ELSEVIER

Journal of Chromatography A, 749 (1996) 247–255

JOURNAL OF CHROMATOGRAPHY A

Separation of pharmaceutically important estrogens by micellar electrokinetic chromatography

Salwa K. Poole[a], Colin F. Poole[a,b,*]

[a]*Zeneca/SmithKline Beecham Centre for Analytical Chemistry, Imperial College of Science, Technology and Medicine, South Kensington, London SW7 2AY, UK*

[b]*Department of Chemistry, Wayne State University, Detroit, MI 48202, USA*

Received 1 February 1996; revised 17 April 1996; accepted 22 April 1996

Abstract

Baseline separation of ten estrogens of the type found in conjugated estrogens tablets from pregnant mares' urine were separated by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing either 75 m*M* sodium cholate and 15 m*M* β-cyclodextrin or 50 m*M* sodium dodecyl sulfate and 20 m*M* γ-cyclodextrin. The system containing sodium cholate was used to identify estrogens in a pharmaceutical tablet formulation of conjugated estrogens and to determine the amounts and ratio of sodium equilin sulfate to sodium estrone sulfate to confirm tablet conformity with regulatory requirements. Baseline separation of the seven ethynyl steroids registered as oral contraceptives in the USA was obtained with the system 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 50 m*M* sodium cholate and 10% (v/v) methanol. Factors that affect selectivity and reproducibility of the above separation systems are discussed.

Keywords: Estrogens; Steroids

1. Introduction

The estrogens are important biologically active substances associated with the development and maintenance of the female sexual organs. As pharmaceutical products they are used mainly as replacements for inadequate supplies of natural hormone to alleviate the symptoms of menopause, in suppressive therapy to counter the effects of other hormones, in postmenopausal women to minimize bone loss due to osteoporosis, and for some treatments of the advanced stages of breast and prostrate cancer [1]. The main estrogens in humans are estrone, 17β-estradiol and estriol; those in other mammals are estrone, equilenin and equilin. When isolated from natural sources, estrogens may be present as a complex mixture that includes not only the principal hormones mentioned, but also their common metabolites. In pregnant mares' urine, a common source of pharmaceutical estrogen formulations, at least nine common conjugated estrogenic substances are found, which after hydrolysis yield the estrogens shown in Fig. 1. The ethynyl steroids are synthetic compounds prescribed extensively as oral contraceptives in the USA and Europe. Their structures are shown in Fig. 2.

Several chromatographic methods have been used for the identification and analysis of estrogens and

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00413-X

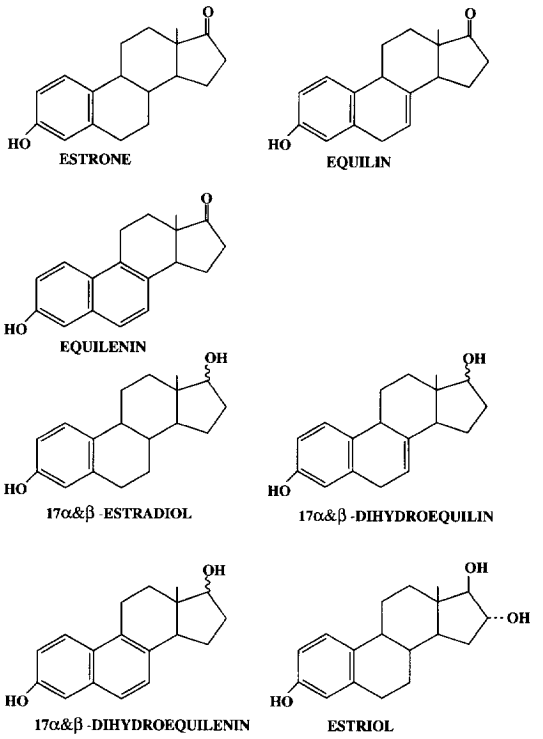

Fig. 1. Structures of equine estrogens.

