# Legacy™

**HPLC columns**
**USP methods**
**Applications**


***"Creating New Dimensions in the World of Chromatography"***

## Introduction

As the HPLC world adapts to scientific advancements in separation technologies, old column technologies can easily become obsolete enough to warrant chromatography companies to stop producing these columns. Sometimes, companies will shut down only that production line, and other times, companies will shutter completely as they are unable to evolve quickly enough with the ever-changing landscape.

Consumers that once depended on these out-of-production columns are now stuck – they can either overhaul entire methods and systems, or they can abandon their projects altogether. With SIELC's Legacy column series, consumers don't have to do either.

SIELC's Legacy column series is based on United States Pharmacopeia's (USP) published chromatographic methods and procedures. A plethora of brands have columns used in USP reference standards and methods, and USP has created different designations to group together columns with similar types of packing and properties in the solid phase. ple replacement. For example, L1 is one of the most popular designations, referring to columns with octadecyl silane (C18) chemically bonded to porous or non-porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter. An L1 column from one manufacturer can be swapped for another L1 column from a different manufacturer with little to no modification of the method. However, not all L1 columns are exactly the same, so relative compound retention and peak shape can be different from one brand's column to another.

For companies that have developed integral methods based on USP columns that are no longer in production, the Legacy column series offers them a simple and easy way to find a compatible replacement.

SIELC offers its Legacy column series in many designations: L1, L3, L7-L14, L16, L18, L20, L26, L28, L44, L78, and L85.

## Validation Criteria

***Accuracy***

USP defines accuracy as "the closeness of test results obtained by that method to the true value," and is calculated as "the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals."

***Precision***

USP defines precision as the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample often expressed as the relative standard deviation. For a method to be considered precise, relative standard deviation must be less than 2%.

***Specificity***

USP defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and matrix components. A method must be able to determine the target components from impurities and noise.

***Detection Limit***

USP defines detection limit as the lowest amount of analyte in a sample that can be detected. The minimum value is three times the signal-to-noise ratio.

***Quantitation Limit***

The quantitation limit of a procedure is the lowest amount of analyte needed in a sample, which can be quantitatively determined with precision and accuracy. This value is a minimum of ten times the signal-noise ratio.

***Linearity and Range***

Linearity is defined as a method's ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sampled within a given range. This is calculated by the correlation coefficient. The range is defined by the interval between the upper and lower levels of the analyte that has been demonstrated to be determined with a suitable level of precision, accuracy and linearity using the method as written.

***Robustness and Ruggedness***

Ruggedness is the degree of reproducibility of the results, while robustness is a measure of the capacity to remain unaffected by small but deliberate variations in methods parameters. Robustness provides an indication of its reliability during normal usage.

**Column:** **Legacy L1**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 30/70%
**Flow Rate:** 1.0 mL/min
**Detection:** UV 254 nm

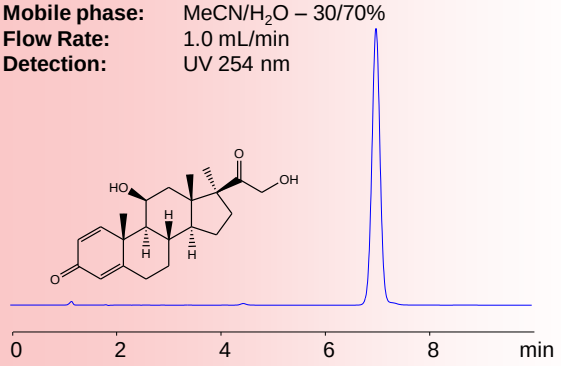


**Figure 1:** Retention of Prednisolone

**Column:** **Legacy L1**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeOH/$H_2O$ – 85/15%
**Buffer:** $NaH_2PO4$/Sodium 1-Octanesulfonate/EDTA 0.05M/519g/45g
**Flow Rate:** 1.0 mL/min
**Detection:** UV 280 nm

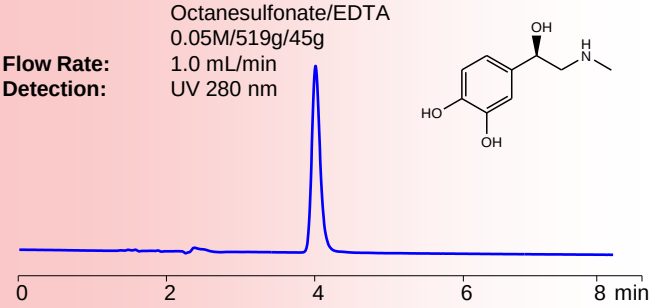


**Figure 2 :** Retention of Epinephrine

**Column:** **Legacy L1**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 43/55%
**Buffer:** $NaH_2PO_4$ – 2%, pH 3.5
**Flow Rate:** 1.0 mL/min
**Detection:** UV 233 nm

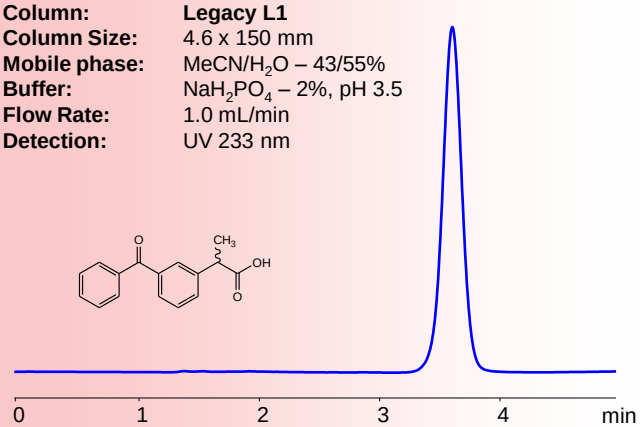


**Figure 3:** Retentino of Ketoprofen

**Column:** **Legacy L1**
**Column Size:** 4.6 x 250 mm
**Mobile phase:** MeOH/$H_2O$ – 5/95%
**Buffer:** AmAc – 20 mM, pH 4.9
**Flow Rate:** 1.0 mL/min
**Detection:** UV 230 nm

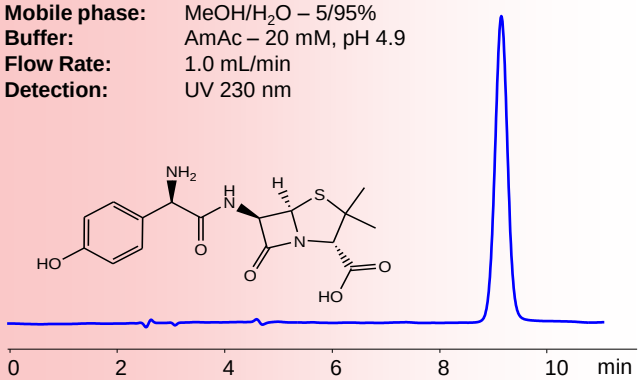


**Figure 4:** Retention of Amoxicilin

The Legacy L1 column is a silica-based, single-mode reversed-phase $C_{18}$ column that meets the USP qualifications for the L1 designation. Compounds are retained based on their hydrophobic interactions with the ligands. The chromatograms in this section, as well as the others, show injections done according to USP standard methods for the given compounds, with modifications to the methods noted where applicable.

Prednisolone is an artificial corticosteroid that is used to treat many chronic ailments, such as colitis, multiple scelerosis, and arthritis. Figure 1 shows retention of Prednisolone on a Legacy L1 column with a mobile phase (MP) of 30% Acetonitrile (MeCN) and 70% water.

Epinephrine, or adrenaline, is a naturally-occurring corticosteroid. In the pharmaceutical industry, it is used to treat severe allergic reactions, heart attacks, and asthma. Figure 2 shows retention of Epinephrine on a Legacy L1 column with a mobile phase mostly consisting of methanol (MeOH) and water, with $NaH_2PO_4$, Sodium 1-Octanesulfonate, and EDTA as buffers.

Amoxicillin is common antibacterial drug derived from penicillin. Figure 3 shows retention of Amoxicillin on a Legacy L1 column with a mobile phase consiting of methanol and mostly water with ammonium acetate as the buffer.

Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) used frequently to help treat arthritis-related pain and gum inflammation caused by severe toothaches. Figure 4 shows retention of Ketoprofen on a Legacy L1 column with a mobile phase consisting of water and acetonitrile and a high concentration of monosodium phophate (MSP, $NaH_2PO_4$).

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Phenylephrine is a popular, multi-faceted drug that can treat congestion, hemorrhoids, low blood pressure, and can dilate the pupil. Figure 5 shows retention of Epinephrine and Phenylephrine on a Legacy L1 column with a mobile phase evenly split between water and MeCN, with 1-octanesulfonic acid as a buffer.

Atenolol is a beta blocker frequently used to treat chest pain and high blood pressure. Figure 6 shows retention of Atenolol on a Legacy L1 column with a mobile phase consisting of mostly water and MeCN, with octanesulfonic acid and sulfuric acid as buffers.

Amitriptyline is often used to treat depression and nerve pain. Figure 7 shows it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN and water with NaH2Po4 as a buffer.

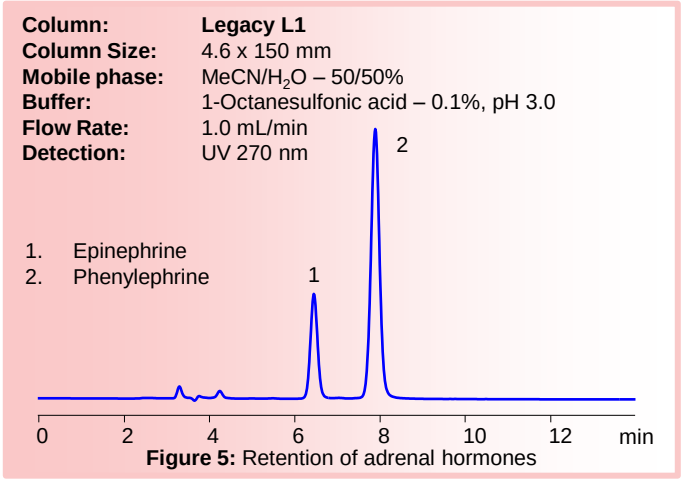

**Figure 5:** Retention of adrenal hormones

Chloramphenicol is an antibiotic frequently used to treat eye infections, as well as meningitis, cholera, and typhoid fever. Figure 8 shows it can be retained on a Legacy L1 column with a mobile phase consisting of methanol, water, and acetic acid as a buffer.

Naproxen is a popular nonsteroidal anti-inflammatory drug used for treating various types of pain. Benzophenone is key building block in many organic reactions. Figure 9 shows how they can be retained and separated on a Legacy L1 column with a mobile phase consisting of MeCN, water, and acetic acid as a buffer.

