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HPLC Separation of Metformin and Seven Combination Drugs on Kinetex® 5 µm EVO C18

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Introduction

Metformin is a generic prescription medication widely used in the treatment of type 2 diabetes. With the increasing number of people being diagnosed with type 2 diabetes worldwide, the use and relevance of Metformin has gained prominence. For better management of type 2 diabetes, Metformin is also available in combination with other drug substances such as Vidagliptin, Sitagliptin, Empagliflozin, Dapagliflozin, Pioglitazone, Glimepiride, and Glimepiride. The HPLC separation of Metformin can be quite challenging as it is a highly polar biguanide molecule, when compared to the other combinational drugs. In this technical note, we have developed a reversed phase based HPLC method for the separation of Metformin and the aforementioned combination drugs. A core-shell Kinetex EVO C18 column (5 µm, 150 mm x 4.6 mm), which has a unique patented organo-silica grafting process providing both 100 % aqueous stability and wider pH stability (pH 1 – 12), was used for the separation. The 100 % aqueous stability has been found to be advantageous in improving retention of problematic polar compounds, such as Metformin, that typically have poor retention under reversed phase conditions on C18 columns. The deposition of a slight positive charge on the surface of the particle further improves basic compound peak shape with repulsion interactions and promotes aqueous stability. This makes Kinetex EVO C18 an excellent choice for the balanced retention and separation of Metformin from its combination drugs in tablets.

Ion-pair reagents are commonly used to facilitate the retention of polar compounds by forming ion-pairs with the ionic analytes. In the method described here, hexane-1-sulfonic acid sodium salt monohydrate was used as an ion-pair reagent to increase the retention of Metformin. The HPLC column was equilibrated with the initial gradient to achieve an efficient, repeatable, and robust method for the analytical separation and quantification of Metformin and the other drug substances present in seven different combination drug products.

LC-UV Conditions

Column: Kinetex 5 µm EVO C18

Dimension: 150 x 4.6 mm

Part No.: [00F-4633-E0](#)

Mobile Phase: A: 2.5 mM sodium 1-hexane sulfonate monohydrate and 10 mM potassium dihydrogen phosphate, pH adjusted to 3.0 with phosphoric acid
B: Acetonitrile

Gradient:	Time (min)	%B
	0	2
	5	2
	10	40
	13	40
	15	65
	17	65
	18	2
	22	2

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Temperature: 30 °C

Detector: UV @ 210 nm

System: Waters® ACQUITY® H-Class HPLC

- Samples:**
1. Galvus Met® (0.15 mg/mL of Metformin HCl and 0.015 mg/mL of Vidagliptin)
 2. Janumet® (0.15 mg/mL of Metformin HCl and 0.015 mg/mL of Sitagliptin)
 3. Jardiance Met® (0.15 mg/mL of Metformin HCl and 0.00375 mg/mL of Empagliflozin)
 4. Gluxit M10® (0.15 mg/mL of Metformin HCl and 0.003 mg/mL of Dapagliflozin)
 5. Glimestar PM2® (0.15 mg/mL of Metformin HCl and 0.0045 mg/mL of Pioglitazone and 0.0006 mg/mL of Glimepiride)
 6. Glykind-M® (0.15 mg/mL of Metformin HCl and 0.024 mg/mL of Glimepiride)
 7. Glinil-M® (0.15 mg/mL of Metformin HCl and 0.015 mg/mL of Glibenclamide)



Krishna Chaitanya Routhu

Outside the lab, Krishna enjoys travelling and finding good places to eat. Krishna loves to go on long walks on the beach with his friends.



Sample Preparation

Diluent 1: Water/Acetonitrile, 50:50 (v/v) , Diluent 2: Mobile Phase A/Mobile Phase B, 98:2 (v/v)

Three tablets of each formulation were crushed to a fine powder. The sample stock was prepared by transferring one tablet equivalent weight into a 50 mL volumetric flask. 35 mL of Diluent 1 was then added and the solution vortexed for 40 minutes. The solution was made up to volume with Diluent 1. The sample stock was further diluted with Diluent 2 prior to injection.

