Ph. Eur. Monograph 2733: Escitalopram Oxalate Related Substances on Luna® 5 µm C18(2) and Luna Omega 5 µm C18

Janja Vasilić[1], Amra Perva Uzunalić[1], Dirk Hansen[2] and Heiko Behr[2]
[1]University of Maribor, Faculty of Chemistry and Chemical Engineering Group for Separation Analysis, Smetanova 17, 2000 Maribor, Slovenia
[2]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

Overview

Escitalopram Oxalate is mainly used for the treatment of major depressive disorder or generalized anxiety disorder. It is administered orally.

In this application note we are showing the effective separation of Escitalopram Oxalate from its impurities and related substances according to Ph. Eur. Monograph 2733. The fully porous Luna 5 µm C18(2) and Luna Omega 5 µm C18 columns met the system suitability requirement for the peak-to-valley ratio between Escitalopram and impurity D (≥ 5.0). Both columns provided a baseline separation between Escitalopram and impurity D. Finally, we are showing the consistency in analytical performance of Luna Omega 5 µm C18 across columns from multiple media batches.

All reference solutions were prepared as indicated in Ph.Eur. monograph 2733 for Escitalopram Oxalate. 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):

- Y0001796 Escitalopram Oxalate CRS
- Y0001797 Escitalopram for system suitability CRS

Experimental

Table 1: Preparation of buffer solution, mobile phase solutions, test and reference solutions

Solution	Composition
Buffer solution	Dissolve 3.4 g KH_2PO_4 in 900 mL water for chromatography. Adjust with phosphoric acid to pH 3.0. Dilute to 1000 mL with water for chromatography.
Mobile phase A	Acetonitrile:**Buffer solution** 10:90 (V/V)
Mobile phase B	**Buffer solution**:Acetonitrile 35:65 (V/V)
Test solution	Dissolve 25 mg of the substance to be examined (for this study: Escitalopram oxalate CRS) in mobile phase A and dilute to 50.0 mL with mobile phase A.
Reference solution (a)	Dilute 5 mg of Escitalopram for system suitability CRS (containing impurity D) in mobile phase A and dilute to 10.0 mL with mobile phase A.
Reference solution (b)	Dilute 1.0 mL of the **test solution** to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

LC-UV Conditions

Columns: Luna 5 µm C18(2) (00G-4252-E0)
Luna Omega 5 µm C18 (00G-4785-E0)
Dimension: 250 x 4.6 mm
Mobile Phase: A = Acetonitrile:**Buffer solution** 10:90 (V/V)
B = **Buffer solution**:Acetonitrile 35:65 (V/V)

Gradient:

Time (min)	%B	Flow Rate (mL/min)
0	5	1.0
2	5	1.0
37	35	1.0
47	100	1.0
47.1	100	2.0
62	100	2.0
62.1	5	1.0
70	5	1.0

Injection: 20 µL
Temperature: 45 °C
Detector: UV @ 237 nm and 254 nm
System: Agilent® 1260 Infinity II

Chromatographic Results

Figure 1: Reference solution (a) on Luna® 5 µm C18(2)