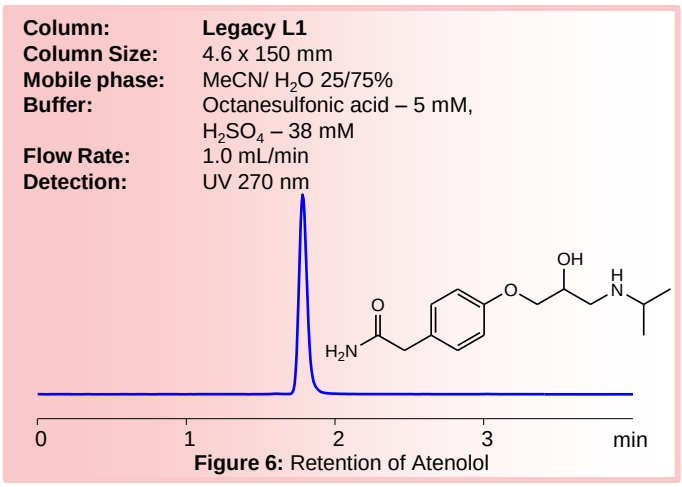

**Figure 6:** Retention of Atenolol

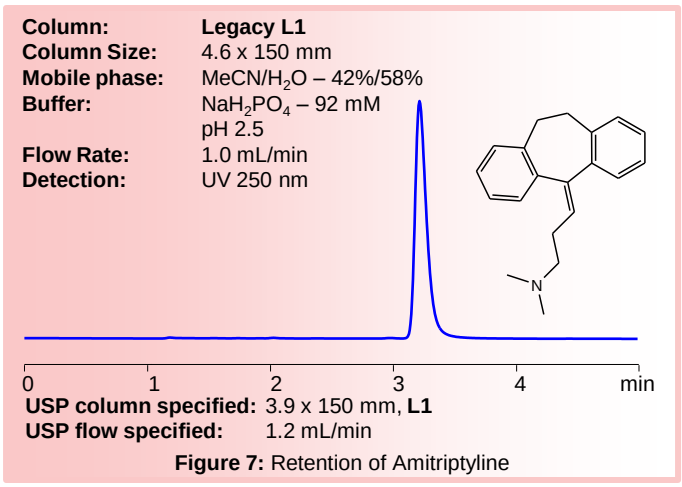

**Figure 7:** Retention of Amitriptyline

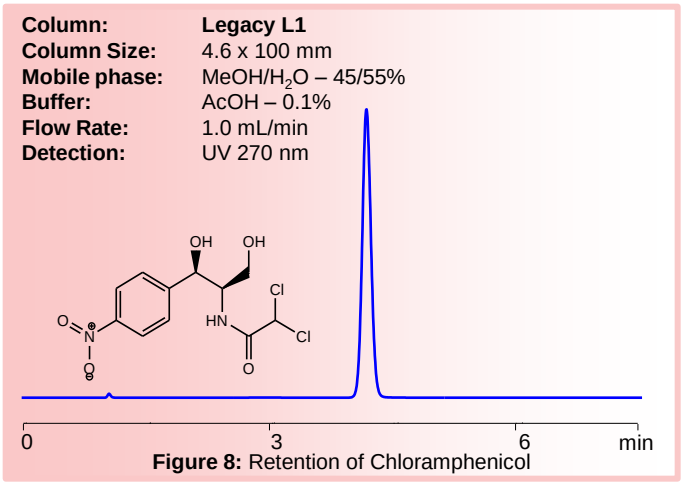

**Figure 8:** Retention of Chloramphenicol

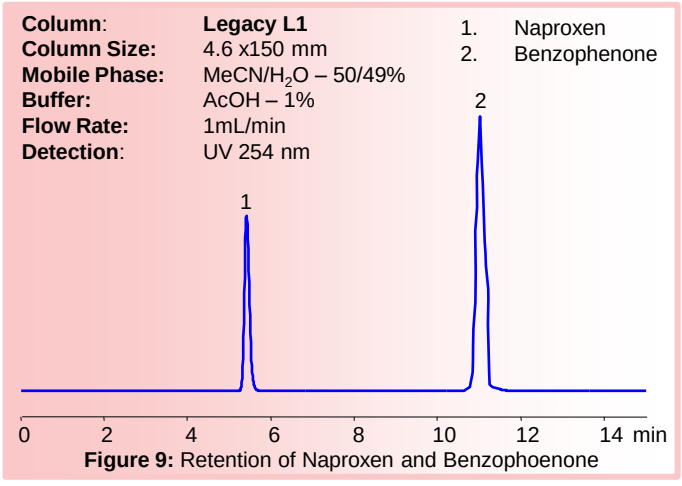

**Figure 9:** Retention of Naproxen and Benzophoenone

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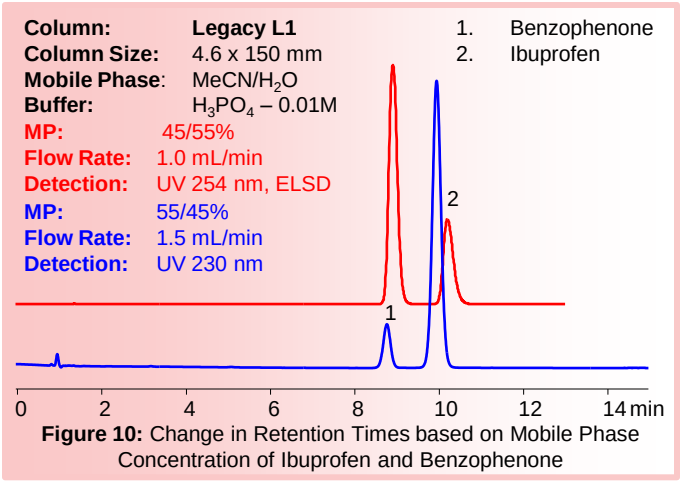


**Figure 10:** Change in Retention Times based on Mobile Phase Concentration of Ibuprofen and Benzophenone

Ibuprofen is another popular nonsteroidal anti-inflammatory drug used for treating various types of pain. Figure 10 shows how benzophenone and ibuprofen can be retained and separated on a Legacy L1 column with a mobile phase consisting of MeCN, water, and phosphoric acid as a buffer.

Guaifenesin is a popular decongestion that is often used to help treat chest congestion. Benzoic acid is a common intermediary in the biosynthesis of metabolites. It also found use as a painkiller and antimicrobial at the turn of the 20th century. Figure 11 shows how they can be retained and separated on a Legacy L1 column with a mobile phase consisting of methanol, water, and acetic acid.

Acetylsalicylic acid, or aspirin, is a common non-steroidal anti-inflammatory drug used as a pain reliever. Salicylic acid is often used to treat acne, dandruff, and other skin conditions, and is a precursor to acetylsalicylic acid. Figure 12 shows how these two compounds can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and an ammonium formate (AmFm) buffer with a pH of 3.0.

Hydrocortisone, or cortisol, is a corticosteroid used to treat a wide variety of ailments, including adrenal gland issues, asthma, eczema, and psoriasis, among many others. Figure 13 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and monosodium phosphate.

Promethazine is an antihistamine used frequently to treat allergies, nausea, and insomnia. Figure 14 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and sodium monophosphate as a buffer.

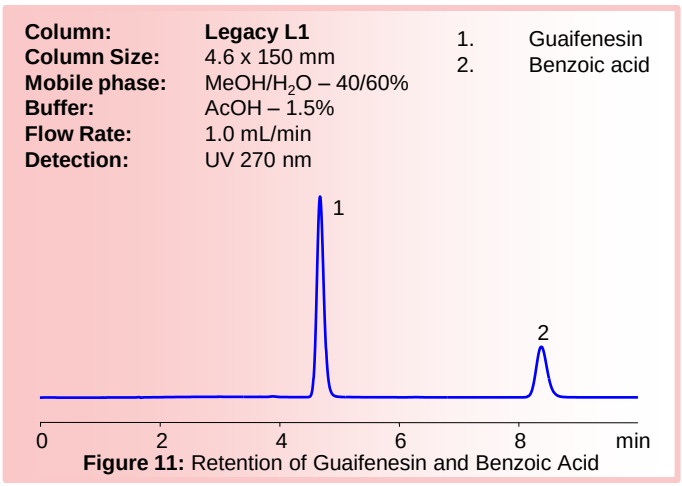


**Figure 11:** Retention of Guaifenesin and Benzoic Acid

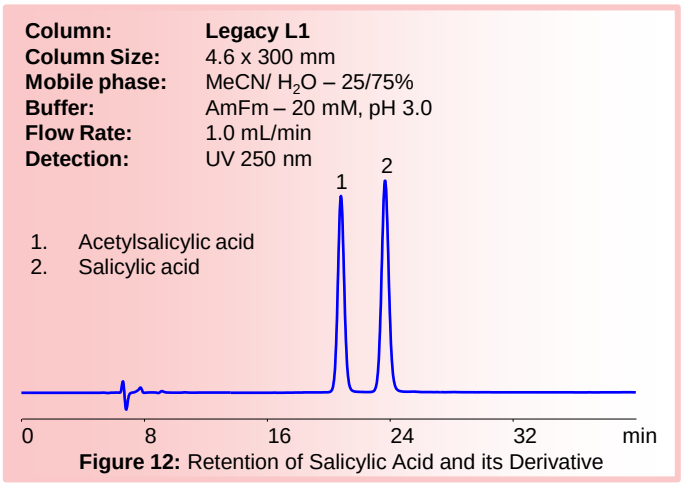


**Figure 12:** Retention of Salicylic Acid and its Derivative

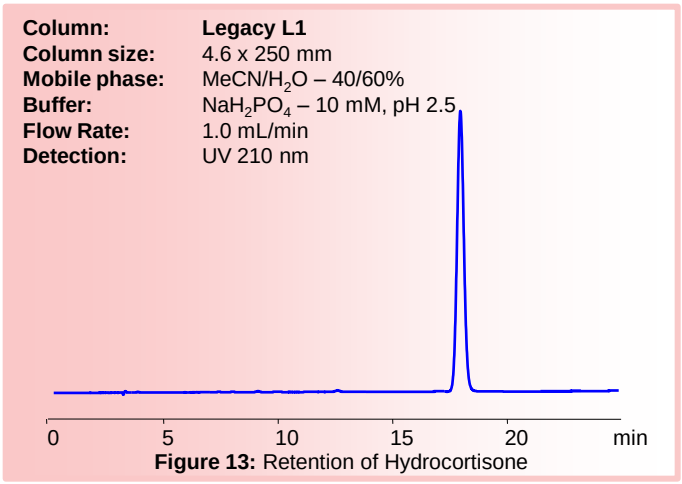


**Figure 13:** Retention of Hydrocortisone

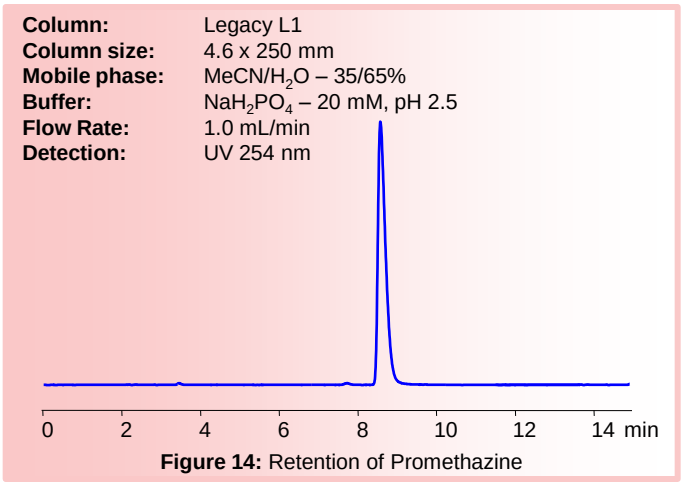


**Figure 14:** Retention of Promethazine

Niacin, commonly known as nicotinic acid or Vitamin $B_3$, is water-soluble and helps treat high cholesterol and triglyceride levels. It can be taken as a supplement or synthesized by the body from tryptophan. Figure 15 shows how niacin can be retained on a Legacy L1 column with a (complex) mobile phase consisting of hexanesulfonate, methanol (MeOH), MeCN, and acetic acid (AcOH). The column parameters and flow rate differ slightly from the USP standard method.