Table 1. Formulation Details Used for Sample Preparation

Brand	Analytes	Dose	Manufacturer
Galvus Met*	Metformin Vidagliptin	Metformin – 500 mg Vidagliptin – 50 mg	Novartis Healthcare Pvt. Ltd
Janumet*	Metformin Sitagliptin	Metformin – 500 mg Sitagliptin – 50 mg	MSD Pharmaceuticals Pvt. Ltd
Jardiance Met*	Metformin Empagliflozin	Metformin – 500 mg Empagliflozin – 12.5 mg	Boehringer Ingelheim Pharma GmbH & Co.
Gluxit M10*	Metformin Dapagliflozin	Metformin – 500 mg Dapagliflozin – 10 mg	MSN Laboratories Pvt. Ltd.
Glimestar PM2*	Metformin Pioglitazone Glimepiride	Metformin – 500 mg Pioglitazone – 15 mg Glimepiride – 2 mg	Mankind India Pvt. Ltd.
Glykind-M*	Metformin Gliclazide	Metformin – 500 mg Gliclazide – 80 mg	Mankind Pharma Ltd.
Glinil-M*	Metformin Glibenclamide	Metformin – 500 mg Glibenclamide – 5 mg	Cipla Ltd.

Results and Discussion

The formulation samples of various combinations were prepared and run on Kinetex® 5 µm EVO C18, 150 x 4.6 mm column using a gradient program. The results demonstrated symmetrical peak shape and excellent resolution between Metformin and its combination drugs (**Figures 1 – 8**).

Table 2. Summary of Results: Metformin Combination Tablets

Sample Name	Analyte	Retention Time (min)	Tailing Factor	Resolution
Galvus Met	Metformin	7.39	0.89	-
	Vidagliptin	9.31	1.36	15.88
Janumet	Metformin	7.31	0.86	-
	Sitagliptin	10.57	1.33	25.53
Jardiance Met	Metformin	7.40	0.94	-
	Empagliflozin	11.68	1.29	35.16
Gluxit M10	Metformin	7.36	0.99	-
	Dapagliflozin	12.51	1.22	38.19
Glimestar PM2	Metformin	7.35	0.91	-
	Pioglitazone	13.36	1.60	35.68
	Glimepiride	16.83	1.25	27.55
Glykind-M	Metformin	7.38	0.95	-
	Gliclazide	15.75	1.22	64.40
Glinil-M	Metformin	7.41	0.91	-
	Glibenclamide	16.67	1.27	74.88



Figure 1. Sample Solution for Assay of Galvus Met® (Metformin and Vidagliptin) on Kinetex® 5 µm EVO C18

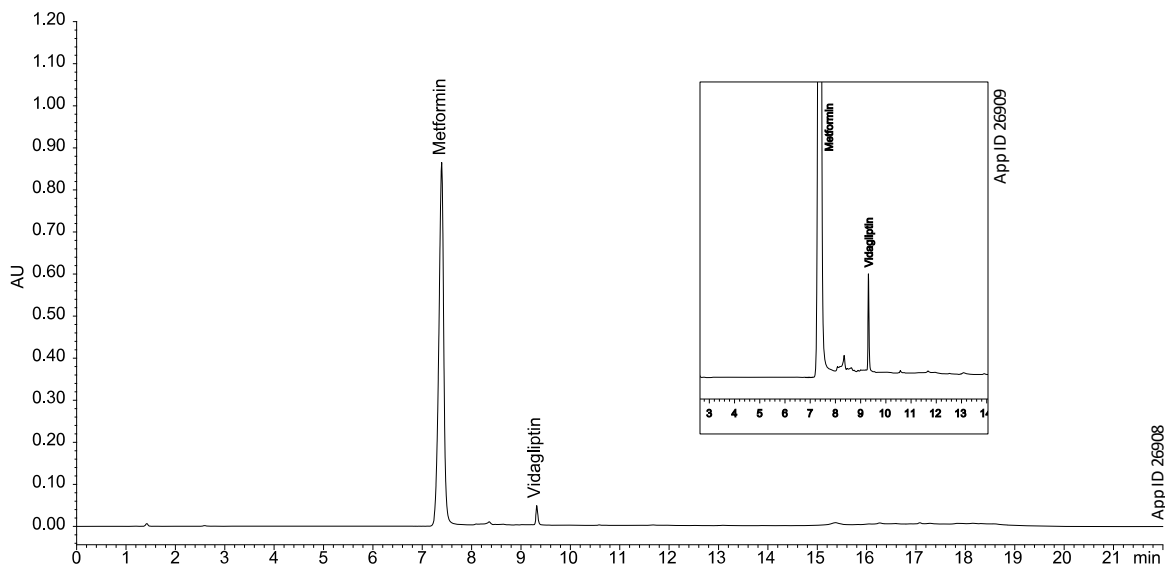


Figure 2. Sample Solution for Assay of Janumet® (Metformin and Sitagliptin) on Kinetex 5 µm EVO C18

