COGENT 2.0
Bidentate C18™

Analysis of Drospirenone/ Ethinyl Estradiol Tablet

Impact of a Small Particle Column on Resolution of These Compounds

Extended Application Note

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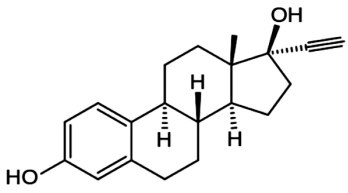
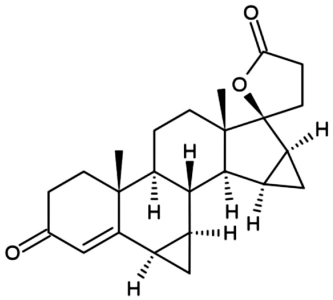


Ethinyl Estradiol



Drospirenone

Introduction

The formulation of drospirenone and ethinyl estradiol, available under the trade name Ocella® among others, is used primarily as a contraceptive, in hormone replacement therapy, and to treat symptoms of premenstrual dysphoric disorder. In terms of a chromatographic assay, the separation is fairly straightforward; both compounds are hydrophobic and retain well in reversed phase. Their structures differ enough to allow for good selectivity.

The main challenge of this analysis is the difference in amounts of drospirenone and ethinyl estradiol in the tablet. In the Ocella® formulation, a tablet contains 3 mg drospirenone but only 0.03 mg ethinyl estradiol. Hence, it may be problematic to adequately detect the ethinyl estradiol peak while simultaneously avoiding overload of the drospirenone peak.

Use of a small particle phase may be able to address this issue. It is known that column efficiency increases significantly with a smaller particle size, and a higher efficiency peak means greater peak height. Therefore, a smaller particle size column can produce greater signal for peaks like ethinyl estradiol, which may be difficult to detect using a standard 4µm stationary phase.

There may be other advantages as well. In an impurities method, attenuated band-broadening of the main peak may make nearby eluting impurity peaks easier to detect. Sometimes these peaks may be lost in the tail portion of the main peak and overlooked.

For these reasons, a simple isocratic method was performed for the separation of drospirenone and ethinyl estradiol in a tablet extract using Bidentate C18™ columns. One column used standard 4µm particles while the other was a 2.2µm phase. Comparisons of the data led to conclusions about the advantages of smaller particle size materials.

Experimental

Materials

Ocella® tablet was obtained from an anonymous source. A binary mixture of 1:1 acetonitrile: DI water (HPLC grade) was obtained from Honeywell (Muskegon, MI, USA).

Instrumentation

a Hewlett–Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector (265 nm) was used. The system was interfaced with Agilent Chemstation (Santa Clara, CA, USA) software. The flow rate was 0.2 mL/min and the injection volume was 4µL. The mobile phase was premixed 50% acetonitrile/ 50% DI water. The analytical columns were as follows:

- Cogent Bidentate C18 2.2µm 120Å, 2.1 x 50 mm
- Cogent Bidentate C18 4µm 100Å, 2.1 x 50 mm

Samples

Ocella® tablet (3 mg strength drospirenone and 0.03 mg strength ethinyl estradiol) was ground with a mortar and pestle and added to a 10 mL volumetric flask containing an acetonitrile diluent. It was sonicated for 10 min and diluted to mark. After mixing, a portion was filtered (0.45µm, nylon) and used for HPLC injections.

Results and Discussion

Use of the Bidentate C18™ column was found to be suitable for assay of these two compounds in the combination formulation. As drospirenone and ethinyl estradiol are hydrophobic molecules, retention and separation by a reversed phase approach was appropriate. The resolution between the two analytes was calculated to be 3.0.

The method is quite simple and the isocratic mobile phase can even be purchased from some suppliers premixed. This is a desirable feature for QC laboratories that might need to perform HPLC assays on drospirenone/ ethinyl estradiol tablets regularly and rapidly. The short 2.1 x 50 mm columns used in this application help reduce the run time, further increasing throughput.

Figure 1 shows an overlay of the full chromatogram view (only the drospirenone peak is visible at this scale). Two features of the comparison are noteworthy. First, the peak's retention time is highly consistent between the two phases. The two Bidentate C18™ materials have the same bonded ligand, so retention should not be significantly different in comparing 4µm vs. 2.2µm particles. This is a beneficial feature for easy method transfer, for example, to a 2.2µm phase if a method exists already using the 4µm column.

The second interesting feature is the advantage that the 2.o™ phase provides in terms of efficiency. Here it can be readily seen how the 2.o™ column gives a peak height significantly greater than that of the 4µm column. It is an inherent advantage of smaller particle size materials to produce superior efficiency and therefore sharper peaks.

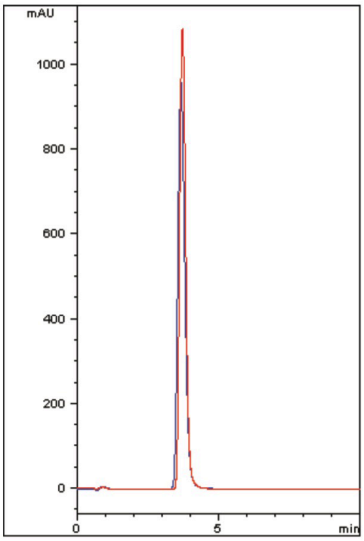


Figure 1

- 4µm BDC18 Blue Trace
- 2.o™ BDC18 Red Trace

Peaks

1. Ethinyl Estradiol
2. Drospirenone
3. Impurity

Zooming in on the chromatograms allows for a clear view of the ethinyl estradiol peak, which is present in the formulation at a much lower amount than drospirenone. When diluted in a 10 mL solution, there is a 3 ppm concentration for ethinyl estradiol in the sample. Figure 2 depicts a zoom-in view of both 4µm and 2.o™ phases for comparison

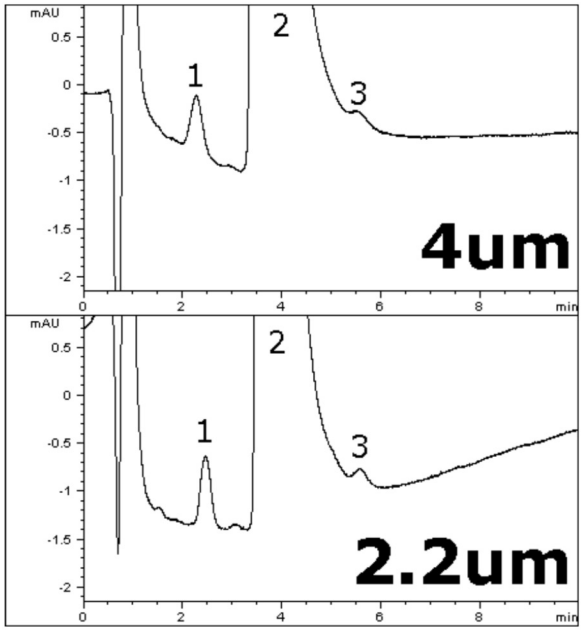


Figure 2

Not only is the ethinyl estradiol peak sharper on the 2.o™ phase, we can also see an impurity peak eluting after the main peak. This peak may be overlooked on the 4µm column, but on the 2.o™ phase it is better resolved due to less tailing from the main peak.

Conclusion

The separation of drospirenone and ethinyl estradiol in a tablet shows a number of advantages for use of 2.2µm particles compared to standard 4µm phases. The peaks are sharper, leading to a stronger signal for detection of low- level components. Reduced tailing of the drospirenone peak led to better resolution of a small impurity peak.

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