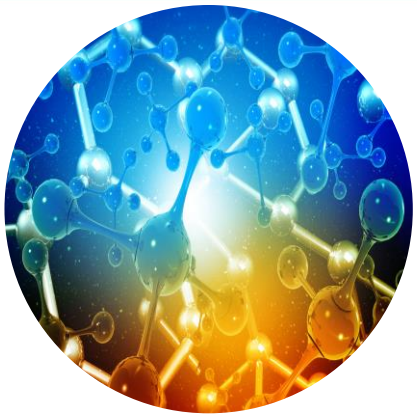


Ph. Eur. Monograph 1075: Budesonide Related Substances and Epimer A

Zandra Baja[1], Zeshan Aqeel[1], Bryan Tackett, PhD[1], James Turner[3], and Heiko Behr, PhD[2]
[1]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany
[3]Phenomenex Ltd., Queens Ave., Hurdsfield Ind. Est., Macclesfield, SK10 2BN, UK

Overview

Budesonide is a corticosteroid that prevents swelling and is used to treat several different lung diseases.

In this application note we demonstrate the effective identification and separation of Budesonide from its related substances following Ph.Eur. Monograph 2232. To meet system suitability requirements for Related Substances Assay, the peak-to-valley ratio must be a minimum of 2.5 between the first peak due to Impurity G and Budesonide Epimer A for reference solution (b). The peak-to-valley ratio is defined as Hp/Hv, where Hp = height above the baseline of first peak due to Impurity G and Hv = height above the baseline of the lowest point of the curve separating this peak from the peak due to Budesonide Epimer A. Also, the peak-to-valley ratio must be a minimum of 3 between the peak due to Impurity L and Budesonide Epimer B for reference solution (b). The peak-to-valley ratio is defined as Hp/Hv, where Hp = height above the baseline of the peak due to Impurity L and Hv = height above the baseline of the lowest point of the curve separating this peak from the peak due to Budesonide Epimer B.

To meet system suitability requirements for Epimer A Assay, the resolution must be a minimum of 1.5 between the 2 principal peaks of Budesonide (Epimer A and B) for reference solution (c). Also, the peak-to-valley ratio must be a minimum of 3 between the first peak due to Impurity L and Epimer B for reference solution (b). The peak-to-valley ratio is defined as Hp/Hv, where Hp = height above the baseline of the peak due to Impurity L and Hv = height above the baseline of the lowest point of the curve separating this peak from the peak due to Budesonide Epimer B.

We used the fully porous Luna 3 µm C18(2) column and compared it to the Hyperclone ODS 3 µm C18 column. The Luna C18(2) column showed a higher peak-to-valley ratio in Reference Solution (b) for the Related Substances Assay of Impurity L compared to the Hyperclone C18 column (12 vs 6.4). However, the Hyperclone C18 column, having a Type A silica, was able to separate Impurity G to a greater extent (34 vs 21). In the Epimer A Assay, the Hyperclone C18 column was able to separate Impurity L in Reference Solution (b) to a greater extent than the Luna C18(2) column (6.7 vs 4.4), as well as the Epimers of Budesonide in Reference Solution (c) with resolutions of 1.95 and 1.57, respectively. Both columns met all system suitability requirements.

All reference solutions were prepared as indicated in Ph. Eur. monograph 1075 for Budesonide. 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):

- B1157300, Budesonide CRS
- Y0001148, Budesonide for System Suitability CRS

LC-UV Conditions

Columns: Luna™ 3 µm C18(2) (00F-4251-E0)
Hyperclone™ 3 µm C18 (00F-4356-E0)

Dimensions: 150 x 4.6 mm

Mobile Phase: Mobile Phase (**Table 1**)

Gradient: **Related Substances Assay:**

Time (min)	%B
0	0
38	0
50	100
60	100

Epimer A Assay:

Time (min)	%B
0	0
21	0
22	100
31	100

Flow Rate: 1.0 mL/min

Injection: 20 µL

Temperature: 50 °C

Detector: UV @ 240 nm

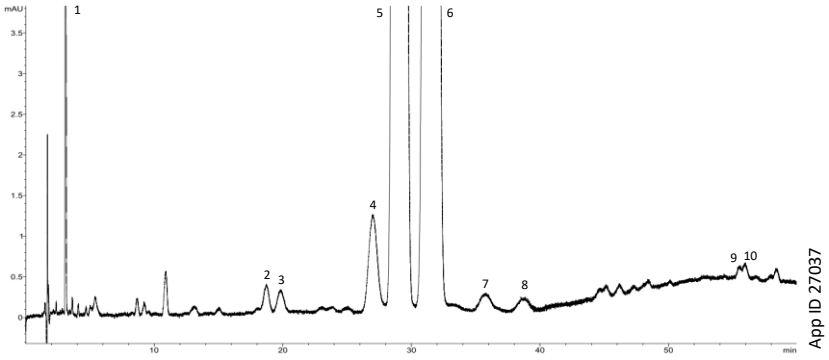
System: Agilent® 1260 Binary UHPLC

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	A: Anhydrous Ethanol / Acetonitrile / Phosphate Buffer Solution, pH 3.2 R (2:32:68, v/v/v) B: Acetonitrile / Phosphate Buffer Solution, pH 3.2 R (50:50, v/v)
Solvent Mixture	Acetonitrile / Phosphate Buffer Solution, pH 3.2 R (32:68, v/v)
Test Solution A	Dissolve 50 mg of Budesonide CRS in 15 mL of Acetonitrile, then dilute to 50 mL with Phosphate Buffer solution, pH 3.2 R
Test Solution B	Dissolve 25 mg of Budesonide CRS in 15 mL of Acetonitrile, then dilute to 50 mL with Phosphate Buffer solution, pH 3.2 R
Reference Solution (a)	Dilute 1.0 mL of **Test Solution A** to 10 mL with **Solvent Mixture**, then dilute 1.0 mL of this solution to 100 mL with **Solvent Mixture**
Reference Solution (b)	Dissolve 5 mg of Budesonide for System Suitability CRS (containing impurities A, D, G, K, and L)in 1.5 mL of Acetonitrile, then dilute to 5 mL with Phosphate Buffer solution, pH 3.2 R
Reference Solution (c)	Same as **Test Solution B**

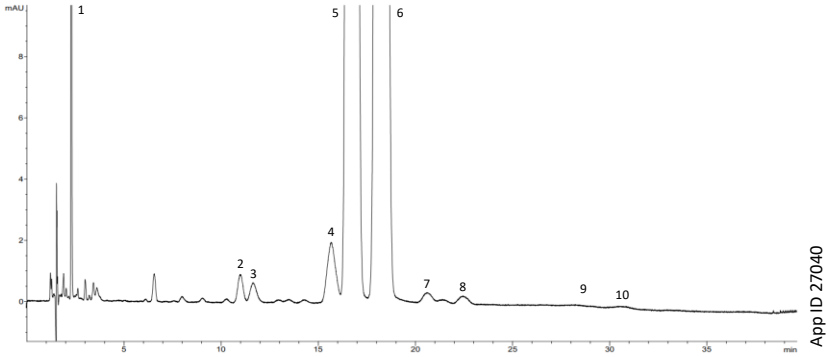
Figure 1. Related Substances Assay Reference Solution (b)

Luna™ 3 µm C18(2) Column



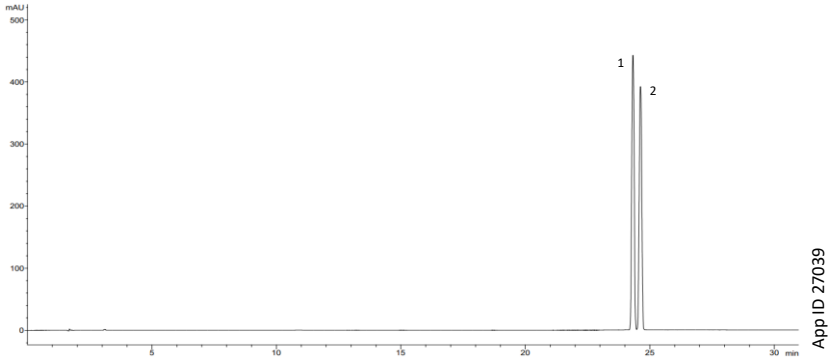
Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Hp/Hv
1	Impurity A	3.102	69.8	17.3	1.018	-
2	Impurity D Epimer 1	18.715	10.1	0.340	1.001	-
3	Impurity D Epimer 2	19.847	9.3	0.280	0.969	-
4	Impurity L	27.005	52.8	1.2	1.004	12
5	Budesonide Epimer B	29.084	5280.4	115.7	1.064	
6	Budesonide Epimer A	31.54	5317.8	105.5	1.035	21
7	Impurity G Epimer 1	35.761	12.5	0.220	0.811	
8	Impurity G Epimer 2	38.828	9.5	0.180	0.411	-
9	Impurity K Epimer 1	55.496	3.7	0.160	0.868	-
10	Impurity K Epimer 2	55.988	4.4	0.200	1.13	-
N = 3 Injections						

