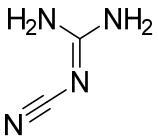
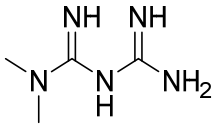
Metformin and Related Impurities

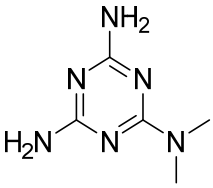
SeQuant® ZIC®-cHILIC



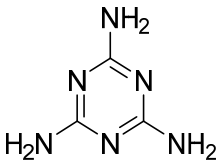
Impurity A



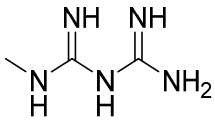
Metformin



Impurity C



Melamine



Impurity B

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Metformin works by suppressing glucose production by the liver.

Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain

Metformin is now 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. It is also present in the World Health Organization Model List of Essential Medicines.

It is marketed by Merck Serono under the brand name Glucophage® and Glucophage XR®.

The current USP assay method for metformin is potentiometric titration wich 0.1 N perchloric acid. Perchloric acid is highly explosive and ammonium perchlorate is used as rocket fuel.

Proposed USP Method Metformin RS

Mobile phase:
Prepare a solution in water, containing 4.62 g of ammonium acetate per L, adjust with glacial acetic acid to a pH of 5.0. Mix acetonitrile and buffer 90/10 (v/v).
Standard solution:
Prepare solutions of USP Metformin Related Compound A, B and C RS in mobile phase having a known concentration of about 0.1 mg per mL. Transfer 5 mL of this solution to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.
Sample solution:
Transfer about 500 mg of Metformin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.
Diluted sample solution:
Transfer 1.0 mL of the Test solution to a 10-mL volumetric flask, dilute with Mobile phase to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.
System suitability solution:
Prepare a solution in water containing about 0.25 mg of metformin hydrochloride and about 0.1 mg of melamine per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with Mobile phase to volume, and mix.

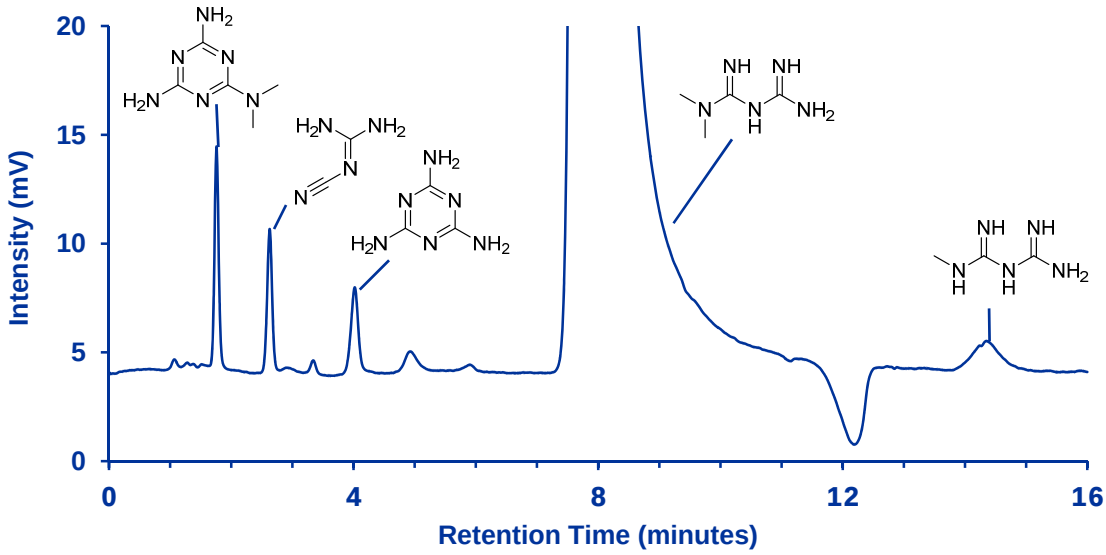
Chromatographic system:
The liquid chromatograph is equipped with a 218-nm detector and a 150x4.6 mm column containing packing ZIC®-cHILIC. The flow rate is 1.5 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution, R, between melamine and metformin is not less than 10.
Procedure:
Separately inject equal volumes (about 5 µL) of the Sample solution, the Standard solution, and the Diluted sample solution into the chromatograph, record the chromatograms for not less than twice the retention time of metformin, and measure the peak areas. Calculate the percentage of metformin related compounds in the portion of Metformin Hydrochloride