Albuterol, also known as salbutamol, is a short—acting $\beta_2$ adrenergic receptor agonist that is commonly inhaled as a treatment for asthma and COPD. Figure 16 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of methanol, water, and a buffer consisting of AcOH and octanesulfonic acid.

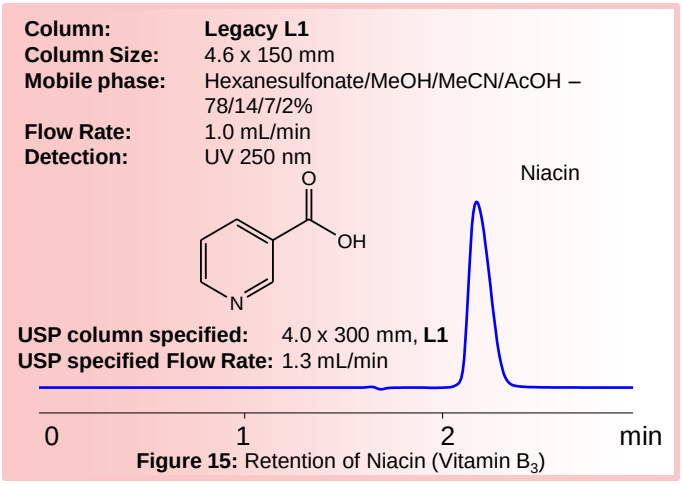


**Figure 15:** Retention of Niacin (Vitamin $B_3$)

Norepinephrine is a hormone and neurotransmitter produced by the adrenal gland. It helps drive the "fight-or-flight" response, and can be taken as a treatment for low blood pressure and heart failure. Figure 17 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeOH, water, and a sodium heptanesulfonate buffer.

Dopamine is another neurotransmitter that is key to how our brains process pleasure and can be used to treat shock symptoms and improve blood flow. Figure 18 shows how benzoic acid and dopamine can be separated on a Legacy L1 column with a mobile phase consisting of MeCN, water, and a buffer consisting of AcOH and octanesulfonic acid.

4-aminobenzoic (PABA) is naturally-occurring compound with some therapeutic effects, such as a treatment for fibrotic skin disorders and irritable bowel syndrome. Pracinamide (PCA) is used in the treatment of cardiac arrhthmias. Figure 19 shows how PABA and can be retained on a Legacy L1 column with a mobile phase consisting of water, MeOH, and triethylamine (TEA) as a buffer.

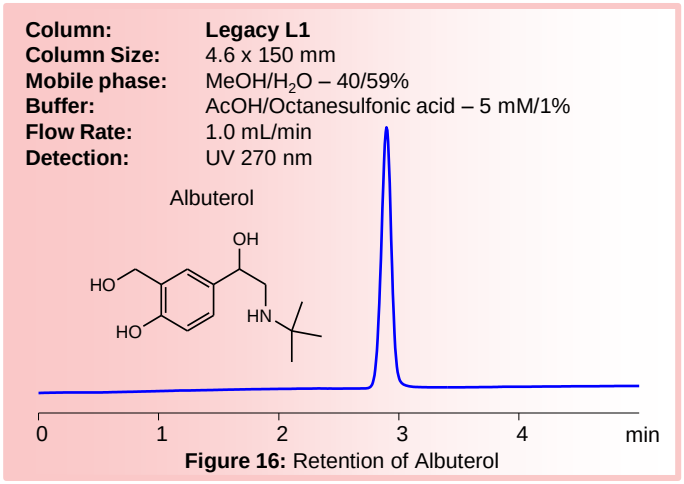


**Figure 16:** Retention of Albuterol

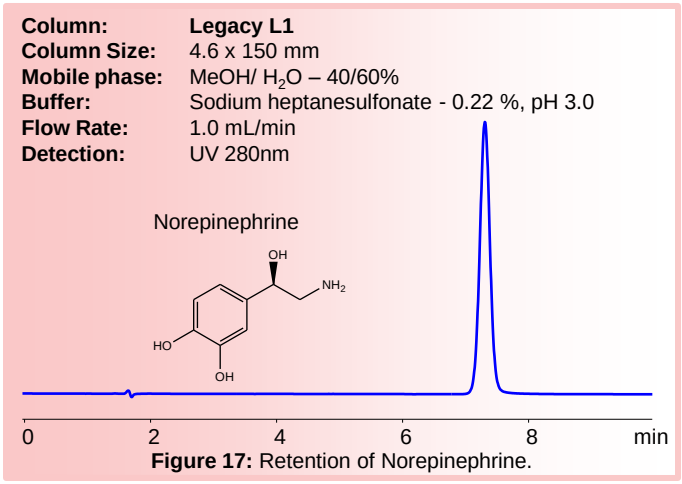


**Figure 17:** Retention of Norepinephrine.

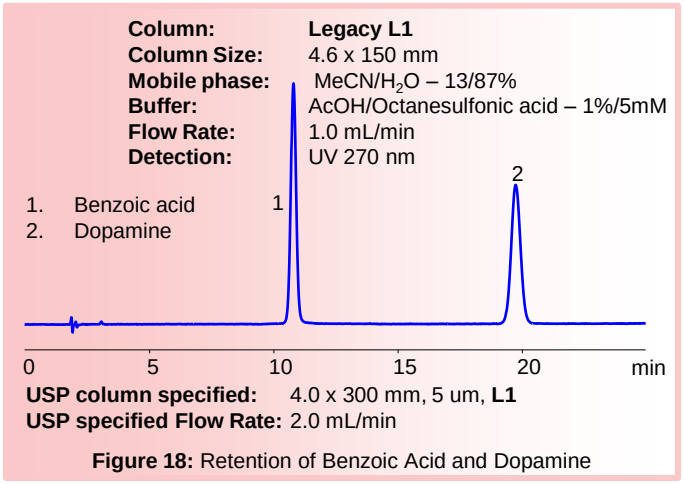


**Figure 18:** Retention of Benzoic Acid and Dopamine

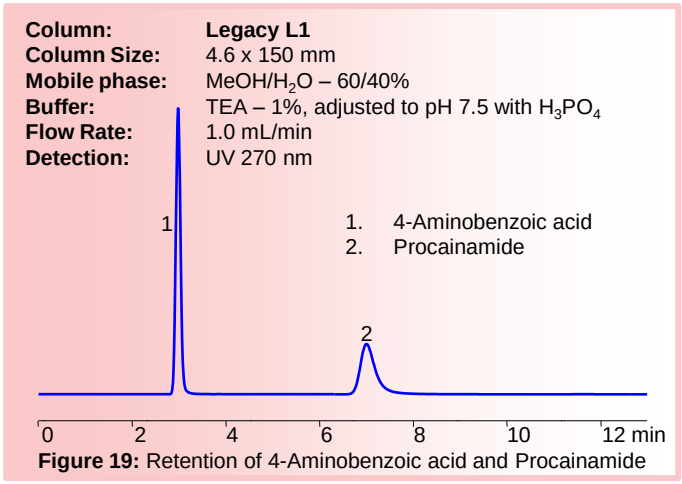


**Figure 19:** Retention of 4-Aminobenzoic acid and Procainamide


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**Column:** **Legacy L1**
**Column size:** 4.6 x 250 mm
**Mobile phase:** MeCN/$H_2O$ – 50/50%
**Buffer:** $NaH_2PO_4$ – 20 mM, pH 2.5
**Flow Rate:** 1.0 mL/min
**Detection:** UV 254 nm

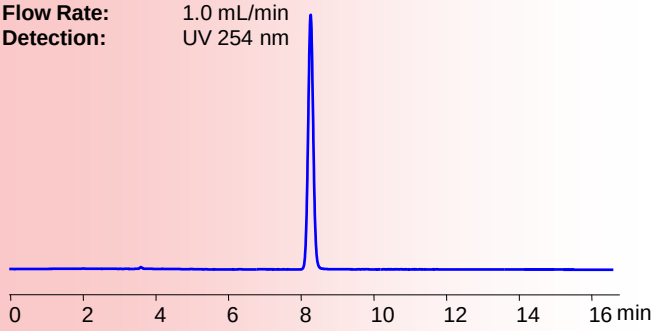


**Figure 20:** Retention of Valsartan

Valsartan is a multi-faceted drug that is sued to treat diabetic kidney disease, high blood pressure, and heart failure. Figure 20 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and MSP as a buffer.

Acyclovir is an anti-viral commonly used to treat shingles, chicken pox, and herpes. Figure 21 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and MSP.

Inosine is an anti-viral commonly used to treat shingles, chicken pox, and herpes. Figure 22 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and MSP.

Praziquantel is an anti-parasitic used for both humans and animals to treat various types of worm-infections. Figure 23 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN and water, without a buffer.

Cefepime is an anti-bacterial of the cefalosporin class that can treat infections beyond the blood-brain barrier. Figure 24 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeOH, water, and MSP as a buffer.

**Column:** **Legacy L1**
**Column size:** 4.6 x 250 mm
**Mobile phase:** MeCN/$H_2O$ – 3/97%
**Buffer:** $NaH_2PO_4$ –10 mM, pH 2.5
**Flow Rate:** 1.0 mL/min
**Detection:** UV 254 nm

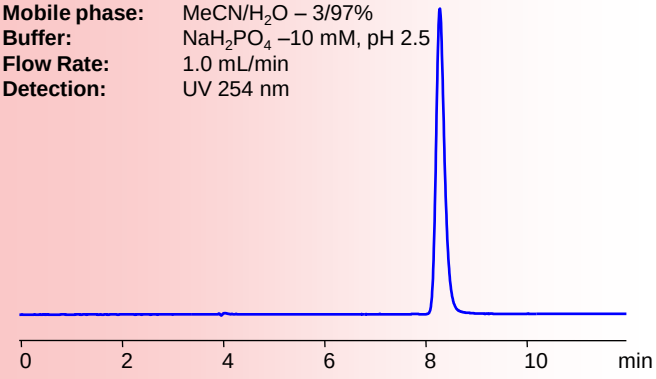


**Figure 21:** Retention of Acyclovir

**Column:** **Legacy L1**
**Column size:** 4.6 x 250 mm
**Mobile Phase:** MeOH/ $H_2O$ – 10/90%
**Flow Rate:** 1.0 mL/min
**Detection:** UV 248 nm

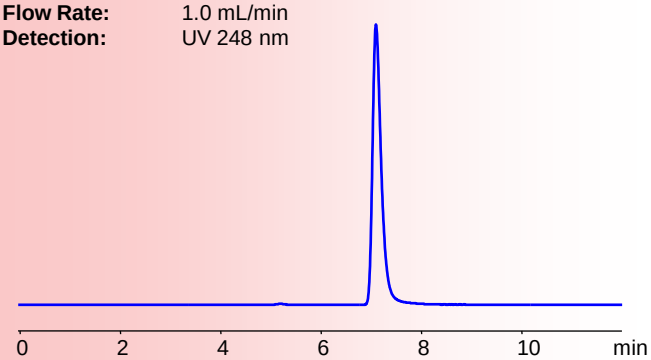


**Figure 22:** Retention of Inosine

**Column:** **Legacy L1**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ 60/40%
**Flow Rate:** 1.0 mL/min
**Detection:** UV 210 nm