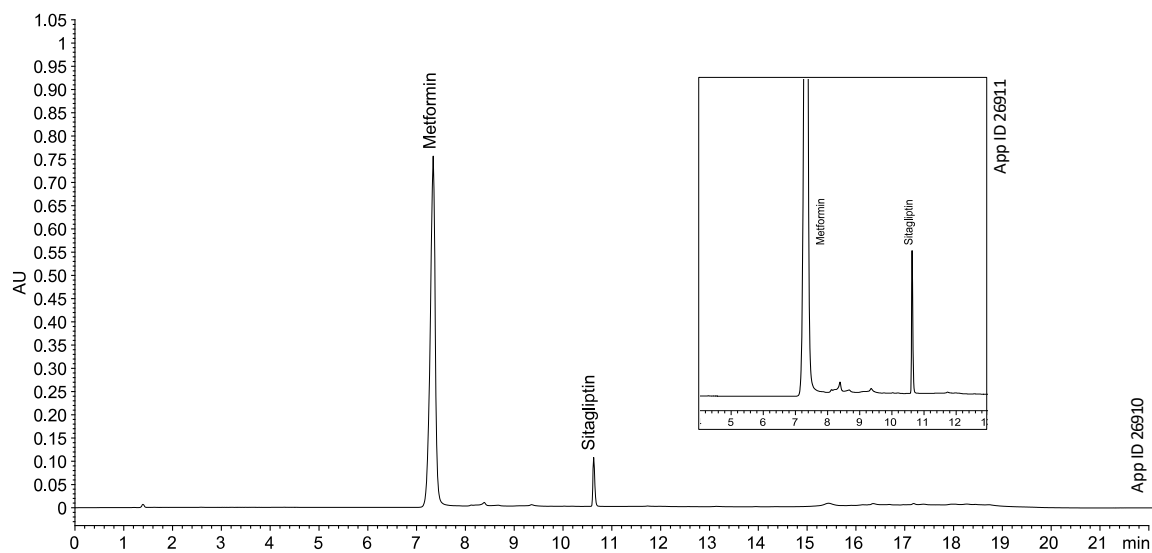


Figure 3. Sample Solution for Assay of Jardiance Met® (Metformin and Empagliflozin) on Kinetex® 5 µm EVO C18

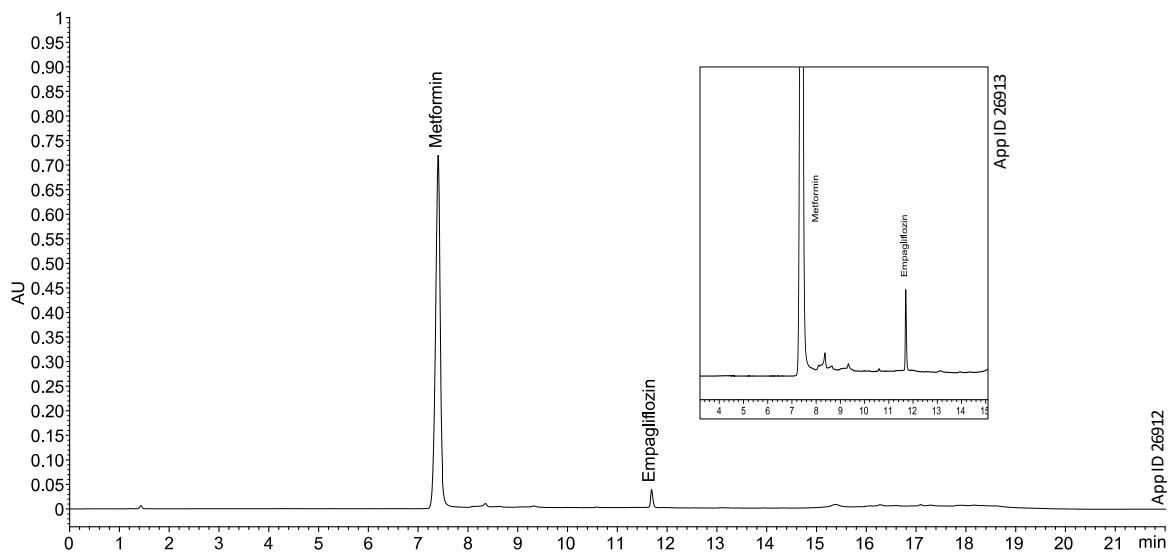


Figure 4. Sample Solution for Assay of Gluxit M10® (Metformin and Dapagliflozin) on Kinetex 5 µm EVO C18

