ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 1153–1157

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Estimation of impurity profiles of drugs and related materials
Part XXI. HPLC/UV/MS study of the impurity profile of ethynodiol diacetate☆

M. Babják *, G. Balogh, M. Gazdag, S. Görög

Chemical Works of Gedeon Richter Ltd., P.O. Box 27, H-1475 Budapest, Hungary

Received 6 June 2001; received in revised form 29 November 2001; accepted 3 December 2001

Abstract

The impurity profile of ethynodiol diacetate was investigated using the HPLC/UV/MS method. Using the slightly modified HPLC method of USP 24 two impurities, earlier isolated by preparative HPLC and investigated by NMR spectroscopy were separated and characterised. The mass spectra amended by the diode-array UV spectra supported the earlier found structures (*E* and *Z* isomers of 17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3′-acetoxy-2′-butenoate). Another, hitherto not described impurity, 17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3-oxo-butanoate) has also been separated and characterised by means of its mass spectrum, NMR and UV spectra. © 2002 Published by Elsevier Science B.V.

Keywords: Ethynodiol diacetate; Impurity profiling; HPLC/MS; Diode-array UV spectra; NMR spectroscopy

1. Introduction

Ethynodiol diacetate (**1**) is one of the first synthetic progestogens used in contraceptive pills. Almost 40 years after its introduction and in possession of new generations of contraceptive gestogens it is still widely used and is official among other pharmacopoeias in USP 24 [3].

Ethynodiol diacetate is prepared from norethisterone (**2**) by reduction of the 3-keto group and the acetylation of the 3β- and 17α-hydroxy groups. For this reason the most probable impurities of **1** are **2**, its 17-acetate (**3**), unacetylated ethynodiol (**4**), its 3-monoacetyl (**5**) and (less likely) 17-monoacetyl (**6**) derivative. (For the structures see Fig. 1.) Due to the not entire stereoselectivity of the reduction of the 3-keto group the epimeric 3α-acetoxy derivative (**7**) is also among the common impurities. The HPLC separation and quantification of the latter was first described by Görög and Herényi [4]. A HPLC method is prescribed in USP 24 [3] for the

☆ For Part XX see Ref. [1]. This paper is Part LV in a series on "Analysis of Steroids"; for Part LIV see Ref. [2].

* Corresponding author
E-mail address: m.babjak@richter.hu (M. Babják).

0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(02)00165-6

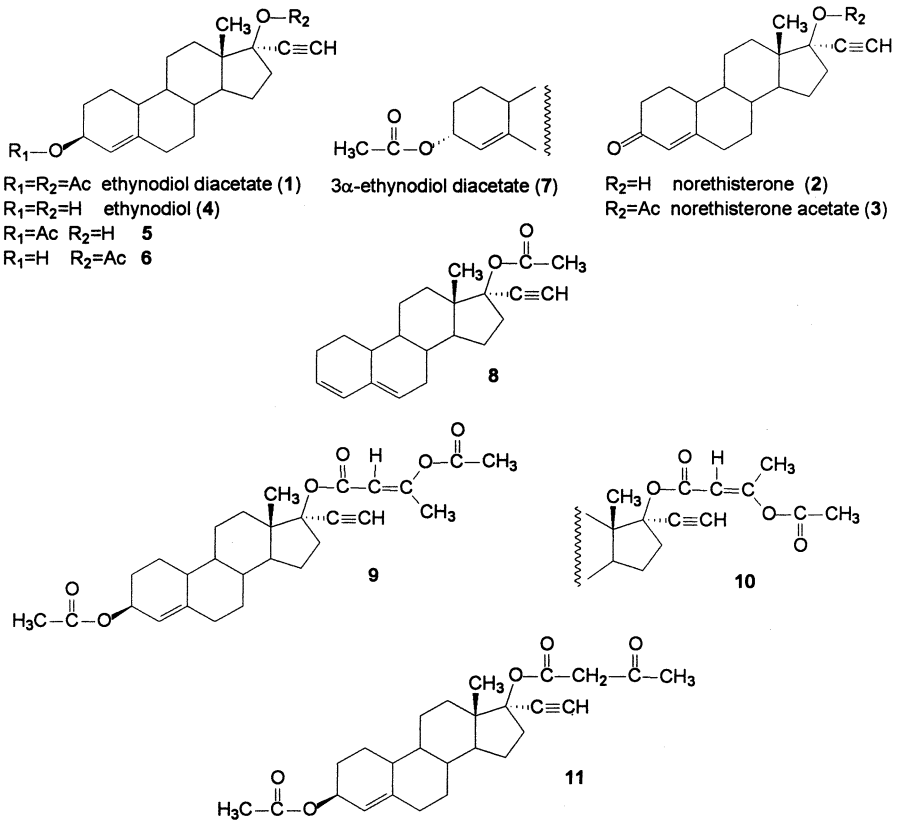

Fig. 1. Structures of ethynodiol diacetate and its impurities.

