Enantioselective Resolution of Triazole Fungicides by HPLC on Hydroxypropyl Beta-Cyclodextrin Chiral Stationary Phase

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Abstract

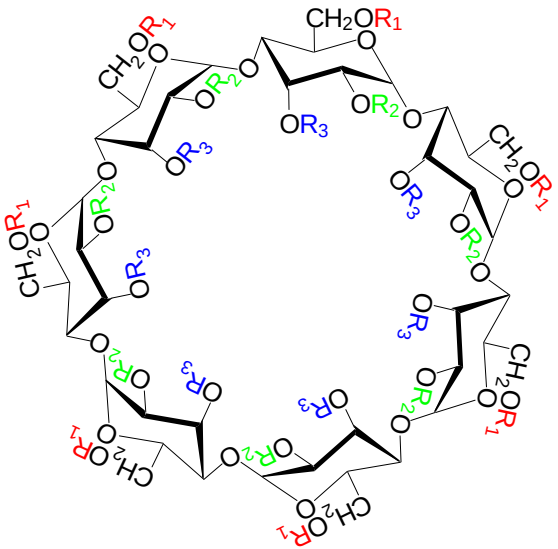
- The global market for antifungal agents is in the billions and continues to grow at a steady rate. This is due to several factors: 1. an increasing number of people living in an immuno-compromised state that it makes them more susceptible to fungal infections; 2. like bacteria-infected diseases, fungi are becoming more resistant to standard therapies; and, 3. many of the standard therapies have a very high toxicity level and adverse side effects.

- Thus, the need to study for effective antifungal agents against specific infections becomes extremely urgent. Furthermore, many of the triazole fungicides are chiral molecules. The pharmacokinetic aspects (absorption, distribution, metabolism and excretion) of each enantiomer need to be realized during drug development stage. Normally, only one enantiomer is biologically effective.

- In this study, enantiomeric resolution of some of the chiral triazole fungicides will be presented. The chiral stationary phase is a newly-improved cyclodextrin phase with pendant hydroxylpropyl groups. The mobile phase design is LC-MS-oriented for future clinical trial studies. The method development is straightforward and the separation conditions are very robust.

Chiral Stationary Phase: New High Performance Hydroxypropylated Beta-Cyclodextrin

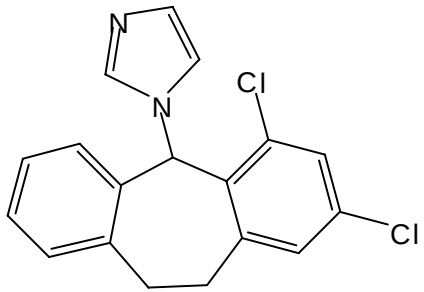
Degree of Substitution = 7



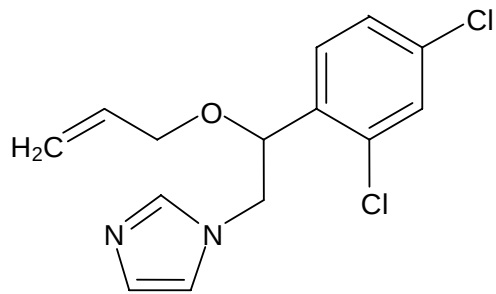
$R_1, R_2, R_3 = H$, or $-CH_2CH(OH)CH_3$

