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STABILITY AND RELIABILITY: NEW APPROACHES IN PREPARATIVE HPLC COLUMN DESIGN

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OVERVIEW-

A proprietary new procedure was developed for the manufacture of preparative columns with inner diameters of 19, 30 and 50 mm, and lengths from 30 to 250 mm. This new procedure ensures columns with optimal bed density, resulting in durable bed stability, better efficiency and higher loadability than other commercially available preparative columns. In addition, separating basic drugs under high pH leads to 11 times higher loadability on the XTerra® MS C_{18} prep column.

INTRODUCTION-

In today's drug purification environment, the demand for timely high purity results places huge emphasis on the integrity and stability of the preparative column. Complex sample starting materials demand high efficiency columns containing smaller particles (< 10 µm) than was conventionally used for purification. The challenge for the column manufacturer is to reproducibly produce analytical columns in preparative dimensions. Waters has developed an innovative procedure for the manufacture of preparative columns. To demonstrate the effectiveness of this new procedure, column resolution and bed capacities of an XTerra® MS C_{18}, 5 µm preparative column were compared with two commercially available preparative columns. In addition, to fully utilize the power of pH for method development, a loading study for basic drugs at high pH was run on an XTerra® MS C_{18}, 5 µm preparative column.

METHOD-

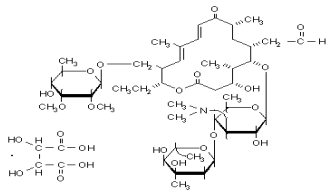
Column Information	Dimensions	Flow Rate
XTerra® MS C_{18}, 5 µm	19 × 100 mm	18 mL/min
Zorbax® CombiHT SB C_{18}, 5 µm	21.2 × 100 mm	22.4 mL/min
Luna® CombiHTS C_{18}, 5 µm	21.2 × 100 mm	22.4 mL/min

EXPERIMENTAL PROCEDURES FOR STABILITY STUDY-

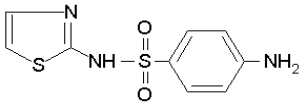
1. Tylosin, sulfathiazole, and ketoprofen samples were continuously injected into the preparative columns. The total injection volume each time is 400 µL. Sample Concentration: 15, 10, 10 mg/mL respectively, in DMSO
2. Same experiments were repeated on three other prep columns.

The QC test was run on all 4 columns after 1000 injections. The USP tailing factor and plate count were calculated and compared with the initial values to see if any severe change occurred. Detection was UV @ 254 nm

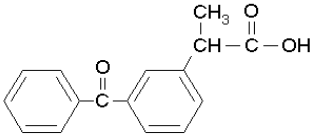
TEST COMPOUNDS FOR STABILITY EXPERIMENTS-



Tylosin



Sulfathiazole



Ketoprofen

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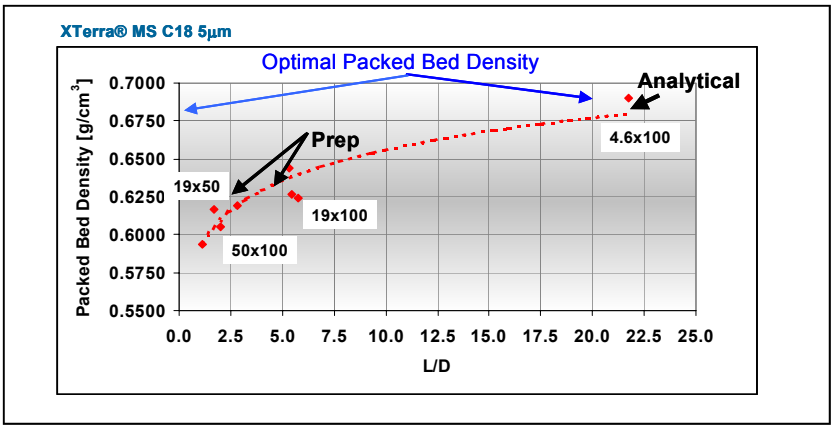
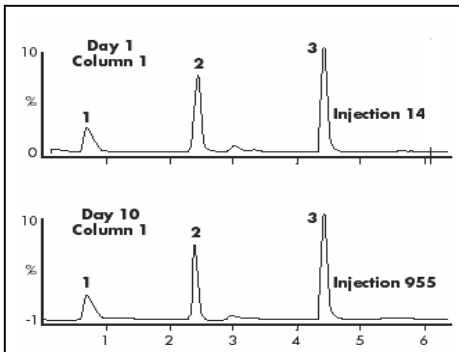
KEY TO COLUMN STABILITY-



1. Packed bed density is the key to achieving reliable bed stability.
2. Standard packing procedures are insufficient to achieve the correct packed bed density.
3. This innovative process enables optimal bed densities (OBD™) in the prep columns that are the same as analytical columns, which ensures a more reliable column.

