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Separation of Zidovudine and Related Substances by Micellar Electrokinetic Chromatography: Comparison with High Performance Liquid Chromatography

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ABSTRACT: Zidovudine (AZT) and 10 related substances including geometrical isomers that may be present as impurities from the synthesis and/or degradation were completely separated using micellar electrokinetic chromatography (MEKC) employing a common surfactant, sodium dodecylsulfate, within a short analysis time. The method was evaluated with respect to repeatability, accuracy, as well as the limit of detection and limit of quantitation for zidovudine and some important impurities.

KEY WORDS: Zidovudine, micellar electrokinetic chromatography, high performance liquid chromatogaphy

INTRODUCTION

Zidovudine is a nucleoside analogue currently approved for the treatment of acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC) (1). It works by inhibiting the viral reverse transcriptase and terminates the viral DNA chain (2). Determination of zidovudine and its metabolites in biological fluids has been investigated using HPLC (3-7) and MEKC (8). With respect to the determination of zidovudine in pharmaceuticals, separation of zidovudine and its potential impurities that may be present from the synthesis and/or degradation of zidovudine by HPTLC and HPLC has been proposed (9-10), as well as the stability study of zidovudine by HPLC in extemporaneous syrup (11). Recently, the B.P. 1996 (supplement) (12) has introduced a monograph for the HPLC determination of zidovudine and the limit tests for the 4 potential impurtities of zidovudine, i.e. deoxythymidine, chlorothymidine, thymine and triphenylmethanol. In this work, the MEKC method is demonstrated for its high potential for the separation of zidovudine and 10 possible impurities including deoxythymidine, chlorothymidine and thymine together with an internal standard paracetamol, employing a common surfactant, SDS. Determination of zidovudine and its degradation product thymine in artificial sample as well as the limit of detection and limit of quantitation of deoxythymidine, chlorothymidine and thymine is also evaluated in an analytical point of view.

Expertimental

Chemicals

Zidovudine or AZT (E*-form) and 10 related compounds; T**-AZT, deoxythymidine, E-chlorothymidine, T-chlorothymidine, thymidine, thymine, cyclothymidine, iminothymidine, 3-isomer and dimer were obtained from ACIC (Canada) Inc. (Ontario, Canada). Their structures are shown in Figure 1. A borate/phosphate buffer solution was prepared by mixing an appropriate volume of 0.02 M sodium tetraborate solution with 0.02 M monobasic sodium phosphate solution to pH 9. Sodium dodecylsulfate (SDS) (Sigma, U.S.A.) was added to the borate/phosphate buffer solution pH 9 at the concentration of 0.05 M. The separation buffer was filtered prior to use. The water used throughout the experiments was tridistilled water.

(* = erythro; ** = threo)

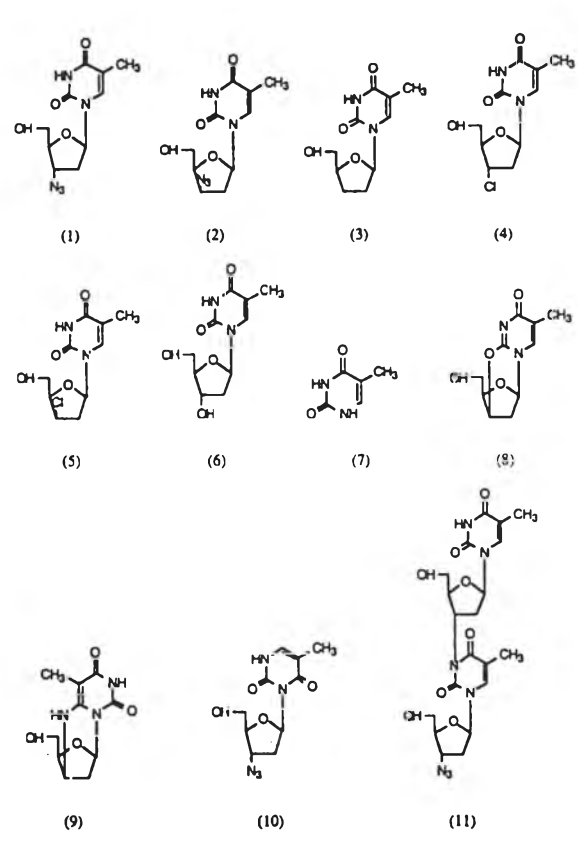

Figure 1 Structure of AZT and 10 related substances
(1) = AZT.(E-AZT), (2) = T-AZT, (3) = deoxythymidine, (4) = E-chlorothymidine, (5) = T-chlorothymidine, (6) = thymidine, (7) = thymine, (8) = cyclothymidine, (9) = iminothymidine, (10) = 3-isomer, (11) = dimer