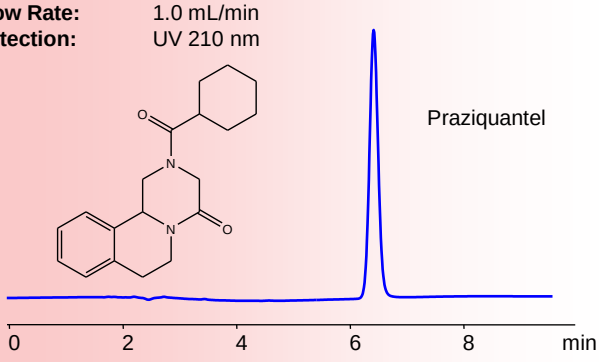


**Figure 23:** Retention of Praziquantel

**Column:** **Legacy L1**
**Column size:** 4.6 x 250 mm
**Mobile Phase:** MeOH/$H_2O$ – 10/90%
**Buffer:** $NaH_2PO_4$ – 5 mM, pH 5.0
**Flow Rate:** 1.0 mL/min
**Detection:** UV 250 nm

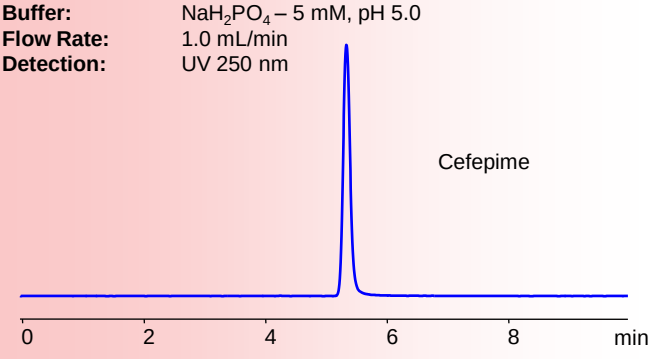


**Figure 24:** Retention of Cefepime

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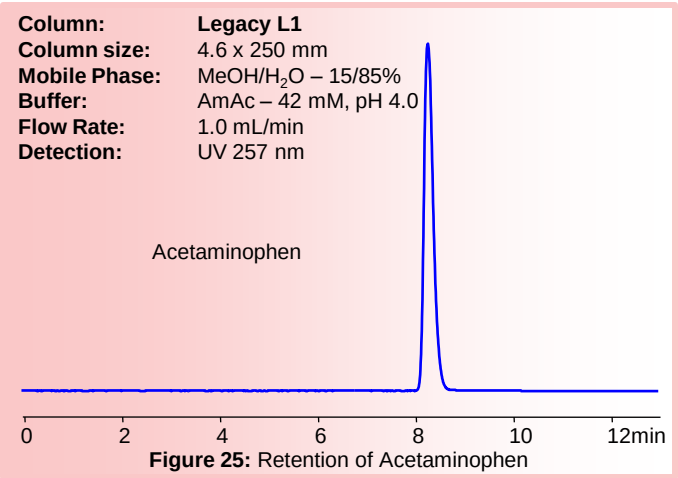


**Figure 25:** Retention of Acetaminophen

Acetaminophen (or paracetamol) is a popular drug with antipyretic and pain-killing properties. Some extra-strength medicines will also include caffeine, a methylxanthine-class stimulant, and/or acetylsalicylic acid, an NSAID, to help with pain relief. Figure 25 shows retention of acetaminophen on a Legacy L1 column with a mobile phase consisting of MeOH, water, and an ammonium acetate (AmAc) buffer.

Figure 26 shows retention of caffeine on a Legacy L1 column using a complex mobile phase consisting of sodium acetate (NaAC), water, MeCN, and tetrahydrofuran (THF) as a buffer.

Figure 27 shows how acetaminophen, caffeine, acetylsalicylic acid, benzoic acid, and salicylic acid can be separated on a Legacy L1 column with a mobile phase consisting of methanol, water, and AcOH as a buffer.

Figure 28 shows how acetaminophen, caffeine, and benzoic acid can be retained and separated on a Legacy L1 column using a mobile phase consisting of MeOH, water, and AcOH as a buffer.

Nitrofurantoin is an anti-biotic commonly used in the treatment of bladder infections. Figure 29 shows how it can be retained on a Legacy L1 column with a mobile phase consisting of MeCN, water, and MSP as a buffer.

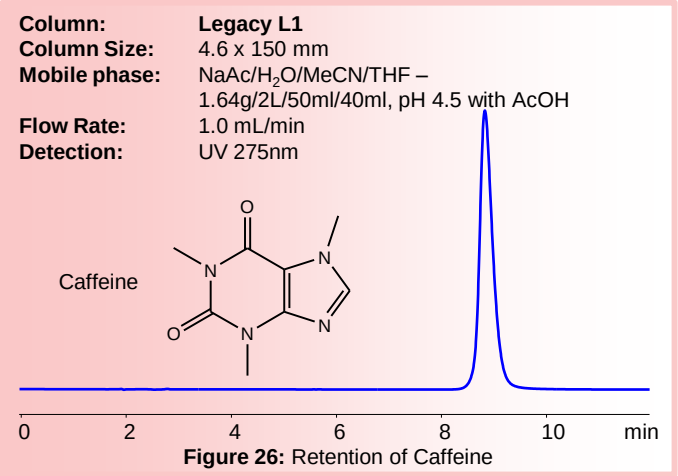


**Figure 26:** Retention of Caffeine

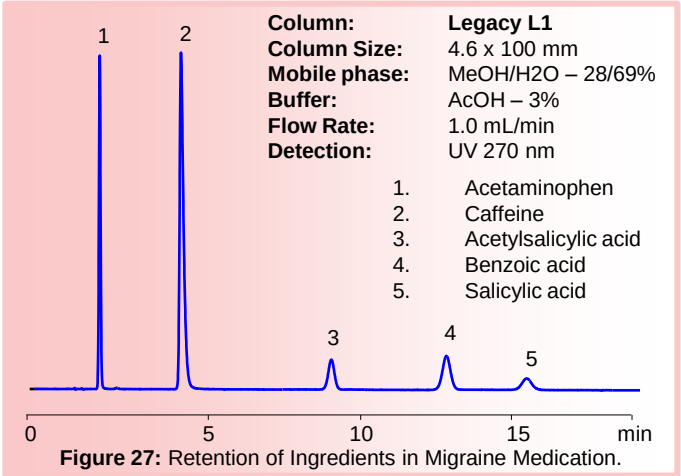


**Figure 27:** Retention of Ingredients in Migraine Medication.

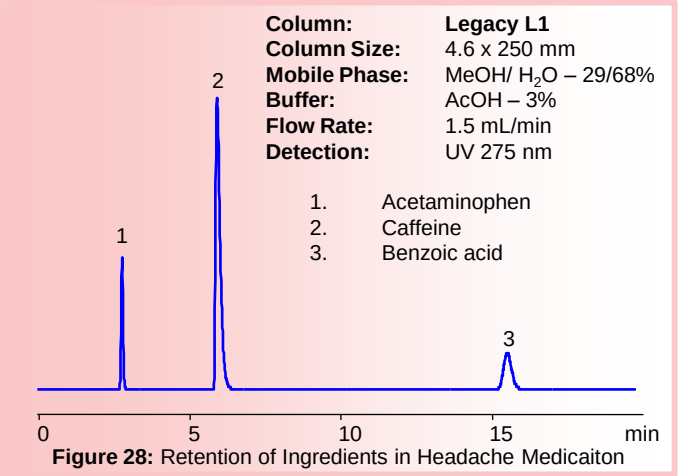


**Figure 28:** Retention of Ingredients in Headache Medicaiton

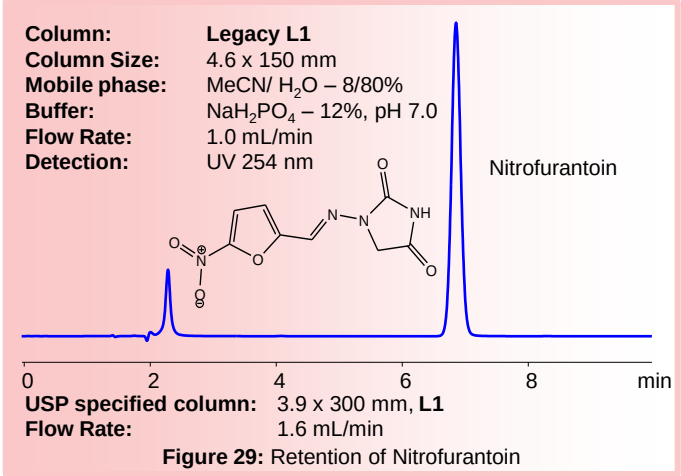


**Figure 29:** Retention of Nitrofurantoin

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The Legacy L3 column is a single-mode, normal phase column that contains porous silica, without any ligands or additional chemistry attached to the surface. The Legacy L3 column meets the USP qualifications for the L3 designation. The chromatograms in this section show injections done according to USP standard methods for the given compounds, with modifications to the methods noted where applicable.

Sulfuric acid is a highly corrosive acid commonly used as a buffer in chromatography. Sulfamic acid is an intermediary between sulfuric acid and sulfamide. Figure 30 shows the separation and retention of sulfuric and sulfamic acid on a Legacy L3 column with a mobile phase consisting of MeCN, water, and formic acid. This separation also used a gradient to change the concentration of MeCN and formic acid over time.

Lecithin s are a class of amphiphilic, fatty, yellow-brownish compounds that occur naturally in plant and animal tissues. Soy lecithin is lecithin derived from soy, and is often used as an emulsifier in food production. Figure 31 shows how commercial-grade lecithin and soy lecithin can be retained on a Legacy L3 column with a mobile phase consisting of MeCN, water, and an ammonium formate buffer using a gradient to control the buffer and MeCN concentrations over the course of the separation.

Ascorbic acid, or Vitamin C, is a vital nutrient for tissue repair and is a key component that helps the immune system function properly. Methylmalonic acid (MMA) is a key intermediary in the Krebs cycle. Succinic acid is another metabolic intermediate that helps the body produce ATP. Figure 32 shows how these acids can be retained on a Legacy L3 column with a mobile phase consisting of MeCN, water, and ammonium acetate as a buffer.

Ritonavir and Ribavirin are both anti-viral compounds. Ritonavir is commonly used to treat HIV, and while Ribavirin is commonly used to treat Hepatitis C. Figure 33 shows how these compounds can be retained on a Legacy L1 column with a mobile phase consisting of water and MeCN, without a buffer.