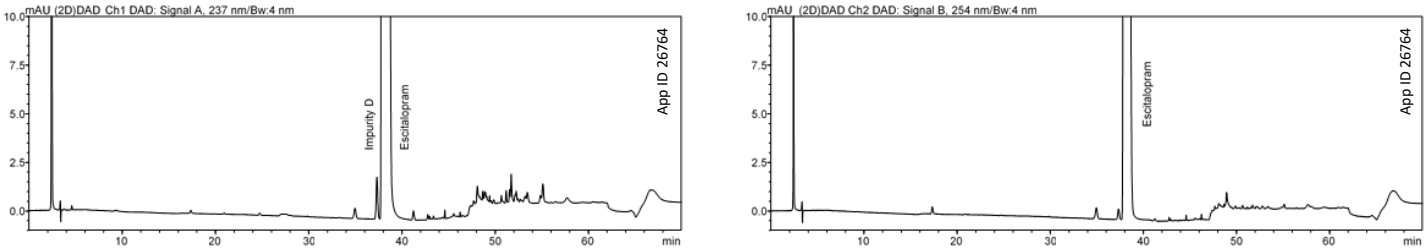

Table 2: Results reference solution (a) on Luna 5 µm C18(2) at 237 nm and 254 nm

No.	Escitalopram Rt (min) at 237 nm	Peak area Escitalopram at 237 nm	Resolution factor (Escitalopram/Impurity D) at 237 nm	Hp/Hv at 237 nm	Impurity D Rt (min) at 254 nm	Impurity D RRt at 254 nm	Peak area impurity D at 254 nm
1	37.917	21090773	1.063	-*	37.331	0.985	25176
2	37.912	21120286	1.057	-*	37.327	0.985	25314
3	37.915	21135675	1.059	-*	37.331	0.985	25119
4	37.925	21132248	1.058	-*	37.341	0.985	24968
5	37.918	21116763	1.060	-*	37.332	0.985	25127
6	37.918	21068527	1.063	-*	37.332	0.985	25143
Average	37.917	21110712	1.06	-*	37.332	0.985	25141
% RSD	0.011	0.12	0.24	-*	0.012	0.003	0.44

*No Hp/Hv ratio could be calculated because of baseline separation between Escitalopram and impurity D.

Figure 2: Reference solution (b) on Luna 5 µm C18(2)

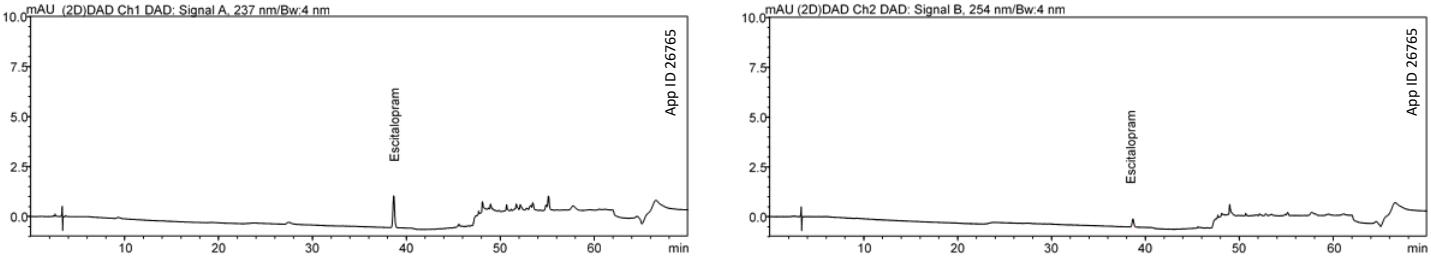

Table 3: Results reference solution (b) on Luna 5 µm C18(2) at 237 nm and 254 nm

No.	Rt (min) at 237 nm	Area of Escitalopram peak at 237 nm	Tailing factor (T) at 237 nm	Rt (min) at 254 nm	Area of Escitalopram peak at 254nm	Tailing factor (T) at 254 nm
1	38.677	20348	1.064	38.681	4778	1.000
2	38.667	20219	1.053	38.669	4912	1.090
3	38.653	20285	1.033	38.654	4951	1.030
4	38.643	20425	1.040	38.642	4701	0.973
5	38.628	20253	1.041	38.629	5020	1.113
6	38.611	20409	1.069	38.601	4863	1.082
Average	38.646	20323	1.050	38.646	4871	1.048
% RSD	0.063	0.42	1.39	0.075	2.4	5.3

Figure 3: Test solution on Luna® 5 µm C18(2)