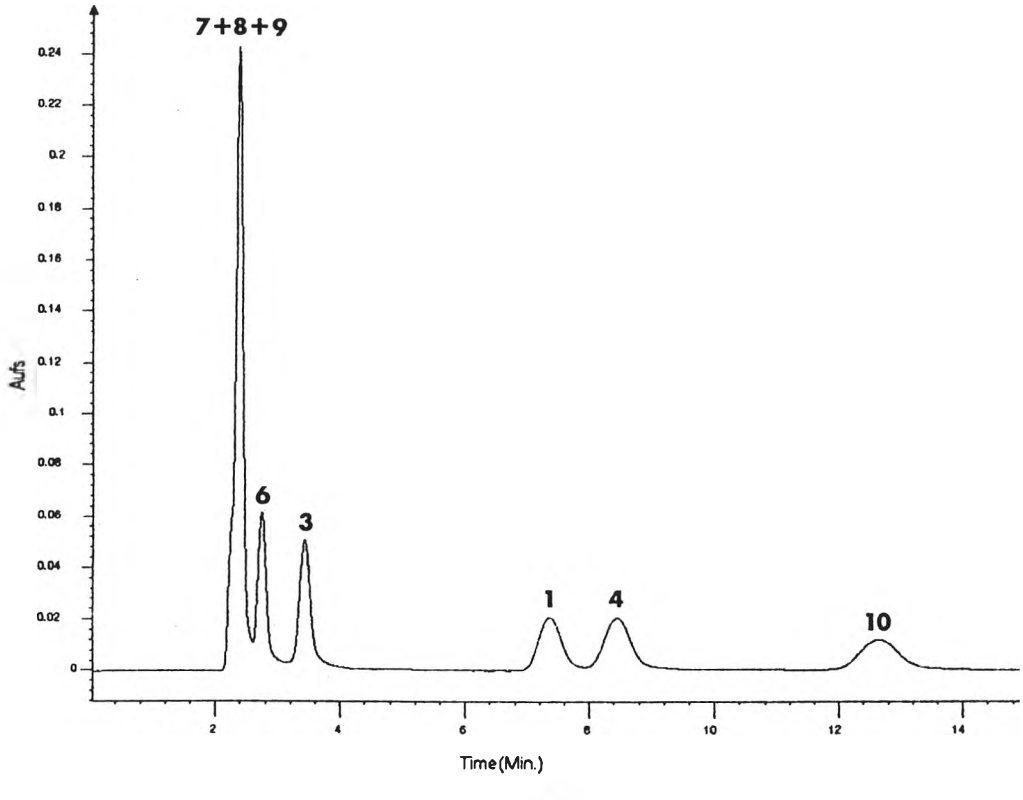

Figure 2 Separation of AZT and its 10 related substances by isocratic HPLC; separation conditions, see text; sample, 100 μg/ml each in 10% methanol, identification numbers, as in Figure 1

Micellar electrokinetic chromatography (MEKC)

Electrokinetic chromatography was performed on P/ACE 5510 system fitted with diode array detector (Beckman, Palo Alto, CA, U.S.A.), using a fused silica capillary of 75 μm I.D., 375 μm O.D., 57 cm long, with the detection window at 7 cm from the capillary outlet. The temperature of the capillary was controlled at 25 °C by a liquid coolant. Integration of the electropherograms was achieved by system Gold Version 810 (Beckman, Palo Alto, CA, U.S.A.)

Samples were introduced by pressure for 1 s, analysed with an applied voltage of 25 kV and detected at the wavelength of 266 nm. Between runs the capillary was flushed for 3 min with buffer and left in equilibrium for 2 min.

High performance liquid chromatography (HPLC)

The HPLC system consisted of a SpectraSYSTEM Gradient Pump constaMetric 4100, a SpectraSYSTEM AS 1000 Autosampler, a SpectraSYSTEM UV 1000 UV/Vis Detector, all from Thermo Separation Products Inc., U.S.A. and data acquisition with BDS (Barspec's Data System) Version 1.2 (Barspec Systems Inc., U.S.A.). A 300 mm × 3.9 mm I.D. Techopak C18 column of 10 μm particle size packing from HPLC Technology Ltd., U.K. was used. Injection volume was 20 μl.