STABILITY OF PREP COLUMN PACKED WITH NEW PROCESS-



1. Tylosin
2. Sulfathiazole
3. Ketoprofen

Conditions
Column: XTerra® MS C_{18} 19x50 mm, 5 µm
Mobile Phase A: 1.0% HCOOH
Mobile Phase B: 1.0% HCOOH in ACN
Flow Rate: 18.0 mL/min
Gradient:

Time (min)	%A	%B
0.0	95	5
4.5	25	75
5.0	25	75

Inj. Vol. 400 µL
Detection: UV @ 254 nm
Instrument:: Waters Purification Factory

Before and After QC Test Comparisons on XTerra® Prep Columns

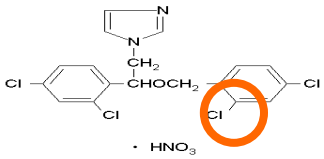
Column	New Columns 21-May-03		Post 1000 Injections 25-Aug-03		% Change in Efficiency
	Plates	USP Tail	Plates	USP Tail	
1	4,278	1.14	3,927	1.17	-8.2
2	4,430	1.05	4,129	1.07	-6.8
3	3,943	1.02	4,054	1.08	2.8
4	4,093	1.03	3,945	1.13	-3.6
Average	**4,186**	**1.06**	**4,014**	**1.11**	**-4.0**

1. We observed no efficiency loss, no peak shape loss, and no pressure increase.
2. The OBD™ prep columns show excellent stability.

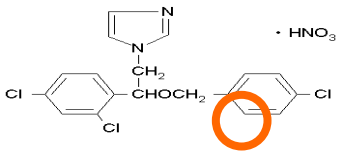
EXPERIMENTAL PROCEDURES FOR EFFICIENCY AND LOADABILITY STUDY-

1. To compare XTerra® columns packed with the old and new procedures, 1000 µL of miconazole and econazole (3.2 mg/mL each in DMSO) were injected into the columns. Resolutions were used to compare efficiency.
2. Various masses of miconazole and econazole samples were injected into these columns at initial R_s of 2, then the total loads were compared to see which column had the highest mass loading.
3. To test the column efficiency with time, samples were injected into these columns under the same unit loading (1000 µL for 19 mm I.D.; 1250 µL for 21.2 mm I.D. columns).
4. Continuous 10 injections were made in daytime from Day 1 to Day 5.
5. Purge columns with ACN:0.1% TFA (70:30, v/v) for approximately 900 column volumes every night.
6. Compare peak shapes and resolution to evaluate how column efficiency changes with time. Repeat steps 4 and 5.

TEST COMPOUNDS FOR EFFICIENCY AND LOADABILITY STUDY-



Miconazole



Econazole

Sample Concentration:	3.2 mg/mL each in DMSO
Mobile phase A:	Water + 0.1% TFA
Mobile phase B:	Acetonitrile + 0.1% TFA
Flow rate:	18 mL/min
Gradient:	10 min linear gradient from 5% B to 95% B.
Detection:	UV @ 280 nm