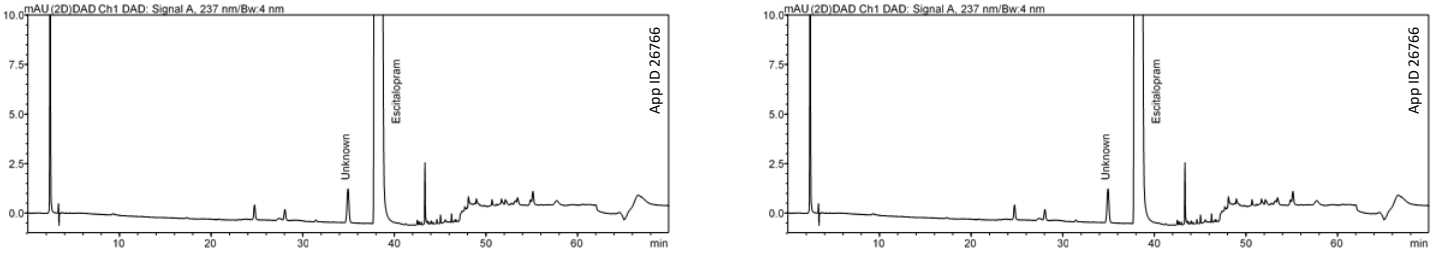

Table 4: Results test solution on Luna 5 µm C18(2) at 237 nm and 254 nm

No.	Escitalopram Rt (min) at 237 nm	Peak area Escitalopram at 237 nm	Unknown Rt (min) at 254 nm	Unknown RRt at 254 nm	Peak area unknown at 254 nm
1	37.928	20558317	34.957	0.922	25174
2	37.935	20551239	34.959	0.922	25671
3	37.925	20552239	34.950	0.922	25841
4	37.924	20550636	34.950	0.922	25997
5	37.907	20549039	34.937	0.922	26402
6	37.906	20546436	34.929	0.921	26634
Average	37.921	20551318	34.947	0.922	25953
% RSD	0.031	0.019	0.034	0.008	2.015

Figure 4: Reference solution (a) on Luna Omega 5 µm C18

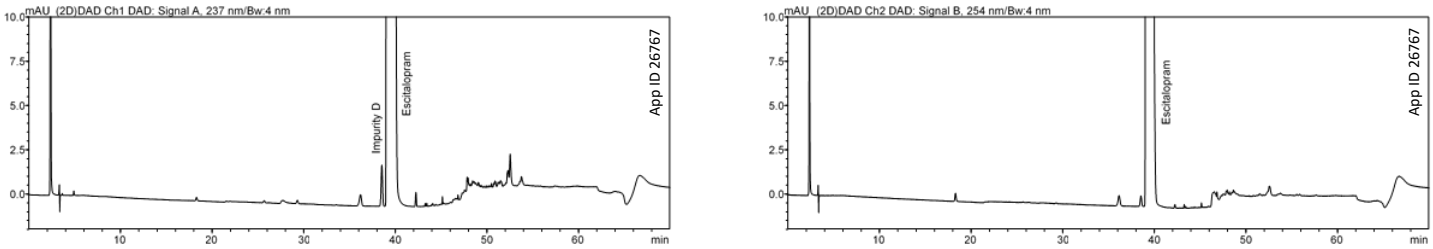

Table 5: Results Reference solution (a) on Luna Omega 5 µm C18 at 237 nm and 254 nm

No.	Escitalopram Rt (min) at 237 nm	Peak area Escitalopram at 237 nm	Resolution factor (Escitalopram/Impurity D) at 237 nm	Hp/Hv at 237 nm	Impurity D Rt (min) at 254 nm	Impurity D RRt at 254 nm	Peak area impurity D at 254 nm
1	39.103	20949728	0.955	-*	38.548	0.986	25058
2	39.092	20940230	0.954	-*	38.538	0.986	25220
3	39.076	20963085	0.952	-*	38.522	0.986	25222
4	39.100	20977182	0.953	-*	38.546	0.986	25077
5	39.102	20959523	0.953	-*	38.548	0.986	25039
6	39.133	20953808	0.955	-*	38.579	0.986	24995
Average	39.101	20957259	0.954	-*	38.547	0.986	25102
% RSD	0.048	0.060	0.128	-*	0.048	0.001	0.382

*No Hp/Hv ratio could be calculated because of baseline separation between Escitalopram and impurity D.

