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Journal of Chromatography B, 744 (2000) 157–163

JOURNAL OF CHROMATOGRAPHY B

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High-performance liquid chromatographic determination of 17β-estradiol and 17β-estradiol-3-acetate solubilities and diffusion coefficients in silicone elastomeric intravaginal rings

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Received 8 October 1999; received in revised form 7 March 2000; accepted 14 April 2000

Abstract

A rapid, sensitive reversed-phase high-performance liquid chromatographic method has been developed for the determination of in vitro release of 17β-estradiol and its ester prodrug, 17β-estradiol-3-acetate, from silicone intravaginal rings. Partial hydrolysis of the acetate under the aqueous conditions provided by the 1% benzalkonium chloride release medium necessitates its conversion to 17β-estradiol prior to HPLC analysis. Both steroid peaks have been fully resolved from the benzalkonium chloride peaks by the reported chromatographic method, which employs a C_{18} bonded reversed-phase column, an acetonitrile–water (50:50, v/v) mobile phase and a UV detection wavelength of 281 nm. The peak area versus 17β-estradiol concentration was found to be linear over the range of 0.0137–1347 μg ml^{-1}. The HPLC method has also been used to determine the silicone solubilities and diffusion coefficients of the two related steroids. The almost 100-fold increase in 17β-estradiol-3-acetate release from the silicone core-type intravaginal rings compared to 17β-estradiol is shown to be due to a 60-fold increase in silicone solubility and a one and a half-fold increase in diffusitivity. The results demonstrate that an effective estrogen replacement therapy dose of 17β-estradiol may be administered from a silicone intravaginal reservoir device containing the labile 17β-estradiol-3-acetate prodrug.

Keywords: 17β-Estradiol; 17β-Estradiol-3-acetate; Silicone

1. Introduction

17β-estradiol (E2) is the most potent of the natural human estrogens [1]. Its principle medical application is in the area of estrogen replacement therapy (ERT) where it is administered, generally as an oral tablet or transdermal patch, to counteract the decreased production of estrogens during and after the menopause as a result of ovarian failure [2–5]. Silicone subcutaneous implants containing E2 have also been developed for ERT releasing between 25 and 100 μg per day (Organon); the disadvantage with these implants is that a minor surgical procedure is required for implantation every 4–8 months. Recently, E2 has been incorporated into silicone intravaginal rings (IVRs) for the treatment of local vaginal atrophy with an in vivo release rate of between 5 and 10 μg/day sustained over a 3-month period [6,7]. The IVR's major advantage over subcutaneous implants for the delivery of drugs is that the device can be self-removed and inserted, thus ensuring better patient compliance and therapy man-

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0378-4347/00/$ – see front matter
PII: S0378-4347(00)00248-6

agement. However, in order to be clinically effective in ERT, controlled release rates of at least 50 μg/day of E2 from an IVR are necessary, which are currently difficult to obtain due to IVR manufacturing limitations and the poor release characteristics of E2 from silicone elastomer. However, the use of reservoir or shell-type IVRs having very thin membranes and releasing up to 200 μg/day of E2 has been reported by several authors [8,9]. Matrix devices have also been investigated [10]. Enhanced release rates from silicone reservoir devices may be obtained by employing certain ester prodrugs of E2 [11]. For example, 17β-estradiol-3-acetate (E3A) not only has increased silicone solubility compared with E2, but it also possesses greater aqueous solubility allowing enhanced penetration through vaginal epithelium [11]. Additionally, it is rapidly hydrolysed in vivo to E2. The silicone diffusion coefficients for these steroids have not been reported, and thus it is unclear as to whether the enhanced release of E3A is a consequence of higher solubility in silicone, a greater diffusion coefficient than for E2, or both. The aims of this study are, therefore, fourfold; to describe an HPLC method for the determination of E2 and E3A release from silicone IVRs, to demonstrate the enhanced release of E3A over E2, to measure the solubility of E3A and E2 in silicone, and to determine the magnitude of the silicone diffusion coefficients.