For isocratic elution, a 20:80 (v/v) mixture of methanol and water was used as the mobile phase. It was degassed by means of ultrasonication prior to use. The flow rate was 1.5 ml/min, and the detection wavelength was at 266 nm.

For gradient elution, the separation started with a 1.5 : 98.5 (v/v) mixture of methanol, degassed with helium, maintained for 6 min at this ratio, then linearly gradient from 1.5 : 98.5 (v/v) to 20 : 80 (v/v) in 2 min, maintained for 8 min at this ratio, then linearly gradient from 20 : 80 (v/v) to 80 : 20 (v/v) in 4 min, maintained at this ratio for the next 10 min. The typical flow rate was 1.5 ml/min, and the detection wave length was at 266 nm.

Sample preparation

An aliquot of sample equivalent to 200 mg of AZT was suspended in 50.0 ml of 10% methanol in water. After shaking and sonicating for 15 minutes and centrifuging until a clear solution was obtained, 1.0 ml of the clear supernatant was transferred into a 25-ml volumetric flask to which 5.0 ml of internal standard solution (paracetamol, 500 μg/ml) were added. The volume was adjusted to 25.0 ml with water. The external standard solution was prepared from a stock solution of AZT at the concentration of 4 mg/ml and treated in the same way as described for the sample.

For the determination of thymine, an aliquot of sample equivalent to 500 mg of AZT was suspended in 25.0 ml of 10% methanol. After sonicating and centrifuging, 5.0 ml of the clear supernatant were transferred into a 10-ml volumetric flask to which 1.0 ml of internal standard solution (paracetamol, 500 μg/ml) was added. The volume was adjusted to 10.0 ml with water. The external standard solution was prepared from a stock solution of thymine at the concentration of 400 μg/ml from which 1 ml was transferred into a 10.0 ml and treated in the same way as described for the sample.

RESULTS AND DISCUSSION

Separation by HPLC

Since AZT and 10 related substances are mostly weak acids having different polarities, employing isocratic LC, a complete separation of 11 compounds could not be obtained. Cyclothymidine, thymine and iminothymidine coeluted together and were the fastest among the analytes. The two pairs of isomers, E-AZT and T-AZT, and T-chlorothymidine and E-chlorothymidine were also coeluted. Moreover, dimer was eluted very late due to its high affinity to the column (Figure 2).

The coelution of thymine, cyclothymidine and iminothymidine could be solved by decreasing the amount of methanol in the mobile phase, whereas dimer could be forced to elute faster by increasing the amount of methanol. Complete separation of 9 out of 11 compounds was achieved by employing the gradient elution using the varying amount of methanol in the mobile phase from 1.5% (v/v) to 80% (v/v) (Figure 3).

Separation by MEKC

Due to either weak acid or neutral properties of AZT and related substances, they need to be separated in MEKC mode. Employing 0.02 M borate/phosphate buffer containing 0.05 M SDS provided a complete separation of AZT and 10 related substances together with an internal standard (paracetamol) with theoretical plate numbers ranging from

140,000 to 670,000 within 8 minutes (Figure 4). It is noted that the two pairs of isomers (E- and T-AZT, and E- and T-chlorothymidine) were separated with high resolution (Rs = 16 and 24 for a pair of E- and T-AZT, and a pair of E- and T-chlorothymidine, respectively).

It is also noted that the order of elution in HPLC was slightly different from that in MEKC especially for thymine. This was partly due to the effect of electrophoretic mobilities of some analytes which were partially ionized, on the net mobilities since the separation was performed under a high pH value. In addition, molecules of geometrical differences might be differently incorporated into the micelle and thereby gave rise to migration differences among these isomers as was clearly seen in cases of E- and T-AZT and E- and T-chlorothymidine, where the T-form showed more partitioning into the micelle than the E-form.