Figure 5: Reference solution (b) on Luna® Omega 5 µm C18

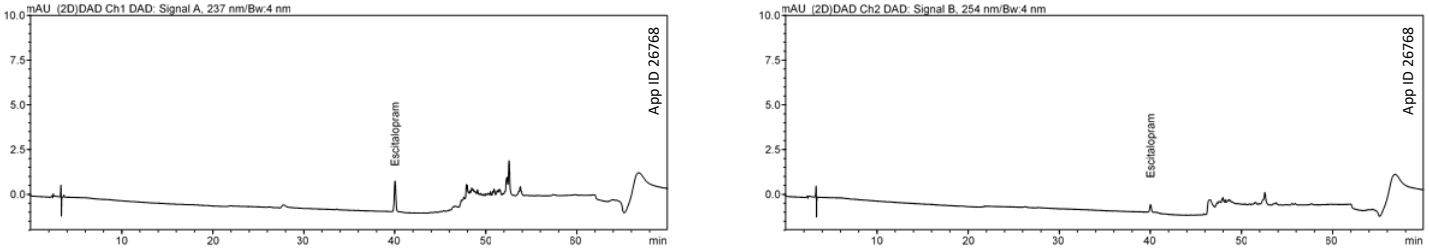

Table 6: Results reference solution (b) on Luna Omega 5 µm C18 at 237 nm and 254 nm

No.	Rt (min) at 237 nm	Area of Escitalopram peak at 237 nm	Tailing factor (T) at 237 nm	Rt (min) at 254 nm	Area of Escitalopram peak at 254nm	Tailing factor (T) at 254 nm
1	40.029	20360	1.025	40.025	4886	1.075
2	40.028	20331	1.024	40.031	4899	1.057
3	40.029	20346	1.009	40.030	4798	1.040
4	40.023	20298	1.023	40.026	4916	1.037
5	40.019	20455	1.024	40.023	4918	0.935
6	40.010	20261	1.038	40.005	4797	1.046
Average	40.023	20342	1.024	40.023	4869	1.032
% RSD	0.019	0.32	0.89	0.023	1.2	4.8

Figure 6: Test solution on Luna Omega 5 µm C18

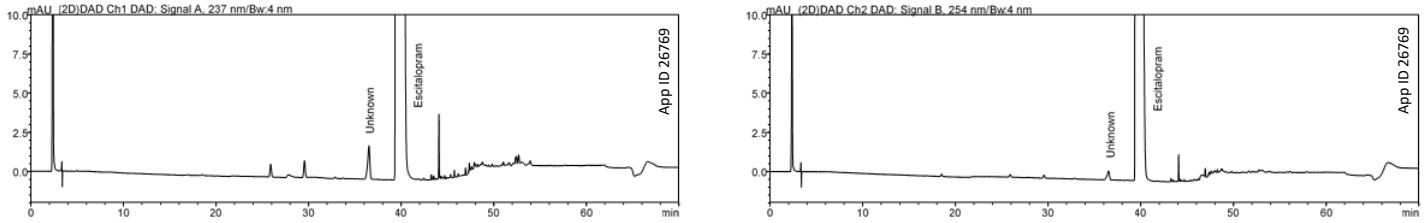

Table 7: Results Test solution on Luna Omega 5 µm C18 at 237 nm and 254 nm

No.	Escitalopram Rt (min) at 237 nm	Peak area Escitalopram at 237 nm	Unknown Rt (min) at 254 nm	Unknown RRt at 254 nm	Peak area unknown at 254 nm
1	39.434	20770725	36.539	0.927	33943
2	39.419	20745765	36.523	0.927	33187
3	39.391	20739482	36.496	0.927	31757
4	39.388	20748513	36.491	0.926	32421
5	39.376	20718286	36.483	0.927	32994
6	39.399	20681838	36.499	0.926	32724
Average	39.401	20734101	36.505	0.927	32838
% RSD	0.054	0.15	0.058	0.008	2.2