2. Experimental

2.1. Reagents and materials

HPLC grade acetonitrile was purchased from LabScan (Dublin, Ireland). Analytical grade water was prepared by passing distilled water through a Milli-Q® reagent water system (Millipore). 17β-Estradiol was obtained from Schering Health (UK). 17β-Estradiol-3-acetate was synthesised and micronised under GMP by Irotech (Cork, Ireland). GPR grade sodium hydroxide pellets, disodium orthophosphate 12-hydrate and sodium dihydrogenorthophosphate monohydrate were purchased from Davidson and Hardy (Belfast, UK). Benzalkonium chloride (BKC), stannous-2-ethyl hexanoate (stannous octoate) and poly(dimethyl siloxane) oil (20 cst, 25°C) were obtained from Sigma (Poole, UK). Silicone elastomer base (MED-6382) and tetrapropoxysilane (TPOS) were purchased from Nusil Technology (Carpinteria, CA, USA).

2.2. Preparation of E2 and E3A silicone reservoir IVRs

A stock silicone elastomer mix was prepared by blending 100 parts silicone elastomer base (MED-6382) with 2.5 parts tetrapropoxysilane. The base contains a mixture of high- and low-molecular-mass (20 000 and 2000) α,ω-hydroxy terminated poly(dimethyl siloxane)s, a reinforcing filler (diatomaceous earth) and a processing fluid. E2 (2.40 g) was added to the elastomer mix (15.0 g) and, following addition of 1 part stannous octoate per 100 parts active mix, injection molded (80°C, 2 min) on a Lab-scale ring-forming machine (Techni-Gal, Galen Holdings, Portadown, UK). A single injection produced two 1.0-g active cores of 2.0-mm cross-sectional diameter and 48.0 mm external diameter. The cores were then encapsulated in two stages with the elastomer mix by injection molding at 80°C, producing IVR reservoir devices having the following measurements: 9.0 mm cross-sectional diameter, 54.0 mm external diameter and 10.8 g. Reservoir IVRs of E3A were manufactured in a similar manner, except 2.84 g E3A were added to 15.0 g of the elastomer mix to produce the active core, thus ensuring that the E2 and E3A cores had an equal number of moles.

2.3. In vitro drug release

The E2 and E3A silicone reservoir IVRs ($n=4$) were placed in stoppered 250-ml conical flasks containing 100 ml of 1.0% BKC solution. The flasks were placed in an shaking orbital incubator (Model 10X400.XX2.C, Sanyo Gallenkamp, Uxbridge, UK) operating at 100 rpm, 32 mm orbit diameter and maintained at a temperature of 37°C. Samples were taken daily for HPLC analysis and the 1% BKC solution was replaced daily to maintain sink conditions.

2.4. E3A sample preparation

Sodium hydroxide solution (0.5 *M*, 50 μl) was added to the daily E3A samples (1000 μl), the

solution vortexed for ~5 s and left to stand for 2 min to afford the fully hydrolysed sample. Neutralising buffer solution (50 μl, pH 6.5) (12.15 g sodium dihydrogenorthophosphate and 8.8 g of disodium hydrogenorthophosphate per 100 ml water), was added to the hydrolysed sample, resulting in a total dilution factor of 1.1. The samples were analysed by HPLC within 24 h of hydrolysis.

2.5. Chromatography

The HPLC instrumentation (Shimadzu, Kyoto, Japan) consisted of a model SIL-10AXL auto injector, a model SCL-10A system controller, a model LC-10AT solvent delivery module, a model FCV-10AL low pressure gradient flow valve, a model GT-154 degassing unit, and a model SPD-10A UV–Vis detector. The ODS (2) Sphereclone column (150×4.6 mm I.D., 5 μm) was obtained from Phenomenex (Cheshire, UK). HPLC analysis was performed at ambient temperature in isocratic mode, with a mobile phase of acetonitrile–water (50:50, v/v) delivered at 1.5 ml min^{-1}. A detection wavelength of 281 nm and injection volumes of 10 μl were used throughout the study. The concentrations of E2 and E3A were calculated using a calibration curve, produced by plotting peak areas versus concentration. Stock solutions of E2 and E3A were prepared in acetonitrile. A series of solutions ranging from 0.137 to 1347 μg ml^{-1} were prepared for the HPLC calibration curve. Each solution was injected in quadruplicate.

