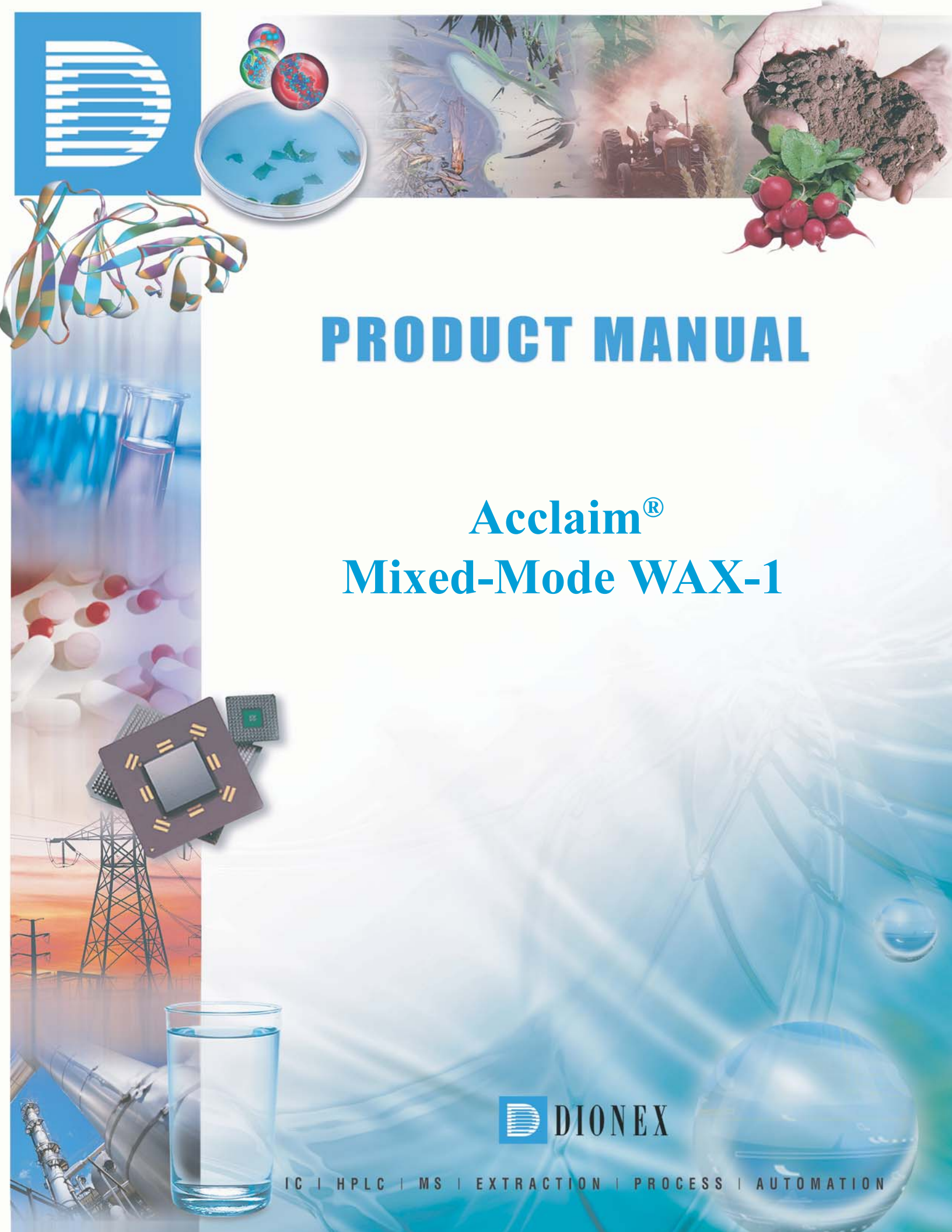


PRODUCT MANUAL

Acclaim®
Mixed-Mode WAX-1

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

for the

Acclaim® Mixed-Mode WAX-1 Columns

4.6 x 150 mm, P/N 064984
4.6 x 250 mm, P/N 064985
2.1 x 150 mm, P/N 067084

Acclaim® Mixed-Mode WAX-1 Guards

4.3 x 10 mm, P/N 064986
2.1 x 10 mm, P/N 067085

Document No. 065165
Revision 02
January 7, 2008

TABLE OF CONTENTS

SECTION 1 – INTRODUCTION

The Acclaim® Mixed-Mode WAX-1 column is based on a new mixed-mode silica-based packing material that incorporates both hydrophobic and weak anion-exchange properties. Unlike traditional reversed-phase stationary phases, the new packing features an alkyl long chain with an ionizable terminus, and demonstrates great potentials for separating a wide range of anionic compounds containing mixtures, including pharmaceuticals, food & beverage, chemical, and more.

1.1. Comparison of Mixed-Mode Chromatography with Reversed-Phase, Ion-exchange and Ion-pairing Chromatography

Reversed-phase (RP) silica columns (e.g. C18) are the most widely used stationary phases for a wide range of liquid chromatography (LC) separations. However, hydrophilic ionic compounds such as small organic acids or inorganic ions are poorly retained and separated on these columns.

Ion exchange columns are used to separate ionic or ionizable compounds such as proteins, nucleic acids, inorganic ions, small organic acids, etc. Because most conventional ion-exchange stationary phases provide inadequate hydrophobic retention for neutral molecules, they have limited applications in small molecules separations.

Ion pairing chromatography is a method for separating ionic or ionizable compounds on a conventional RP medium, which requires hydrophobic ionic compounds, typically comprised of an alkyl chain with an ionizable terminus, are added to the mobile phase. Generally, retention of neutral analytes is nearly unaffected, while analytes with charges complementary to the ion pairing reagent are retained for a longer period of time and analytes with the same charge as the ion pairing reagent are retained for a shorter period of time. Limitations of ion pairing chromatography include long column equilibration times and the quantity of solvent and time needed to elute the ion pairing reagent from the column.

Mixed-mode chromatography combines aspects of ion exchange chromatography and conventional reversed-phase chromatography. A mixed-mode stationary phase has both hydrophobic and ion-exchange properties. These two strong interactions of the phase with analytes allow for controlling retention of ionizable and neutral molecules independently. As a result, many application challenges involving hydrophilic ionizable compounds that are difficult for C18 columns, can be easily tackled on a mixed-mode column.

1.2. Features

1. Adjustable selectivity.
2. Selectivity complementary to reversed-phase columns.
3. Simultaneous separation of acidic, basic, and neutral molecules.
4. High capacity and unique selectivity for anionic molecules.
5. Multi-mode retention mechanisms: reversed-phase, anion-exchange, and HILIC modes.

1.3. Specifications and Recommended Operating Conditions

Flow Rate Range: 4.6mm ID 0.5 - 4 mL/min (not to exceed backpressure limit)
Flow Rate Range: 2.1mm ID 0.1 - 0.8 mL/min (not to exceed backpressure limit)
Shipping Solution 60 / 40 Acetonitrile / 100mM Ammonium Acetate
Storage Solution: 60 / 40 Acetonitrile / 100mM Ammonium Acetate

Buffer pH Range: pH 2.5 - 7.5
Temperature Range < 50°C
Pressure Limit 4000 psi (266 bar)

1.4. Physical Characteristics

Bonding Chemistry: Proprietary alkyl
Silica Substrate: Spherical, high-purity
Particle size 5 µm
Surface area 300 m^2/g
Pore size 120 Å

1.5. Acclaim Mixed-Mode WAX-1 Products

Part number Description Dimensions

064984	Acclaim Mixed-Mode WAX-1, 5µm	4.6 x 250mm
064985	Acclaim Mixed-Mode WAX-1, 5µm	4.6 x 150mm
067084	Acclaim Mixed-Mode WAX-1, 5µm	2.1 x 150mm
064986	Acclaim Mixed-Mode WAX-1, 5µm, guard (set of 2)	4.3 x 10mm
067085	Acclaim Mixed-Mode WAX-1, 5µm, guard (set of 2)	2.1 x 10mm
059526	Guard Holder and Coupler Kit	

SECTION 2 – INSTALLATION: Step-by-Step User Guide

Step 1 – Validating Column performance

Dionex recommends that you perform an efficiency test on your Acclaim Mixed-Mode HILIC column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box (also included in Figures 1, 2 and 3 of this manual). Repeat the test periodically to track the column performance over time. Note that slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique. The steps below outline the steps in preparing your system and using your column.