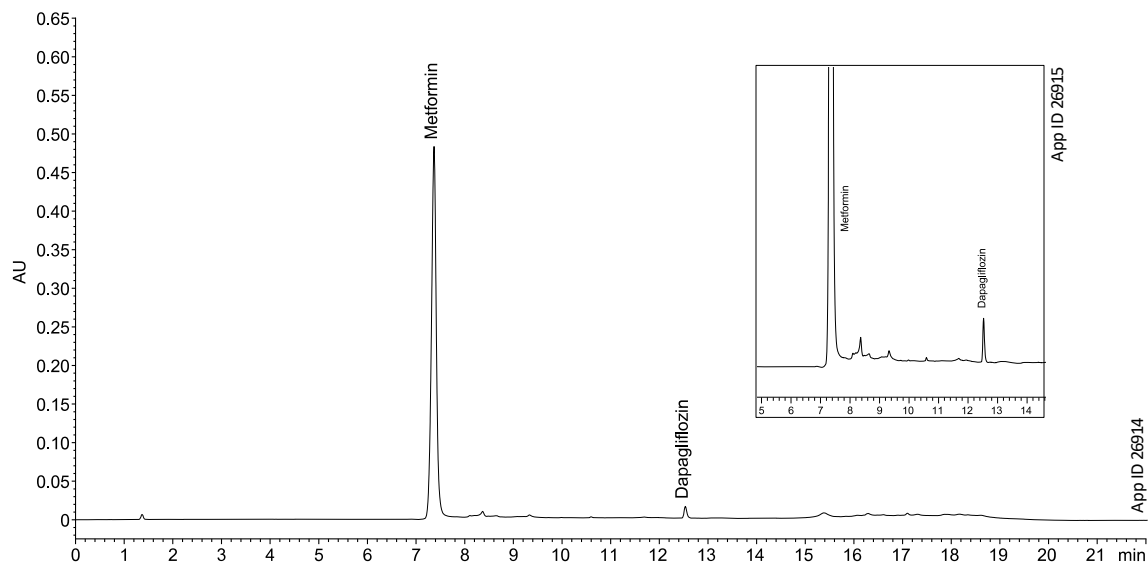


Figure 5. Sample Solution for Assay of Glimestar PM2® (Metformin, Pioglitazone and Glimepiride) on Kinetex® 5 µm EVO C18