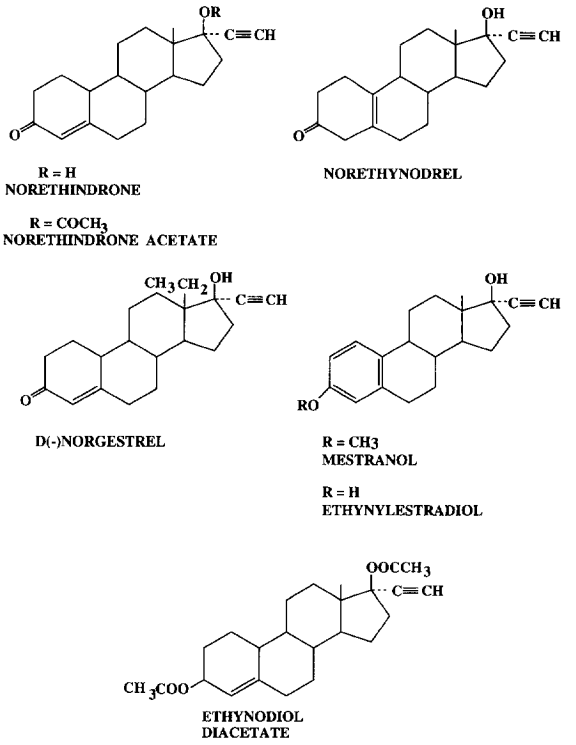

Fig. 2. Structures of ethynyl steroids.

ethynyl steroids related to the estrogens in pharmaceutical preparations. Thin-layer chromatographic methods are the most economical for routine screening procedures and all seven of the ethynyl steroids [2] and the major estrogens subject to regulatory control in tablet formulations [3,4] have been separated by this technique. Underivatized equine estrogens have been separated on mixed phase [5] and serially coupled open tubular columns [6] by gas chromatography although for a more complete separation of major and minor components formation of oxime-trimethylsilyl [7,8], trimethylsilyl [8,9] or *tert.*-butyldimethylsilyl [8] derivatives is required. Reversed-phase liquid chromatography has emerged as the preferred technique for the separation of the intact conjugated estrogens [10,11] and is widely used for the quality control aspects of determining the conformity with dosage indications of the ethynyl steroids in oral contraceptives [2,12]. Complete separation of all ten free estrogens indicated in Fig. 1 has been achieved by reversed-phase liquid chromatography using complicated mobile phases for adequate resolution of critical pairs which has tended to restrict its routine use [13–16]. β-Cyclodextrins have been investigated as mobile phase additives to improve the separation of 17α- and 17β-estradiol and estrone [17], and estrone, equilin and three estrone derivatives [18], by reversed-phase liquid chromatography. In these studies evidence is presented for inclusion complexation between the model estrogens and β-cyclodextrin leading to improved resolution and shorter separation times. Potter et al. [19] separated estrone, 17β-estradiol and estriol using capillary zone electrophoresis with an aqueous–methanol buffer. Ji et al. [20] reported the separation of the same three estrogens with equilenin as an internal standard by micellar electrokinetic chromatography using sodium cholate (75–90 m*M*) in a 5 m*M* borate–5 m*M* phosphate buffer (pH 8.6). Chan et al. [21] separated ten estrogenic compounds, including estrone, 17β-estradiol and estriol by micellar electrokinetic chromatography using a 10 m*M* borate buffer (pH 9.2) containing 50 m*M* sodium dodecyl sulfate and 20 m*M* γ-cyclodextrin. Nine of

the ten same compounds were separated by a 10 m*M* borate buffer (pH 9.2) containing 100 m*M* sodium cholate, the critical pair 2-hydroxyestrone and 4-hydroxyestrone being unseparated in this system.

The objective of this work was to develop a separation system based on electrophoretic methods for the baseline separation of the pharmaceutically important estrogens in Figs. 1 and 2 and to apply this method to their determination in pharmaceutical products. These mixtures have not been separated previously by electrophoretic methods which offers the possibility of improved resolution and speed over liquid chromatographic methods and shorter separation times without the need for derivatization over gas chromatography. The estrogens in Fig. 1 represent a difficult separation problem because of their small structural differences and number of stereoisomers as indicated by the limited number of studies where a complete separation has been achieved and the general complexity of the separation systems employed. It is hoped to provide in this paper a more rugged system for their detailed analysis as may be required by future regulatory guidelines [22].

2. Experimental

2.1. Materials

Sodium tetraborate, sodium phosphate, sodium dodecyl sulfate, cholic acid sodium salt, taurocholic acid sodium salt hydrate, taurodeoxycholic acid sodium salt monohydrate, and α-, β-, and γ-cyclodextrin were obtained from Fluka (Gillingham, Dorset, UK). Estrone, equilin, 17α-estradiol, 17β-estradiol, estriol, norethindrone, norethindrone acetate, norethynodrel, *d*(−)norgestrel, 17α-ethynylestradiol 3-methyl ether (mestranol), and 17α-ethynylestradiol were obtained from Sigma-Aldrich (Poole, Dorset, UK). Equilenin was purchased from Steraloids (Wilton, NH, USA). Ethynodiol diacetate was a gift from Searle (Skokie, IL, USA). 17α-Dihydroequilin, 17β-dihydroequilin, 17α-dihydroequilenin and 17β-dihydroequilenin were a gift from Wyeth-Ayerst Research (Princeton, NJ, USA). Premarin tablets (Wyeth-Ayerst) containing 2.5 mg of conjugated estrogens were obtained from Rite Aid (St. Clair Shores, MI, USA). All solvents were high-performance liquid chromatography grade from Merck (Poole, Dorset, UK).