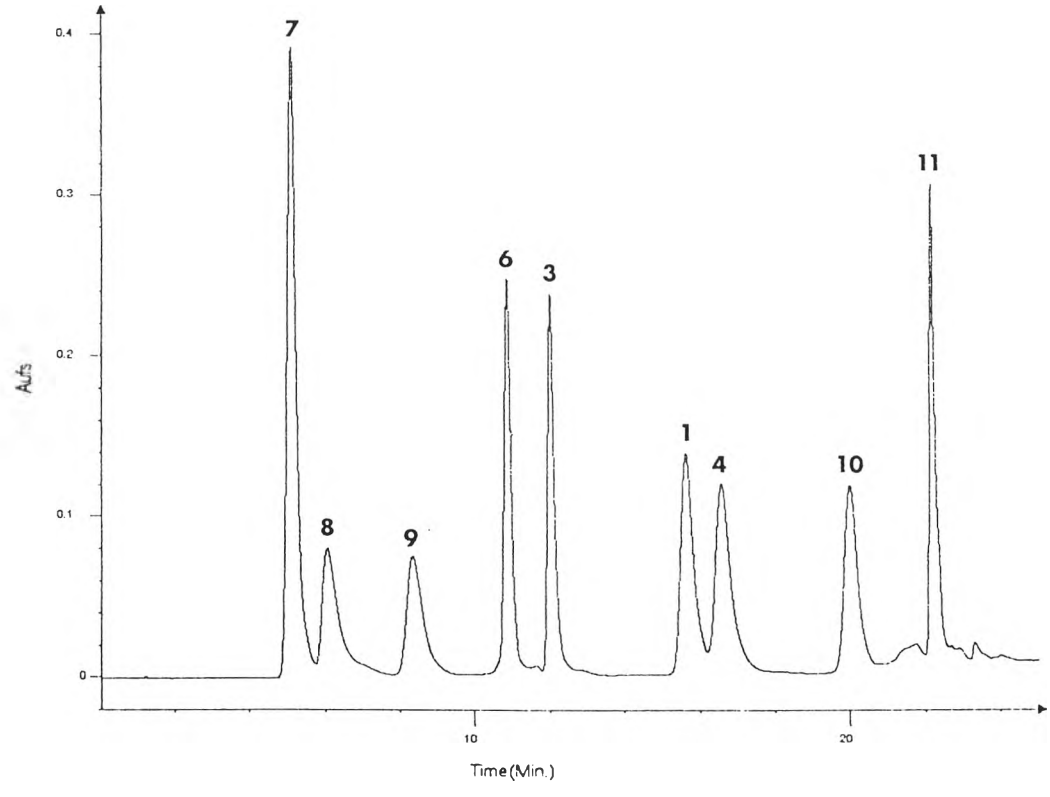

Figure 3 Separation of AZT and its 10 related substances by gradient HPLC; separation conditions, see text; sample, 100 μg/ml each in 10% methanol, identification numbers, as in Figure 1

