TN-1350

Driving More Sensitive and Selective Quantitation of Highly Potent Inhaled Corticosteroids in Human Plasma Using Accurate Mass Spectrometry

Rahul Baghla[1], Eshani Nandita, PhD[1], and Bryan Tackett, PhD[2]
[1]AB Sciex LLC, 500 Old Connecticut Path, Framingham, MA 01701, USA
[2]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Introduction

For long-term asthma control, inhaled corticosteroids are highly effective anti-inflammatory medications. Fluticasone Furoate, Fluticasone Propionate, and Mometasone Furoate are inhaled corticosteroids commonly used to treat allergic conditions such as asthma and allergic rhinitis.

Since the daily dose of inhaled corticosteroids is low, drug circulation in the blood occurs at low concentration levels. As a result, pharmacokinetic studies require highly sensitive and selective assays to quantify inhaled corticosteroids at pg/mL levels in biological matrices.

Here, a method to quantify Fluticasone Furoate, Fluticasone Propionate, and Mometasone Furoate using the Kinetex 2.6 µm EVO C18 column coupled to a ZenoTOF 7600 system is presented. The accurate mass spectrometer provides exceptional selectivity for accurate and precise drug quantitation in complex biological matrices.

This technical note presents a selective and sensitive approach to quantify the potent inhaled corticosteroids Fluticasone Furoate, Fluticasone Propionate, and mometasone Furoate in human plasma using a Kinetex EVO C18 column and accurate mass spectrometry. This method successfully achieved a lower limit of quantitation (LLOQ) of 1 pg/mL for Fluticasone Propionate and Fluticasone Furoate and 2 pg/mL for Mometasone Furoate in human plasma (**Figure 1**).

For further detailed discussion of the ZenoTOF 7600 system, refer to SCIEX technical note MKT-28776-A.

Sample Preparation

All 3 corticosteroids were spiked into 300 µL of human plasma at concentrations ranging from 1 to 1000 pg/mL. A 700 µL aliquot of 30 % (v/v) Methanol in water was added to the sample and vortexed. Samples were centrifuged at 9400 rcf for 5 minutes. The supernatant was extracted using Strata™-X 33 µm Polymeric Reversed Phase, 30 mg 96-well plates (Part No.: 8E-S100-TGB). The plates were conditioned with 1 mL of Methanol and 1 mL of water. Following sample loading, the plates were washed with 1 mL of water, 2 mL of 50% (v/v) Methanol in water and eluted with 1 mL of Acetonitrile. The eluent was dried under a nitrogen stream at 40 °C. The dried samples were reconstituted in water with 100 µL of 50% (v/v) Methanol in water. A 25 µL sample injection was used for analysis.

LC Conditions

Column: Kinetex™ 2.6 µm EVO C18
Dimensions: 150 x 3.0 mm
Part No.: 00B-4725-AN
Mobile Phase: A: 1 mM Ammonium Trifluoroacetate in Water
B: Methanol

Gradient:

Time (min)	Flowrate (mL/min)	%B
0.00	0.3	30
0.20	0.3	30
6.00	0.3	80
6.10	1.0	98
9.00	1.0	98
9.10	0.3	30
10.0	0.3	30

Injection Volume: 25 µL
Temperature: 50 °C
LC System: SCIEX® ExionLC™
Detection: TOF MS and MRM^{HR}
Detector: SCIEX ZenoTOF 7600

MS Conditions

	TOF MS	MRM^{HR}
Polarity:	Positive	Positive
Source Temperature:	600 °C	600 °C
GS1:	60 psi	60 psi
GS2:	65 psi	65 psi
CUR:	40 psi	40 psi
CAD:	9	9
IS:	5500 V	5500 V
Declustering Potential:	80 V	80 V
Precursor Ion:	N/A	501.2 m/z (Fluticasone Propionate)
	N/A	539.2 m/z (Fluticasone Furoate)
	N/A	521.2 m/z (Memetasone Furoate)
Start Mass:	100 m/z	100 m/z
Stop Mass:	600 m/z	600 m/z
Q1 Resolution:	N/A	Unit
Accumulation Time:	0.05 s	0.03 s
Collision Energy:	10 V	24 V
CE Spread:	0 V	0 V
Zeno Trap:	N/A	ON
ZOD Threshold (CID):	N/A	20,000 cps
Time Bins to Sum:	8	8

Revision: 0
PHEN-RUO-00212

Results and Discussion

Quantitation was performed using the Zeno MRM^{HR} mode on the ZenoTOF 7600 system. Here, the selected precursor ions were fragmented in Q2 and sent to the Zeno trap. The Zeno trap provides control of the ion beam from the collision cell into the TOF accelerator. All ions then arrive in the TOF accelerator at the same time and location, improving the overall MS/MS sampling efficiency. The overall MS/MS intensity is enhanced 4–25x compared to traditional MRM^{HR} workflows.