.

Please see the example Quality Assurance Report in Figures 1, 2 and 3.

Step 2 – Mobile phase preparation

Obtaining reliable, consistent and accurate results require mobile phases that are free of ionic and spectrophotometric impurities. Chemicals, solvents and de-ionized water used to prepare mobile phase should be of the highest purity available. Maintaining low trace impurities and low particle levels in mobile phases helps to protect your columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

<u>De-ionized Water</u>

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade Water, or HPLC Grade Water. The de-ionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE

Degas the aqueous component of the mobile phase and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents, if possible, since the volatile solvent can be 'boiled' off from the solution.

<u>Solvents</u>

The solvents used must be free from ionic and UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent.

<u>Mobile Phase for Column Performance Test:</u>

Depending on specific application, the mobile phase system consists of an organic modifier (e.g. acetonitrile or methanol) and a buffer (e.g. phosphate buffer).

Example A. Preparation of 50 mM, pH phosphate buffer.

1. Weigh 13.6 g potassium monobasic phosphate and 0.5 g sodium pyrophosphate*.
2. Completely dissolve above two salts in 2000 g of D.I. water.
3. Carefully adjust the pH to pH 6 with HCl or NaOH.

* Pyrophosphate is used to eliminate metal interference from stainless steel instrument (pump head, autosampler, tubings, etc) and column hardware.

Example B. Preparation of a mobile phase containing 50 mM, pH 6 phosphate buffer and acetonitrile (50:50 v/v).
Pre-mixed: mix 500 g of 50 mM, pH 6 phosphate buffer and 390 g of acetonitrile.
Proportioning valve generated: 50/50 v/v 50 mM, pH 6 phosphate buffer/acetonitrile.

NOTE ***These two mobile phases could give slightly different results due to the ways they are prepared.***

Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a UV detector, and an injector (or an auto-sampler). The system should be thoroughly primed before use.

Step 4 – Condition the column

The column is shipped in an acetonitrile-water mixture, therefore when a new column is used for the first time, it should be washed thoroughly with the mobile phase (e.g., for at least 45 min at the normal flow rate) before any injection is made. When switching to a new mobile phase, make sure that the new mobile phase is compatible with the previous mobile phase in the column to avoid column clogging due to precipitation. The column should be fully conditioned before any injection is made (e.g. at least 45 min at the normal flow rate).

Step 5 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column performance test using the conditions described in the Quality Assurance Report (see Figures 1, 2 and 3) and compare the result with the one in the report. After the column is fully equilibrated, multiple injections should be made until the reproducible retention is obtained. Keep a record of the column performance for future reference.

NOTE ***Due to various reasons, such as differences in LC systems, mobile phases oven temperature control, etc, you may observe slightly different retention time for the iodide peak from that in the report.***

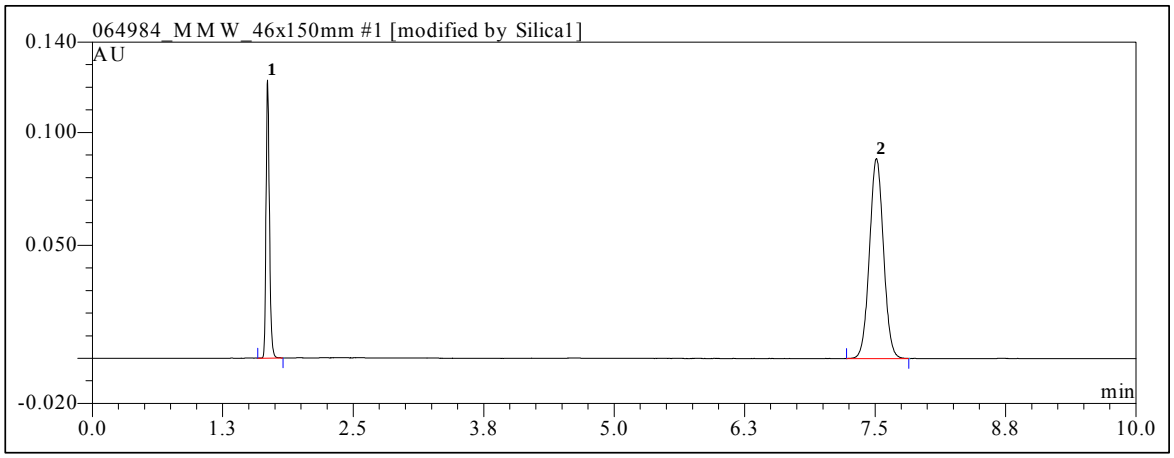
Step 6 – Real sample analysis

Contamination of the column by particulate matter in the sample is a leading cause of column failure. Symptoms may include high pressure, poor symmetry, extraneous peaks, or low efficiency. Pass the sample through a 0.5µm or smaller porosity filter before injection. Biological samples are especially troublesome due to dissolved proteins that may precipitate some time after the initial preparation.

Acclaim® Mixed-Mode WAX-1
5µm 120Å (4.6 X 150 mm)
Product No. 064984

Date: **18-Dec-06 11:21**
Serial No. : **000010**
Lot No. : **PO-3**

Mobile Phase: 50/50 v/v Acetonitrile/50 mM Phospate buffer, pH 6.0
Flow Rate: 1.00 mL/min **Injection Volume:** 5.0 µL
Detection: 220 nm **Temperature:** 30 °C
Storage Solution: Mobile Phase



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Plates/Column (EP)	Concentration (ppm)
1	Uracil	1.68	1.20	13660	100.0
2	Iodide	7.51	1.03	15152	100.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=11,294	Passed
Iodide	Asymmetry	0.95-1.32	Passed
	Pressure	<=1320	720

Production Reference:
Datasource: QAR
Directory: Acclaim\Mixed-Mode_WAX-1
Sequence: 064984_MMW_46x150mm
Sample No: 1

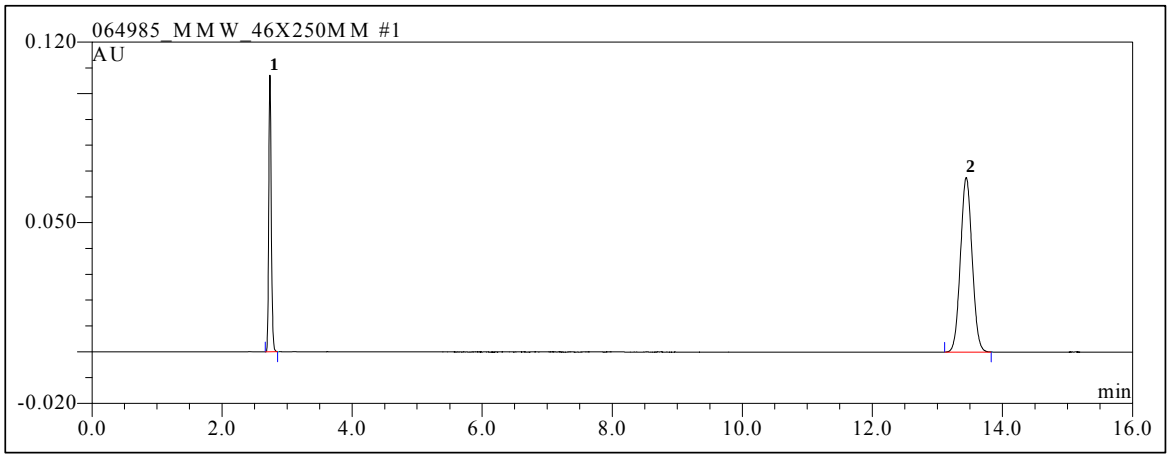
6.80 Build 2212 (Demo-Installation)

