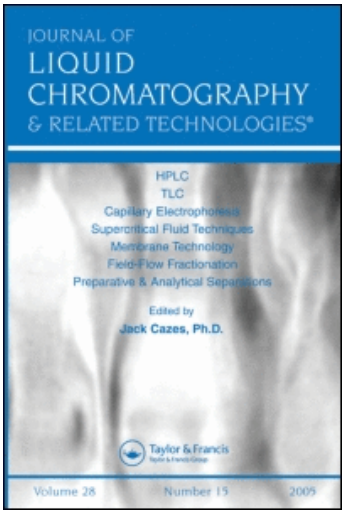

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Anti-Hormonal Agents. V. HPLC of Anordrin

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ANTI-HORMONAL AGENTS. V. HPLC OF ANORDRIN

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ABSTRACT

Procedures are described for the reverse phase HPLC analysis of the contraceptive A-nor steroid Anordrin, including the separation of its alpha- and beta-isomers, present in formulated tablets (Chinese Vacation Pill).

INTRODUCTION

There has been a decline in popularity of the traditional, daily, oral contraceptives in recent years and, at the same time, a growing interest in the development of post-ovulatory and post-coital contraceptive methods for women and of systemic contraceptive agents for men. Recently, a number of new drugs have been reported which prevent or interfere with implantation and which are now the subject of laboratory investigations and clinical trials.[1] There is a requirement for suitable analytical methods which

will provide assurance of the purity and content of these new drugs and their formulations and provide a basis for pharmacokinetic and metabolic studies in animal and human subjects.

We have recently described suitable methods for the analysis of 11-aminophenyl steroids such as RU 38486, ZK 98734 and ZK 98299, which are powerful anti-progestational anti-implantation agents in women [2,3], for the analysis of the anti-estrogen tamoxifen and its metabolites [4] and for the analysis of cyproterone acetate, which is an anti-spermatogenic agent in men [5]. Chinese scientists have reported that the A-nor steroid anordrin (1) is a very effective contraceptive agent (Chinese No. 53 Pill, Vacation Pill or Visiting Pill) which has anti-implantation activity and has been used in China by many thousands of women: the chief side effect is reported to be a variable degree of nausea associated with undesirable estrogenic character [6-8]. Despite the considerable amount of laboratory and clinical work already carried out on anordrin, to date there have been no reports of the HPLC analysis of this drug. In this paper we present details of such a method and its application to the analysis of the drug in a tablet formulation.

EXPERIMENTAL

General methods and procedures for the analytical HPLC have been described previously [2-4]. Analyses were performed on a 25 cm x 4.5 mm i.d. column packed with 5 um ODS-Hypersil, eluted at 2.0 ml/min with degassed, HPLC-grade solvents (MeCN: far UV grade) using a Cecil 1100 pump. Injections of steroids, dissolved in MeCN, were made via a Rheodyne 7125 valve

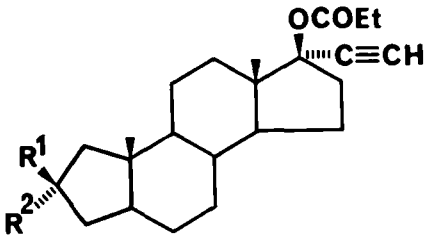
fitted with a 20 ul loop. Detection on a Cecil CE 1220 UV monitor was at 208 nm x 0.164 AU fsd. Peak areas were measured with a Trivector TRIO computing integrator.

Purification of anordrin: 10 anordrin tablets were ground and extracted with CH_2Cl_2 and the extract filtered, evaporated and redissolved in 200 ul MeCN. Preparative HPLC was performed by injection through a Rheodyne 7125 injector fitted with a 0.5 ml loop onto a 33 cm x 22 mm i.d. column packed with 5 um ODS-Hypersil, eluted at 17 ml/min with MeOH-H_2O (43:57 v/v). Fractions across the main peak (8 min - 12 min) were collected and evaporated under vacuum for HPLC analysis: all were pure and were combined for NMR.

Analysis of anordrin tablets: each tablet was ground and extracted with MeCN (2.00 ml), which was centrifuged to spin down excipients prior to injection of 20 ul onto HPLC. Standards containing 0.8-5.0 mg/ml of anordrin in MeCN were injected for calibration (a linear calibration graph was obtained) and the reproducibility for 5 repeat injections of alpha-anordrin standard was RSD 1.17% by automatic peak area measurement (1.32% by manual peak height measurement).

RESULTS AND DISCUSSION

The synthesis of anordrin involves the reaction of potassium acetylide with a steroid precursor, 2,17-diketo-A-nor-androstane, followed by esterification of the resulting diol with propionic anhydride [9]. The attack of acetylide ion occurs exclusively from the alpha face at C-17, but on both the alpha- and beta-face of the A ring at C-2 [9]. Consequently, anordrin can be generated in two isomeric forms (Figure 1).