Triazole Compound Structures

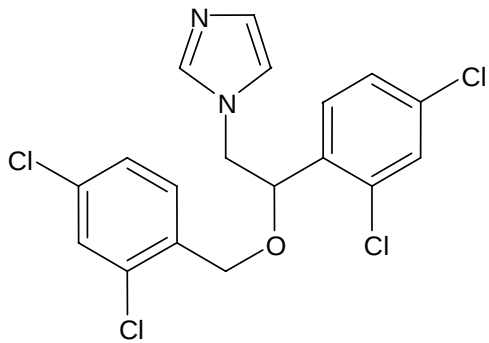
Eberconazole



Enilconazole

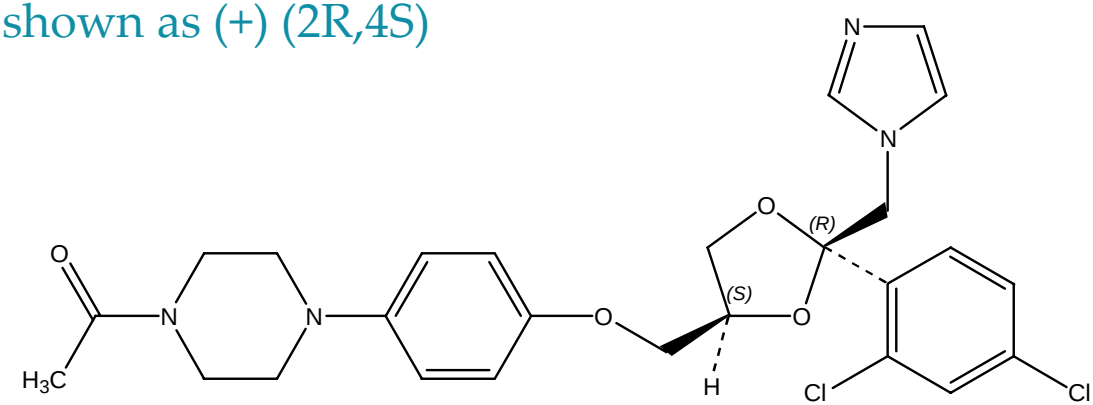


Miconazole

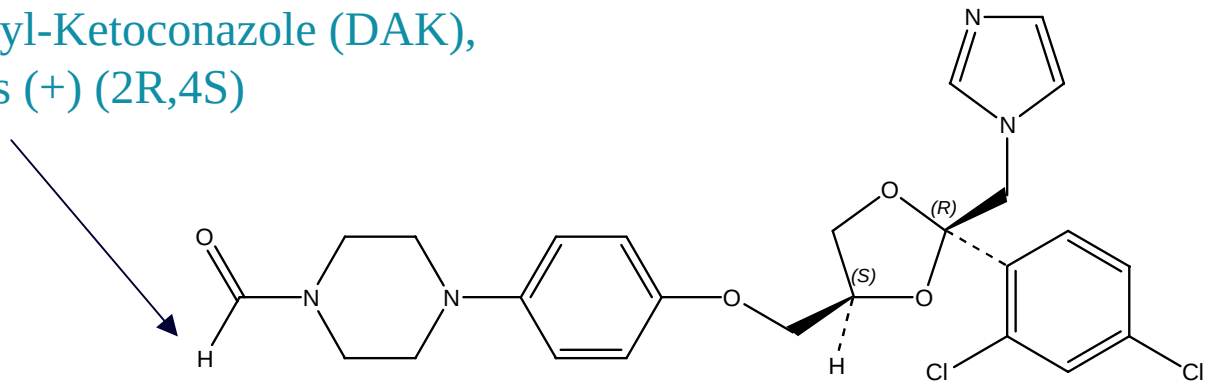


Triazole Compound Structures

Ketoconazole, shown as (+) (2R,4S)



DesAcetyl-Ketoconazole (DAK), shown as (+) (2R,4S)