2.2. Hydrolysis of conjugated estrogens tablets

The sugar coating layer of the conjugated estrogens tablets was carefully removed with a moist paper towel down to the shellac layer and the tablets dried on filter papers. Twenty tablets were weighed and finely powdered in a mortar and pestle to pass a 60 mesh sieve. An amount of powder containing 25 mg of conjugated estrogens was mixed with 20 ml of methanol, sonicated, centrifuged, and the methanol layer was collected.

To a round bottom flask fitted with a reflux condenser were added the methanol extract, deionized and distilled water (20 ml), concentrated hydrochloric acid (4 ml), and a few boiling chips. The mixture was then heated under reflux for 30 min and allowed to cool to room temperature.

After acid hydrolysis, the free estrogens were extracted with chloroform (3×10 ml) and the chloroform extracts combined, washed with water (5 ml), passed through a short column of anhydrous sodium sulfate, and evaporated to dryness on a rotary evaporator. The residue was taken up with 20 ml of methanol and diluted to 25 ml with water. The standard stock solution was divided into 5 ml vials and stored in a refrigerator at 4°C until used.

2.3. Instrumentation

All separations were performed with a Hewlett-Packard ^{3D}CE system (Stockport, Cheshire, UK) with a UV diode array detector and laser jet printer. The fused-silica separation capillaries were 48.5 cm long (effective length 40 cm) and 50 μm internal diameter from Hewlett-Packard. Prior to each separation the capillaries were flushed with 0.1*M* sodium hydroxide for 2 min followed by the separation buffer for 5 min. In the case of systems containing sodium dodecyl sulfate it was necessary to flush the capillary with water for 3 min after each separation then follow the normal conditioning cycle. Unless otherwise noted separations were performed at 25°C and +20 kV with recording of chromatograms at 210 nm.

Standard solutions of estrogens and ethynyl ster-

oids were prepared in methanol at a concentration of 1–2 mg/ml and stored at 4°C when not in use. Injection solutions were prepared by diluting the stock solution with water to give a final solution containing 20% (v/v) water in methanol. All sample solutions and buffers were filtered through 0.2-μm poly(propylene) syringe filters (Whatman, Clifton, NJ, USA) prior to use. Samples were introduced into the capillary by applying pressure of 50 mbar for 1–2 s at the anode end.

3. Results and discussion

Initial attempts were made to separate the estrogens by capillary zone electrophoresis at alkaline pH in aqueous and partially aqueous buffers. All attempts failed, however, because the electrophoretic mobilities of the compounds in Fig. 1 are too similar. The addition of an ionic surfactant above its critical micelle concentration to the buffer system provides an additional separation mechanism based on partitioning of the analytes between the micelles and solution phase. The velocity at which the micelles migrate through the capillary is usually different to the electroosmotic flow such that the separation of hydrophobic compounds is enhanced by differences in their residence times (solubility) in the micelles. Sodium dodecyl sulfate and bile salts have been used successfully in micellar electrokinetic chromatography for the separation of steroids [20,21,23,24]. The composition, structure, and capacity to absorb organic solvent from the solution phase all influence the distribution constant for solutes between the micellar (pseudostationary phase) and mobile phase in a generally unpredictable manner [25–28]. In these studies we investigated the use of sodium dodecyl sulfate, sodium cholate, sodium taurocholate and sodium deoxytaurocholate as the micelle phase for the separation of estrogens with a variety of experimental conditions.