Figure 1

Acclaim® Mixed-Mode WAX-1
5µm 120Å (4.6 X 250 mm)
Product No. 064985

Date: **19-Dec-06 12:20**
Serial No. : **000008**
Lot No. : **PO-1**

Mobile Phase: 50/50 v/v Acetonitrile/50 mM Phospate buffer, pH 6.0
Flow Rate: 1.00 mL/min **Injection Volume:** 5.0 µL
Detection: 220 nm **Temperature:** 30 °C
Storage Solution: Mobile Phase



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Plates/Column (EP)	Concentration (ppm)
1	Uracil	2.74	1.15	25505	100.0
2	Iodide	13.44	1.05	26879	100.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=16,941	Passed
Iodide	Asymmetry	0.95-1.32	Passed
	Pressure	<=1980	1180

Production Reference:
Datasource: QAR
Directory: Acclaim\Mixed-Mode_WAX-1
Sequence: 064985_MMW_46X250MM
Sample No: 1

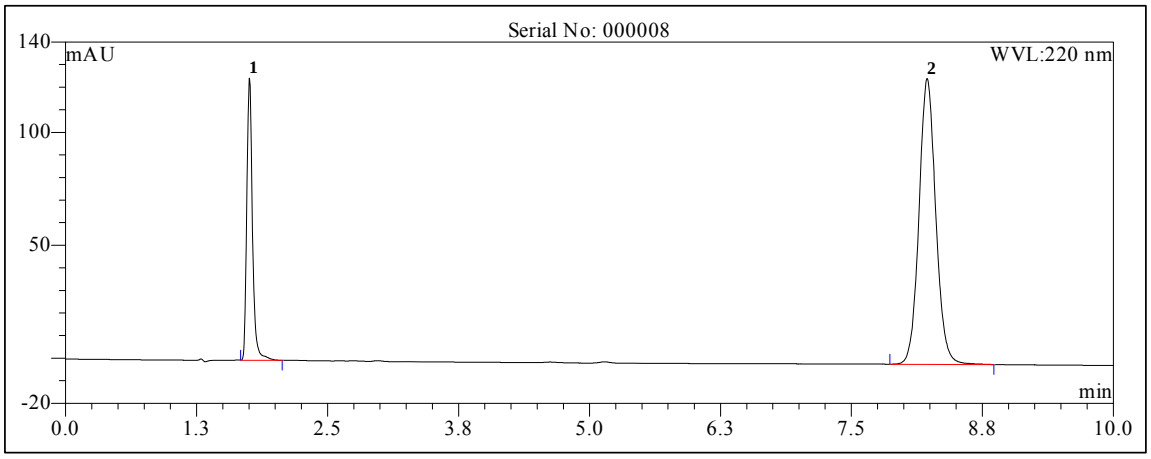
6.80 Build 2212 (Demo-Installation)

Figure 2

Acclaim® Mixed-Mode WAX-1
5µm 120Å (2.1 X 150 mm)
Product No. 067084

Date: **01-Nov-07 16:58**
Serial No. : **000008**
Lot No. : **07-12-53**

Mobile Phase: 50/50 v/v Acetonitrile/50 mM Phospate buffer, pH 6.0
Flow Rate: 0.21 mL/min **Injection Volume:** 1.0 µL
Detection: 220 nm **Temperature:** 30 °C
Storage Solution: Mobile Phase



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Plates/Column (EP)	Concentration (ppm)
1	Uracil	1.75	1.27	5630	100.0
2	Iodide	8.22	1.07	13300	100.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=10,353	Passed
Iodide	Asymmetry	0.95-1.32	Passed
	Pressure	<=1320	746

Production Reference:
Datasource: Silica
Directory: Silica2\Silica2_1
Sequence: 067084_10-25-2007
Sample No: 33

6.80 SP2 Build 2284 (Demo-Installation)

Figure 3

SECTION 3 – METHOD DEVELOPMENT

To optimize chromatographic methods, mobile phase ionic strength, pH, and organic modifier are three key variables that can be adjusted either independently or concurrently.
(Figure 4 see pg. 11, Figure 5 see pg.12, Figure 6 see pg.13)

3.1. Ionic Strength

Ionic strength is crucial for changing retention of charged molecules.
An increase in ionic strength can create the following results:
a) Retention decrease for acidic molecules
b) Retention increase for basic molecules
e) Minimal effect for neutral molecules

3.2. Organic Modifier

Hydrophobic retention is markedly affected by organic modifier composition in the mobile phase. In general, all types of molecules (acids, bases, and neutrals) are less retained on this column with increased organic content in the mobile phase, when keeping other conditions constant (e.g. ionic strength, pH, temperature, etc).

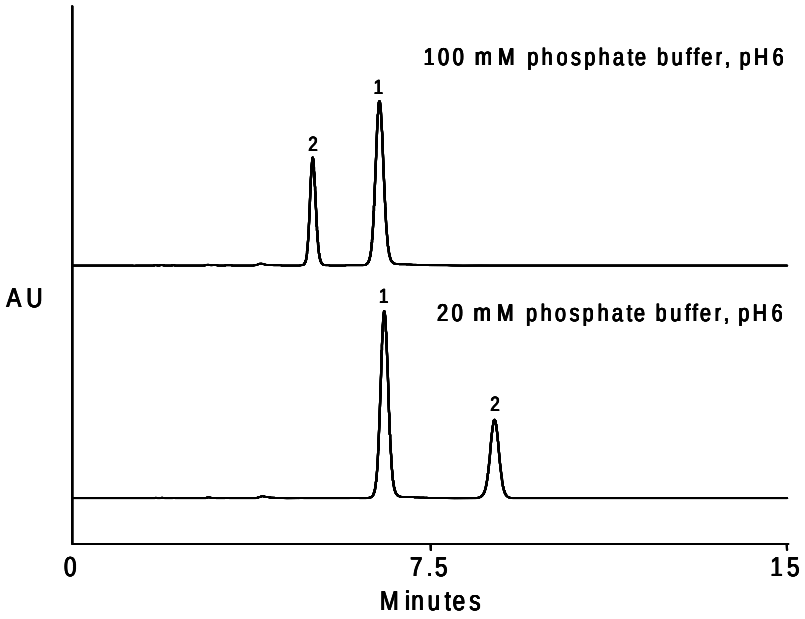
3.3. Mobile Phase pH

Although pH has little effect on retaining neutral molecules, it affects anionic molecules significantly. For example, with pH decrease, molecules containing carboxylic groups are less negatively charged, giving rise to decreased ion-exchange retention.

3.4. Isocratic vs. Gradient

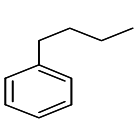
For many applications that involve small number of analytes, it is usually easier to develop an isocratic method on the Acclaim Mixed-Mode WAX-1 column than a reversed-phase column. For more complicated separations, such as one that concerns a mixture of molecules with different types and numbers of charge, as well as different hydrophobicity, a gradient method could be advantageous. In practical, ionic strength gradient, organic modifier gradient, or a combination of both has proven to be satisfactory with respect to reproducibility and simplicity.