	R^1	R^2
1-alpha	OCOEt	HC≡C
1-beta	HC≡C	OCOEt

Figure 1 Structures of isomers of anordrin (1)

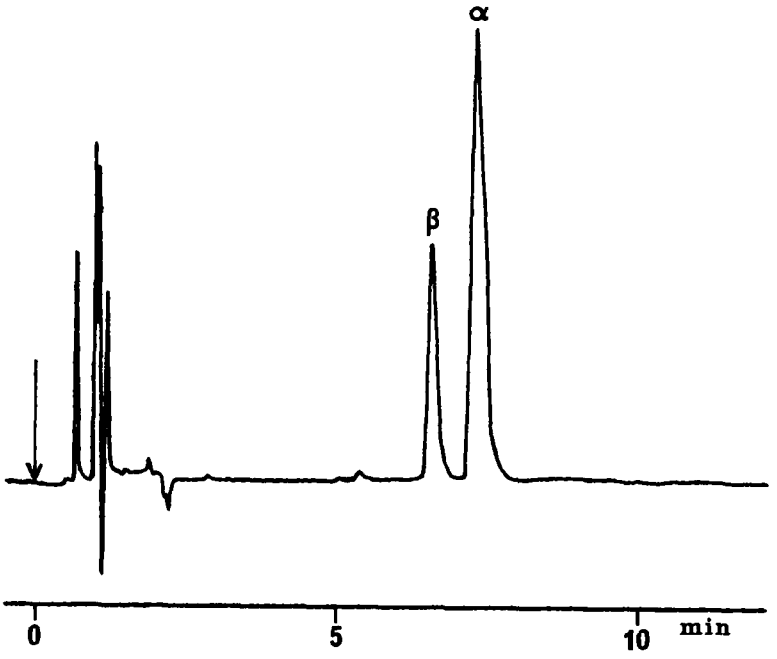


Figure 2 **HPLC separation of alpha- and beta-anordrin** 25 cm x 4.5 mm i.d. 5 um ODS-Hypersil column eluted at 2.0 ml/min with MeCN-H_2O 82:18 v/v. Injection: anordrin (3 mg/ml) in 20 ul MeCN. Detection: UV 208 nm x 0.164 AU fsd.

Since the anti-implantation effect resides mainly in the alpha-isomer [10], an analytical method is required which can distinguish between the two isomeric forms.

Anordrin has only a weak, short-wavelength absorbance band in its UV spectrum, for the non-conjugated ester groups. HPLC detection was therefore carried out at 208 nm. We found that the two stereoisomers of anordrin can be resolved by reverse-phase HPLC (Figure 2), with the less active beta-isomer eluting first. Relative amounts of more than 0.1% of the beta-isomer in alpha-anordrin could be detected.

Blister-packs of Chinese No. 53 Pill (Shanghai Sine Pharmaceutical Plant, China; declared content 7.5 mg anordrin per tablet) were obtained from a pharmacy outlet in Shanghai. The identity of the steroid component was confirmed by isolating a sample by extraction, purification on preparative reverse-phase HPLC and comparison of its NMR with authentic material.

Analysis of several of the tablets showed them to contain 95% $\pm$5% of the declared content as alpha-anordrin. In addition, they contained a small amount of the beta-anordrin isomer (1.5% $\pm$ 0.5%).

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REFERENCES

[1] Van Look, P.F.A. Post-Ovulatory Agents for Fertility Regulation, in: **World Health Organization, Biennial Report 1986-7**, WHO, Geneva, 1988, 153.

[2] Matlin, S.A. and Abderabbani, N. **J. High Resolution Chromatog. & Chromatographic Commun.** 10, 401, 1987.

[3] Matlin, S.A. and Wu, Z.Y. **J. High Resolution Chromatog. & Chromatographic Commun.** 10, 557, 1987.

[4] Matlin, S.A., Wu, Z.Y. and Kianchehr, Y.S. **J. High Resolution Chromatog. & Chromatographic Commun.** 11, 602, 1988.

[5] Matlin, S.A. and Jiang, L.X. **J. Liquid Chromatogr.** in the press, 1989.

[6] Chihping, K., Ming-Kang, C., Hsui-Chuan, C., Shihsing, C., Tawei, P. and Kang, T. **Sci. Sinica** 18, 262, 1975.

[7] Gu, Z. and Chang, M.C. **Contraception** 20, 549, 1979.

[8] Van Look, P.F.A. **World Health Organization, 14th Annual Report 1984**, WHO, Geneva, 1985.

[9] Li, R.L. and Ma, R.H. **Recent Advances in Synthetic Chemistry for Fertility Regulating Agents 1981; J. Reproduction and Contraception (Shanghai)**, 38, 1982.

[10] Crabbe, P., Fillion, H., Letourneaux, Y., Diczfalusy, E., Aedo, A.R., Goldzieher, J.W., Shaikh, A.A. and Castracane, V.D. **Steroids** 33, 85, 1979.