2.6. Silicone solubility of E2 and E3A

E2 and E3A (100 mg) were each added to poly(dimethyl siloxane) oil (6.0 ml) and placed in the shaking orbital incubator at 37°C for 3 days to achieve saturation. The saturated solutions were filtered through Nalgene® disposable syringe filters (0.45 μm, PTFE membrane, Davidson and Hardy) before extracting 1 ml into 10 ml GPR-grade acetonitrile. The E2 acetonitrile solution was directly analysed by HPLC; the E3A solution was first hydrolysed according to the method outlined in Section 2.4. Silicone solubilities were thus readily determined.

2.7. Stability profiles of E2 and E3A in BKC and acetonitrile

BKC solutions (1%) of E2 and E3A (10.2 and 11.9 mg in 100 ml, respectively) were placed in an orbital incubator. Samples were taken daily over 14 days and the percentage purity determined by HPLC. Reference samples of E2 and E3A in acetonitrile were used as controls.

3. Results and discussion

No previous HPLC method has been published in the literature for the analysis of E3A. However, HPLC determination of 17β-estradiol from a wide range of samples including plasma [12], urine [13], and health foods [14] has been reported.

3.1. Chromatographic method

The in vitro assay reported here produced E2 and E3A retention times of 2.0 and 4.5 min, respectively. Under these conditions the E2 and BKC peaks (0–2 min) were fully resolved with a R_s value of 2.9 for E2 and the closest BKC peak. A total chromatographic run time of <5.0 min was possible. A typical chromatogram obtained from an E3A IVR release sample is presented in Fig. 1A and shows two main peaks, corresponding to E3A and the E2 hydrolysis product. The extent of hydrolysis is not as large as the relative peak areas might suggest since E2 and E3A have significantly different molar absorptivities at the 281 nm detection wavelength (1962 and 70 $mol^{-1}\,dm^3\,cm^{-1}$, respectively, in acetonitrile–water, 50:50, v/v). Subsequent hydrolysis of the same E3A sample according to the procedure outlined resulted in the chromatogram of Fig. 1B, where no E3A peak is detectable and the area under the E2 peak increases. Due to the larger molar absorbtivity of E2 the *y*-axis of the E3A chromatogram has been enlarged by a factor of five to facilitate comparison of the two chromatograms.

3.2. Estradiol calibration curve

A standard calibration curve for E2 based on mean peak areas was constructed over the concentration range 0.137–1347 μg ml^{-1}. A linear curve, $y =$

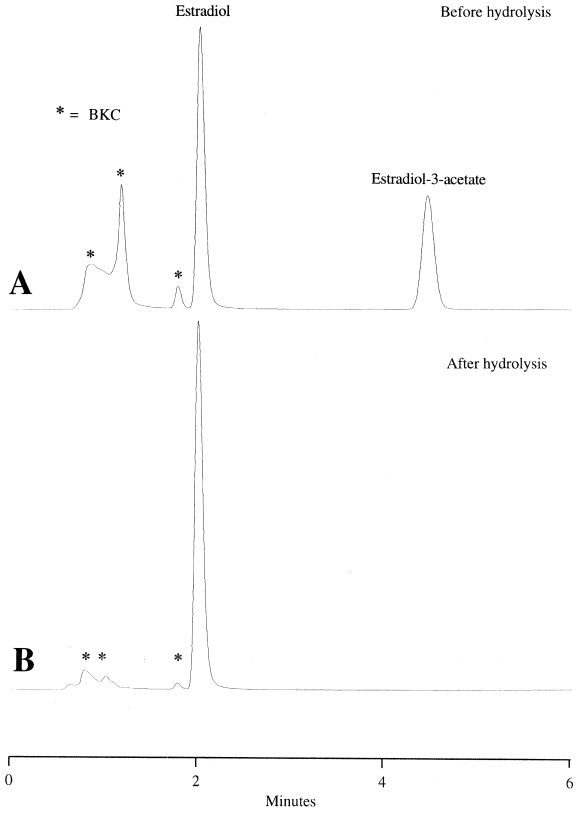

Fig. 1. Representative chromatograms of in vitro 17β-estradiol-3-acetate release from a silicone IVR; (A) before hydrolysis; (B) after hydrolysis.