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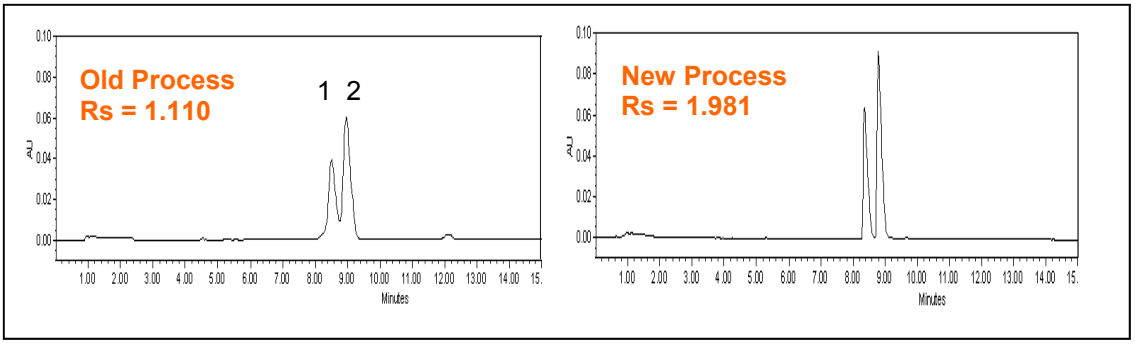
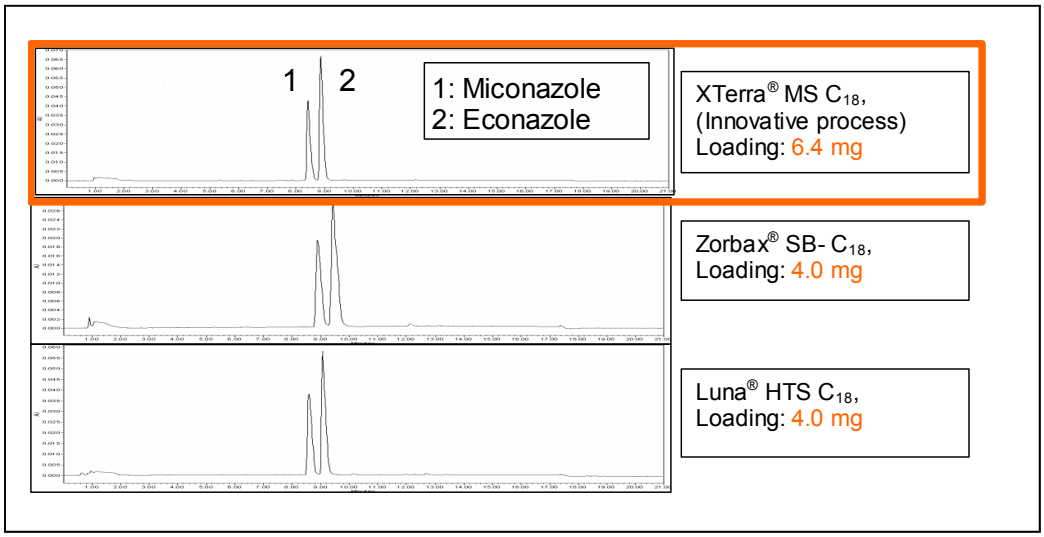
COMPARISON OF PREP COLUMNS MANUFACTURED BY NEW AND OLD PROCESS-



Analytes: 1. Miconazole; 2. Econazole.

XTerra® MS C_{18} column packed with the innovative process exhibits better resolution (efficiency) than the column with traditional process.

COMPARISON OF TOTAL COLUMN LOADINGS UNDER SIMILAR RESOLUTIONS-



XTerra® MS C_{18} column packed with the innovative process exhibits highest mass loading while maintaining the same resolution although it has the smallest dimensions.

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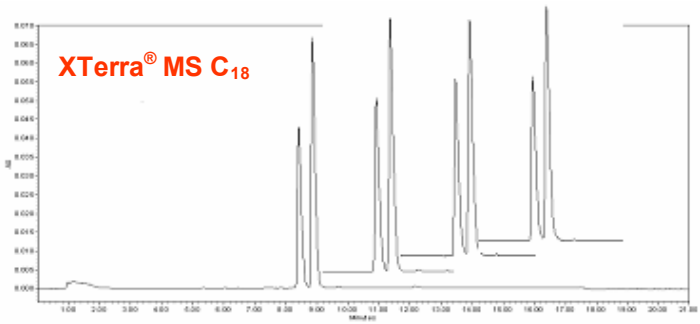
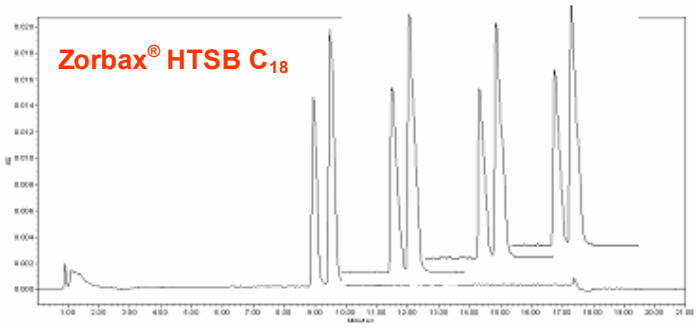
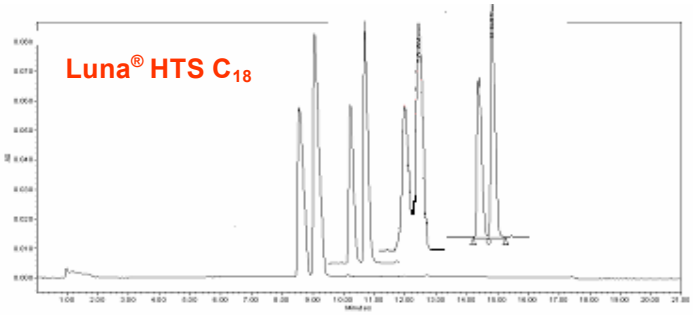
CHANGE OF EFFICIENCY WITH TIME UNDER THE SAME UNIT LOADING-