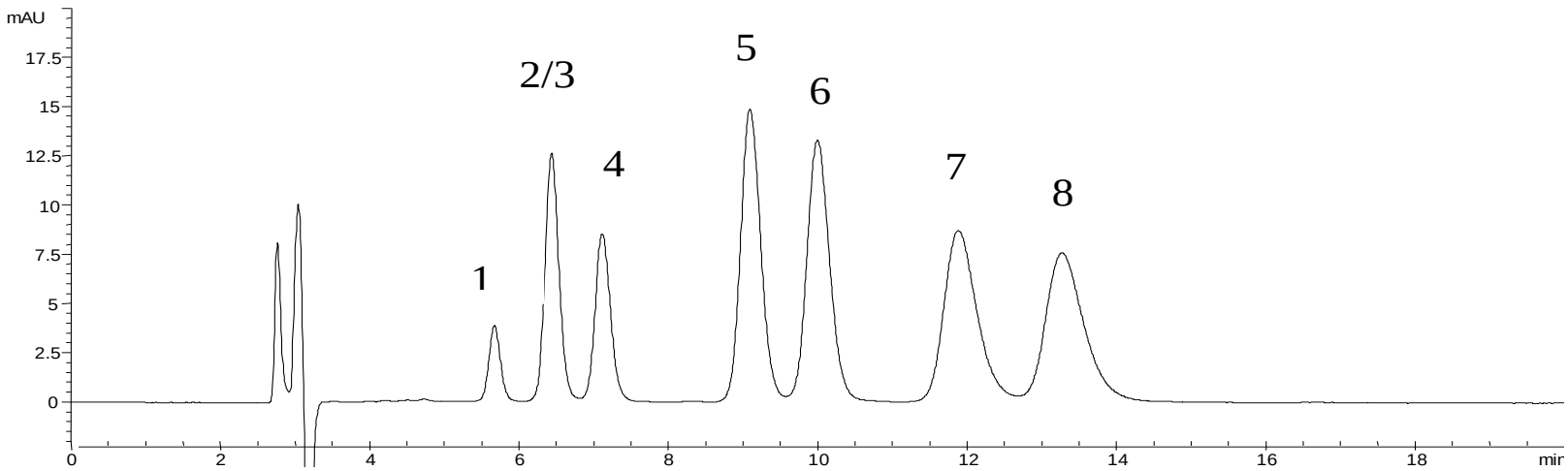
Methods Development

Sample:	10-100 μg/mL
Injection:	1-5 μL
Detection:	UV-230 nm
Flow Rate:	1 mL/min
Temperature:	23°C

Starting Mobile Phase: 30/70: ACN/0.1% HCOOH (LC-MS compatible)
Step 1. Set % ACN
Step 2. Decrease flow rate for increased Rs.

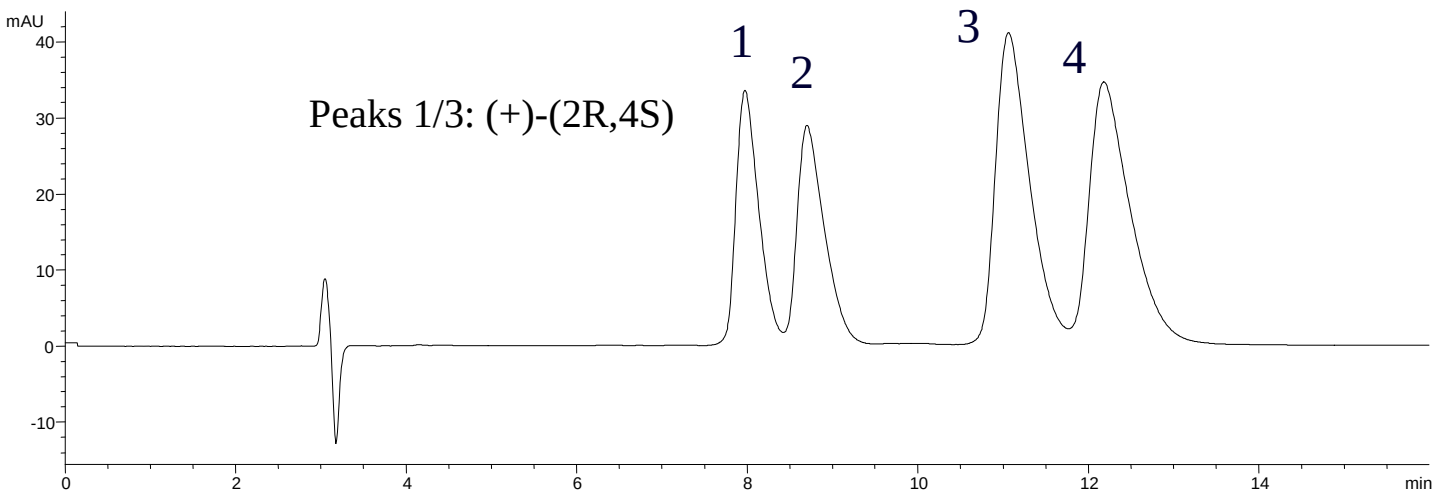
Simultaneous Separation- 4 Triazole Compounds

