

Preparative Chromatography Mix Standard

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I. INTRODUCTION

The Quality Control Reference Material (QCRM) portfolio is a unique collection of standards and mixtures. These products allow users to evaluate and benchmark the chromatography system before analysis of critical material. The products in the portfolio are all precisely formulated based on the expertise of Waters scientist.

The preparative chromatography mix is 5 mg/mL each of Diclofenac sodium salt, Diphenhydramine hydrochloride, and Flavone in DMSO.

This standard mix should be used to confirm the benchmark performance of your Preparative/purification system. This particular QCRM is a precisely formulated mix that includes a void marker, neutral, acidic and basic compounds. These compounds were vigorously tested and evaluated and chosen because they provide the following advantages:

- Well-separated
- Easily visually identified
- Acceptable for use on a variety of column

a. The Purpose of a QCRM:

Waters recommends to benchmark your chromatographic system with a QCRM prior to system usage when there is confidence your system is in good working order. It is recommended to run and save the initial results and continue to compare your QCRM results to the previous benchmark any critical assay is run, and after any hardware, column or mobile phase changes.

The QCRM benchmark result will be specific to the performance of the system it is run on. All chromatographic systems have some minor level of variability from run to run. Trending the benchmark results over time will provide an understanding of system typical variability. Trending of the same QCRM result on multiple systems will provide the typical variability of those systems. Trending of the same QCRM result on systems in laboratories in different locations will provide the typical variability from across locations. Setting specification for QCRM results of a system, multiple systems or

between laboratories should not be done without sufficient data trending. Once variability is understood, QCRM results will help determine the capability of the system to provide reliable results.

Determining your QCRM Criteria:

QCRM criteria should be determined based on specific requirements. As mentioned above, specifications should not be set until the variability of the system population is understood. The criteria and specifications should allow it to be determined if the QCRM results indicate that the system is functioning as expected or outside of expectation. Typical criteria might include any of the following: retention time reproducibility, peak area reproducibility, peak tailing plate count, peak resolution, mass accuracy range, sensitivity or response.

b. What affects your QCRM result:

The goal of the QCRM specifications and criteria will be to indicate that the system is functioning as expected or outside of expectation.

The system is comprised of many interdependent components working together to produce results to an expected specification. An issue with any one component can produce erroneous final results. All components performing correctly will produce results within an expected variability. Any changes or technical issues within any one of the system components (hardware, software, or chemical) may add variability to the QCRM result. Potential causes of variability in QCRM results may include the following: mobile phase preparation, column performance, tubing size, system component performance (pump, injector, detector), temperature control, data collection rate, integration.

Differences in any of the components mentioned can result in system to system variability of results even when each system's components are functioning correctly.

II. STORAGE AND STABILITY:

The compounds are stable through the expiration date listed as provided in 1 mL amber ampule before opening. This product is for one time usage. The integrity of the standard can not be guaranteed if stored after first use.

III. USING THE PREPARATIVE CHROMATOGRAPHY MIX

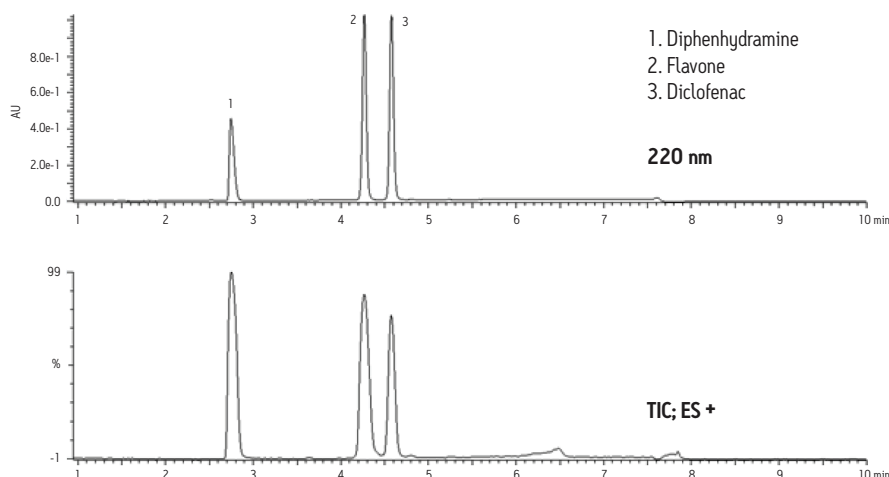
For preparative chromatographic analysis on a 19 x 50 mm column the Preparative Chromatography Standard mix was injected at 10 μ L. The injected quantity should be scaled for other column diameters.

