

Waters column

A Reprint from

*Volume VI
Issue 4, 1997*

*If you would like a subscription,
or more information,
please contact your nearest
Waters Office.*

*Check the Waters website
for up-to-date information:
<http://www.waters.com>*

Published by Waters Corporation
34 Maple Street
Milford, MA 01757 USA
Tel: 508-478-2000
Toll-free: 1-800-252-4752
Fax: 508-872-1990

Editor: Uwe D. Neue, Ph.D.
ISSN #1084-0540

Evaluation of Imitation μ Bondapak™ C₁₈ Columns

Waters μ Bondapak™ C₁₈ column was introduced in 1973 as the first 10 micron particle size bonded reversed-phase column. Twenty-four years later, it remains one of the most widely used HPLC columns, with applications in many different scientific fields. The selectivity of the μ Bondapak™ C₁₈ column is unique, the result of a carefully controlled combination of hydrophobicity and silanol activity. μ Bondapak™ C₁₈ columns are manufactured only at Waters' ISO 9002-certified Material Synthesis Facility in Taunton, Massachusetts, using proprietary procedures.

The popularity of Waters μ Bondapak™ C₁₈ columns has inspired many attempts at imitation. To determine how successful these attempts have been, we recently characterised three imitation columns using a sensitive chromatographic selectivity test. The results are described in this article.

Experimental

The columns evaluated were all 3.9 x 300 mm steel columns, except for the 4.6 x 216 mm Imitation C column. The reference column was an authentic Waters μ Bondapak™ C₁₈ 3.9 x 300 mm column. The chromatography was carried out using a Waters 625 LC System, a 717plus Autosampler, a 490 Programmable Multiwavelength Detector, and a Millennium® Workstation (all from Waters Corporation). The columns were thermostatted at 23.4 (± 0.5) °C using a Mistral® column thermostat.

Results and Discussion

The columns were first tested for efficiency using acenaphthene as the marker, and a mobile phase of 60/40, v/v, acetonitrile/water flowing at 2.0 mL/min. The results are summarised in Table 1.

Table 1: Efficiencies and Tailing Factors for Authentic Waters μ Bondapak™ C₁₈ and Imitation C₁₈ Columns

Column	Efficiency ¹	USP Tailing
μ Bondapak™ C ₁₈	30,200	1.01
Imitation A C ₁₈	20,800	0.92
Imitation B C ₁₈	22,100	0.93
Imitation Ct C ₁₈	21,900	0.98

¹ Plates per meter, measured using 5 sigma method

Table 2: Retention Factors and Relative Retentions for Authentic Waters μ Bondapak™ and Imitation C₁₈ Columns

	μ Bondapak™	Imitation A	Imitation B	Imitation C
Retention Factors				
Propyl paraben	1.47	1.29	1.17	0.98
Toluene	2.43	2.40	1.90	1.72
Propranolol	4.52	6.35	1.61	13.1
Acenaphthene	7.60	7.69	6.23	5.09
Dibutyl phthalate	12.3	11.4	9.61	7.85
Relative Retention versus Acenaphthene				
Propyl paraben	0.193	0.167	0.188	0.193
Toluene	0.320	0.312	0.305	0.339
Propranolol	0.594	0.825	0.258	2.57
Dibutyl phthalate	1.62	1.48	1.54	1.54

The authentic Waters μ Bondapak™ C₁₈ column was found to be the most efficient of the four columns, and also gave the best peak symmetry. The imitation columns were found to have efficiencies that were 25–30% lower, and all showed significant peak fronting (USP Tailing < 1). This is an indication that the imitation columns are not as well packed as the authentic Waters μ Bondapak™ C₁₈ column.

To evaluate the selectivity of the columns we used a test mixture containing acidic, basic, and non-ionisable compounds (see Figure 1). The mobile phase contained methanol (65% v/v) and a pH 7.20 mM potassium phosphate buffer. Under these conditions, the strong base, propranolol ($pK_a \approx 9.6$) is protonated, and interacts with ionised silanol groups

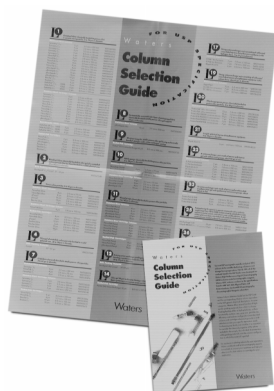
through an ion-exchange mechanism. This causes the propranolol peak to tail noticeably.