Adjustable Selectivity - Ionic Strength Effect

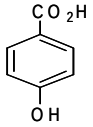


Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 50/50 v/v acetonitrile/20 mM phosphate buffer
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 2 µL
Detection: UV @ 210 nm
Peaks:

1. Butylbenzene (0.1 mg/mL)
2. 4-Hydroxybenzoic acid (0.5 mg/mL)



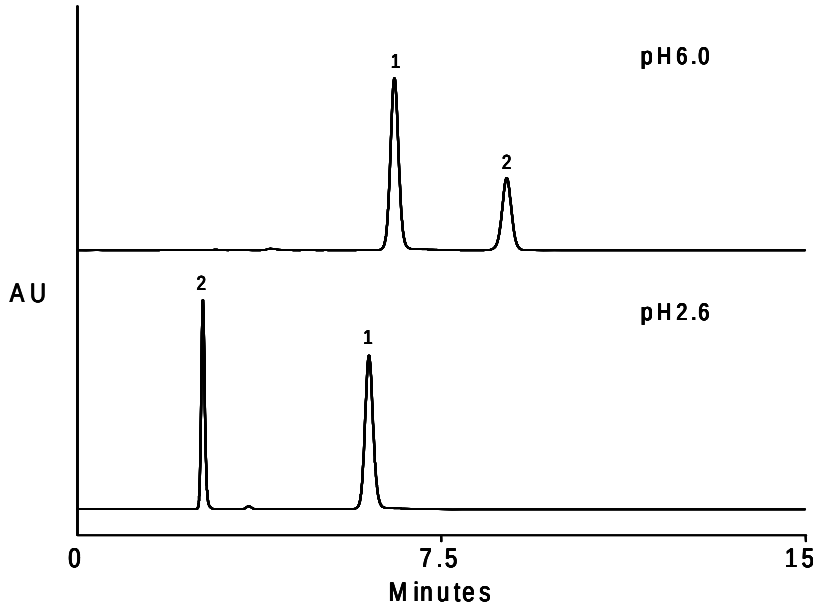
Butylbenzene



4-Hydroxybenzoic acid

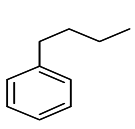
Figure 4

Adjustable Selectivity - pH Effect

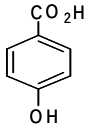


Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 50/50 v/v acetonitrile/20 mM phosphate buffer
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 2 µL
Detection: UV @ 210 nm
Peaks:

1. Butylbenzene (0.1 mg/mL)
2. 4-Hydroxybenzoic acid (0.5 mg/mL)



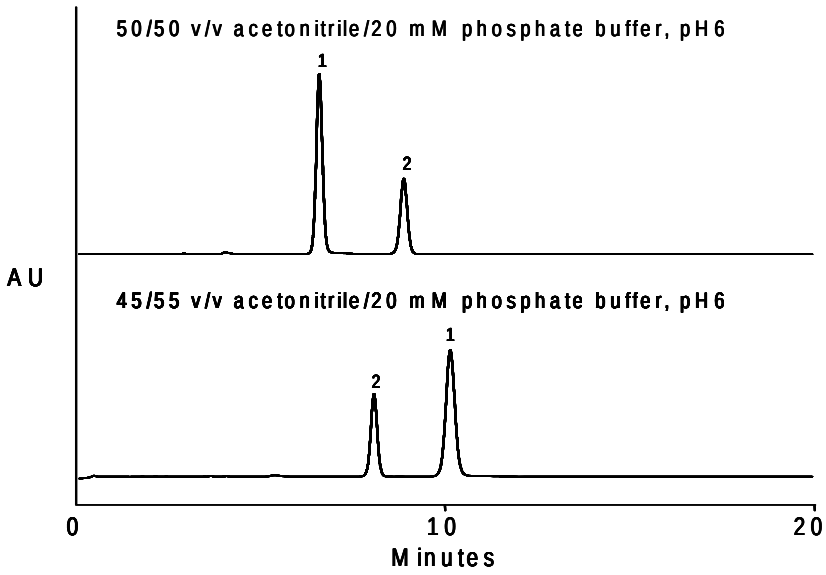
Butylbenzene



4-Hydroxybenzoic acid

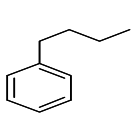
Figure 5

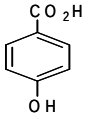
Adjustable Selectivity- Organic Modifier Effect



Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: see chromatograms
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 2 µL
Detection: UV @ 210 nm
Peaks:

1. Butylbenzene (0.1 mg/mL)
2. 4-Hydroxybenzoic acid (0.5 mg/mL)





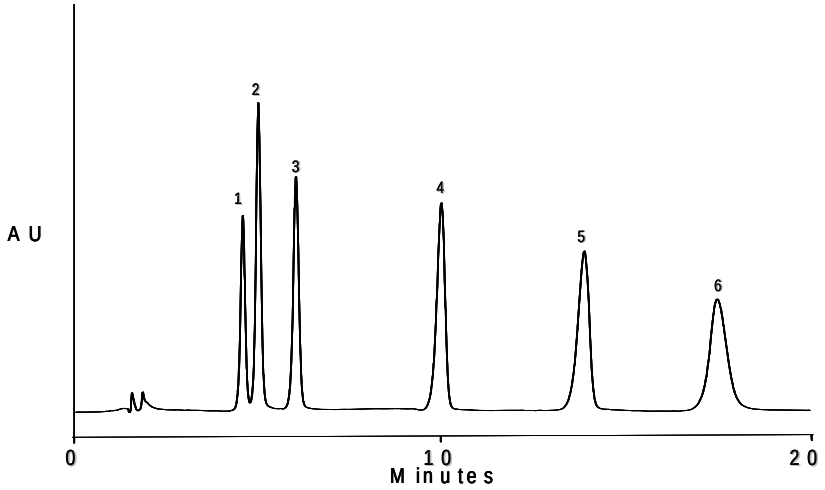
Butylbenzene **4-Hydroxybenzoic acid**

Figure 6

3.5. HILIC Mode

The Acclaim Mixed-Mode WAX-1 column can operate in HILIC mode. In this mode, acetonitrile (not methanol) should be used in a range of 70 to 100% acetonitrile. The elution power can be modified by the employment of a polar solvent, such as an aqueous buffer. Using this column in HILIC mode provides increased retention for highly polar molecules. In general the higher the organic content in mobile phase, the longer the retention for a highly polar compound.

Separation of Amino Acids in HILIC Mode



Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions: 4.6 x 150 mm
Mobile phase: 25/75 v/v 25 mM phosphate buffer, pH6.0/acetonitrile
Temperature: 30°C
Flow rate: 1 mL/min
Injection vol.: 10 µL
Detection: UV, 210 nm

Peaks:
1. Leucine
2. Isoleucine
3. Valine
4. Alanine
5. Serine
6. Glycine

Figure 7

3.6. Buffer Types

The Acclaim Mixed-Mode WAX-1 column should not be used with non-buffered aqueous mobile phase, whether alone or with an organic modifier. Phosphate buffers have proven to be useful for most HPLC applications. An acetate or formate buffer may also be applicable, depending on applications.

SECTION 4 – COLUMN CARE

4.1. Mobile phases

Mobile phases should be freshly prepared. All chemicals and solvents should be at the highest available quality. All mobile phases should be filtered before use. In-liner filters are recommended.

4.2. Guard cartridges

It is highly recommended that a guard cartridge be used with the analytical column, and replaced periodically depending on the nature of the sample. Failing to do so may result in rapid deterioration of column performance, and short column lifetime.