Figure 2 illustrates the sensitivity gains achieved using the Kinetex™ 2.6 µm EVO C18 column and Zeno MRM^{HR} for all 3 corticosteroids analyzed. Compared with conventional MRM^{HR}, Zeno MRM^{HR} enabled a 7x improvement in S/N ratio for Mometasone Furoate and Fluticasone Furoate and a 5.5x improvement in S/N ratio for Fluticasone Propionate. The Kinetex 2.6 µm EVO C18 column, based on core-shell particle technology, provides high chromatographic efficiency thereby generating narrower and taller peaks which results in an increased peak sensitivity leading to better peak shape and the increased S/N ratio.

The Zeno MRM^{HR} workflow is designed to gather all MS/MS information for each sample analyzed (**Figure 3**). The accessibility of the entire MS/MS spectrum can be advantageous as post-acquisition data decisions can be made on which measured fragments can be utilized for Zeno MRM^{HR}. For Zeno MRM^{HR}, quantitation can be performed using a single fragment ion or by summing multiple dominant fragment ions. When multiple highly abundant fragment ions are generated from the target analyte, summed XICs can further enhance assay sensitivity. Lower LLOQs were achieved using the summation approach compared to a single fragment ion for quantitation, as shown in **Figure 4**. Using summation of 2 highly abundant fragment ions, a 2x improvement in LLOQ was observed for Fluticasone Propionate and Fluticasone Furoate, while a 2.5x improvement in LLOQ was achieved for Mometasone Furoate (**Figure 4**).

A calibration curve was analyzed for concentrations ranging from 1 to 1000 pg/mL. To evaluate reproducibility, each concentration was analyzed in triplicate. The LLOQs achieved for Fluticasone Furoate, Fluticasone Propionate, and Mometasone Furoate in human plasma were accurately measured at 1 pg/mL, 1 pg/mL and 2 pg/mL, respectively. No interferences were observed in the blank matrix, as shown in **Figure 1**.

Linearity was achieved across a range of concentrations from 1 to 1000 pg/mL with correlations of determination (r^2) of 0.992, 0.992, and 0.993 for Fluticasone Propionate, Fluticasone Furoate, and Mometasone Furoate, respectively (**Figure 5**). A linear dynamic range (LDR) of 3 orders of magnitude was reached for all 3 corticosteroids analyzed.

Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80 % and 120 % at the LLOQ and between 85 % and 115 % at higher concentrations. To ensure a robust assay, the %CV of the calculated mean concentration should be below 20 % at the LLOQ and below 15 % at all higher concentrations.

The assay accuracy was within ±15 % of the nominal concentration and the %CV was <15 % (**Table 1**). The calculated percent accuracy and %CV values were within the acceptance criteria at each concentration level.

A 5 pg/mL matrix extracted sample was also analyzed in MRM mode using a SCIEX nominal mass spectrometer. Data were compared using the sum of 2 ions from Zeno MRM^{HR} with the single most intense transition and the sum of 2 ions using MRM on a SCIEX® nominal mass spectrometer.

Higher isobaric background interference was observed using the sum of 2 transitions on a SCIEX nominal mass spectrometer, as shown in **Figure 6**. As a result, a lower S/N ratio was observed when compared to using a single transition on a SCIEX nominal mass spectrometer. The peak-to-peak S/N ratio was determined by measuring the deviation in the baseline width compared to the height of the analyte peak.

Higher selectivity and S/N ratio were achieved using Zeno MRM^{HR} on the ZenoTOF 7600 system compared to both MRM experiments on a SCIEX nominal mass spectrometer (Figure 6).

Given the higher selectivity, the sum of multiple ions approach is more effective on the ZenoTOF 7600 system. Additionally, method development is more streamlined, as less ion path tuning is needed, and the user can access the full product ion profile.

Figure 1. Representative Extracted Ion Chromatograms for Fluticasone Propionate, Fluticasone Furaote, and Memtasone Furoate in Human Serum.