The chromatograms obtained on the four columns are shown in Figure 2. The retention factors and several relative retentions are summarised in Table 2. The imitation columns clearly do not match the separation obtained using the authentic Waters μ Bondapak™ C₁₈ column. The largest differences are observed for the strong base, propranolol (marked with arrow). On the authentic Waters μ Bondapak™ C₁₈ column, propranolol elutes midway between toluene and acenaphthene. On the Imitation A C₁₈ columns, however, propranolol partially coelutes with acenaphthene.

This indicates that the silanol activity of the alphaBond™ column is higher than that of an authentic Waters µBondapak™ C₁₈ column. On the Imitation C C₁₈ column, the silanol activity is so much higher that propranolol elutes last, as a barely discernable peak. In contrast, on the Imitation B C₁₈ column propranolol elutes before toluene, indicating reduced silanol activity. Clearly, none of the imitation columns matches the selectivity of an authentic Waters µBondapak™ C₁₈ column.

Conclusions

These results show that there are significant differences between authentic Waters µBondapak™ C₁₈ columns and the imitation columns. The imitation columns were found to have lower efficiencies, and produced fronting peaks. In addition, we found large differences in selectivity, particularly evident in the retention of basic compounds relative to neutral compounds. This is not surprising, because controlling the retention of basic compounds has been one of the most difficult problems in the synthesis of C₁₈-silica packings. Research has shown that base retention is affected not only by the bonding processes, but also by many properties of the silica support. In the final analysis, no imitation column matches the performance of an authentic Waters µBondapak™ C₁₈ column.



For your copy of the Waters Column Selection Guide for USP Specifications, please check box 7 on the business reply card.

Figure 1: Structures of Test Compounds

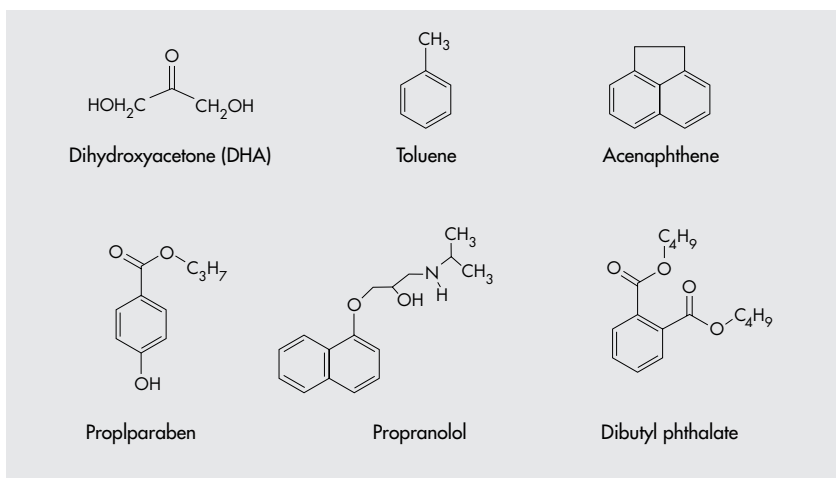
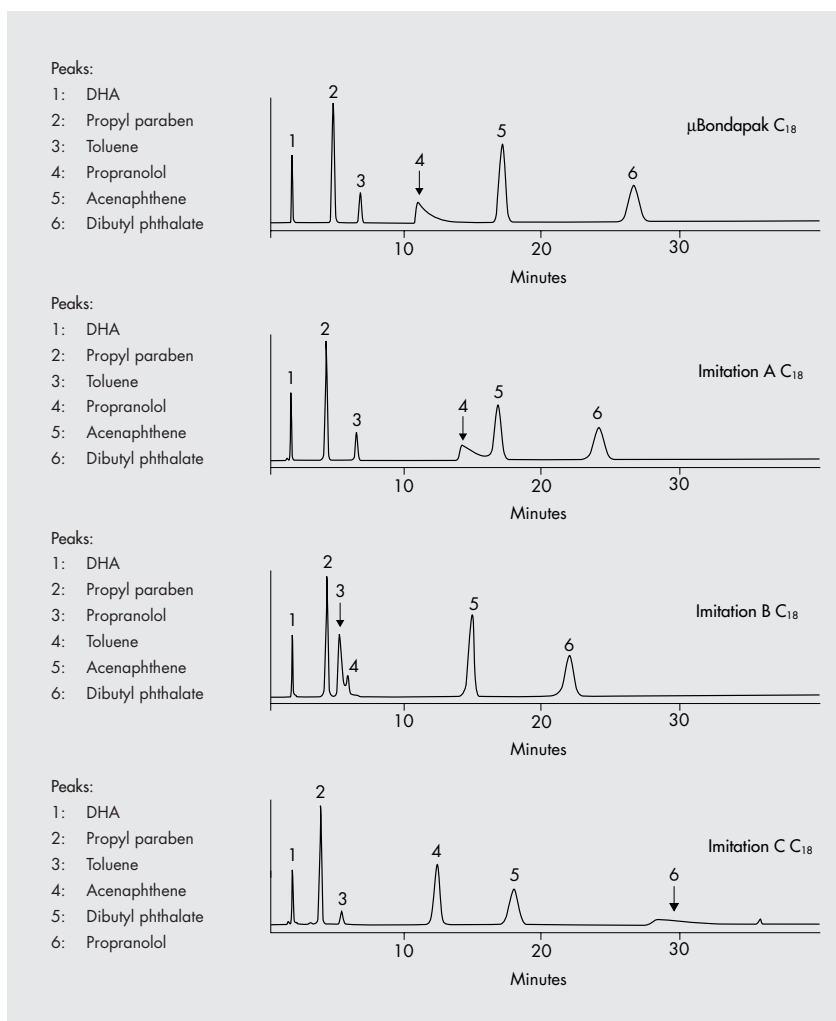