Resolution:
1.98 (Day 1)
1.75 (Day 2)
1.65 (Day 3)
1.63 (Day 4)



Resolution:
1.86 (Day 1)
1.59 (Day 2)
1.45 (Day 3)
1.42 (Day 4)



Resolution:
1.43 (Day 1)
1.72 (Day 2)
0.49 (Day 3)
1.62 (Day 4)

- XTerra® prep column with innovative process has good reproducibility from day to day.
- Zorbax® prep column has very low loading and its resolution drops dramatically with time.
- Luna® prep column needs a long time to rehydrate in order to achieve good separations. Day to day performances are not very reliable either.

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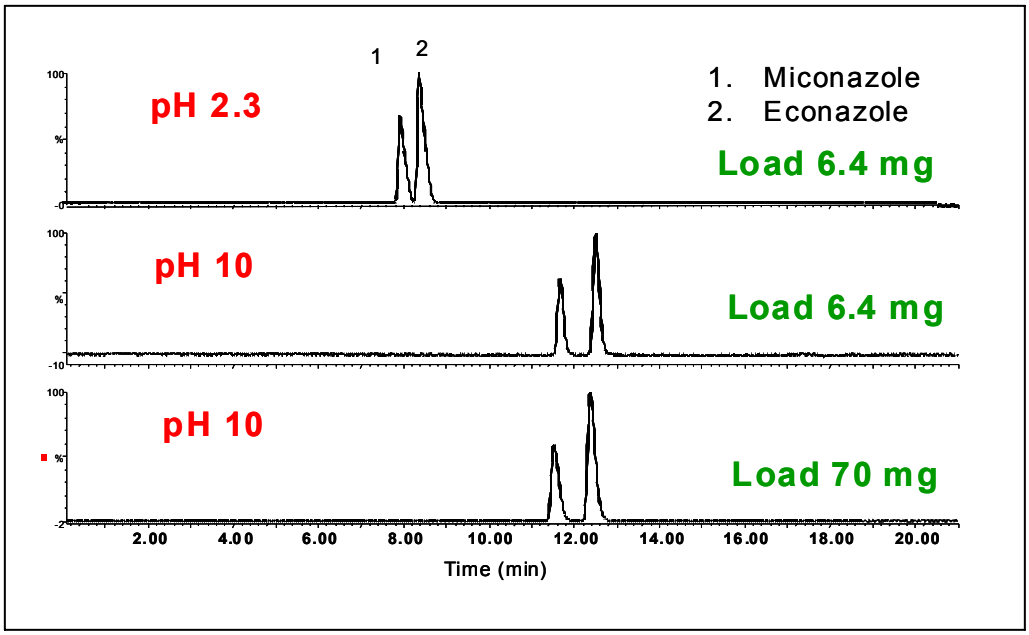
MAXIMIZING LOADING VIA CHANGING PH-



Test Conditions

Column: XTerra® MS C_{18} 19 × 100 mm, 5 µm

Detection: UV @ 280 nm

Instrument: Waters AutoPurification™ System

1. For basic analytes, the peak shapes improve at high pH as well as retention time increase at high pH.
2. 11 fold more material was load on XTerra® MS C_{18} columns at high pH.