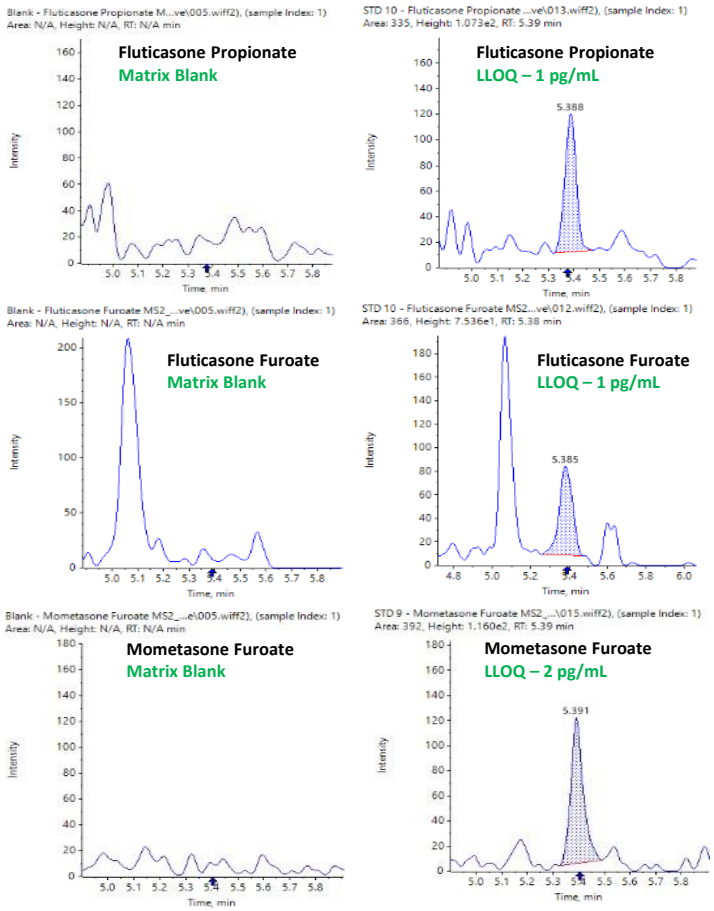


Figure 2. Chromatograms Showing Sensitivity Gains Based on S/N Ratio Using Zeno MRM^{HR} for All 3 Analyzed Corticosteroids.

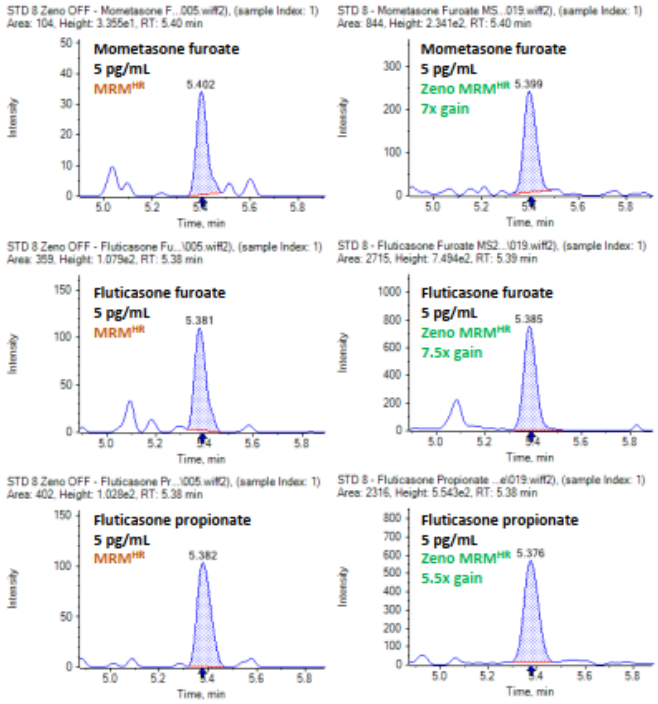


Figure 3. MS/MS Spectra for Fluticasone Propionate Using Zeno MRM^{HR}.

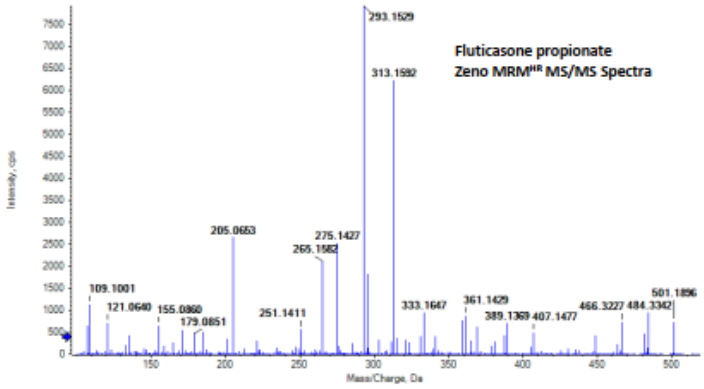


Table 1. Summary of the Quantitative Performance.