Figure 2: Chromatograms for Selectivity Test Mixture Separated on Authentic Waters µBondapak™ C₁₈ and Imitation Columns



Ordering information

Column	Particle Size	Pore Size	Dimensions	Part No.
μBondapak C ₁₈	10 μm	125Å	2.1 mm x 300 mm	WAT086609
			3.9 mm x 150 mm	WAT086684
			3.9 mm x 300 mm	WAT027324
			4.6 mm x 150 mm	WAT044370
Bondapak C ₁₈	15-20 μm	125Å	3.9 mm x 300 mm	WAT025875
μBondapak C ₁₈ cartridge column (requires endfittings)	10 μm	125Å	4.6 mm x 250 mm	WAT052860
Endfittings (includes 2 sets of reusable endfittings, 2 c-clips and o-rings)				WAT037525

How to Reach Us

Sales Offices

Austria and European Export
(Central Europe, CIS,
Middle East, India and India
Subcontinent)
Waters Ges.m.b.H.
Hietzinger Hauptstrasse 145
A-1130 Wien
Tel: 1 877 1807
Fax: 1 877 1808

Australia Waters Australia Pty.
Limited
Unit 3, 38-46 South Street
P.O. Box 84
Rydalmere NSW 2116
Australia
Tel: 2 9933 1777
Fax: 2 9898 1455

Belgium and Luxemburg
Waters S.A.-N.V.
Roketstraat 60
Rue de la Fusee 60
1130 Brussels
Tel: 02 726 1000
Fax: 02 726 1100

Brazil Waters Comercial LTDA
Avenida Morumbi
8411 - 1 Andar (1st floor)
CEP. 04703-004 Sao Paulo
S.P. Brazil
Tel: 011 55 11 543 7788
Fax: 011 55 11 530 6413

Canada Waters Limited
3687 Nashua Drive, Unit 8
Mississauga, Ontario
L4V 1V5
Tel: 800 252 4752
Fax: 905 678 9237

CIS Representation Office,
Moscow
Ul. Miklukho-Miklaia 16/10
117871 Moscow
Tel: 7 095 336 7000
Fax: 7 095 931 9193

Czech Republic
Waters Ges.m.b.H.
Ohnivcova 11
14700 Praha 4 Branik
Tel: 42 2 472 8672
Fax: 42 2 472 8023