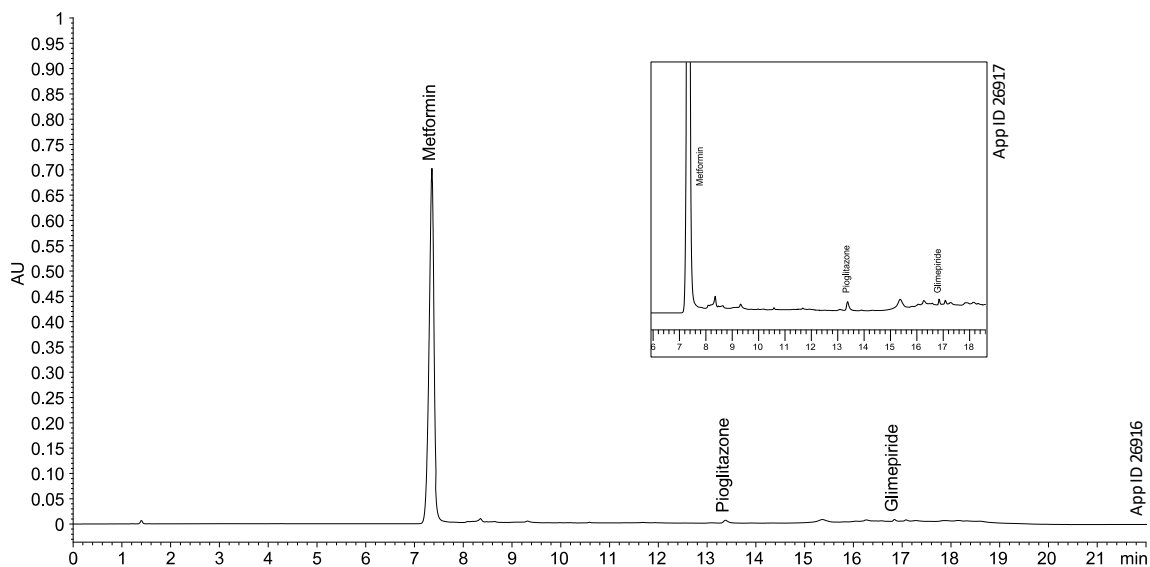


Figure 6. Sample Solution for Assay of Glykind-M® (Metformin and Glimepiride) on Kinetex 5 µm EVO C18

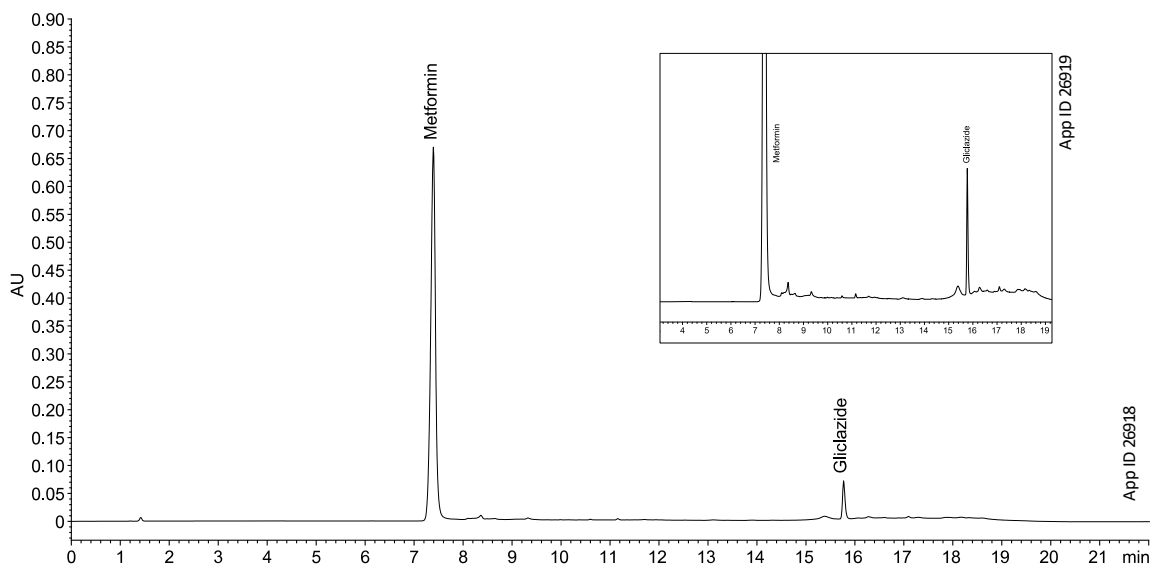


Figure 7. Sample Solution for Assay of Glinil-M® (Metformin and Glibenclamide) on Kinetex® 5 µm EVO C18