$4640x + 176$, $r^2 = 0.999$, was fitted to the data. The calibration curve data is summarised in Table 1. At higher concentrations (1912, 2391 and 2869 $\mu g\,ml^{-1}$) deviation from linearity was observed. Estradiol concentrations as low as 10 $ng\,ml^{-1}$ were detectable although reproducibility was poorer. Both within-day and day-to-day precision and accuracy of the calibration curves yielded coefficients of variance less than 2%. Both E2 and E3A silicone IVRs provided E2 release values within the linear region of the calibration curve.

3.3. Hydrolysis of E3A to E2

The rate of hydrolysis of E3A in aqueous (1% BKC) and non-aqueous (acetonitrile) solution was monitored by HPLC over a period of 14 days. E3A did not hydrolyse in the non-aqueous environment, maintaining a 99.6% purity profile over the study period (Fig. 2). The 0.4% impurity was verified to be E2. However, in the 1% aqueous BKC solution, the E3A slowly hydrolysed (Fig. 2), confirming the need to pre-hydrolyse the E3A samples before HPLC analysis. The in vitro plasma half life of the 3-acetate

Table 1
Calibration data for E2, yielding a line of equation $y = 4640x + 176$, $r^2 = 0.999$

Estradiol concentration ($\mu g\,ml^{-1}$)	Area under curve (±SD)
0.090	416.1±6.26
0.137	626.7±7.77
0.685	3091.3±29.6
1.37	6268.0±31.4
13.7	61 997±161
68.5	310 174±801
137	628 780±1511
269	1 257 493±4744
404	1 902 116±1391
674	3 110 373±13 520
898	4 164 211±37 595
137	6 262 836±34 243

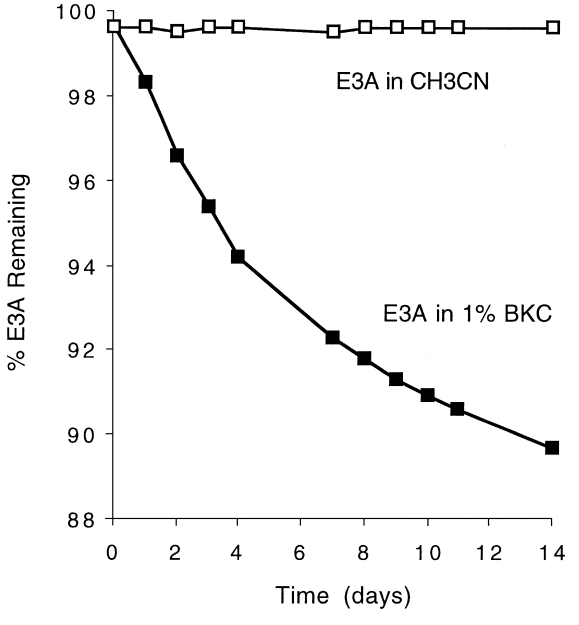

Fig. 2. Stability profiles for 17β-estradiol-3-acetate in acetonitrile and 1% BKC solution.

has previously been determined to be >1 min [11]. E2 was found to be stable both in BKC and organic solution.

3.4. Release of E2 and E3A from IVRs

Eq. (1) describes release from a polymer membrane permeation-controlled drug delivery device under sink conditions [15,16]

$$Q = \frac{D_m}{h_m} C_p t \quad (1)$$

where Q is the amount of drug released per unit area of the device (mg/cm^2), C_p is the solubility in the polymer (mg/cm^3), D_m is the diffusion coefficient for the drug through the polymer (cm^2/day), h_m is the thickness of the membrane (cm), and t is time (days). The IVR surface area, s, was calculated using Eq. (2), and the values of Q/t determined (Table 2).

$$s = 4\pi^2 bc \quad (2)$$

where b is the cross-sectional radius and c is the external radius.

