Long-Acting Contraceptive Agents: HPLC of Levonorgestrel Cyclopentylcarboxylate Oxime and Its Hydrolysis Products

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1 Introduction

Since 1975, the World Health Organization Special Program of Research, Development and Research Training in Human Reproduction has promoted a multinational research program involving the synthesis and testing of a large number of steroidal esters with progestational activity, in the search for new, long-acting injectable contraceptive agents [1–7]. Preliminary evidence has indicated that levonorgestrel cyclopentylcarboxylate oxime (1), as a mixture of *syn* and *anti* isomers, may have the desired hormonal characteristics and avoid some of the unwanted disturbances in bleeding patterns and clinical trials sponsored by the World Health Organization are planned for the near future. A requirement for initiating these trials is the availability of suitable analytical procedures for establishing the pharmacokinetics of the drug following injection in women. The oxime ester may undergo *in vivo* hydrolysis by two separate pathways (**Figure 1**), via either the ester (2) or the levonorgestrel oxime *syn* and *anti* isomers (3), leading to levonorgestrel (4), all of which will have different levels of intrinsic progestational activity. Antibodies to levonorgestrel are expected to show a substantial amount of cross-reactivity to this series of levonorgestrel derivatives, thus precluding the direct use of radio-immunoassay (RIA) for determination of plasma levels. It was therefore desired to devise a method which could separate all of these compounds, including the isomers, for the determination of their concentrations in plasma samples. In this paper we report the development of an HPLC separation method which is unusual in requiring the use of a column containing mixed stationary phases.

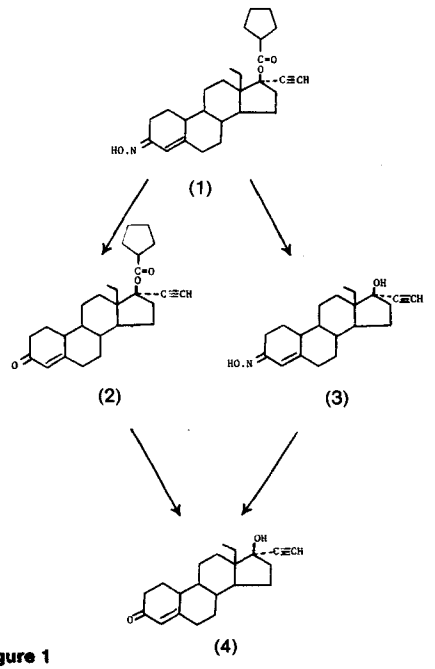

Figure 1

Hydrolysis pathways for levonorgestrel cyclopentylcarboxylate oxime.

2 Experimental

Samples of levonorgestrel and levonorgestrel oxime were provided by the World Health Organization. Levonorgestrel cyclopentylcarboxylate and levonorgestrel cyclopentylcarboxylate oxime were synthesized by Palmer Research Laboratories, Mostyn, UK.

General methods and procedures for the analytical HPLC have been described previously [2–4]. Analyses were performed on 25 cm × 4.5 mm i.d. columns packed with 5 μm spherical particles of ODS-Hypersil and/or Phenyl-Hypersil, eluted at 1.0 ml/min with degassed, HPLC-grade solvents. Injections of steroids, dissolved in MeCN, were made via a Rheodyne 7125 valve fitted with a 20 μl loop. Detection on a Cecil CE1220 UV monitor was at 238 nm × 0.5 AU fsd (0.005 AU fsd for detection limit determination).

3 Results and Discussion

Separation of compounds (1)–(4) was initially investigated on ODS-Hypersil (25 cm × 0.45 mm i.d.). Using MeOH-H_2O (85:15 v/v) as mobile phase, the isomers of the ester-oxime (1) were resolved (k'_{syn} 4.63; k'_{anti} 5.07). However, under these conditions the isomers of levonorgestrel oxime (3) showed no separation at all. A variety of different solvent ratios and combinations with MeOH, MeCN, and water were tried, but none gave any resolution of the isomers of (3).