Sample chromatography for the Preparative Chromatography Standard is shown in Figure 1. Note that the use of different column stationary phases and/or column dimensions will have a effect on the separation. On different column chemistries or dimensions, the method may need to be modified or re-developed to obtain sufficient resolution. To properly transfer the separation across column dimensions, use the Prep Calculator. www.waters.com/prepcalculator. The table below indicated the approximate retention times obtained for the compounds when using the specified chromatographic method in Figure 1, as well as the m/z criteria for each compound.

Compound	Type	MS (M+H)	Approximate RT (min) (XSelect™ CSH™ C ₁₈ , 5 μ m, 19 x 50 mm) 220 nm
Diclofenac sodium salt	Acid	296.02	4.6
Diphenhydramine hydrochloride	Base	256.17	2.8
Flavone	Neutral	223.07	4.3

Table 1: Preparative Mix UV and MS

Figure 2 shows an example of the chromatography obtained for the preparative mix via UV and MS when the method in Figure 1 is using an XSelect™ CSH C₁₈, 5 μm, 19 x 50 mm.



IV. QCRM TESTING

The use of reference standards for QCRM testing should allow the analyst to track important instrument analytical parameters such as peak width, peak area, retention time, and peak resolution. Each of these important parameters can be tracked and evaluated using control charts. The use of a high quality reference standard allows the analyst to reliably measure and track these parameters.

QCRM testing should be performed on a regular basis for each instrument/analyst combination or instrument per test method. The data should be collected and entered into a control chart allowing the analyst to evaluate the system performance over time. The use of performance control charts has been a staple of analytical chemistry quality control. The most common form of the control charting is to track the analytical results and statistically analyze the data to a 99% (3 standard deviations) or 95% confidence interval (2 standard deviations) confidence interval around the mean of the data to establish upper control limits (UCL) and lower control limits (LCL).

The initial criteria to establish a mean, standard deviation and control limits involves analyzing a reference material a minimum of 7 times to establish an initial estimate of precision and bias. This provides the analyst with sufficient data to be statistically valid. The analyses should be carried over the course of several days to provide a more realistic view of the system variability. The frequency of analyzing system performance will be dependent on the stability of the analysis and the analytes. QCRM should always be evaluated after maintenance has been performed, or when changes to the system or analytical procedure have been made.

The example in Table 1 uses retention time monitoring to establish a set of control limits for the purpose of monitoring on-going system performance.

Table 1: Reference Standard Retention Time Data Example

Analysis Peak	Retention Time (mins)
1	7.10
2	7.11
3	7.12
4	7.09
5	7.08
6	7.10
7	7.11
8	7.13
9	7.10
10	7.11
Mean 7.11	
Standard Deviation 0.0136	
LCL 7.08	
UCL 7.13	

The standard reference material was analyzed 10 times yielding the above retention times. The mean retention time and standard deviation were calculated and from this the UCL and LCL limits were determined. The control limits represent a 95% confidence interval (2 standard deviations) for the data. The control chart in Figure 2 was then produced to establish that the instrument retention times are in control.

Figure 2: Retention Time Control Chart

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	25.00	95.0	5.0	Initial
2	0.10	25.00	95.0	5.0	6
3	6.10	25.00	5.0	95.0	6
4	7.10	25.00	5.0	95.0	6
5	7.20	25.00	95.0	5.0	6
6	10.10	25.00	95.0	5.0	6

A = 0.1% TFA in water
B = 0.1% TFA in acetonitrile

The establishment of control limits provides data as to the current capabilities of the system. Control charting allows the quality professional to compare instrument performance to the required method specifications.