Thus, a plot of Q versus t yields a straight line of gradient $D_m C_p / h_m$. The daily and cumulative release profiles for E2 and E3A are shown in Figs. 3 and 4 respectively. Both profiles exhibit an initial burst, followed by a release rate following zero-order kinetics. The burst effect can be attributed to two factors. Firstly, having encapsulated the drug-containing silicone core with the drug-free silicone membrane, the drug concentration gradient that is established ensures drug transport from core to membrane. Secondly, the silicone curing chemistry produces propanol as a by-product (Fig. 5), in which the steroids are highly soluble. The propanol dissolves some of the drug at the core/membrane interface and, on subsequent evaporation, deposits it within the outer membrane layer. The high concentration of drug in the membrane produces the observed burst over 1 or more days. From the linear (zero-order) region of the cumulative release plot, the average daily release rates were determined (Table 2). E3A was observed to release 92 times faster from the rings than E2.

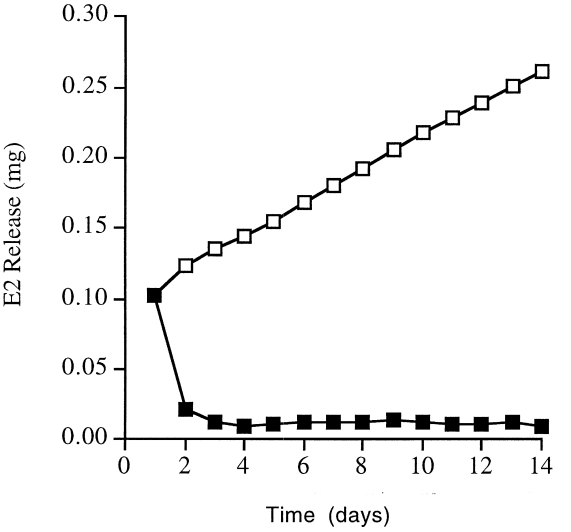

Fig. 3. Daily (closed squares) and cumulative (open squares) release of 17β-estradiol from a silicone reservoir IVR containing 17β-estradiol.

3.5. Silicone solubilities and diffusion coefficients

The solubilities of E2 and E3A in silicone oil at 37°C were measured by the reported HPLC method to approximate the solubility in the silicone elas-

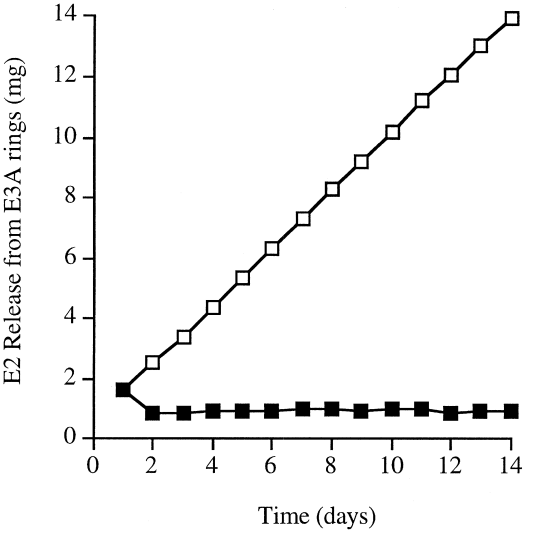

Fig. 4. Daily (closed squares) and cumulative (open squares) release of 17β-estradiol from a silicone reservoir IVR containing 17β-estradiol-3-acetate.