**Column:** **Legacy L3**
**Column Size:** 4.6 x 150 mm
**Gradient:** 10 min, 2 min hold
**Mobile phase:** MeCN/$H_2O$ – 95/5 to 50/50%
**Buffer:** Formic acid – 0 to 0.2%
**Flow Rate:** 1.0 mL/min
**Detection:** ELSD, 40 ºC

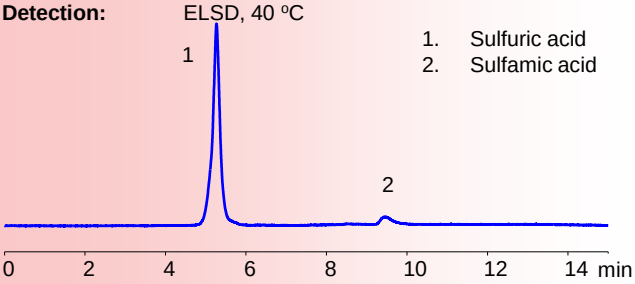


**Figure 30:** Retention of Sulfuric Acid and Sulfamic Acid

**Column:** **Legacy L3**
**Column Size:** 4.6 x 150 mm
**Gradient:** 20 min
**Mobile phase:** MeCN/$H_2O$ – 92/8% to 75/25%
**Buffer:** AmFm – 8 mM to 20 mM, ph 3.0
**Flow rate:** 1.0 mL/min
**Detection:** ELSD, 40 ºC

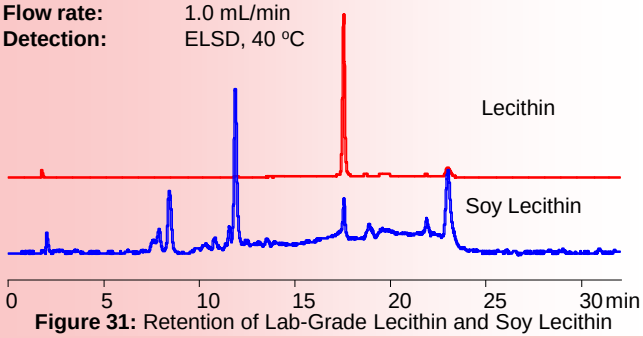


**Figure 31:** Retention of Lab-Grade Lecithin and Soy Lecithin

**Column:** **Legacy L3**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/ $H_2O$ – 75/25%
**Buffer:** AmAc – 15 mM, no pH
**Flow rate:** 1.0 mL/min
**Detection:** ELSD

1. Ascorbic Acid
2. Methylmalonic Acid
3. Succinic Acid

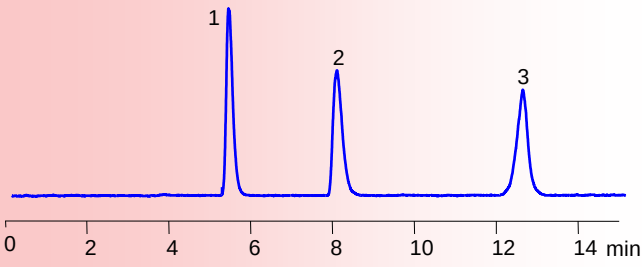


**Figure 32:** Retention of Organic Acids

**Column:** **Legacy L3**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 97/3%
**Flow Rate:** 1.0 mL/min
**Detection:** UV 250 nm

1. Ritonavir
2. Ribavirin

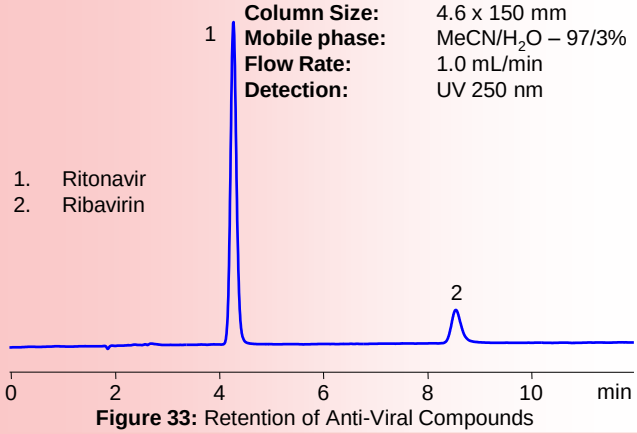


**Figure 33:** Retention of Anti-Viral Compounds

Uracil is one of the four main nucleobases that makes up RNA (replacing thymine from DNA). Uridine has a uracil group and is one of the main nucleosides that make up nucleic acids. Figure 34 shows how they can be separated and retained on a Legacy L3 column with a mobile phase consisting of MeCN and water without a buffer.

Saccharin and sorbitol are two different kinds of sweeteners. Saccharin can often be found in a pink packet at your local diner, and is known for its sweetness but has a slight metallic aftertaste. Sorbitol is a sugar alcohol added to foods as a preservative or a sweetener. Figure 35 shows how these sugar subsitutes can be retained on a Legacy L3 column with a mobile phase consisting of MeCN, water, and an ammonium formate buffer.

Glycerol, or commonly known as glycerine, is a renaissance compound with a wide variety of uses, from an antimicrobial and antiviral to a sweetener as a food additive to antifreeze and hair gel. To prepare glycerol for HPLC, it first needs to be mixed with two different derivatization agents. Instructions on how to make these reagents is listed below. Figure 36 shows how glycerol can be retained on a Legacy L3 column with a mobile phase consisting of MeCN and water without a buffer.

Carbamide peroxide, or hyperol, is a solid compound consisting of equal parts hydrogen peroxide ($H_2O_2$) and urea ($NH_4O$). This crystalline mixture is commonly used as a disinfectant and bleaching agent, and is frequently found in dental offices to help whiten teeth. Figure 37 shows how the component compounds, hydrogen peroxide and urea, can be separated and retained on a Legacy L3 column with a mobile phase consisting of water and mostly MeCN without a buffer.

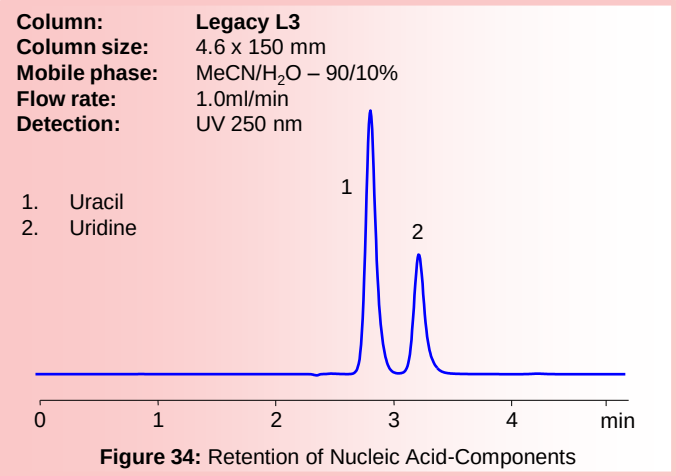

**Figure 34:** Retention of Nucleic Acid-Components

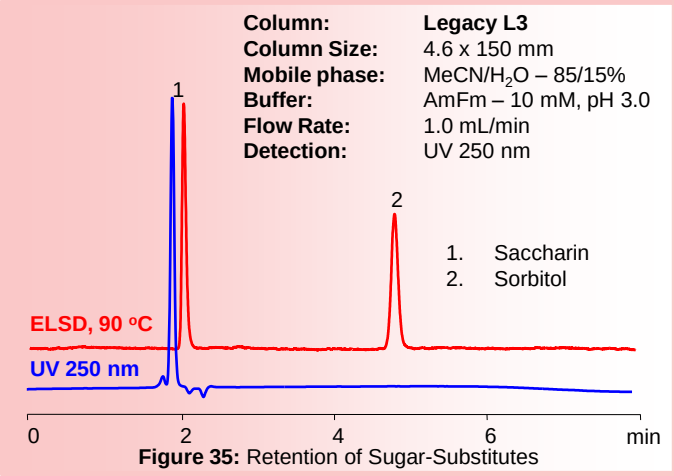

**Figure 35:** Retention of Sugar-Substitutes

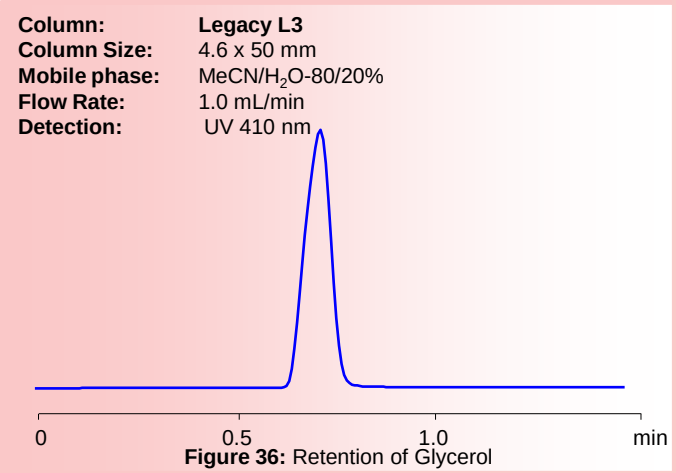

**Figure 36:** Retention of Glycerol

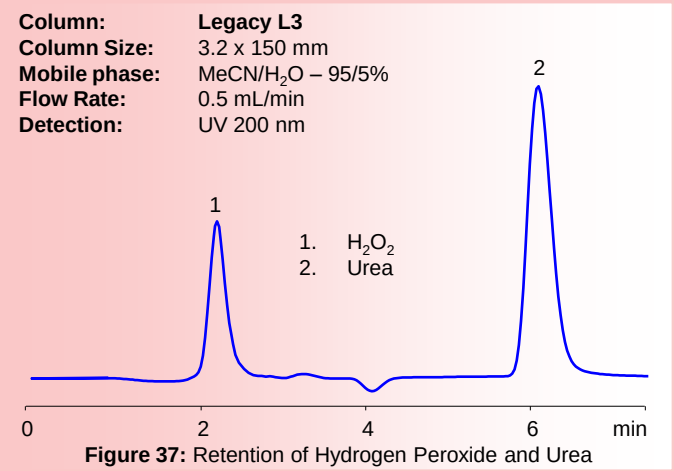

**Figure 37:** Retention of Hydrogen Peroxide and Urea

**Reagent A:** (Periodate reagent): Add 6.5 mg NaIO4 to 9 mL of water, add 1 mL acetic acid, mix, then add 0.77 g ammonium acetate.
**Reagent B:** (Acetylacetone reagent): Add 0.25 mL of Acetylacetone to 24.75 mL of isopropanol, mix, then store in the dark.
**Procedure:** Add 1 mL of **Reagent A** .75 mL of glycerine solution (0.05% glycerol/$H_2O$) to hydrolysate and keep 5 min at room temperature. Add 2.5 mL **Reagent B** , mix and keep 20 min.