A number of other stationary phases were then examined for the separation of the oxime isomers of (3), including aminopropyl, cyanopropyl, short alkyl, nitroalkyl, and phenyl phases. Only the last of these (25 cm × 0.45 cm i.d. Phenyl-Hypersil, eluted with

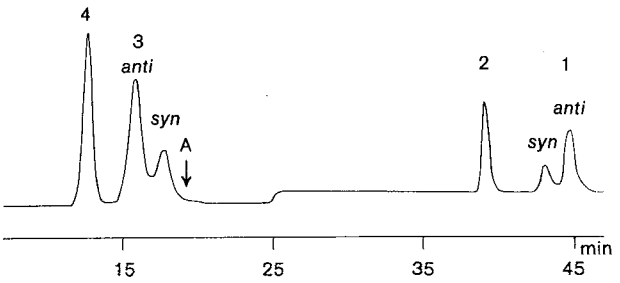

Figure 2

Separation of levonorgestrel derivatives (1) – (4) on a mixed-phase HPLC column.

Conditions: 30 cm × 0.45 cm i.d. column containing 5 μm Phenyl-Hypersil and 5 μm ODS-Hypersil, 2::1 w/w, eluted initially with MeOH-MeCN-H_2O, 55 : 5 : 40 v/v/v, switched at point A to 58 : 20 : 22 v/v/v, at 1.0 ml/min. Detection at 238 nm × 0.64 AU fsd.
Compounds: (1) cyclopentylcarboxylate oxime, (2) cyclopentylcarboxylate, (3) oxime, (4) levonorgestrel.

MeOH-H_2O, 60:40 v/v) gave an adequate separation (k'_{syn} 4.55; k'_{anti} 3.55). Disappointingly, however, when the full range of compounds (1)–(4) was examined on this stationary phase it was found that there was no visible resolution of the ester oxime isomers (1).

It was thus apparent that no single bonded phase available was able to achieve the desired separation of all the compounds and it was concluded that a combination of the Phenyl and ODS phases would be necessary. When columns of these two phases were coupled together, in either order, a partial separation of the compounds and the isomer pairs in a mixture containing (1)–(4) was achieved. However, it became apparent that the ratio of phase volumes of the two bonded phases would require adjustment in order to achieve an optimum separation. It was decided that this would be better achieved by co-mixing the phases in a single column than by coupling columns of varying lengths, thus standardizing the column size and also avoiding band spreading effects due to the inter-column junction.

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Coupled HPLC-GC for Determining PCBs in Fish 10685

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1 Introduction

Use of HPLC for sample clean-up is attractive because of the high separation power available and the simple, direct control over the elution of the fraction of interest by on-line detection using the HPLC detector (at least in mixtures of standards or in spiked samples), allowing accurate cuts. For other applications, like the one described below, coupled HPLC-GC is primarily attractive due to the simplicity of the method, integrating the main sample preparation step into the final analysis [1]. Instead of cleaning the sample, *e.g.*, by a Florisil column, the raw extract, *e.g.* of fish, is injected onto HPLC, followed by on-line transfer to GC. During GC analysis the HPLC column is reconditioned allowing the next sample to be run as soon as GC analysis is completed. Of course, the attractiveness of the method will be greatly enhanced with the availability of automated HPLC-GC equipment as described by *Munari et al.* [2]. Raw extracts, *e.g.*, from foods, will be placed in an HPLC autosampler and the analysis, including reconditioning of the HPLC column, carried out automatically.

The first application of coupled HPLC-GC for the analysis of PCBs was described by *Cortes et al.* [3,4]. Packed capillary HPLC columns (0.32 mm i.d.) were used for isolating some PCBs from coal tar. However, there is little purpose in generating high HPLC separation power when analyzing PCBs as very modest HPLC columns (modified or unmodified silica gel) already separate different individuals of the class, and there is no point in separating a sample well and recombining the separated components for transfer to GC. Furthermore, packed HPLC capillary columns would not be suitable for the determination of PCBs in foods because only small volumes of sample material can be loaded onto such columns.