Various concentrations of surfactant were added to 20 m*M* sodium tetraborate–sodium phosphate buffer (pH 8); as based on previous experience this pH provided reasonable separation times and a wide separation window within which to optimize the selectivity of the separation. All estrogens migrated with the electroosmotic flow at 75 m*M* sodium taurocholate and at 400 m*M* were poorly separated with a migration time of 8.6 to 9.0 min. Sodium taurodeoxycholate at 50 m*M* separated estriol, 13.0 min, from the other estrogens which were poorly separated with a migration time of 16.0 to 17.0 min. With 50 m*M* sodium dodecyl sulfate, estriol migrated in 14.0 min with the remaining estrogens at about 20 min. At concentrations in the range 50–150 m*M* sodium cholate, the estrogens were separated into three groups; estriol migrated first, followed by the estrogens with two hydroxyl groups, and finally the estrogens with a ketone group at C_{17}. Within group separations were not particularly good and the resolution obtained depended on the surfactant concentration. The best separation was obtained with a sodium cholate concentration of 130 m*M*, Fig. 3. Equilin is nearly baseline resolved from estrone/equilenin, estriol and 17α-estradiol are well separated from the other estrogens, but 17β-dihydroequilin and 17β-dihydroequilenin and 17α-dihydroequilin and 17α-dihydroequilenin are unresolved with the latter group only partially separated from 17β-estradiol.

To improve the separation of the estrogens it is necessary to introduce some additional selectivity into the separation systems developed above. The addition of organic solvents increased the separation

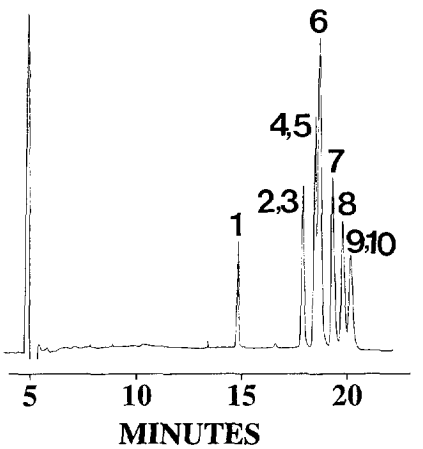

Fig. 3. Separation of estrogens by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 130 m*M* sodium cholate. Peak identifications: 1 = estriol; 2 = 17β-dihydroequilin; 3 = 17β-dihydroequilenin; 4 = 17α-dihydroequilin; 5 = 17α-dihydroequilenin; 6 = 17β-estradiol; 7 = 17α-estradiol; 8 = equilin; 9 = estrone; 10 = equilenin.

times with only modest gains in resolution in the most favorable cases. The addition of cyclodextrins, cyclic oligosaccharides containing 5, 6, or 7 glucose units (designated α-, β-, and γ-cyclodextrin, respectively) were more successful. The estrogens are known to form inclusion complexes with cyclodextrins [17,18]. The cyclodextrins have the shape of a hollow truncated cone with a cavity diameter determined by the number of glucose units. The cavity is relatively hydrophobic while the external surface is hydrophilic. The solubility of the cyclodextrins in the micelles is assumed to be low because of their hydrophilic external surface (although surfactant molecules may be complexed by the cyclodextrins). Therefore, when the estrogens are incorporated in the cyclodextrin cavity they migrate with the velocity of the solution phase, and when they are incorporated into the micelle they migrate with the micelle velocity, accordingly they will be separated depending on their distribution between the micelle and the solution phase augmented by the complexing capacity of the cyclodextrins.

When 20 m*M* β-, or γ-cyclodextrin were added to the pH 8 buffer containing 50 m*M* sodium dodecyl sulfate, significant improvements in the resolution of the estrogens was obtained. The addition of α-cyclodextrin did not change the results obtained with sodium dodecyl sulfate alone, Fig. 4A, except that the migration time was shorter. This should be contrasted with the results obtained by adding 20 m*M* β-cyclodextrin to the buffer containing 50 m*M* sodium dodecyl sulfate, Fig. 4B, where nine estrogens are separated almost to baseline (17α-dihydroequilin and 17β-estradiol are about 70% separated and 17α-estradiol and equilin co-migrate) with a shorter separation time. When γ-cyclodextrin was used in place of β-cyclodextrin significant changes in the migration order were observed and baseline resolution of all ten estrogens was obtained, Fig. 4C. The α-cyclodextrin cavity is too small for the formation of inclusion complexes with the estrogens but β- and γ-cyclodextrins are capable of forming inclusion complexes of significantly different stability with the estrogens providing complementary properties for their separation.

Again, when 20 m*M* γ-cyclodextrin was added to the 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 130 m*M* sodium cholate significant changes in migration time and migration order of the estrogens were observed (compare Fig. 3 with Fig. 5). Baseline separation for eight of the estrogens was achieved with 17α-estradiol and 17α-dihydroequilin co-migrating. Changing the concentration of sodium cholate (70–150 m*M*) or γ-cyclodextrin (10–20 m*M*) resulted in significant changes in resolution and, in some cases, in migration order but did not improve on the separation given in Fig. 5.