	Mometasone Furoate		Fluticasone Furoate		Fluticasone Propionate	
Concentration (pg/mL)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
1	N/A	N/A	3.43	98.3	6.20	96.7
2	14.3	99.8	9.18	105	8.25	108
5	6.26	104	8.77	98.8	3.52	98.9
20	6.69	86	5.37	85.5	4.32	86.8
40	1.81	100	4.23	107	3.22	102
100	1.75	96.9	1.66	95.2	2.54	95.0
250	1.40	105	2.50	103	0.43	103
500	4.24	107	2.67	105	1.38	104
1000	0.55	100	1.95	102	3.08	105

Figure 4. Representative Extracted Ion Chromatograms at the LLOQ Using the Sum of Multiple Ions Compared to a Single Most Intense Fragment Ion for All 3 Corticosteroids.

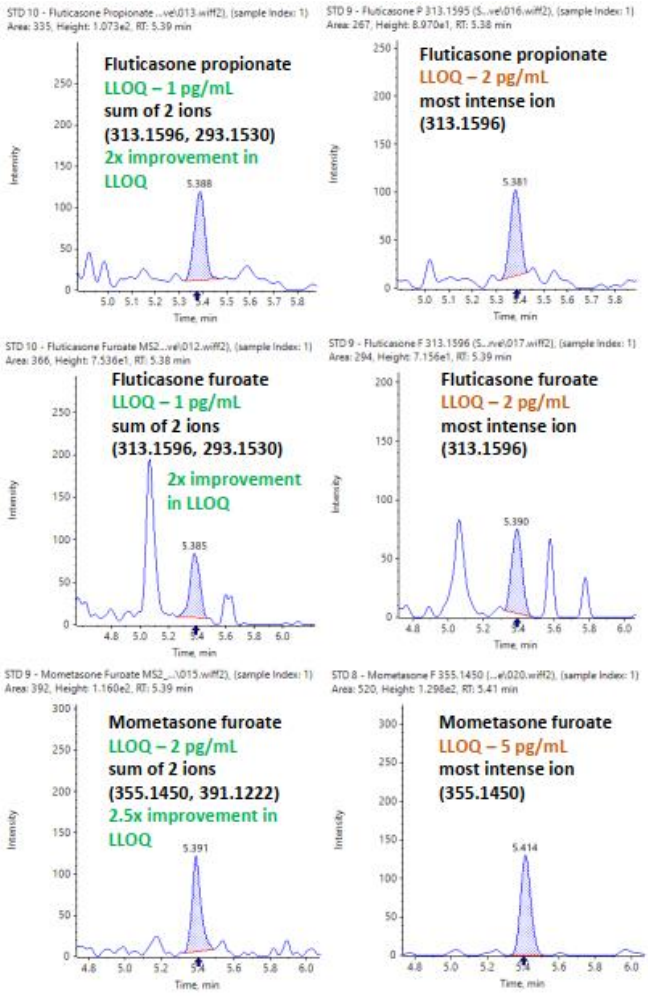


Figure 5. Calibration Curves for the Quantitation of Fluticasone Furoate, Fluticasone Propionate, and Mometasone Furoate in Human Plasma.