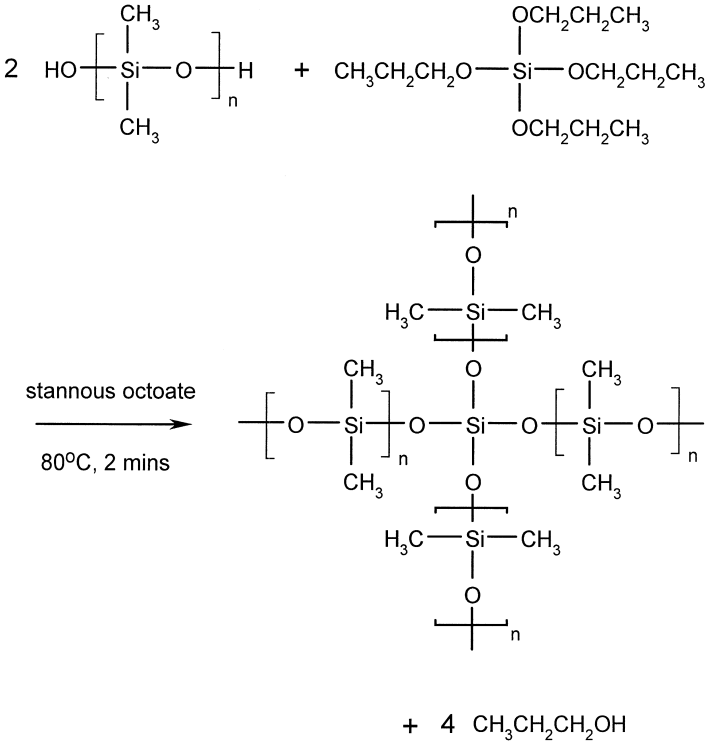

Fig. 5. Condensation curing mechanism of hydroxyl-terminated silicones with tetrapropoxysilane.

tomer. Solubility in the elastomer is expected to be very similar to that measured in the oil owing to the very low glass transition temperature of poly(dimethylsiloxane), $T_g = -123$°C, and the low degree of crosslinking in the elastomer, ensuring that the polymer chain segments between cross-linking sites are almost as mobile and fluid-like as those in the oil. The values for the solubilities of E2 and E3A in silicone oil are presented in Table 2. The presence of the acetate group of E3A results in a 60-fold increase in solubility compared with E2. The actual values obtained (237 and 4 $\mu g/cm^3$ respectively) are of a similar magnitude to those measured for other structurally related steroids, where solubilities ranged from 1 to 1500 $\mu g/cm^3$ depending on chemical structure [15]. According to Eq. (1), the enhanced release for E3A is a consequence of a greater silicone solubility (C_p) and/or a larger silicone diffusion coefficient (D_m) compared with E2. The diffusion coefficients were calculated from the corresponding silicone oil solubilities using Eq. (1), with $h_m = 0.35$ cm, and are also presented in Table 2. The

Table 2
Mean daily IVR release rates, silicone solubilities and silicone elastomer diffusion coefficients for 17β-estradiol and 17β-estradiol-3-acetate

Steroid	Release rate (μg/day)	Q/t ($\mu g/cm^2$/day)	Silicone solubility C_p ($\mu g/cm^3$)	Diffusion coefficient D_m (cm^2/day)
E2	12.1±0.2	0.252±0.004	4.0	$2.55 \cdot 10^{-7}$
E3A	1113±21 (964±18)[a]	23.2±0.4 (20.1±0.4)[a]	237	$3.96 \cdot 10^{-7}$

[a] This value, corresponding to the average daily concentration of E2 in the release medium post-hydrolysis, has been determined from the gradient of the cumulative release plot of Fig. 4. The unbracketed value, representing the actual release of E3A from the IVRs, has been calculated from the bracketed value using the relative molecular mass ratio for the two steroids.

silicone diffusion coefficient for E3A is 1.5 times greater than for E2, the individual values again within a range determined for other steroid molecules [15].

4. Conclusions

The HPLC method described in this paper allows the determination of in vitro release rates of 17β-estradiol and 17β-estradiol-3-acetate from silicone intravaginal rings. The values of the silicone solubilities and diffusion coefficients were thus determined. The results demonstrate that the enhanced solubility and diffusitivity of the acetate prodrug compared with 17β-estradiol make it a better candidate for the provision of controlled-release estrogen replacement therapy from a silicone intravaginal ring.

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