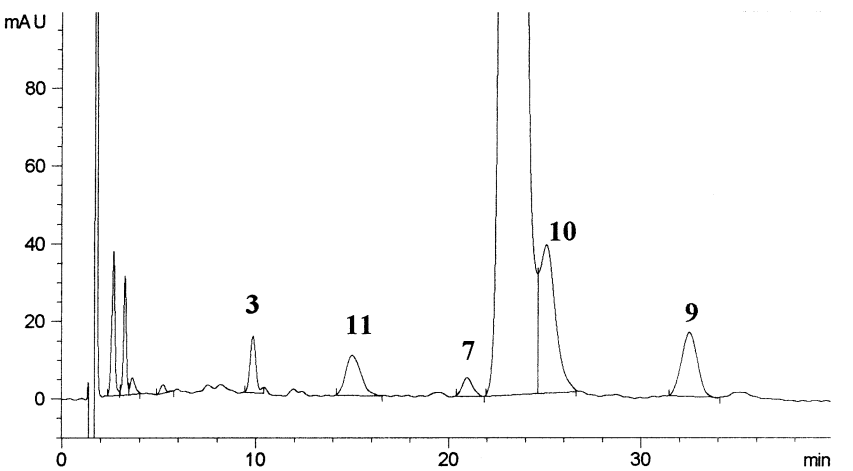

Fig. 2. Chromatogram of a bulk ethynodiol diacetate sample. For the chromatographic conditions see System B in Section 2.2. For the key see Fig. 1.

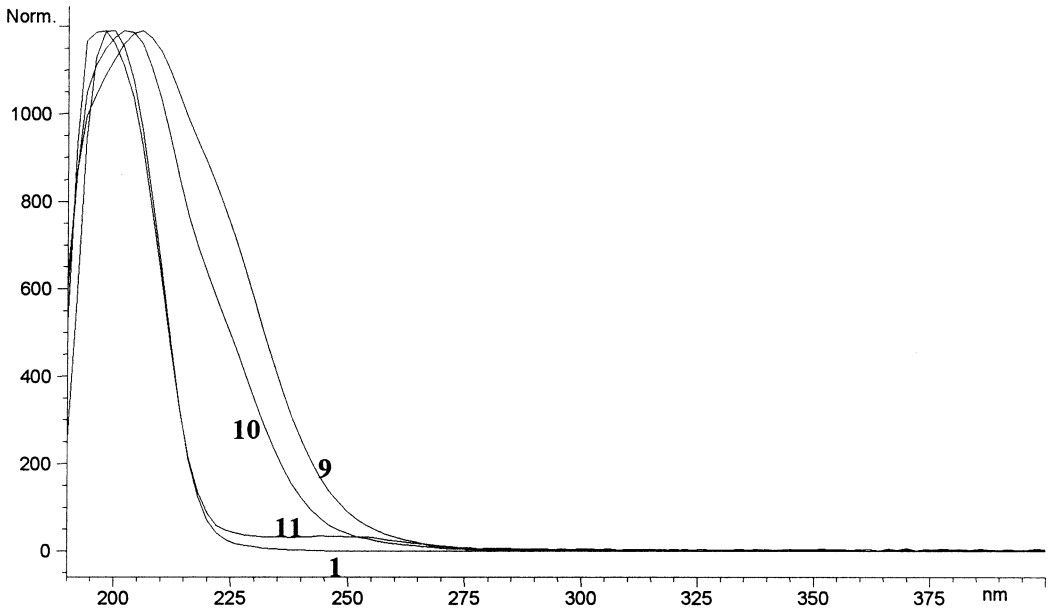

Fig. 3. Diode-array UV spectra of ethynodiol diacetate (**1**) and impurities **9**, **10** and **11**.

determination of **7** (limit 1.5%). Another common impurity is 17α-ethinyl-estra-3,5-diene-17-ol acetate (**8**), which is the product of the acid-catalysed degradation of **1**. Due to the high UV-activity of **8** and the poor UV-activity of **1** the determination of **8** in **1** is carried out in USP 24 [3] by direct UV spectrophotometry at 236 nm.