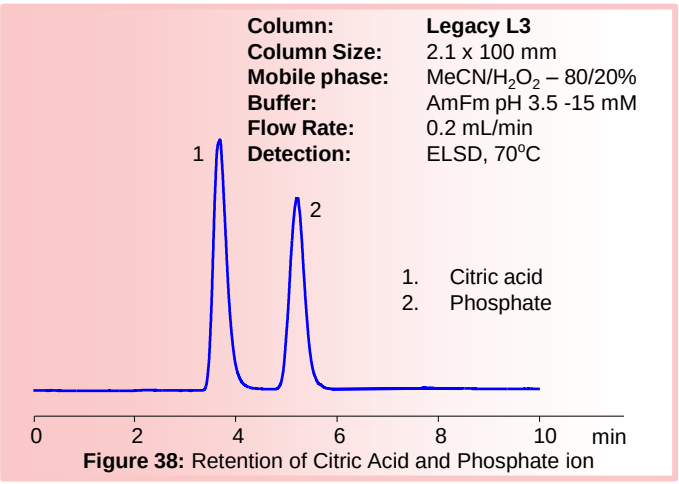


**Figure 38:** Retention of Citric Acid and Phosphate ion

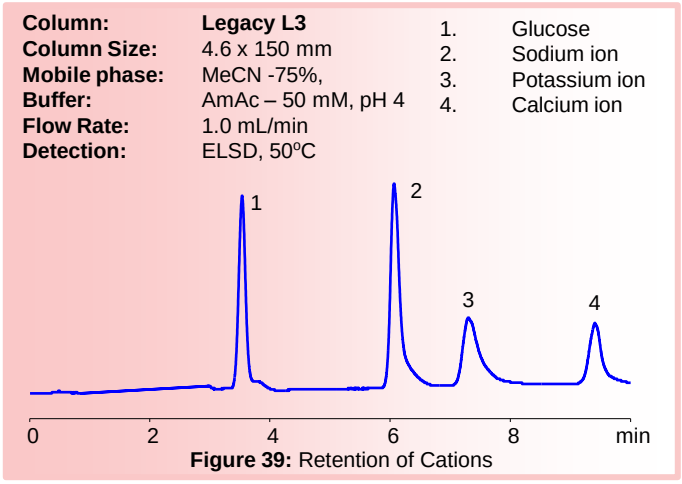


**Figure 39:** Retention of Cations

## **L6 -** Sulfonated Fluorocarbon

The L6 designation is a strong cation-exchange packed column with sulfonated fluorocarbon polymers coated onto a solid spherical core, 30 to 50 µm in diameter. SIELC's Legacy L6 column utilizes a coated silica gel (with 50 µm particles) instead of coated polymers to achieve similar chromatography and interactions, but with improved resilience at higher pressures. This column is useful for separating basic compounds and neutral polar compounds.

## **L8 -** Aminopropylsilane

The Legacy L8 column is a single-mode, ion-exchange column with aminopropylsilane (AP) groups chemically bonded to totally porous silica gel micro-particles. The Legacy L8 column meets the UPS standard for the L8 designation. The AP groups allow for both cation-exclusion and hydrophilic interaction chromatography (HILIC).

Short-chain peptides can often be highly polar, and when they include lysine moieties, they can be very basic as well. Short-chain lysine-based peptide oligomers have been found to have their best peak shape in HILIC/cation-exclusion mode. Figure 40 shows how these oligomers of different sizes (one made from 3 lysine moieties and one made from 6) can be retained on a Legacy L8 column using a gradient mobile phase consisting of MeCN, water, and phosphoric acid as a buffer.

Polylysine, significantly longer-chain polymers made out of many more lysine units, are easily produced by batcerial fermentation. Since any given sample can have differently sized polymers, it is often difficult to obtain a single, sharp peak. Figure 41 shows, however, that a relatively sharp peak can be obtained on a Legacy L8 column with a gradient mobile phase consisting of MeCN, water, and phosphoric acid as a buffer.

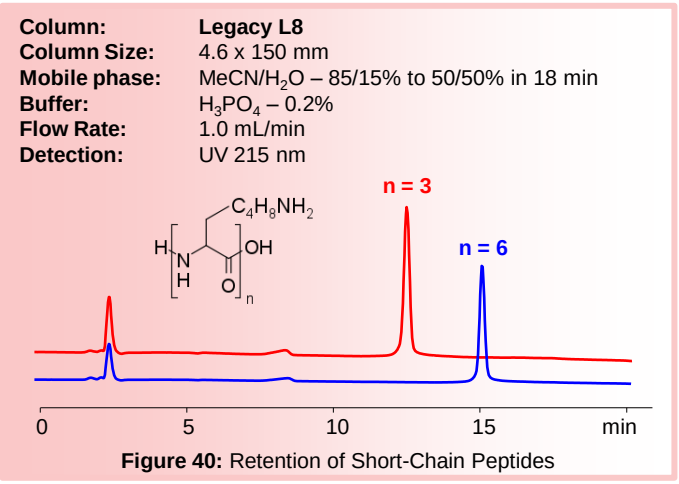


**Figure 40:** Retention of Short-Chain Peptides

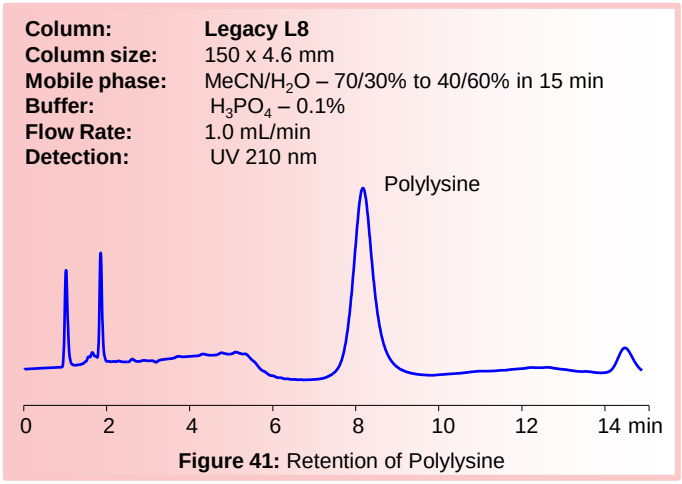


**Figure 41:** Retention of Polylysine

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**Column:** **Legacy L8**
**Column Size:** 4.6 x 150 mm
**Flow Rate:** 1.0 mL/min
**Mobile phase:** MeCN/$H_2O$ – 80/20% to 55/45% in 15 min
**Buffer:** $H_2SO_4$ – 0.2%
**Detection:** UV 215 nm

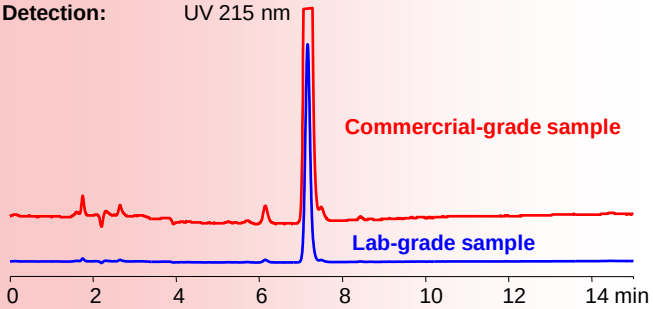


**Figure 42:** Retention of m-Xylylenediamine

*m*-Xylylenediamine (MXDA) is popular industrial compound used as a curing agent for various epoxy resins. Figure 42 shows how a commercial-grade and lab-grade sample it can be retained and identified on a Legacy L8 column with a mobile phase gradient consisting of MeCN, water, and sulfuric acid as a buffer.

## **L9 –** Cation-Exchange

The Legacy L9 column is a silica-based column that consists of strongly a acidic, cation-exchange coating on the surface of totally porous silica particles and meets the qualifications for the L9 designation. The column is capable of both cation-exchange and hydrophilic interaction (HILIC) chromatography.

Acid anhydrides are compounds derived from acids by the removal of water. Examples include Succinic Anhydride (from succinic acid), Benzoic Anhydride, Diglycolic Anhydride, and Maleic Anhydride. Figure 43 shows how these acid anhydrides can be retained on a Legacy L9 column with a mobile phase consisting of just MeCN without any buffer or aqueous component.

Toluenesulfonic acid or tosylic acid (TsOH) is a very strong organic acid with applications in several syntheses that require an organic-soluble acid. Naphthalenedisulfonic acid, or Armstrong's acid, is a fluroescent strong acid commonly used in the pharmaceutical industry to synthesize drugs. 3,5-Dihydroxybenzoic Acid, or 3,5-DHBA, is another organic acid and is a metabolite involved in the body's synthesis of resorcinolic lipds. Figure 44 shows how these organic acids, including ascorbic acid, can be retained and separated using cation-exchange chromatography on a Legacy L9 column with a mobile phase consisting of MeCN, water, and ammonium acetate as a buffer.

**Column:** **Legacy L9**
**Column size:** 4.6 × 250 mm, 5 µm 100A
**Mobile phase:** MeCN -100%
**Flow rate:** 1.0 mL/min
**Detection:** UV 210 nm

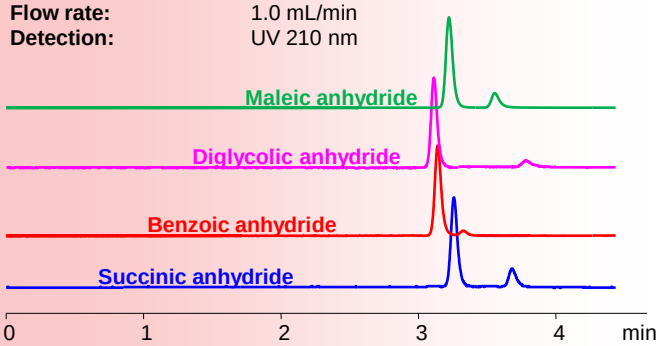


**Figure 43:** Retention of Acid Anhydrides

**Column:** **Legacy L9**
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 85/15%
**Buffer:** AmAc –15 mM, pH 5.0
**Flow:** 1.0 mL/min
**Detection:** UV 250 nm

1. TsOH
2. Naphthalenedisulfonic acid
3. 3.5 DHBA
4. Ascorbic acid (Vitamin C)

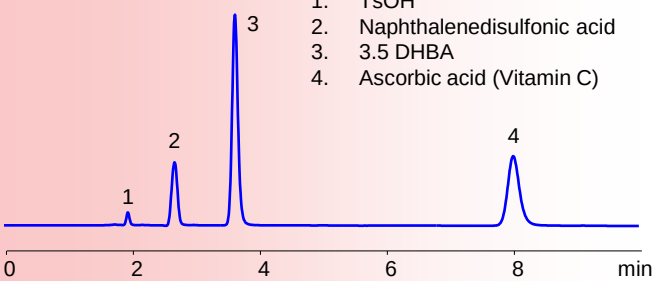


**Figure 44:** Retention of Organic Acids

## **L16 –** Dimethylsilane

The Legacy L16 column is a column with dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm in diameter. This column is useful for separating very hydrophobic molecules or large molecules with multiple points of interaction with the stationary phase. Compounds such as lipids in high organic MP can be analyzed using this column.

The Legacy L78 column is a silica-based, mixed-mode column that consists of both reversed-phase ligands (alkyl chains longer than 8 Carbons, or C8) and anion-exchange (primary, secondary, or tertiary amino) functional groups chemically bonded to porous silica that meets the USP qualifications for the L78 designation. As a mixed-mode column, it can be tuned to retain and separate a wide variety of compounds based on several different interactions, including ion-exchange, hydrophobic, and hydrophilic interactions.

Amitryptiline is a tricyclic compound with anti-depresseant and pain-relief properties (particularly for nerve pain). Figure 45 shows how it can be retained on a Legacy L78 column with a mobile phase consisting of MeCN, water, and trifluoroacetic acid (TFA).

Vitamin E is a fat soluble vitamin that the body uses as an antioxidant. Vitamin E acetate, a synthetic form of Vitamin E, has gained notoriety for being a harmful thickening additive in vaping cartridges that has led to a recent rise in vaping-associated pulmonary injury. Figure 46 shows how these two similar compounds can be retained on a Legacy L78 column with a mobile phase consisting of MeCN, water, and no buffer.