Hyperclone™ ODS 3 µm C18 Column



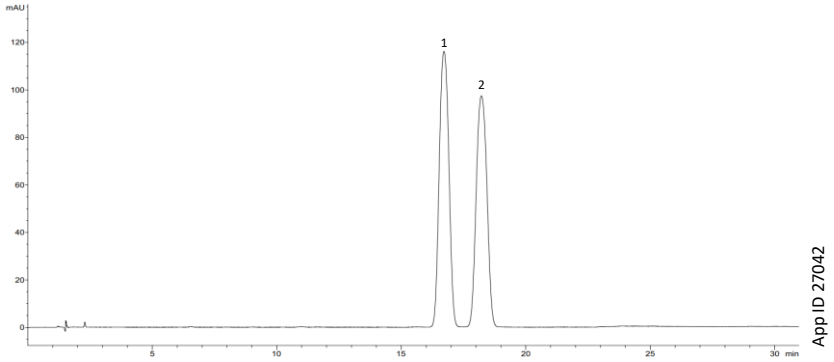
Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Hp/Hv
1	Impurity A	2.293	71.6	23.7	0.933	-
2	Impurity D Epimer 1	10.991	18.1	0.940	0.957	-
3	Impurity D Epimer 2	11.652	16.1	0.670	0.868	-
4	Impurity L	15.663	58.1	2	0.916	6.4
5	Budesonide Epimer B	16.723	5457.9	205.2	0.959	
6	Budesonide Epimer A	18.232	5503.3	185.8	0.93	34
7	Impurity G Epimer 1	20.619	10.8	0.340	0.727	
8	Impurity G Epimer 2	22.443	9.6	0.280	0.521	-
9	Impurity K Epimer 1	28.471	4.9	0.073	0.654	-
10	Impurity K Epimer 2	30.681	4.7	0.086	0.383	-
N = 3 Injections						

Luna™ 3 µm C18(2) Column



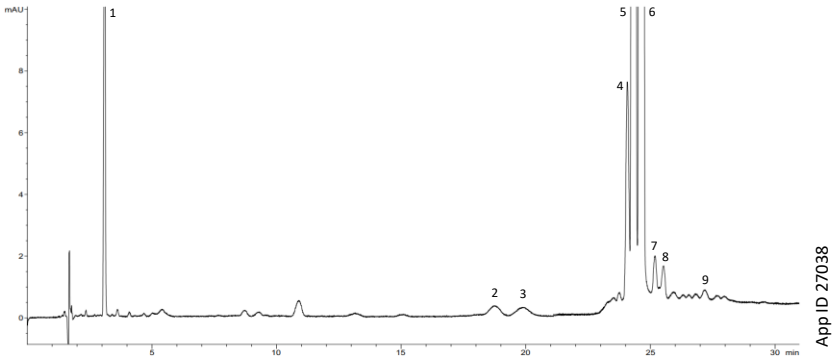
Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Resolution
1	Budesonide Epimer B	24.32	3017.93	446.3	0.99	1.57
2	Budesonide Epimer A	24.62	2813.53	395.67	0.99	
N = 3 Injections						

Hyperclone™ ODS 3 µm C18 Column



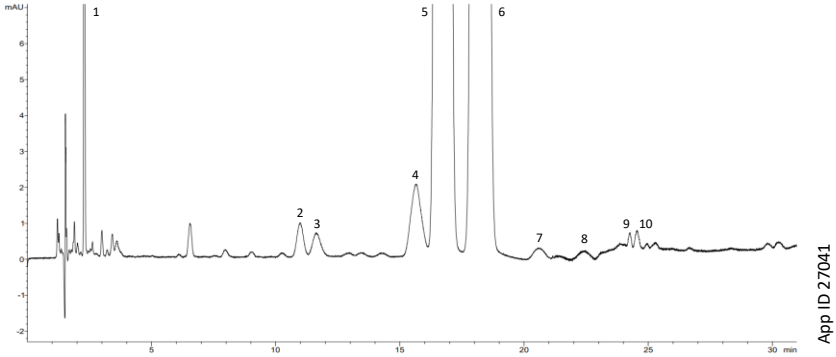
Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Resolution
1	Budesonide Epimer B	16.72	3117.53	117.53	0.95	1.95
2	Budesonide Epimer A	18.23	2909.67	98.87	0.93	
N = 3 Injections						

Luna™ 3 µm C18(2) Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Hp/Hv
1	Impurity A	3.098	70.2	17	1.024	-
2	Impurity D Epimer 1	18.766	9.3	0.320	1.032	-
3	Impurity D Epimer 2	19.874	10.2	0.290	0.882	-
4	Impurity L	24.074	55.9	7	0.907	4.4
5	Budesonide Epimer B	24.327	5195.8	729.8	0.981	
6	Budesonide Epimer A	24.633	5228.5	696.6	0.926	-
7	Impurity G Epimer 1	25.186	10.8	1.2	1.038	-
8	Impurity G Epimer 2	25.531	10.7	1	1.731	-
9	Impurity K	27.194	4.3	0.320	1.199	-
N = 3 Injections						

Hyperclone™ ODS 3 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Height	Symmetry Factor	Hp/Hv
1	Impurity A	2.292	71.4	24	0.932	-
2	Impurity D Epimer 1	10.997	18	0.960	1.155	-
3	Impurity D Epimer 2	11.617	15.5	0.680	0.684	-
4	Impurity L	15.642	59.4	2	0.887	6.7
5	Budesonide Epimer B	16.712	5500.8	209.4	0.968	
6	Budesonide Epimer A	18.217	5548.1	189.8	0.934	-
7	Impurity G Epimer 1	20.571	9.1	0.320	0.789	-
8	Impurity G Epimer 2	22.483	7.7	0.270	1.54	-
9	Impurity K Epimer 1	24.268	2.9	0.390	1.371	-
10	Impurity K Epimer 2	24.541	5.3	0.490	0.751	-
N = 3 Injections						

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Austria
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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
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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
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United Kingdom
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