Further to the above listed impurities two unusual impurities (*E* and *Z* isomers of 17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3′-acetoxy-2′-butenoate), **9** and **10** were described by Görög et al. [5,6]. This study was based on the detection of the impurities by RP-HPLC, their isolation by normal-phase preparative HPLC and structure elucidation by NMR spectroscopy. The 3′-acetoxy-2′-butenoate moiety which can be considered as a 'trimerised' acetyl group is a side product of the acetylations catalysed by 4-dimethylaminopyridine [7].

HPLC/MS was not involved in the above-mentioned study [5,6]. Since in the meantime this technique has become the key method in drug impurity profiling [8,9a], the aim of this study was the re-evaluation of the impurity profile of ethynodiol diacetate supported by HPLC/MS.

2. Experimental

2.1. Instruments

A Hewlett-Packard 1100 LC/MSD instrument, equipped with a diode-array UV detector, was used.

The NMR studies were performed on a Varian $^{UNITY}INOVA$-500 instrument in $CDCl_3$ solution at 30 °C ($\delta_{TMS} = 0.00$ ppm). Assignments were corroborated by the concerted use of two-dimensional $^1H-^1H$ (gradient enhanced phase-sensitive double-quantum filtered COSY) and $^1H-^{13}C$ (GHSQC, GHMBC) correlation experiments as well as NOE (1D DPFGSE-NOE) experiments.

2.2. Chromatographic conditions

System A (USP 24 [3]). Column: LiChroCART 125 × 4 mm i.d. (Merck); eluent: water–acetonitrile 59:41 v/v; flow rate: 2 ml min^{-1}; temperature: 40 °C; UV detector: 200 nm.

System B. The same as System A except for the eluent which was 5 mM aqueous ammonium acetate–acetonitrile 35:65 v/v at a flow rate of 0.5 ml min^{-1}.

2.3. MS conditions

Electrospray positive ionisation mode (API-ES); drying gas temperature: 100 °C; nebulizer pressure 340 kPa; capillary voltage: 4000 V.

3. Results and discussion

In the course of the above mentioned earlier studies [4–6] mixtures of methanol and water were used as the eluents for the RP-HPLC separation of impurities **2**–**10**. It has to be noted that under these conditions **10** was only poorly separated from ethynodiol diacetate (**1**).

In this study the system of USP 24 was modified by incorporating ammonium acetate into the eluent in order that the soft electrospray (API-ES) ionisation can be used for the investigation of the thermally labile parent drug and its similarly labile impurities. A typical chromatogram of a bulk ethynodiol diacetate sample is shown in Fig. 2. In the mass spectrum of ethynodiol diacetate (**1**) obtained under the conditions described in Section 2 the water adduct m/z $(M+18)=402$ is well detectable. The most intense peaks are derivable from the loss of acetic acid (m/z $(M+H)^+-60$) and the splitting of the acetyl group (m/z $(M+H)^+-43$), respectively. The two geometrical isomers of 17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3′-acetoxy-2′-butenoate), (**9** and **10**) were well separated from the main component using both system A and B; relative retention times in System B: 1.41 and 1.09, respectively. These can be characterised by their abundant $(M+18)=486$ peaks and—as in the case of the main component—by peaks, characteristic of the loss of acetic acid and acetyl group as well. These data furnish further evidence to the structures determined earlier by means of NMR spectroscopy after preparative HPLC separation [5,6].

The UV spectra obtained by the diode-array detector are also in accordance with these structures. The evaluation of these, easily obtainable spectra can often greatly contribute to the success of impurity profiling [9b,10]. In this case, as seen in Fig. 3, the λ_{max} value of the main component is at 199 nm which is characteristic of its isolated double bond. The slight bathochromic shift of this and especially the shoulder around 220 nm in the spectra of **9** and **10** are characteristic of the second double bond in their molecules which is in conjugation with the ester-carbonyl group of the 'trimerised' acetyl group [11].