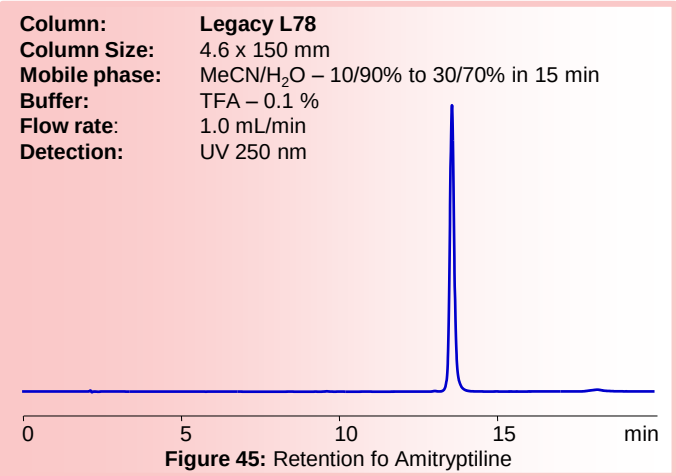


**Figure 45:** Retention fo Amitryptiline

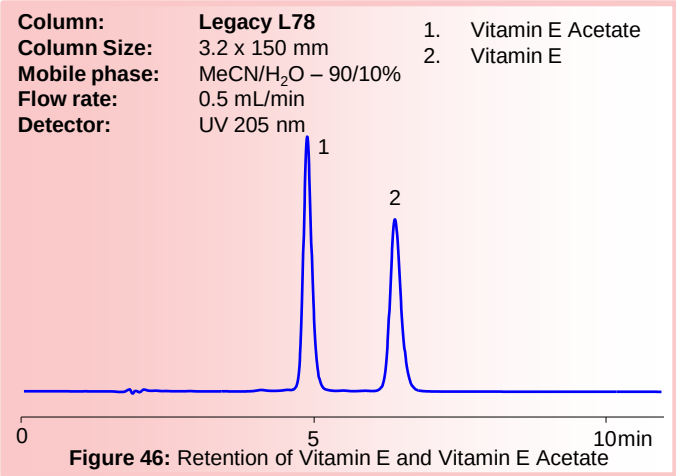


**Figure 46:** Retention of Vitamin E and Vitamin E Acetate

Quinidine and Quinine have the same chemical formula and only differ in the position of two functional groups; thus, they are diastereoisomers. Quinine has fever-reducing and anti-inflammatory analgesic properties, while quinidine has anti-arrhythmic and anti-parasitic properties. Figure 47 shows how these two nearly identical compounds can be retained and separated on a Legacy L78 column with a mobile phase gradient consiting of MeCN, water, and trifluoroacetic acid as a buffer.

Vitamin C and Vitamin $B_2$ are both water-soluble vitamins that require daily replacement through our diets. Figure 48 shows how these vitamins can be retained and separated on a Legacy L78 column with a mobile phase consisting of MeCN, water, and acetic acid.

Cidofovir is a pyrimidine-based phosphate with anti-viral properties. Figure 49 show how Cidofovir and Cyclo-cidofovir can be retained and separated on a Legacy L78 column with a mobile phase consisting of MeCN, water, and acetic acid as a buffer.

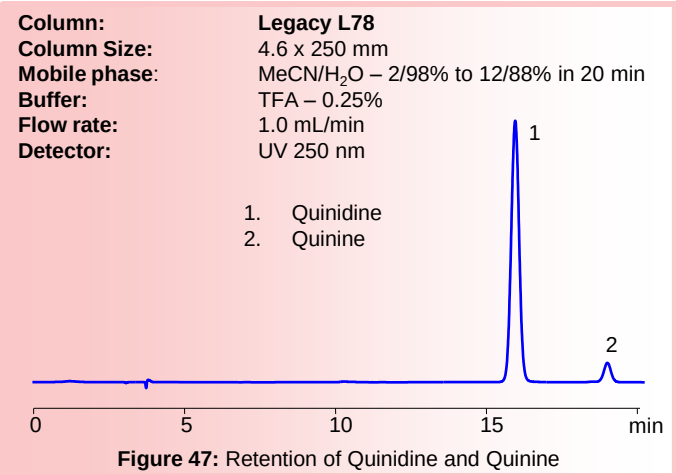


**Figure 47:** Retention of Quinidine and Quinine

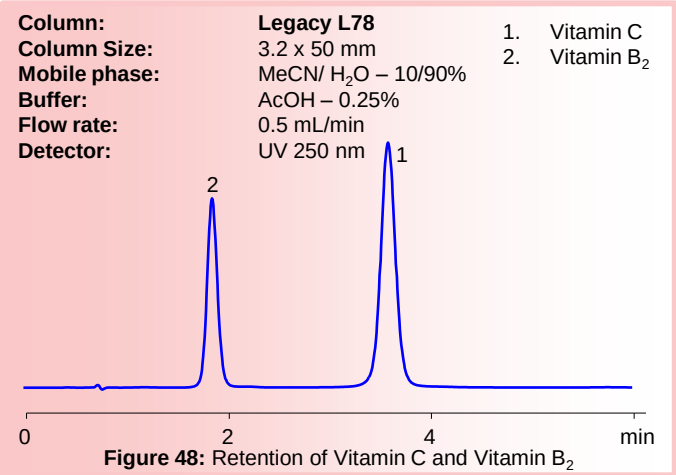


**Figure 48:** Retention of Vitamin C and Vitamin $B_2$

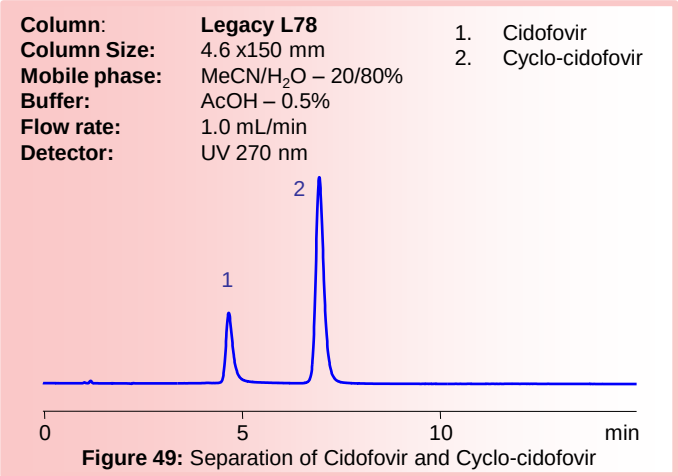


**Figure 49:** Separation of Cidofovir and Cyclo-cidofovir

## **L85 –** Mixed-Mode, Negative Surface

The Legacy L85 column is a silica-based, mixed-mode column that consists of both reversed-phase ligands (alkyl chains longer than 8 Carbons, or C8) and cation-exchange (carboxyl) functional groups chemically bonded to porous silica that meets the USP qualifications for the L85 designation.

Guanidine is a strong basic compound used in the production of explosives and plastics. Figure 50 shows how it can be retained on a Legacy L85 column with a mobile phase consisting of MeCN, water, and trifluoroacetic acid as a base.

Amoxicillin is an antibacterial used to treat strep throat, pneumonia, urinary tract infections, and many others. Figure 51 shows how they can be separated via electrostatic, hydrophobic, and polar interactions on this mixed-mode Legacy L85 column using a mobile phase consisting of MeCN, water, and trifluoroacetic acid (TFA) as a buffer.

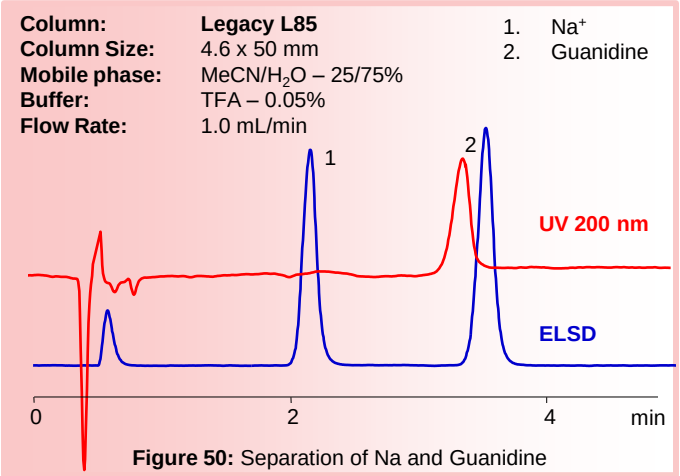


**Figure 50:** Separation of Na and Guanidine

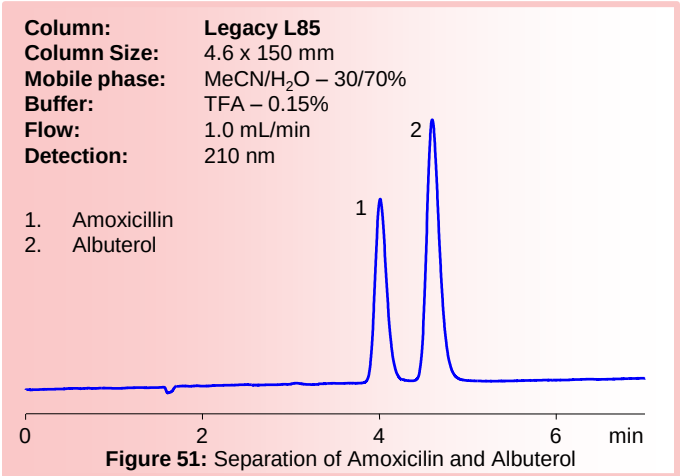


**Figure 51:** Separation of Amoxicilin and Albuterol

Figure 52 show how Cidofovir and Cyclo-cidofovir can be retained and separated on a Legacy L85 column with a mobile phase consisting of MeCN, water, and sulfuric acid as a buffer. Notice how the retention order is reversed from Fig. X with a Legacy L78 column. This is due to the surfaces of the two different columns being oppositely charged, so the acidic compounds retain in the opposite order when the surface charge is switched.

The catecholamine neurotransmitters are a set of neurotransmitters essential to the nervous system that have dual functionality as hormones in the endocrine system. These neurotransmitters, such as SOPA, epinephrine, norepinephrine, dopamine, and phenylaline, are all amino-acid derivatives of tyrosine. Figure 53 shows how these compounds can be retained and separated on a Legacy L85 column

Figure 54 shows the separation of a mixture of amino acids with an acid (buffer) gradient. Where a mobile phase gradient can help separate compounds with significantly different polarities, an acid gradient can help separate compounds with significantly different pKa values within a single run through the column.