4.3. Column storage

The column can be stored in the mobile phase for a short period of time. For long term storage, use a buffered solution with higher organic content, such as 70/30 v/v acetonitrile (or methanol)/20 mM phosphate buffer at a pH between 3 to 6, or an ammonium acetate buffer at a pH between 3.8 to 5.8.

4.4. Column pH range – pH 2.5 to 7.0

To obtain better column lifetime, it is highly recommended to use "silica friendlily" mobile phases. While the pH limit of the column is pH 2.5 to 7 the recommended operating pH range is between 2.8 - 6.5.

4.5. Recommended operating temperature limit <50 °C

Although our experimental results indicated that the column could be used at 50 °C, the separation is usually optimized by modifying mobile phase ionic strength, pH, and/or organic modifier content. Elevated temperature is not recommended and should be avoided.

4.6. Flow rate and pressure limit

Usually, good column efficiency for a 4.6mm internal diameter column can be obtained at 1 mL/min while a higher flow rate (2 mL/min) can be used for fast analysis provided that the pressure limit is not exceeded. The 2.1mm internal diameter columns are normally used at 0.1 to 0.8 mL/min provided that the pressure limit is not exceeded. It is extremely important not to impose sudden column pressure surge. Thus increase flow rate gradually from 0.5 mL/min up to the desired flow rate. The pressure limit for the column is 4000 psi.

4.7. Column washing procedure

When the column washing practice is needed, such as deteriorated column performance and/or excessively high backpressure, the following procedure can be used as a guideline:

1. Wash the column with 20 mM phosphate buffer, pH 3/acetonitrile v/v 50/50 for 3 column volumes at a flow rate between 0.5 to 1 mL/min.
2. Wash the column with 150 mM phosphate buffer, pH 3 /acetonitrile v/v 50/50 for 30 column volumes at a flow rate between 0.5 to 1 mL/min **(to remove strongly retained anionic compounds)**.
3. Wash the column with 20 mM phosphate buffer, pH 3 /acetonitrile v/v 50/50 for 3 column volumes at a flow rate between 0.5 to 1 mL/min.
4. Wash the column with 20 mM phosphate buffer, pH 3 /acetonitrile v/v 75/25 for 30 column volumes at a flow rate between 0.5 to 1 mL/min **(to remove strongly retained hydrophobic compounds)**.
5. Equilibrate the column with the mobile phase.

Before any injection is made, the column should be equilibrated with a mobile phase for at least 30 column volumes.
If the above treatment fails to improve the column performance, replace it with a new one.
For a 2.1 mm i.d. column, the flow rate should be reduced to 20% of that for 4.6 mm i.d. column.
If the above treatment fails to revive the column, the column should be replaced.

SECTION 5 – FREQUENTLY ASKED QUESTIONS

1. What is the Acclaim Mixed-Mode WAX-1 column?
The Acclaim Mixed-Mode WAX-1 column is a new mixed-mode silica column that incorporates both hydrophobic and weak anion-exchange properties. Its surface chemistry features an alkyl long chain with a weak anion-exchange terminus. This column has demonstrated great potentials for separating a wide range of anionic compounds containing mixtures, including pharmaceuticals, food & beverage, chemical, and more.

2. Why do I need the Acclaim Mixed-Mode WAX-1 column?
The mixed mode separation mechanism of the Acclaim Mixed-Mode WAX-1 column allows for controlling retention of ionizable and neutral molecules by change mobile phase ionic strength, pH, and organic composition, either independently or collaboratively. As a result, many application challenges involving hydrophilic ionizable compounds that are difficult for C18 columns, can be easily accomplished on this column.

3. When do I need the Acclaim Mixed-Mode WAX-1 column?
Here are some situations among others you may consider using the Acclaim Mixed-Mode WAX-1 column:

1) Separation of hydrophilic organic acids.
   (Figures 8 see pg.18, Figure 9 see pg.19, Figure 10 see pg.20)
2) Simultaneous separation of acidic, neutral, and hydrophobic pharmaceuticals.
   (Figures 11 see pg.21, Figure 12 see pg.22)
3) Fast analysis of soft drinks. **(Figure 13 see pg. 23)**
4) When you need selectivity orthogonal to a reversed-phase column.
   (Figure 14 see pg.24, Figure 15 see pg.25)

4. What factors should I consider for method development using this column?
There are three main factors that affect column selectivity: mobile phase ionic strength, mobile phase pH, and mobile phase organic composition. You can optimize your separation by changing one, two, or all three factors. See Section 3 - Method Development for details.

5. What mobile phases should I use with this column?
Phosphate buffer works satisfactorily in many applications. Depending on the application, the buffer concentration range can be between 10 mM to 250 mM, and pH range should be in the range 2.5 to 7. When an organic modifier is used, make sure to keep it miscible with the buffer solution. A formate or acetate buffer can also be used in some applications.

6. What should I do before starting using Acclaim Mixed-Mode WAX-1 column?
Read this User Guide carefully, and contact Dionex Technical Support if you have any questions regarding using this column.

7. Can I use this column for separating hydrophilic organic acids?
Yes. You can use this column to separate a wide range of organic acids that are difficult to separate on reversed-phase columns.

8. How to store the column?
Refer to “Section 4.3. Column storage” for details.

9. Can I use this column to analyze basic molecules?
This column is suitable to analyze basic molecules with intermediate to high hydrophobicity under proper chromatographic conditions. Good peak shape is usually expected on this column.

10. Can I use this column to analyze neutral molecules?
Yes. This column provides intermediate hydrophobic retention so that neutral molecules with intermediate to high hydrophobic retention can be retained sufficiently. For highly hydrophilic/polar molecules, a HILIC mode separation using this column should be considered.

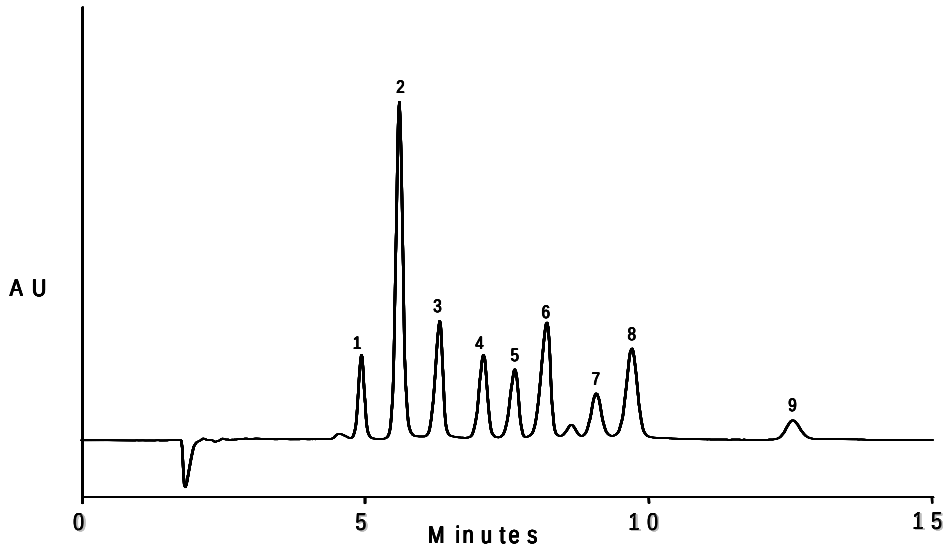
11. Can I use this column to separate a mixture of basic, acidic, and neutral molecules?
Yes. As shown in Figures 11 and 12 the Acclaim Mixed-Mode WAX-1 separates a mixture of basic, neutral, and acidic molecules in a single, with excellent peak shape and resolution. It provides higher degree of flexibility for application method development compared to both conventional reversed-phase and ion-exchange columns.

