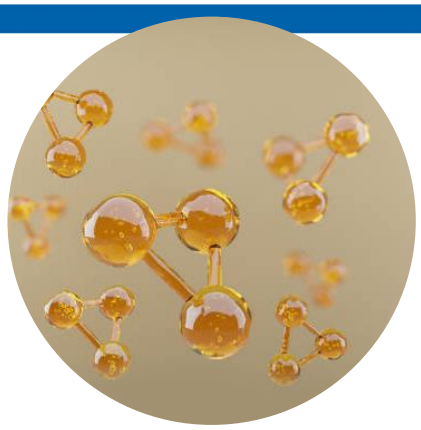


Ph. Eur. Monograph 1593: Megestrol Acetate Assay and Related Substances on Luna™ Omega 3 µm C18 Column

Grace Guo[1], Bryan Tackett, PhD[1], and Heiko Behr, PhD[2]
[1]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

Overview

Megestrol Acetate is a progestin hormone that is used for anorexia, cachexia, or serious unexplained weight loss.

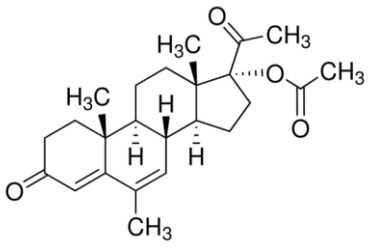
In this application note we show the separation of Megestrol Acetate from its related substances following Ph. Eur. Monograph 1593. We used a Luna Omega 3 µm C18 column and compared it to the Intersil 3 µm ODS-3 column originally used in the monograph. System suitability per Ph. Eur. Monograph for Megestrol Acetate is the same for both Assay and Related Substances: Minimum peak-to-valley ratio of 5.0 between the peak due to Impurity A and the peak due to Impurity D for reference solution (b). The peak-to-valley ratio is defined as H_p/H_v, where H_p = height above the baseline of the peak due to Impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity A.

The Luna Omega 3 µm C18 column used for this study met the system suitability criteria for Assay and Related Substances according to Ph. Eur. monograph 1593 for Megestrol Acetate. The Luna Omega C18 column provided a superior H_p/H_v ratio relative to the Inertsil ODS-3 column under the monograph conditions. Interestingly, an unspecified impurity eluted next to Impurity I but was only observed at 245 nm in Reference Solutions (b) and (c), which were used to identify the impurities. This unknown impurity was observed on each column used. A blank injection did not contain this unknown impurity, suggesting that it was an impurity present in the reference standards used to prepare the Reference Solutions.

All reference solutions were prepared as indicated in Ph. Eur. monograph 15933 for Megestrol Acetate. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0001514, Megestrol Acetate CRS
- Y0001507, Megestrol Acetate for Peak Identification CRS
- Y0001524, Megestrol Acetate for System Suitability CRS
- Y0001594, Megestrol Acetate for Impurity K Identification CRS

Figure 1. Megestrol Acetate



LC-UV Conditions

Columns: Luna Omega 3 µm C18 (00F-4784-E0)
Inertsil® 3 µm ODS-3
Dimensions: 150 x 4.6 mm
Mobile Phase: Mobile Phase (**Table 1**)
Gradient:

Time (min)	%B
0	30
16	30
42	70
49	70

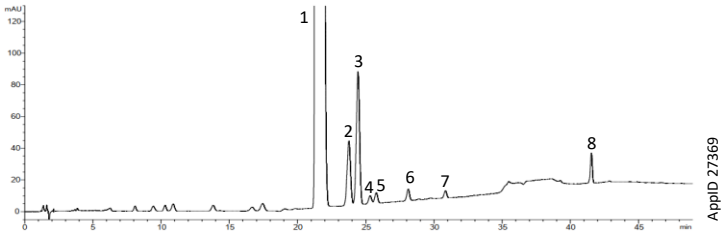
Flow Rate: 1.0 mL/min
Injection: 20 µL of Test Solution (a) and Reference Solutions (a), (b), (c), and (e)
Temperature: 40 °C
Detector: UV @ 245 nm and 210 for Impurity J
System: Agilent® 1260

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	A: Tetrahydrofuran R / Acetonitrile R1 / Water R (7.5:12.5:80, v/v/v) B: Water R / Tetrahydrofuran R / Acetonitrile R1 (20:30:50, v/v/v)
Solvent Mixture	Acetic Acid R / Water R / Acetonitrile R1 (0.1:20:80, v/v/v)
Test Solution (a)	Dissolve 0.100 g of Megestrol Acetate CRS in the Solvent Mixture and dilute to 10.0 mL with Solvent Mixture.
Test Solution (b)	Dissolve 50.0 mg of Megestrol Acetate CRS in the Solvent Mixture and dilute to 50.0 mL with the Solvent Mixture.
Reference Solution (a)	Dilute 1.0 mL of Test Solution (a) to 100.0 mL with the Solvent Mixture. Dilute 1.0 mL of this solution to 10.0 mL with the Solvent Mixture.
Reference Solution (b)	Dissolve 10 mg of Megestrol Acetate for System Suitability CRS (containing Impurities A, D, G, H, I, J, and L) in 1.0 mL of the Solvent Mixture.
Reference Solution (c)	Dissolve 10 mg of Megestrol Acetate for Peak Identification CRS (containing Impurities B, C, and E) in 1.0 mL of the Solvent Mixture.
Reference Solution (d)	Dissolve 50.0 mg of Megestrol Acetate CRS in the Solvent Mixture and dilute to 50.0 mL with the Solvent Mixture.
Reference Solution (e)	Dissolve the contents of a vial of Megestrol Acetate for Impurity K Identification CRS in 1.0 mL of the Solvent Mixture.