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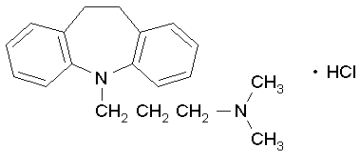
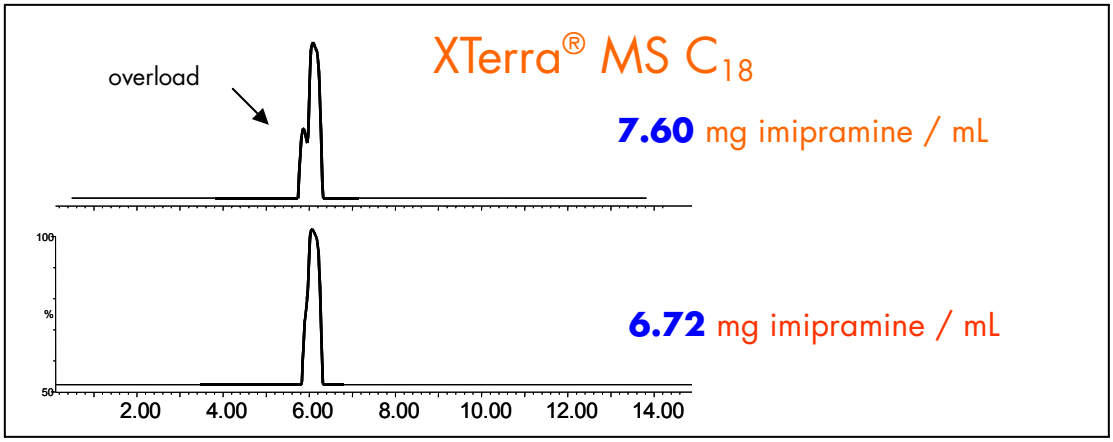
EXPERIMENTAL PROCEDURES FOR BED CAPACITY STUDY-

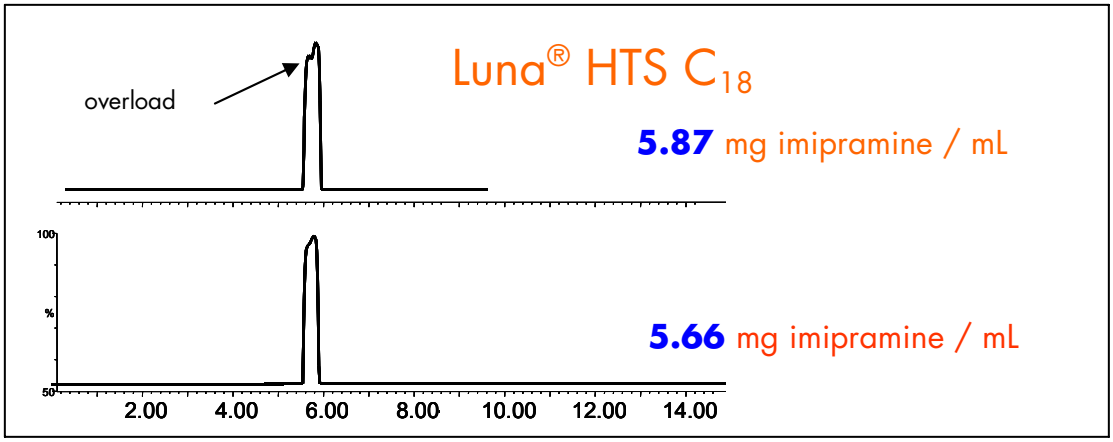
1. Imipramine was dissolved in DMSO at concentration of 50 mg/mL.
2. Gradually increase the injection volume of imipramine onto the XTerra® and Luna® columns until peak shape exhibits overloading.
3. Bed capacity of each column was calculated based on mass balance.

TEST COMPOUND FOR BED CAPACITY STUDY-



Sample Name: Imipramine
Sample Concentration: 50 mg/mL in DMSO
Detection: UV @ 320 nm





XTerra® MS C_{18} column has higher bed capacity than Luna® HTS C_{18} prep column when imipramine was loaded onto the column.

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CONCLUSION-

- We have found that optimal bed density (OBD™) is the key to manufacturing more efficient, stable and reproducible preparative columns with small particles.
- Over the two-year research program, we developed an innovative packing procedures that combines the influences of hardware, particle type, and packed bed density. This new packing process ensures achieving OBD for both analytical an preparative columns.
- The XTerra® columns manufactured with the innovative process exhibit excellent bed stability even after large numbers of injections.
- The XTerra® column manufactured with the innovative process have better efficiency than the old process.
- The XTerra® columns manufactured with the innovative process have better reproducibility and higher loading than the competitors' columns.
- The XTerra® column has higher bed capacities than the Luna® column.
- Under high pH conditions, the total loadings of these two antifungal drugs increased by 11 fold.

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