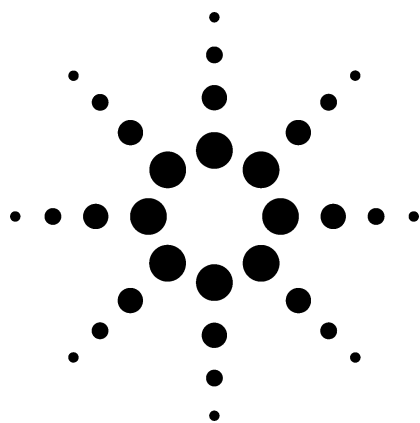




HPLC Separation of Norethindrone and Mestranol Using Progesterone as an Internal Standard on Eclipse XDB-CN (L10 - USP) and Eclipse XDB-C8 (L7 - USP) Columns

Application

Drug Testing/Development/Discovery

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Introduction

HPLC is the method of choice for dissolution and assay testing of norethindrone/mestranol tablets. A cyano column (USP-L10) is specified in the dissolution method for norethindrone/mestranol tablets published in the USP Formulary (USP 27 - NF 22) [1]. Although a C8 column (USP-L7) is specified for the assay analysis for norethindrone/mestranol tablets in the same publication, the results presented below indicate that the L10 column may provide the optimal separation for both analyses.

Experimental

All separations were performed isocratically at 1 mL/min and 25 °C, with the eluent compositions specified in Figure 1. An Agilent 1100 HPLC system was used, consisting of a binary pump, autosampler, and diode array detector (DAD). Organic and aqueous solvents were mixed online. Detection was at 205 nm. Five-microliter injections were made with the autosampler. The HPLC columns are specified in the figures.

Steroid standards were purchased from Sigma, Inc. and dissolved in the eluent corresponding to each column. Sample concentrations were 0.514 µg/µL (norethindrone), 0.407 µg/µL (progesterone), and 0.057 µg/µL (mestranol).

Results and Discussion

Figure 1 shows the separation of norethindrone, progesterone [internal standard (ISTD) for USP assay method], and mestranol on the Eclipse XDB-CN (Figure 1A) and Eclipse XDB-C8 (Figure 1B).

Highlights

- Either Eclipse XDB-CN or XDB-C8 can be used for steroid analyses.
- Eclipse XDB-CN provides faster, lower cost, and higher-resolution separations for both dissolution and assay analyses.



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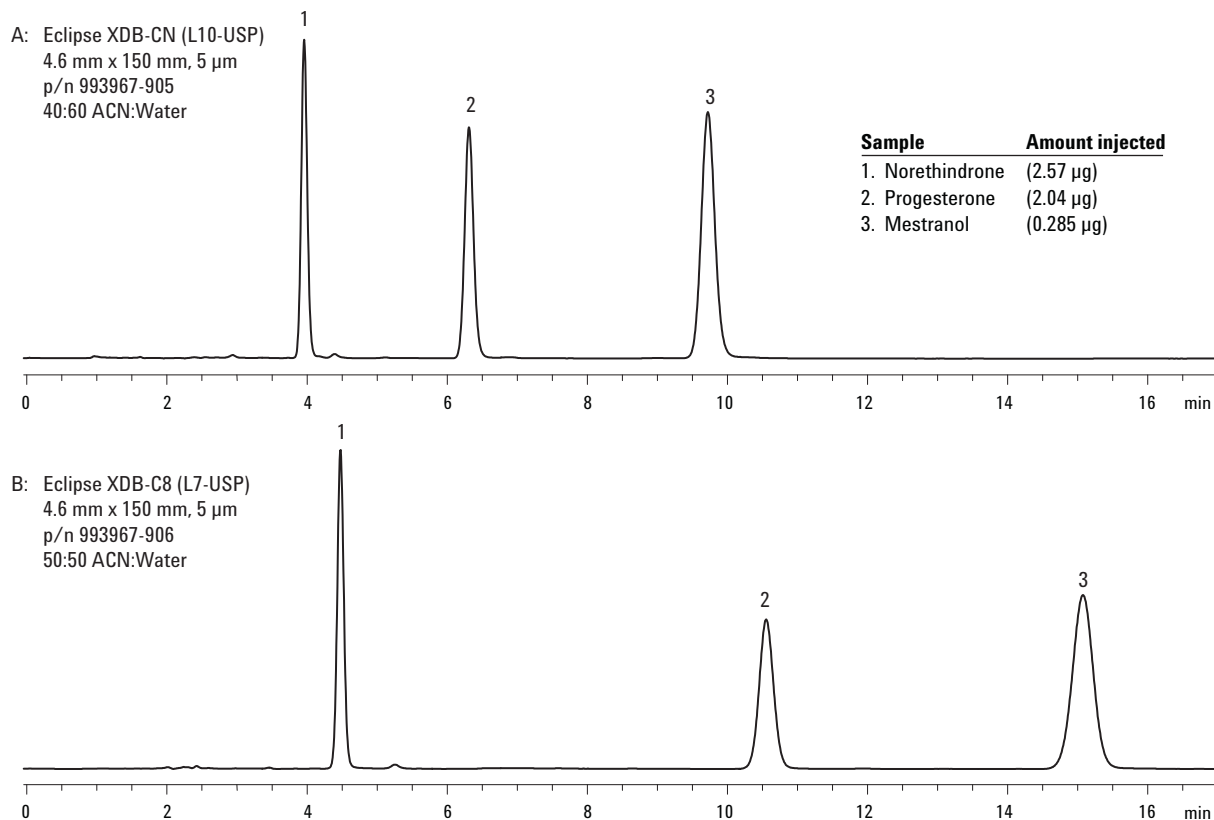


