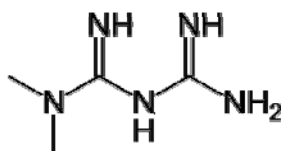


Metformin

- Type 2 Diabetes medication

Metformin (sold as Glucophage) is an oral antidiabetic drug. It is the first-line treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Metformin is the only antidiabetic drug that, convincingly, prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. Metformin is believed to be the most widely prescribed antidiabetic drug in the world; in the United States alone, more than 48 million prescriptions were filled in 2010 for its generic formulations. Metformin is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production by the liver.

Previous analytical methods typically involve separations on reversed phase columns with ion pair reagents, or ion exchange columns. Both these approaches are unsuitable for mass spectrometry detection (MS). HILIC is on the other hand very suitable for connection to MS and gives a simplified sample preparation for plasma and other biomatrices. HILIC is also suitable for the metformin impurity assay which by recent guidelines from FDA now involves melamine analysis. We present here a rapid bioanalytical method using HILIC-MS combined with simple protein crash sample preparation that allow a high sample-throughput, yet sensitive and with excellent recoveries. An external calibration curve were constructed in the range 0.05–1.0 ppm. The new method was, furthermore, also tested for plasma analysis against an alternative HILIC column having a lower amount of bonded stationary phase which also turned out to be more unstable, leading to higher background and thus lower sensitivity and accuracy.



Metformin

Metformin in Plasma

SeQuant® ZIC®-HILIC

Recommended column:

SeQuant® ZIC®-HILIC (5µm, 200Å) PEEK 100 × 2.1 mm (1.50452.0001)

Recommended solvents and reagents

Acetonitrile: hypergrade for LC-MS LiChrosolv® (1.00029)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Ammonium acetate (HPLC grade) or in-situ prepared buffer from ammonia and acetic acid

Ammonia solution 28–30% for analysis EMSURE® ACS, Reag. Ph Eur (1.05423)

Acetic acid 96% for analysis EMSURE® (1.00062)

HPLC Sample Preparation

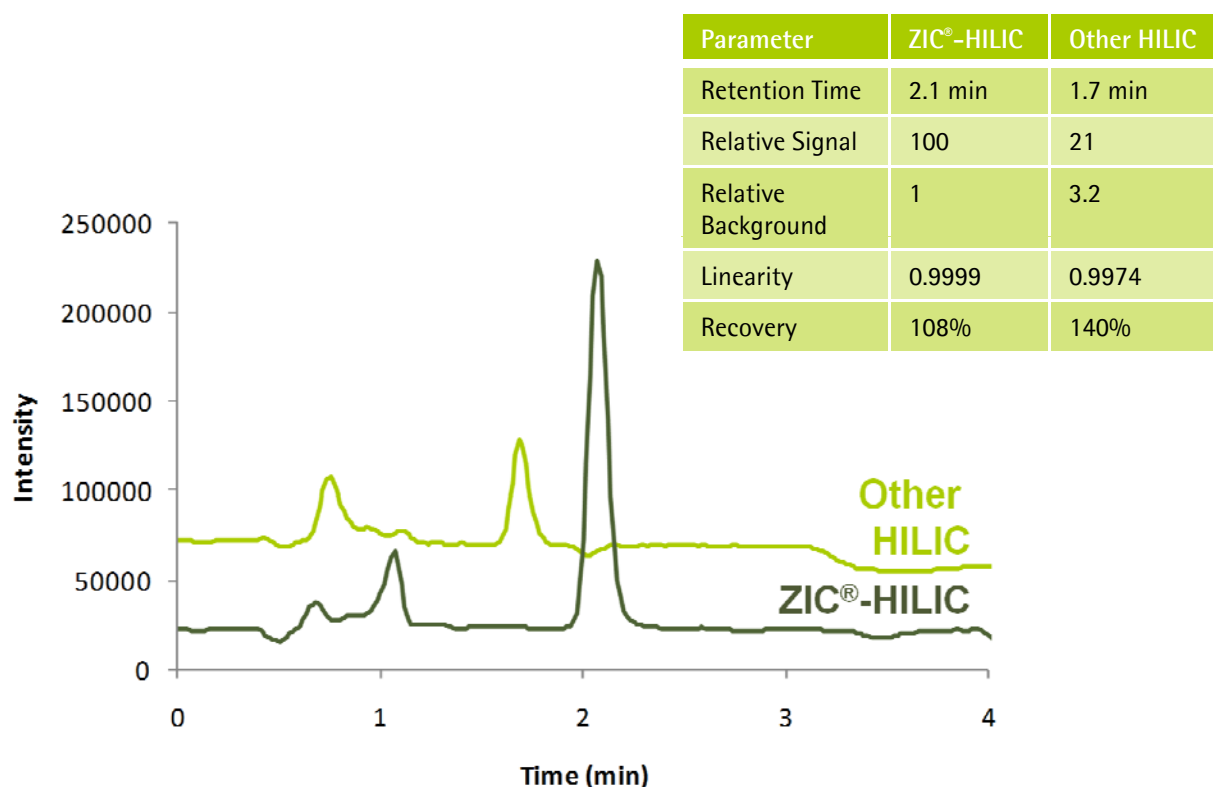
Sample preparation by dilution of plasma/serum in acetonitrile (1:4), followed by centrifugation at 6400 rpm (15 °C) and direct injection of 1 µL supernatant in the LC/MS instrument.

Metformin in Plasma

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column: SeQuant® ZIC®-HILIC (5µm, 200Å) PEEK 100 × 2.1 mm (1.50452.0001)
 Injection: 1 µL of supernatant from protein precipitation (acetonitrile and Milli-Q water; 80:20 v/v)
 Detection: LC-MS, positive ESI mode, SIM; m/z 130
 Flow Rate: 0.5 mL/min
 Mobile Phase: Acetonitrile and 10 mM ammonium acetate, pH 6.8 (85:15 v/v). Total ionic strength: 1.5 mM
 Temperature: 30 °C
 Sample: Patient plasma sample treated according to sample preparation protocol.



Chromatographic Data

No.	Compound	Retention Time (min)	m/z
1	Void volume	0.4	-
2	Metformin	2.1	130