A.A. Maes, J. Chromaogr., 21 (1976) 243.
[9] M.G. Donato, W. Baeyens, W. Van Den Bossche and P. Sandra, J. Pharm. Biomed. Anal., 12 (1994) 21.
[10] C.W. Maboundou, G. Paintaud, M. Berad and P.R. Bechtel, J. Chromatogr. B, 657 (1994) 173.
[11] Q.X. Dang, Z.P. Sun and D.K Ling, J. Chromatogr., 603 (1992) 259.
[12] Q.X. Dang, L.X. Yan, Z.P. Sun and D.K. Ling, J. Chromatogr., 630 (1993) 363.
[13] W.G. Kuhr and C.A. Monnly, Anal. Chem., 64 (1992) 389R.
[14] C.A. Monnly and R.T. Kennedy, Anal. Chem., 66 (1994) 208R.
[15] R.L. Chien and D.S. Burgi, Anal. Chem., 64 (1992) 409A.
[16] D.S. Burgi and R.L. Chien, Anal. Chem., 63 (1991) 1354.
[17] R. Szücs, J. Vindevogel, P. Sandra and L.C. Veyhagen, Chromatographia, 36 (1993) 323.

[18] R. Weinberger, E. Sapp and S. Moring, J. Chromatogr., 516 (1990) 271.
[19] Z.Y. Liu, P. Sam, S.R. Sirimanne, P.C. McClure, J. Grainger and D.G. Patterson, Jr., J. Chromatogr. A, 673 (1994) 125.
[20] K.R. Nielsen and J.P. Foley, J. Chromatogr. A, 686 (1994) 283.
[21] British Pharmacopoeia, 1993, p. 665.
[22] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall K.A. Pittman and S. Spector, J. Pharm. Sci., 81 (1992) 309.