Figure 1. HPLC separation of norethindrone and mestranol using progesterone as an ISTD on an Eclipse XDB-CN (L10 - USP) column versus an Eclipse XDB-C8 (L7 - USP) column.

This mixture of steroids is well separated on both columns, including a small impurity eluting after norethindrone, using the elution conditions specified for each column in the USP Formulary (USP 27 - NF 22) for the dissolution (L10 column) and assay (L7 column) of norethindrone/mestranol tablets. Although the dissolution method does not specify the use of an ISTD, the progesterone peak is also well separated on the L10 (Eclipse XDB-CN) column.

The dissolution method (USP) for norethindrone/mestranol tablets specifies that the minimum number of theoretical plates (N) for the mestranol peak is 4000, that the tailing factors (Tf) for the norethindrone and mestranol peaks do not exceed 1.5, and that the relative retention times (RRT) are about 0.4 for norethindrone and 1.0 for mestranol.

The assay method (USP) for norethindrone/mestranol tablets specifies that the minimum number of theoretical plates (N) for the mestranol peak is not less than 6000, the Resolution (Rs) between the progesterone and mestranol peaks is not less than 5.0, and that the RRT are about 1.0 for norethindrone and 2.5 for mestranol.

Table 1 indicates that the Eclipse XDB-CN (L10) column easily meets these requirements for the dissolution method and that the Eclipse XDB-C8 (L7) column easily meets these requirements for the assay method. The RRT for the mestranol peak on the Eclipse XDB-C8 column actually exceeds the requirement specified in the assay method but this simply indicates greater selectivity and higher resolution on the Eclipse XDB-C8 column.

Table 1. USP Performance Measures for Norethindrone/Mestranol Methods

Column	Steroid	N	Tf	RRT	RRT	Rs
Eclipse XDB-CN (L10)	Norethindrone	10,200	1.09	0.41	1.00	–
	Progesterone	12,100	1.08	0.65	1.59	12.2
	Mestranol	12,300	1.08	1.00	2.45	11.7
Eclipse XDB-C8 (L7)	Norethindrone	11,100	1.06	1.00	–	–
	Progesterone	12,800	1.04	2.36	–	22.3
	Mestranol	12,300	1.00	3.36	–	9.8

The chromatograms in Figure 1 and data in Table 1 also suggest that the Eclipse XDB-CN might be suitable for both dissolution and assay methods. It easily meets the requirements for both methods and has the advantages of a shorter analysis time (33% shorter), with subsequent lower solvent consumption per analysis, as well as using a lower percentage of acetonitrile, which is also a cost savings.

Conclusions

The Eclipse XDB-CN (L10) and Eclipse XDB-C8 (L7) columns meet or exceed all the performance requirements for the USP methods for dissolution and assay, respectively, of norethindrone/mestranol tablets. In addition, the results for the Eclipse XDB-CN column suggest that it might be suitable for both the dissolution and assay methods, which would result in a faster (33%), and lower cost assay method.

Reference

1. USP Method "Norethindrone and Mestranol Tablets", USP27 - NF22.

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