Metformin and Related Impurities

SeQuant® ZIC®-cHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-cHILIC (3 µm, 100 Å) PEEK 150 × 4.6 mm	1.50661.0001
Injection:	5 µL	
Detection:	Shimadzu LC-10, UV 218 nm	
Cell:	8 µl	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 4.62 g of ammonium acetate in 1000 ml water (60 mM). Adjust buffer to pH 5 using glacial acetic acid. Mix Acetonitrile and Buffer 90:10 (v/v)	
Temperature:	30 °C	
Diluent	Mobile phase	
Sample:	5000 ppm metformin & 1 ppm of each impurity: A, C and melamine & 5 ppm of impurity B in mobile phase	
Pressure Drop:	105 bar (1522 psi)	

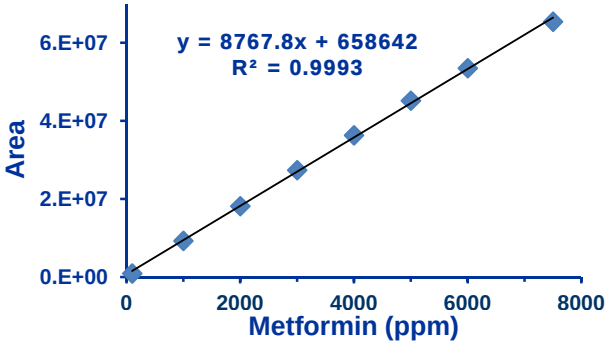


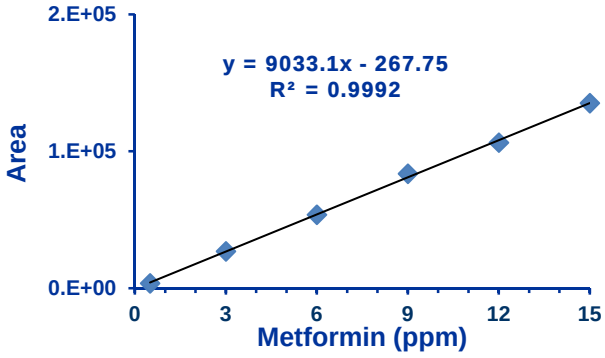
Chromatographic Data

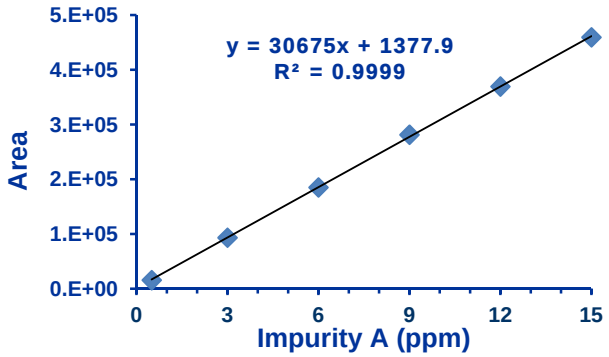
No.	Compounds	Retention Time (min)	k'	Resolution	Area (%)	Theoretical Plates	Tailing Factor
1	C: Dimethylmelamine	1.8	0.6	-	0.1	3100	1.1
2	A: Cyanoguanidine	2.6	1.4	6.3	0.1	4900	1.0
3	Melamine	4.0	2.7	7.4	0.1	5000	1.1
4	Metformin	8.0	6.5	10.0	99.6	3100	0.8
5	B: Methylbiguanide	14.4	12.3	9.1	0.1	4900	1.2