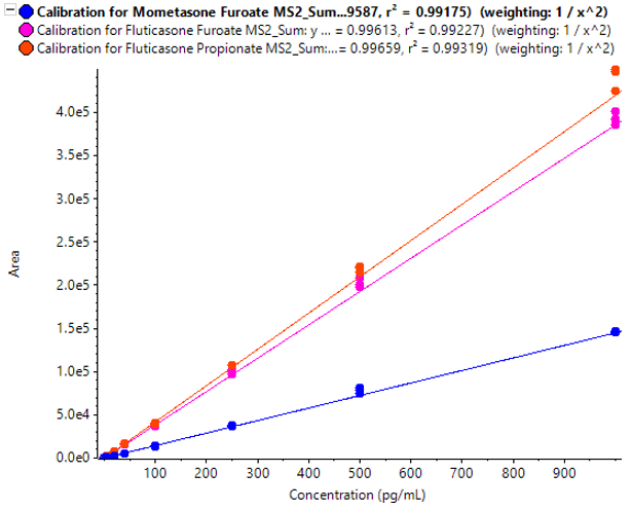
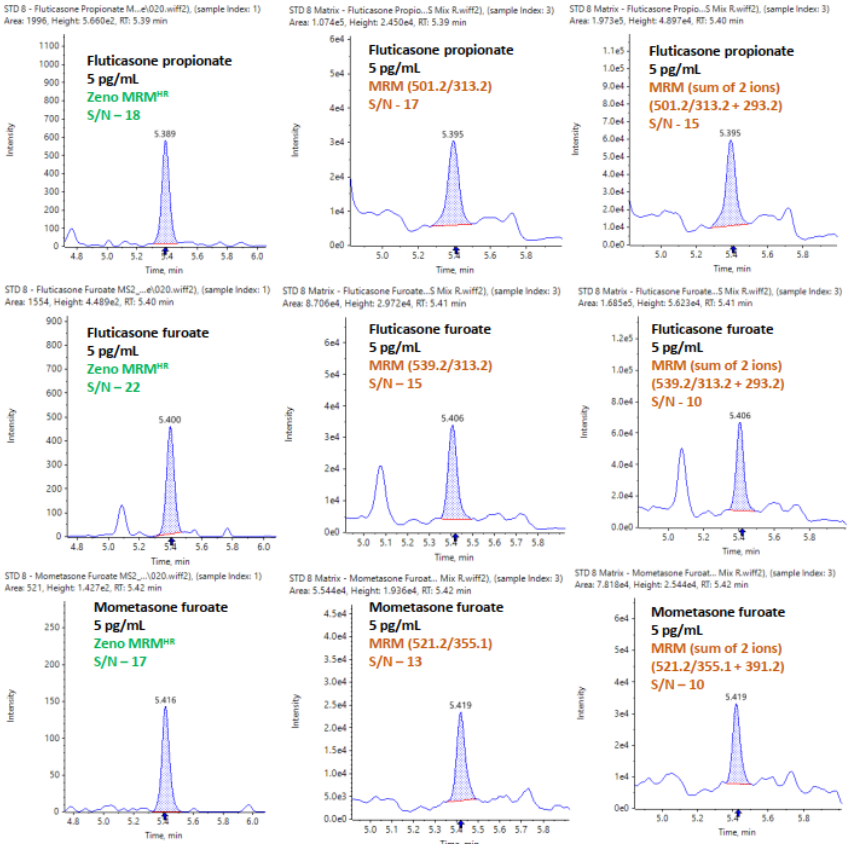


Figure 6. Representative Extracted Ion Chromatograms from a 5 pg/mL Sample of the Corticosteroid Analytes Analyzed Using Zeno MRM^{HR} and MRM.



Conclusions

LLOQs of 1 pg/mL, 1 pg/mL, and 2 pg/mL were achieved in human plasma for Fluticasone Furoate, Fluticasone Propionate, and Mometasone Furoate, respectively. High mass accuracy and resolution from an accurate mass spectrometer and the high efficiency and sensitivity of the core-shell Kinetex EVO C18 HPLC column yielded significant gains in selectivity and S/N ratio and reduced background noise compared to a nominal mass spectrometer. Linearity was achieved for the concentration range of 1 pg/mL to 1000 pg/mL with r^2 values of 0.992, 0.992, and 0.993 for Fluticasone Propionate, Fluticasone Furoate, and Mometasone Furoate, respectively. Method development time was reduced by using the Kinetex EVO C18 column and with less ion path tuning using MRM^{HR}-based quantitation. Increased data processing flexibility was achieved with access to the entire product ion profile. The method demonstrated accurate and highly reproducible (%CV <15 %) quantitative performance at all concentration levels.

Ordering Information

2.6 µm Midbore™ Columns (mm)						SecurityGuard™ ULTRA Cartridges (mm)‡
Phases	**30 x 3.0**	**50 x 3.0**	**75 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

for 3.0 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Strata™-X Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK**	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ**	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/b0x)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S100-HDG	12 mL (20/box)
	1 g	8B-S100-JDG	12 mL (20/box)
	1 g	8B-S100-JEG	20 mL (20/box)
	2 g	8B-S100-KEG	20 mL (20/box)
	5 g	8B-S100-LFF	60 mL (16/box)
Teflon® Tube			
	200 mg	8B-S100-FBJ-T	3 mL (50/box)
	200 mg	8B-S100-FDG-T	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S100-AGB	2 Plates/Box
	30 mg	8E-S100-TGB	2 Plates/Box
	60 mg	8E-S100-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S100-4GA	ea

**Tab-less tubes available. Contact Phenomenex for details.

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