The process of continuous quality improvement can also be tracked using control charts. When improvements are made to a method, control charts allow you to see that the changes you have made are effective and having the desired impact. The control chart will also allow you to track trends over time. By observing the data trending higher or lower over time, you can take preventative action prior to having an out of specification result.

Control charting can be employed for each QCRM criteria; peak retention time, peak area, peak width, and peak resolution. Control charts allow quality control professionals to establish statistically significant criteria to monitor and control their HPLC analyses thereby avoiding criteria that are too stringent or set arbitrarily.

Summary

The use of high quality reference standards specifically designed for the system analysis, provide a controlled, consistent, and reliable measure of system performance. Regular use of reference standards and control charting the data provides improved monitoring of system performance and system robustness, while at the same time providing assurance that any results produced are high quality, reliable, and reproducible.

References

- 1) Taylor, J.K., "Quality Assurance of Chemical Measurements", Lewis Publishers, 1987
- 2) Smith, G.M., "Statistical Process Control and Quality Improvement", 3rd edition, Prentice Hall, 1998
- 3) Ahuja, S. and Dong, M.W., "Handbook of Pharmaceutical Analysis by HPLC", Elsevier Inc., 2005

V. TROUBLESHOOTING:

The Preparative Chromatography Standard contains an acidic, basic and neutral compound. Due to the vastly different properties of stationary phases, not all compounds will behave similarly on each column. For instance, basic compounds often have poor peak shape on reversed-phase columns at neutral pH due to increased interactions between the charged bases and silanols on the surface of the stationary phase. For this reason, it is very important to benchmark the performance of the Preparative Chromatography Standard on a new column and functioning system. This will help to identify whether poor peak shape issues are due to compound interactions on the column, or failing column/system performance.

Failure to meet QCRM criteria will result in the need to troubleshoot the system. Some chromatographic issues may be easily resolved, for instance, a missing peak may simply be due to co-elution of two peaks. However, most issues such as poor peaks shape, tailing peaks, retention time shifts and poor peak response, to name a few, may be due to a variety of causes that can be difficult to pinpoint. For a detailed and comprehensive guide to troubleshooting, please refer to the HPLC Troubleshooting Guide (WA20769) on the Waters website.

VI. ORDERING INFORMATION

Description	Part Number
Preparative/Purification Chromatography Mix Standard	186006703

Thank you for choosing a QCRM from Waters. The standards are manufactured in our ISO 9001 ISO 17025 facility. Each standard is manufactured to ensure optimal reproducibility from lot to lot. A Waters QCRM can be depended on for its' accuracy. This removes one variable from your system variability and provides you the most dependable starting point for your testing.

If the QCRM box shows significant damage, notify the carrier and your supplier at once and retain evidence of shipping damage so that a claim can be made.