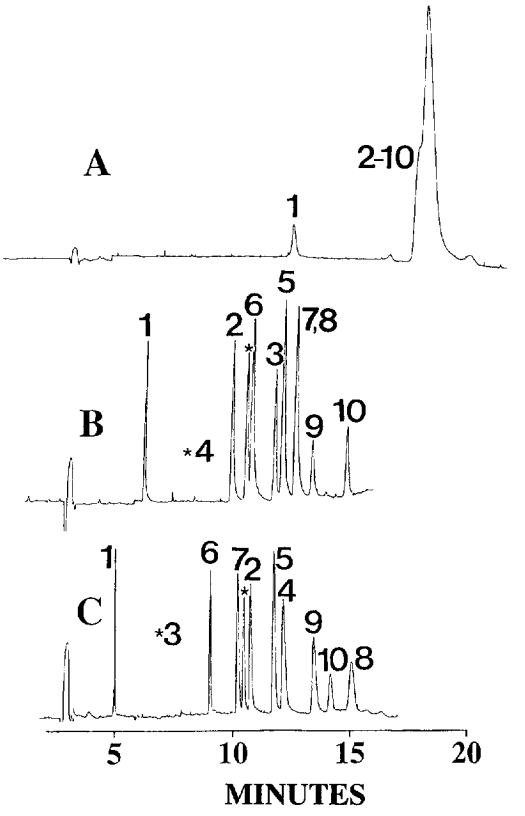

Fig. 4. Separation of estrogens by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 50 m*M* sodium dodecyl sulfate and 20 m*M* cyclodextrin. A = α-cyclodextrin; B = β-cyclodextrin; C = γ-cyclodextrin. Peak identifications are given in the legend to Fig. 3.

The addition of 15 m*M* β-cyclodextrin to the 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 130 m*M* sodium cholate separated eight of the estrogens with 17α-dihydroequilenin and 17β-dihydroequilenin partially separated, Fig. 6A. With the exception noted the peak shape and peak to peak separation was very good. Changing the con-

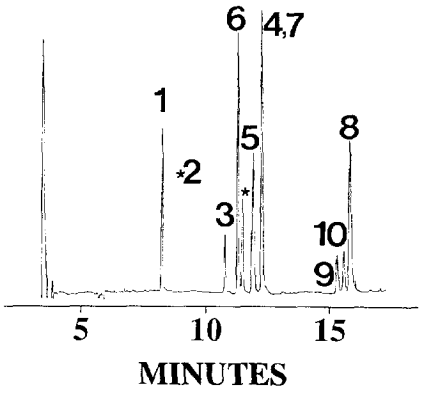

Fig. 5. Separation of estrogens by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 130 m*M* sodium cholate and 20 m*M* γ-cyclodextrin. Peak identifications are given in the legend to Fig. 3.

centration of β-cyclodextrin (10–20 m*M*) affected the separation less than changing the concentration of sodium cholate. With 100 m*M* sodium cholate, 17α-dihydroequilenin, 17β-dihydroequilenin and 17α-estradiol co-migrate while reducing the amount further to 75 m*M* sodium cholate resulted in 17α-estradiol migrating faster than 17α-dihydroequilenin and 17β-dihydroequilenin, all three of which are now almost baseline separated, Fig. 6B. Comparing Fig. 6A and Fig. 6B, improved separation of 17α-dihydroequilenin and 17β-dihydroequilenin by reducing the concentration of sodium cholate is achieved at the expense of increased peak tailing so that in those cases were information concerning the concentration of individual dihydroequilenin isomers is not required (they are normally trace components in pharmaceutical preparations derived from pregnant mares' urine) the system represented in Fig. 6A would be preferred.

To ensure good reproducibility in migration times for the above systems it was necessary to replenish the buffer after every two or three separations, otherwise a gradual increase in migration time with each subsequent injection was observed. The reproducibility of the migration time for the system containing 75 m*M* sodium cholate and 15 m*M* β-cyclodextrin was 0.4 to 1.0% R.S.D. ($n=7$). For 50 m*M* sodium dodecyl sulfate and 20 m*M* γ-cyclodextrin between 1.0–2.2% R.S.D. ($n=7$) for migration time. The retention factors (capacity factors) for the 10 estrogens were calculated using methanol as the electroosmotic flow marker and phenyloctane as the micelle marker. The results are summarized in Table 1 and indicate good reproducibility.