Column: CYCLOBOND I *2000* HP-RSP, 250x4.6mm
Mobile phase: 30/70: ACN/0.1% HCOOH, isocratic
Sample: 100 µg/mL each
Injection: 1 µL
Detection: UV: 230 nm
Flow rate: 1 mL/min
Peaks 1,2 Enilconazole; Peaks 3,4 Eberconazole
Peaks 5,6 Miconazole; Peaks 7,8 Ketoconazole



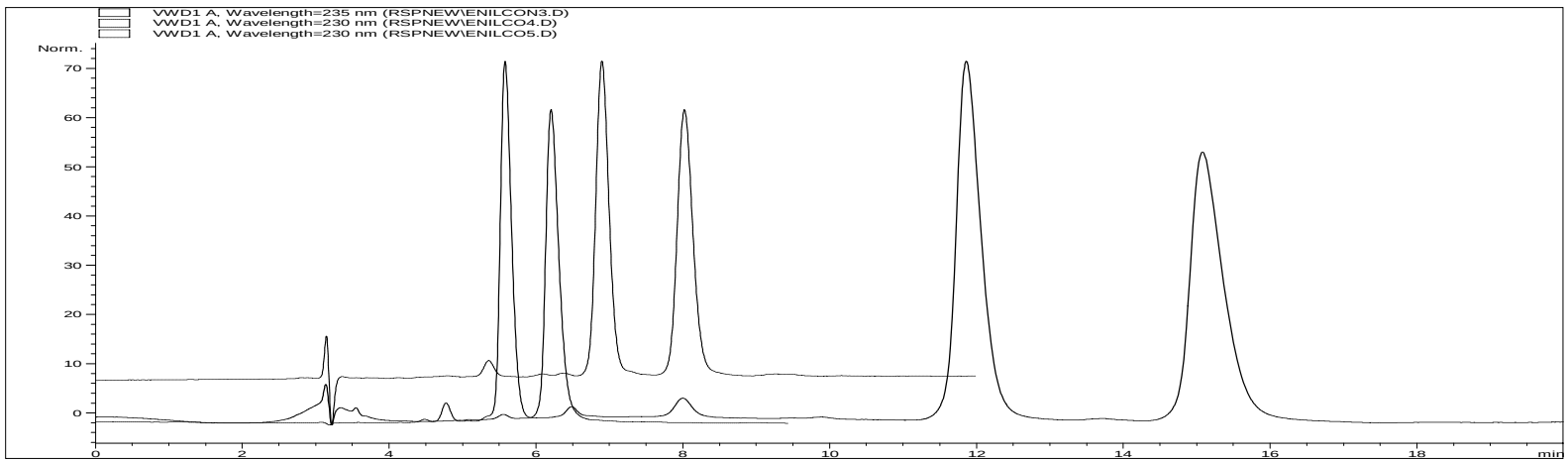
Simultaneous Separation- Ketoconazole + its N-derivative

Column: CYCLOBOND I *2000* HP-RSP, 250X4.6mm
Mobile phase: 30/70: ACN/0.1% HCOOH, isocratic
Sample: 100 µg/mL each
Injection: 1 µL
Detection: UV-230 nm
Flow rate: 1 mL/min
Peaks 1,2 Desacetyl ketoconazole; Peaks 3,4 Ketoconazole