Related Products

XSelect Preparative Columns

Description	Part Number
XSelect CSH C ₁₈ OBD, 5 µm, 19 x 50 mm	186005420
XSelect CSH C ₁₈ OBD, 5 µm, OBD 19 x 100 mm	186005421
XSelect CSH C ₁₈ OBD, 5 µm, OBD 19 x 150 mm	186005422
XSelect CSH C ₁₈ OBD, 5 µm, OBD 19 x 250 mm	186005492
XSelect CSH C ₁₈ OBD, 5 µm, OBD 30 x 50 mm	186005423
XSelect CSH C ₁₈ OBD, 5 µm, OBD 30 x 75 mm	186005424
XSelect CSH C ₁₈ OBD, 5 µm, OBD 30 x 100 mm	186005425
XSelect CSH C ₁₈ OBD, 5 µm, OBD 30 x 150 mm	186005426
XSelect CSH C ₁₈ OBD, 5 µm, OBD 30 x 250 mm	186005493
XSelect CSH C ₁₈ OBD, 5 µm, OBD 50 x 50 mm	186005494
XSelect CSH C ₁₈ OBD, 5 µm, OBD 50 x 100 mm	186005495
XSelect CSH C ₁₈ OBD, 5 µm, OBD 50 x 150 mm	186005496
XSelect CSH C ₁₈ OBD, 5 µm, OBD 50 x 250 mm	186005497
XSelect CSH Fluoro-Phenyl OBD, 5 µm, 19 x 50 mm	186005433
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 19 x 100 mm	186005434
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 19 x 150 mm	186005435
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 19 x 250 mm	186005499
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 30 x 50 mm	186005436
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 30 x 75 mm	186005437
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 30 x 100 mm	186005438
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 30 x 150 mm	186005439
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 30 x 250 mm	186005500
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XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 50 x 100 mm	186005502
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 50 x 150 mm	186005503
XSelect CSH Fluoro-Phenyl OBD, 5 µm, OBD 50 x 250 mm	186005504
XSelect CSH Phenyl-Hexyl OBD, 5 µm, 19 x 50 mm	186005446
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 19 x 100 mm	186005447
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 19 x 150 mm	186005448
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 19 x 250 mm	186005506
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 30 x 50 mm	186005520
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 30 x 75 mm	186005450
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 30 x 100 mm	186005451
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 30 x 150 mm	186005452
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 30 x 250 mm	186005507
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 50 x 50 mm	186005508
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 50 x 100 mm	186005509
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 50 x 150 mm	186005510
XSelect CSH Phenyl-Hexyl OBD, 5 µm, OBD 50 x 250 mm	186005511

XBridge Preparative Columns

Description	Part Number
XBridge BEH C ₁₈ OBD, 5 µm, 19 x 50 mm	186002977
XBridge BEH C ₁₈ OBD, 5 µm, 19 x 100 mm	186002978
XBridge BEH C ₁₈ OBD, 5 µm, 19 x 150 mm	186002979
XBridge BEH C ₁₈ OBD, 5 µm, 19 x 250 mm	186004021
XBridge BEH C ₁₈ OBD, 5 µm, 30 x 50 mm	186002980
XBridge BEH C ₁₈ OBD, 5 µm, 30 x 75 mm	186002981
XBridge BEH C ₁₈ OBD, 5 µm, 30 x 100 mm	186002982
XBridge BEH C ₁₈ OBD, 5 µm, 30 x 150 mm	186003284
XBridge BEH C ₁₈ OBD, 5 µm, 30 x 250 mm	186004025
XBridge BEH C ₁₈ OBD, 5 µm, 50 x 50 mm	186003933
XBridge BEH C ₁₈ OBD, 5 µm, 50 x 100 mm	186003937
XBridge BEH C ₁₈ OBD, 5 µm, 50 x 150 mm	186003929
XBridge BEH C ₁₈ OBD, 5 µm, 50 x 250 mm	186004107
XBridge BEH C ₁₈ OBD, 10 µm, 19 x 50 mm	186003893
XBridge BEH C ₁₈ OBD, 10 µm, 19 x 100 mm	186003901
XBridge BEH C ₁₈ OBD, 10 µm, 19 x 150 mm	186003894
XBridge BEH C ₁₈ OBD, 10 µm, 19 x 250 mm	186003895
XBridge BEH C ₁₈ OBD, 10 µm, 30 x 75 mm	186004711
XBridge BEH C ₁₈ OBD, 10 µm, 30 x 100 mm	186003930
XBridge BEH C ₁₈ OBD, 10 µm, 30 x 150 mm	186003896
XBridge BEH C ₁₈ OBD, 10 µm, 30 x 250 mm	186003897
XBridge BEH C ₁₈ OBD, 10 µm, 50 x 50 mm	186003898
XBridge BEH C ₁₈ OBD, 10 µm, 50 x 100 mm	186003902
XBridge BEH C ₁₈ OBD, 10 µm, 50 x 150 mm	186003899
XBridge BEH C ₁₈ OBD, 10 µm, 50 x 250 mm	186003900