Denmark Waters A/S
Baldersbuen 46
2640 Hedehusene
Tel: 46 598080
Fax: 46 598585

Finland Waters Oy
Kuparitie 1
00440 Helsinki
Tel: 90 506 4140
Fax: 90 5064 1411

France Waters S.A.
B.P. 608
78056 St. Quentin en Yvelines
Cedex
Tel: 1 30487200
Fax: 1 30487211

Germany Waters GmbH
Hauptstraße 87
D-65760 Eschborn
Tel: 06196 40 06 00
Fax: 06196 48 23 88

Hong Kong Waters China Ltd.
Unit 1804, Seaview Commercial
Bldg
21-24 Connaught Road West
Sheung Wan, Hong Kong
Tel: 852 2964 1800
Fax: 852 2549 6802

Hungary Waters kft
Váci ut 202
H-1138 Budapest
Tel: 36 1 270 5086
Fax: 36 1 270 5087

India Waters Pvt. Ltd.
No. 36A, II Phase
Peenya Industrial Area
Bangalore 560080
Tel: 80 8394799
Fax: 80 3392157

Italy Waters S.p.A.
Via Achille Grandi, 27
20090 Vimodrone MI
Tel: 02 2500565
Fax: 02 2501827

Japan Nihon Waters K.K.
No. 5, Koike Bldg.
1-3-12 Kitashinagawa
Shinagawa-ku, Tokyo
Tel: 3 3471 7191
Fax: 3 3471 7116

Malaysia Waters Asia Ltd.
51A Jalan SS25/2
Taman Bukit Emas
47301 Petaling Jaya
Selangor, Malaysia
Tel: 3 704 8600
Fax: 3 704 8599

Mexico Waters S.A. DE C.V.
Adolfo Prieto No. 1634
Col. Del Valle
03100 Mexico, D.F.
Tel: 525 524 7636
Fax: 525 524 7643

The Netherlands
Waters Chromatography B.V.
Postbus 379
Florijnstraat 19
4870 AJ ET TEN-LEUR
Tel: 076 508 7200
Fax: 076 508 7280

Norway Waters AS
Hvamstuppen 17
2013 Skjetten
Tel: 47 638 46 050
Fax: 47 638 46 051

Peoples Republic of China - Beijing
Waters China Ltd.
Suite 709-711, Xinzhong Building
No. 2 Xinzhong Xiejie
Gongti Beilu, Dongcheng District
Beijing, 100027 P.R. China
Tel: 860 10 6592 8917
Fax: 860 10 6506 0344

Poland Waters Sp. z o.o.
ul. Lektykarska 25 m 10
01-687 Warszawa
Tel: 48 22 33 44 00
Fax: 48 22 33 09 87

Puerto Rico
Waters Technologies Corporation
P.O. Box 6870
Caguas, Puerto Rico 00726-6870
Tel: 787 747 8445, 790 2225
Fax: 787 747 8448

Singapore Waters Asia Ltd.
(Asia Headquarters)
No. 12 Prince Edward Road
#01-03 Podium A
Bestway Building
Singapore 079212
Tel: 225 4855
Fax: 225 4655

Spain Waters Chromatografia S.A.
Entenza 24- planta baja
E-08015 Barcelona
Tel: 3 325 9616
Fax: 3 325 9896

Sweden Waters Sverige AB
Box 485, Turebergsvägen 3
S-191 24 Sollentuna
Tel: 08623 0090
Fax: 08623 0095

Switzerland Waters AG
Dorfstrasse 10
5102 Rupperswil
Tel: 062 889 2030
Fax: 062 889 2059

Taiwan Waters Asia Ltd.
Room #603, 6th Floor, No.
136
Sec. 3, Jen-Ai Road
Taipei, Taiwan, 10628, R.O.C.
Tel: 02 706 8766
Fax: 02 706 9704

UK and Ireland Waters Ltd.
The Boulevard
Blackmoor Lane
Watford, Hertfordshire
WD1 8YW
Tel: 01 923 816700
Fax: 01 923 219012

All Other Countries
Waters Corporation
34 Maple Street
Milford, MA 01757 USA
Tel: 508 478 2000
Toll-free: 1 800 252 4752
Fax: 508 872 1990
<http://www.waters.com/>