In the course of this study one more, hitherto unknown impurity (**11**) of ethynodiol diacetate was also detected and characterised. Its relative retention time in System B related to the main component is 0.65. From the $(M+18)$ peak at m/z 444 and $(M+H)^+$-60 and $(M+H)^+$-43

Table 1
^{1}H and ^{13}C NMR data for **11** [17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3-oxo-butanoate)]

Position	^{13}C NMR [δ (ppm)]	^{1}H NMR [δ (ppm)]
C-1	25.2	1.20 m[a] and 2.02 m[a]
C-2	27.7	1.42 m and 2.04 m[a]
C-3	70.3	5.22 m
C-4	120.1	5.34 m
C-5	144.7	
C-6	35.0	2.05 m[a] and 2.27 m
C-7	31.4	0.96 m and 1.72 m[a]
C-8	41.2	1.28 m
C-9	49.4	0.71 m
C-10	41.6	1.80 m[a]
C-11	25.7	1.16 m[a] and 1.84 m[a]
C-12	33.0	1.66 m and 1.81 m[a]
C-13	47.9	
C-14	47.7	1.52 m
C-15	23.4	1.34 m and 1.75 m[a]
C-16	37.2	2.08 m and 2.73 m
C-17	85.8	
C-18	13.5	0.89 d
–C≡	82.7	
≡CH	75.5	2.63 s
O–(*CO*)–CH_3	170.9	
O–(CO)–*CH_3*	21.4	2.04 s
O–(*CO*)–CH_2 –(CO)–CH_3	165.5	
O–(CO)–*CH_2* –(CO)–CH_3	50.5	3.42 m
O–(CO)–CH_2 –(*CO*)–CH_3	200.3	
O–(CO)–CH_2 –(CO)–*CH_3*	30.1	2.27 s

[a] Chemical shift determined from the relevant cross-peak position in an HSQC experiment.

peaks at 367 and 384, respectively, a molecular mass of 426 and a structure where a 'dimerised' rather than 'trimerised' acetyl group i.e. 3-oxo-butanoate (acetoacetate) is linked to the 17-hydroxy group is derivable. As its diode-array spectrum shows, this does not contain a second (conjugated) double bond. This indicates that it is not enolised: it is present in the oxo form. This was confirmed also by the NMR study of the synthesised impurity **11**; (see Table 1). This impurity can be regarded either the precursor or the degradation product of impurities **9** and **10**.

It has to be noted that the quantity of impurities **9**, **10** and **11** in the investigated batches of bulk ethynodiol diacetate were far below the limit set by USP 24 [3] for individual impurities (0.5%).

References

[1] S. Görög, Acta Pharm. Hung. 70 (2000) 131–137.
[2] S. Görög, M. Babják, G. Balogh, J. Brlik, F. Dravecz, M. Gazdag, P. Horváth, A. Laukó, K. Varga, J. Pharm. Biomed. Anal. 18 (1998) 511–525.
[3] United States Pharmacopoeia 24, USP Convention Inc., Rockville, Md., 2000, p. 698.
[4] S. Görög, B. Herényi, J. Chromatogr. 152 (1978) 240–242.
[5] S. Görög, Zs. Halmos, B. Herényi, A. Georgakis, G. Balogh, É. Csizér, Z. Tuba, in: S. Görög (Ed.), Advances in Steroid Analysis, Akadémiai Kiadó, Budapest, 1991, pp. 323–329.
[6] S. Görög, G. Balogh, M. Gazdag, J. Pharm. Biomed. Anal. 9 (1991) 829–833.
[7] G. Höfle, W. Steglich, H. Vorbrüggen, Angew. Chem. 90 (1978) 602–615.
[8] W.M.A. Niessen, Liquid Chromatography—Mass Spectrometry, 2nd ed., Marcel Dekker Inc, New York, Basel, Hong Kong, 2001, pp. 405–463.
[9] S. Görög (Ed.), Identification and Determination of Impurities in Drugs, Elsevier, Amsterdam, 2000, pp. 267–298
(a) L. Tollsten, HPLC/MS for drug impurity identification, pp. 266–298
(b) S. Görög, The role of diode-array UV spectra in the identification of impurities, pp. 252–265.
[10] S. Görög, M. Bihari, É. Csizér, F. Dravecz, M. Gazdag, B. Herényi, J. Pharm. Biomed. Anal. 14 (1996) 85–92.
[11] S. Görög, Ultraviolet–Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, Boca Raton, 1994, p. 55.