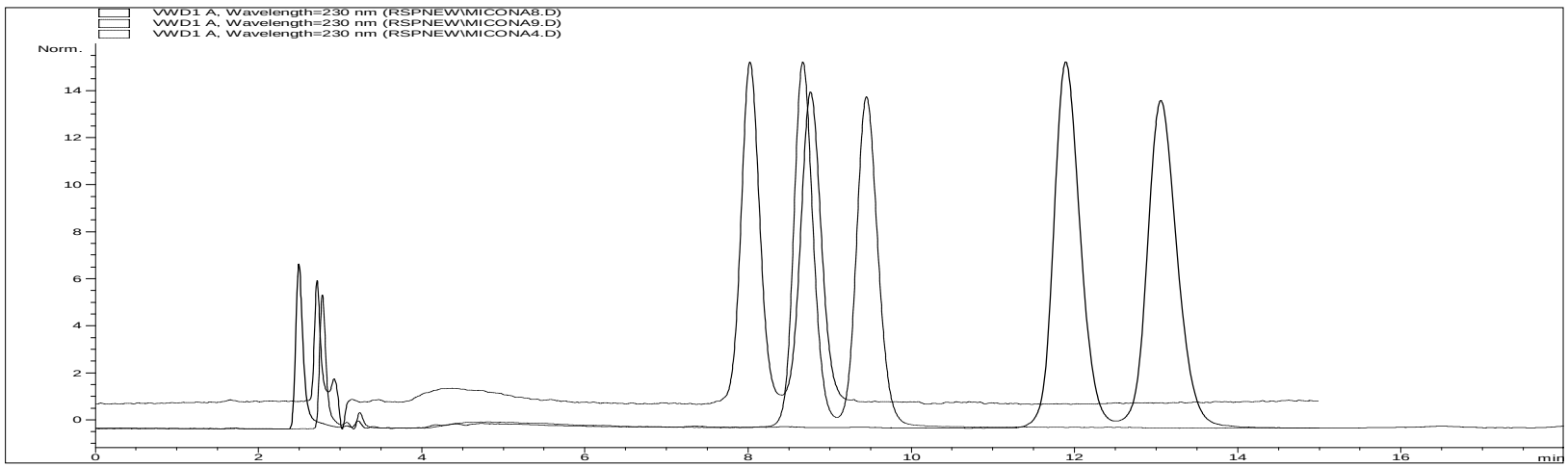
ACN Concentration Effect: Enilconazole

Column: CYCLOBOND I *2000* HP-RSP, 250x4.6mm
Mobile phase: ACN/0.1% HCOOH
Flow rate: 1 mL/min
Screen result: ---------- 30/70: α= 1.24 Rs= 1.6
Better results: …… 25/75: α= 1.29 Rs= 2.2
———— 20/80: α= 1.35 Rs= 3.0



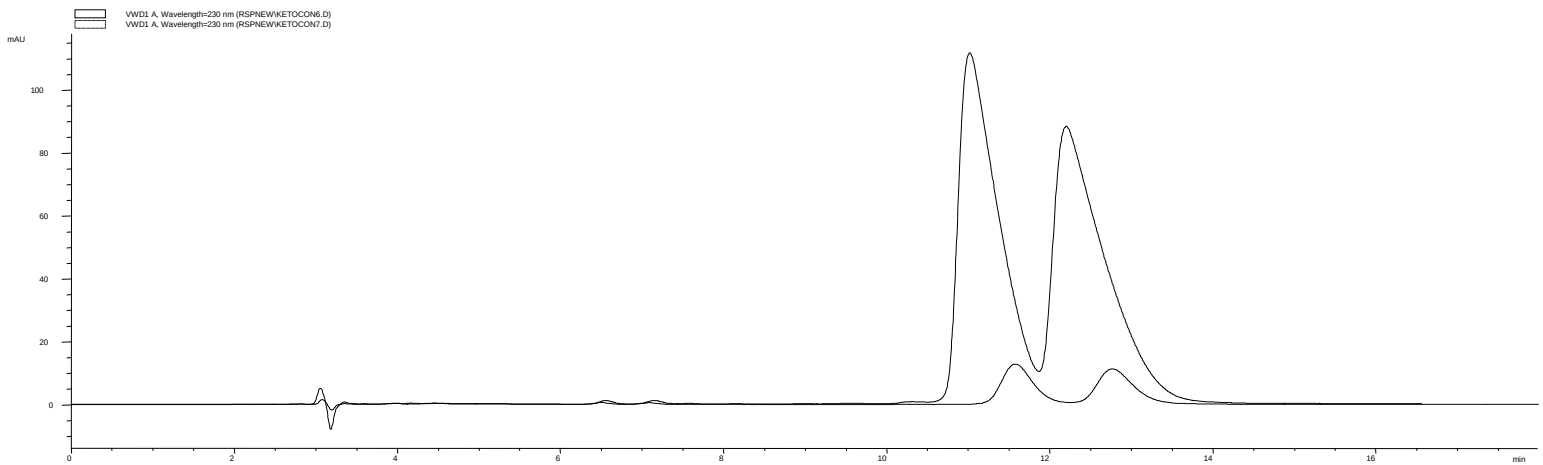
HCOOH Concentration Effect

Column:	CYCLOBOND I *2000* HP-RSP, 250x4.6mm
Mobile phase:	30/70: ACN/HCOOH
Flow rate:	1 mL/min
Detection:	UV-230 nm
Sample:	Miconazole, 100 µg/mL
Injection:	1 µL
————	0.02%
……	0.1%
----------	0.2%