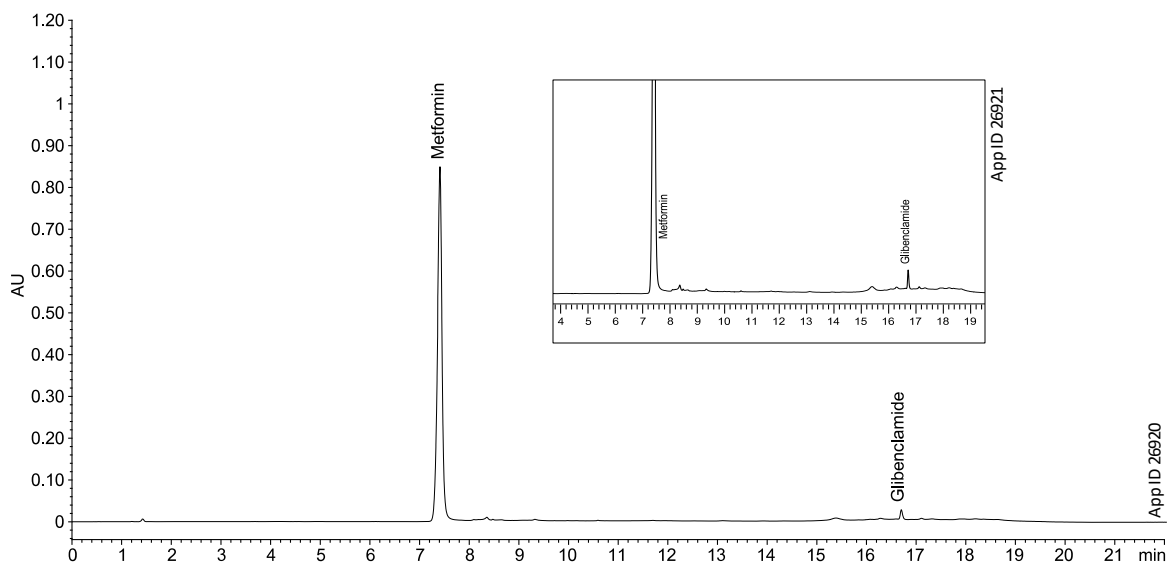
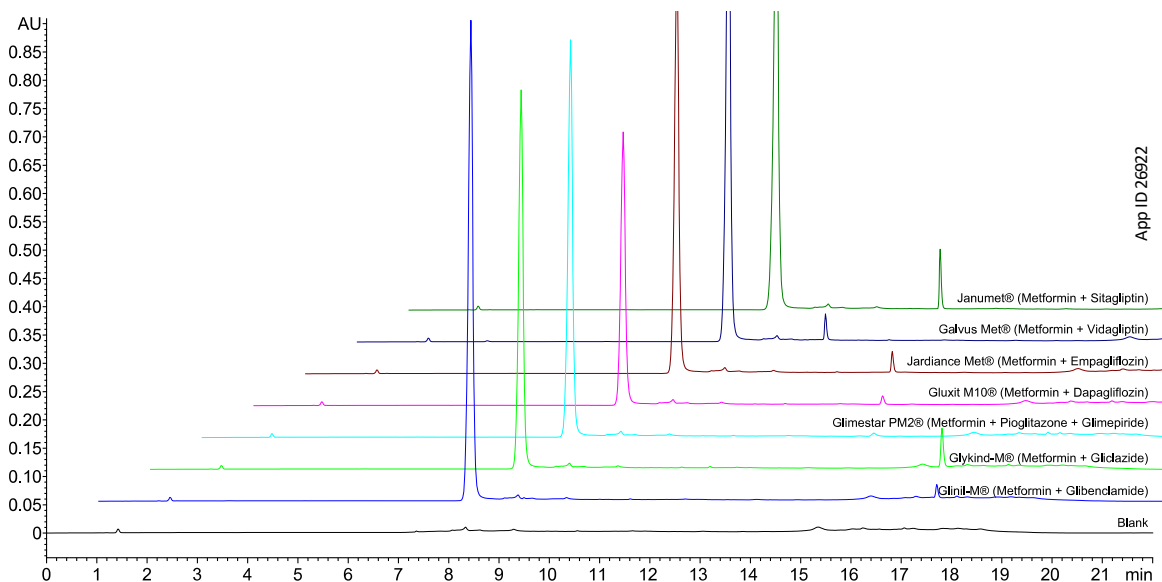


Figure 8. Overlay Chromatogram of All Seven Formulations with Blank



Conclusions

The results successfully demonstrate a simple gradient HPLC method has been developed for the assay of Metformin and other drug substances used in seven different combination drug products. The study clearly shows that Metformin, a highly polar molecule can be well retained and separated from drug substances which are used in combination in different drug products containing Vidagliptin, Sitagliptin, Empagliflozin, Dapagliflozin, Pioglitazone, Gliclazide, Glibenclamide, and Glimepiride. The excellent resolution and peak shape obtained on Kinetex EVO C18 column makes it the best choice for the assay analysis of Metformin and its combination drug products.



Kinetex® Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ Ultra Cartridges (mm) [‡]
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
HILIC	—	—	00F-4606-E0	00G-4606-E0	AJ0-8772

for ID: 4.6 mm ID

[‡]SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#)

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