Several factors that might affect the resolution in the system containing 20 m*M* sodium borate–sodium phosphate buffer (pH 8), 75 m*M* sodium cholate and 15 m*M* β-cyclodextrin were investigated. There was no significant change in the resolution between pH 8 to 9. Above pH 9, 17α-dihydroequilin and 17α-estradiol co-migrate. Addition of methanol (5–15%, v/v) was detrimental to the separation and increased the separation time. Temperatures between 15–30°C were evaluated. A temperature of 25°C was optimum. At 15–20°C the resolution of the faster migrating peaks increased at the expense of expanding the migration time. At 30°C 17α-estradiol, 17α-dihydroequilenin and 17β-dihydroequilenin co-mi-

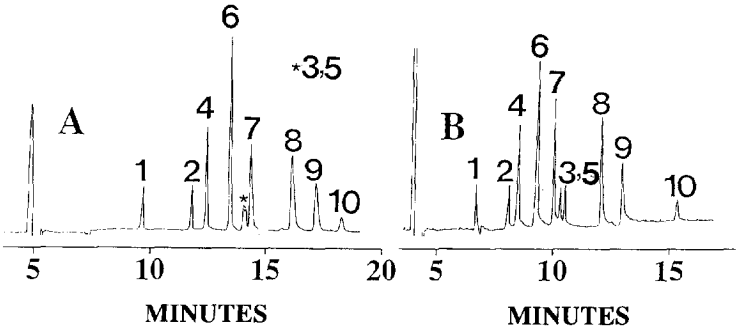

Fig. 6. Separation of estrogens by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 15 m*M* β-cyclodextrin and 130 m*M* sodium cholate (A) or 75 m*M* sodium cholate (B). Peak identifications are given in the legend to Fig. 3.

Table 1
Within-day precision of the retention factors for estrogens in the system 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 75 m*M* sodium cholate and 15 m*M* β-cyclodextrin ($n = 10$)

Estrogen	Retention factor	
	Mean	R.S.D. (%)
Estriol	0.77	1.2
17β-dihydroequilin	1.19	1.3
17α-dihydroequilin	1.35	1.4
17β-estradiol	1.69	1.5
17α-estradiol	1.99	1.3
17α-dihydroequilenin	2.10	1.2
17β-dihydroequilenin	2.17	1.3
Equilin	3.07	1.8
Estrone	3.75	1.2
Equilenin	5.81	1.8

grated. Voltages between 15–30 kV were evaluated. At 15 kV the estrogens containing hydroxyl groups were well separated, however, the migration time for the ketone-containing compounds was increased substantially. Above 25 kV the resolution among the estrogens with hydroxyl groups deteriorated dramatically but the ketone-containing compounds remained well separated. Varying temperature and voltage simultaneously proved to be useful for the fast separation of individual groups. A temperature of 15°C and 15 kV were the best choice for the separation of the estrogens with hydroxyl groups whereas a temperature of 30°C and 25 kV enabled the ketone-containing estrogens to be separated in about 7 min.

The separation system containing 20 m*M* sodium borate–sodium phosphate buffer (pH 8), 75 m*M* sodium cholate and 15 m*M* β-cyclodextrin was used to identify the estrogens in a tablet formulation of conjugated estrogens of the type excreted in pregnant mares' urine. Quite a good separation of the conjugated estrogens was obtained with no detectable amounts of free estrogens, Fig. 7A. Estrogen sulfate is indicated on the figure but a lack of suitable standards prevented other compounds from being identified. After acid hydrolysis of the conjugated estrogens, estrone, equilin, equilenin, 17α-dihydroequilin, 17α-estradiol, and 17α-dihydroequilenin were identified based on their migration time and coincidence of their UV spectra, Fig. 7B. The amount of estrogen per tablet was found to be 1.23 mg (R.S.D. = 3.1%) and of equilin was 0.46 mg (R.S.D. = 2.4%) for ten determinations. As defined in the current US Pharmacopoeia conjugated estrogens tablets are to contain not less than 73% and not more than 95% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate with the ratio of sodium equilin sulfate to sodium estrone sulfate not less than 0.35 and not more than 0.65 [29,30]. For the tablets analyzed the amount of

