USP Method for HPLC
Analysis of Methotrexate

Application

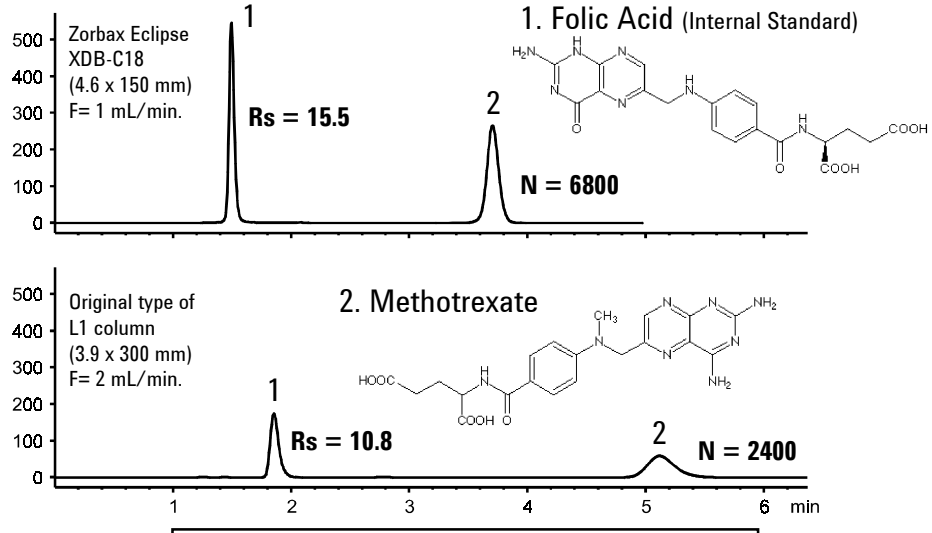
Pharmaceutical

Robert Ricker

According to the United States Pharmacopeia (USP), L1 column packing is defined as "octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter". Some USP methods specifying an L1 column use intermediate pH, and many of these methods were done on a 10 µm, 3.9 x 300 mm column. Until recently there was no modern alternative to the older 10 µm C18 column for intermediate pH-range applications.

Now ZORBAX Eclipse XDB-C18 is available as a state of the art L1 alternative, available in 3.5 and 5.0 µm particle sizes and various column dimensions.

Below is a comparison of the USP method for methotrexate performed on the original brand of L1 column and on a modern L1 column, ZORBAX Eclipse XDB-C18. Methotrexate is used as an antineoplastic and an antirheumatic. The USP Method uses a pH 6.0 acetonitrile: phosphate/citrate buffer (10:90) mobile phase and a flow rate of 2.0 ml/min. Intermediate pH unreacted silanols impart a negative charge to the stationary phase which may be detrimental to peak shape. The 2.0 ml/min. flow rate is standard on the older 3.9 x 300 mm columns. On 150 mm columns flow rate is typically 1.0 ml/min.



Conditions: LC: Hewlett Packard Series II 1090
Column: ZORBAX Eclipse XDB-C18, Agilent P/N: 993967-902
Mobile Phase: ACN : pH 6.0 phosphate / citrate buffer (10:90)
Buffer: 0.2 M K_2HPO_4 : 0.1 M citric acid (63:37)
Temp: 23°C; Inj Vol: 10 µL (0.1 µg / µL); UV: 302 nm

Highlights

- *Improved peak shape using ZORBAX Eclipse XDB-C18.*
- *Higher efficiency of ZORBAX Eclipse XDB-C18.*
- *Using ZORBAX Eclipse XDB-C18 as a state of the art L1 column offers:*
  - *Greater sensitivity*
  - *Reduced analysis time*
  - *Reduced back pressure*
  - *Shorter length offers reduced solvent use per analysis*
- *USP performance is easily surpassed using ZORBAX Eclipse XDB-C18.*
  - *Resolution between methotrexate and the internal standard, folic acid is greater on the ZORBAX Eclipse XDB-C18 because the methotrexate peak is much sharper than on the original L1 column.*
  - *Relative retention meets specs (methotrexate is 1, folic acid is 0.35)*

Agilent Technologies

Robert Ricker is an application chemist based at Agilent Technologies, Wilmington, Delaware.

For more information on our products and services, visit our website at:
www.agilent.com/chem

Printed in the USA
April 25, 2002
5988-6394EN

Agilent Technologies