**Column:** Legacy L85
**Column Size:** 3.2 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 20/80%
**Buffer:** $H_2SO_4$ – 0.05%
**Flow Rate:** 0.5 mL/min
**Detection:** 270 nm

1. Cidofovir
2. Cyclo-cidofovir

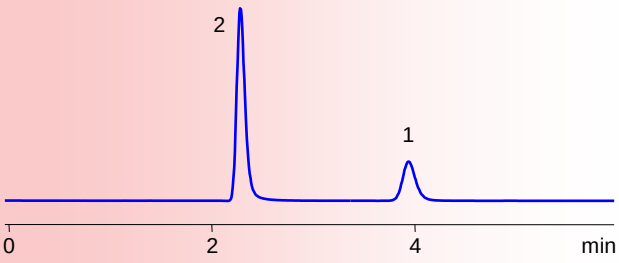


**Figure 52:** Separation of Cidofovir and Cyclo-cidofovir

**Column:** Legacy L85
**Column Size:** 4.6 x 150 mm
**Mobile phase:** MeCN/$H_2O$ – 10/90%
**Buffer:** $Na_2HPO_4$ – 20 mM, pH 3.0
**Flow Rate:** 1.0 mL/min
**Detection:** UV 210 nm

1. Dopa
2. Tyrosine
3. Phenylalanine
4. Norepinephrine
5. Epinephrine
6. Dopamine

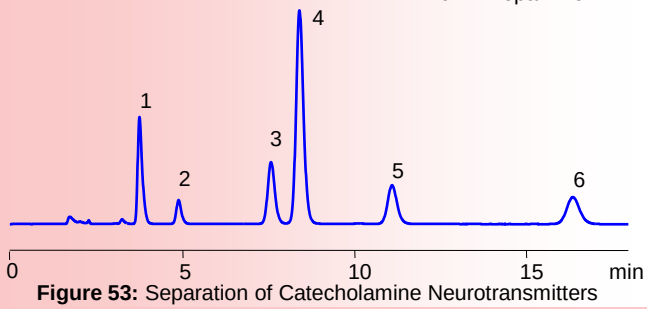


**Figure 53:** Separation of Catecholamine Neurotransmitters

The Legacy L85 column can interact with a wide variety of analytes in a number of ways, depending on the properties of the mobile phase, such as the buffer pH, organic and buffer concentrations, and the gradient type (if applicable). Below in Figures 55 thru 57, the column was 'tuned' to an ion-exchange mode in order to retain and separate positively charged ions. Notice that the relative concentration of MeCN is fairly high in Figures 55 and 56, and the gradient increases in MeCN concentration in Figure 57; since the ions are not as soluble in MeCN as they are when in water, they favor interacting with the stationary phase and it's embedded charged functional group and thus are able to be retained and separated.

When a complex mixture is analyzed using Legacy L85 columns, two or more interaction mechanisms help to tune the separation. Elution order and retention time can be adjusted in accordance with your analytical needs. The typical combinations of the mechanisms are reverse-phase – ion-exchange; reverse phase – ion exclusion; hydrophilic interaction – ion-exchange; chelating - reverse phase.

**Column:** Legacy L85
**Column Size:** 4.6 x 250 mm
**Mobile phase:** MeCN/$H_2O$ – 30/70%
**Buffer:** TFA – 0.05% to 0.2% in 25 min
**Flow:** 1 mL/min
**Detection:** ELSD

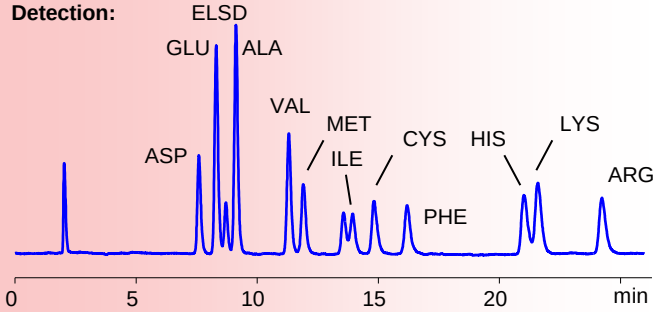


**Figure 54:** Separation of Common Collection of Amino Acids

**Column:** Legacy L85
**Column Size:** 4.6 x 250 mm
**Mobile phase:** MeCN/$H_2O$ – 20/80%
**Buffer:** TFA – 0.2%
**Flow Rate:** 1.0 mL/min
**Detection:** ELSD

1. $Li^+$
2. $Na^+$
3. $NH_4^+$
4. $K^+$

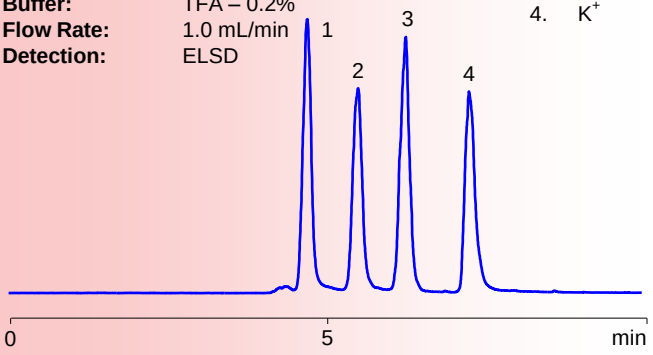


**Figure 55:** Separation of Cations on a Mixed-Mode Column

1. $Li^+$
2. $Na^+$
3. $K^+$
4. $Mg^{2+}$
5. $Ca^{2+}$
6. $Zn^{2+}$

**Column:** Legacy L85
**Column Size:** 250 x 4.6 mm
**Mobile Phase:** MeCN/$H_2O$ – 70/30%
**Buffer:** TFA – 0.2%
**Flow rate:** 0.5 mL/min
**Detection:** ELSD

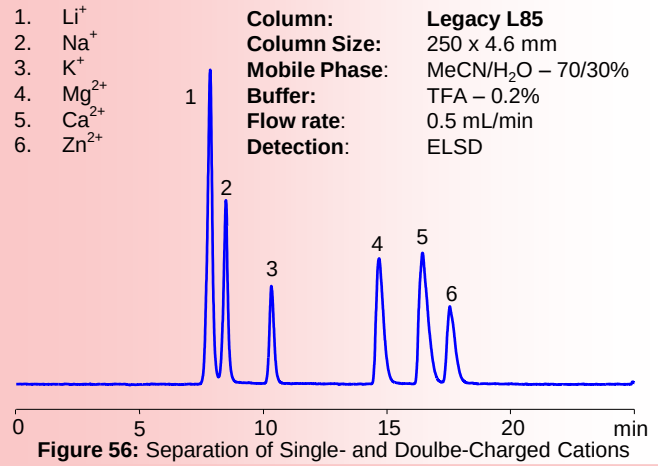


**Figure 56:** Separation of Single- and Doulbe-Charged Cations

**Column:** Legacy L85
**Column Size:** 4.6 x 150 mm
**Gradient:** 12 min with 4 min hold
**Mobile phase:** MeCN/$H_2O$ – 5/95 to 50/50%
**Buffer:** TFA – 0.03 to 0.2%
**Flow Rate:** 1.0 mL/min
**Detection:** ELSD

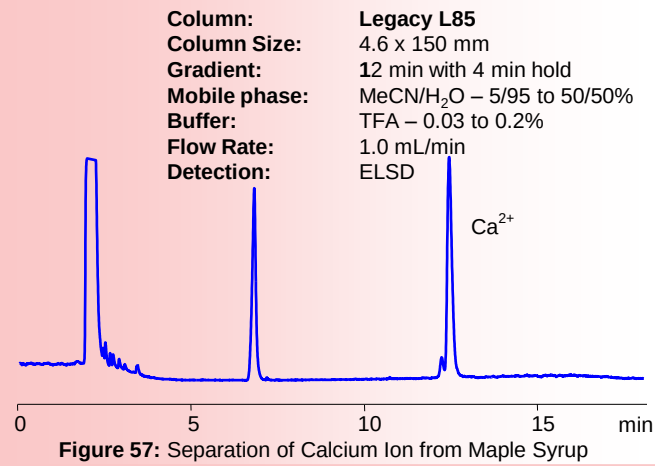


**Figure 57:** Separation of Calcium Ion from Maple Syrup

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## Specifications Legend

The table below shows all the Legacy columns beyond the ones shown in this brochure. If you have any questions or would like to place an order, please contact us so we can help you determine which column type is best for you.

| SIELC Column | USP Specification |
|---|---|
| **Legacy L1** | Octadecyl silane chemically bonded to porous silica or ceramic particles (3 to 10µm) |
| **Legacy L3** | Porous silica microparticles 1.5 to 10µm in diameter |
| **Legacy L6** | Strong cation-exchange packing - sulfonated fluorocarbon polymer coated on a solid spherical core, 30 to 50 µm in diameter. |
| **Legacy L7** | Octyl silane ($C_8$) chemically bonded to porous silica particle- 3 to 10µm in diameter |
| **Legacy L8** | An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 3-10µm diameter |
| **Legacy L9** | Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter. |
| **Legacy L10** | Nitrile groups chemically bonded to porous silica particles 3 to 10µm in diameter |
| **Legacy L11** | Phenyl groups chemically bonded to porous silica particles 3 to 10µm in diameter |
| **Legacy L12** | A strong anion-exchange packing made by chemically bonding a quaternary amine to a solid silica spherical core, 3 to 50 µm in diameter |
| **Legacy L13** | Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter |
| **Legacy L14** | Silica gel, 5 to 10µm in diameter, having a chemically bonded, strongly basic quaternary ammonium anion exchanger (SAX) coating. |
| **Legacy L16** | Dimethylsilane ($C_2$) chemically bonded to totally porous silica particles - 5 to 10µm. |
| **Legacy L18** | Amino and cyano groups chemically bonded to porous silica particles, 5 to 10µm in diameter |
| **Legacy L20** | Dihydroxypropane (diol) groups chemically bonded to porous silica or hybrid particles, 3 to 10µm in diameter. |
| **Legacy L26** | Butyl silane ($C_4$) chemically bonded to porous silica particle- 3 to 10µm. |
| **Legacy L28** | A multifunctional support which consists of a high purity, 100A, spherical silica substrate that has been bonded with anionic (amine) functionality in addition to conventional reversed-phase $C_8$ functionality. |
| **Legacy L44** | A multifunctional support, which consists of high purity, 100A, spherical silica substrate that has been bonded with a cationic exchanger, sulfonic acid functionality in addition to a convention reversed-phase $C_8$ functionality |
| **Legacy L78** | A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and anion-exchange (primary, secondary, or tertiary amino groups) functional groups chemically bonded to porous or non-porous or ceramic micro-particles, 3 to 50µm in diameter or a monolithic rod |
| **Legacy L85** | A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and weak cation-exchange (carboxyl groups) functional groups chemically bonded to porous or non-porous particles, 3 to 50µm in diameter |

All data were obtained in SIELC Technologies labs.

## Legacy Column Part Number Generator

**L1 - 46 . 150 . 0510**

**Type of packing**

| | |
|---|---|
| Legacy L1 | **L1** |
| Legacy L3 | L3 |
| Legacy L7 | L7 |
| Legacy L8 | L8 |
| Legacy L9 | L9 |
| … | … |
| Legacy L78 | L78 |
| Legacy L85 | L85 |

**Column id**

| | |
|---|---|
| 50 mm | 500 |
| 30 mm | 300 |
| 22 mm | 220 |
| 10 mm | 100 |
| 4.6 mm | **46** |
| 3.2 mm | 32 |
| 2.1 mm | 21 |
| 1.0 mm | 10 |
| 0.5 mm | 05 |

**Column length**

| | |
|---|---|
| 250 mm | 250 |
| 150 mm | **150** |
| 100 mm | 100 |
| 50 mm | 50 |
| 25 mm | 25 |
| 10 mm | 10 |
| Guard | G |

**Packing size**

| | |
|---|---|
| 3 µm | 03 |
| 5 µm | **05** |
| 10 µm | 10 |

**Pore size**

| | |
|---|---|
| 100 Å | **10** |

SIELC

To order a Column or ask a question send your message to **sales@sielc.com** or call us at : **+1 (847) 229-2629**