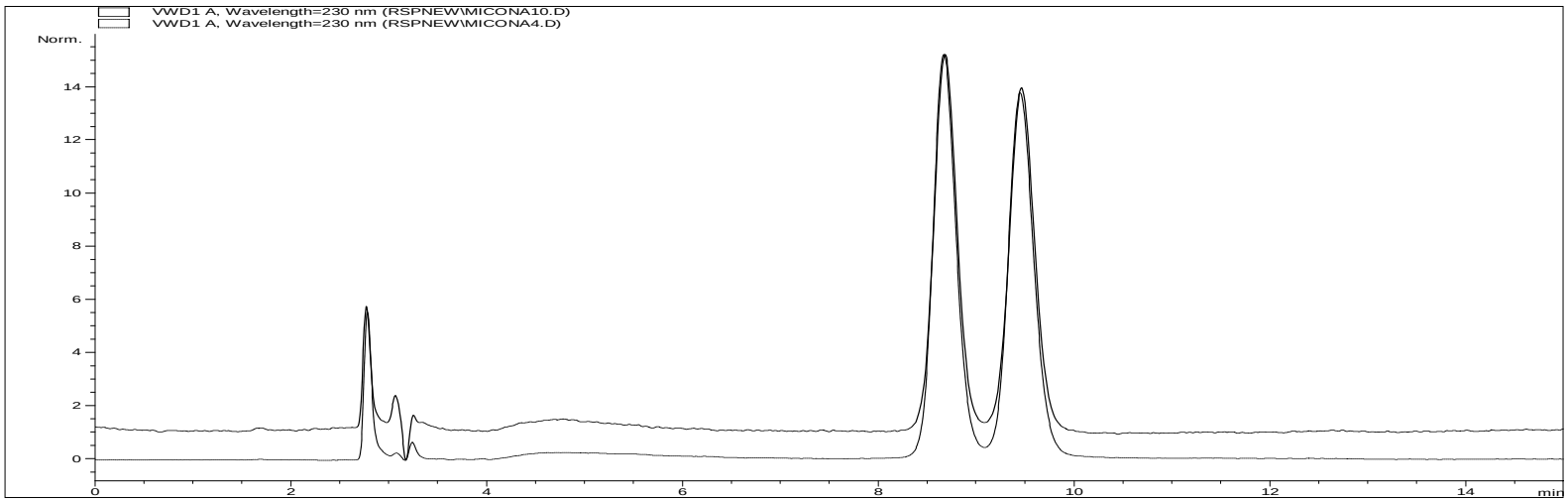
Sample Load-Effect Ketoconazole

Column:	CYCLOBOND I *2000* HP-RSP, 250x4.6mm
Mobile Phase:	30/70: ACN/0.1% HCOOH
Flow Rate:	1 mL/min
Detection:	UV-230 nm
Sample:	100 µg/mL in mobile phase
Injection:	———— 10 µL
	…….. 1 µL



Stability Tests

Column:	CYCLOBOND I *2000* HP-RSP, 250x4.6mm
Mobile phase:	30/70: ACN/0.1% HCOOH
Flow rate:	1 mL/min
Detection:	UV-230 nm
Sample:	Miconazole, 100µg/mL in mobile phase
Injection:	1 µL
_____	Day one
----------	After 2000 mL of mobile phase



Conclusions

- CYCLOBOND I *2000* HP-RSP is extremely stable, even under acidic buffer conditions.
- The method development and subsequent optimization is straight forward.
- Typically, lower ACN concentration has higher selectivity.
- Higher % HCOOH does not affect selectivity but reduces retention time.
- The mobile phase conditions are LC-MS compatible.