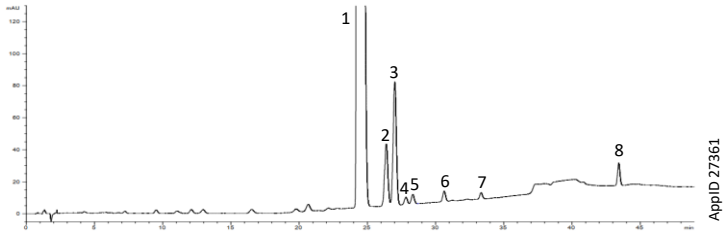
Figure 2. System Suitability Test for Assay and Related Substances Using Reference Solution (b), UV at 245 nm

Luna™ Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H_p/H_v
1	Megestrol Acetate	21.40	68741.1	2742.4	0.304	-
2	Impurity D	23.76	718.4	40.8	1.054	31.4
3	Impurity A	24.43	1389.4	83.8	1.039	
4	Unknown Impurity	25.33	79.3	5	1.073	-
5	Impurity I	25.77	102.3	6.7	1.067	-
6	Impurity G	28.11	104.4	7.3	1.072	-
7	Impurity H	30.84	62.3	4.7	1.07	-
8	Impurity L	41.54	201.9	19	1.215	-

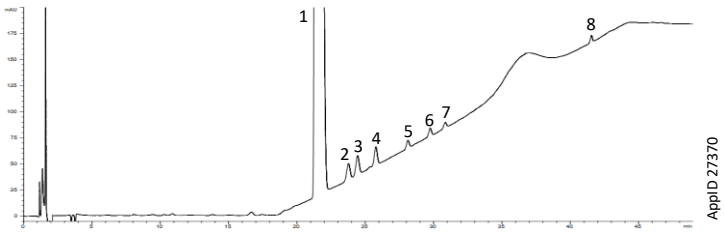
Inertsil® 3 µm ODS-3 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H_p/H_v
1	Megestrol Acetate	24.35	63244.2	2761	0.435	-
2	Impurity D	26.39	652.4	38.8	0.99	19.4
3	Impurity A	27.01	1260.6	77.1	0.974	
4	Unknown Impurity	27.83	70.6	4.6	1	-
5	Impurity I	28.33	93.2	6	0..982	-
6	Impurity G	30.63	96.3	6.6	0.986	-
7	Impurity H	33.34	56.6	4.1	0.972	-
8	Impurity L	43.43	173.2	14	0.956	-

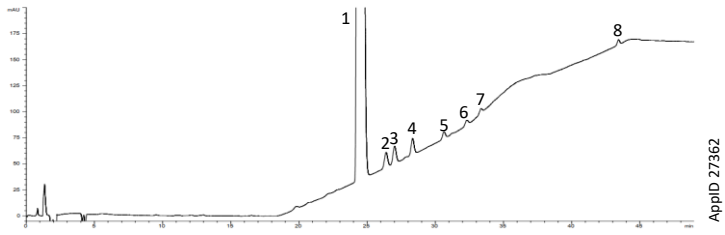
Figure 3. System Suitability Test for Assay and Related Substances Using Reference Solution (b), UV at 210 nm

Luna Omega 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H_p/H_v
1	Megestrol Acetate	21.40	65650.6	2400.8	0.31	-
2	Impurity D	23.77	263.6	15.4	1.15	31.4
3	Impurity A	24.44	298	18.2	1.08	
4	Impurity I	25.77	286	17.4	1.33	-
5	Impurity G	28.12	105.1	7.5	1.26	-
6	Impurity J	29.75	114.2	7.7	1.38	-
7	Impurity H	30.85	74.5	5.1	1.56	-
8	Impurity L	41.55	69.1	6.7	1.27	-

Inertsil 3 µm ODS-3 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	H_p/H_v
1	Megestrol Acetate	24.35	59294.6	2432.4	0.41	-
2	Impurity D	26.39	242	14.6	1.06	19.4
3	Impurity A	27.01	268.8	16.7	1.02	
4	Impurity I	28.33	233.8	15.4	1.01	-
5	Impurity G	30.64	92.7	6.5	1.21	-
6	Impurity J	32.30	69.6	4.7	1.02	-
7	Impurity H	33.34	61.5	4.4	1.00	-
8	Impurity L	43.44	61.1	4.9	1.26	-

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com