12. Do I need a guard cartridge with an Acclaim Mixed-Mode WAX-1 analytical column?
Yes. It is highly recommended to use guard cartridges with an Acclaim Mixed-Mode WAX-1 analytical column. The guard cartridge protects the more expensive analytical column by trapping highly retained components and particulates from the mobile phase or the sample.

13. What should I do if the column shows deteriorated performance?
Refer to "Section 4.7. Column washing procedure" for details.

14. What should I do if the column exhibits excessively high backpressure?
First, make sure that the mobile phase is freshly prepared and filtered before use, and that the sample are free of particulates. Then, back flush the column for certain amount of time while monitoring the change in column pressure. If problem persists, try to replace the inlet bed support. If all above fail, purchase a new column.

Separation of Mono-Valent Carboxylic Acids

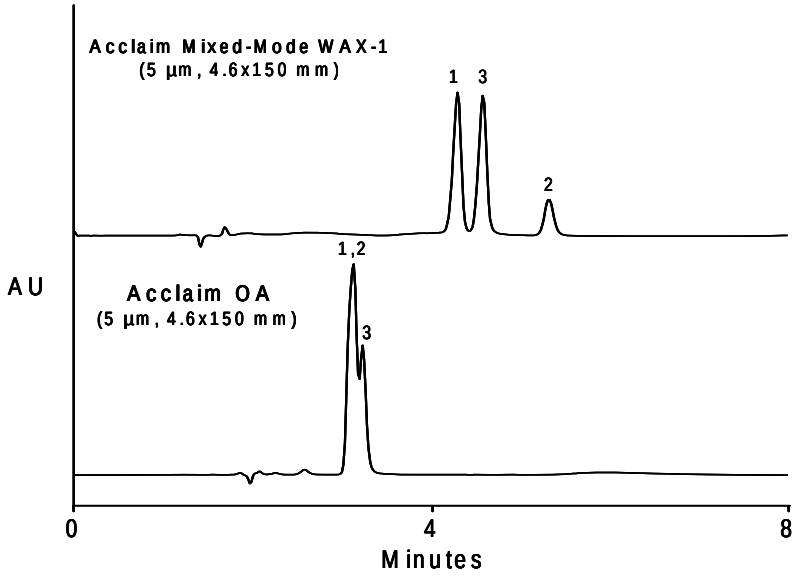


Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions: 4.6 x 150 mm
Mobile phase: 25 mM phosphate buffer, pH 6
Temperature: 30°C
Flow rate: 0.8 mL/min
Injection vol.: 10 µL
Detection: UV @ 210 nm
Peaks:

1. Quinic acid
2. Shikimic acid
3. Glycolic acid
4. Lactic acid
5. Acetic acid
6. Formic
7. Ascorbic acid (Vitamin C)
8. Iso-ascorbic acid
9. Propionic acid

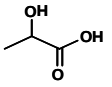
Figure 8

Seperation of Lactate, Acetate and Ascorbate

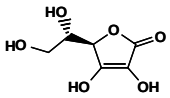


Mobile Phase: 20 mM phosphate buffer, pH6.0 for Acclaim Mixed-Mode WAX-1
50 mM phosphate buffer, pH2.6 for Acclaim OA
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV @ 210 nm
Peaks:

1. Lactic acid (0.8 mg/mL)
2. Ascorbic acid (Vitamin C) (0.25 mg/mL)
3. Acetic acid (0.8 mg/mL)



Lactic acid

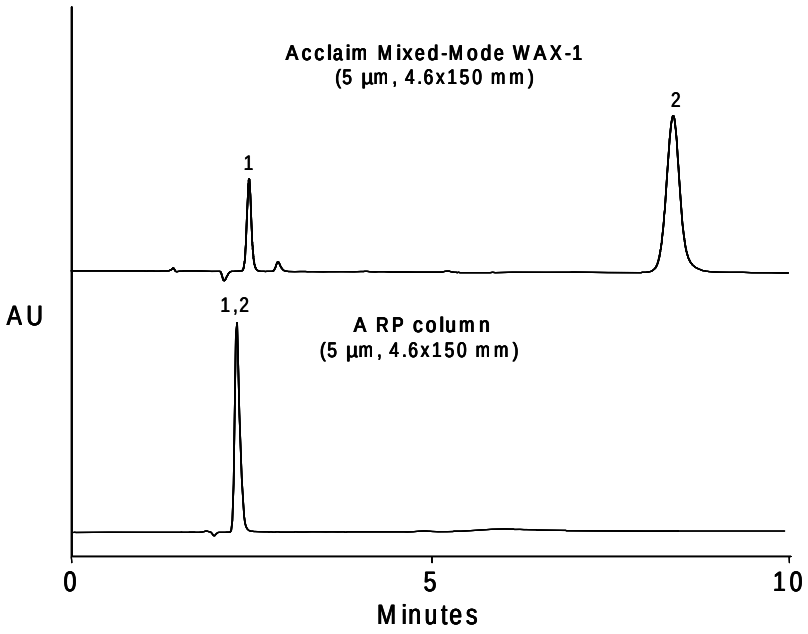


Ascorbic acid (Vitamin C)

Acetic acid

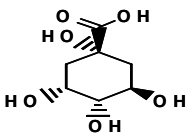
Figure 9

Separation of Quinic Acid and Tartaric Acid



Mobile Phase: 40/60 v/v acetonitrile/50 mM phosphate buffer, pH 6.0 for Acclaim Mixed-Mode WAX-1
50 mM phosphate buffer, pH 2.6 for a RP column
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV @ 210 nm
Peaks:

1. Quinic acid (1 mg/mL)
2. Tartaric acid (1 mg/mL)


Quinic acid

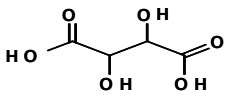
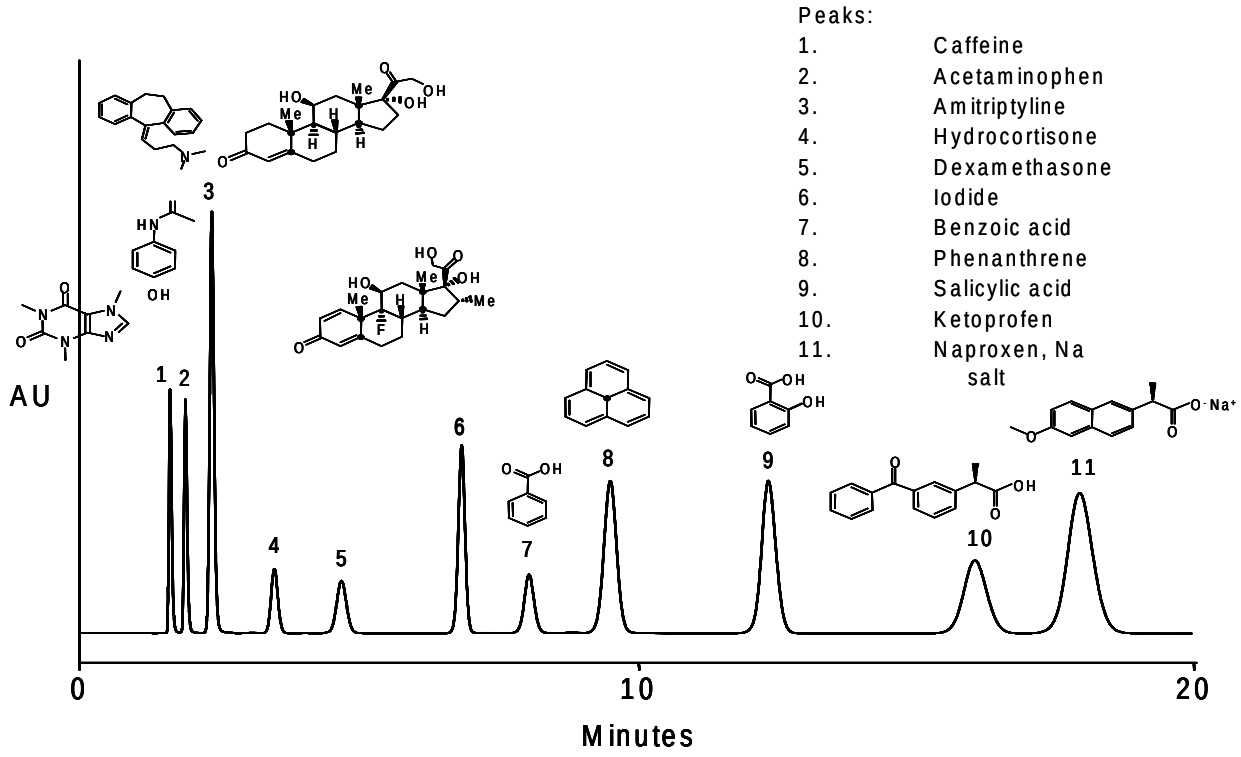