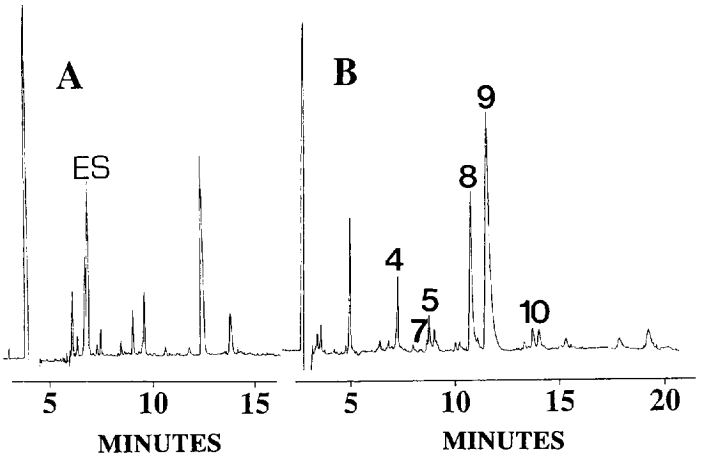

Fig. 7. Separation of estrogens in an unhydrolyzed (A) and hydrolyzed (B) conjugated estrogens tablet formulation by micellar electrokinetic chromatography. The separation conditions are given in Fig. 6(B) and the peak identification in the legend to Fig. 3; ES = estrone sulfate.

estrogens as the total of sodium estrone sulfate and sodium equilin sulfate was 87.9% of the labeled amount and the ratio of sodium equilin sulfate to sodium estrone sulfate, 0.41, which conforms to the specifications stated in the US Pharmacopoeia for conjugated estrogens tablets.

An attempt was made to separate the seven ethynyl steroids, Fig. 2, registered for use as oral contraceptives in the USA with the same separation system developed for the estrogens. A good separation was obtained except for norethynodrel and norgestrel, Fig. 8A, which co-migrated. In the absence of β-cyclodextrin, norethynodrel and norgestrel were separated but with a reduction in the resolution between norethindrone acetate and 17α-ethynylestradiol. The concentration of sodium cholate was determined to be most important in controlling the zone spacing in the separation. All seven ethynyl steroids were baseline resolved with 50 m*M* sodium cholate, Fig. 8B. The addition of 10% (v/v) methanol to the separation system improves the peak shapes with little change in the migration time, Fig. 8C. The reproducibility of the separation system is very good as indicated by the data in Table 2.

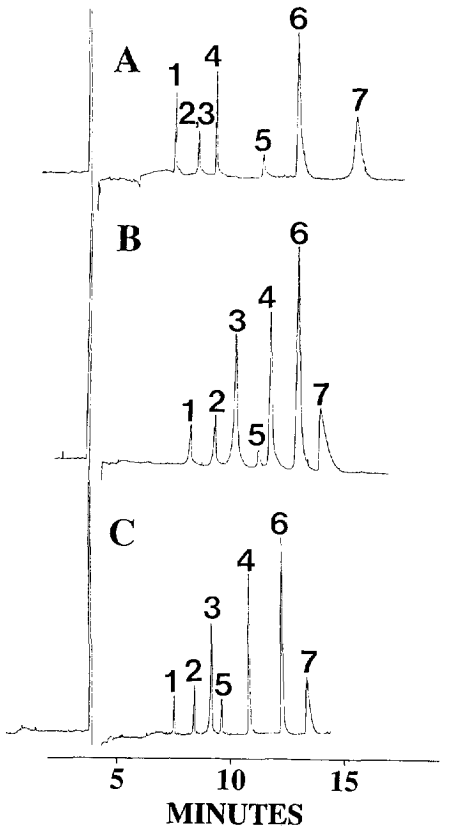

Fig. 8. Separation of ethynyl steroids by micellar electrokinetic chromatography with a 20 m*M* sodium borate–sodium phosphate buffer (pH 8) buffer containing 15 m*M* β-cyclodextrin and 130 m*M* sodium cholate (A); 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 50 m*M* sodium cholate (B); system (B) containing 10% (v/v) methanol (C). Peak identification: 1 = norethindrone; 2 = norgestrel; 3 = norethynodrel; 4 = 17α-ethynylestradiol; 5 = norethindrone acetate; 6 = mestranol; 7 = ethynodiol diacetate.