SunFire Preparative Columns

Description	Part Number
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 19 x 50 mm	186002566
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 19 x 100 mm	186002567
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 19 x 150 mm	186002568
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 19 x 250 mm	186004027
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 30 x 50 mm	186002570
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 30 x 75 mm	186002571
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 30 x 100 mm	186002572
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 30 x 150 mm	186002797
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 30 x 250 mm	186003969
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 50 x 50 mm	186002867
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 50 x 100 mm	186002869
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 50 x 150 mm	186003941
SunFire C ₁₈ OBD Prep Column, 100Å, 5 µm, 50 x 250 mm	186003970
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 19 x 150 mm	186002668
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 19 x 250 mm	186002669
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 30 x 50 mm	186003854
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 30 x 100 mm	186003971
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 30 x 150 mm	186002670
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 30 x 250 mm	186002671
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 50 x 50 mm	186002871
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 50 x 100 mm	186003972
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 50 x 150 mm	186002672
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 50 x 250 mm	186002673
SunFire C ₁₈ OBD Prep Column, 100Å, 10 µm, 100 x 250 mm	186003928
SunFire C ₁₈ Prep Column, 100Å, 5 µm, 10 x 50 mm	186002561
SunFire C ₁₈ Prep Column, 100Å, 5 µm, 10 x 100 mm	186002562
SunFire C ₁₈ Prep Column, 100Å, 5 µm, 10 x 150 mm	186002563
SunFire C ₁₈ Prep Column, 100Å, 5 µm, 10 x 250 mm	186002564
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SunFire C ₁₈ Prep Column, 100Å, 10 µm, 30 x 250 mm	186002671
SunFire C ₁₈ Prep Column, 100Å, 10 µm, 50 x 50 mm	186002871
SunFire C ₁₈ Prep Column, 100Å, 10 µm, 50 x 100 mm	186003972
SunFire C ₁₈ Prep Column, 100Å, 10 µm, 50 x 150 mm	186002672
SunFire C ₁₈ Prep Column, 100Å, 10 µm, 50 x 250 mm	186002673
SunFire C ₁₈ Prep Column, 100Å, 10 µm, 100 x 250 mm	186003928

SunFire Preparative Scouting Columns

Description	Part Number
SunFire C ₁₈ Column, 100Å, 10 µm, 4.6 x 150 mm	186003390
SunFire C ₁₈ Column, 100Å, 10 µm, 4.6 x 250 mm	186003391

SymmetryPrep Columns

Description	Part Number
Symmetry C ₁₈ Prep Column, 100Å, 5 µm, 7.8 x 50 mm	186000208
Symmetry C ₁₈ Prep Column, 100Å, 5 µm, 7.8 x 100 mm	186000209
Symmetry C ₁₈ Prep Column, 100Å, 5 µm, 19 x 50 mm	186000210
Symmetry C ₁₈ Prep Column, 100Å, 5 µm, 19 x 100 mm	186000211
Symmetry C ₁₈ Prep Column, 100Å, 5 µm, 30 x 100 mm	186000236
Symmetry C ₁₈ Prep Column, 100Å, 7 µm, 7.8 x 150 mm	WAT066288
Symmetry C ₁₈ Prep Column, 100Å, 7 µm, 7.8 x 300 mm	WAT066235
Symmetry C ₁₈ Prep Column, 100Å, 7 µm, 19 x 150 mm	WAT066240
Symmetry C ₁₈ Prep Column, 100Å, 7 µm, 19 x 300 mm	WAT066245

Symmetry300 Columns

Description	Part Number
Symmetry C ₁₈ Prep Column, 300Å, 5 µm, 19 x 50 mm	186001848
Symmetry C ₁₈ Prep Column, 300Å, 5 µm, 19 x 100 mm	186001849
Symmetry C ₁₈ Prep Column, 300Å, 5 µm, 19 x 150 mm	186001850

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