Tartaric acid

Figure 10

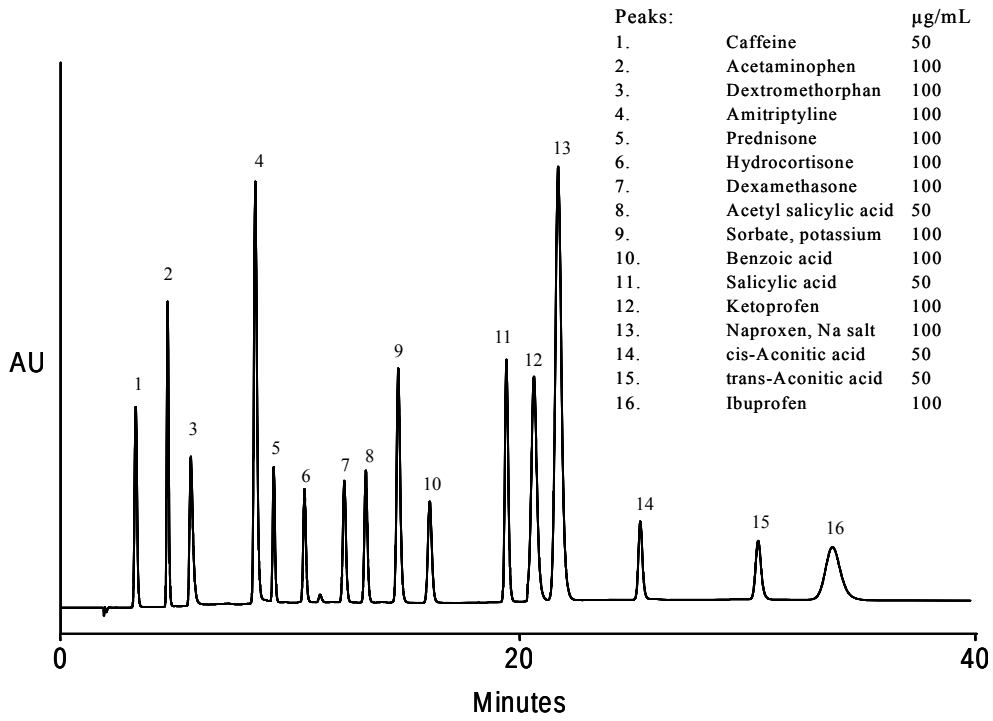
Isocratic Separation of Basic, Neutral and Acidic Molecules



Column:	Acclaim Mixed-Mode WAX-1, 5 µm
Dimension:	4.6 x 150 mm
Mobile Phase:	50/50 v/v Acetonitrile/buffer (6.8 g potassium monophosphate and 0.5 g pyrophosphate in 1000 g D.I. H_2O, pH is adjusted to 6.0 with NaOH)
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV @ 220 nm

Figure 11

Gradient Seperation of Basic, Neutral and Acidic Pharmaceticals



Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 15 µLColumn:Acclaim Mixed-Mode WAX-1, 5 µm
Dimension: 4.6 x 150 mm
Mobile Phase: A – Acetonitrile; B – D.I. water;C – 150 mM phosphate buffer, pH6.0

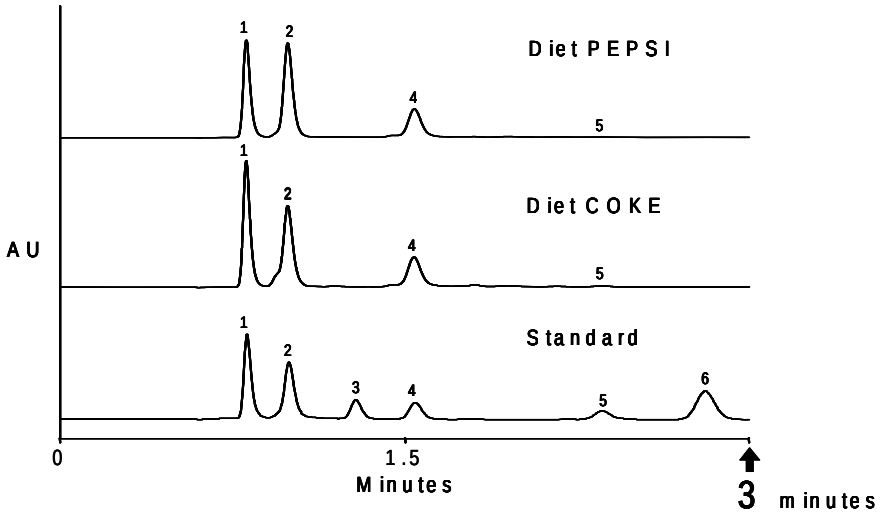
Gradient:

Time	%**A**	**% B**	**% C**
-15	10	80	10
0	10	80	10
10	50	40	10
12	50	40	10
25	50	0	50
40	50	0	50

Detection: UV @ 220 nm

Figure 12

Rapid Analisys of Soft Drinks



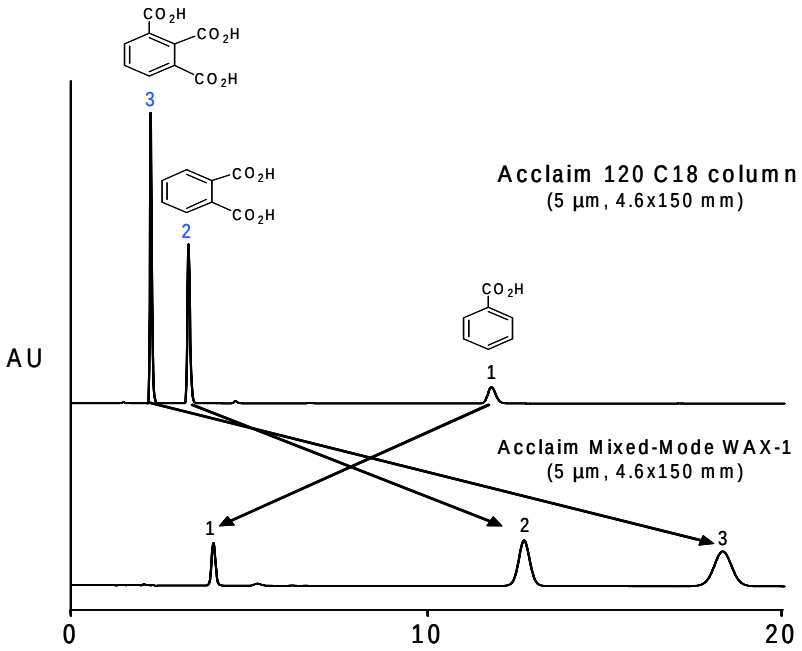
Column: Acclaim Mixed-Mode WAX-1, 5 µm
Dimension: 4.6 x 150 mm
Mobile Phase: 57/43 v/v Acetonitrile/ 120 mM phosphate buffer, pH 2.9
Temperature: 30 °C
Flow Rate: 2 mL/min
Inj. Volume: 2.5 µL
Detection: UV @ 210 nm
Sample: Direct injection of degassed sample
Peaks:

1. Caffeine
2. Aspartame
3. Sorbate
4. Benzoate
5. Citrate
6. Acesulfame

Diet Pepsi is a registered trademark of Pepsi-Cola Company.
Diet Coca-Cola is a registered trademark of the Coca-Cola Company.