Table 2
Within-day precision of the retention factors for ethynyl steroids in the system 20 m*M* sodium borate–sodium phosphate buffer (pH 8) containing 50 m*M* sodium cholate and 10% (v/v) methanol ($n = 10$)

Ethynyl steroid	Retention factor	
	Mean	R.S.D. (%)
Norethindrone	1.28	1.7
Norgestrel	1.77	1.8
Norethynodrel	2.38	2.2
Norethindrone acetate	2.62	2.4
17α-Ethynylestradiol	4.69	2.1
17α-Ethynylestradiol 3-methyl ether	8.94	2.0
Ethynodiol diacetate	16.26	2.8

4. Conclusions

The above work demonstrates the separation of complex mixtures of hydrophobic steroids of pharmaceutical interest by micellar electrokinetic chromatography. The separation of the estrogens is superior to those that have been obtained by column liquid chromatography and are preferable to gas chromatography since derivatization is not required and the separation time is shorter. The identification of the estrogens in a tablet formulation of conjugated estrogens of the type isolated from pregnant mares' urine demonstrates the practical utility of micellar electrokinetic chromatography for composition conformity analysis of these complex natural products. In addition the separation of seven ethynyl steroid oral contraceptives is demonstrated using a simple

modification of the separation system developed for the separation of the estrogens.

References

[1] A.G. Gilman, L.S. Goodman and A. Gilman, Goodman and Gilman's The Pharmacological Basis of Therapeutics, Macmillan, New York, 1980.
[2] J.A. Berndt and C.F. Poole, J. Planar Chromatogr., 1 (1988) 174.
[3] J. Novakovic, J. Kubes and I. Nemec, J. Planar Chromatogr., 3 (1990) 521.
[4] S.K. Poole, M.T. Belay and C.F. Poole, J. Planar Chromatogr., 5 (1992) 16.
[5] J. Novakovic and E. Tvrzicka, J. High Resolut. Chromatogr., 14 (1991) 495.
[6] J. Novakovic, F. Tvrzicka, V. Vsetecka, V. Pouzar and L. Feltl, Collect. Czech. Chem. Commun., 60 (1995) 813.
[7] J.S. Zweig, R. Roman, W.B. Hagerman and W.J.A. van den Heuvel, J. High Resolut. Chromatogr., 3 (1980) 169.
[8] A. Jayatilaka and C.F. Poole, J. Chromatogr., 617 (1993) 19.
[9] G.W. Lyman and R.N. Johnson, J. Chromatogr., 234 (1982) 234.
[10] B. Flan and B. Lodge, J. Chromatogr., 402 (1987) 273.
[11] R.W. Townsend, V. Keuth, K. Embil, G. Mullersman, J.H. Perrin and H. Derendorf, J. Chromatogr., 450 (1988) 414.
[12] R.W. Roos, J. Assoc. Off. Anal. Chem., 63 (1980) 80.
[13] B. Desta, J. Chromatogr., 435 (1988) 385.
[14] J. Novakovic, E. Tvrzicka and V. Pacakova, J. Chromatogr. A, 678 (1994) 359.
[15] S.Y. Su, K. Shiu, J.E. Simmons and J.P. Skelly, Int. J. Pharm., 67 (1991) 211.
[16] J.-T. Lin and E. Heftmann, J. Chromatogr., 212 (1981) 239.
[17] H. Lamparczyk and P.K. Zarzycki, J. Pharm. Biomed. Anal., 13 (1995) 543.
[18] B.J. Spencer and W.C. Purdy, J. Liq. Chromatogr., 18 (1995) 4063.
[19] K.J. Potter, R.J.B. Allington and J. Algaier, J. Chromatogr. A, 652 (1993) 427.
[20] A.J. Ji, M.F. Nunez, D. Machacek, J.E. Ferguson, M.F. Iossi, P. C. Kao and J.P. Landers, J. Chromatogr. B, 669 (1995) 15.
[21] K.C. Chan, G.M. Muschik, H.J. Issaq and P.K. Siiteri, J. Chromatogr. A, 690 (1995) 149.
[22] D. Farley, FDA Consumer, 25 (1991) 16.
[23] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 513 (1990) 279.
[24] H. Nishi and M. Matsuo, J. Liq. Chromatogr., 14 (1991) 973.
[25] R.O. Cole, M.J. Sepaniak, W.L. Hinze, J. Gorse and K. Oldiges, J. Chromatogr., 557 (1991) 113.
[26] S. Yang and M.G. Khaledi, Anal. Chem., 67 (1995) 499.
[27] S. Yang and M.G. Khaledi, J. Chromatogr. A, 692 (1995) 301.
[28] M.H. Abraham, H.S. Chadha, J.P. Dixon, C. Rafols and C. Treiner, J. Chem. Soc., Perkin Trans. 2, (1995) 887.
[29] United States Pharmacopoeia, Revision XXII, United States Pharmacopeial Convention, Rockville, MD, 1990, pp. 535–537.
[30] Association of Official Analytical Chemists, Official Methods of Analysis, AOAC, Washington, DC, 15th ed., 1990, pp. 764–766.