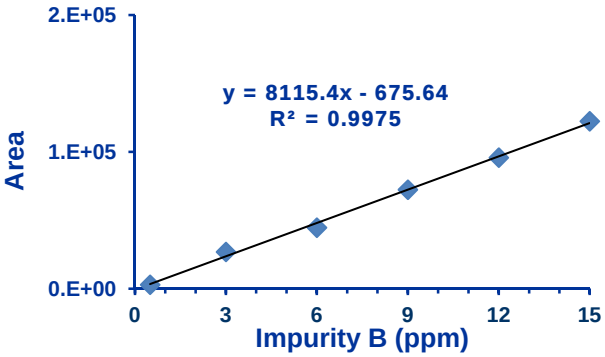
Metformin and Related Substances

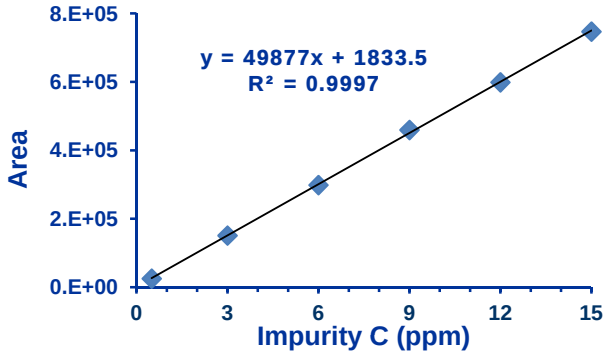
SeQuant® ZIC®-cHILIC

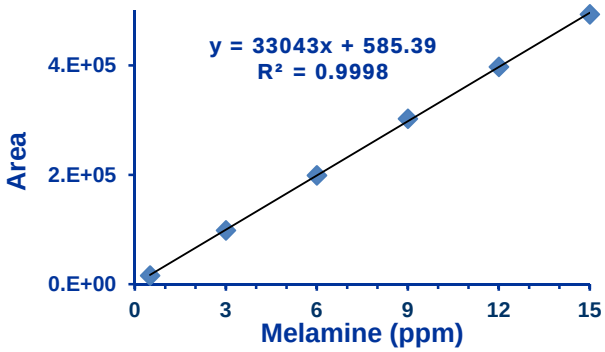












USP Method – Metformin RS (USP36-NF31 S1)

Column: L9 classification - Partisil 10 SCX Related compounds 4.6 mm x 25 cm, 10 µm. (Not official USP-NF text; and thereby not bound to specific brand)

Mobile phase:
Prepare a solution in water, containing 17 g of monobasic ammonium phosphate per L, adjust with phosphoric acid to a pH of 3.0, and mix.

Standard solution:
Prepare a solution of USP Metformin Related Compound A RS in water having a known concentration of about 0.2 mg per mL. Transfer 1.0 mL of this solution to a 200-mL volumetric flask, dilute with Mobile phase to volume, and mix. (note—Metformin related compound A is 1-cyanoguanidine)

Sample solution:
Transfer about 500 mg of Metformin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Diluted sample solution:
Transfer 1.0 mL of the Test solution to a 10-mL volumetric flask, dilute with Mobile phase to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.

System suitability solution:
Prepare a solution in water containing about 0.25 mg of metformin hydrochloride and about 0.1 mg of melamine per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Chromatographic system:
The liquid chromatograph is equipped with a 218-nm detector and a 250x4.6 mm column containing packing L9. The flow rate is about 1.0 to 1.7 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: **the resolution, R, between melamine and metformin is not less than 10**.

Procedure:
Separately inject equal volumes (about 20 µL) of the Sample solution, the Standard solution, and the Diluted sample solution into the chromatograph, **record the chromatograms for not less than twice the retention time of metformin**, and measure the peak areas. Calculate the percentage of metformin related compound A in the portion of Metformin Hydrochloride