Figure 13

Orthogonal Selectivity to Reversed-Phase Columns - I

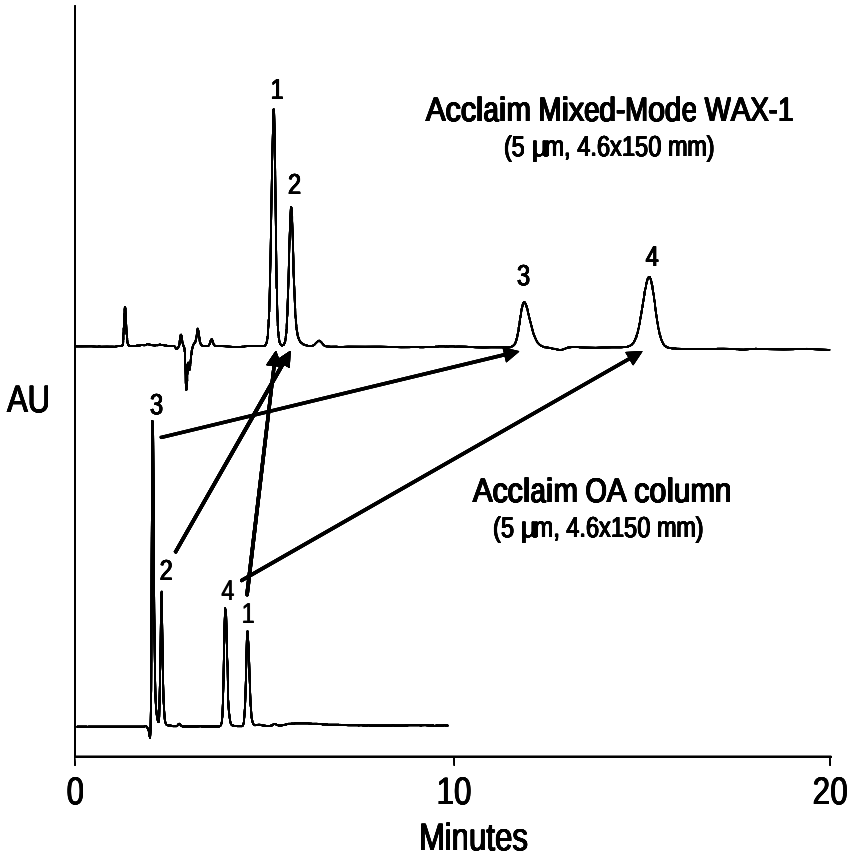


Mobile Phase: 50/50 v/v acetonitrile/50 mM phosphate buffer, pH 2.8 for Acclaim Mixed-Mode WAX-1
20/80 v/v acetonitrile/50 mM phosphate buffer, pH 2.8 for Acclaim 120 C18 column
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 2 µL
Detection: UV @ 220 nm
Peaks:

1. Benzoic acid (0.2 mg/mL)
2. Phthalic acid (0.2 mg/mL)
3. 1,2,3-Tricarboxylic benzene (0.2 mg/mL)

Figure 14

Orthogonal Selectivity to Reversed-Phase Column - II

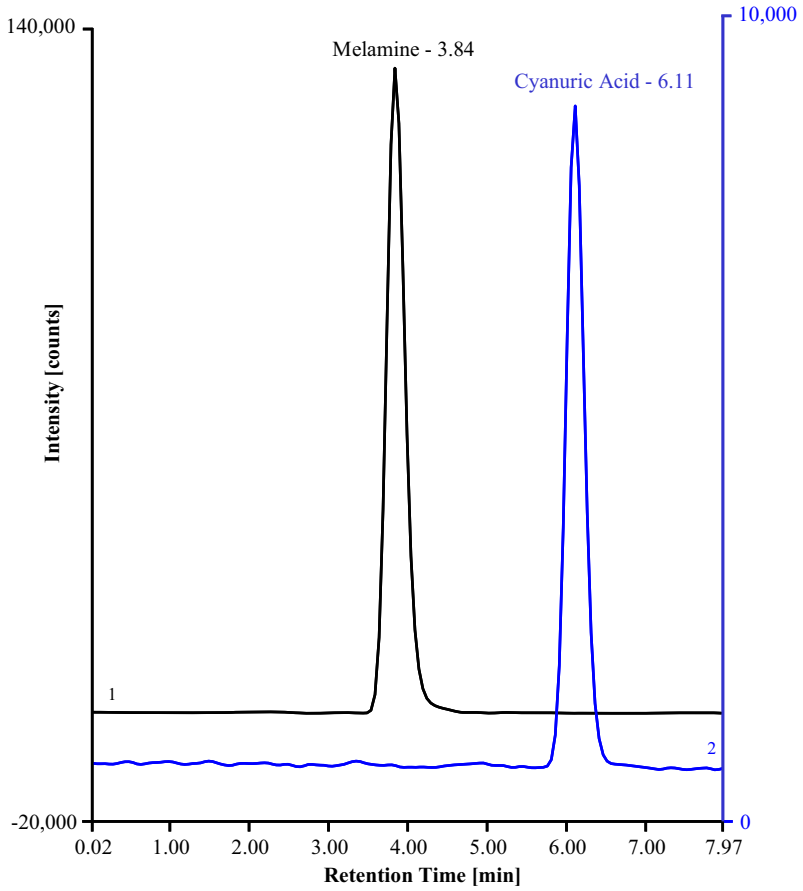


Mobile Phase: 50/50 v/v acetonitrile/100 mM phosphate buffer, pH 6.0 for Acclaim Mixed-Mode WAX-1
50 mM phosphate buffer, pH2.6 for Acclaim OA column
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV @ 210 nm
Peaks:

1. Succinic acid (0.5 mg/mL)
2. Tartaric acid (0.5 mg/mL)
3. Oxalic acid (0.1 mg/mL)
4. Citric acid (0.5 mg/mL)

Figure 15

Melamine and Cyanuric Acid on Mixed-Mode WAX-1



Column:	Mixed-Mode WAX-1,
Dimensions:	2.1 x 150 mm, 5µm
Mobile Phase:	90/4/6 v/v acetonitrile/50 mM ammonium formate buffer (pH 4)/water
Flow Rate:	0.25 mL/min
Column Temp.:	30 °C
Inj. Volume:	5 µL
Detector:	MSQ Plus mass spectrometer with Selected Ion Monitoring (SIM)
Melamine SIM:	Positive ESI +127 *m/z* @ 40V
Cyanuric Acid SIM:	Negative ESI -128 *m/z* @ 40V
Dwell time:	0.5 second

Figure 16