Figure 7: Overlap Reference solution (a) on 3 Luna® Omega 5 µm C18 columns from 3 different batches at 237 nm

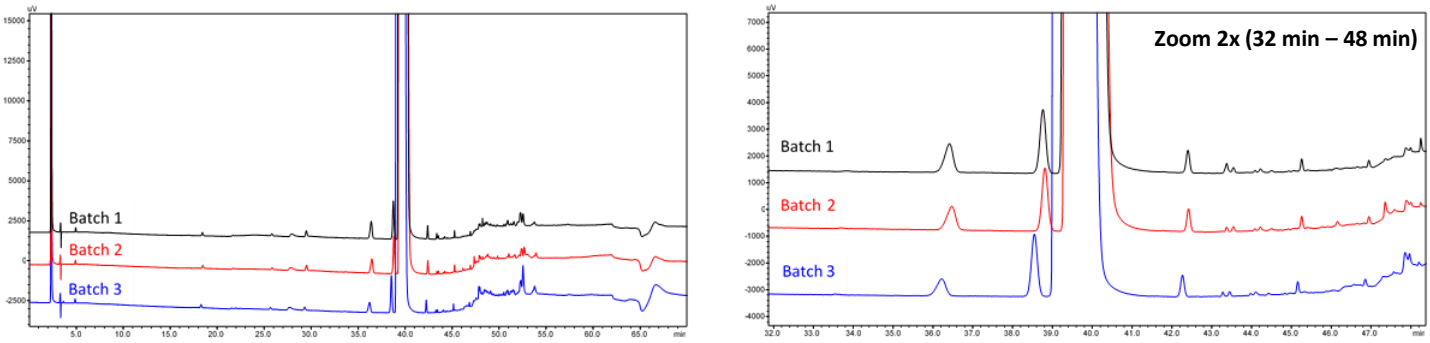

Table 8: Results reference solution (a) on 3 Luna Omega 5 µm C18 columns from 3 different batches at 237 nm

No.	Batch 1			Batch 2			Batch 3		
	Escitalopram Rt (min)	Impurity D Rt (min)	Resolution	Escitalopram Rt (min)	Impurity D Rt (min)	Resolution	Escitalopram Rt (min)	Impurity D Rt (min)	Resolution
1	39.314	38.763	0.956	39.366	38.814	0.953	39.103	38.548	0.955
2	39.309	38.759	0.956	39.310	37.758	0.953	39.092	38.538	0.954
3	39.309	38.757	0.959	39.321	38.769	0.952	39.076	38.522	0.952
4	39.304	38.753	0.958	39.320	38.767	0.956	39.100	38.546	0.953
5	39.298	38.747	0.957	39.305	38.755	0.953	39.102	38.548	0.953
6	39.267	38.716	0.958	39.308	38.757	0.953	39.133	38.579	0.955
Average	39.300	38.749	0.956	39.322	38.770	0.953	39.101	38.547	0.954
%RSD	0.044	0.044	0.11	0.057	0.057	0.13	0.048	0.048	0.13

Conclusion

The Luna 5 µm C18(2) (**Figure 1** and **Table 2**) and Luna Omega 5 µm C18 (**Figure 4** and **Table 5**) columns tested in this study fulfilled the system suitability requirements of Ph. Eur. Monograph 2733. In addition, the high batch-to-batch reproducibility of the Luna Omega 5 µm C18 column has been verified by the comparison of separation results for 3 columns from 3 different batches (**Figure 7** and **Table 8**).

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/LiveChat.

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 5010 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: +65 800-852-3944
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
info@phenomenex.com

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

www.phenomenex.com

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