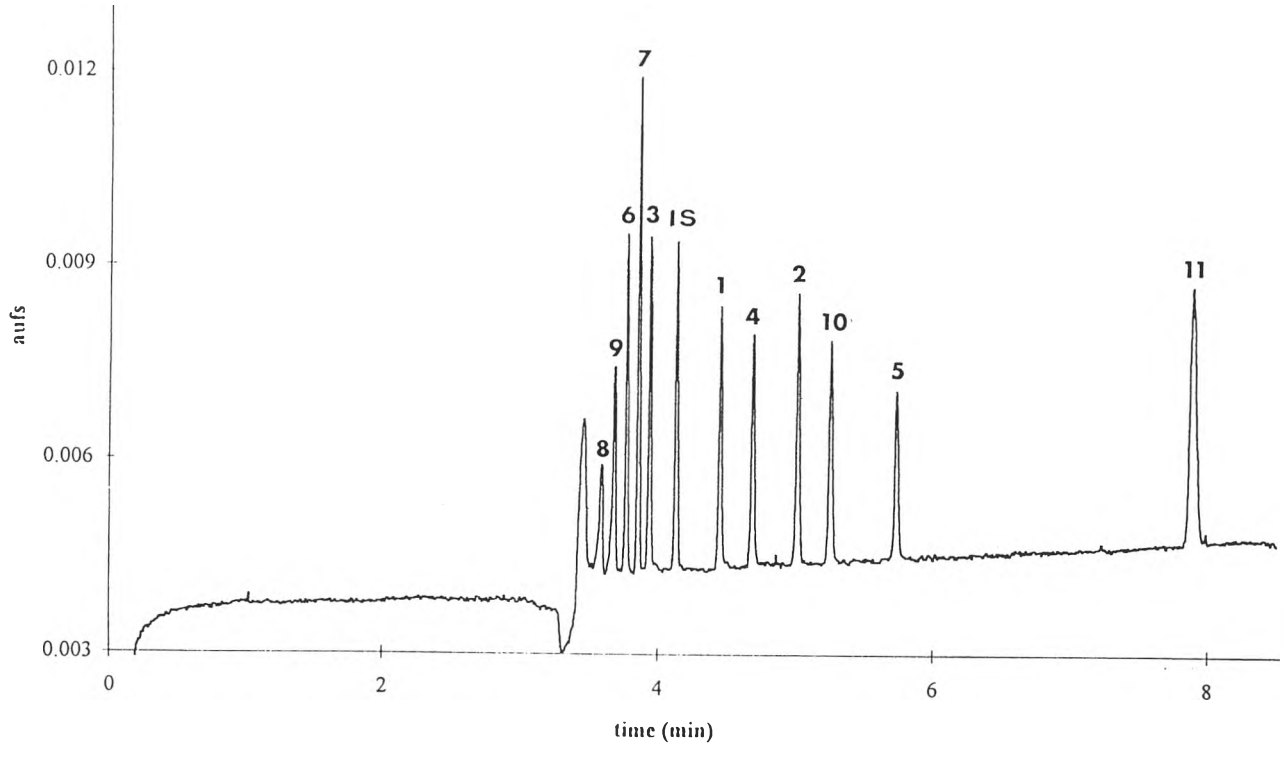

Figure 4 Separation of AZT and 10 related substances by MEKC; separation conditions, see text; sample, 100 μg/ml each in 10% methanol, identification numbers, as in Figure 1, IS = paracetamol

Linearity, Repeatability, Precision and Accuracy

Calibration graphs were constructed for AZT and its degradation product thymine from the concentration ranges of 50–200 μg/ml for AZT and 20–80 μg/ml for thymine. Linear regression lines of the ratio of the peak areas to that of the internal standard as a function of the concentration were calculated using the least squares method. Good linearity (r = 0.9978–0.9994) was obtained for both compounds in the range studied with intercepts of the regression lines not significantly different from zero.

The repeatability as well as the accuracy of the method was evaluated by determining AZT and thymine in an artificially prepared mixture composed of 49.5% AZT and 0.5% thymine added to a mixture of capsule excipients composed of lactose, starch and magnesium stearate. Samples were treated as previously described in the procedure for sample preparation. The final concentration of AZT in the sample solution as well as in the external standard solution was 160 μg/ml for the determination of AZT and 10 mg/ml for the determination of thymine. Six replicate samples were analysed by comparison with an external standard solution of either AZT or thymine. Relative standard deviations (RSD) of the migration times of thymine, paracetamol and AZT were < 0.5%. Mean recoveries are given in Table 1, together with the relative standard deviations (RSD) and 95% confidence intervals (C.I.) of the means. Acceptable repeatability (RSD ≤ 3%) and good accuracy were obtained for both compounds.

Limit of detection and limit of quantitation of thymine were found to be 1.2 μg/ml (signal-to-noise ratio = 3) and 4 μg/ml (signal-to-noise ratio = 10; RSD = 4.83%), respectively, corresponding to 0.012% and 0.04% of AZT, respectively.

For deoxythymidine, limit of detection and limit of quantitation were found to be 2.1 μg/ml and 7.0 μg/ml (RSD = 3.22%), respectively, corresponding to 0.021% and 0.07% of AZT, respectively.

For T-chlorothymidine, limit of detection and limit of quantitation were found to be 3.0 μg/ml and 10.0 μg/ml (RSD = 2.11%), respectively, corresponding to 0.03% and 0.1% of AZT, respectively.

Table 1 Recovery ($\bar{X}$), repeatability (RSD) and accuracy [95% confidence interval (C.I.)] for artificial samples (n = 6)

component	$\bar{X}$ (%)	RSD (%)	95% C.I. (%)
AZT	100.71	0.75	99.91-101.51
thymine	101.79	3.07	98.51-105.07

CONCLUSION

Micellar electrokinetic chromatography employing SDS as micelle forming surfactant has been shown for its high potential for the separation of zidovudine and 10 possible impurities including isomers over HPLC. The analytes were completely separated from each other in a short analysis time, with high efficiency. In HPLC, the separation required gradient elution in order to achieve separation of all compounds except for isomers. The MEKC method showed high repeatability and good accuracy evaluated from spiked zidovudine and thymine in capsule excipients. The limit of detection and the limit of quantitation were ≤ 0.03% and ≤ 0.1%, respectively, for the impurities deoxythymidine, T-chlorothymidine and thymine.

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